EFFECTS AND CHANGES OF ZEARALENONE AND FUMONISIN CONTAMINATION IN CORN-BASED BIOETHANOL PROCESS

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Bioethanol production is a growing industry nowadays. In this work dry-grind ethanol production was carried out from different corn samples (uncontaminated; zearalenone; fumonisin B_1+B_2 contaminated) and the changes of the ratio of the solid-liquid phase as well as toxin concentrations were examined in laboratory scale. The ethanol yields of mycotoxin-contaminated corn-mashes were 27% lower, due to 10% less produced glucose-concentrations from these raw materials, compared to uncontaminated ones. By the end of the whole process, the initial 20% solid content was reduced below 7% both in contaminated and uncontaminated corn-mashes. Differences were observed in the concentration changes of examined toxins. Zearalenone was localized in the solid phase, and its concentrations did not alter during the ethanol production process. Fumonisin concentration increased 3 times at the end of the process, and it dissolved in the liquid phase in significant amount.

Keywords: Mycotoxins, bioethanol, zearalenone, fumonisin

Introduction

There is a growing significance of corn usage for industrial purpose along the human and animal nutrition since this can be produced relatively cheaply. For this reason the mainly used source for bioethanol production is corn. The mouldy attach of cereals is not only a yield influencing factor. The mycotoxin producing Deuteromycota (mainly *Aspergillus, Fusarium, Penicillium* and *Alternaria* species)* found both on fields and during crop-storage can cause serious food safety and health risk to vertebrates. The effects of the most important fungi metabolites – aflatoxins, fusarium toxins (trichotechenes, zearalenone, fumonisin), ocharoxin A – on the living organisms are well studied and this is a continuously developing field of science, as well as their analyzing techniques and decreasing possibilities of their occurrence [1-6].

Several Fusarium fungi, which are common soil fungi, produce different mycotoxins of the class of trichothecenes (T-2 toxin, HT-2 toxin, deoxynivalenol (DON) and nivalenol and some other toxins (zearalenone and fumonisins). The Fusarium fungi are probably the most prevalent toxin-producing fungi of the northern temperate regions and are commonly found on cereals grown in the temperate regions of America, Europe and Asia. Fusarium toxins have been shown to cause a variety of toxic effects in both experimental animals and livestock. On some occasions these mycotoxins also been suspected to cause toxicity in humans [7].

Zearalenone (ZEA) – also known as F-2 toxin –, a lactone of the derivative of the resorcylic acid *(Fig. 1)* has estrogenic effect [8]. It is found worldwide in a number of cereal crops such as maize, barley, oats, wheat, rice and sorghum, and also in bread. ZEA is a stable compound, both during storage/milling and the processing/cooking of food, and it does not degrade at high temperatures. Wet milling of corn concentrates ZEA in the gluten fraction (2-7 fold concentration) [9]
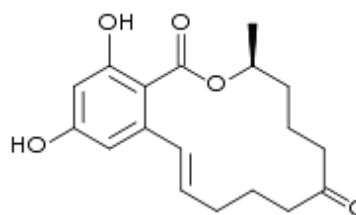

Figure 1: Structure of zearalenone
(www.fermentek.co.il)

Fumonisin B_1 (FB_1) *(Fig. 2)* – discovered in 1988 – belongs to fumonisins, which have been reported to cause various diseases in animals, such as liver and kidney cancer in rodents, leukoencephalomalacia in equines, pulmonary oedema in pigs.

Fumonisin B_2 *(Fig. 3)* is more cytotoxic than Fumonisin B_1, both are carcinogenic mycotoxins. They are produced by *Fusarium verticilloides* that commonly contaminate maize. FB_1 has been found as natural contaminant in maize and maizebased food worldwide.
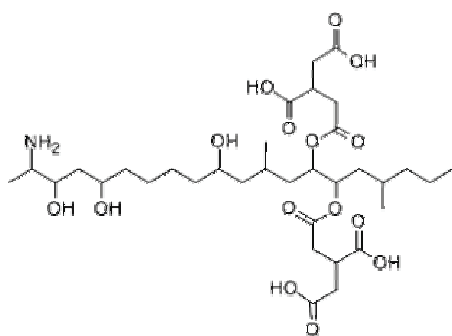
Figure 2: Molecular structure of FB$_1$
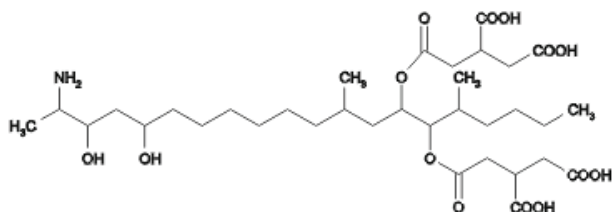(www.fermentek.co.il)

