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Evaluation of mycotoxins in imported wheat from SAGO Al-Jouf

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Abstract
The quality of cereals is very important for both human and animal nutrition. Mycotoxins are secondary metabolites of filamentous fungi that occur naturally in food and feed. The presence of these compounds in the food chain is of high concern for human health due to their properties to induce severe toxicity effects at low dose levels. Trichothecenes, zearalenone (ZEN) and fumonisin are the major Fusarium mycotoxins occurring in cereal grains, animal feeds and forages. Conditions that predispose to mycotoxin production by *Fusarium* species include humidity, temperature, aeration and substrate type. Even if a great number of fungal metabolites have been designated as mycotoxins, a small number are known to have significant animal/human health and economic significance. In this context, the aim of this study is evaluate the Mycotoxicological quality of SAGO (Saudi Grain Organization) wheat grains and wheat products wheat flour for Aflatoxins, Wheat, mix wheat and flour samples were analyzed for Aflatoxins (AF), deoxynivalenol (DON), Zearalenone (ZON), T-2/HT-2 toxins and fumonisin (FM) contamination. Results reported in table 3.1 showed that deoxynivalenol (DON) and T-2/HT-2 toxins contamination was mainly observed maximum level of samples analyzed (wheat sample 160 and 101) with a maximum level of contamination of 1400 and 0165ppb.
For mix wheat (113+161) analysis, the levels measured varied from 0018ppb to 0052ppb. The high level was obtained in mix wheat (sample (113+161)). Wheat flour extraction 171(IWW) was the most contaminated with T-2/HT-2 toxins with 0021 ppb. Flour samples constituted of wheat flour extraction didn't show any contamination by Aflatoxins (AF), deoxynivalenol (DON), Zearalenone (ZON) and fumonisin (FM) compared to other samples from imported wheat.

Keywords: mycotoxins, wheat, SAGO Al-Jouf

1. Introduction
As a result of its nutritional properties, wheat (*Triticumaestivum L.*) is one of the most frequently eaten cereals all over the world by both humans and animals (Vieira, 2006) [1].
Members of three fungal genera, *Aspergillus, Fusarium*, and *Penicillium,* are the major mycotoxin producers. While over 300 mycotoxins have been identified, six (Trichothecenes, zearalenone, fumonisins, ochratoxins, and patulin) are regularly found in food, posing unpredictable and ongoing food safety problems worldwide.
In plantations, wheat can be contaminated by various diseases because of weather conditions, soil type and crop susceptibility. One of the best-known diseases that commonly affect this cereal is *Fusarium* head blight, triggered by infection of *Fusarium* fungi, which not only cause diseases in plants but also produce toxic substances known as mycotoxins through their secondary metabolism (Calori-Domingues *et al*., 2007) [2].
Mycotoxins are naturally-occurring toxic compounds, produced by a variety of fungal species that grow on agricultural products, during their growth in the field and in storage, as well as in processed food and animal feed (Scussel, 2002) [3]. They cause significant economic impact because they reduce plant and animal productivity, and also toxicological impact, with clinical manifestations in both humans and animals (Santos 2009) [4].
Maximum permitted limits have been set by European Union legislation for DON and ZON (Anon. 2006) [4]. EC regulations for DON and ZON apply not only to the unprocessed wheat both before or after cleaning (1250 and 100 µg kg-1 respectively) but to milled intermediate products eg flour (750 and 75 µg kg-1 respectively), finished products (500 and 50 µg kg-1 respectively) and to infant food (200 and 20 µg kg-1 respectively).
Mycotoxins are secondary fungal metabolites that can develop on a range of important food commodities. Several mycotoxins often occur in wheat and other cereals; those commonly found in UK grown cereals being deoxynivalenol (DON), nivalenol (NIV), HT-2 toxin (HT2),

T-2 toxin (T2) and zearalenone (ZON), although DON occurs most frequently in wheat and at the highest levels (MacDonald *et al*. 2004; Edwards 2009) [5, 6]. The toxicology of the most important mycotoxins have been assessed internationally in order to protect the consumer against mycotoxins in the food supply.

