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Microbiology and Safety of Bran from Latvia

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Abstract. Scientific work was undertaken to establish the microbiological status of Latvian wheat (*Triticum aestivum L*) and rye *(Secale cereal L)* bran the prevalence of microorganisms in the four bran samples. Bran's was obtained from industrial mills Stock Company "Rigas dzirnavnieks", SC "Jelgavas dzirnavnieks" and SC "Dobeles dzirnavnieks", harvested period was summer of 2012.
The highest microbial contaminations with yeasts on the bran samples were found in wheat bran with large particle size "Dobeles dzirnavnieks" (WLSD), its approximate contamination is 10 ln CFU g^{-1}, in the second place are wheat bran with large particle size "Rigas dzirnavnieks" (WLSR) - 8 ln CFU g^{-1}. Assessing microbial contaminations with moulds it's possible to conclude that the more polluted bran is (WLSD) – 8 CFU g^{-1}. Investigated bacterial impurity of bran, it can be stated that there are not significant differences between the samples ($p>0.05$), bacterial impurity ranged from 8 to 10 ln CFU g^{-1}.

Keywords: Deoxynivalenol, mycotoxins, bran safety, microbiology

1. Introduction

Mycotoxins occurring in food commodities are secondary metabolites of filamentous fungi, which can contaminate many types of food crops throughout the food chain. Although hundreds of fungal toxins are known, a limited number of toxins are generally considered to play important roles in food safety. Around a quarter century back itself, the World Health Organization estimated that approximately 25% of the world's grains were contaminated by mycotoxins. This figure has most certainly grown since then due to an increase in global import and export of grains and cereals and the changing environmental and weather patterns. (C.S. Reddy, 2011)

Most common micotoxins contamination in cereals is Aspergillus, Penicillium, Fusarium and Claviceps (Finnegan D, 2010). Mycotoxins are formed during cereal growth or in post-harvest storage during the wet season, sun drying practiced by most farmers may not adequately reduce the moisture content in grains. As a result grains with moisture content higher the permissible level enter the storage system.

Thus, wheat and rye grains with moisture content higher than the desired level enter the storage system. In this way appears favourable environment for the reproduction different type of fungus, as a result in the future storages they produce important quantities of mycotoxins (Finnegan D, 2010).

The aim of this scientific work is estimate wheat and rye bran microbiological contamination with yeast, moulds and bacteria and made connectedness with mycotoxins quantity.

2. Materials and Methods

2.1. Bran Samples

Summer wheat (*Triticum aestivum*) and rye *(Secale cereale)* bran samples were collected from industrial mills in Latvia:

1) Stock Company (SC) 'Dobeles dzirnavnieks'-wheat bran with large particle size bran (**WLSD);**

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2) SC ‘Dobeles dzirnavnieks’-small particle size wheat bran **(WSSD);**
3) SC ‘Rigas dzirnavnieks’-large particle size wheat bran **(WLSR);**
4) SC ‘Jelgavas dzirnavnieks’-small particle size rye bran **(RSSJ).**

2.2. Bran Microbiological Contamination

Microbiological evaluation of grain bran was performed according to the standard LVS EN ISO 7218:2007, LVS EN 4833:2003 (bacteria) and ISO 21527-1:2008 (yeasts and moulds) (Fig. 1).