Figure 3: Molecular structure of FB$_2$
(www.fermentek.co.il)

FB$_1$ is stable during most types of processing. Dry milling of maize results in the distribution of FB$_1$ into the bran, germ and flour. FB$_1$ is stable in polenta (maize porridge). However, the concentration of FB$_1$ is reduced during the manufacture of cornstarch by wet milling, since FB$_1$ is soluble in water [10].

The simultaneous occurrence of two or more toxins in cereals has also been frequently documented in the literature, e.g. maize contaminated with FB$_1$, ZEA, DON and T-2 toxin [11].

There are different limit values recommended of several legislations in food safety. *Table 1* indicates the EU health regulation of the mycotoxins studied in this work. Zearalenone has a stronger impact on vertebrates so its regulation limit is lower than fumonisin's.

Table 1: Recommended limit values of mycotoxins

	Upper limit (µg/kg crop)	
	ZEA	Fumonisin B$_1$ and B$_2$
Grains for human consumption	350 [12]	EU: 1,000 (B$_1$+B$_2$) [12]; US: 2-4,000 (B$_1$+B$_2$+B$_3$) [13]
Ground grains (>500 µm) for not direct human consumption (19041010 KN-code)	200 [12]	1,400 (B$_1$+B$_2$) [12]
Feed	3,000* [5]	20,000 (swine, B$_1$+B$_2$+B$_3$) [14]; 60,000* (B$_1$+B$_2$) [5]

* reference value

Wet-milling and dry-grinding are the two major technologies used to convert corn grain into ethanol [15]. The wet-milling process is designed for the best utilization of the corn grain while dry-grinding process is designed to be cost effective. In wet-milling process corn is fractionated into its individual components of starch, protein, fibre, germ and soluble, so there are more products beyond the ethanol (e.g. corn syrup, dextrose, glucose). However, in conventional dry-grinding process there is no fractionation; the whole corn kernel is ground, mixed with water and convert to alcohol.

Distillers grains with solubles (DGS) are the main byproduct feeds produced, which have a good nutritional value. Its starch content less than 5% of dry matter, These can be marketed in the original wet form (~33% dry matter, WDGS), dried partially and called modified (~48% dry matter, MDGS), or dried (~90% dry matter, DDGS). Nutrient composition of DGS, consequently its quality can be highly variable. It consists of mainly protein and fibre (lignocellulose, hemicellulose, pectin): approximately 31% CP (crude protein) (70% undegradable intake protein), 11.9% ether extract, 33% NDF (non-degradable fibre), 4.5% ash, 0.84% P, and 0.77% S [16].

The current study was undertaken to examine the changes of the mycotoxin content in both the solid and liquid phase of the naturally contaminated substrate during the dry-grinding bioethanol process. We also compared the maximum ethanol yield achieved to the end of the fermentation from naturally contaminated and control corns.

Materials and methods

Preparation and fermentation of corn

For the experiments uncontaminated and naturally mycotoxin contaminated corns (1,170 µg/kg zearalenone and 760 µg/kg fumonisin B$_1$+B$_2$) were processed. Corn grains were ground in two steps: at first step it was ground in a hammer mill with a 2 mm screen size, and then in a coffee grinder under the particle size of 1 mm of the ground flour.

The starch-hydrolysis was carried out from 2,000 g ground corn slurry (20% solids content) in a completely-stirred glass reactor. For the starch liquefaction α-amylase (Liquozyme SCDC, NOVOZYME) was used in the amount of 0.88 mg/g dry corn source. The saccharification was performed with gluco-amylase (Spirizyme Fuel, NOVOZYME) in the amount of 1.1 mg/g dry corn source. For the pH adjustment 20% NaOH and 25% H$_2$SO$_4$ were used.

The fermentation process was achieved in Biostat Aplus fermentor with 2 dm^3 useful volume, with Ethanol Red® (FERMENTIS, product-code: 42138) dry alcohol yeast in 7 g/kg dry corn source during 62 hours on 34 °C.

