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## The Influence of *Fusarium* Infection on Wheat (*Triticum aestivum* L.) Proteins Distribution and Baking Quality

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Under artificial *Fusarium* infection the total glutenin content determined by chromatographic (RP-HPLC) method was significantly reduced in comparison to gliadins which were increased. Among protein types,  $\alpha$ -GLI and HMW-GS were the highest affected. Artificial *Fusarium* infection significantly increased GLI/GLU ratio when compared with the natural infected samples. Artificial *Fusarium* infection dramatically decreased the dough mixing tolerance and had a considerable negative effect on dough energy, maximum resistance, and resistance/extensibility ratio. Disturbed GLI/GLU ratio and an increased amount of mycotoxin DON under artificial *Fusarium* infection showed a strong negative impact on affected functional properties of dough and bread. Total and  $\gamma$ -GLI as well as GLI/GLU ratio were significantly positively affected by mycotoxin DON in contrast to total GLU, HMW-GS and LMW-GS which were negatively affected. Results indicated that the stability of baking quality parameters of cultivars more tolerance to the *Fusarium* infection can be well define by lower accumulation of mycotoxin DON.

**Keywords:** wheat, *Fusarium culmorum*, wheat proteins, baking quality, RP-HPLC

### Introduction

*Fusarium* head blight (FHB) or scab is a destructive fungal disease of small-grain cereals which occurs worldwide (Lemmens et al. 2004). The report on the molecular identification of *Fusarium* species isolated from naturally infected wheat ears in East Croatia showed that the dominant species were *F. graminearum*, *F. culmorum* and *F. avenaceum* (Spanic et al. 2010). The capability of *Fusarium* species to produce mycotoxins represents a potential risk to human and animal health (Nightingale et al. 1999). In Europe, trichothecane mycotoxins such as deoxynivalenol (DON) and its derivatives (3-ADON, 15-ADON) and zearalenone (ZEA) are the most commonly found in *Fusarium* spp. infected grains (Bottalico and Perrone 2002).

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The viscoelastic properties of wheat dough make it suitable for various leavened bakery products. The gluten proteins play an important role in determining the wheat processing quality (Shewry and Halford 2002; Torbica et al. 2007; Horvat et al. 2013). According to the solubility, Osborne classified wheat proteins into albumins and globulins (AG) (soluble in water and dilute salt), gliadins (GLI) (alcohol soluble) and glutenins (GLU) (soluble in dilute acid or alkali). Gluten constitutes 80–85% of total protein in mature wheat grain and are composed of GLI and GLU and their single protein types ( $\omega$ -,  $\alpha$ - and  $\gamma$ -GLI and high molecular weight-glutenin subunits (HMW-GS) and low molecular weight-glutenin subunits (LMW-GS) (Wieser et al. 1998). In the evaluation of baking quality the glutenin polymers which are covalently linked into large elastic networks are of particular importance. It is well established that flour with higher gluten strength contain favourable HMW-GS (1 and 2 at the Glu-1 locus, 7+8 at the Glu-B1 locus and 5+10 at the Glu-D1 locus) and higher proportions of HMW-GS. During the dough formation the gliadins act as a ‘plasticizer’, promoting viscous flow and extensibility which are important rheological characteristics of dough (Wieser and Kieffer 2001; Shewry and Halford 2002). AG constitute 10–22% of total flour protein and have mainly metabolic activity or structural functions with minor effects on wheat processing quality (Horvat et al. 2007; Gao et al. 2009).

There have been several studies (Meyer et al. 1986; Dexter et al. 1996; Nightingale et al. 1999; Prange et al. 2005; Wang et al. 2005) focusing on the influence of *Fusarium* infection on the baking properties in relation to protein changes but these works were mainly focused only on the major components of gluten (total GLI and GLU, HMW-GS and LMW-GS) using a different biochemical separation technique (SDS-PAGE, RP-HPLC, SE-HPLC and turbidimetry). Eggert et al. (2010, 2011) were the only studies of the influence of natural and artificial *Fusarium* infection on the protein fractions and their single protein types according to the detailed classification of Wieser et al. (1998) using RP-HPLC.