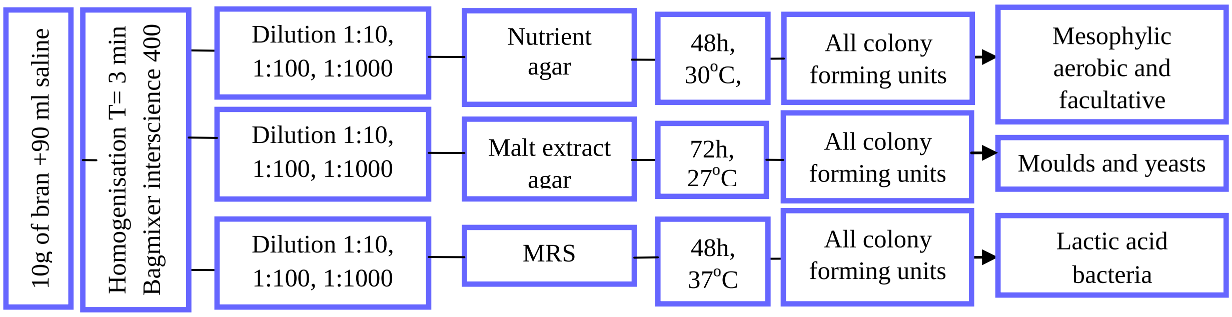

Fig. 1: Scheme of microbiological testing of wheat and rye bran

2.3. Detection of Microscopic Fungi

Detection of microorganisms was performed using the moist chamber method. Samples of bran samples were placed in Petri dishes, moisturized and grown in a chamber for seven days at 25 °C. Developed fungi were identified by their morphological features according to previously described methods (Malcolm, Shurtleff 1995; Sinclair, Dhingra 1995).

Microbiological contamination was analysed under the triocular microscope Axioskop 40. Pictures were taken by digital compact camera Canon PowerShot A620 via 16×10 or 16×40 magnification of the microscope and processed with software Axiovision Le Rel 4.7.

Moisture content was analysed with *Precisa XM 120* at temperature 110 ± 1 °C in five reiterations.

2.4. Statistical Analysis

Statistical differences were considered significant at $p<0.05$. Microsoft office software version 2007 was used to determine significant differences between the samples.

3. Results and Discussion

Wheat and rye bran contained from 8.8 – 24.8% and 17.1 – 28.3% starchy endosperm, respectively. Accordingly to other scientific works particle size diameters of wheat starchy endosperm approximate is from 10 to 40 µm (Kamal-Eldin et al., 2009), but rye starchy endosperm have two types of starch granules: A - type granules (>10 µm – 85%) and B – type granules (>10 µm – 15%) (Karlsson et al.,1983). Accordingly with this information and dates from (Table I) it’s possible to conclude that the highest content of small particle sizes was in WSSD, it’s explainable by large amount of starchy endosperm with particle size <160 µm.

Table I: Relative particle size distribution of different bran samples

	WSSD	WLSD	WLSR	RSSJ
Relative particle size distribution (µm)			%	
750	0.05	57.35	89.00	4.35
450	8.05	20.65	5.60	25.25
315	23.05	13.66	1.30	41.55
250	17.25	3.80	0.20	18.15
200	17.85	1.23	0.05	5.20
160	13.85	0.43		0.55
<160	15.1	0.25		0.35

Considered tables dates of RSSJ particle size distribution can be explained by A – types starchy endosperm dispersion with particle size >10 µm in this bran samples, on the other hand particle size distribution can be affected by grain type, and varietal heterogeneity as well as differences in milling processing. Wheat and rye bran with a larger particle size is rich in different bran layers, such as pericarp-rich fractions or aleurone-rich fractions.

We have two bran samples (Table I) contained 57.35 - WLSD, 89.0% - WLSR, of particles whose diameter were 750 µm, only one samples has 15.1% - WSSD, of particles whose diameter were <160 µm.

The particle size dispersion were higher in the RSSJ: it contained more medium particles (315 µm), than the WLSR (1.30%) and WLSD (13.66%). More homogeneous distribution of particle sizes were in WSSD, there is 8.05% - 450 µm, 23.05% - 315 µm, approximate amount of particles with diameter 250 – 200 µm (17.25, 17.85), and 13.85%- 160 µm.

One of most important factor for fungal contamination and developments is cereal harvesting period and storage conditions. Temperature impact closely related to grain moisture. Fungi produce mycotoxins. Synthesis is highest when product humidity is above 13% and temperature is between 24 °and 37 °C. That is why warm and wet geographic regions are the most favorable environments for mycotoxins and usually are affected (Jakić-Dimić1 D et al., 2009).

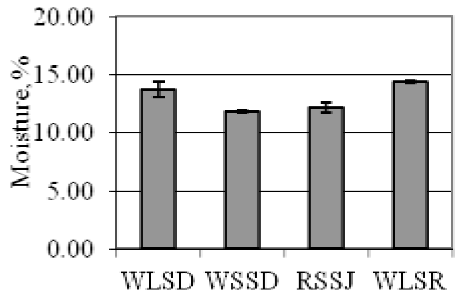

Fig. 2: Moisture content of different bran samples, %

Milling industries used different type of cereal drying and milling technologies, which can be affected on bran moisture content. Not sufficiently know only moisture content of bran, another important marker is water activity (a_w) which can influence on fungal development.