The European Commission (EC) has asked the European Food Safety Authority (EFSA) for scientific opinion on the risk to human and animal health related to the presence of T-2 and HT-2 toxin in food and feed. In particular the opinion should consider any new results of toxicological studies published since the assessment by the SCF in 2001 [7], in order to assess if the combined t-TDI of 0.06 µg/kg B.W. for T-2 and HT-2 toxin is still appropriate.

1.1 Aflatoxin

Aflatoxin is considered by many to be the most potent naturally-occurring carcinogen known. It has been linked to a variety of health problems in both humans and animals. Aflatoxin is a by-product of mold growth in a wide range of commodities. Two molds that are major producers of aflatoxin are *Aspergillus flavus* and *A. parasiticus*. These fungi can be found virtually everywhere in the world. They are soil-borne, but like to grow on high-nutrient seeds. Their toxins are produced pre-harvest in the field, and post-harvest in storage. In both cases, damage due to insects, mishandling or environmental stress can enable the fungi to invade the seed.

When aflatoxin contaminated feed is eaten, it can cause many health and performance problems. Often it is not suspected as a cause of poor performance in livestock and poultry. In many cases, other diseases develop that are then diagnosed as the cause of poor performance. Because aflatoxin is not suspected as the primary cause, contaminated feed can continue being fed long after problems develop and substantial loss has resulted.

The U.S. Food and Drug Administration has set a maximum allowable level of total aflatoxins at 20 parts per billion (ppb). Commodities used for human and animal consumption must be tested to ensure that aflatoxin levels are below this number. The foreign markets regularly inspect loads and reject shipments of commodities with levels higher than 4 to 15 ppb.

1.2 Ochratoxin

Ochratoxin, commonly produced by the molds *Aspergillus ochraceus* and *Penicilliumviridicatum*, can be found in corn, barley, milo, wheat, dried fruits and green coffee. Ochratoxin may be present in conjunction with aflatoxin, one of the most potent naturally-occurring carcinogens. In fact, ochratoxin is a suspected carcinogen.

Ochratoxin affects kidneys in animals exposed to naturally-occurring levels of this mycotoxin. Turkeys and other poultry exhibited lower productivity levels during field outbreaks of ochratoxicosis. Symptoms included retarded growth and decreased feed conversion. It has also been known to affect egg production in laying hens. Although there has been no advisory or regulatory level for ochratoxin issued by the Food and Drug Administration, many agree that levels between 10–20 parts per billion (ppb) for commodities destined for human or animal consumption may cause health problems and economic losses. Some foreign markets have set regulation limits ranging from 5 to 50 ppb.

The best protection against mycotoxins is monitoring for their presence in feeds and foods. That means testing all along the pathway from initial harvest of grains to the finished product.

1.3 Deoxynivalenol (DON)

Deoxynivalenol (DON), a member of the trichothecene family, is produced most commonly by the pink mold *Fusarium graminearum* living on cereal commodities such as wheat, corn, and barley. The toxicological effects attributed to DON include: nausea, vomiting, feed refusal, gastroenteritis, diarrhea, immunosuppression and blood disorders. Pigs have been shown to be highly sensitive to DON. They will refuse to eat feeds when DON levels of >1 ppm are present. The toxin and its analogs cause toxic effects in other species as well, with varying degrees of sensitivity. DON has been implicated as causing problems in processed food, including off flavor in ready-to-eat cereals and adverse effects on dough quality. Accurate determination of the presence of the toxin is of major importance to those monitoring the quality of feed and food. The FDA has issued advisory levels for DON in grains: humans, 1 ppm; dairy cattle, 5 ppm total ration; beef cattle, 10 ppm total ration; swine, 5 ppm in 20% of diet; chickens, 10 ppm in 50% of diet; all other animals, 5 ppm in 40% of diet.

