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GUSHING POTENTIAL OF WHEAT MALT INFECTED WITH *FUSARIUM CULMORUM*

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Abstract

Gushing is uncontrolled, eruptive foaming of beer after a bottle has been opened, without previous shaking. This is a phenomenon that affects breweries worldwide and all brands and types of beer. Gushing can be divided into primary (secretion of hydrophobins class II and other fungal metabolites from *Fusarium* fungi) and secondary gushing (calcium oxalate precipitations and metal ions). One of the methods to reduce the risk of gushing is the early detection of gushing potential of raw materials such as wheat or barley malt. The aim of this study was to determine the impact of wheat infection by *Fusarium culmorum* on the gushing potential of wheat malt.

Standard micro-malting procedure (MEBAK) was performed using wheat variety „Lucija" (less susceptible to infections by *Fusarium*), the sample „A" was the control sample, the sample "B" was treated with fungicide, sample "C" was infected with *F. culmorum* and treated with fungicide and sample "D" was infected with *F. culmorum* and was not treated with fungicide. Analyses were performed on dried and stabilized wheat malt samples in order to determine the correlation between the *F. culmorum* infection and the malt quality parameters (the share of soluble N in malt, Kolbach index, total proteins, soluble proteins and the gushing potential of malt). All analyses were done according to the methods from Analytica EBC (European Brewery Convention).

Results obtained from this research indicate that the infection of wheat by *F. culmorum* significantly affects and increases the gushing potential of wheat malt, and this is particularly evident in samples "C" and "D" (high gushing potential). By overlooking the selected malt quality parameters (the share of soluble N in malt which ranges from 0.92% in sample "A" to 0.98% in sample "C"; Kolbach index which ranges from 49 in sample "A" to 51 in sample "D"; total proteins range from sample "A" - 11.8% to sample "C" - 12.5%; soluble proteins range from 5.75% in sample "A" to 6.12% in sample "C") it is evident that they are correlated with *Fusarium* infection, as that they are increasing in samples infected with *Fusarium*.

In conclusion, results obtained from this research indicate that the infection of wheat by *F. culmorum* significantly affects and increases the gushing potential of wheat malt, which is particularly evident in samples "C" and "D".

***Key words**: Wheat, Wheat malt, Gushing potential, Fusarium culmorum.*

1. Introduction

Fusarium infected grains may pose a serious problems for malting and brewing industry, not only because *Fusarium* species produce mycotoxins but also because they have been linked to the gushing phenomenon (Gjertsen *et al*. [1]; Haikara [2]; Niessen *et al*. [3]; Sarlin *et al*. [4]; Sarlin *et al*. [5]; Sarlin *et al*. [6]; Christian *et al*. [7]). Gushing is eruptive and spontaneous over foaming of carbonated beverages directly after a bottle or a can has been opened (Christian *et al*. [7]). Apart from causing massive economic losses to malting and brewing industry, beer gushing also drives the consumers away from the product giving the brewery a bad image (Blechová *et al*. [8]).

There are two types of beer gushing: primary and secondary. Primary gushing is induced by fungal activity, especially *Fusarium* species, but it has also been linked to species of genera *Aspergillus, Rhizopus, Penicillium* and *Nigrospora* (Sarlin *et al*. [5]). Secondary gushing is caused by manufacturing factors, such as coarse bottle surface, CO_2 over saturation, increased oxalate concentrations, etc. (Havlova *et al*. [9]).

Previous studies tried to correlate the gushing effect with mycotoxin content in malt. Schwarz *et al*. [10], reported that approximately 90 % of all malts containing deoxynivalenol (DON) are prone to manifest some gushing.

Recent studies indicate that hydrophobins, a small fungal proteins characteristic for filamentous fungi, are responsible for gushing (Haikara *et al*. [12]; Kleemola *et al*. [13]; Wessels [14]). Hydrophobins act as potent surfactants that stabilize the CO_2 bubbles in beer by forming a layer around the micro-bubbles thus causing gushing (Wessels [14]; Pellaud [15]). One of the roles of hydrophobins produced by fungi is to adhere to all types of surfaces by reducing the surface tension of water, which gives the fungi a better grip on the grain (Linder *et al*. [11]). Since they are termostable, 10% of hydrophobins will survive the temperatures applied during the brewing process and transfer to beer causing gushing (Műller *et al*. [16]). The minimal reported concentration of hydrophobins that can cause gushing is 0.003 ppm (Sarlin [17]).

Gushing potential of barley can be predicted by quantifying the presence of *Fusarium* species and their antigens, but some researchers claim that the actual level of *Fusarium* is not a precise method to foreknow the gushing potential of malt (Munar [18]; Manke and Rath [19]; Schwarz *et al*. [10]).

The influence of fungicides on gushing potential of malt is also under review by many researchers. It has been discovered that even though the *Fusarium* induced disease (*Fusarium* head blight, FHB) was reduced significantly after the application of some fungicides, they did not suppress the production of respective mycotoxins (Suproniene *et al*. [20]). Similar discovery, considering gushing was reported by Havlova *et al*. [9], indicating that application of some fungicides can induce the gushing potential of malt. Namely, the fungicide treatment stimulated the production of hydrophobins by fungi in order to sustain on grain surface.

