Original scientific paper
UDC 664.641.019:582.28(498)

CONTAMINATION WITH DEOXYNIVALENOL IN THE MILLING - BAKERY INDUSTRY UNDER THE INFLUENCE OF CLIMATIC CONDITIONS FROM ROMANIA

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Abstract

Climatic changes expected for Romania (increase of temperature with 3 - 5 ^{0}C and decrease of rainfalls during summer) require assessments of mycotoxins incidence and trends in the agri-food chain. The purpose of research was to determine deoxynivalenol in the milling and bakery industry, under the influence of the agro-climatic factors in period 2014-2015.

From the Vel Pitar Group some product categories (47 samples: common wheat, wheat and rye flour, wheat and rye bread, toast, cake) were sampled and analyzed for deoxynivalenol by the enzyme-linked immunosorbent assay. In Romania, the maximum levels for deoxynivalenol in foodstuffs are according to Commission Regulation (EC) No. 1881/2006. Meteorological (rainfall, temperatures) and geographical data for the place of cultivation, storage and processing of grain and food were taken into consideration.

Deoxynivalenol ranged from <18.5…963.86 µg/kg (mean 528.04 ± 83 µg/kg), incidence of positive samples was 17% (8/47s), no sample was registered above the maximum limits (0%). Although in May and June, abundant rainfall were recorded in Transylvania and Southern Hilly Area, deoxynivalenol was not detected above the maximum limit in any sample of the raw grains, intermediate cereal products and cereal foods. Conditions of extreme, strong or moderate drought were recorded in Moldavia, Southern Plain and Dobrogea, and Oltenia Plain regions, resulting a very low incidence or no positive samples. A tendency of deoxynivalenol contamination was observed for Transylvania (Brasov county) and Southern Hilly Area (Gorj and Valcea counties), confirming the data for temperate and humid regions of Romania according to the climate change scenarios.

To maintain the safety of food products, Vel Pitar Group is partner in research projects regarding risk assessment of mycotoxins in agri-food chain under the influence of climate change predictions for Romania.

***Key words**: Deoxynivalenol, Agrifood chain, Climate change, HACCP system, Vel Pitar Grup, Romania.*

1. Introduction

Cereal contamination with *Fusarium* sp. and deoxynivalenol (DON) occurs globally, especially in temperate regions (Miller [1]), being the most influenced by climatic factors in the flowering period (Skrbic *et al.*, [2]). Fungal infections result in reduced yields and quality of grain, beans are smaller and white - pink (van der Fels - Klerx *et al.*, [3]). Wheat batches contaminated with *Fusarium* sp. and deoxynivalenol represent a potential danger for the milling and bakery industry because infection can continue during the technological process (milling, flour storage, dough's kneading and baking) and food distribution system (Amra *et al.*, [4]; Paterson *et al.*, [5]; Lemmens and Krska [6]).

Contamination can be reduced by physical (milling), chemicals (preservatives, essential oils) and biological (sourdoughs) methods. Milling process has the most important role because it removes the external layers

of the grain and contaminants adhering to its surface, thus influencing the dough and bread quality (Rios *et al.*, [7]; Berghofer *et al.*, [8]; Weidenbörner *et al.*, [9]). Reducing contamination with fungi and mycotoxins can be greater than 65% for the new milling technologies that include a conditioning step (Posner and Hibbs [10]). Contaminated flour from the atmosphere of production spaces lead to recontamination with molds of the baked goods (Bullerman and Hartung [11]).

Consumption of grain and food contaminated with deoxynivalenol can cause gastrointestinal (diarrhea, vomiting, inflammation), hematologic (Königs *et al.*, [12]), headache and fever (Sobrova *et al.*, [13]) and carcinogenic (Fink-Gremmels [14]) disorders.

