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Original research paper

MYCOPOPULATIONS OF GRAIN AND FLOUR OF WHEAT, CORN AND BUCKWHEAT

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ABSTRACT: According to the nutritive characteristics, whole grain flour is a high quality product, due to its high vitamin, mineral, and dietary fiber content. However, the cereal grains are susceptible to the series of contamination during the ripening, harvesting, processing and storage.
The aim of this work was to determine mold presence in grains and flour of wheat, corn and buckwheat. The determination of total number and identification of isolated genera and species of molds were the subject of this research.
All samples were contaminated with the molds. The total number of molds per 100 cereal grains was between 60 cfu (wheat) and 120 cfu (buckwheat). The total number of molds in the samples of flour ranged from $6.0x10^1$ cfu/g in white wheat flour to $5.0 x10^2$ cfu/g in buckwheat whole grain flour (DG18 medium). Eight fungal genera (*Alternaria, Aspergillus, Cladosporium, Chrysonilia, Fusarium, Penicillium, Rhizopus* and *Scopulariopsis*) and fifteen species were isolated. The largest number of species of molds was isolated from the genus *Aspergillus*. About 66.7% of isolated fungi belonged to potentially toxigenic species.
The results pointed out the necessity of grain surface treatment, preceding the milling of grains in wheat, corn and whole grain buckwheat flour production.

Key words: *wheat, corn, buckwheat, flour, molds*

INTRODUCTION

Since ancient times, cereals present the most important group of agricultural products, considering the human nutrition. Cereals are rich in carbohydrates, proteins, minerals, vitamins and other nutritious substances, and they are characterized by the high nutritional value.

The most frequent cereals in human diet are wheat, corn and rice, while the other species such as oat, barley, rye, triticale, millet and pseudocereals – buckwheat and sorghum are present in a smaller amount.

Whole grains of cereals and their products are an important part of human nutrition. Whole grains of cereals contain some very important phytochemicals. The most important phytochemicals that originate from whole grain are polyphenolic compounds, carotenoides, vitamin E, lignans, ß-glucans and inulin (Bunzel, et al., 2001; Sedej, 2011).

The basic technological procedure of cereal processing is the milling process. In the case of this process the basic raw ma-

terial is whole grain, and the products are flour, grits, bran and etc. which are separated during the process (Stojanović and Psodorov, 2007). By their nutritional characteristics, whole grain cereals have a high quality due to the high content of vitamins, minerals and especially dietary fibers. On the other hand, the cereal grains are susceptible to different contaminations during the maturation, harvesting, storaging and processing.

Microorganisms are constant contaminants of grain flours, because they originate from the cereals vegetation period, and they are an integral part of the grain mass. Under unfavorable conditions they are inactive and do not present a potential hazard. However, in the moment of the condition change, the microorganisms get active and could cause an enormous material and economic loss (Žeželj, 1995). Healthy grains posses their natural protection.

The damaged grain, atacked by microorganisms is lighter, without glow and has inadequate quality for direct use, also as a raw material for the further processing. The level of the flour microbiological contamination during the storaging depends on the quantity of damaged grains, type and the quantity of impurities, level of aeration, average moisture and the temperature of grain mass, moisture and temperature in the storages (Žakula, 1980; Škrinjar and Tešanović, 2007).

Molds which are the most frequent contaminants of flour mostly originate from cereal grains. Molds are only partly removed during the milling process, esspecialy if they penetrate inside the grain tissue. Most frequently isolated molds from the milling products belong to the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Cladosporium*, *Mucor*, *Rhizopus*, *Eurotium* and *Emericella* (Kljusurić, 2000; Pitt and Hocking, 2009; Kocić-Tanackov, 2012; Krulj et al., 2016).

The aim of this investigation was to determine mold presence in grains and flour from wheat, corn and buckwheat. The determination of total number and identification of isolated genera and species of molds were the subject of this research.

