EFFICIENCY OF SOME SCREENING METHODS USED IN MONITORING THE QUALITY OF VEGETABLE SUBSTRATES AND THE PRESENCE OF MYCOTOXINS

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Abstract

The purpose of this study was to evaluate the effectiveness of screening methods applied in the analysis of plant products (cereal seeds, feed, hay, etc.) used in agriculture and animal husbandry. Their screening can be done both during the harvesting season aswell as during their storage for the cold season. Information regarding the physico-chemical parameters and mycotoxicological load may be obtained by applying this rapid screening techniques. Near-infrared spectroscopy (NIRS) screening techniques can provide a quick result regarding the quality of plant products. In this study, the efficiency of using the Perten FT-NIR Analyzer in determining the physico-chemical parameters was tested. The sample were scanned and the values were provided shortly after. The analyzed products were also tested from a mycotoxicological point of view by identifying the presence of different types of mycotoxins in plant products by applying thin layer chromatography (TLC). The use of fast screening methods leads to lower costs, elimination of toxicity and shortening of the time to results.

Key words: screening, plant products, mycotoxins, NIRS, TLC.

Feed quality control is of biological and economic importance, which is why it is desirable to be performed in every production unit. Near-infrared spectroscopy (NIRS) is a well-known technology in the agricultural sector, providing information regarding the physico-chemical parameters of different samples in a very short time. Being an easy-to-use tool, it provides fast and simultaneous analysis of many components in a clean, non-invasive and non-destructive technology, being suitable for field implementation as well. The disadvantages of this technique are the relatively high price of the device or the necessity of calibration models for standardization. Furthermore, the NIRS technique has recently become a widely used method for determining the quality of plant products. Currently, in agriculture, there are new analytical tools based on developed spectroscopic technologies (Dale L.M. *et al*, 2011). The NIRS technique is used in various fields such as: agricultural and agro-industrial (animal feed, feed analysis, plant protection, food quality and safety) (Dale L.M. *et al*, 2012). However, the presence of bacteria, moulds and/or some of their metabolites, such as mycotoxins, must be taken into consideration due to the effects on feed production and the health of animals and humans. Mycotoxins are secondary fungal metabolites, with the potential of bioaccumulation that leads to their transfer into fluids, organs and tissues (Ariton A.M. *et al*, 2020). Evidence of fungi and mycotoxins presence in different plant products from various geographical areas and the persistence of fungi and mycotoxins during storage confirms the need to monitor the quality of plant products (Cheli F. *et al*, 2013). The most important mycotoxins that occur naturally in human food and feed are: aflatoxins, ochratoxin, deoxynivalenol, zearalenone and fumonisin. Thus, for an informative analysis regarding their presence in plant products can be used. This technique provides useful information by enabling the continuous monitoring of possible types of mycotoxins presence.

MATERIAL AND METHOD

Material and method. vegetable products (corn grains, alfalfa hay, corn silage, etc.) from farms in Iași County, chromatographic plates, petroleum ether, acetonitrile, chloroform, ethyl acetate, toluene, formic acid, disposable gloves, automatic analyzer - FT-NIR (*Instruments DA7200*

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Perten), oven, laboratory mill, rotary evaporator, chemical niche, development tank, UV radiation generating lamp with wavelength between 254-366 nm.

Determination of physico-chemical parameters using the FT-NIR Automatic Analyzer - The sample submitted to analysis was very well homogenized and placed into the analysis tray. Subsequently, the tray with the sample was fixed in the apparatus and the analysis was carried out. The results were displayed on the screen in about one minute after the sample scan was performed (*figure 1*).

Thin layer chromatography (TLC) - is a simple technique, often used as a screening test to determine mycotoxins. It consists of the extraction of mycotoxins with organic solvents and their separation by thin layer chromatography. The identification of mycotoxins is done by separating in ultraviolet light (254 - 366 nm), from the standard substance and confirming the presence of mycotoxins on the chromatographic plate. ***Preparation of the sample for analysis*** – according to the method presented by Porosnicu I. *et al,* in 2021.