Romania is an important regional producer of wheat, ranking third in the Central Europe after Serbia and Hungary (Alexa *et al.*, [15]). By 2011, contamination of cereals, food and feed with fungi and mycotoxins were investigated for specific regions of Romania (Banu *et al.*, [16]; Tabuc *et al.*, [17]; Alexa *et al.*, [15]), being highlighted the incidence and level of contamination and toxic effects on humans and animals. Research performed by Gagiu *et al.*, [18, 19] on cereal harvests during 2012 - 2014 period (2829 samples: common wheat, durum wheat, triticale, rye) showed that agricultural regions of the Western Plain (Banat), Northwest Transylvania and Southern Hilly Area present a potential risk of contamination with deoxynivalenol under the influence of agroclimatic conditions (rainfall, temperature, floods, soil). Ittu *et al.*, [20] showed that wheat contamination with deoxynivalenol vary by the location, the year and the interaction between genotype and environment.

Assessment of incidence and tendencies of contamination with mycotoxins on the agrofood chain is very important in the context of climate change predicted for Romania (temperature rise by 3…5 ^{0}C and reduced rainfall in summer) that will be more evident at regional level, especially in intermountain regions (IPCC [21], Sandu and Mateescu [22], Busuioc *et al.*, [23]). Entry of cereal lots contaminated with fungi and mycotoxins in the processing chain affect food safety and security and lead to financial losses (Magan *et al.*, [24]; Tirado *et al.*, [25]).

The aim of research was to assess the incidence and tendency of contamination with deoxynivalenol on the milling - baking chain of the Vel Pitar Group under the influence of agroclimatic factors of 2014 - 2015 agricultural year. It is the first time in Romania when evaluate contamination with deoxynivalenol on the milling - bakery chain related to the local and regional climatic conditions. This study is a continuation of crop assessment for contamination with deoxynivalenol under the influence of agroclimatic conditions during 2011 - 2014 period, when all six agricultural regions of Romania (Western Plain, Transylvania, Moldavia, Southern Hilly Area, Southern Plain and Dobrogea, Oltenia Plain) were assessed.

2. Materials and Methods

2.1 Vel Pitar Group

Vel Pitar Group (SC Sapte Spice SA, and SC Vel Pitar SA) is the national leader, absolutely in every respect: size, geographical coverage, turnover, profit level, number of customers, the level of implementation of modern technologies and compliance with European legislation.

SC Sapte Spice SA owns four automatic mills with a capacity of 170 - 250 tons/day and supplies with flour the twelve bakery factories of the SC Vel Pitar SA.

Mills and bakery factories are located in five of the six agricultural regions of Romania, which shows different agroclimatic conditions (Transylvania, Moldavia, Southern Hilly Area, Southern Plain and Dobrogea, Oltenia Plain) (Figure 1).

Figure 1. Working points (w. p.) of the Vel Pitar Group located in counties from the agricultural regions of Romania with different agroclimatic conditions. Agricultural regions (County - Working point of the Vel Pitar Group):
1. Transylvania (Cluj county - Cluj w. p.; Brasov county - Brasov w. p.); 2. Moldavia (Iasi county - Iasi w. p.; Galati county - Tecuci w. p.); 3. Southern Hilly Area (Arges county - Pitesti w. p.; Gorj county - Targu Jiu w. p.; Valcea county - Ramnicu Valcea w. p.); 4. Southern Plain and Dobrogea (Giurgiu county - Giurgiu w. p.; Ilfov county - Bucharest w. p.); 5. Oltenia Plain (Dolj county - Craiova w. p.; Olt county - Bals w. p.)

2.2 Cereal and food samples

Cereal and food were sampled by qualified personnel of the Vel Pitar Group according to the internal procedures and standards in force.

Product categories (53 samples) were: raw cereals (4 samples: common wheat), flour (8 samples: wheat and rye flour), cereal based food (41 samples: wheat and rye bread, toast, cake).

Flour and food batches come from the grain ground at the nearest mills of the SC Sapte Spice SA.

2.3 Deoxynivalenol testing

Deoxynivalenol was tested by IBA Bucharest using the enzyme-linked immunosorbent assay based on the Ridascreen DON kit and RidaWin software (R-Biopharm, Germany) and Sunrise spectrophotometer with an absorbance filter of 450 nm (Tecan, Austria Ges.m.b.H.). Analytic laboratory is accredited according to EN ISO 17025.

In Romania, the maximum levels (MLs) for mycotoxins in foodstuffs are according to the Commission Regulation (EC) No. 1881 from 2006 [26].

