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The fate of spirotetramat and its metabolite spirotetramat-enol in apple samples during apple cider processing

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ABSTRACT

The fate of spirotetramat and its metabolite spirotetramat-enol in apple samples during apple cider processing was assessed. The residues were determined by ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) after each step including washing, peeling & coring, juicing, primary filter, enzymolysis, secondary filter, sterilization, and fermentation. Results showed that the concentration of spirotetramat and spirotetramat-enol residues significantly decreased in apple cider after processing. The processing factors (PFs) of apple samples after each step were generally less than 1 except that the PF of spirotetramat-enol after sterilization was larger than 1. Spirotetramat and spirotetramat-enol were proved to be mostly retained in apple peels. The peeling & coring process caused the loss of 76.4% of spirotetramat and 62.9% of spirotetramat-enol from apples, with the processing factor of peeling & coring at 0.14 and 0.22 respectively.

1. Introduction

Apple is one of the major food crops grown in the world (Pennell, 2006). And China is the largest producer of apples which are not only eaten for fresh fruit, but also processed into different products such as apple sauce, juice, cider, brandy, and distilled spirits (Brown, 2012). The apple peels have been found to have a potent antioxidant activity which can greatly inhibit the growth of liver cancer and colon cancer cells (Eberhardt, Lee, & Liu, 2000; Wolfe, Wu, & Liu, 2003). Moreover, several lines of evidences suggest that apple products possess a wide range of biological activities which may contribute to health beneficial effects against cardiovascular disease, asthma and pulmonary dysfunction, diabetes, obesity and cancer (Boyer & Liu, 2004; Gerhauser, 2008). However, pests are still the greatest impact on apple production. Therefore, a large number of pesticides are applied to control these pests, which increases the potential risk for human exposure (Kong, Shan, et al., 2012). As a new two-way systemic insecticide, spirotetramat is a popular product widely used to control various sucking pests on apple (Nauen, Reckmann, Thomzik, & Thielert, 2008). This compound is mobile within the phloem of the plants and can control hidden pests (Mohapatra, Deepa, & Jagadish, 2012). However, the US Environmental Protection Agency (EPA) reported that spirotetramat is toxic to bees and aquatic invertebrates and oysters (EPA, 2008). Moreover, spirotetramat is an irritant to the eyes and exhibits a skin-sensitization potential in animals and humans (EPA, 2008). The primary metabolite of spirotetramat in plants produced by hydrolysis is spirotetramat-enol (Mohapatra, Deepa, Lekha, et al., 2012), which is equivalent to its parent compound in toxicity. In addition, repeated dosing with spirotetramat and spirotetramat-enol can produce male reproductive toxicity in rats (EPA, 2008). So the large number of spirotetramat applications associated with apple production may increase the potential risk for human exposure and harmful impact to the environment. In addition, there are few methods described in literature to analyze spirotetramat and its metabolite residues in fruits or fruit juice. Mohapatra, Deepa, and Jagadish (2012) conducted the analysis of spirotetramat and its metabolite spirotetramat-enol in mango and cabbage by HPLC. Tran et al. (2012) found spirotetramat in fashionable fruit juices by LC–MS/MS. Mohapatra, Deepa, Lekha, et al. (2012) studied the dynamics of spirotetramat and imidacloprid in mango and soil. However, the residue analytical method for

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spirotetramat and its metabolite spirotetramat-enol in apples has not been reported to date. To ensure food safety for consumers, monitoring the fate of spirotetramat and spirotetramat-enol residue in apples is still indispensable.

The food security issue has received global attention (Barrett, 2010; Godfray et al., 2010). And the issue induced by food contamination concerning pesticide residues is becoming more and more important (Ling et al., 2011). Most agricultural products are not only consumed fresh, but also consumed after commercial processing (Keikotlhaile, Spanoghe, & Steurbaut, 2010). The processing techniques (e.g. washing, peeling, cooking) usually result in some decrease of pesticide residues in foods, which may reduce impact on human health (Abou-Arab, 1999; Chavarri, Herrera, & Arino, 2005; Kaushik, Satya, & Naik, 2009; Kong, Dong, et al., 2012; Zhang, Liu, & Hong, 2007). However, in some cases (e.g. fruit drying or production of unrefined vegetable oil) increase of residues in product may also occur (Amvrazi & Albanis, 2008). Many studies have been carried out on the removal of pesticide residues in apples during home preparation and commercial processing (Hercegova, Domotorova, Hrouzkova, & Matisova, 2007; Hwang, Cash, & Zabik, 2002; Rasmusssen, Poulsen, & Hansen, 2003; Sanz-Asensio, Martinez-Prado, Plaza-Medina, Martinez-Soria, & Perez-Clavijo, 1999; Zabik, El-Hadidi, Cash, Zabik, & Jones, 2000). However, no paper has been reported on the fate of spirotetramat and spirotetramat-enol residues during apple cider making. Therefore, to obtain a good knowledge of the process effect, processing experiments employing apples with field incurred residue of spirotetramat were conducted.