According to the information from fourth edition of ‘Wheat chemistry and technology’ (Khan and Shewry, 2009), the moisture content increases in outer layers. This increase in bran probably results from a plasticizing effect of water, which reflects a phase transition within the cell walls and more particularly within the most hydrophobic regions, which are rich in cutin (Evers and Reed, 1988).

Our findings imply that the increase of moisture content of bran might be caused by the increase of particle sizes in bran that leads to intensive growth and reproduction of fungi spores.

Analysing data of bran moisture (Fig. 2) it’s possible to conclude that the highest moisture content was in WLSR wheat bran with large particle size (14.37±0.11%), but the lowest in WSSD wheat bran with small particle size (11.85±0.10%). One-way Anova showed there were significant differences between the four bran samples ($p<0.05$). Moisture content varied from 11.85±0.10% to 14.37±0.11%.

3.1. Level of fungal contamination of bran samples

The highest concentration of moulds were observed in (WLSD) bran samples 8 ln CFU g^{-1}, the lowest in (RSSJ) 5 ln CFU g^{-1} Bran showed not significant level of fungal contamination (Fig. 3). Apparently, this mycobiota contains various species of fungi like representatives of ephytic microflora in plant grains.

Fungi were detected in all bran samples, as well as in samples of wheat and rye barn, with the genus *Penicillium* being the most frequent. Fungal counts ranged from 5 to 8 CFU g^{-1}. Water activities of the samples ranged from 0.75 to 0.80 for wheat and rye bran. According to (Adams M. R. and Moss M. O., 2000), the minimum water activities of some common field and storage fungus are 0.89 – *Fusarium spp.* 0.88 – *Alternaria spp.* 0.80-0.82 – *Penicillium spp.* 0.75-0.78 – *Aspergillus spp*.

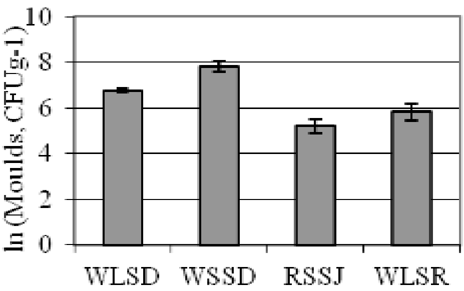

Fig. 3: Number of particular microbiological contamination with moulds.

In our study, we found that all samples were mostly contaminated with *Penicillium spp.* this is explained by the fact that the most appropriate water activity (a_w) for *Penicillium spp.* These results agree with findings of Li and Yoshizawa (2000), who reported a higher frequency of species of the genera *Drechslera*, *Penicillium*, *Fusarium*, and *Aspergillus*, among others.

Fig. 4: A) *Penecillium* genera on Malt extract agar, B) Penecillium genera ×100.

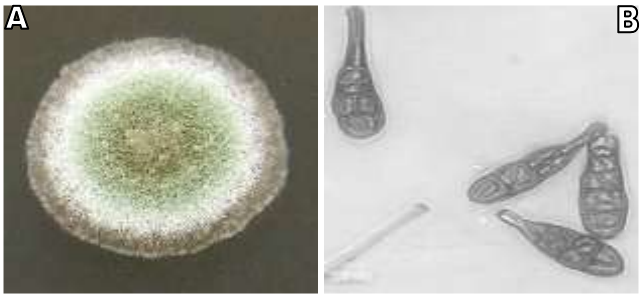

Fig. 5: A) *Alternaria* genera on Malt extract agar, B) *Alternaria* genera ×100.

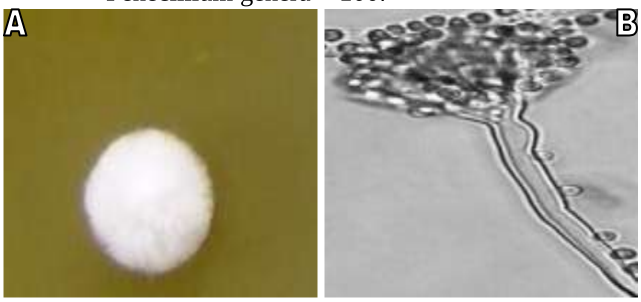

Fig. 6: A) *Aspergillus* genera on Malt extract agar, B) *Aspergillus* genera ×100.

