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EFFECTS OF MOULDS ON THE SAFETY AND PROCESSING QUALITY OF *TRITICUM AESTIVUM**

ABSTRACT: Wheat and wheat products are frequently subjected to mould infestations. Many of them are potential producers of various mycotoxins. Some of the consequences, due to the infestations by genus *Fusarium* and *Alternaria*, are mostly: yield loss, decrease of biological and technological quality, and unacceptable quality of infected kernels for the production and processing into human food because of the possible presence of mycotoxins.

It is unknown whether and how the contaminated grains are distributed during milling into various flour streams and finished products. Wholegrain flours and related products contain all anatomic parts of kernels, including mycotoxins. It is a known fact that mycotoxins are resistant to thermal degradation, so they do not loose their toxicity during processing. Moulds from genus *Fusarium* spp. and *Alternaria* spp. synthesize mycotoxins, mostly zearalenon and ochratoxin A.

The aim of the investigation was to examine mould contamination of wheat grain, as well as to identify the isolated species, especially those capable of producing toxins, and to determine their impact on technological quality, safety and sanitary condition of wheat.

Six varieties of wheat, contaminated with moulds, were investigated. Each sample was separated manually into four fractions: sound kernels, black germ kernels, kernels infected slightly and those infected severely with *Fusarium* spp.

KEY WORDS: moulds, mycotoxins, ochratoxin, technological quality, wheat, zearalenon

INTRODUCTION

Agricultural production is a complex processing cycle susceptible to contamination with field moulds and/or toxic metabolites, in all phases of production, transport, storage, and processing. The researches have been conducted to emphasize the necessary changes needed in comprehending that wheat is

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staple food and the most important chain in the biological cycle of the migration of hazardous contaminants, posing serious health concerns to humans.

Cereals, especially wheat, represent a strategic raw material from the aspect of human nutrition. However, cereals and cereal products can be contaminated with moulds in any phase of a processing cycle: in fields, during harvest, storage, processing, transport, and over a period between production and consumation. Many of the moulds are potential producers of mycotoxins (Šarić et al., 1973; Šarić et al., 2004).

The aim of the investigation was to examine mould contamination of wheat grain and wheat flour, as well as to identify the isolated species, especially those capable of producing toxins, and to determine their impact on technological quality, safety and sanitary condition of wheat.

MATERIALS AND METHODS

The paper presents results of investigation of 6 wheat varieties infested severely with *Fusarium* spp. and *Alternaria* spp. Kernels were separated on the basis of sensory properties, mycological and mycotoxical tests.

The contaminated kernels were separated into three fractions:

— **Black germ fraction** (Figure 1) comprises kernels with altered colour of outer layers, mostly around germ and crease of the wheat kernel. The colour changes are caused by different mould species or, so called, "black moulds", mostly belonging to the genera *Alternaria* and *Helminothosporum* (Šarić et al., 1973; Šarić et al., 2004; Šarić et al., 2004a; Šarić et al., 1980). The content of these kernels is not considered impure according to JUS E.B1.200 standard (1992).

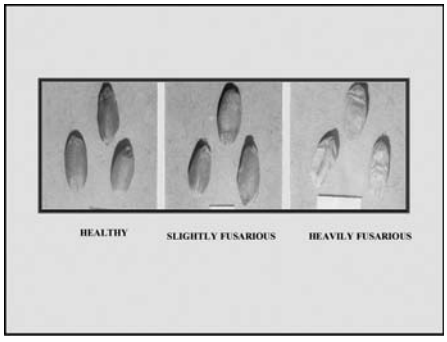


Fig. 1 — Kernels infested with moulds from genus *Fusarium*

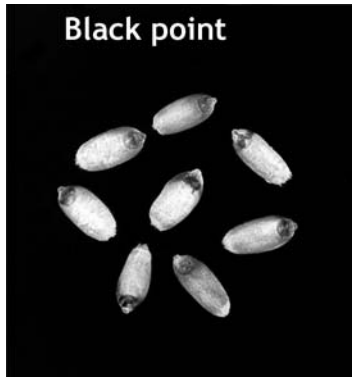


Fig. 2 — Kernels with blask germ

— **Fraction of kernels moderately contaminated with** *Fusarium* spp. consists of less shrivelled kernels with slightly expressed white or pink layers on the surface. These kernels are mostly contaminated with *Fusarium* spp. during waxy or full maturity of the wheat. It is not possible to separate this frac-