MATERIAL AND METHODS

Three types of cereal species were investigated: wheat, corn and buckwheat grains, also as white wheat flour, whole grain wheat flour, corn flour and whole grain buckwheat flour.

Mycological researches contained the determination of the total number of molds (cfu) and identification of molds.

The total number of molds in wheat, corn and buckwheat was determined by the direct inoculation of grains (25 grains/Petri dish Ø14 mm) on Dichloran 18% glycerol agar (DG18, pH 5.6 ± 0.2)(Biokar Diagnostics, France) (SRPS ISO 21527-2, 2011).

The total number of molds in flour samples was determinated by the Koch method of dilutions. For dilutions preparation, 0.1% of sterile solution of peptone water was used.

Isolation and determination of the total number of molds were conducted on the two surfaces:

1. Dichloran 18% glycerol agar (DG18) (Biokar Diagnostics, France) for isolation of xerotolerant molds grows below 0.9 a_w;
2. Malt yeast 50% glucose agar (MY50G)(malt extract 10g, yeast extract 2.5g, agar 10g, glucose 500g, distilled water 500ml, pH 5.3 ± 0.2) for isolation of extremely xerophile molds grows below 0.7 a_w (Samson et al., 2004; Pitt and Hocking, 2009).

The seeded media were incubated at 25 °C. The results were obtained after 5 and 7 days. Samples were analized in triplicates.

Monocultivation of molds was conducted in the way that conidia and fragments of hypha from mold colonies were translated on Czapek yeast (autolisate) extract agar (CYA) [$NaNO_3$ 3g, K_2HPO_4 1g, KCl 0.5g, $MgSO_4x7H_2O$ 0.5g, $FeSO_4x7H_2O$ 0.01g, yeast extract 5g, sucrose 30g, solution of microelements 1ml ($ZnSO_4x7H_2O$ 1g, $CuSO_4x7H_2O$ 0.5g, distilled water 100ml), agar 20g, distilled water 1000ml,pH 6.0 - 6.5] or Malt extract agar (MEA) (malt extract 20g, peptone 1g, glucose 20g,

agar 20g, distilled water 1000ml, pH 5.6 ± 0.2) or Potato dextrose agar (PDA, pH 5.6 ± 0.2) (Himedia, India). In accordance with macromorphological characteristics colonies which were presumed to belong to the genera *Penicillium* and *Aspergillus* were seeded on CYA, and others on MEA and PDA. Seeded media were incubated 7 days at 25 °C. The criteria described by Samson et al. (2004), Samson and Frisvad (2004) and Pitt and Hocking (2009) were used for species identification.

RESULTS AND DISCUSSION

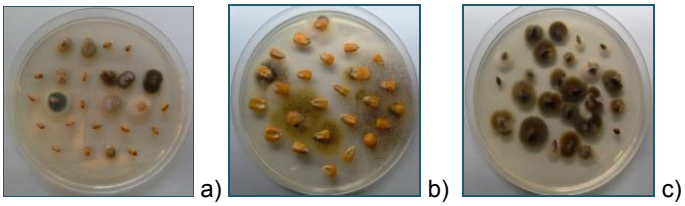
The presence of molds was determinated in every grain and flour sample of wheat, corn and buckwheat. The total number of molds per 100 wheat grains was between 60 cfu (wheat) and 120 cfu (buckwheat) (Table 1 and Figure 1).

The total number of molds in the samples of flour on DG18 surface ranged from $6.0x10^1$ cfu/g in white wheat flour to $5.0 x10^2$ cfu/g in whole grain buckwheat flour (Table 2 and Figure 2). The lowest contamination on MY50G surface was spotted in the samples of white wheat flour and whole grain buckwheat flour ($3.0x10^1$ cfu/g), and the largest was in corn flour ($2.7x10^2$ cfu/g). The DG18 surface is usually used for researches, while the MY50G surface is suitable for the quantitative and qualitative determination of xerophile types of molds due to the reduced water activity (a_w). Mycopopulation was most abundant in the whole grain buckwheat flour samples.