The present study is focused on the degradation of proteins and baking properties of wheat under artificial *Fusarium* infection in comparison with natural infected samples. The obtained results could enlarge the current knowledge about diminishing of processing quality of wheat in relation to protein components degradation under *Fusarium* infection.

## Materials and Methods

### *Plant material and experimental design*

Twelve wheat cultivars were grown at the experimental field of the Agricultural Institute Osijek, Croatia (45°32"N, 18°44"E, 94 m above sea level, eutric cambisol) during 2008/2009 and 2009/2010 growing seasons. French cultivar Soissons was included in the experiment because it is well distributed at Croatian wheat market due to well-balanced agronomic and baking properties. The included cultivars differed in their susceptibility against *Fusarium* infection and in baking performance (Table 1).

The field trials were set up as randomized complete block (RCB) design with three repetitions which were bulked and homogenized after the harvest and used for further analysis. The area of one experimental plot was 7.56 m<sup>2</sup> with sowing rate of 330 seeds m<sup>-2</sup>. The

wheat samples were artificially inoculated with a *Fusarium culmorum* spore suspension ( $10 \times 10^4$  spores  $\text{ml}^{-1}$ ) by spraying the inoculum directly on the wheat spikelet at a time when 50% of the wheat ears per plot were at flowering. The average annual temperatures during growing period in 2008/2009 and 2009/2010 were 10.8 and 10.3°C, respectively, while the sum of precipitation were 368.6 and 846.6 mm, respectively. In this study the field observation of the *Fusarium* infection severity were not made and no kernel was rated; only the content of mycotoxin DON was measured.

#### *Baking quality evaluation and deoxynivalenol (DON) assay*

The wheat grain samples were conditioned at 15.5% moisture before milling on a Brabender Quadromat Senior Mill (ash content 0.50%–0.60%). Protein content (PC) (Nx5.7, dry matter) was measured by NIT technology (Infratec 1241, Foss Tecator), while the determination of thousand-kernel weight (TKW) was obtained by counting (Contador, Pfeuffer) and weighing.

Table 1. Wheat cultivars origin and HMW-GS\* composition at the Glu-1 loci

Cultivars	Origin**	Year of release	HMW-GS			GLU-1 score***	Susceptibility**** to Fusarium
Olimpija	AIO	2009	2*	7+9	5+10	9	MR
Divana	JS	1995	2*	7+9	5+10	9	MR
Srpanjka	AIO	1989	N	7+8	2+12	6	MR
Seka	AIO	2006	1	7+9	5+10	9	MR
Žitarka	AIO	1985	N	7+8	2+12	6	MS
Aida	AIO	2006	1	17+18	5+10	10	MR
Felix	AIO	2007	2*	7+8	5+10	10	S
Soissons	FD	1987	2*	7+8	5+10	10	MR
Zlata	AIO	2007	2*	7+9	5+10	9	MS
Ilijija	AIO	2008	2*	7+8	5+10	10	MR
Sana	BC	1983	2*	6+8	2+12	6	S
Golubica	AIO	1997	N	7+9	2+12	5	S

\* HMW-GS = high molecular weight-glutenin subunits.

\*\* AIO = Agricultural Institute Osijek, Croatia; JS, Jost-Seed, Croatia; BC, Bc Institute, Croatia; FD, Florimond Desprez, France.

\*\*\* According to the Payne and Lawrence nomenclature (1987).

\*\*\*\* MR = moderately resistant; MS = moderately susceptible; S = susceptible.