tion during milling cleaning so it enters flour. The highest contamination is usually observed in the aleuronic layer (Šarić et al., 1973; Šarić et al., 2004; Šarić et al., 2004a; Šarić et al., 1980).

— **Fraction of kernels severely contaminated with** *Fusarium* spp. (Figure 2) includes shrunk, and scabby kernels, coloured in white or light red. Such kernels are consequence of mould infestation in early stages of wheat maturity. During milling cleaning, these kernels are only partially removed, and mostly enter further processing phases (Šarić et al., 1973; Šarić et al., 2004; Šarić et al., 2004a; Šarić et al., 1980).

— Control sample consisted of **fraction of sound wheat kernels** from the corresponding wheat sample.

At collection terminals, harvested wheat usually contains all of the described fractions. Wheat bulk is sampled, evaluated regarding sensory properties and impurity content in order to establish its price. Only kernels severely contaminated with *Fusarium* spp. are classified into organic black impurity category, i.e. the category of deteriorated kernels. There is very little information on the distribution of the contaminated kernels during milling cleaning and the influence of milling processing on the safety of flour and other end-products. Particularly hazardous is preparation of wholegrain flour, because the whole kernels, including contaminated ones, are milled and processed into bread and bakery goods.

Each kernel category was analyzed using standard mycological, mycotoxicological, physico-chemical and rheological methods (Pitt and Hocking, 1985; Moresu, 1995; *Official Methods AOAC*, 1990; Kaluđerski and Filipović, 1998; *Pravilnik*, 1980; Marasas et al., 1984; Škrinjar et al., 1997).

RESULTS AND DISCUSSION

Target fungal species were detected in all of the investigated samples at varying levels according to the total fungal count per wheat kernel (Table 1). The highest contamination was observed in the category of grains severely contaminated with *Fusarium* spp. Overall, 9 fungal species were isolated, with *Fusarium* being the most dominant genus detected in higher numbers (78% of total isolated mycopopulations). There were eleven species (identified from *Fusarium* genus which could produce toxic metabolites like zearalenon (ZEA) and trihoteceron (Moresu, 1995; Marasas et al., 1984). *Fusarium oxysporum* was the most dominating mould, comprising 38% of the contaminated samples.

Table 1 — Average content of mold number per kernel of wheat fraction pattern

Grain fraction	1	2	3	4	5	6
Sound	0,92	0,73	0,83	1,15	0,75	1,11
Black germ	2,00	2,97	2,94	1,52	2,51	1,95
Moderatcly infested with *Fusarium spp.*	2,87	3,12	3.21	2,21	2,62	2,94
Severely infested with *Fusarium spp.*	3,21	3,25	3,25	2,58	3,21	3,14

Besides the most commonly isolated *Fusarium* spp., moulds from genus *Alternaria* were also present in the fraction of all the investigated wheat varieties, accounting for 14% of the samples (*Pravilnik*, 1980; Marasas et al., 1984; Šarić et al., 1997).

Investigating the physical properties of wheat fractions, it was concluded that test weights decreased depending on the infestation level (Šarić and Sekulić, 1981; Šarić et al., 2001) (Table 2, Figures 3, 4). Only fraction of sound kernels satisfied the minimum quality requirements regulated by JUS E.B1.200 standard (1992).