After fermentation, ethanol was distilled from the stillage by a laboratory distillation apparatus equipped with a 20 cm long separating column.

Samples were taken for analyze glucose, toxin and ethanol concentrations. The known weight samples were centrifuged (12,000 rpm, 4 °C, 1 h) and the ratio of their liquid and solid phase was measured. The whole process as well as sampling points (S.I.-S.V.) according to *Table 2* are shown in *Fig. 4*.

Table 2: Analyzed parameters at different sampling points

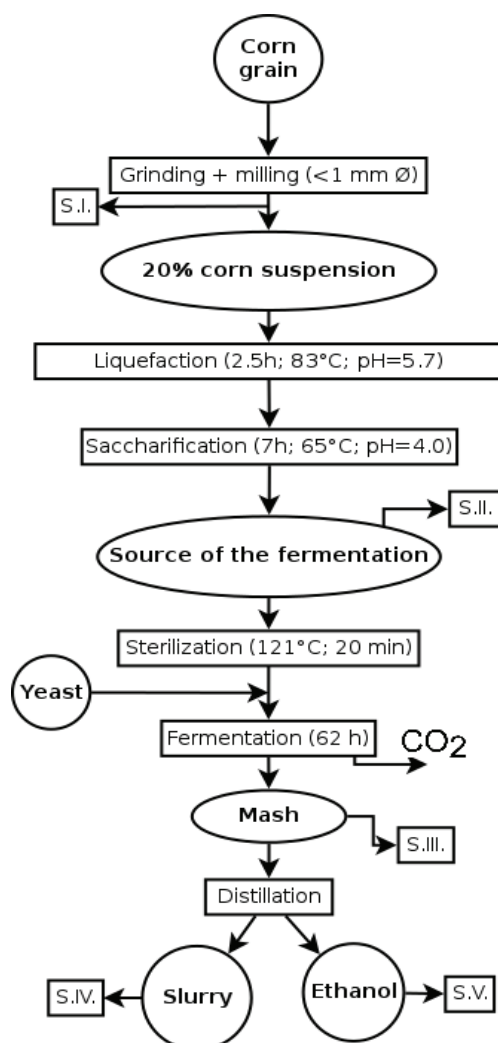| Analyzed parameters	S. I.	S. II.	S. III.	S. IV.	S. V.
Glucose	-	+	-	-	-
Toxins	+	+	+	+	+
Ethanol	-	-	+	+	+

- not analyzed; + analyzed

Figure 4: Flowchart of the bioethanol process

Analyses

Glucose concentrations were determined by high-performance liquid chromatography (HPLC). Samples for HPLC analyses were prepared by filtration through a 0.2 μm pore size cellulose acetate filter (Sartorius Stedim Biotech GmbH, Germany). For the analysis YL 9100 type of liquid chromatograph (Young Lin, Rep. Korea), Aminex-87P column and pre-column HyperREZ XP Pb were used at 35 °C with bidistilled water as mobile phase at 0.3 mL/min flow rate. A refractive index detector was applied for detection of monosaccharides.

Ethanol concentrations of the produced mash and alcohol distillate, as well as the remained alcohol content of the WDGS were determined by gas chromatography (GC). Samples for GC analyses were prepared by

filtration through a 0.2 μm pore size cellulose acetate filter (Sartorius Stedim Biotech GmbH, Germany). For the analysis YL ACME 6000 type of gas chromatograph (Young Lin, Rep. Korea) equipped with flame ionization detector and autosampler was used. DB-FFAP (Agilent J&W) GC column was used at 200 °C (injector) and 250 °C (detector) with N_2 carrier (6 mL/min, 20:1 split ratio). During the run an oven temperature program was applied [60 °C (3 min) → 10 °C/min → 200 °C (10 min)].

MSZ EN 12955:2000 sample preparative method, AOAC 985.18.:1988 (ZEA) and MSZ EN 13585:2002 (FB1+B2) analysis standards were used for measuring the mycotoxin contents.

Results and Discussion

Fermentation

Glucose concentration after the starch-hydrolysis of mycotoxin contaminated corn stock was 10% lower than of uncontaminated ones. Similar conversion efficiencies could be observed at the end of the fermentation process, though the specific ethanol yield was 27% lower in the case of contaminated corn stock *(Table 3)*.