1.4 Fumonisin

Discovered in 1989, fumonisins are a family of mycotoxins produced by the molds *Fusarium verticillioides*. These molds commonly infect corn (in fact, they are considered ubiquitous in corn) and rice, hence the potential for fumonisins to be found in feed and foodstuffs is high. Fumonisins are a group of mycotoxins produced by various *Fusarium* sp., and are frequently found in maize worldwide, mainly associated to *Fusarium verticillioides*, a member of *Fusarium fujikuroi* species complex. Structurally, they are long-chained aliphatic amines carrying methyl and hydroxyl groups at various positions of the aliphatic chain. Two of the hydroxyl groups are esterified with tricarballylic acids. Although a large number of fumonisins have been identified so far, the B group (i.e. fumonisin B1 to B4 (FB1-4) is the most prevalent in food and feed commodities. Fumonisins affect various animals differently and have been linked to esophageal cancer in humans. The Environmental Protection Agency classifies fumonisins as Category II-B carcinogens. Horses are extremely sensitive to low amounts of fumonisin, which can cause leukoencephalomalcia (liquefaction of the brain). In swine, research has shown fumonisin attacks the cardiopulmonary system causing pulmonary edema, as well as liver and pancreatic lesions. The FDA has issued the following guidelines for total fumonisins (FB1+FB2+FB3) in corn and corn by-products in food and animal feeds: humans, 2 ppm; equids and rabbits, 5 ppm, no more than 20% of diet; swine, 20 ppm, no more than 50% of diet; all other livestock species and pet animals: 10 ppm, no more than 50% of diet.

1.5 Zearalenone

Zearalenone is primarily produced by the mold *Fusarium graminearum*, which also commonly produces deoxynivalenol. Hence, there is evidence that if zearalenone is detected, there is a high probability that other fusarial mycotoxins may be present. Zearalenone is classified as an estrogenic mycotoxin because it frequently causes estrogenic responses in animals.

When Zearalenone contaminated feed or grain is eaten by livestock, it can cause a wide variety of reproductive problems. In swine, it causes vulvovaginitis, low birth

weights, fetal re-absorption, aborted pregnancies, reduced litter sizes, abnormal estrus and feminization of males. Zearalenone can delay the breeding process and cost the producer significant economic and physical losses.

1.6 T-2/HT-2 Toxins

T-2/HT-2 toxins are trichothecene mycotoxins produced by several species of *Fusarium* molds. As T-2 toxin is readily metabolized to HT-2 toxin, and the toxins have been shown to produce numerous adverse effects on many animals, these two mycotoxins are frequently evaluated together.

Animals affected by the toxins include swine, dairy cattle, poultry, dogs, cats and horses. Effects of the toxins include digestive disorders, hemorrhage, edema, oral lesions, dermatitis, and blood disorders. Damage caused by the toxins to the digestive track is irreversible. In the most severe cases, these toxins will cause death. T-2 toxin is the principal causal toxin in the human disease alimentary toxic aleukia.

Poultry studies have shown T-2 intoxication has led to a reduction in weight gain and other problems such as beak lesions, poor feathering, motor function impairment and increased susceptibility to *Salmonella* spp.

The best protection against these mycotoxins is monitoring for their presence in feeds and foods. That means testing all along the pathway from initial harvest of grains to the finished product. T-2 toxin is metabolised in the intestines and by liver and other tissues. T-2 toxin is rapidly metabolized to a range of different compounds in rodents, one of the major metabolites being HT-2 toxin. Metabolic transformations of T-2 toxin include deacetylation, acetylation, hydroxylations, de-epoxidation and glucuronide conjugations (Figure 18). Carboxylesterases have been shown to be responsible for the transformations of T-2 toxin to HT-2 toxin and neosolaniol in white blood cells (Johnsen *et al*., 1988) [8].

Table 1.1: Toxic secondary metabolites produced by fungi in the associated mycobiota of cereals and cereal products (Samson and Frisvad 2004, Samson *et al*. 2010) [9, 10]

