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Mitigation of enniatins in edible fish tissues by thermal processes and identification of degradation products

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ABSTRACT

Emerging mycotoxins, such as enniatins and beauvericin, are common contaminants in vegetal matrices, but recently, the occurrence of mycotoxins in foodstuffs from animal origin has been also reported as they can be present in edible tissues of animals fed with contaminated feedstuffs. Sea bass, sea bream, Atlantic salmon and rainbow trout from aquaculture analyzed in the present survey showed contamination by emerging *Fusarium* mycotoxins enniatins (ENs). ENs were extracted from raw and cooked fish with acetonitrile and analyzed by Liquid Chromatography coupled to Mass Spectrometry. In this study, the stability of ENs was evaluated during food processing by the application of different cooking methods (broiling, boiling, microwaving and baking treatments). All treated samples showed a reduction in mycotoxin levels with different percentages depending on the type of EN and the fish species. Thus, the reduction obtained ranged from 30 to 100%. The thermal treatments have shown to be a good strategy to mitigate ENs content in edible fish tissues. On the other hand, some ENs degradation products originated during the application of thermal treatments were identified.

1. Introduction

Emerging mycotoxins, such as enniatins (ENs) and beauvericin (BEA), are depsipeptide molecules produced by the secondary metabolism of fungi from *Fusarium* genera. These mycotoxins occur as contaminants mainly in cereals, such as wheat, barley and maize, but also in oats, rye and rice. However, their presence has been also reported in other matrices from both vegetal and animal origin (Tolosa et al., 2013). The most prevalent ENs in food are ENA, ENA_1, ENB and ENB_1 (Serrano et al., 2012). The presence of these contaminants and their metabolites in products from animal origin, such as meat, milk, eggs and cheese could be consequence of a carry-over of these compounds into animal tissues after feeding of contaminated feed (Zhao et al., 2015).

In the last years, several studies have been focused in the development of strategies to reduce mycotoxin levels during food and feed production. As reported by other authors, food processing of cereals has effects on mycotoxins, leading to less-contaminated food compared to the raw materials (Hu et al., 2014a,b). Different industrial processes have shown to be effective practices to reduce mycotoxin contents. In this sense, the content of some mycotoxins can be reduced due to thermal food processing applied, such as cooking, boiling, baking, frying, roasting and pasteurization (Kabak, 2009).

The content reduction achieved depends on several parameters. Some parameters are related with the nature and chemical structure of the mycotoxins and with the initial level of contamination in food. Other parameters are related with the treatment applied, such as temperature, time, pH, moisture content, etc (Bretz et al., 2006; Ryu et al., 2008; Serrano et al., 2013).

Regarding reduction achieved on other "traditional" *Fusarium* mycotoxins, Bretz et al. (2005) reported nivalenol (NIV) degradation at high temperatures and prolonged heating time. Beyer et al. (2009) have reported mycotoxin reduction depending on the interaction with other components such as sugar, starch and protein model. In the study conducted by these authors, the fate of T-2 toxin under typical food-processing conditions was assessed by applying different heating experiments. T-2 degradation was observed under all conditions, accelerating with rising temperatures, but the strongest degradation was observed in the protein model. However, studies performed with fumonisins (FMs) showed higher reduction percentages in sugar models (Bullerman and Bianchini, 2007). Most of the data indicate that FMs levels are decreased during heating, baking, frying, roasting, nixtamalizing

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and extrusion cooking of foods, concluding that reduction is directly related to time and temperature applied, as foods reaching temperatures greater than 150 °C during processing may have lower FMs levels (De Girolamo et al., 2016).

Limited data is available on the effects of food processing on ENs and BEA contents. As reported in the EFSA (European Food Safety Authority) Scientific Opinion on the risks to human and animal health related to the presence of BEA and ENs in food and feed, more data is necessary in order to assess the role of temperature applied in the mycotoxin content reduction and other factors related present in different foodstuffs (SCF, 2014).

Thus, the content reduction of *Fusarium* mycotoxins ENs and beauvericin (BEA) by thermal treatments have been reported by some authors (Meca et al., 2012; Serrano et al., 2013; García-Moraleja et al., 2015). Furthermore, recent studies have evidenced that ENs contents are reduced through common industrial processes, such as bread-making (Vaclavikova et al., 2013; Hu et al., 2014a,b), beer-making, brewing or malting processes (Meca et al., 2013; Hu et al., 2014a,b) and pasta production (Tittlemier et al., 2014; García-Moraleja et al., 2015; Serrano et al., 2016). Concerning fish, thermal treatments are applied to cook food; however, no data is available related with the employment of thermal processes to reduce mycotoxin contents in fish fillets. Furthermore, literature about natural occurrence of mycotoxins in edible fish fillets is still scarce (Tolosa et al., 2014).

Nevertheless, other studies have focused on the isolation and characterization of new mycotoxin products generated after thermal treatment application to food, as well as in the evaluation of the toxicity of these compounds. In most cases, degradation products are less toxic than their original molecules (Bretz et al., 2005; Beyer et al., 2009). In this sense, Shams et al. (2011) reported a new less-toxic derivative of diacetoxyscirpenol after thermal treatment applied to potatoes.

