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Stability of nitrofuran residues during honey processing and nitrofuran removal by macroporous adsorption resins

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ABSTRACT

There is increasing concern that the presence of antibiotics such as nitrofurans in animal-derived food products is harmful to human. This study originally assessed the effects of different honey processing steps on the stabilities of four nitrofuran metabolites (3-amino-2-oxazolidone, 1-aminohydantonin, semicarbazide and 3-amino-5-morpholinomethyl-2-oxazolidone). Macroporous adsorption resins (MARs) were evaluated for the removal of these residues. Nitrofuran metabolites were analysed by LC–MS/MS after each processing step. The results revealed that honey processing reduced nitrofuran metabolites in honey and the total loss was from 56.6% to 90.4%. Furthermore, LS-901 was the optimum MAR with adsorption rates of 69.9–91.8% for four metabolites. After removing nitrofuran metabolites, the honey could be safely used as winter feed for honeybees.

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1. Introduction

Nitrofurans belong to a class of broad spectrum antibiotics that contain 5-nitrofuran rings. They are used as feed additives to promote growth and reduce the incidence of gastrointestinal and dermatological infections in cattle, swine, poultry, fish, shrimp, and bees (Cheng et al., 2009). The concentration of nitrofurans administered to animals is 8–400 mg/kg body weight (Thongsrisomboon, Liawruangrath, Liawruangrath, & Satienperakul, 2010). Common nitrofurans include furazolidone (FZD), nitrofurantoin (NFT), nitrofurazone (NFZ) and furaltadone (FTD). Following administration, FZD, NFT, NFZ and FTD are rapidly metabolised to 3-amino-2-oxazolidone (AOZ), 1-aminohydantonin (AHD), semicarbazide (SEM) and 3-amino-5-morpholinomethyl-2-oxazolidone (AMOZ), respectively (Fig. 1). These protein-bound metabolites, which are stable in the body, can be detected even several weeks post-administration. Due to potential carcinogenicity and mutagenicity, nitrofurans and their metabolites have been banned from use in food producing animals (Annex IV of the Council Regulation 2377/90; Leitner, Zöllner, & Lindner, 2001; O'Keeffe et al., 2004). However, in several developing countries, nitrofurans are still administered to food producing animals because they are inexpensive and effective. Therefore, a minimum required performance limit (MRPL) of 1 μg/kg has been established for all protein-bound nitrofuran metabolites in edible animal tissues (Commission Regulation (EC), 1995).

Food products from animal origin (e.g., milk, eggs and honey) are prone to contain nitrofurans. Several studies have reported that nitrofurans could be present in milk and eggs obtained from domestic and farm animals fed nitrofurans-containing feed or water (Barbosa, Freitas, Mourão, Silveira, & Ramos, 2012; Díaz, Cabanillas, Valenzuela, Correa, & Salinas, 1997; Li, Liu, & Wang, 2009; Rodziewicz, 2008). Honey is a natural, nutritious, and healthy food produced by honeybees from nectar of plants or honeydew. It can easily be polluted from the antibiotics used to protect and cure the usual diseases in beekeeping. Toxic nitrofurans have already been detected in honey and pollen products (Bargańska, Ślebioda, & Namieśnik, 2011; Cooper & Kennedy, 2005; Moreno-Bondi, Marazuela, Herranz, & Rodriguez, 2009).

However, commercial honey can be achieved by several stages such as melting, filtration, concentration and pasteurisation. These steps can potentially reduce nitrofuran residues. Even though the effects of food processing on undesirable compounds residues have been reported (Xu et al., 2012; Zhu et al., 2010), there is little information available on the reduction of nitrofuran residues in honey. Therefore, it is important to evaluate the effects of honey processing on nitrofuran metabolites to determine whether the residue levels can be reduced. The information obtained from this study