Fungus Species	Important toxic secondary metabolites produced (Mycotoxins)	Food/feed affected
Aspergillus		Barley, oat, rye, wheat
A. flavus	Aflatoxin B1, B2, aspergillic acid, cyclopiazonic acid, 3-nitropropionic acid	Barley, oat, rye, wheat, Millets, sorghum
A. niger	Ochratoxin A, fumonisin B2, B4	Maize
A. ochraceus	Ochratoxin A, penicillic acid, xanthomegnin, viomellein, vioxanthin	Maize
A. parasiticus	Aflatoxins B1, B2, G1, G2, aspergillic acid, parasiticolide, Cyclopiazonic acid	Barley, oat, rye, wheat
A. versicolor	Sterigmatocystin	Barley, oat, rye, wheat
A. terreus	Citreoviridin, occationallyterritrem	Barley, oat, rye, wheat
Byssoclamus		
B. fulva	Patulin (?)	Apple juice, jams, cider, barley, wheat, corn and milk
B. nivea	Patulin	
Fusarium		
F. graminearum	Trichothecenes (Type A: DAS, T-2,	cereals (wheat, maize)
F. culmorum	HT-2; Type B: DON, NIV)	
F. langsethiae	Trichothecenes (HT-2, T-2, DAS)	
F. crookwellense	Zearalenone	cereals, rice, beer, silage
F. semetectum	Zearalenone	
F. moniliforme	Fumonisins(B1 to B4), fusaric acid, moniliformin	
F. subglutinans	Fumonisins(B1 to B4), fusaric acid	
F. tricinctum	Fusarin C	
F. verticilloides	Fumonisins(B1 to B4), fusaric acid, moniliformin	Maize
F. proliferatum	Fumonisins(B1 to B4), fusaric acid	Maize
Penicillium		
P. verrucosum	Ochratoxin A, Citrinin	cereals (wheat, barley)
P. aurantiogriseum	Pencillic acid, Citreoviridin,verrucosidin, terrestric acid, nephrotoxic glycopeptides	Barley, oat, rye, wheat
P. citrinum	Cyclopiazonic acid, PenitremA,Citrinin	nuts, fruit
P. expansum	Patulin	fruit and vegetables, silage
P. brevicompactum	Botryodiploidin	Barley, oat, rye, wheat
P. carneum	Patulin, penitrem A	cereals, maize, nuts
P. chrysogenum	PR toxin, secalonic acids D, F,	Maize
P. freii	Viomellein	Barley, oat, rye, wheat
P. hordei	Roquefortine C	Barley, oat, rye, wheat
P. paneum	Botryodiploidin, patulin	Rye bread
P. polonicum	Penicillic acid, verrucosidin, terrestric acid, nephrotoxic glycopeptides	Barley, oat, rye, wheat
P. viridicatum	Penicillic acid, viomellein, xanthoemegnins, viomellein	Barley, oat, rye, wheat
P. glandicola	Patulin, penitrem A, roquefortine C	cereals, coffee, fruit, nuts, beer

The maximum level is designed to prevent the occurrence of each mycotoxin at levels considered harmful to human and/or animal health. Selected examples of maximum values for some mycotoxins in wheat are given in Table 1.2.

Table 1.2–EU maximum levels (MLs) for mycotoxins in wheat grain and wheat products for human consumption (EU, 2017a) [11]. If not mentioned otherwise, the MLs are based on the product “as is.”

Mycotoxin	Foodstuffs	Maximum
		levels [µg/kg]
Aflatoxin B1	Wheat and all products derived from wheat, including processed wheat products (excluding foods for infants and young children and dietary foods for special medical purposes intended specifically for infants)	2
	Processed cereal-based foods and baby foods for infants and young children[c,d]	0.1
	Dietary foods for special medical purposes intended specifically for infants[c,e]	0.1
Aflatoxins B1+B2+G1+G2	Wheat and all products derived from wheat, including processed wheat products	4
Deoxynivalenol	Unprocessed durum wheat and oats	1750
	Cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption	750
Zearalenone	Unprocessed cereals other than maize	100
	Cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption	75
HT-2 and T-2 toxin	Unprocessed wheat	100
	Wheat grains for direct human consumption	50
Fumonisins (B1+B2)	Not mentioned for wheat	

2. Materials and Methods

2.1 Samples

A total of 17 samples of wheat (106, 172, 171, 165, 161, 160, 113, 107, 101), mix wheat (155+165, 113+161, 106+156, 156+161) and wheat flour (extraction 70%, 80%, 95%, IWW) were collected from SAGO (Saudi Grain Organization) Al-Jouf Saudi November 2018, and analyzed according they were acquired. Samples were directly taken to Laboratory for microbiological analysis and mycotoxin evaluation.