Table 1.
The total number of molds in wheat, corn and buckwheat grains*

Sample	Total number of molds (cfu)
	per 100 grains
Wheat	60
Corn	80
Buckwheat	120

Legend:- the result presents the average value of three repetitions*



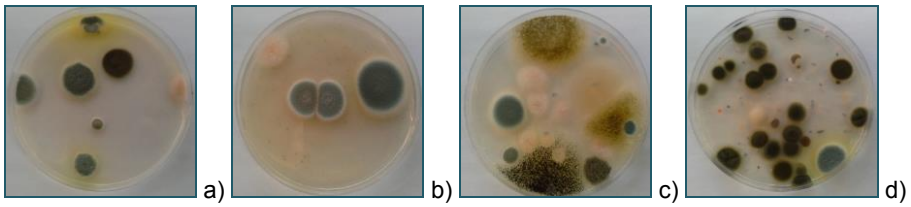
a) b) c)

Figure 1. Total number of molds per grain samples: a) wheat, b) corn and c) buckwheat (DG18, 25 ºC, 7 days)

Table 2.
The total number of molds in flour samples*

Sample	Total number of molds (cfu/g)	
	DG18	MY50G
Wheat flour	$6.0x10^1$	$3.0x10^1$
Whole grain wheat flour	$1.3x10^2$	$4.0x10^1$
Corn flour	$3.4x10^2$	$2.7x10^2$
Whole grain buckwheat flour	$5.0x10^2$	$3.0x10^1$

Legend:- the result presents the average value of three repetitions*



a) b) c) d)

Figure 2. Total number of molds: a) wheat flour, b) whole grain wheat flour, c) corn flour and d) whole grain buckwheat flour (DG 18, 25 ºC, 7 days)

The results are in accordance with expectations, considering the fact that whole grain buckwheat flour was examined, namely with the bran. Due to the structure and appearance of grain, buckwheat grain is a suitable environment for establishing the surface mycopopulation which is afterward present in the flour. The reason of higher surface contamination of grains is the presence of a fine hairs or "villus". These „villi" retain the dust particles and microorganisms.

The controlled conditions of storaging, as well as the procedures for wheat preparation before the milling, could significantly lower the number of molds, thus the constitutiens of the mycopopulation. According to the research of Doolotkeldieva (2010), during grain processing without tempering with moisture a decrease in occurred fungal contamination was almost double in comparison to the wheat grains before processing.

Terzi et al. (2014) reported that application of required technological procedures for the remolval of contaminated grain could significantly decrease the total contamination. By the application of the device for an intensive grain surface treatment, the presence of harmful substances could be decreased, such as microorganisms and their metabolites. Demin (2007) proved that multiple grain treatment could significantly lower the presence of molds, up to the amount of 21%. Most of the impurities are concentrated in removed parts of the grain after the coarse and fine scrubbing.

The presented results are in accordance with our earlier researches (Plavšić et al., 2007), whereas the maximal number of molds in the samples of different types of wheat flour was $1.5x10^3$ cfu/g. Alborch et al. (2012) determined the presence of molds in the samples of corn flour within the interval of $<10 – 8.8x10^4$ cfu/g. Demirel and Sariozlu (2013) investigated the presence of molds in the samples of flour from various cereals. The total number of molds was between $2.0x10^4$ and $2.0x10^4$ cfu/g. Furthermore, the results of these authors showed that the total number of molds in the samples of whole grain wheat flour exceeded the value of $1.0x10^4$ cfu/g. Rezazadeh et al. (2013) determined the presence of molds in 31.5% samples of flour in larger number than 10^4 cfu/g. According to studies of Asadzadeh et al. (2014) in the samples of wheat flour the presence of molds was determined in the interval from 10^2 to 10^4 cfu/g.

From all examined samples of wheat, corn and buckwheat grain numerous species of molds were isolated, which are sorted in 7 genera and 11 species (Table 3).

