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Determination of trichothecenes in chicken liver using gas chromatography coupled with triple-quadrupole mass spectrometry

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ABSTRACT

Chicken meat and edible offal are included in a wide number of preparations in the worldwide cuisine. Among the offal, the liver is one of the most consumed in some countries like Egypt since it is rich in proteins and is an economical source of many essential nutrients for humans. Mycotoxins, secondary toxic metabolites produced by fungi, have the potential to enter human food chain not only with cereal consumption but also through edible tissues from animals fed with mycotoxin-contaminated feed. Accordingly, a rapid method for the determination of eight trichothecenes in chicken liver was developed based on a quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction and gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) determination. The proposed method was successfully validated according to the European guideline recommendations with satisfactory trueness (recovery range from 85.1 to 108.4%), precision (relative standard deviation $< 8\%$) and limits of quantitation in a range from 0.25 to 0.75 μg/kg. The method was applied to twenty chicken liver samples collected from Egypt markets. 65% of samples showed mycotoxin contamination in a concentration range from 1.3 to 25.0 μg/kg. Up to four mycotoxins were detected, being deoxynivalenol the most commonly detected mycotoxin.

1. Introduction

Mycotoxins are secondary toxic metabolites produced by various fungi (mainly *Aspergillus*, *Penicillium* and *Fusarium*), that represent an important group of contaminants particularly in food. Trichothecenes are a toxic group of mycotoxins produced by different species of *Fusarium*. Trichothecenes type-A: HT-2 toxin (HT-2), T-2 toxin (T-2) and neosolaniol (NEO); and type-B: nivalenol (NIV), deoxynivalenol (DON), 3-acetyl-DON (3-ADON), 15-acetyl-DON (15-ADON) and fusarenon-X (FUS-X) are commonly found in grains, animal feed and human food derived from contaminated grains (Anater et al., 2016; Hernández-Rodríguez, Rodríguez-Carrasco, Berrada, & Ruiz, 2016; Neme & Mohammed, 2017). Mycotoxins are generally stable, resistant to various food processing methods, and could even remain in cooked food (Adekoya, Obadina, Phoku, Nwinyi, & Njobeh, 2017; Cheli et al., 2010; Stuper-Szablewska, Szablewski, Buśko, & Perkowski, 2016). Consequently, human exposure to mycotoxins may result from consumption of both plant-derived foods that are contaminated with mycotoxins, and the carry-over of mycotoxins and their metabolites in animal products (Marin, Ramos, Cano-Sancho, & Sanchis, 2013).

From a toxicological point of view, mycotoxins pose health risk to human and livestock that comprise nephrotoxicity, hepatotoxicity, neurotoxicity, mutagenicity, carcinogenicity, immunosuppression and adverse estrogenic effects (Escrivá, Font, & Manyes, 2015; Payros et al., 2016). Signs of toxicity may vary among species, based on mycotoxin concentration and exposure time. For instance, the effects of DON and other trichothecenes have been investigated in different species laboratory animals, poultry and pigs. Among other species, pig react most sensitively to the exposure of trichothecenes and are particularly interesting as a target species for mycotoxins as recently reviewed by Escrivá et al. (2015). On the other hand, the co-occurrence of different mycotoxins may lead to their interactions affecting their toxicity (e.g. synergism, addition, etc), and individual factors such as age, sex, diet or overall condition may also have influence on mycotoxins toxicity (Grenier & Oswald, 2011).