All of mycotoxins analyses were conducted on the premises of the Laboratory of Mycotoxicological Analyses SAGO (Saudi Grain Organization) Al-Jouf Saudi November 2018.

2.2 Chemicals and reagents

2.3 Equipment and supplies

1. Test Strips
2. 100 µL pipet and pipet tips
3. 300 µL pipet and pipet tips
4. 100 to 1000 µL variable volume pipet or 1.0 mL pipet and pipet tips
5. 250 mL graduated cylinder
6. Balance
7. Methanol
8. Deionized or distilled water
9. Micro-centrifuge tubes
10. Mini-centrifuge
11. Charm EZ-M reader
12. Printer for Charm EZ-M reader (optional)
13. ROSA Incubator
14. Sample extraction containers
15. Sample grinder
16. Storage bottle
17. Transfer pipets (optional)
18. 10-30% KOH (w/v) in water
19. Conical tubes
20. pH paper or pH meter
21. GF/CA syringe filters (Phenomenex Part No AF0-8A09-12)
22. Syringes
23. Charm EZ-M reader
24. Printer for Charm EZ-M reader (optional)
25. ROSA Incubator
26. Sample extraction containers
27. Sample grinder
28. Storage bottle
29. Transfer pipets (optional)
30. 10-30% KOH (w/v) in water
31. Conical tubes
32. pH paper or pH meter
33. GF/CA syringe filters (Phenomenex Part No. AF0-8A09-12)
34. Syringes

2.4 Standards and solvents

2.5 Mycotoxin detection by ROSA (Rapid One Step Assay) lateral flow technology

ROSA FAST Aflatoxin Quantitative Test is an immunoreceptor assay utilizing ROSA (Rapid One Step Assay) lateral flow technology. Aflatoxin is extracted from the sample using 70% methanol in water. Aflatoxin interacts with colored beads in the lateral flow test strip and the color intensity in the test and control zones is measured by the ROSA-M Reader or Charm EZ-M reader and interpreted as parts per billion (ppb) aflatoxin.

2.6 Extraction procedures

Procedure for corn, barley, brewer's rice, brown rice, corn flour, corn gluten meal, corn meal, corn screenings, corn/soy blend, flaking corn grits, hominy, milled rice, millet, oats, popcorn, rough rice, rye, sorghum, soybean meal, wheat, and wheat flour:

(1) Weigh 50 ± 0.2 grams ground samples into a Whirl-pak bag.
(2) Add 100 mL extraction solvent.
(3) Shake vigorously for 1 minute (use within 30 minutes).
(4) Transfer 1 to 1.5 mL extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (use within 2 hours).
(5) Repeat for additional samples.

2.7 Sample Preparation for Quantification

This test kit uses different testing sensitivity ranges (Diluted Extract and Second Diluted Extract) for reporting aflatoxin measurements for grain and commodities.

2.8 Sample Preparation of Diluted Extract for 20 to 100 ppb quantitation:

(1) Pipet 1.0 mL AFQ Dilution Buffer into a clean micro-centrifuge tube.
(2) Pipet 300 µL Diluted Extract or filtered Diluted Extract to

micro-centrifuge tube containing 1.0 mL AFQ Dilution Buffer, cap, mix (5 times inverting up and down), and label. This tube contains the Second Diluted Extract.

2.2 Zearalenone quantification

The Charm ROSA FAST5 Zearalenone Quantitative Test kit is an immunoreceptor assay utilizing ROSA (Rapid One Step Assay) lateral flow technology. Zearalenone is extracted from the sample using 70% methanol (MeOH) in water. Zearalenone interacts with colored beads in the lateral flow test strip and the color intensity in the test and control zones is measured by the ROSA-M Reader or Charm EZ-M and displayed as parts per billion (ppb) zearalenone.

2.2.1 Extraction procedures

Extraction Procedure for: corn, barley, brewer's rice, flaking corn grits, milled rice, oats, rough rice, sorghum, wheat, wheat flour.