RESULTS AND DISCUSSIONS

Determination of physico-chemical parameters using the FT-NIR Analyzer

In this study, a series of sample results from vegetable and zootechnical farms in Iasi County were exemplified. Figure 1 shows the Perten FT-NIR analyzer. After scanning each sample, the result was displayed on the device screen (*figure 2*).

Figure 1 **Analizor FT-NIR Perten**

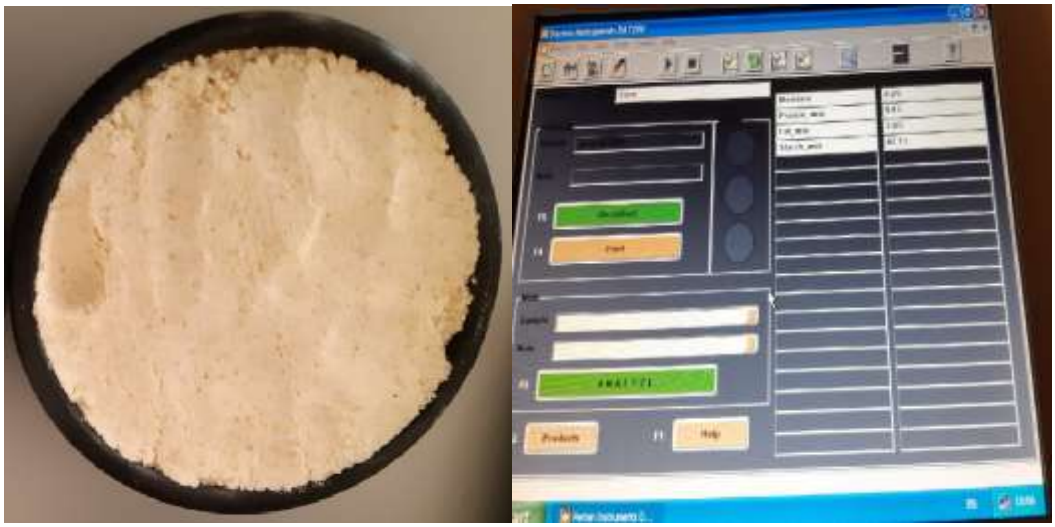

Figure 2 **Presentation of results for maize flour**

The results of the analyzes may be influenced by the time of harvest, the variety of the sample analyzed, or the composition of the analyzed mixture. The NIRS technique does not require the use of chemicals and no prior preparation of samples is needed, being considered "clean technology" - in accordance with the requirements of sustainable agriculture. Near-infrared spectrometry (NIRS) is a modern (non-destructive) technique with applications in the food and agriculture industry (identification of mycotoxins from seeds, hay, fodder, feed rations, etc.), being a rapid method used for quantitative and qualitative characterization of vegetable products. This technique presents several advantages such as the possibility of analyzing several physico-chemical parameters at the same time, reduction of toxicity and laboratory working time, reproducibility of results.

Determination of mycotoxins in plant substrates using TLC

Contamination of plant products and foods with mycotoxins leads to significant economic losses as well as undesirable trade barriers for raw materials and consumables (Escrivá L. *et al,* 2017).

Determination of mycotoxins with TLC can be considered a semi-quantitative method because after identifying their presence on the chromatographic plate in a dark space, using the UV light, the migration distance of all spots is noted, expressed in cm. Mycotoxins are appreciated and identified based on the color of the spot fluorescence and the Rf value.

$$Rf = \frac{d}{D};$$

Rf =retention factor calculated based on the ratio of the migration distance of the compound to the migration distance of the solvent front; d=*distance in cm of the spot migration; D=the migration distance in cm of the developing fluid.*

The concentration of each type of mycotoxin present in the test sample can be determined by the Rf value. Figure 3 shows the development tank with solvent system for the silica gel chromatographic plate.

