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Fate of deoxynivalenol and nivalenol during storage of organic whole-grain wheat flour

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ABSTRACT

This study was carried out to determine the level of retention of the mycotoxins deoxynivalenol (DON) and nivalenol (NIV) during 120 days of storage (aging) of flours produced from organic wheat grain naturally infected with *Fusarium* fungi. Three types of flour (standard white flour prepared by a roller-grinder mill – IRG, whole-grain flour produced by a hammer–crusher mill – IHC and whole-grain flour prepared by a millstone – OMS) were packaged in food-grade paper or polypropylene plastic bags and stored at two different storage temperatures (constant 10 °C or 25 °C). The concentrations of DON and NIV were measured prior to and after storage by means of HPLC-UV detection methods. After 120 days of storage, the concentrations of DON and NIV decreased between 0% and 29% compared to the initial measurements, depending on the combination of experimental factors. The greatest decrease in mycotoxin concentration was observed in the IHC and OMS flours packaged in paper bags and stored at 25 °C. The smallest decrease in mycotoxin concentration was observed in the IRG flours packaged in sealed plastic bags and stored at 10 °C. Statistical analysis showed that the level of retention of DON and NIV depended significantly on the type of packaging material, but did not depend on the type of flour or the storage temperature.

1. Introduction

In recent years, there has been an enormous increase in consumer demand for traditionally produced organic cereal products in European markets. Several types of organic cereal products are marketed. The first group includes products that are baked in industrial bakeries from flours produced from organic cereal grain using industrial methods. The second group of products is offered by small boutique organic bakeries that blend several types of whole-grain and organic flours in specific ratios. The third group consists of products that are baked at organic farms from whole-grain flours using traditional methods. Many consumers accept only products of the third type as organic.

Organic producers who bake cereal products at farm-level bakeries lack information regarding the influence of simple mid-duration storage methods for whole-grain flours on the rheological and food safety microbiological quality of flours. These organic producers are often concerned about mycotoxin contamination of stored flours. They do not have sophisticated storage equipment, facilities or educated food safety personnel for quality monitoring of the stored flour.

Mycotoxins produced by fungi belonging to the *Fusarium* genus are one of the major microbiological concerns in the organic milling and baking industry. They occur very frequently in bakery products, and have been shown to have adverse and chronic health impacts (FAO, 2002; Murphy et al., 2006; Scudamore, 2008). The likelihood of occurrence of inappropriately high concentrations of *Fusarium* mycotoxins, especially deoxynivalenol (DON) and nivalenol (NIV), in whole-grain products prepared at the organic farm level is slightly higher than that of whole-grain industrial bakery products (Schollenberger et al., 2005; Lešnik et al., 2008).

There are many studies regarding the fate of mycotoxins during the storage and processing of wheat grain. However, currently there is insufficient information about their fate during the storage of flours. Mycotoxins are very stable compounds that decompose only during long exposure to high temperature processing treatments or after exposure to high concentrations of bases or other aggressive chemicals (Wolf-Hall et al., 1999; Hazel and Patel, 2004; Bretz et al., 2006). There are also several microbes that have been shown to decompose mycotoxins (Völkl et al., 2004; Zhou et al., 2008).

Flour is a mixture of complex organic substances and microbes. Because of the low moisture content (m.c.) under normal storage

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conditions, microbes are not in an active state in flour. Nevertheless, whole-grain flours are especially high in microbial load, because a significant number of the microbes that colonize the grain surface enter the flour during the milling process (Chelkowski and Perkowski, 1992; Berghofer et al., 2003; Lugauskas et al., 2006). There are many chemical processes that occur during the storage of the flour. Two processes that occur during the storage of flour are the oxidation of essential compounds by lipases and proteases and the development of flour rancidity. Substances that are released during those processes could interact with mycotoxins and change their chemical and biological properties. Complex processes during flour aging are influenced by flour type, storage conditions, packaging materials, microbial activity and many other factors (Sur et al., 1993; Cenkowski et al., 2000; Nishio et al., 2004).

