VALIDATION OF A METHOD FOR THE CONTROL OF ERGOT ALKALOIDS IN OAT-BASED FUNCTIONAL FOODS

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INTRODUCTION

Ergot alkaloids (EAs) are mycotoxins produced mainly by fungi of the *Claviceps* genus, as *Claviceps purpurea*. The fungus infects the seed heads of living plants, specially cereals, at the time of flowering replacing the developing grain or seed with specialized fungal structures known as sclerotium (or ergot body), which contains alkaloid substances. Although the sclerotia can be mechanically removed during the harvesting process, EAs can be found in cereal-based food, and their ingestion might cause adverse health effects in humans. The European Comission has established a maximum content of 0.5 g/kg of ergot sclerotia in most unprocessed cereals; however, the maximum content for EAs in food is still under study.

Ergot body

EFSA has stated that collection of analytical data are required in order to define the variability of EAs in food and feed commodities, paying special attention to processed foods

AIM OF THE WORK

As functional foods containing cereals as ingredients have been scarcely explored so far, in this work, we propose the extraction and quantification of the main 6 EAs; Ergometrine (Em), Ergosine (Es), Ergotamine (Et), Ergocornine (Eco), Ergorkriptine (Ekr) and Ergocristine (Ecr), and their corresponding epimers; Ergometrinine (Emn), Ergosinine (Esn), Ergotaminine (Etn), Ergocorninine (Econ), Ergokriptinine (Ekrn), Ergocristinine (Ecrn) in different oat-based products using QuEChERS combined with UHPLC-MS/MS.

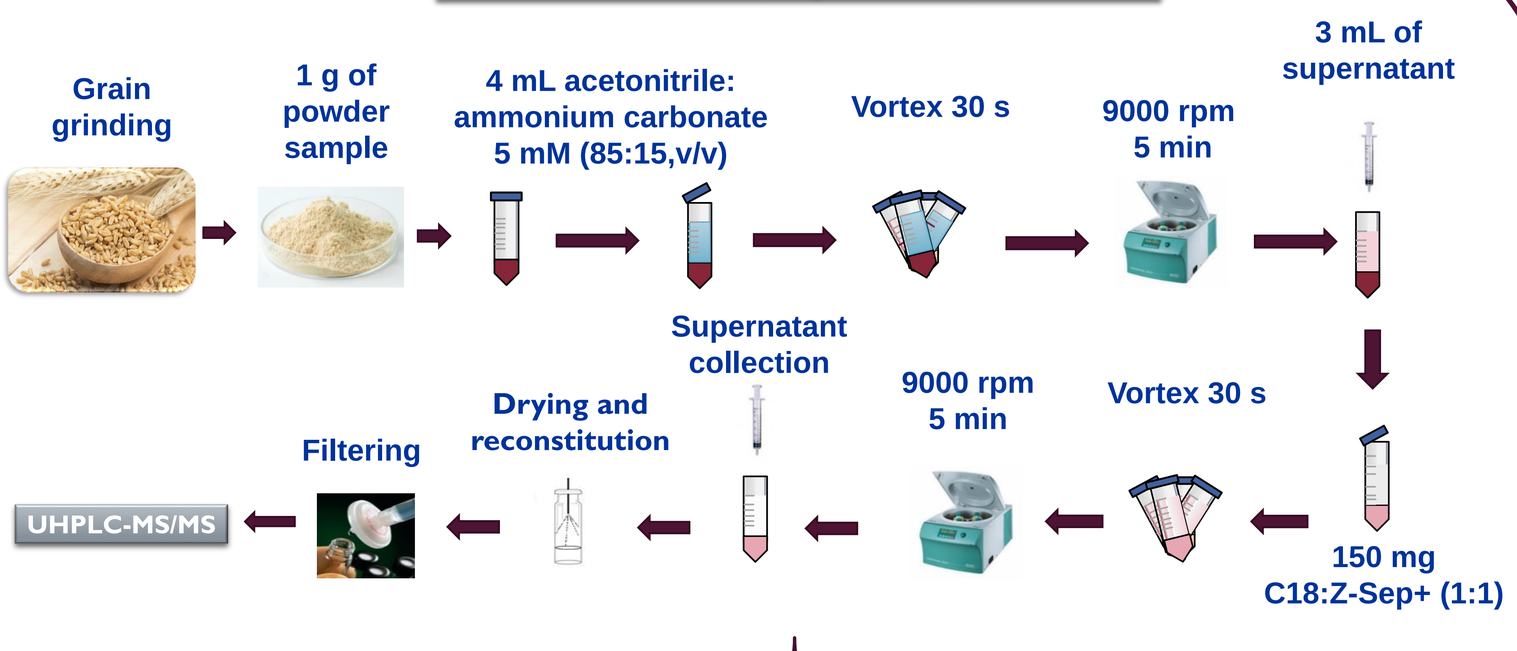
ANALYTICAL METHOD

Chromatographic conditions	
Column	Zorbax Eclipse Plus RRHD C18 (50×2.1 mm, 1.8 µm)
Organic solvent (B)	MeOH + 0.3% formic acid
Aqueous solvent (A)	Ultrapure water + 0.3% formic acid
Gradient	0-6 min 30-60% B; 6-9 min 60% B; 9-10 min 60-30% B; 10-12 min 30% B
Column temperature	35 °C
Flow rate	0.4 mL/min
Injection volume	5 µL

MS conditions	
Ionization mode and polarity	ESI +
Scan type	MRM
Target scan time	1s

The monitored ions were the protonated molecules $[M+H]^+$ for all of them, except for Esn, Etn, Econ, Ecrn and Ekrn, where the signal at *m/z* corresponding to $[M-H_2O+H]^+$ was higher than that of the protonated molecules.