The ratio of residue levels in processed products and their respective raw products is called the processing factor. The PFs assist in the dietary intake assessment of related pesticides in processed commodities (Amvrazi & Albanis, 2008; Christensen, Granby, & Rabolle, 2003). They are also used in recommending MRLs for processed products with an existing Codex commodity code, but only if the processing leads to an increase of the residue level (Gonzalez-Rodriguez, Rial-Otero, Cancho-Grande, Gonzalez-Barreiro, & Simal-Gandara, 2011). To our knowledge, no study has been conducted to evaluate the PFs of spirotetramat and spirotetramat-enol in apples during apple cider processing. Therefore, it is very significant to clarify the PFs on spirotetramat and spirotetramat-enol in apples during apple cider processing.

The present work was designed to investigate the fate of spirotetramat and spirotetramat-enol residues in field-treated apples after systematic processing, and throw light on the PFs in apple and its products during apple cider processing.

2. Materials and methods

2.1. Materials

The analytical standard spirotetramat (99.2% purity) and its metabolite spirotetramat-enol (99.1% purity) were obtained from the Bayer Crop Science AG (Frankfurt, Germany). Commercial 240 g/L spirotetramat suspension concentrate (SC) was obtained from Bayer Crop Science (Beijing, China). Acetonitrile, anhydrous magnesium sulfate and sodium chloride for pesticide residue analysis were of analytical grade and purchased from Beijing Chemical and Reagent (Beijing, China). Acetonitrile (Chromatography grade) were obtained from Honeywell International Inc. (New Jersey, USA). Octadecylsilane (C_{18}, 40 μm) was purchased from Agela Technologies (Tianjin, China). Ultra-pure water was obtained from a Milli-Q system (Bedford, MA, USA).

Standard stock solutions of spirotetramat (100 μg/L) and spirotetramat-enol (100 μg/L) were prepared in acetonitrile. The standard solutions required for construction of a calibration graph (0.01, 0.05, 0.1, 0.5, and 1 μg/L) were prepared from stock solutions by serial dilution with acetonitrile. Correspondingly, matrix-matched standard solutions were obtained at 0.01, 0.05, 0.1, 0.5, and 1 μg/L concentrations by adding blank apple, apple juice, apple cider sample extracts to each serially diluted standard solution, respectively. All solutions were stored in a refrigerator in the dark at 4 °C, and the working standard solutions underwent no degradation for 3 months.

2.2. Field trials

The field trials were conducted in a commercial orchard located in Beijing, China, which was investigated and determined free of spirotetramat and spirotetramat-enol prior to the experiment. Trees were planted 5 years before the experiment with 4.0 m spacing between rows and 1.5 m between individual trees in the rows. Three replications were taken for the treatment and each trial plot contained three trees. All four sides of the plots were protected by blank trees. According to the OECD guideline for the pesticide residues in processed commodities, spirotetramat 240 g/L SC was applied on apple trees at triple higher dosage of commercial recommendation of 360 g active ingredient hectare^{-1} (a.i./ha) (OECD, 2008). The recommendation dosage was 120 g a.i./ha. The pesticide was sprayed three times with an LP-605 (Agrolex, Singapore) manual sprayer on August 26, September 2 and 9, 2012. Twenty five kilograms of apple samples were sampled at 3 days after the last treatment. All samples were placed in polyethylene bags and transported to the laboratory in the same day. Following, the apple samples were processed immediately and all the subsamples were kept deep-frozen (−20 °C) until analysis.

2.3. Sample preparations

In general, the production procedures of apple cider include eight steps: washing, peeling and coring, juicing, primary filter, enzymolysis, secondary filter, sterilization, and fermentation, as shown in Fig. 1. In current study, the samples in different processing steps were collected to determine the variation of pesticide residue during the processing procedure. The detailed processing procedure is as follows:

Washing: the raw apples were washed with tap water for 10 min.

Peeling and coring: apples were peeled by hand with a peeler and 2 mm apple peel was removed. Meanwhile, the core and stalk of the apples were removed by knife.

Juicing: a JE2233 automatic juice extractor (Beijing Petrus Electrical Co., Ltd, China) was fed with processed apples (which were cored, peeled and cut into quarters) to produce juice and pomace.

Primary filter: the juice was primary filtered with gauze.

Enzymolysis: the pectinase (Jiangsu RuiYang BioTech Co., Ltd, China) was used to process the primary filtered juice for 30 min.

Secondary filter: the enzymolysis processed juice was secondary filtered with FT15 Disc Bowl Centrifuge (Armfield Ltd, England).

Sterilization: the juice was subjected to sterilization at 140 °C for about 10 s through Ultra Heat Treated (UHT) (Shanghai Triowin Automation Machinery Co., Ltd, China).

Fermentation: the apple juice was fermented at 25 °C for about 7 days in a closed fermentation tank to produce apple cider.

2.4. Instrumentation and LC–MS/MS analytical conditions

Chromatographic separation was carried out on a Waters Acquity UPLC binary solvent manager equipped with a BEH-C_{18} column (2.1 mm × 50 mm, 1.7 μm particle size) (Milford, MA, USA). This