Egypt (North Eastern Africa) is a largely agricultural country characterized by high humidity and high-temperature climate, which favor the contamination of crops by molds and their specific mycotoxins production. Egypt human population is approximately 90 million, and poultry production is one of the fastest developing industries as it has

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been encouraged by local governments to guarantee a stable supply of animal products. In Egypt, the broiler meat trade is rather diverse, accounting for more than 70% of total broiler production (Hosny, 2008). Chicken is the staple product of livestock production which reached 1,035,193 tons in 2014, (84% of total broiler production in Egypt) (FAO, 2014). In addition to chicken meat, there are the giblets, edible viscera of domestic fowl including liver, heart and gizzard. These edible offal, especially liver, are considered valuable in Middle East countries and give rise to a number of preparations in the Egyptian cuisine. Chicken muscle and liver are widely consumed, because they are rich in proteins, minerals, vitamins, and they are also an economical source of many essential nutrients for humans (Abu-Salem & Abou Arab, 2010; Terrasa, Dello Staffolo, & Tomás, 2016). According to FAO statistical database (FAOSTAT), the per capita annual consumption of poultry meat (14.43 kg) and edible offal (3.56 kg) are calculated as ~18 kg (FAO, 2013).

As far as the legislation is concerned, the European Union set in the Commission Regulation (CE) 1881/2006 the maximum limits for some mycotoxins in foodstuffs (Commission Regulation, No 1881/2006). Up to now, aflatoxin M1 is the only mycotoxin regulated in foodstuffs from animal origin. However, trichothecenes have the potential to enter human and animal food chains through edible tissues from livestock and poultry animals that are fed with trichothecenes-contaminated feed. In recent years some studies have elucidated the occurrence of some mycotoxins in different animal tissues (Broekaert et al., 2014; Kongkapan, Giorgi, Poapolathep, Isariyodom, & Poapolathep, 2016; Rodríguez-Carrasco, Mañes, Berrada, & Font, 2015; Tolosa, Font, Mañes, & Ferrer, 2014; Yang et al., 2015). Therefore, it is of great interest to develop convenient, sensitive and straightforward methods to determine mycotoxins in animal-derived food.

Analysis of mycotoxins in meat and liver samples is a difficult task because of their complex matrices: containing fats, proteins, carbohydrates, vitamins and inorganic salts. To maximize recovery of mycotoxins and minimize matrix effects, the use of an appropriate extraction solvent and clean-up is of critical importance. Among the extraction solvents used, acetonitrile exhibited the best extraction efficiency due to its high extraction recovery for mycotoxins and its significant reduction in co-extracts from various samples in comparison with other organic solvents. Several extraction procedures have been reported in previous works, including liquid-liquid- extraction (LLE; recovery range: 42–94%, RSD < 15%, LODs: 0.02–10.14 ng/mL) (Flores-Flores & González-Peñas, 2017), solid-phase extraction (SPE; recovery range: 94–101%, RSD < 6%; LODs: 00.03–0.65 ng/mL) (Winkler, Kersten, Meyer, Engelhardt, & Dänicke, 2014), LLE-SPE (recovery range: 40–126%, RSD < 23%; LODs: 0.01–0.63 ng/mL) (De Baere et al., 2011; Osselaere et al., 2013), LLE-SPE (recovery range: 87–97%, RSD < 6%; LODs: 0.02 μg/kg and 0.007 μg/kg) (Zou et al., 2012), salting-out liquid-liquid extraction (SALLE; recovery range: 72–109%, RSD < 13%, LODs: 0.12–4 ng/mL) (Rodríguez-Carrasco, Moltó, Mañes, & Berrada, 2014), pressurized liquid extraction (PLE; recovery range: 71–100.5%, RSD < 17.5%, LODs: 0.01–0.69 ng/mL) (Cao et al., 2013), dispersive liquid–liquid microextraction (DLLME; recovery range: 79–113%, RSD < 12%, LODs: 0.005–2 ng/mL) (Escrivá, Manyes, Font, & Berrada, 2017) and QuEChERS (acronym of Quick, Easy, Cheap, Effective, Rugged and Safe; recovery range: 55–105%, RSD < 17.5%, LODs: 0.01–0.69 ng/mL) (Zhu et al., 2015) for the simultaneous determination of mycotoxins in biological samples. The QuEChERS comprise in an acetonitrile partitioning and dispersive solid-phase extraction (d-SPE) technique. Moreover, the QuEChERS method has several advantages in comparison with most traditional methods of analysis such as: the quickness, the usage of smaller quantities of organic solvents and the satisfactory recoveries obtained for all compounds (Juan, Mañes, Font, & Juan-García, 2017).

