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Mini Review

Enniatins: An Emerging Food Safety Issue

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Abstract

Mycotoxins are significant contaminants in human foodstuffs and feeds, and have demonstrated serious effects in both humans and animal's health. They are produced as secondary metabolites by different genera of fungi. Enniatins are *Fusarium* mycotoxins which belong to the category of 'emerging' mycotoxins, mainly contaminated cereals. Last decade has greatly increased the research on this class of mycotoxins due to their constantly increasing presence in the entire food chain. The main analytical technique that used for the analysis and identification of Enniatins is the liquid chromatograph - mass spectrometry. Enniatins have biological activities, that indicate the potent toxicity of these toxic metabolites. The European Commission has not set maximum permitted levels of Enniatins in foods and feeds.

Keywords: *Enniatins; Analytical techniques; Biosynthesis; Production; Safety; Foods*

Introduction

Enniatins (ENs) are mycotoxins that were first isolated from *Fusarium oxysporum* in 1947 [1]. Although different genera of fungi such as, *Fusarium*, *Verticillium*, *Alternaria* and *Halosarpheia* produce these secondary metabolites, the main producers for ENs are the strains of *Fusarium subglutinans*, *Fusarium tricinctum* and *Fusarium proliferatum* [2]. They are identified and isolated until now, 29 enniatins. As single compounds have been isolated 18 enniatins and the others as five mixtures of homologs. The enniatins which mostly detected in foods and feed are enniatin A, (ENA), enniatin A1 (ENA1), enniatin B (ENB) and enniatin B1 (ENB1). Figure 1 and Table 1 indicate the chemical structure and the substituents related to the general structure of enniatins. The most well-known biological activities of enniatins are the cytotoxic, antiinsectan, antibiotic and antifungal. For this reason, last decade, more and more researchers continue to work strongly in this area of research against many biological targets [3]. In 2014 the European Commission asked the European Food Safety Authority (EFSA) to express its scientific opinion of the human and animal risk related to the presence of ENs in food and feed. EFSA failed to express the risk assessment, because of the luck of adequate data mainly concerning in vivo toxicity regarding human and animal exposure. According to EFSA, the most significant contributors to the chronic dietary exposure to the enniatins ENA, ENA1, ENB, and ENB1 were grains and grain-based products especially pasta (raw), fine bakery wares, and bread and rolls. According to EFSA, the prevalence and concentrations of ENA and ENA1 in grains are generally lower than those of ENB and ENB1 [4]. Until now maximum levels for ENs have not been regulated yet from the European Commission. ENs are also known as emerging mycotoxins, something that indicates the great concern about the risk of ENs worldwide, as the occurrence of these mycotoxins is steadily increased in foods and feeds [5].

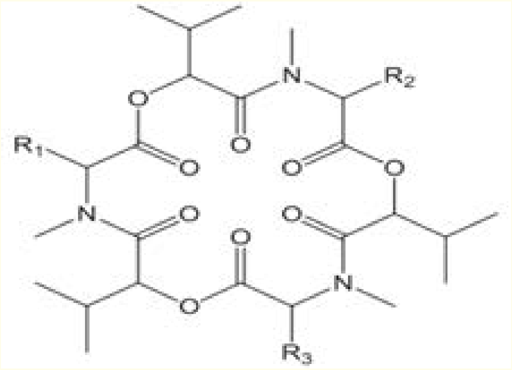

Figure 1: *Chemical structure of enniatins.*

Enniatin	R1	R2	R3
Enniatin A	$-CH(CH_3)CH_2CH_3$	$-CH(CH_3)CH_2CH_3$	$-CH(CH_3)CH_2CH_3$
Enniatin A1	$-CH(CH_3)CH_2CH_3$	$-CH(CH_3)CH_2CH_3$	$-CH(CH_3)_2$
Enniatin B1	$-CH(CH_3)_2$	$-CH(CH_3)_2$	$-CH(CH_3)_2$
Enniatin B1	$-CH(CH_3)_2$	$-CH(CH_3)_2$	$-CH(CH_3)CH_2CH_3$

Table 1: *Substituents related to the general structure of enniatins.*

ENs are mostly detected in cereals grains, like corn, rice, wheat, rye and other grain based food, like pasta, flour, biscuits and bread [5-8]. Moreover, ENs have contaminated different kinds of agricultural products such as medicinal herbs [9], vegetable oils, dried fruits and coffee [4].

Chemical structures and biosynthesis of enniatins

ENs are cyclohexadepsipeptides (CHDPs), composed of three a-hydroxy acids residues typically hydroxyisovaleric acid (Hiv) linked alternatively to N-methyl-a-amino acid or three a-amino acids residues commonly valine (Val), leucine (Leu), and isoleucine (Ile), and give an 18-membered cyclic skeleton. Enniatin synthetase is the enzyme that takes part in the biosynthesis of enniatins. The adenylation is the first step of the biosynthesis of enniatins. The formation of the peptide bond is through the N-methylation through which result a dipeptidol. The following concentration of dipeptides with two others forming a linear hexadepsipeptide. Finally, hexadepsipeptide cyclizes and gives the final product, the Enniatin Although the most ENs are formed naturally for the various fungi, it also important that these substances could produce by altering the combination of the amino acids and hydroxy acids [3].