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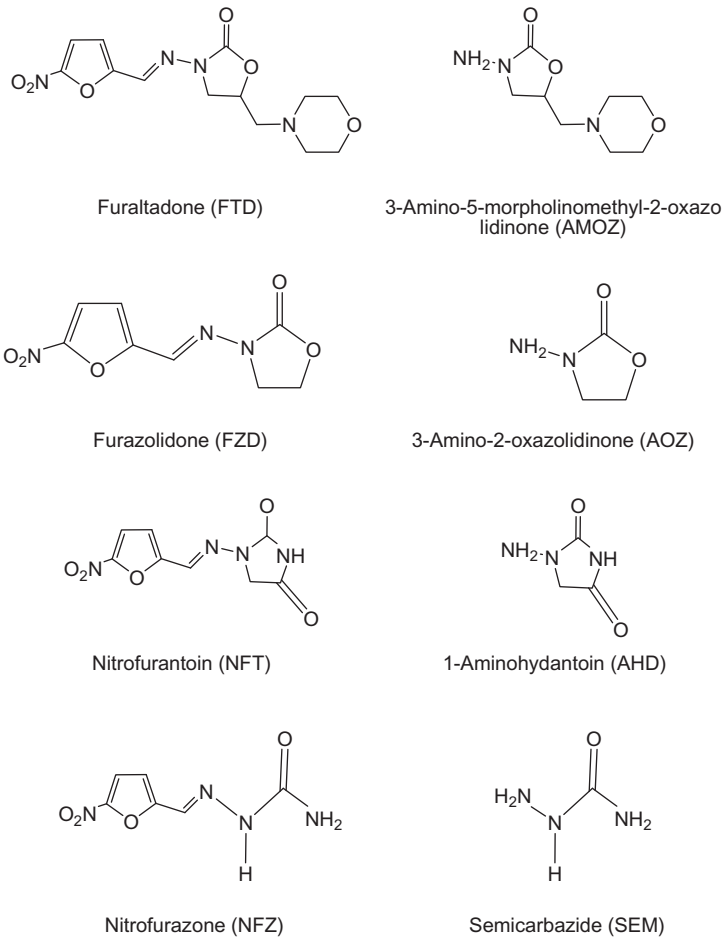

Fig. 1. Chemical structures of four nitrofurans and their side-chain metabolites.

will be useful for the development of an effective food safety program for honey.

Generally, honey is discarded when the concentration of nitrofuran metabolites is >MRPLs. Yet this way may cause a lot of wastes retaining beneficial substances underutilised. If processing conditions do not reduce nitrofurans levels, additional treatments need to be performed. Studies have reported that several methods including Fenton's advanced oxidation, electro-oxidation, electron beam irradiation and photodegradation can remove antibiotics from water and soil (Elmolla & Chaudhuri, 2010; Haidar, Dirany, Sirés, Oturan, & Oturan, 2013; Liu, Song, Yang, & Zhang, 2006; Péraz-Moya et al., 2010; Trovó, Nogueira, Agüera, Fernandez-Alba, & Malato, 2011). However, these methods have disadvantages such as high production costs and formation of toxic compounds. To reduce nitrofurans in honey, it is necessary to establish reliable, efficient and economical methods. Recently, macroporous adsorption resins (MARs) have been used as adsorbents due to their high efficiency, long life, environmental friendliness, high adsorption capacity and selectivity, favourable physicochemical stability and convenient regeneration treatments (Liu et al., 2010). Previous studies have shown that MARs can purify rutin, quercetin and lycopene; decolor levan; and remove chloramphenicol and parathion from honey (Cheng et al., 2012; Liu et al., 2010; Xu et al., 2012; Zhao, Dong, Wu, & Lin, 2011; Liu et al., 2010).

Accurate analytical methods for the determination of nitrofuran residues are very important. Few methods have been reported for the determination of nitrofuran residues in honey, such as HPLC-FLD (Du et al., 2014), UHPLC–MS/MS (Radovnikovic, Moloney, Byrne, & Danaher, 2011; Valera-Tarifa, Plaza-Bolaños, Romero-González, Martínez-Vidal, & Garrido-Frenich, 2013), and LC–MS/MS (Bock, Gowik, & Stachel, 2007; Leston et al., 2011; O'Mahony et al., 2011; Palaniyappan et al., 2013; Tribalat, Paisse, Dessalces, & Grenier-Loustalot, 2006; Wood et al., 2005). Among these methods, LC–MS/MS provides precise results in accordance with EU requirements (2002/657/EC, 2002).

The objectives of this study were to investigate the effects of different processing steps like preheating, filtration, vacuum concentration and pasteurisation on AOZ, SEM, AHD and AMOZ levels and to develop a novel method based on MARs for removing these four metabolites from honey.

2. Experimental

2.1. Chemicals

In this study, AOZ, AHD, SEM, AMOZ and 2-nitrobenzaldehyde (2-NBA) standards (>99.5% pure) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Analytical grade acetonitrile, ethyl acetate, formic acid and *n*-hexane were purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA).

2.2. Standard solutions

AHD, AMOZ, AOZ and SEM stock solutions in acetonitrile (50 mg/L) were stored at −18 °C (these stock solutions were stable for 3 months). Intermediate solutions containing all four analytes

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