For some organic bakery products, whole-grain flours are processed fresh (i.e., unleavened and sourdough), whereas for many others, the flours must be aged prior to use (similar to most standard industrial flours). During the aging process, the flours are exposed to chemical changes and microbial spoilage. Industrial millers can speed up the flour aging process by adding chemicals such as bleaching agents. Organic millers and bakers are not allowed to use chemical additives if they want to offer so-called "unfortified organic products". Organic millers and bakers must age the flour by traditional methods via storage. The dynamics of the aging process within organic whole-grain flours and standard industrial flours are similar but not identical.

Due to the lack of data describing the dynamics of DON and NIV mycotoxin levels during storage (aging) of organic whole-grain flours, we carried out this study to establish the influence of mid-duration flour storage by organic farms on the retention of trichothecene mycotoxins (DON and NIV). Our hypothesis was that storage of whole-grain organic wheat flours in organic farm-level conditions (20–25 °C, 60–70% relative humidity (r.h.), 13% m.c. and packaged in paper or plastic bags) for several months does not significantly change the concentrations of trichothecene mycotoxins in flours.

2. Materials and methods

2.1. Production of flour before starting the storage experiment

Prior to the start of the storage experiment, flour was produced from wheat grain (Insengrain cultivar) in an industrial mill belonging to the Mlinarstvo Sajko milling company, as well as by a traditional millstone at the Kvas organic farm. Both are located in Slovenia. The chosen organic farm is typical of Slovenian conditions and has a long tradition of preparing stone-milled whole-grain flours.

The initial concentrations of DON and NIV in the experimental lots of wheat grain naturally infected with *Fusarium* fungi under field conditions were determined by HPLC assay. The initial concentration ranged from 1400 to 2400 μg kg^{-1} for DON and from 130 to 200 μg kg^{-1} for NIV. The grain lot with the highest concentration of mycotoxin was chosen for trial performance for easier comparison of the experimental effects and laboratory measurements. The m.c. of the grain prior to cleaning and milling was 13.2%. Three different types of flours were produced from the same lot of wheat grain: IRG flour, IHC flour and OMS flour. Characteristics of the flours at the beginning and end of the storage period are presented in Tables 1 and 2. Determination of basic flour characteristics was performed in the laboratory of the local milling company using standard procedures (ISO, 1998, 2003, 2004, 2007). IRG standard bread flour was produced from grain cleaned using industrial cleaning devices (a combination of separators and aspirators) and milled in a standard industrial roller-grinder mill. IHC flour was produced after cleaning the grain in an industrial cleaning device and milling in an industrial hammer–crusher mill. Wheat grain used for producing OMS flour on the organic farm was first cleaned using a primitive traditional seed cleaner and afterwards milled by a traditional millstone.

Table 1
Flour properties prior to storage.

Type of flour	Moisture (%)	Ash (%)	FN	Acidity (mmol/kg)	Particle size distribution (%)		
					>250 μm	>200 μm	>132 μm
IRG flour	13.4	0.530	202	31.0	0.8	3.5	24.0
IHC flour	13.2	1.715	218	42.9	18.6	25.9	45.0
OMS flour	13.3	1.670	295	33.7	22.3	33.2	56.2

IRG – industrial standard flour, IHC – industrial whole-grain flour, OMS – organic whole-grain flour, FN – falling number.

The initial concentrations of DON and NIV in the different types of flours were not identical, as they were influenced by the different milling methods (Tables 3 and 4). As a result of milling, five lots of IRG, IHC and OMS flour were prepared. From each of the flour lots, samples weighing 10 kg each were packaged and stored. In order to compare the mycotoxin concentrations at the beginning of the storage experiment with those at the end, 3 kg samples were also taken for analysis of DON and NIV.