Occurrence of enniatins, in various food commodities and feed matrices

Most recently, published data have shown that the most important four enniatins mainly investigated for the potent contamination of cereals and cereals based foods and feeds. As it mentioned above, ENs can contaminate various agricultural products including dried fruits, nuts [10], beans [4], and wine [11].

Bryła., *et al*. analyzed 147 samples of wheat, oat, barley and triticale grain, cultivated in Poland for the occurrence of 26 mycotoxins. ENA1, ENB, and ENB1 were found in all analyzed triticale, barley and oat samples and ENA was also found in the 70% of triticale samples, and 38% of barley samples. As it concerns wheat samples, were reported in the 37% ENA, 47% ENA1, 94% ENB and 84% ENB1 of all tested samples. Concentrations of ENs were ranged between 1-3328 $\mu g \cdot Kg^{-1}$ [12]. Zinedin., *et al*. have reported that from the analysis of 98 couscous samples, commercialized in Morocco, ENA1, ENB, and ENB1 were found at the highest concentrations, that were ranged between 0.7-592 $\mu g \cdot Kg^{-1}$ [13]. Pamel., *et al*. have reported the occurrence mainly of ENB and ENB1 in maize silage samples, collected in Belgium [14]. Nazari., *et al*. also reported from the analysis of 65 rice samples from Iran the occurrence only of ENA1 [15]. Habler., *et al.* detected the occurrence of ENB in 15 out of 61 beer samples from Germany and all over the world [16].

It is worth mentioning that recently [17], from the analysis of 52 barley and malt samples from Czech Republic, the results indicated that the ENs were detected in all analyzed samples. From another study [9], from a total of 60 analyzed medicinal herbs from China, have reported that 25 samples were contaminated with at least with one of the ENA ENA1, ENB, and ENB1.

Developments in enniatins analysis

Nowadays, a broad range of methods are used for the food contaminant analysis, including mycotoxins, with the most privileged to be the liquid chromatography. All the methods that take places in the field of mycotoxins analysis require to be validated, to have repeatability, sensitivity, and to detect quantities with low concentrations at the scale of nanograms. The co-occurrence of many mycotoxins in the same food commodity is another significant issue in mycotoxins analysis. One method is succeeded when manage to simultaneously determine as much as possible mycotoxins and their metabolites with only a single elution procedure. These approaches may achieve with the help of a high performance liquid chromatograph coupled with a mass spectrometer (LC-MS). On the other hand, food matrices are very complex, so it is very crucial to eliminate all the interfering substances with the step of purification. The proper sample preparation prior to chromatographic analysis is required, in terms to achieve good chromatographic separations. Techniques such as solid phase extraction (SPE) and liquid-liquid extraction (SLE) are mostly used before chromatographic analysis [12]. In many publications the QuEChERS (quick, easy, cheap, effective, rugged, safe) extraction procedure [7,12-13,18], have been developed, for purification of ENs analysis, as it concerns a flexible methodology with minimum sample preparation.

Also, the use of relatively expensive immunoaffinity columns (IAC) for purification of many mycotoxins compromise a common practice. Many mycotoxins such as aflatoxins, zearalenone, deoxinivalenol, ochratoxin, fumonisins are purified with these columns, before chromatographic analysis. Until now some kind of proper columns for mycotoxins enniatins are not available in the market [5]. ENs analysis with LC-MS/MS but without the purification step has reported by the EFSA or with purification by SPE cartridge [4].

Moreover, an ultra-high-performance liquid chromatography UHPLC-MS/MS method have been used for the determination of ENs in *in vitro* transdermal experiments by Taevernier., *et al*. [19], and for the detection of ENs in maize silage [14]. The UHPLC method uses smaller particle size as columns packing materials and develops higher pressures than in traditional HPLC, resulting in better chromatographic separations.

Except for chromatographic techniques, there are other available analytical techniques, such as, the immunochemical techniques the optical techniques and the biosensors approaches [20]. Until now according to the EFSA [4] nor the immunochemical techniques or some other technique for the analysis of ENs in foodstuffs and feeds are used.

Risk assessment and toxic effects in humans and animals

There are no published data with any kind of human toxicosis, coming from ENs from contaminated foodstuffs from all over the world. From data, related with *in vitro* experiments, it seems that, ENs are absorbed and metabolized to a range of uncharacterized metabolites. ENs have been detected at low concentrations from feed to skin, liver, eggs and meat of poultry, something that indicates the very small contribution to the human exposure from the consumption of poultry meat. Moreover, toxicity in vitro is caused by ENs, in many cell lines. The relative mechanism of action is believed to be associated with the formation of specific channels for transport of monovalent and divalent cations through biological membranes and the accumulation of abnormal concentrations in cells, leading to toxic actions [4].