Most frequently isolated species belonged to the genera *Fusarium*, *Alternaria* and *Cladosporium*. Species from those genera are field molds and they require higher content of moisture in the substrate and lower temperatures for their growth (Dimić, 1999; Maletić, 2005; Sinovec et al., 2006; Kocić-Tanckov and Dimić, 2013, Oliveira et al., 2014).

Mycopopulations of wheat, corn and buckwheat flour were sorted in 7 genera and 14 species. The most dominant genera were *Aspergillus* and *Penicillium*, that were equally presented with 4 species each (Table 4, Figure 3).

The presence of *Alternaria, Fusarium, Cladosporium* and *Rhizopus*, or so called „field molds" should not be neglected. Most probably, it refers to inadequate grain surface treatment before the milling. From the results of our research on mycopopulation composition of wheat flour samples, a similarity in composition and the frequency of appearance can be noticed, as Weidenbörner et al. (2000) also reported. These authors reported that in the samples of whole grain wheat flour most dominant genera were *Aspergillus* (84%), and *Penicillium* (77.3%). Demirel and Sariozlu (2013) pointed on the significant presence of species from the genera *Aspergillus* (42.82%) and *Penicillium* (42.65%) in flour. According to the results of Plavšić (2015) and Plavšić et al. (2015), most frequently isolated species of molds in the samples of flour were also *Aspergillus* (93.33%) and *Penicillium* (73.33%), followed by *Eurotium* and *Paecilomyces* (46.67%), *Rhizopus* (33.33%).

Table 3.
Mold species isolated from wheat, corn and buckwheat grain

Molds species	Sample of grains		
	Wheat	Corn	Buckwheat
Alternaria alternata	+		+
Aspergillus flavus		+	
A. niger		+	
Cladosporium cladosporioides	+		+
Fusarium proliferatum	+	+	
F. sporotrichioides	+	+	+
Penicillium aurantiogriseum	+		
P. expansum	+		
P. oxalicum		+	
Rhizopus stolonifer		+	
Scopulariopsis brevicaulis			+

Legend: + - presence of mold species in the samples

Table 4.
Mold species isolated from flour samples

Molds species	Sample			
	Wheat flour	Whole grain wheat flour	Corn flour	Whole grain buckwheat flour
Alternaria alternata				+
Aspergillus flavus			+	
A. fumigatus				+
A. niger			+	
A. versicolor	+			
Cladosporium cladosporioides	+	+		+
Chrysonilia sitophila				+
Fusarium proliferatum		+	+	
F. sporotrichioides	+		+	
Penicilium aurantiogriseum	+			+
P. commune			+	
P. expansum	+	+		
P. oxalicum			+	
Rhizopus stolonifer			+	