2.2. Packaging material and flour storage conditions

Flours were packaged in 3 different ways and stored for 120 days in 2 different storage environments. The aim was to mimic the storage environments of organic farms. Two-layered kraft paper bags (AeroPapiroti d.o.o, Slovenia) and food-grade polypropylene bags (Thermoplast d.o.o., Slovenia) were used for flour storage. Paper bags were declared by the producer to be suitable for storage of flour in industrial storage facilities and trade warehouses. Polypropylene transparent bags (0.2 mm thick) were labeled as suitable for packaging all kinds of food products. Each bag was filled with 10 kg of flour. Paper bags and one experimental group of plastic

Table 2
Means (±*SE*) for moisture content and acidity of flour samples at the beginning (BS) and end of 120-day storage (AS) in relation to type of flour (TF), packaging material (PM) and storage temperature (ST).

TF	ST	PM	Moisture content (% ± *SE*)		Acidity (mmol/kg ± *SE*)	
			BS	AS	BS	AS
OMS	10 °C	Pa	13.1 ± 0.1	14.2 ± 0.2*	33.5 ± 0.1	34.0 ± 0.1
OMS	10 °C	Pl1	13.2 ± 0.1	13.8 ± 0.1*	33.5 ± 0.1	33.7 ± 0.1
OMS	10 °C	Pl2	13.2 ± 0.1	13.4 ± 0.1	33.3 ± 0.2	33.7 ± 0.1
OMS	25 °C	Pa	13.3 ± 0.0	14.2 ± 0.1*	33.5 ± 0.1	34.9 ± 0.1*
OMS	25 °C	Pl1	13.3 ± 0.1	13.2 ± 0.1	33.7 ± 0.2	34.3 ± 0.2
OMS	25 °C	Pl2	13.3 ± 0.1	13.2 ± 0.1	33.7 ± 0.2	34.1 ± 0.2
IHC	10 °C	Pa	13.0 ± 0.1	13.7 ± 0.2*	42.6 ± 0.3	46.6 ± 0.5*
IHC	10 °C	Pl1	13.0 ± 0.1	13.2 ± 0.1	43.3 ± 0.7	45.3 ± 0.4*
IHC	10 °C	Pl2	13.0 ± 0.1	13.0 ± 0.0	43.8 ± 0.5	44.5 ± 0.3
IHC	25 °C	Pa	13.2 ± 0.1	13.5 ± 0.1	43.0 ± 0.4	47.8 ± 0.3*
IHC	25 °C	Pl1	13.2 ± 0.1	13.2 ± 0.0	43.3 ± 0.2	44.4 ± 0.2*
IHC	25 °C	Pl2	13.1 ± 0.1	13.2 ± 0.1	43.6 ± 0.2	43.2 ± 0.2
IRG	10 °C	Pa	13.4 ± 0.1	13.8 ± 0.1	30.4 ± 0.4	31.4 ± 0.3
IRG	10 °C	Pl1	13.4 ± 0.1	13.6 ± 0.1	30.3 ± 0.2	31.4 ± 0.3
IRG	10 °C	Pl2	13.5 ± 0.1	13.5 ± 0.1	30.8 ± 0.1	30.7 ± 0.1
IRG	25 °C	Pa	13.4 ± 0.1	13.4 ± 0.1	30.9 ± 0.2	32.7 ± 0.1
IRG	25 °C	Pl1	13.5 ± 0.1	13.5 ± 0.1	31.0 ± 0.3	32.1 ± 0.2
IRG	25 °C	Pl2	13.4 ± 0.1	13.5 ± 0.2	30.5 ± 0.2	30.7 ± 0.1

IRG – industrial standard flour, IHC – industrial whole-grain flour, OMS – organic whole-grain flour, Pa – paper bag, Pl1 – plastic bag open, Pl2 – plastic bag sealed.
*AS values were significantly different from BS values according to a paired sample *t*-test ($N = 5$, $P \leq 0.05$) for the specific combination of experimental factors.