Fusarium infection affects other malt quality parameters as well. Some of the problems linked to *Fusarium* infected grains include: lower grain weight and germinative capacity, yield loss, endosperm protein and starch degradation, etc. (Schwarz *et al*. [21]; Vaughan *et al*. [22]).

The aim of this study was to investigate the changes in malt produced using *Fusarium culmorum* infected wheat, and to determine the impact of different fungicide treatments of wheat on the malt quality parameters. Furthermore, the overall aim was to correlate the *Fusarium* infection with gushing potential of malt.

2. Materials and Methods

Samples used in this experiment were obtained from Agricultural Institute Osijek and were treated as stated: the sample "A" was the control sample, the sample "B" was treated with fungicide, sample "C" was infected with *F. culmorum* and treated with fungicide and sample "D" was infected with *F. culmorum* and was not treated with fungicide.

Inoculum production

To produce macroconidia of *F. culmorum*, a mixture of wheat and oat grain (3 : 1 by volume) was soaked in water overnight using 250 mL glass bottles, then water was decanted and seeds autoclaved. After the inoculation procedure was performed by adding the *F. culmorum* conidial suspension to the glass bottles, the seeds were kept in the dark for 2 weeks at 25 ^{0}C. Concentration of conidial suspension was 10^5 mL^{-1} which was prepared according to Snijders and Van Eeuwijk [23].

Field experiment and inoculation treatment

Research was conducted during 2009/2010 using winter wheat variety Lucija (a domestic variety obtained by Agricultural Institute Osijek) in Osijek, Croatia. To control seed borne diseases the seed was treated with Vitavax 200 (thiram + carboxin) at a rate of 200 g 100 kg^{-1}. Genotypes were sown in eight row plots of 7 m length and 1.08 m wide in October at a sowing rate of 330 seeds m^{-2}. Wheat was grown according to standard agronomic practice. The plot area had sufficient nitrogen and other nutrients for normal crop growth. Spray inoculations were performed individually for each genotype at flowering (Zadok's scale 65) (Zadoks *et al*. [24]) using a tractor back-sprayer. Inoculations were performed in the late afternoon and repeated two days later. To maintain moisture at ears we sprayed water with tractor back-sprayer on several occasions during the day. The second treatment (three replications) were control plots which were left to natural infection. Symptoms of the disease were recorded at 22 day after the first inoculation in the treatment where it was carried out artificial infection. Values for the entire area within the plot were visually rated using a linear scale from 0 (no infection) to 100 (100 % infection).

Micromalting

Micromalting was performed according to standard procedure (MEBAK [25]) in a laboratory incubator (ClimaCell, MMM Medcenter Einrichtungen, München, Germany). However, the micromalting procedure (MEBAK [25]) was adjusted to wheat, since wheat grain has no husk, hence it can adsorb water much quicker. This is why soaking time had to be shortened and relative humidity of air had to be decreased. Five hundred grams of wheat was soaked in 500 mL of tap water according to the standard procedure described by MEBAK [25] (Table 1). The kilning of green malt was also performed according to the MEBAK [25] protocol. After drying, malt was transferred into paper bags and

kept at room temperature for three days for moisture equilibration.

Malt analyses

The share of soluble nitrogen in malt (3.1.4.5.2.1.), Kolbach index (3.1.4.5.3.), total proteins (3.1.4.5.1.1.), soluble proteins (3.1.4.5.2.1.) and the gushing potential of malt (3.1.4.21.2.) were analyzed according to the Analytica EBC methods [26].

Table 1 General micromalting scheme of wheat samples

1st day	Immersion steeping for 5 h at 14.5 °C; Dry steeping for 19 h at 14.5 °C, relative air humidity 85%.	
2nd day	Immersion steeping for 4 h at 14.5 °C; Dry steeping for 20 h at 14.5 °C, relative air humidity 85%.	
3rd day	Immersion steeping for 2 h at 14.5 °C, relative air humidity 85%.	
3rd- 6th day	Germination was carried out according to the scheme: 96 h at 14.5 °C; Relative air humidity in each procedure was 85%.	
7th day	Kilning was performed for 19 h, according to standard procedures for pale malt, after last germination hour.	50 °C for 16 h 60 °C for 1 h 70 °C for 1 h 80 °C for 1 h
	Malt degermination; Packing in paper bags and storage.	

3. Results and Discussion

The results presented in Table 1 indicate that wheat infected with *F. culmorum* significantly affects the gushing potential of wheat malt. This is particularly evident in samples "C" and "D" which exhibit a high gushing potential. Even when fungicide was applied, as in case of sample "C", gushing was still increased comparing to the control sample "A". This is in accordance with previous research conducted by Havlova *et al*. [9].