Table 2 — Physical properties of kernels from various fractions

Grain fraction	Test weight (kg/m^3)	Mass of 1000 kernels (g)
Average sample	710	27,4
Sound kernels	760	32,4
Black germ kernels	720	31,0
Moderatcly infested with *Fusarium spp.*	690	26,9
Severely infested with *Fusarium spp.*	550	19,4

Considering mycotoxicological analyses (Table 3), ochratoxin A (OA) was present in significant numbers of samples, mostly in the fractions of moderately and severely infested kernels with *Fusarium* spp., at concentrations in the range 11—48 mg/kg, while ZEA was detected in very high concentrations, ranging from 170 to 500 mg/kg. According to Regulation (*Pravilnik*, 1992), the maximum level of OA is 10 mg/kg, and 1 mg/kg for ZEA (*Official Methods AOAC*, 1990). These samples would be rendered unacceptable with respect to hygiene and safety, especially because toxins do not deteriorate after thermal processing (baking).

Tabele 3 — Contamination of wheat grains with mycotoxins (μg/kg)

Grain fraction	Variety	Mycotoxin (μg · kg^{-1})	
		Ohratoxin A	Zearalenon
Sound	1	0	0
Black germ	1	0	0
Moderatcly infested with *Fusarium spp.*	1	0	0
Severely infested with *Fusarium spp.*	1	8	280
Sound	2	16	0
Black germ	2	32	0
Moderatcly infested with *Fusarium spp.*	2	32	0
Severely infested with *Fusarium spp.*	2	32	0
Sound	3	32	0
Black germ	3	0	0
Moderatcly infested with *Fusarium spp.*	3	0	0
Severely infested with *Fusarium spp.*	3	32	400
Sound	4	0	0
Black germ	4	34	200
Moderatcly infested with *Fusarium spp.*	4	11,5	0
Severely infested with *Fusarium spp.*	4	34	500

Sound	5	11	0
Black germ	5	11	170
Moderatcly infested with *Fusarium spp.*	5	16	250
Severely infested with *Fusarium spp.*	5	48	350

Depending on the stage of kernel maturity, fungal infestations affect the filling of kernels, since the mass of 1000 kernels decreases regularly from sound to infested fractions. The kernel dimension is an important factor that affects flour yielding.

The highest flour yield was obtained in sound fractions, and the lowest in the severely infected fractions (Figure 3), that is in an inverse proportion with mineral content of kernels (Figure 4). The severely infested fractions showed the lowest flour yield, and the highest ash content, which is an unfavourable characteristic for milling processing.