2.4 Meteorological data

Meteorological data were recorded by the METEO-Romania using automated MAWS stations with CERES - Wheat and DSSAT v.3.5 software. Meteorological parameters (precipitations and temperatures) were recorded for the 1 September 2014 - 31 August 2015 agricultural year.

2.5 Research methodology

Meteorological data (rainfall, temperature), climate aridity and geographical coordinates (northern latitude, eastern longitude) for the place of cultivation, storage and processing of grain and food were taken into consideration to assess contamination with deoxynivalenol on the milling - bakery chain of the Vel Pitar Group.

2.6 Statistical analysis

Statistical analysis was performed using Microsoft Excel 2013. Student test (T-test, p) was reported to the maximum levels of deoxynivalenol in cereal, flour and bread.

3. Results and Discussions

3.1 Monitoring of contamination with deoxynivalenol on the milling - bakery chain of the Vel Pitar Group

3.1.1 Milling - bakery chain contamination

On milling - bakery chain of the Vel Pitar Group contamination with deoxynivalenol ranged < 18.5 - 963.86 µg/kg (mean 528.04 ± 83 µg/kg) and incidence of positive samples was 15.1% (8/53 s); no sample (0%) was recorded above the maximum levels (wheat: 1250 µg/kg; flour 750 µg/kg; bread 500 µg/kg).

3.1.2 Cereals (common wheat) contamination

For cereals (common wheat) from SC Sapte Spice SA, contamination with deoxynivalenol ranged < 18.5 - 963.86 µg/kg (mean 468.5 ± 520.86 µg/kg) ($p = 0.058$ determined by very different climatic conditions of agricultural regions) and incidence of positive samples was 50% (2/4 s); no sample was recorded above the maximum level (1250 µg/kg).

Only two samples of common wheat registered higher concentrations of deoxynivalenol, namely a sample from Brasov mill (963.86 µg/kg) and the other from Ramnicu Valcea mill (872.03 µg/kg), but both samples were appropriate (Figure 2).

For Brasov area, Ittu *et al.*, [27]) recorded a maximum cereal contamination with deoxynivalenol of 0 - 1720 µg/kg (mean 690 µg/kg) in 2006 year and 0...80 µg/kg (mean 30 µg/kg) in 2007 year, while Gagiu *et al.*, [18, 19]) recorded a cereal contamination between 36.16 - 4345.70 µg/kg (mean 608.25 µg/kg) in 2012 year and 1306.20 µg/kg in 2014 year, without contamination in harvest of 2013 year.

For Ramnicu Valcea area, Gagiu *et al.*, [18] recorded cereal contamination with deoxynivalenol of 960.25 - 3592.66 µg/kg (mean 1814.75 µg/kg) only in harvest of 2014 year. These results confirmed that wheat contamination with deoxynivalenol varied by location, year, weather and interaction genotype - environmental conditions (Ittu *et al.*, [20]; Gagiu *et al.*, [18], [19]).

3.1.3 Processed cereals (wheat and rye flour) contamination

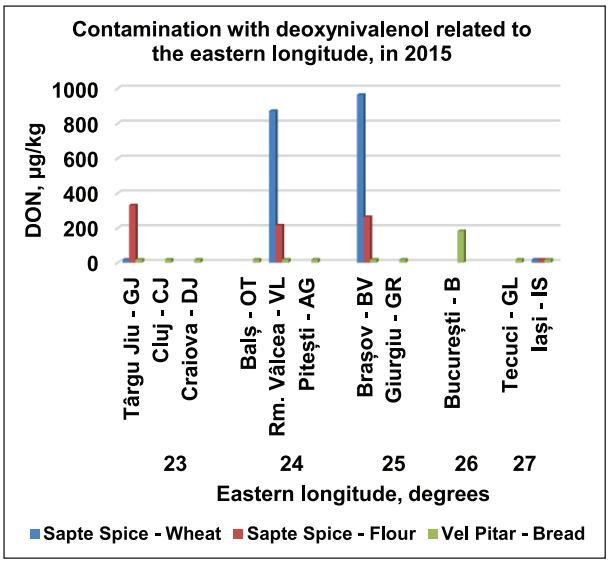
In processed cereals (wheat and rye flour) from SC Sapte Spice SA, contamination with deoxynivalenol ranged < 18.5 - 331.42 µg/kg (mean 154.99 ± 122.3 µg/kg) ($p < 0.05$) and the incidence of positive samples was 63% (5/8 s); no sample was recorded above the maximum level (750 µg/kg) (Figure 2). Wheat samples with a higher rate of extraction (flour type 650, respectively type 1350) showed contamination with deoxynivalenol between 178.34 - 263.66 µg/kg.