Fig. 7: A) *Fusarium* genera on Malt extract agar, B) *Fusarium* genera ×100

The microbiology of cereals, during growth, harvest and storage is dominated by the moulds The most important genera of the storage fungi are *Penecillium* and *Aspergillus*, although species of *Fusarium* may also be involved in spoilage when grain is stored under moist conditions. Water activity and temperature are the most important environmental factor influencing the mould spoilage of cereals, and the possible production of mycotoxins (Adams M. R. and Moss M. O., 2000). In further studies we paid more attention to the isolates of *Aspergillus* and *Penicillium* genera as producers of the most important mycotoxins responsible for mycotoxicosis of animals and human.

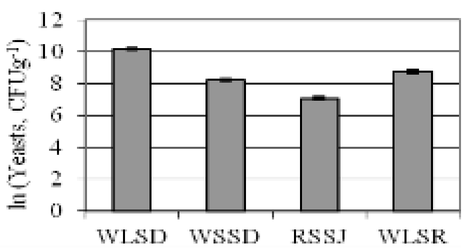

Fig. 8: Level of particular microbiological contamination with yeasts.

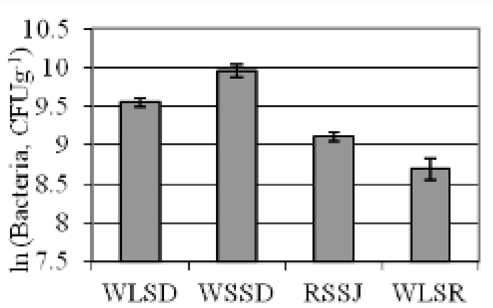

Fig. 9: Number of particular microbiological contamination with bacteria.

In consequence of the study of all the data's it's possible to conclude that the most resistant to the yeast as well as fungi is (RSSJ). The higher tolerance of rye to disease results from its higher growth rates and from the fact its grain-filling assimilates are photosynthesized mainly in the stalk and head (Czembor J and Sowa W, 2001). Further bran samples investigation of contamination with yeast, was found that most polluted is wheat bran with large particle size " Dobeles dzirnavnieks" (WLSD) , number of bacteria is 10 ln CFU g^{-1}, in turn, the smallest yeast content was found in (RSSJ) 7 ln CFU g^{-1}.

From the three of wheat and one of rye bran samples, the results (Fig. 9) showed contamination with bacteria. The initial study showed that the most contaminated sample by bacteria is WSSD, amount of bacteria ranged from 8 to 10 ln CFU g^{-1}. The most common pathogenic bacteria are *Xantahmonas campestris*, which causes bacterial leaf stripe. *Pseudomonas syringae*, which causes bacterial leaf blight and *Corynebacterium spp*., which cause yellow ear rot and wrinkled or twisted leaves (Czembor H.J and Sowa W 2001).

In our scientific work we don't isolates genera's from bran species, purpose of this work was to determine the number of bacteria.

This study was designed to screen wheat and rye bran obtained from cereal grain for mycotoxin contamination.