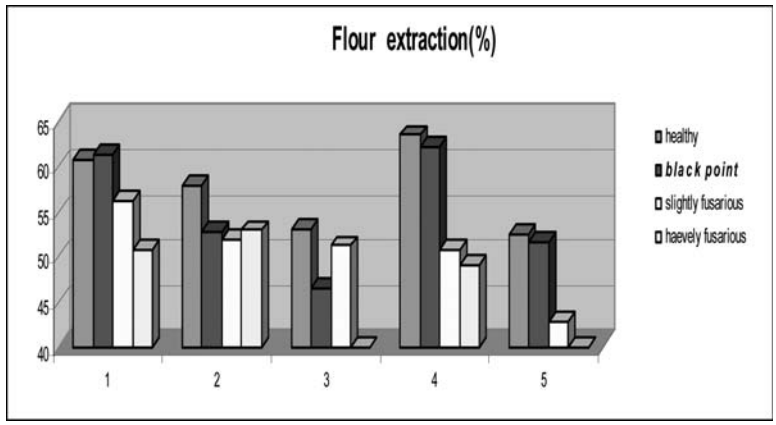


Fig. 3 — Flour yields of wheat fractions

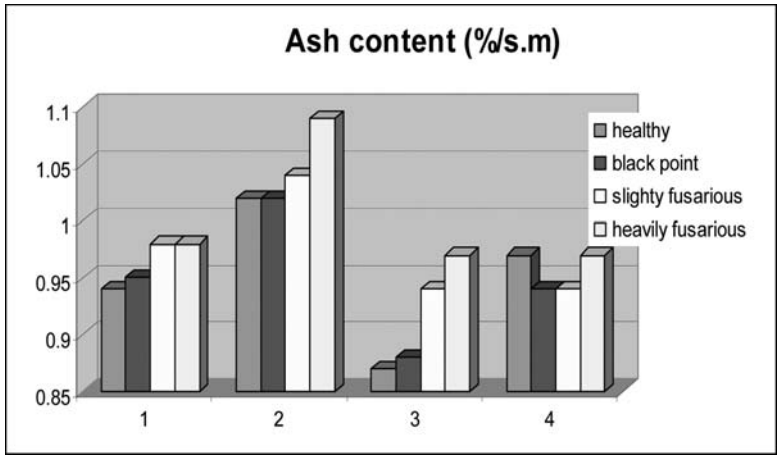


Fig. 4 — Mineral contents of wheat fractions

Chemical methods (protein content, wet gluten content, etc.) clearly revealed the breakdown of wheat gluten/starch conglomerate provoked by fungal infestation.

Table 4 — Protein content of wheat fractions

Variety	Protein content (%/dry basis)			
	Sound kernels	Black germ kernels	Moderatcly infested with *Fusarium spp.*	Severely infested with *Fusarium spp.*
1	13,3	13,7	13,8	14,0
2	13,6	14,0	14,5	15,0
3	14,0	14,6	14,8	15,1
4	14,8	15,0	14,9	15,5
5	14,3	14,8	14,9	15,1
6	13,5	14,0	14,3	14,8

All the investigated wheat varieties had relatively high protein content at a level of the first technological class, with the highest contents determined within the severely infested fractions (Table 4). This is the consequence of infestation with fungi belonging to genus *Fusarium* that resulted in prematuration and insufficient kernel filling, thus disturbing the ratio of endosperm to aleurone cell layer, in advantage to aleurone cell layer. This disturbance arose because moulds use carbohydrate components as a substrate for growth. Since the fungal biomass consists of 40% protein, the higher content of proteins in the infested fractions presumably comes from fungal mycelia present in significant amounts in the infested kernels.

From rheological measurements, the determination of quality and content of gluten was carried out. The wet gluten content significantly varied in the fractions of wheat varieties. The black germ fractions were the highest in this parameter, while the severely infested fractions were the lowest (Table 5). This is probably the consequence of gluten degradation, especially of gliadin, the major contributor to dough elasticity (Šarić et al., 1997).

Table 5 — Wet gluten content in wheat fractions

Variety	Wet gluten content (%)			
	Sound kernels	Black germ kernels	Moderatcly infested with *Fusarium spp.*	Severely infested with *Fusarium spp.*
1	32	33	30	26
2	33	35	31	27
3	34	36	32	30
4	35	37	34	32
5	35	36	33	29
6	34	33	31	26

Besides the difference in wet gluten content between fractions, there was a significant variation in the quality of gluten as well. Structural Berliner's number (Q_o) more or less regularly decreased from sound to the severely infested fractions of the examined wheat varieties (Figure 5).

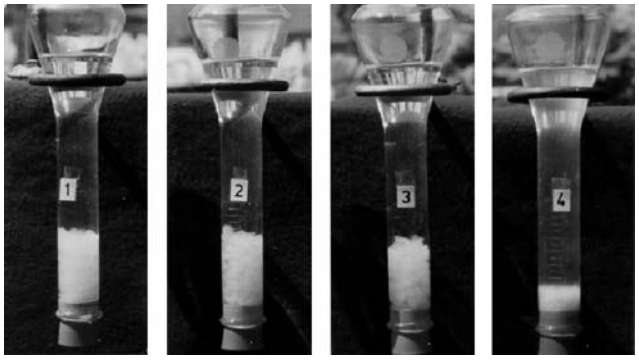
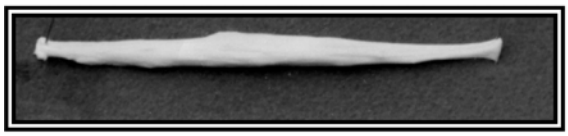


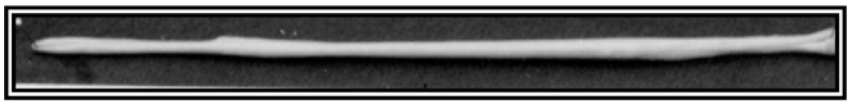
Fig. 5 — Changes in the structural Berliner's number (Q_0)

To investigate the gluten quality, gluten extensibility test was performed (Kaluđerski and Filipović, 1998). Gluten extensibility varied from fraction to fraction (Figure 6).