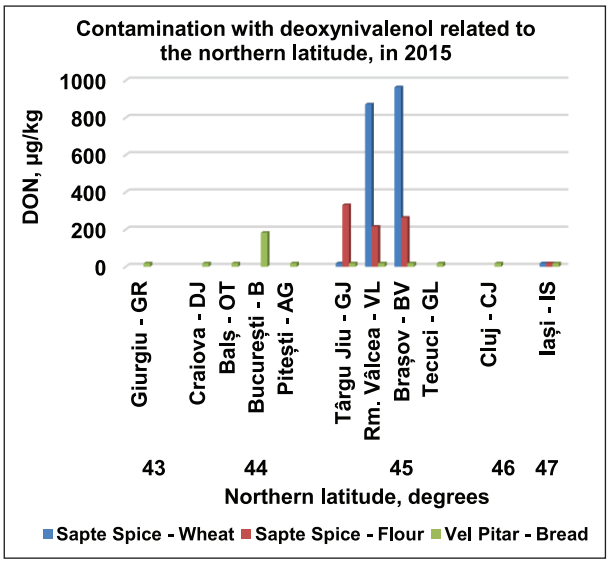
Decreasing deoxynivalenol concentration by milling technology was 75% for Ramnicu Valcea mill (wheat: 872.03 µg/kg; flour from whole wheat: 215.35 µg/kg) and 73% for Brasov mill (wheat: 963.86 µg/kg; flour type 1350: 263.33 µg/kg) due to wheat conditioning step (Posner and Hibbs [10]).

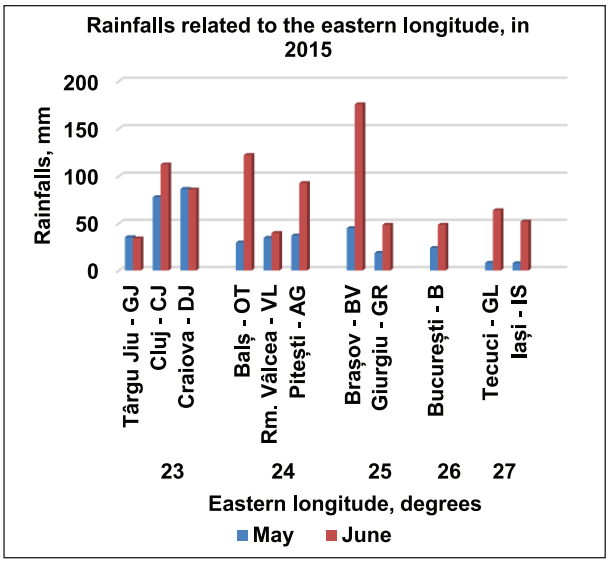
In the cereals based food (wheat and rye bread, toast, cake) from the SC Vel Pitar SA, deoxynivalenol concentration ranged < 18.5 - 182.59 µg/kg (mean 22.8 ± 26.6 µg/kg) ($p < 0.05$) and incidence of positive samples was 2.4% (1/41 s); no sample was recorded above the maximum limit (500 µg/kg) (Figure 2).