Figure 3 **Development tank with solvent system for the chromatographic plate**

In the first instance, the sample can be placed under the lamp as such in a Petri dish, and if the amount of mycotoxins is high, different colours appear on the tested plant substrate. Figure 4 shows a sample of alfalfa hay in a Petri dish that has been placed under the UV lamp.

After examination of the plate, it may assumed that there are two types of mycotoxins on the surface of alfalfa hay: the red-dark color indicates the presence of sterigmatocystin (areas marked with a circle in the figure), while the blue color indicates the presence of ochratoxin in the plate (areas marked with rectangle in the figure).

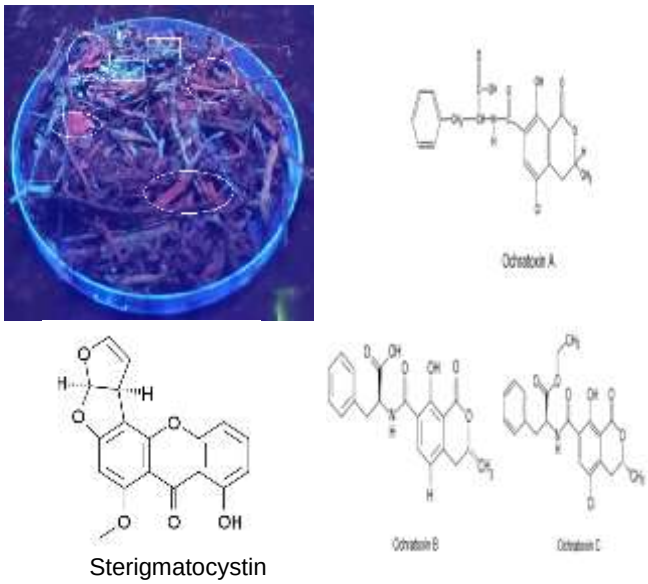

Figure 4 **Alfalfa hay sample and the structural formulas of the identified mycotoxins**

Several types of mycotoxins were identified on the surface of the chromatographic plate shown in figure 5. In the samples of maize grains (1), alfalfa hay (2) and corn silage (3), aflatoxins, ochratoxins and sterigmatocystin were identified,

and in the feed ratio (4) and maize grains (5) aflatoxins (B1, G1) and zearalenone were identified. In such situations, specialists in the field must find solutions to apply prevention/decontamination strategies and a continuous monitoring of the quality of plant products and storage conditions.

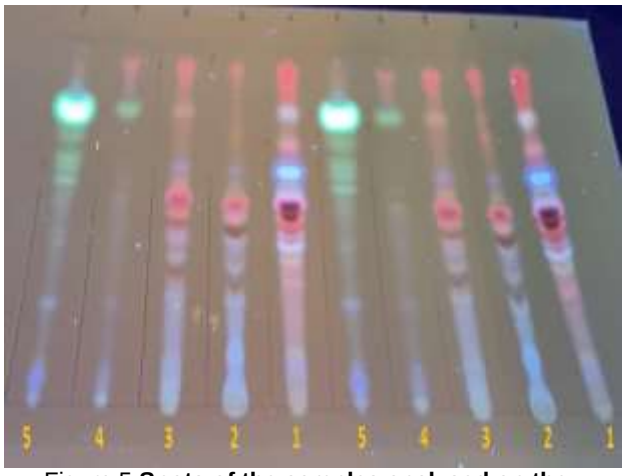

Figure 5 **Spots of the samples analysed on the chromatographic plate**

1- corn grains, 2- alfalfa hay, 3- corn silage, 4 - feed ration, 5 - concentrates

CONCLUSIONS

The NIRS technique can be successfully applied for assessing the quality of plant substrates, respectively for the determination of different parameters such as the content of crude protein, crude ash, crude fat, crude cellulose, NDF content, ADF content, lignin content, digestibility of organic matter plant products.

Food industry needs concerted and ongoing efforts to monitor the production of mycotoxin, inhibit the growth of toxic mounds in food and feed, and develop rapid and cost-effective detection techniques to improve food safety.

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