QuEChERS procedure

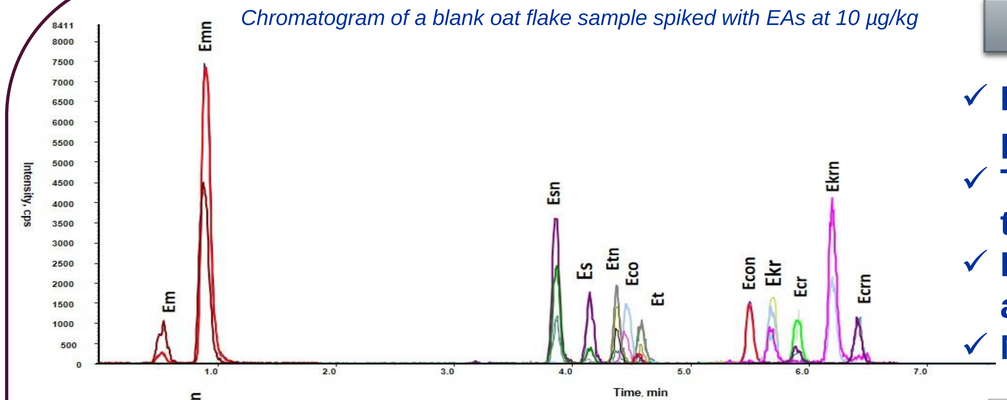


Some improvements were achieved

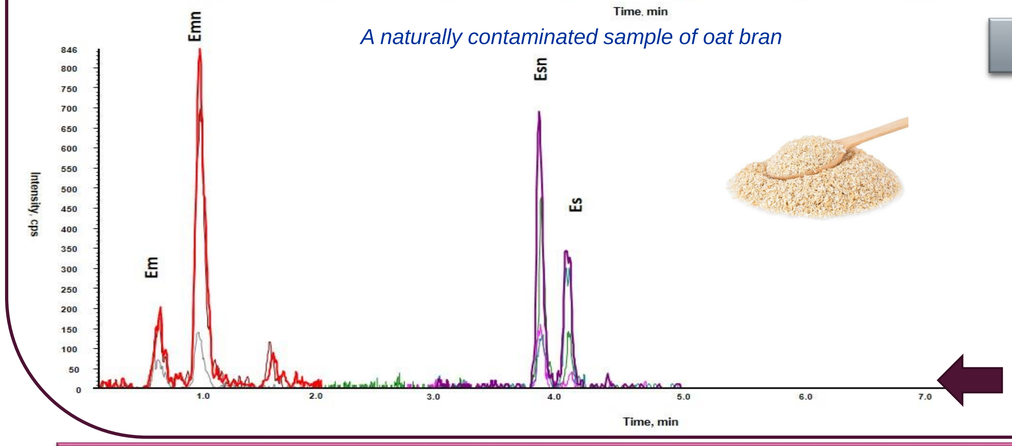
- **Lower consumption organic solvents by reducing the volume of the extraction mixture to 4 mL, which leads to an increase in sensitivity.**
- **Different dispersive sorbents (C18, PSA and Z-Sep+) were evaluated. The best clean-up of the matrix without affecting the recoveries was achieved using a mixture of C18:Z-Sep+ (1:1).**

RESULTS

Chromatogram of a blank oat flake sample spiked with EAs at 10 µg/kg



A naturally contaminated sample of oat bran



Method Characterization

- ✓ **Procedural calibration curves were established with LODs and LOQs between 0.1-1.0 µg/kg and 0.2-3.20 µg/kg, respectively. Good linearity was obtained (R>0.994).**
- ✓ **The precision, evaluated in terms of repeatability and intermediate precision, was lower than 15% RSD in all cases.**
- ✓ **Recovery experiments were carried out on oat samples at two concentration levels (5 and 50 µg/kg). The recoveries ranged between 90 and 106%.**
- ✓ **Matrix effects were lower than 20% in most cases.**

Sample analysis

25 oat-based samples with different presentations were analyzed.

Flakes | Bran | Flavours | Grass | Extracts | Juices | Tablets

One sample of oat bran was contaminated with Em, Emn, Es and Esn in the range of 1.1-7.2 µg/kg, with a total content of EAs of 10.7 µg/kg.

CONCLUSIONS

- ❑ **For the first time EAs have been explored in such a variety of oat-based functional foods.**
- ❑ **The modifications carried out in the QuEChERS procedure allowed to increase the sensitivity of the method and to reduce the matrix effects.**
- ❑ **Despite the improvements in industrial grain processing, contamination of EAs must be considered, especially in cereal-based processed foods**

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