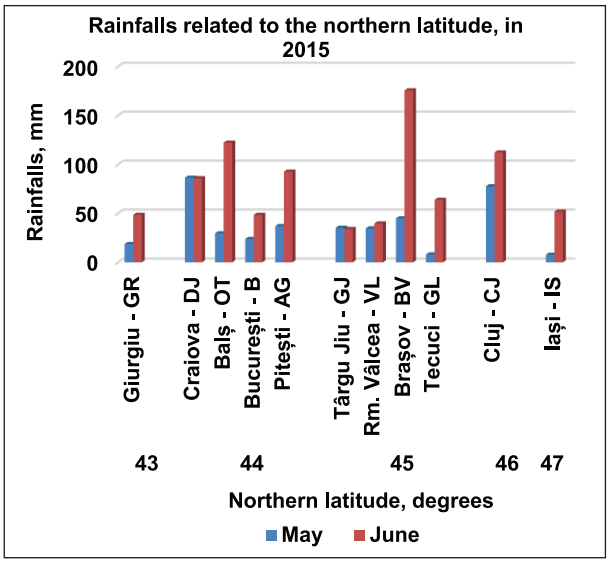
The high efficiency of automatic mills to reduce flour's contamination with deoxynivalenol (73 - 75%) and the control of hygiene and technological process led to safety food for consumers' health.

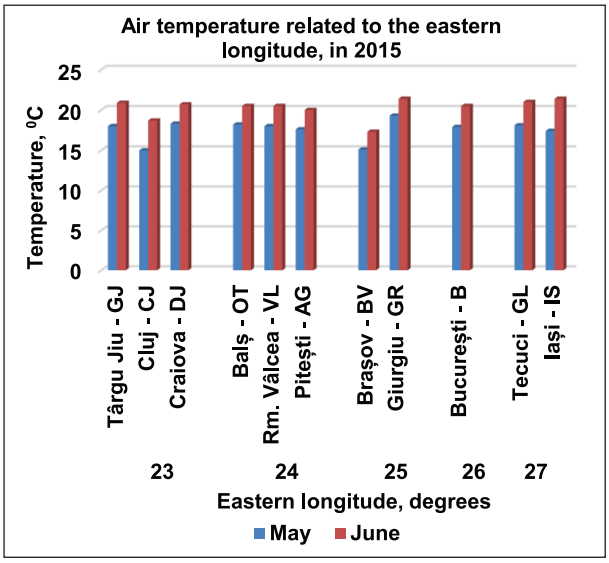
These results have consolidated the external controls performed by the Vel Pitar Group in the period January 2012 - December 2014, when the legal compliance of food products was proven for contamination with deoxynivalenol (45 samples: wheat, flour, bread; deoxynivalenol < 18.5 - 446.78 µg/kg, mean 44.48 µg/kg) (data pending publication).











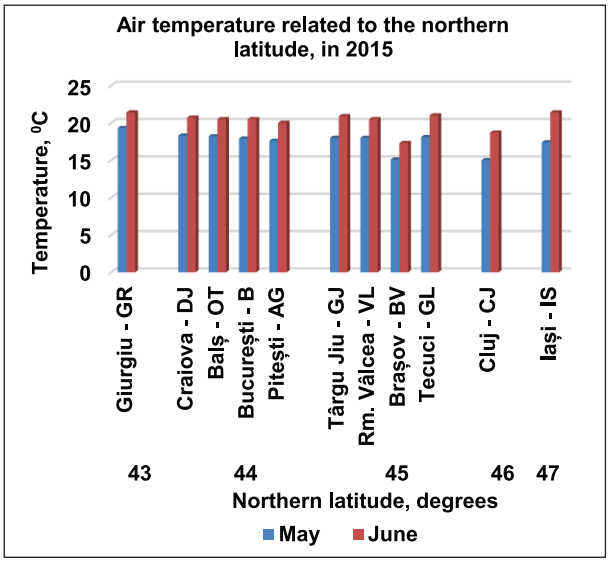


Figure 2. Cereal and food contamination with deoxynivalenol at working points of the Vel Pitar Group related to agroclimatic conditions (meteorological paramenters: rainfalls and temperature; continental position: latitude and longitude) of 2015 year. In Romania, the maximum levels (MLs) for deoxynivalenol in foodstuffs are according to the Commission Regulation (EC) No. 1881 from 2006 (wheat - 1250 µg/kg; flour - 750 µg/kg; bread - 500 µg/kg)

3.2 Contamination with deoxynivalenol on the milling - bakery chain of the Vel Pitar Group

3.2.1 Agroclimatic conditions in the working points area

Working points of the Vel Pitar Group are located in five regions with different agroclimatic conditions:

- Moderately arid continental climate (Olt, Dolj, Giurgiu and Ilfov counties: 43 - 44^{0}N latitude, 23 - 27^{0}E longitude).