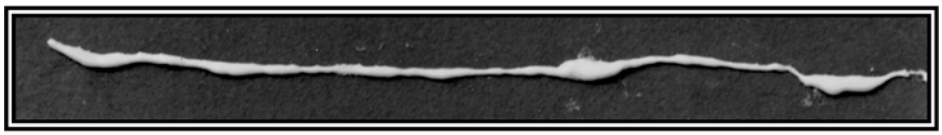
Sound grain fraction



Black germ grain fraction



Moderatcly infested with *Fusarium spp.* grain fraction



Severely infested with *Fusarium spp.* grain fraction

Fig. 6 — Changes in the properties of gluten

The sound fractions of all the investigated wheat varieties had moderately extensible gluten which was firm, slightly sticky, and elastic. The slightly infested fractions had moderately extensible, elastic, soft and mucous gluten. The

severely infested fractions had moderately to extremely extensible, non-elastic, sticky, and mucous gluten. In most of the cases, the incoherent mechanical structure enabled the gluten to return into the primary position after the extension.

CONCLUSION

— Moulds are frequent contaminants of wheat and related products with potential to produce mycotoxins;

— Fungal contamination by field moulds, especially from genus *Alternaria*, deteriorates the quality of wheat grains, depending on the level of infestation and the content of infested kernels;

— Field moulds, by their filaments, degrade the kernels, decreasing their processing quality and sanitary condition;

— Moulds and their metabolites, and mycotoxins cannot be detected organoleptically in grains, flour and bread, but they tend to accumulate in human organism, posing a health risk by causing severe diseases.

Especially emphasized is the contamination with moulds and their toxic metabolites which are resistant to heat degradation, thus representing potential silent killers of a living world. The basic recommendations coming from this paper are directed towards the strict enforcement of laws regarding microbiological and mycotoxicological control of cereals, as well as to encourage the changes and the necessary accommodation of valid regulations concerning toxin limits and introduction of obligatory control of newly recognized toxins.

ACKNOWLEDGMENT

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УТИЦАЈ ПЛЕСНИ НА ЗДРАВСТВЕНО БЕЗБЕДНУ ИСПРАВНОСТ И ТЕХНОЛОШКИ КВАЛИТЕТ *TRITICUM AESTIVUM*

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Резиме

Пшеница и прерађевине од ње намењене хуманој исхрани веома често подложне су инфекцији различитих плесни. Многи од њих су потенцијални продуценти широке лепезе микотоксина. Као последица напада плесни из родова *Fusarium* и *Alternaria* већином су губици у приносу, пад биолошког и технолошког квалитета, неподобност инфицираних зрна за производњу и прераду у људску храну због евентуалног садржаја микотоксина. Непознато је како и да ли се контаминирана зрна плеснима одвајају у млинској чистионици и распоређују у одређене типове брашна, као и у финалне производе. Интегрално брашно и производи од њега садрже све анатомске делове зрна, самим тим и микотоксине. Познато је да су микотоксини изразито термостабилни и не губе токсичност при термичкој обради, односно производњи финалних производа. Плесни из родова *Fusarium* spp и *Alternaria* spp синтетишу у зрну жита микотоксине и то већином зераленон и охратоксин А.

Циљ испитивања је био да се утврди присуство плесни у пшеничним зрнима. Посебно је важно да се изврши идентификација изолованих врста и то пре свега токсигених врста као и да се одреди њихов утицај на технолошки квалитет и здравствено безбедну исправност пшенице.

Испитано је шест сорти пшенице контаминираних плеснима и сви узорци су сензорно раздвојени на четири фракције зрна: здрава, тамноклична, мало и јако фузариозна.