The dough rheological properties were evaluated by the Farinograph and Extensograph (Brabender, Germany) in accordance with ICC method No 115/1 and No 114/1, respectively. The chemical and rheological analysis of grain and flour were done in duplicate. The baking test was performed by adding water following the farinographic method, 1.5% salt, 1.86% sugar, 1.8% compressed yeast and 0.005% ascorbic acid (based on flour weight). The components were mixed in a Diosna SP 12 spiral mixer (Dierks and Söhne GmbH, Germany). Dough pieces were scaled, rounded, rested 30 min, sheeted and moulded, placed in tins, proofed for 50 min (30°C, 87% RH) and baked in a Miwe BackCombi oven (Miwe Michael Wenz GmbH, Germany) for 30 min at a temperature

gradient from 240 to 220°C. Bread volume ( $V_{\text{SPEC}}$ ) was measured using a volumeter (Chopin Technologies, France) after 60 min of cooling using the millet displacement method. The crumb porosity was scored according to the Dallmann's pore table. The shape of bread slice was defined by height/diameter ratio (H/D). The measurements were carried out in triplicate. Bread crust colour was determined by measuring the lightness ( $L^*$ -value) of bread sample with the Minolta Chroma Meter CR-400 (Minolta, Japan).  $L^*$ -value was measured at five different points of loaf surface. The measurement was conducted on three loaves and mean value was calculated. DON content was determined in duplicate by Veratox for DON 5/5 enzyme-linked immunosorbent assay (ELISA) kits (Neogen Corporation, USA) on 10 g of flour.

#### *Proteins characterization*

Composition of HMW-GS was analysed by sodium-dodecyl-sulphate-polyacrylamide-gel electrophoresis (SDS-PAGE) using a Hoefer SE 600 vertical slab gel unit (Amersham Biosciences, Germany). The nomenclature system of Payne and Lawrence (1983) was used for the HMW-GS identification. The wheat proteins extraction from 100 mg of flour sample was done stepwise accordingly to the procedure of Wieser et al. (1998). Proteins separation was carried out using Perkin Elmer LC 200 chromatograph controlled by Total-Chrom software (Perkin Elmer Instruments, USA) on a Discovery Bio Wide Pore C18 column (300 Å pore size, 5 µm particle size, 4.6×150 mm i.d.) (Sigma-Aldrich Chemie GmbH, Germany). A 0.1% trifluoroacetic acid (TFA) in water (v/v) and 0.1% TFA in acetonitrile (ACN) were applied as mobile phase and 20 µl samples were injected for analyses. AG, GLI and GLU fractions were eluted with a linear gradient from 24% to 58% ACN over 30 min at flow 1 ml/min using a column temperature of 50°C. All determinations were made in duplicate. The peak areas under AG, GLI and GLU chromatograms were summed and used as a direct measure of total content of extractable wheat proteins. Consequently the proportions (%) of protein fractions and single protein types were calculated.

#### *Statistics*

The results were statistically evaluated by the ANOVA with subsequent Tukey's test, ( $P < 0.05$ ). Principal component analysis (PCA) was applied to effectively reduce large set of data into lower dimension of latent variables amenable for analysis (Kurtanjek et al. 2008). Statistical analysis was performed using STATISTICA 8.0 (StatSoft Inc., USA) software.

## **Results**

The year had significant effect on nearly all of the analysed parameters, but due to the large amount of data, the results are expressed as mean values of obtained data under natural and artificial *Fusarium* infection, since the effect of the *Fusarium* treatment was more pronounced when compared to the effect of the years included in this study.

*The influence of Fusarium infection on baking properties*

The grain PC varied between 12.0 and 19.0% and averagely was not significantly affected by the artificial *Fusarium* infection, while the TKW was significantly decreased (7.3%) (Table 2).

The content of mycotoxin DON showed a significant increase in artificial infected flour (3.98 mg/kg) when compared to natural infection (0.69 mg/kg). The range of DON content values indicates that the extent of the *Fusarium* effect appears to be cultivar (Fig. 1, Table 2) and year specific. During the growing season 2008/2009 DON was not detected in flour samples under natural infection, while in 2009/2010, which was more susceptible for *Fusarium* infection, only three cultivars had DON content below the EU limit value of 0.75 mg/kg that refers to the flour for direct human consumption.