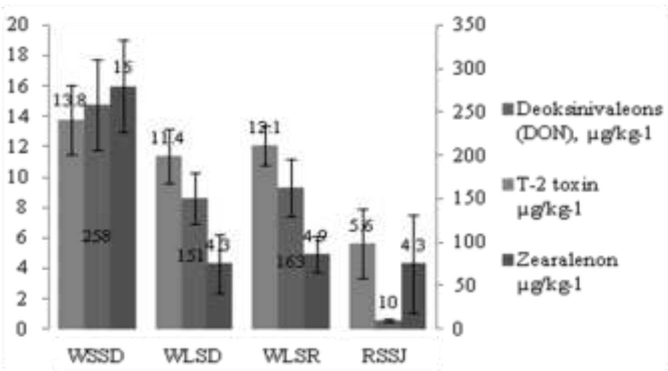

Fig. 10: Most common Fussarium mycotoxin of wheat and rye bran, µg/kg^{-1}

Analyses of 4 - summer bran samples harvested period is 2012 showed all samples contain traces of mycotoxin (Fig. 10). DON, ZEA and T-2 toxin were found in all of samples. Concentration of DON varied from 10 ± 2 µg/kg^{-1} to 258 ± 52 µg/kg^{-1}. Considered concentration of T-2 toxin amount it's could be possible to conclude: in all of bran samples T-2 toxin is less than <20 µg/kg^{-1}. ZEA concentration in bran samples ranged from 4.3 ± 3.2 µg/kg^{-1} to 16 ± 3 µg/kg^{-1}. Comparing all of bran samples RSSJ rye bran with small particle size were found to be least contaminated with mycotoxins. Although European Committee established a tolerable daily intake (TDI) µg/kg^{-1} body weight day^{-1} for DON, T-2 toxin and ZEA. About 6.7 times less than TDI for DON, 1.44 times less than TDI for T-2 toxin, 4.6 for ZEA.

4. Conclusions

This study with the wheat and rye bran samples asserts that fungal infection occurs during harvest as well as grain milling technologies and storage conditions. This might be caused by the differences in their moisture contents as well as bran water ability (a_w). Bran samples showed not so high levels of fungal contamination, but contamination increases due to increase of moisture content.

Our experimental findings showed:

1. Mycotoxin concentration increases due to increase of moisture content in bran samples;
2. During this study 4 different genera was isolated from bran samples: *Aspergillus spp*., *Alternaria spp*., *Penicillium spp*., *Fusarium spp.*

5. Acknowledgements

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6. References

[1] A.D. Evers, M. Reed. Some novel observations by scanning electron microscopy on the seed coat and nucellus of the mature wheat grain. *Cereal Chem.* 1988, 65: 81–85.

[2] C. Malcolm, C.W. Shurtleff. The Plant Disease. *Clinic and Field Diagnosis of Abiotic Diseases*. APPS Press, 1995.

[3] C.S. Reddy. Mycotoxin Contamination of Rice. *Rice. Know. Managt. Por.* 2011, 7: 2-21.

[4] Commision Regulation (EC) No 2005/38 of analysis for the official control of the levels of Fusarium toxins in foodstuffs (2005) Official Journal of the European Union, L 143/18 – L143/26: Available at: http://eur lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:143:0018:0026:EN:PDF

[5] D. Finnegan. Mycotoxins in cereals: sources and risks: 2010, Available at http://services.leatherheadfood.com/eman/FactSheet.aspx?ID=72

[6] D. Jakic-Dimic, K. Nesic, M. Petrovic. Contamination of cereals with aflatoxins, metabolites of fungi Aspergillus flavus. *Biotechnology in Animal Husbandry*. 2009, 25 (5-6): 1203-1208.

[7] H.J. Czembor and W. Sowa. Diseases and Pests of Rye. In: W. Bushuk, (eds.). *Rye: Production, Chemistry, and Technology.* Canada: Department of Food Science University of Manitoba Winnipeg, Manitoba. 2001, pp. 37-52.

[8] H.J. Czembor and W. Sowa. Diseases and Pests of Rye. In: W. Bushuk, (eds.). *Rye: Production, Chemistry, and Technology.* Canada: Department of Food Science University of Manitoba Winnipeg, Manitoba. 2001, pp. 37-52.

[9] J.B. Sinclair, O.D. Dhingra. Basic Plant Pathology Methods. CRC Press, 1995.

[10] K. Khan, P.R. Shewry. Wheat kernel structure. In: K. Khan, P.R. Shewry, (eds.). *Wheat chemistry and tehnology.* Minnesota: AACC International St. Paul. 2009, pp. 81-145.

[11] K.E. Afaf, H.N. Lærke, K.E.B. Knudsen, A.L. Lampi, V. Piironen, H. Adlercreutz, K. Katina, K. Poutanen, P.A. Mand. Physical, microscopic and chemical characterization of industrial rye and wheat brans from the Nordic countries. *Food Nutr. Res*, 2009, 53 (0): 11.

[12] M.R. Adams. and M. O. Moss. Food microbiology. In: M. R. Adams. and M. O. Moss, (eds.). *Factor Affecting the Growth and Survival of Micro-organisms in Food.* Cambridge: CB4 0WF, UK. 2000, pp. 37-38.

[13] R. Karlsson, R. Olered, A.C. Eliasson. Changes in starch granule size distribution and starch gelatinization properties during development and maturation of wheat, barley and rye. *Starch/Staerke*, 1983, 35: 335-340.