Legend: + - presence of mold species in the samples

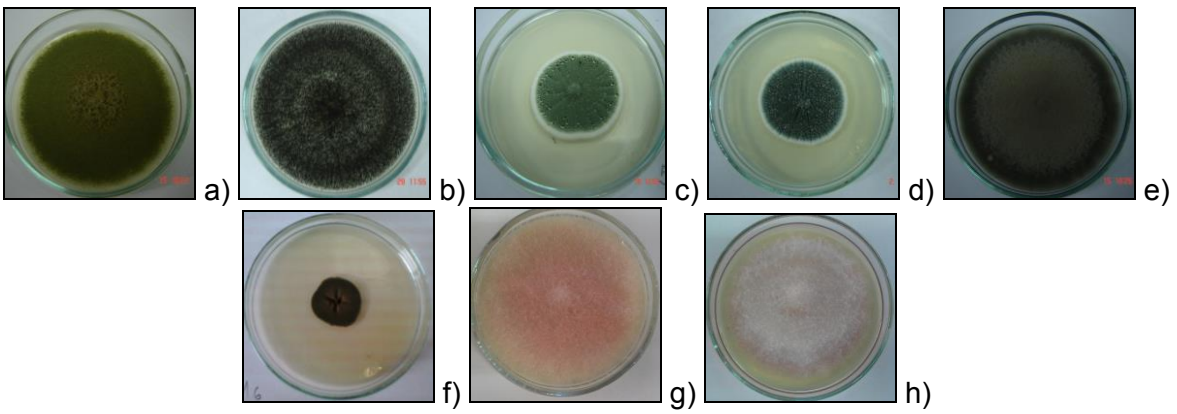


Figure 3. Most frequently isolated species: a) *Aspergillus flavus*, b) *Aspergillus niger*, c) *Penicillium aurantiogriseum*, d) *Penicillium expansum,* e) *Alternaria alternata*, f) *Cladosporium cladosporioides,* g) *Fusarium sporotrichioides* and h) *Fusarium proliferatum* (a-d CYA, e-h PDA, 25 ºC, 7 days)

Mycological analysis of Mariotti et al. (2011) showed that maize grains were contaminated with many yeasts and fungal genera such as *Mucor* spp., *Alternaria* spp. and *Rhizopus* spp., but *Penicillium* spp. and *Aspergillus* spp. were the most abundant isolated genera of fungi. From 15 species of molds isolated during the present investigation (Tables 3 and 4), in accordance with Pitt & Hocking (2009), 10 of them are potentialy toxigenic, which means 66.7% of the total mycopopulation of tested cereal grains and flour samples. According to the same authors *C. sitophila*, *C. cladosporioides*, *R. stolonifer* and *S. brevicaulis* are not potentialy toxigenic.

CONCLUSIONS

All examined samples were contaminated with different molds. The total number of molds per 100 of cereal grains was between 60 cfu (in wheat) and 120 cfu (in buckwheat). The total number of molds in the samples of flour ranged from $6.0x10^1$ cfu/g in white wheat flour to $5.0 x10^2$ cfu/g in whole grain buckwheat flour on DG18 medium. Isolated molds were classified in 8 genera (*Alternaria, Aspergillus, Cladosporium, Chrysonilia, Fusarium, Penicillium, Rhizopus* and *Scopulariopsis*) and 15 species. The results are pointing out the necessity of the grain surface treatment, preceding the milling of grains in wheat, corn and whole grain buckwheat flour production.

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МИКОПОПУЛАЦИЈЕ ЗРНА И БРАШНА ПШЕНИЦЕ, КУКУРУЗА И ХЕЉДЕ

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Сажетак: Према нутритивним карактеристикама интегрално брашно је висококвалитетно, због изузетно великог садржаја витамина, минерала, а посебно дијетних влакана. Међутим, зрно житарица је такође подложно и низу контаминација у току сазревања, жетве, складиштења и прераде. Циљ овога рада био је испитивање присуства плесни на зрну и у брашну пшенице, кукуруза и хељде. Извршено је испитивање укупног броја плесни и детерминација изолованих родова и врста плесни. Сви узорци су били контаминирани плеснима. Укупан број плесни на 100 зрна кретао се од 60 cfu код пшенице до 120 cfu код хељде. Укупан број плесни у узорцима брашна кретао се од $6.0x10^1$ cfu/g код белог пшеничног брашна до $5.0 x10^2$ cfu/g код интегралног хељдиног брашна на DG 18 подлози. Из анализираних узорака изоловано је осам родова плесни (*Alternaria, Aspergillus, Cladosporium, Chrysonilia, Fusarium, Penicillium, Rhizopus* и *Scopulariopsis*) и петнаест врста. Највећи број изолованих врста припадао је роду *Aspergillus.* Од укупно изолованих врста плесни око 66,7% сврстава се у потенцијално токсигене врсте. Добијени резултати указују на неопходност површинске припреме зрна која претходи млевењу у поступку добијања интегралног пшеничног, кукурузног и хељдиног брашна.

Кључне речи: *пшеница, кукуруз, хељда, брашно, плесни*

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