Dough development time (DDT), degree of softening (DS), farinograph quality number (FQN), energy (E), maximum resistance ( $R_{MAX}$ ) and resistance to extensibility ratio (R/EXT) varied between weak to very strong implying cultivar and year specific differences (Table 2). WA capacity in *Fusarium* infected samples was decreased by 1.6%. *Fusarium* infection decreased DDT by 17.0% and dramatically decreased the dough mixing tolerance (DS increased by 81.0%) (Table 2). *Fusarium* infection had a considerable negative effect on E,  $R_{MAX}$  and R/EXT and these parameters were decreased by 57.7%,

Table 2. Baking properties of wheat and DON content under natural and artificial *Fusarium* infection

Parameters	Natural		Artificial		Difference Artificial/Natural (%)
	Mean* $\pm$ sd	Range	Mean $\pm$ sd	Range	
PC** (%)	14.6 <sup>ns</sup> $\pm$ 1.7	12.0–18.9	14.5 $\pm$ 1.6	12.3–19.0	–0.9
TKW (g)	39.7 <sup>a</sup> $\pm$ 8.8	22.6–54.0	36.8 <sup>b</sup> $\pm$ 6.4	27.5–51.8	–7.3
WA (%)	59.8 <sup>a</sup> $\pm$ 2.5	53.8–64.4	58.8 <sup>b</sup> $\pm$ 2.4	53.9–63.8	–1.6
DDT (min)	3.8 <sup>a</sup> $\pm$ 3.0	1.5–11.5	3.2 <sup>b</sup> $\pm$ 2.2	1.2–9.6	–17.0
DS (FU)	52 <sup>b</sup> $\pm$ 26.2	15–125	94 <sup>a</sup> $\pm$ 49.1	0.0–211	81.0
FQN	85 <sup>a</sup> $\pm$ 54.7	27–200	68 <sup>b</sup> $\pm$ 38.8	23–160	–19.9
E (cm <sup>2</sup> )	98 <sup>a</sup> $\pm$ 37.2	42–167	42 <sup>b</sup> $\pm$ 35.1	0.0–108	–57.7
$R_{MAX}$ (EU)	434 <sup>a</sup> $\pm$ 162.2	191–739	182 <sup>b</sup> $\pm$ 146.5	0.0–462	–58.2
R/EXT	1.6 <sup>a</sup> $\pm$ 0.5	0.8–2.4	0.8 <sup>b</sup> $\pm$ 0.5	0.0–1.7	–50.2
DON (mg/kg)	0.69 <sup>b</sup> $\pm$ 1.09	0.00–3.80	3.98 <sup>a</sup> $\pm$ 2.12	1.30–8.90	476.8
$V_{SPEC}$ (cm <sup>3</sup> /g)	3.6 <sup>ns</sup> $\pm$ 0.4	2.8–4.4	3.4 $\pm$ 0.9	0.0–4.8	–6.6
H/D	0.66 <sup>a</sup> $\pm$ 0.1	0.5–0.9	0.56 <sup>b</sup> $\pm$ 0.2	0.0–0.8	–16.2
L*	62.0 <sup>a</sup> $\pm$ 9.5	45.0–74.1	57.9 <sup>b</sup> $\pm$ 13.7	41.2–73.1	–6.6
POR	6.8 <sup>a</sup> $\pm$ 0.7	5.0–7.5	5.6 <sup>b</sup> $\pm$ 1.3	3.0–7.5	–17.6

\* Mean  $\pm$  standard deviation of 12 cultivars during two years ( $n = 24$ ); means followed by the same letter in the row are not significantly different at  $P < 0.05$  according to Tukey's test (ns = not significant).

\*\* PC = crude protein content on dry matter basis; TKW = thousand-kernel weight; WA = water absorption; DDT = dough development time; DS = degree of softening; FQN = Farinograph quality number; E = dough energy;  $R_{MAX}$  = maximum resistance to extension; R/EXT = ratio of resistance and extensibility; DON = deoxynivalenol;  $V_{SPEC}$  = specific bread volume; H/D = height/diameter ratio; L\* = lightness of bread crust; POR = crumb porosity according to the Dallmann's pore table (1–8), where 1 represents the largest pores and 8 the smallest ones.

