Microextraction of ochratoxin A in raw wheat with supramolecular solvents

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Ochratoxin A (OTA) is one of the most widespread and hazardous mycotoxins contaminating foodstuffs. It is produced by several fungi (*Aspergillus* and *Penicillium* species) in a variety of plant products, such as cereals, pulses, coffee, wine, grape juice, dried fruits and spices. OTA is considered a potent nephrotoxic and genotoxic agent and has been classified as a possible human carcinogen (group 2B) by the International Agency for Research on Cancer[23]. Monitoring OTA in cereals has become an important issue worldwide due to both the impact on human health and the high economic losses associated to crop production. In fact, among the most frequently contaminated food commodities, cereals are the main European dietary source of OTA (44%), increasing this value in the case of Spain (60%), according to a 2002-study[24]. Consequently, the European Union has established maximum residue levels (MRLs) of OTA in raw cereals and derivative products of 5 and 3 μg Kg^{-1}, respectively[25].

For surveillance on OTA residues in cereals, several official AOAC and ISO methods are available and a number of methodologies have been reported in literature as good alternatives in terms of sensitivity, reproducibility or simplicity. Detection and quantification is performed by liquid chromatography with fluorescence detection (LC-FL), although other techniques such as LC coupled with mass spectrometry and enzyme linked immunosorbent assay (ELISA) have been applied for multi-toxins determination and screening purposes, respectively. Due to the complexity of the matrices, sample preparation is essential in OTA analysis. The most common extraction step is solvent extraction. The volume of toxic organic solvent consumed per sample extracted is relatively high (50-250ml) and after extraction, further clean-up is usually needed. With this aim, immunoaffinity chromatography (IAC) is the most widely used procedure. Although IAC has been proven to be applicable in a wide range of matrices, allowing clean extracts due to high selectivity and consequently low limits of quantification (around 0.1 μg Kg^{-1}) and reproducible results (<5%), it presents important disadvantages for routine analysis: immunoaffinity columns are expensive and not recyclable, have a limited storage time and, in some cases, show cross-reactivity with Ochratoxin C.

This research presents a new rapid simple and low-cost method for the analysis of OTA in wheat based on supramolecular solvents. Supramolecular solvents made up of reverse

[23] International Agency for Research on Cancer. Monograph on the Evaluation of Carcinogenic Risks to Humans, some Naturally Ocurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins; International Agency for Research on Cancer: Lyon, France, vol. 56, 1993, 489–521.
[24] Scientific Cooperation (SCOOP) Task Report 3.2.7, Assessment of dietary intake of Ochratoxin A by the population of EU Member States, 2002, http://europa.eu.int/comm/food/fs/scoop/index en.html.
[25] Commission Regulation (EC) no 123/2005 of 26 January 2005 amending regulation (EC) no 466/2001 as regards ochratoxin A, Off. J. Eur. Union L25 (2005) 3.

micelles of decanoic acid were proposed to extract ochratoxin A (OTA) from raw wheat trough hydrophobic and hydrogen bond interactions, prior to determination by liquid chromatography-fluorescence detection. The method was optimised on the basis of extraction efficiency, detection and quantification limits and operationality. Decanoic acid, tetrahydrofuran (THF) and sample amount were the most influential parameters, being 300 mg, 0.5 mL and 0.3 g the optimum selected values, respectively. The procedure was robust under the selected conditions and the extractions were not significantly influenced by the nature or concentration of matrix components. OTA recoveries from different wheat samples ranged between 74 and 91%, while the precision of the method, expressed as relative standard deviation, was about 2%. The detection limit of the method was 0.5 μg kg^{-1} and was far below the threshold limit established for OTA in raw cereals by EU directives (5.0 μg kg^{-1}). Sample treatment was simple, no clean-up of the extracts was needed, it took about 30 min and several samples could be simultaneously treated using conventional lab equipment. The approach developed was successfully applied to the determination of OTA in different wheat and synthetic wheat hybrids from crops harvested in the South of Spain. OTA was not detected in any of the analysed samples.