(1) Obtain a representative sample according to official procedures.
(2) Grind/mill to a 20-mesh particle size according to official procedures.
(3) Mix thoroughly and transfer 50 ± 0.2 grams ground sample into a clean extraction container.
(4) Add 100 mL extraction solvent
(5) Shake vigorously for 1 minute by hand.
(6) Clarify sample extract.
(a) Allow sample to settle for 1 minute to obtain settled extract.
(b) After settling, centrifuge to clarify extract. Using transfer pipet, add 1 to 1.5 mL sample extract into a clean micro-centrifuge tube and label. Centrifuge in mini-centrifuge for 10 seconds.
(7) Prepare additional sample extracts (up to 4 for quad incubator) following steps 1 – 6.
(8) Proceed to Sample Preparation section.

2.2.2 Sample preparation

This test kit uses different testing sensitivity ranges (Diluted Extract and Second Diluted Extract) for reporting zearalenone measurements for grains and commodities.

2.2.3 Sample Preparation of Diluted Extract from the Diluted Extract for 300 to 1000 ppb quantitation.

(1) Pipet 300 µL of filtered Diluted Extract to a predispensed (1.0 mL ZEAR Dilution Buffer) micro-centrifuge tube, cap, mix, and label.
(2) Repeat for additional samples.
(3) Use Second Diluted Extract as your test sample in Sample Analysis found in Test Procedures section.

2.3 DON (deoxynivalenol) quantification

ROSA FAST5 DON Quantitative Test is an immunoreceptor assay utilizing ROSA (Rapid One Step Assay) lateral flow technology. DON (deoxynivalenol or vomitoxin) is extracted from the samples using water. DON interacts with colored beads in the lateral flow test strip and the color intensity in the test and control zones is measured by the Charm EZ-M reader and interpreted as parts per billion (ppb) or parts per million (ppm) DON. To convert results in ppb to ppm divide by 1000 (e.g., 5000 ppb = 5 ppm).

2.3.1 Extraction procedures

2.3.1 Procedure for barley, corn, malted barley, milled rice, oats, rough rice, sorghum, wheat, wheat bran, and wheat flour:

(1) Weigh 50 ± 0.2 grams ground samples into a clean extraction container.
(2) Add 250 mL deionized or distilled water.
(3) Shake vigorously for 1 minute.
(4) Allow sample to settle for 1 minute to obtain settled extract (use within 30 minutes).
(5) Transfer 1 to 1.5 mL settled extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (use within 2 hours).
(6) Repeat steps 1 to 5 for additional samples.

2.3.2 Sample preparation for quantification

2.3.3 Sample Preparation of Diluted Extract for 1.0 to 5.0 ppm quantitation.

(1) Pipet 1.0 mL DONQ-FAST5 Dilution Buffer into a clean micro-centrifuge tube.
(2) Pipet 300 µL Diluted Extract to micro-centrifuge tube containing 1.0 mL DONQ-FAST5 Dilution Buffer, cap, mix (5 times inverting up and down), and label.
(3) Repeat for additional samples.

2.4 Fumonisin quantification

ROSA FAST5 Fumonisin Quantitative Test is an immunoreceptor assay utilizing ROSA (Rapid One Step Assay) lateral flow technology. Fumonisinis extracted from the sample using 70% methanol in water. Fumonisin interacts with colored beads in the lateral flow test strip and the color intensity in the test and control zones is measured by the ROSA-M Reader and interpreted as parts per billion (ppb) or parts per million (ppm) Fumonisin.

2.4.1 Extraction procedures

2.4.1 Procedure for corn, barley, flaking corn grits, millet, oats, rough rice, sorghum, and wheat:

(1) Weigh 50.0 ± 0.2 grams ground samples into a clean extraction container.
(2) Add 100 mL extraction solvent.
(3) Shake vigorously for 1 minute (use within 30 minutes).
(4) Allow sample to settle for 1 minute to obtain sample extract.
If particles are present after settling, centrifuge to clarify extract. Transfer 1 to 1.5 mL extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (use extract within 30 minutes or within 2 hours if centrifuged).
(5) Repeat for additional samples.