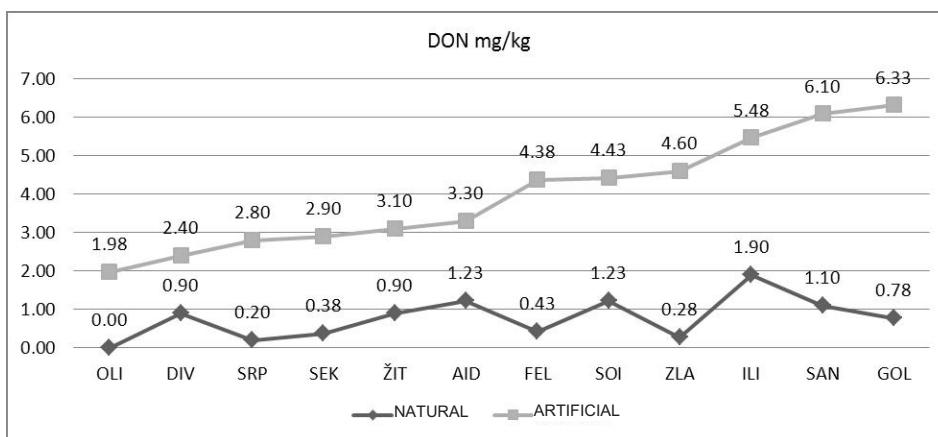


Figure 1. Content of mycotoxin DON in wheat cultivars under natural and artificial *Fusarium* infection. Cultivar abbreviations for natural infection: OLI = Olimpija, DIV = Divana, SRP = Srpanjka, SEK = Seka, ŽIT = Žitarka, AID = Aida, FEL = Felix, SOI = Soissons, ZLA = Zlata, ILI = Ilirija, SAN = Sana, GOL = Golubica

58.2% and 50.2%, respectively (Table 2). The artificial *Fusarium* infection averagely showed non-significant reduction of  $V_{SPEC}$ , while a significant differences in bread shape (H/D) were observed as well as a more intensive browning (decreased  $L^*$ -value by 6.6%) of the bread crust (Table 2). Under *Fusarium* infection crumb porosity scored by Dallman's was more inconsistent and random (decreased POR by 17.6%) (Table 2).

#### *The influence of Fusarium infection on wheat proteins*

The percentage distribution of analysed proteins showed that AG was not significantly affected by artificial *Fusarium* infection. The proportions of total GLI and  $\alpha$ - and  $\gamma$ -GLI significantly increased after artificial infection (6.9%, 9.0% and 6.9%, respectively), while total GLU showed a significant reduction (10.0%) (Table 3). Concerning GLU main sub-units, the proportion of HMW-GS and LMW-GS was reduced by 11.5% and 9.4%, respectively (Table 3). The reduction of GLU fraction was related to infection level, in the way the reduction of HMW-GS in the severely infected samples varied between 20.4% and 25.5%. *Fusarium* treatment had a significant influence on GLI/GLU ratio (Table 3). The increase of GLI/GLU ratio in the severely infected samples varied from 25.8% to 42.9%. The GLI/GLU ratio had a strong impact on DS, E,  $R_{MAX}$ , R/EXT and bread shape (H/D) (Fig. 2). The particular cultivars data are not shown.

#### *The relationship among DON and baking and proteins quality*

The obtained results showed that H/D, DS, E,  $R_{MAX}$ , R/EXT were strongly negatively influenced by DON content that represents an indirect measure of level of *Fusarium* infection. Total and  $\gamma$ -GLI as well as GLI/GLU ratio were significantly positively affected by DON in contrast to total GLU, HMW-GS and LMW-GS which were negatively affected (Fig. 2).