Considering the lack of data related to ENs and BEA degradation during food processing, the purpose of this study is to provide information on the fate of ENs and BEA during the fish cooking process and to determine the content reduction of emerging *Fusarium* mycotoxins in fish tissues achieved by four different thermal processes commonly used to cook fish (conventional oven, microwave, broiled and boiled treatments). Analyses were carried out by liquid chromatography coupled to tandem mass spectrometry with linear ion trap (LC-MS/MS-LIT).

2. Materials and methods

2.1. Materials

All solvents (acetonitrile (MeCN) and methanol (MeOH)) were purchased from Merck (Darmstadt, Germany). Deionized water (<18 MΩ/cm resistivity) was obtained from a Milli-Q water purification system (Millipore Corporation, Bedford, MA, USA). Ammonium formate (HCO_2NH_4, 97%) was supplied by Sigma-Aldrich (St. Louis, USA). All solvents were passed through a 0.22 μm cellulose filter from Membrane Solutions (Texas, USA).

ENs and BEA toxin solutions were provided by Biopure (Tulln, Austria). Individual stock solutions (1 mg) were diluted in 1 mL of MeCN (1000 mg/L). Then, intermediate solutions with decreasing concentration were prepared by dilution from the stock (from 1000 mg/L to 100 mg/L, from 100 mg/L to 10 mg/L, and from 10 mg/L to 1 mg/L). They were stored in glass-stoppered bottles and in darkness conditions at −20 °C. Working standard solutions (ranged from 0.01 to 100 μg/L) were prepared by the suitable dilution from 1 mg/L solution and were kept in darkness at 4 °C.

2.2. Sampling

The species included in the survey were selected due to their important production in aquaculture. Atlantic salmon is economically the most important farmed fish in Europe, although other commercially reared species include rainbow trout, sea bass, sea bream, cod, halibut, tuna, eel and turbot. In this sense, fourty fish samples of sea bass, sea bream, Atlantic salmon and rainbow trout (*Dicentrarchus labrax* (n = 10), *Sparus aurata* (n = 10), *Salmo salar* (n = 10) and *Oncorhynchus mykiss* (n = 10), respectively) were purchased from different supermarkets located in Valencia (Spain), all of them from aquaculture farming. The origin of the sea bass and sea bream samples were Spain and Greece, respectively, which is the main producer of sea bream in European aquaculture, while Atlantic salmon was from Norway and rainbow trout belonged from Spain. Samples were previously deboned and beheaded. All samples were stored in a dark and dry place at −20 °C until analysis. After their packages had been opened they were analyzed within the same day.

2.3. Thermal treatments (cooking techniques)

Fish fillets were divided into 5 different parts, one of them was analyzed raw. Results for ENs contents in raw samples of sea bass and sea bream were previously reported by Tolosa et al., 2014. The other four parts were reserved for further processing. Four different thermal treatments were applied to those positive raw samples in all the species analyzed in the survey. The treatments applied were those most common employed to cook fish: boiled (BO), broiled (BR), baking in a conventional oven (CO) and baking in a microwave oven (MO). Parameters and thermal procedures were set based in literature reviewed about the effect of thermal treatments on nutritional parameters in different fish species (Nurhan, 2007; Ersoy & Özeren, 2009; Hosseini et al., 2014). No salt, oil or other ingredients were added. Each fillet was cooked separately and raw fillets were used as the reference for calculating the percentage reduction achieved by the different thermal treatments applied. Thus, levels in raw fillets were considered to be the initial concentration.

Broiling (BR): Fish fillets were cooked in a pan for a total of 10 min (5 min each side) in a preheated pan. The temperature reached in the center of the fillet ranged between 64 and 70 °C.

Microwave oven-cooked (MO): The fish fillet was placed in the microwave and cooked for 3 min at regular power (650 W). The temperature reached in the center of the fillet ranged between 69 and 74 °C.

Boiling (BO): Fish fillets were boiled in 500 mL of tap water for 5 min. The temperature reached in the center of the fillet ranged between 63 and 68 °C.

Baking in a conventional oven (CO): Fillets were cooked in a conventional oven at 180 °C for 30 min. The temperature reached in the center of the fillet ranged between 64 and 69 °C.

2.4. Mycotoxin extraction procedure

Sample preparation was performed according to a previous study (Tolosa et al., 2014). In brief, 10 g of homogenized fish sample was mixed with 50 mL of MeCN for 30 min and 30 °C using a Branson 5200 ultrasonic bath (Branson Ultrasonic Corp., CT, USA). The extract was evaporated to dryness at 30 °C using a Büchi Rotavapor R-200 (Flawil, Switzerland). The solution is reconstituted in 10 mL of MeCN-MeOH 50:50 v/v (MeOH with 20 mM of ammonium formate) and centrifuged at 3500 g for 15 min and 5 °C. The supernatant was purified using C_{18} cartridges (Waters, Milford, Massachusetts) by applying a slight vacuum. The extract was