Conclusions

The high occurrence of ENs in various food and feed commodities has been reported in many countries around the world including European countries. Although they have been categorized as 'emerging' mycotoxins, ENs are not regally legislated mycotoxins yet, by the European Commission, in contrast with other mycotoxins. The contamination from ENs seems to be more frequent in cereals and cereals based foods, resulting in their presence in the entire human and animal food chain. According to these, more geographical regions with different climatic conditions from all over the world should be considered for the presence of these mycotoxins. Moreover, further studies are needed, for toxicity and potential chronic effects.

Bibliography

1. Gaumann E., *et al.* "Ionophore antibiotics produced by the fungus Fusarium orthoceras var. enniatum and other". *Fusaria Experientia* 3 (1947): 202-203.
2. Cuomo V., *et al.* "Production of enniatins A, A1, B, B1, B4, J1 by Fusarium tricinctum in solid corn culture: Structural analysis and effects on mitochondrial respiration". *Food Chemistry* 140 (2013): 784-793.
3. Sy-Cordero AA., *et al.* "Revisiting the enniatins: a review of their isolation, biosynthesis, structure determination, and biological activities". *The Journal of antibiotics (Tokyo)* 65.11 (2012): 541-549.
4. EFSA CONTAM Panel. "Scientific Opinion on the risks to human and animal health related to the presence of beauverivin and enniatins in food and feed". *EFSA Journal* 12.8 (2014): 3802.
5. Yoshinari T., *et al.* "Occurrence of beauvericin and enniatins in wheat flour and corn grits on the Japanese market, and their co-contamination with type B trichothecene mycotoxins". *Food Additives and & Contaminants: Part A* 33.10 (2016): 1620-1626.
6. Malachova A., *et al.* "Deoxynivalenol, deoxynivalenol-3-glucoside, and enniatins: the major mycotoxins found in cereal-based products on the Czech market". *Journal of Agriculture and Food Chemistry* 59.24 (2011): 12990-12997.
7. Capriotti AL., *et al.* "Multiclass analysis of mycotoxins in biscuits by high performance liquid chromatography-tandem mass spectrometry. Comparison of different extraction procedures". *Journal of Chromatography A* 1343 (2014): 69-78.
8. Juan C., *et al.* "Evaluation of beauvericin and enniatins in Italian cereal products and multicereal food by liquid chromatography coupled to triple quadrupole mass spectrometry". *Food Chemistry* 140.4 (2013): 755-762.
9. Hu L and Rychlik M. "Occurrence of enniatins and beauvericin in 60 Chinese medicinal herbs". *Food Additives & Contaminants: Part A* 31.7 (2014): 1240-1245.
10. Tolosa J., *et al.* "Nuts and dried fruits: natural occurrence of emerging Fusarium mycotoxins". *Food Control* 33.1 (2013): 215-220.
11. Wang Z., *et al.* "Wine contamination by mycotoxin enniatin B from Fusarium tricinctum (Corda)". *Journal of Food, Agriculture & Environment* 9.1 (2011): 182-185.
12. Bryła M., *et al.* "Occurrence of 26 Mycotoxins in the Grain of Cereals Cultivated in Poland". *Toxins* 8.6 (2016): E160.
13. Zinedine A., *et al.* "Multi-mycotoxin contamination of couscous semolina commercialized in Morocco". *Food Chemistry* 214 (2017): 440-446.

14. Pamel EV., *et al.* “Ultrahigh-Performance Liquid Chromatographic Tandem Mass Spectrometric Multimycotoxin Method for Quantitating 26 Mycotoxins in Maize Silage”. *Journal of Agriculture and Food Chemistry* 59.18 (2011): 9747-9755.

15. Nazari F., *et al.* “Evaluation of Emerging Fusarium mycotoxins beauvericin, Enniatins, Fusaproliferin and Moniliformin in Domestic Rice in Iran”. *Iranian Journal of Pharmaceutical Research* 14.2 (2015): 505-512.

16. Habler K., *et al.* “Multi-mycotoxin stable isotope dilution LC–MS/MS method for Fusarium toxins in beer.” *Food Chemistry* 218 (2017): 447-454.

17. Bolechová M., *et al.* “Determination of seventeen mycotoxins in barley and malt in the Czech Republic”. *Food Control* 47 (2015): 108-113.

18. Malachova A., *et al.* “Deoxynivalenol, Deoxynivalenol-3-glucoside, and Enniatins: The Major Mycotoxins Found in Cereal-Based Products on the Czech Market”. *Journal of Agriculture and Food Chemistry* 59.24 (2011): 12990-12997.

19. Taevernier L., *et al.* “UHPLC-MS/MS method for the determination of the cyclic depsipeptide mycotoxins beauvericin and enniatins in in vitro transdermal experiments”. *Journal of Pharmaceutical and Biomedical Analysis* 100 (2014): 50-57.

20. Turner NW., *et al.* “Analytical methods for determination of mycotoxins: An update (2009-2014)”. *Analytica Chimica Acta* 901 (2015:) 12-33.

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