Table 3. Proteins distribution (%) in wheat samples under natural and artificial *Fusarium* infection

Protein fractions (%)*	Type	Natural		Artificial		Difference Artificial/Natural (%)
		Mean** ± sd	Range	Mean ± sd	Range	
AG***	Total	14.8 <sup>ns</sup> ± 2.9	11.0–23.4	14.4 ± 2.4	10.0–20.8	-2.3
GLI	Total	52.5 <sup>b</sup> ± 3.5	42.2–59.8	56.1 <sup>a</sup> ± 4.1	49.3–63.6	6.9
	ω-	4.1 <sup>ns</sup> ± 1.1	2.3–7.6	3.8 ± 0.8	2.4–6.9	-7.4
	α-	27.6 <sup>b</sup> ± 3.0	19.8–32.1	30.1 <sup>a</sup> ± 3.3	23.2–37.2	9.0
	γ-	20.7 <sup>b</sup> ± 2.9	16.7–29.3	22.2 <sup>a</sup> ± 2.9	17.5–29.7	6.9
GLU	Total	32.7 <sup>a</sup> ± 2.8	26.4–37.1	29.4 <sup>b</sup> ± 4.2	21.6–37.2	-10.0
	HMW-GS	9.2 <sup>a</sup> ± 1.3	5.5–13.1	8.2 <sup>b</sup> ± 1.5	5.0–11.8	-11.5
	LMW-GS	23.4 <sup>a</sup> ± 2.2	18.2–28.9	21.2 <sup>b</sup> ± 3.0	16.6–26.8	-9.4
GLI/GLU		1.63 <sup>b</sup> ± 0.2	1.23–2.26	1.97 <sup>a</sup> ± 0.5	1.34–2.90	20.9

\* The peak areas under AG, GLI and GLU chromatograms were summed and used as a direct measure of total extractable proteins and consequently the proportion of protein fractions and single protein types were calculated.

\*\* Mean ± standard deviation of 12 cultivars during two years (n = 24); means followed by the same letter in the row are not significantly different at P < 0.05 according to Tukey's test (ns = not significant).

\*\*\* AG = albumins and globulins; GLI = gliadins; GLU = glutenins; HMW-GS = high molecular weight-glutenin subunits; LMW-GS = low molecular weight-glutenin subunits; GLI/GLU = gliadins/glutenins ratio.

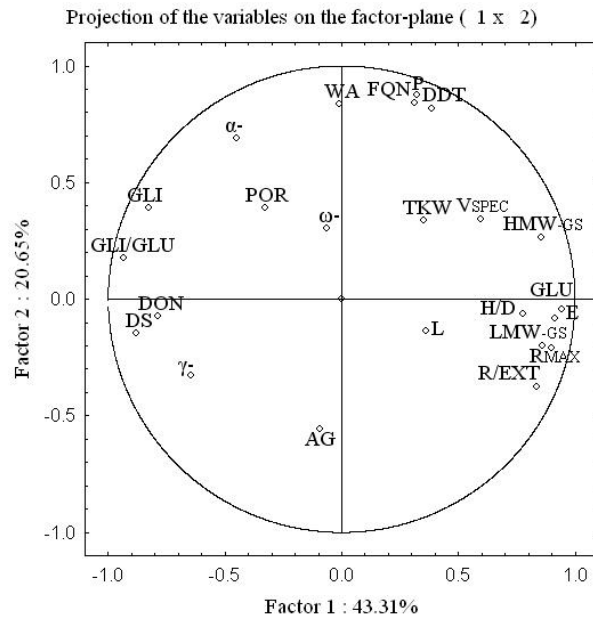


Figure 2. Projections of DON, baking quality and proteins data under natural and artificial *Fusarium* infection onto the first two principal components