2.4.2 Sample preparation for fumonisin quantification

This test kit uses different testing sensitivity ranges (Diluted Extract and Second Diluted Extract) for reporting Mycotoxin measurements for grain and commodities.

2.4.3 Sample Preparation of Diluted Extract for 1 to 5 ppm quantitation:

(1) Pipet 1.0 mL FUM Dilution Buffer into a clean micro-centrifuge tube.
(2) Pipet 300 µL Diluted Extract or filtered Diluted Extract to micro-centrifuge tube containing 1.0 mL FUM Dilution Buffer, cap, mix (5 times inverting up and down), and label. This tube contains the Second Diluted Extract.

3 Result and Discussion

Table 3.1: Qualitative test for mycotoxins of cereal contamination by aflatoxins (AF), deoxynivalenol (DON) and Fumonisin (FM) Zearalenone and T-2/HT-2 toxin.

Qualitative test for mycotoxins						
S.No.	**Wheat Samples**	**Aflatoxin**	**Deoxynivalenol (DON)**	**Fumonisins**	**Zearalenone**	**T-2/HT-2 toxins**
1	106	+	+	ND	NT	NT
2	172	+	NT	ND	+	+
3	171	+	NT	ND	+	+
4	165	NT	NT	ND	+	+
5	161	NT	+	ND	+	+
6	160	NT	+	ND	+	+
7	113	NT	NT	ND	+	+
8	107	NT	NT	ND	+	+
9	101	NT	+	ND	+	+
10	155+156	NT	NT	ND	NT	+
11	113+161	NT	NT	ND	NT	+
12	106+156	NT	NT	ND	NT	+
13	156+161	NT	NT	ND	NT	+
14	160(70%)	NT	NT	ND	NT	+
15	165(80%)	NT	NT	ND	NT	+
16	101(90%)	NT	NT	ND	NT	+
17	171(IWW)	NT	NT	ND	NT	+

***ND:** Not Determinate, NT: Not Testing

Table 3.2: Levels of cereal contamination by Aflatoxins (AF), deoxynivalenol (DON) and Fumonisin (FM) Zearalenone and T-2/HT-2 toxin.

Quantitative test for mycotoxins											
		Aflatoxin		**Deoxynivalenol (DON)**		**Fumonisins**		**Zearalenone**		**T-2/HT-2 toxins**	
S.No.	**Wheat Samples**	**ppb**	**ppm**	**ppb**	**ppm**	**ppb**	**ppm**	**ppb**	**ppm**	**ppb**	**ppm**
1	106	0012	0.012	0250	0.25	ND	ND	NT	NT	NT	NT
2	172	NT	NT	NT	NT	ND	ND	0045	0.045	0020	0.02
3	171	NT	NT	NT	NT	ND	ND	0035	0.035	0019	0.019
4	165	0016	0.016	NT	NT	ND	ND	0040	0.04	0069	0.069
5	161	0019	0.019	0800	0.8	ND	ND	0041	0.041	NT	NT
6	160	NT	NT	1400	1.4	ND	ND	0037	0.037	0022	0.022
7	113	NT	NT	NT	NT	ND	ND	0048	0.048	0014	0.014
8	107	NT	NT	NT	NT	ND	ND	0051	0.051	0013	0.013
9	101	NT	NT	0800	0.8	ND	ND	0044	0.044	0165	0.165
10	155+156	NT	NT	NT	NT	ND	ND	NT	NT	0020	0.02
11	113+161	NT	NT	NT	NT	ND	ND	NT	NT	0052	0.052
12	106+156	NT	NT	NT	NT	ND	ND	NT	NT	0019	0.019
13	156+161	NT	NT	NT	NT	ND	ND	NT	NT	0018	0.018
14	160(70%)	NT	NT	NT	NT	ND	ND	NT	NT	0010	0.01
15	165(80%)	NT	NT	NT	NT	ND	ND	NT	NT	0015	0.015
16	101(90%)	NT	NT	NT	NT	ND	ND	NT	NT	0019	0.019
17	171(IWW)	NT	NT	NT	NT	ND	ND	NT	NT	0021	0.021