- Arid continental climate (Iasi and Galati counties: 45 - 47^{0}N latitude, 27^{0}E longitude).

- Carpathian humid continental climate (Gorj, Valcea, Brasov and Cluj counties: 45 - 46^{0}N latitude, 23 - 25^{0}E longitude).

In the period May 1st to June 30th, 2015 (the critical period for wheat contamination with *Fusarium* sp. and deoxynivalenol - Figure 3), the average amounts of rainfalls were:

- Dry (6 - 100 mm) in Moldavia (Iasi and Galati counties), Southern Plain and Dobrogea (Ilfov and Giurgiu counties).

- Moderate drought (101 - 150 mm) in Southern Hilly Area (Arges county).

- Optimal (151 - 200 mm) in Southern Hilly Area (Gorj and Valcea counties) and Oltenia Plain (Dolj county).

- Abundant (201 - 269 mm) in southeastern Transylvania (Brasov county) and southeastern Oltenia (Olt county) (Figure 3).

3.2.2 Contamination with deoxynivalenol related to the agroclimatic conditions at the working points

Figure 2 shows the cereal and food contamination with deoxynivalenol at the working points of the Vel Pitar Group, related to the agroclimatic conditions (rainfall, temperature, aridity) and continental coordinates (43 - 47^{0}N latitude; 23 - 27^{0}E longitude).

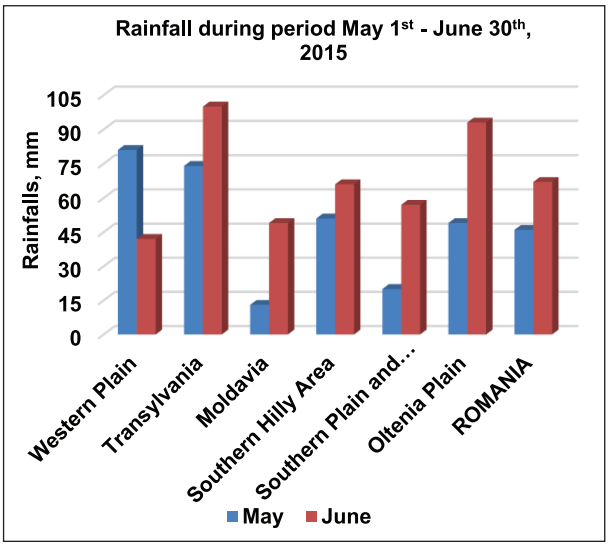
There were no overruns for cereal and food contamination with deoxynivalenol, although in the Carpathian region were recorded abundant rainfall in the May 1st - June 30th period (Transylvania - Brasov county: 132 and 184 mm; Southern Hilly Area - Arges county: 127 and 135 mm).

A higher incidence of positive samples was recorded for agricultural regions Transylvania (25%; < 18.5 - 963.86 µg/kg; mean 167.32 µg/kg) and Southern Hilly Area (30%; < 18.5 - 872.03 µg/kg; mean 118.52 µg/kg), data confirming the incidence and tendency of cereal contamination in these regions (Gagiu *et al.* [18, 19]; Ittu *et al.* [20]).

Wheat samples contaminated with deoxynivalenol (963.86 µg/kg and 872.03 µg/kg) are produced by the mills from the counties Brasov (Transylvania) and Ramnicu Valcea (Southern Hilly Area), which are regions with humid and temperate continental climate (T_{min} <20 ^{0}C, low aridity index) (Miller [1]; Cotty and Jaime Garcia [28]; Paltineanu *et al.*, [29]).

Extreme, strong or moderate drought conditions (low rainfalls, temperatures > 25 ^{0}C) have resulted in a very low incidence and range of contamination with deoxynivalenol (Moldavia: 0%, < 18.5 µg/kg; Southern Plain and Dobrogea: 1%, < 18.5 - 182.59 µg/kg; Oltenia Plain: 0%, < 18.5 µg/kg).