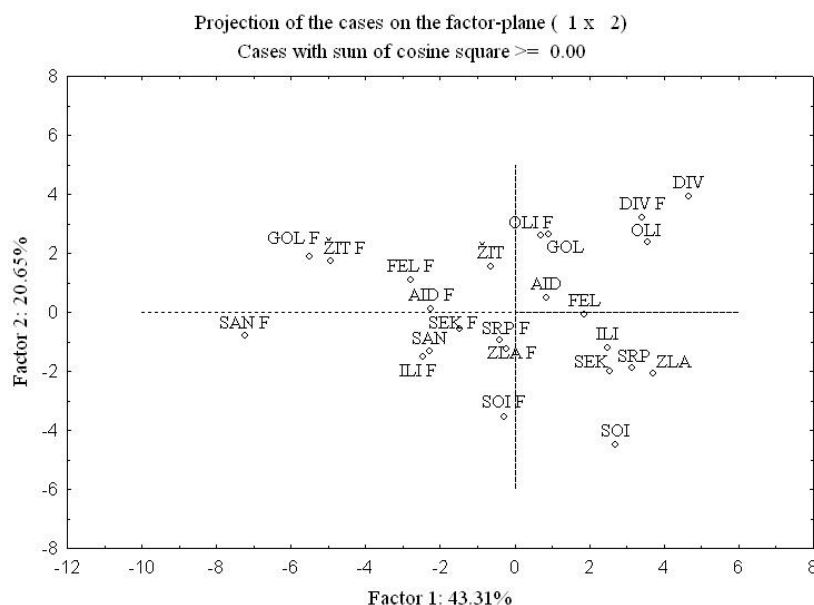


Figure 3. Projections of wheat cultivars growing under natural and artificial *Fusarium* infection onto the first two principal components. Cultivar abbreviations for natural infection: SRP = Srpanjka, ŽIT = Žitarka, DIV = Divana, AID = Aida, FEL = Felix, ZLA = Zlata, ILI = Ilirija, SAN = Sana, SEK = Seka, GOL = Golubica, SOI = Soissons, OLI = Olimpija. Cultivar abbreviations for artificial infection: SRP F, ŽIT F, DIV F, AID F, FEL F, ZLA F, ILI F, SAN F, SEK F, GOL F, SOI F, OLI F

#### Cluster analysis of wheat samples under natural and artificial *Fusarium* infection

A clear distinction between the two sets of cultivars growing under natural and artificial *Fusarium* infection may be observed, resulting in the formation of several groups based on their reaction to *Fusarium* infection and their baking and proteins quality (Fig. 3). The Croatian cultivars Divana and Olimpija with favourable HMW-GS (2\* 7+9 5+10) (Table 1) showed a distinct position in Figure 3, obtaining the best quality attributes for dough elasticity properties, high proportion of total GLU (35.7% and 34.4%, respectively), and the lowest GLI/GLU ratio (1.46 and 1.55, respectively). These cultivars showed the smallest difference between natural and artificial infection.

The high yielding cultivar Sana had a severe reaction to *Fusarium* infection (Fig. 1) and shows a distinct position in the Figure 3. This cultivar had a very weak baking quality due to unfavourable HMW-GS composition (2\* 6+8 2+12) (Table 1), very low total gluten (24.6%) and HMW-GS (5.6%), as well as a high GLI/GLU ratio (2.49). Also, the cultivars Žitarka (N 7+8 2+12) and Golubica (N 7+9 2+12) (Table 1) with moderate baking characteristics had lower tolerance to *Fusarium* infection (Fig. 1 and Fig. 3). French cultivar Soissons also takes up an isolated position in Figure 3 due to a favourable HMW-GS composition (2\* 7+8 5+10) (Table 1), higher proportion of GLU (34.4%) and lower GLI/GLU



ratio (1.43). This cultivar did not demonstrate significant disruption of the functional properties of dough and bread under *Fusarium* infection regardless of the increased contamination of flour by DON (Fig. 1). The particular cultivars proteins and quality data are not shown.