As the concentration of trichothecenes in edible tissues of animals is lower than those in the grain products, low concentrations (μg/kg) in tissue samples need to be detected. Thus optimizing the sensitivity of the MS method is important. In these sense, both gas chromatography (GC) and liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) techniques, have been developed for the determination of mycotoxins as recently reviewed by Turner et al. (2015) and Escrivá et al. (2017). In spite of the fact that GC methods require an additional step (derivatization) when compared to LC methods, GC-MS/MS allow simultaneously a very useful and relatively inexpensive analytical performance with some advantages such as lower detection limits and greater selectivity (Rodríguez-Carrasco et al., 2014). Thus GC-MS/MS was proposed as a useful analytical alternative tool for further exploration.

Therefore, the purpose of this research was to develop a reliable and sensitive method for the simultaneous determination of trichothecenes (DON, 3-ADON, 15-ADON, NIV, FUS-X, NEO, HT-2 and T-2) in chicken liver samples using gas chromatography-triple quadrupole mass spectrometry (GC-QqQ-MS/MS) and to evaluate the occurrence of these contaminants in twenty chicken liver samples collected from Egypt for the first time.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile (AcN), hexane, and methanol were acquired from Merck KGaA (Darmstadt, Germany). Anhydrous magnesium sulfate (thin powder) was acquired from Alfa Aesar GmbH & Co (Karlsruhe, Germany), and C18-E (50 μm, 65 A) was obtained from Phenomenex (Torrance, USA). The derivatization reagent composed of BSA (N,Obis (trimethylsilyl)acetamide) + TMCS (trimethylchlorosilane) + TMSI (N-trimethylsilyimidazole) (3:2:3) was purchased from Supelco (Bellefonte, USA). Disodium hydrogen phosphate and sodium dihydrogen phosphate, used to prepare phosphate buffer, were acquired from Panreac Quimica S.L.U. (Barcelona, Spain).

The standards solutions of the type A and B trichothecenes namely DON, 3-ADON, 15-ADON, ^{13}C-labeled deoxynivalenol (internal standard, I.S), NIV, FUS-X, NEO, T-2 and HT-2 were obtained from Sigma-Aldrich (St. Louis, USA). All stock solutions were prepared by dissolving 1 mg of the mycotoxin in 1 mL of pure methanol, obtaining a 1 mg/mL (1000 mg/L) solution. The stock solutions were diluted with AcN in order to obtain the appropriate multi-compounds working standard solutions. All standards were stored in darkness and kept at −20 °C.

2.2. Sampling and sample preparation protocol

Twenty samples of chicken liver were randomly purchased between March and July 2017, from different retailers, including local markets and smaller shops from central cities of Sharkia province, Egypt. All samples were produced in Sharkia province and at the moment of acquisition they were stored at refrigeration temperature. These samples were then collected in 15 mL Falcon tubes and kept at −20 °C until analysis.

To achieve a good compromise between simplicity of extraction and sample clean-up, the procedure proposed by Rodríguez-Carrasco et al. (2016) to extract emerging *Fusarium* toxins in mice was adopted as a starting point with slight modifications.

In this work, 0.5 ± 0.05 g of chicken liver sample was used to reach appropriate levels of mycotoxin in final extract. The sample was weighed into 2 mL Eppendorf Safe-Lock Microcentrifuge Tube and spiked with 10 μL of ^{13}C-labeled deoxynivalenol at 2 μg/mL. Later, 1.5 mL of AcN was added and. the mixture was completely grounded using an Ultra-Turrax T8 (IKA Labortechnik, Staufen, Germany) for 1 min. Then, the sample was centrifuged at 4000 rpm for 3 min at 4 °C (Sigma Centrifuge 3K15; Sigma, Germany), and the supernatant was collected into new centrifuge tubes. The supernatant was subjected to a dispersive solid-phase extraction (d-SPE) with a mixture of 150 mg $MgSO_4$ and 50 mg C18, vortexed for 30 s and centrifuged again at

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