Table 1 Results of gushing potential test

Lucija malt					
	Unit of measurement	Sample			
		A	B	C	D
Gushing potential	-	**potential**	**potential**	**high**	**high**

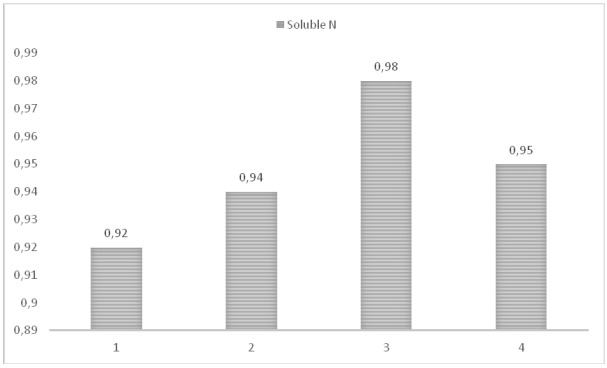

Figure 1. Soluble N content in wheat malt Lucija

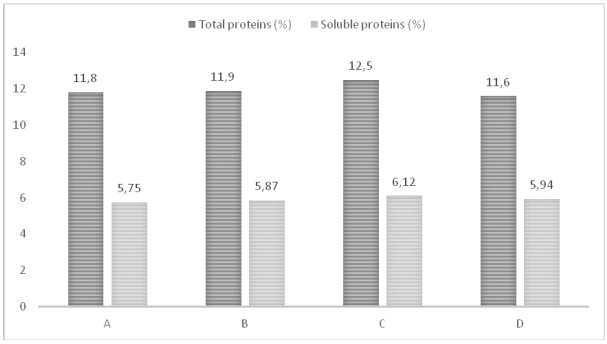

Figure 2. Total and soluble proteins in wheat malt Lucija

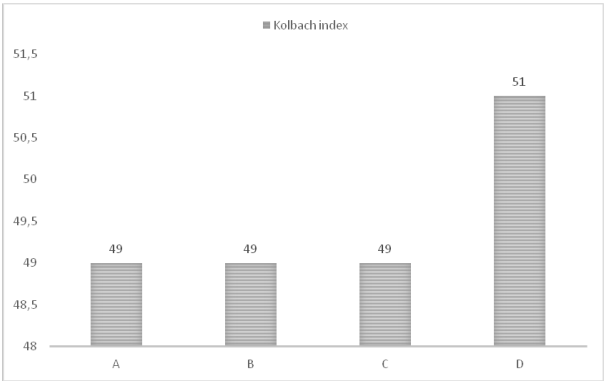

Figure 3. Kolbach index in wheat malt Lucija

The malt quality parameters were selected as indicators of proteolitic activity of malt and in this research their values show an increase with the applied *Fusarium* infection. Schwarz *et al*. [21] reported the same changes in malt during their research. The share of soluble nitrogen (Figure 1) in malt increases from 0.92% in control sample "A" to 0.98% in sample "C". This suggests that *Fusarium* proteinases have degraded wheat proteins already in the field or during malting. *F. culmorum* can act as an activator of many pathogen-related proteins which are a part of the plants' protective mechanisms (Sarlin *et al*. [4]; Oliveira *et al*. [27]). This also applies to the increase of total proteins (Figure 2) ranging from

sample "A" - 11.8% to sample "C" - 12.5%; and soluble proteins ranging from 5.75% in sample "A" to 6.12% in sample "C". A slight decrease of monitored parameters was noticed in the last sample, "D", the one inoculated with *F. culmorum* spores but not treated with fungicide. This indicates that fungicide, when applied during inoculation, may stimulate the fungi to secret hydrophobins. These results agree with the research conducted by Havlova *et al*. [9], stating that the application of some fungicides can lead to the increase of gushing potential in comparison to the control sample. This could be explained by the postulation that the fungi are forced to produce more hydrophobins and proteolitic enzymes to fight the effect of fungicide in the field. Also, some studies have shown that some fungicides can stimulate *Fusarium* to produce more mycotoxins (Blandino *et al*. [28]; D'Mello *et al*. [29]).

It should be noted that the wheat sample "D" showed very low germination rate. Furthermore, the respective malt was extremely contaminated with *F. culmorum* and during malting a specific white, cotton-like mycelium appeared on the grains. Since *Fusarium* infection was so severe in wheat sample "D", wheat grain was in extremely bad condition and one could expect that it is not suitable for malting.

Kolbach index (Figure 3), an indicator of protein degradation (ratio of soluble and total nitrogen), ranged from 49 in sample "A" to 51 in sample "D". This increase in Kolbach index values is in accordance with previous research conducted by Schwarz *et al*. [30].

It is evident that these malt quality parameters are correlated with *Fusarium* infection, as that they were increasing in samples infected with *Fusarium* fungi.

4. Conclusions

- According to the results obtained by this research, it can be presumed that *F. culmorum* produces hydrophobins, proteins that cause gushing of beer. In order to obtain more information on this subject, a further investigation should be conducted considering identification of the type of hydrophobins involved in gushing.

- Also, further research is needed in order to establish the impact of other fungal species of genus *Fusarium* on gushing potential of infected malt (both wheat and barley), as well as to establish the possible correlation between gushing and mycotoxin production. The impact of fungicide application on gushing should also be considered.

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