### Discussion

The grain P content generally was not affected by the *Fusarium* infection (Table 2) what is in accordance with other studies (Dexter et al. 1996; Prange et al. 2005; Terzi et al. 2007). After *Fusarium* infection, at eight analysed cultivars a slight decrease of P content was noted, what is in agreement with Siuda et al. (2010), while some others (Gartner et al. 2008; Papoušková et al. 2011) observed the opposite effect. *Fusarium* infection resulted in a decrease of TKW what is in agreement with the earlier work of Dexter et al. (1996), Wang et al. (2005), Dvojković et al. (2007) (Table 1). The shrunken and lighter infected kernels show a tendency towards a decrease in the endosperm to bran ratio due fungal carbohydrate consumption (Wang et al. 2005).

Concerning the WA capacity in *Fusarium* infected samples; the obtained decreasing is in agreement with Dexter et al. (1996). In contrast, Gartner et al. (2008) showed that WA was not affected by *Fusarium* infection, while Wang et al. (2005) observed a slight increase possibly due to higher proportion of damaged starch in infected kernels. Regarding the significant degradation of functional properties of dough in our study, Wang et al. (2005) emphasizes that the fungal protease produced by *Fusarium culmorum* are active over a wide range of temperature (10–100°C) and pH values (4.5–8.5) and may impair dough functionality and bread properties throughout the entire processing procedure. The dough of cultivars Felix, Sana and Golubica under artificial *Fusarium* infection in 2009 was not possible to stretch on the Extensograph because this method has long fermentation time which enables efficiently proteolytic degradation of gluten. In addition, from cultivar Ilirija under artificial infection in 2010 was not possible to make the bread because of dough stickiness. After artificial *Fusarium* infection the trend of  $V_{SPEC}$  reduction was noticed what is in accordance with Meyer et al. (1986), Dexter et al. (1996), Nightingale et al. (1999), Gartner et al. (2008) and Papoušková et al. (2011) (Table 2). Several authors observed an increase of  $V_{SPEC}$  under *Fusarium* infection (Prange et al. 2005; Wang et al. 2005; Gartner et al. 2008). These contradictions may be caused by different baking quality of analysed cultivars, with different fermentation time applied during bread production and intensity of *Fusarium* infection (Gartner et al. 2008).

Eggert et al. (2010) are the only ones investigated the impact of *Fusarium* infection on the AG and in agreement with our results they also noted that this fraction was not changed by *Fusarium* infection. As well as in our study, Wang et al. (2005) reported that in seriously infected samples the total GLU and HMW-GS were clearly decreased while total GLI increased. Eggert et al. (2010) also confirmed that in contrast to GLI, the GLU main subunits HMW-GS and LMW-GS showed in the artificially infected samples a significant reduction. These authors emphasized the importance of the enzymatic activity of fungal proteases in the degradation of HMW-GS and LMW-GS. Eggert et al. (2011) showed that

*Fusarium graminearum* proteases *in vitro* were able to degrade both GLI and GLU with pronounced degradation of HMW-GS because of their relatively higher quantity of lysine. The incomplete biosynthesis of GLU subunits during later stages of kernel development and the presence of mycotoxins as protein synthesis inhibitors may also lead to their reduced content in infected samples (Eriksen and Pettersson 2004). The obtained a significant influence of *Fusarium* treatment on GLI/GLU ratio are in accordance with Wang et al. (2005) and Eggert et al. (2010). Determined a strong impact of GLI/GLU ratio on DS, E, R<sub>MAX</sub>, R/EXT is in accordance with Wieser and Kieffer (2001), Kurtanjek et al. (2008) and Horvat et al. (2013).

In conclusion, it can be stated that the reduction of GLU fractions and deteriorated GLI/GLU ratio under *Fusarium* infection had a significant impact on the disrupted functional properties of dough and bread. The extent of the *Fusarium* infection effects appeared to be a cultivar specific. Results indicated that the stability of baking quality parameters of cultivars with higher *Fusarium* resistance is higher than that of those with high susceptibility. The more resistant cultivars can be defined by a lower accumulation of mycotoxin DON.

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