***ND:** Not Determinate, NT: Not Testing.

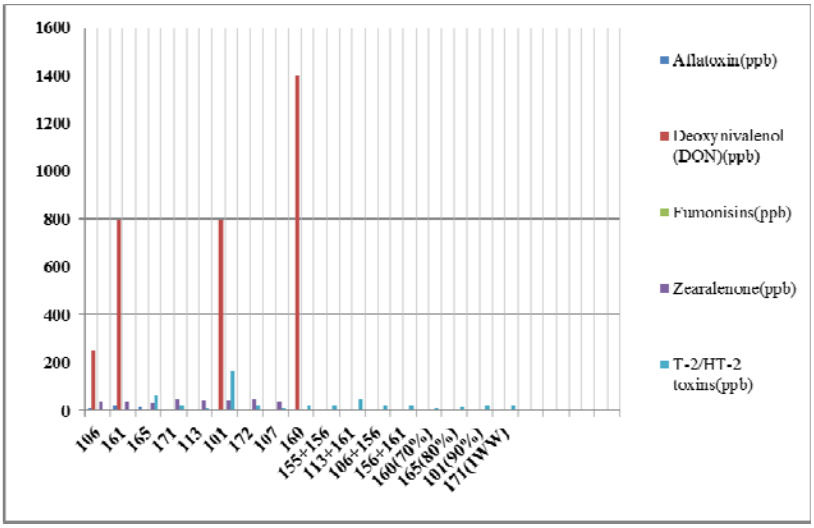

Fig 3.1: Showed Levels of cereal contamination by Aflatoxins (AF), deoxynivalenol (DON) and Fumonisin (FM) Zearalenone and T-2/HT-2 toxin

Wheat, mix wheat and wheat flour samples were analyzed for aflatoxins (AF), deoxynivalenol (DON), Zearalenone (ZON), T-2/HT-2 toxins and fumonisin (FM) contamination. Results reported in table 3.1 showed that deoxynivalenol (DON) and T-2/HT-2 toxins contamination was mainly observed maximum level of samples analyzed (wheat sample 160 and 101) with a maximum level of contamination of 1400 and 0165ppb.

For mix wheat (113+161) analysis, the levels measured varied from 0018ppb to 0052ppb. The high level ofT-2/HT-2 toxins was obtained in mix wheat (sample (113+161)).Wheat flour extraction 171(IWW) was the most contaminated with T-2/HT-2 toxins with 0021 ppb. Flour samples constituted of wheat flour extraction didn't show any contamination by fumonisin (FM) compared to other samples from imported wheat. We showed that deoxynivalenol (DON) and T-2/HT-2 toxins were very frequent contaminants in our samples.

We reported in table 3.1 showed that Zearalenone (ZON) contamination was observed low level of samples analyzed (wheat sample 160 and 171) with a minimum level of contamination of 0035 and 0037 pp band observed maximum level of samples analyzed (wheat sample 107 and 113) with a maximum level of contamination of 0051 and 0048 ppb.

4. Conclusions and Future Perspectives

Food security and food quality are important issues in the context of globalization. The European Union is one of the world's biggest cereal producers, but it is also a big cereals consumer, wheat being the most important in this category. *Fusarium* genus produce an extraordinary diversity of biologically active secondary metabolites, some of which are harmful to animals and humans, like mycotoxins. Health risks associated with the consumption of cereal products, contaminated with *Fusarium* mycotoxins are worldwide recognized. Trichothecenes, zearalenone, T-2/HT-2 toxins and fumonisins are distributed widely in cereals, including wheat. The problem of co-occurrence of Fusarium mycotoxins in wheat is a recurring feature, raising the question of interactions, synergistic or antagonistic actions in the manifestation of toxicity, which can be the future in this field of research. The relationship between climate change in Europe and mycotoxin development is an accepted idea by most scientists. Global warming will possibly increase the fungi development, but will definitely also produce the growth of new fungi species, and consequently new mycotoxins, in crops. Following this, an increase of analytical methods for food control and measures that influence food security, such as Good Practice, HACCP and others are required. To avoid negative impacts on humans and animal health, compliance with EU regulations and the development of programs of risk assessment based on hazard and exposure evaluation are necessary.

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