For agricultural regions with warm and arid climate of Romania, the incidence of positive samples for deoxynivalenol was 71.6% in the Southeast (Southern Plain and Dobrogea - Baragan area) (Tabuc *et al.*, [17]) and 85% in East (Moldavia) (Banu *et al.*, [16]), but results were presented without correlation with weather conditions.



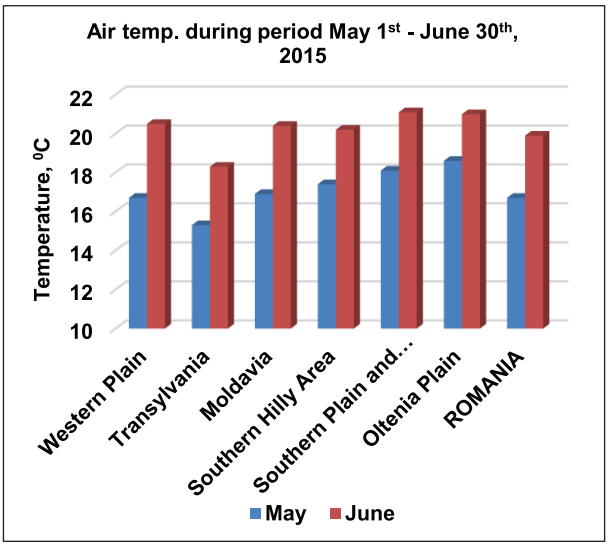


Figure 3. Climatic parameters (rainfall and temperature) in the critical period of wheat, in all agricultural regions of Romania (Western Plain, Transylvania, Moldavia, Southern Hilly Area, Southern Plain and Dobrogea, Oltenia Plain)

In terms of continental coordinates, wheat mills of the Vel Pitar Group are located at 45^{0}N latitude and 23…25^{0}E longitude, this Carpathian area having humid and temperate climate conditions. Although rainfalls were optimal and abundant (151 - 269 mm) in the period May 1st to June 30th, 2015, the Brasov and Gorj mills decreased by 73 - 75% deoxynivalenol level in wheat flour.

In this way, flour quality contributed to the hygienic conditions on technological flow and guaranteed production of bread safe for human consumption (DON < 18.5 µg/kg) (Figure 2).

4. Conclusions

- The study showed a low contamination with deoxynivalenol on the milling - bakery chain of the Vel Pitar Group and all 53 samples (wheat, flour, bread) were legally appropriate, then safe for consumers' health. The tendency of contamination with deoxynivalenol presented a local and interregional variation being most evident at the milling working points, but can also occur in south (regions with arid and temperate continental climate) depending on the annual and local agroclimatic conditions.

- Assessment of incidence and tendency of contamination with deoxynivalenol on the milling - bakery chain under the influence of local and regional agroclimatic conditions contributes to the good functioning of the HACCP (Hazard analysis and critical control points) system of the Vel Pitar Group by avoiding entry of the contaminated cereal lots in technological flow and maintaining hygienic conditions for safety food. The results from the period 2012 - 2015 showed proper functioning of the food safety systems at the Vel Pitar Group, both in dry (2012, 2013 and 2015) and rainy (2014) years from Romania.

- Knowledge of influence of the agroclimatic conditions and the climate change scenarios on cereal and food contamination with mycotoxin contribute to ensuring food safety, as well as increasing company revenue. To maintain food safety, Vel Pitar Group is a partner in some research projects on risk assessment of contamination with mycotoxins on the agrofood chain under the influence of climatic changes projected for Romania.

Acknowledgment

This paper was achieved through the Nucleu Programme, with the support of the National Authority for Scientific Research and Innovation, project no. PN 12 48 01 05. V. G., L. D. and E. M. designed the experiments, analyzed the data and wrote the manuscript; I. S, M. E. C. and A. A. D. contributed to deoxynivalenol analysis; O.O. contributed to meteorological factors; E. I. and N. B. revised the manuscript and has given final approval of the version to be published.

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