Table 3: Fermentation results

Corn	Initial glucose concentration ($g \cdot dm^{-3}$)	Ethanol conversion (%)	Specific ethanol yield (g EtOH· g corn dry matter^{-1})
Uncontaminated	169	85	0.34
	171	82	0.32
ZEA+FB$_1$+B$_2$-contaminated	151	84	0.25

The ratio of solid and liquid phase of both contaminated and uncontaminated corn suspensions changed similarly in each technological steps *(Fig. 5)*. Dry matter content decreased from 20% to 5-7% during the bioethanol production process.
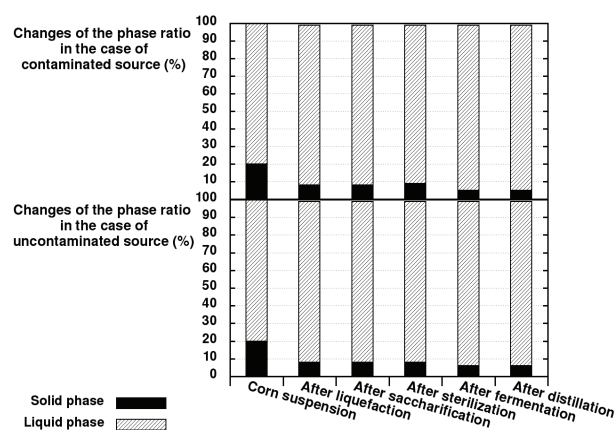

Figure 5: Changes of the ratio of solid and liquid phase at the end of each technological steps

Mycotoxin monitoring

Zearalenone was localized principally in the solid phase of corn mash due to hydrophobic characteristics of the toxin. Its concentration did not alter *(Fig. 6)*, what shows thermal stability and low chemical reactivity of the molecule under applied circumstances.

Fumonisin concentration increased three times at the end of distillation *(Fig. 7)*. The "multiplication" can be explained by appearance of starch-bound fumonisins after saccharification and getting more concentrated suspension after distillation, since there was no any toxin in distillate. Maize like other cereals is a complex matrix, where polysaccharides, proteins and lipids can form different bonds with FBs changing the structure and toxicity of the molecule. Accurate knowledge of this masking mechanism is not available yet [17].

In contrast to ZEA, significant amount of FB getting out in the liquid phase can be due to the chemical structure of this toxin molecule: the four carbonyl groups, amino and hydroxyl groups located on carbon structure also facilitate good solubility in water.

Zearalenone and fumonisin concentrations of dried out solid phase increased 1,402 to 3,173 µg/kg and 911 to 3,434 µg/kg, respectively.
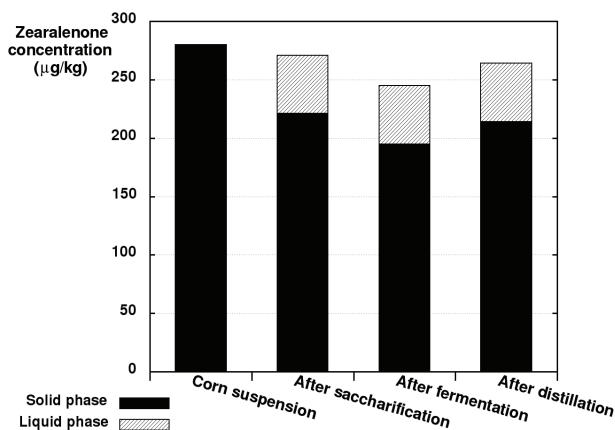

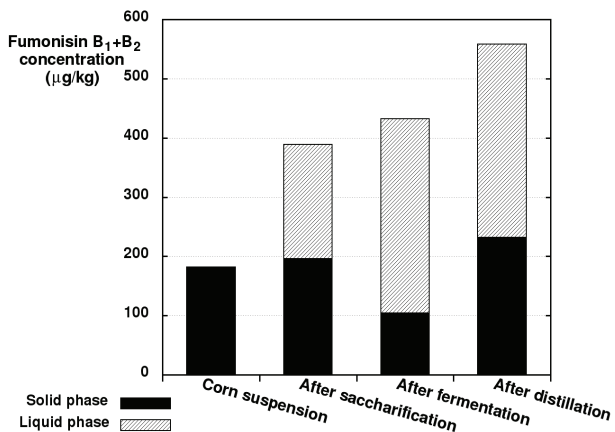*Figure 6*: Changes of Zearalenone concentrations calculated to corn suspension

Figure 7: Changes of Fumonisin concentrations calculated to corn suspension

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