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9,10-Anthraquinone deposit in tea plantation might be one of the reasons for contamination in tea

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ABSTRACT

9,10-Anthraquinone (AQ) was a new contaminant, with unknown sources, occurred globally in tea. European Union (EU) fixed the maximum residue limit (MRL) of 0.02 mg/kg. The pollution source of AQ in tea was traced from the view of AQ deposit on tea crop by simulation. The possible contamination pathway and main factors to decrease AQ were explored in tea cultivation- tea manufacture- tea infusion, on the basis of AQ analytical methods by using solvent extraction and gas chromatography-tandem mass spectrometry (GC–MS/MS) quantification. 58.8–84.6% of AQ degraded in tea processing, and drying played a key role to reduce the AQ contamination. Certain concentration of AQ deposited on tea shoots could resulted in AQ beyond the MRL of 0.02 mg/kg in tea. AQ leaching into tea brew (about 10%) could lead to the possible health risk. AQ deposit on tea crop during the tea cultivation might cause the AQ contamination in tea.

1. Introduction

Tea comes from the leaves of the plant *Camellia sinensis* (L.) Kuntze (family Theaceae). As one of the most popular beverages, the world tea production reached 5.13 million tonnes in 2014. The world tea consumption increased to 4.95 million tonnes and the international exports was 1.73 million tonnes at the same year. In order to safeguard consumer's health and ensure the quality of tea products, it is of prime important to control and minimize the level of contaminants in tea.

The primary task is to ascertain where they come from. Pesticide residues in tea usually come from the chemical application, not only from directly spray in tea garden, but also from the air drift caused by operation nearby agricultural fields (Chen, Ruan, Cai, & Zhang, 2007). The heavy metal in tea is mainly resulted in element concentration by tea plant from soil (Ruan & Wong, 2001; Karak et al., 2015), fertilizer (Owuor, Gone, Onchiri, & Jumba, 1990) and atmosphere (Jin et al., 2005). It is difficult and complicated to trace some specific contaminants, accidentally emerged in tea. Investigations are needed to conduct in the whole tea chain, because they may occur in the tea plantation, tea processing, tea package, tea storage or other procedures. Tang (Tang, Chen, Liu, Luo, & Lou, 2007) identified that high level of octachlorodipropyl ether in Chinese tea came from the absorption from the smoke of the mosquito coils in the workshop of tea processing. The ppm level of Dichlorodiphenyltrichloroethane (DDT) residues in tea (Chen & Yue, 1981) in the 1990's was demonstrated from the DDT impurity in dicofol formulation, after DDT had been banned in the 1970's in China, which was evidenced by the following studies (Gurusubramanian, Rahman, Sarmah, Ray, & Bora, 2008; Qiu, Zhu, Yao, Hu, & Hu, 2005; Turgut, Gokbulut, & Cutright, 2009).

As a new contaminant, AQ (CAS 84–65-1) has been detected in tea from the main tea-producing countries recently, especially from China, India and Sri Lanka. Since the first notification of AQ in tea was issued by EFSA in 2012 (RASFF), the AQ residues in tea are of increasing global concerns: 3 notifications in 2012, 15 notifications in 2014, and there were already 7 notifications until August in 2016. The AQ level were in the range of 0.021–0.19 mg/kg in defective tea reported by EFSA, which inhibited international tea trade seriously. The most important thing is that it is not clear where AQ in tea comes from. AQ is used as a raw chemical in paper, pulp and dye-industry. It indicated that AQ contributed to the carcinogenicity potentially (Wei et al., 2010), and the probability of cancer were correlated with the concentration of AQ. Although there is no information on registration and application of AQ in crops in China, United States of America (Werner et al., 2015), EU (Commission Directive 2007/565/EC) and other countries, the insecticidal activity of anthraquinones extracted from natural substances and bacteria have been increasing (Georges,

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Jayaprakasam, Dalavoy, & Nair, 2008; Werner et al., 2015) in recent years, AQ may be as a promising ingredient in chemical formulation applied in agricultural production in the future. AQ is ubiquitous in the environment, and has been detected in the ambient air at the concentration ranging from 0.0009 ng/m^3 to 52 μg/m^3 in France, Canada, Spain, Germany, Belgium, Japan, USA, etc. (Albinet, Leoz-Garziandia, & Budzinski, 2008; Oda, Maeda, & Mori, 1998; Galceran & Moyano, 1993; María del Rosario Sienra, 2006; Sklorz, Briedé, & Schnelle-Kreis, 2007; Ligocki & Pankow, 1989). The AQ in ambient air can be removed by wet or dry deposition, and then precipitated on the interface on the plant's leaf and ground (Lunde, 1976; Ligocki, Leuenberger, & Pankow, 1985; Pankow, Isabelle, & Asher, 1984).

Given the researches on deposition of AQ from air and the possible future use of AQ in agriculture, a simulated field experiment was carried out to estimate the possible source of AQ on tea, quantitative analysis the transfer of AQ and find out the key factors to decrease the AQ level on tea, which is helpful to improve the tea quality and safety.

2. Experimental

2.1. Reagents, chemicals and materials

AQ (99.0%) was provided by Dr. Ehrenstorfer GmbH Company (Augsburg, Germany). 9,10-anthracenedione-d8 (d8-AQ, 98.6%) as the internal standard was provided by C/D/N Isotopes (Quebec, Canada). Sodium sulfate anhydrous (Na_2SO_4) and magnesium sulfate ($MgSO_4$) were baked for 12 h at 120 °C to remove residual water. Florisil (60–100 mesh) was purchased from Wenzhou Organic Chemicals Company (Wenzhou, China). Standard stock solution of AQ and d8-AQ of 200 mg/L were prepared by accurately weighing and dissolving 10 mg of the chemicals in 50 mL acetone, respectively. Working standard solutions were obtained via further dilution with acetonitrile. Matrix-matched calibration solutions of desired concentration were prepared freshly using extracts from blank samples for each experiment.

2.2. Field trial

For simulating the deposit of AQ on tea crop (Longjing 43, a variety of tea grew widely in China) and obtaining a series of tea shoots with different level of AQ, the field experiment was carried out in Hangzhou (120.2°E, 30.3°N), China. AQ was sprayed at the dosage of 2.1 g a.i. ha^{-1}. About 1 kg tea shoots (one bud and two leaves) were plucked randomly at 0 (2 h), 1, 3, 6, 10, 14 and 21 days after AQ was sprayed.

2.3. Sample preparations

Tea shoots were processed according to the following steps: withering (28 °C, 2–3 h), rolling (28 °C, 30 min), fermentation (30 °C, 4–5 h) and drying (120 °C, 30 min) for black tea, while de-enzyme (220 °C), rolling (28 °C, 30 min), and drying (120 °C, 30 min) for green tea. The heat was supplied with electricity. All the samples collected in each step were stored at −20 °C until further analysis. 3 g of tea were infused in 150 mL water for 5 min, then the water extract was filtered, and cooled for AQ detection.

2.4. Sample extraction and clean-up

Tea samples (tea shoots, withered leaves, rolled leaves, fermented leaves, de-enzymed leaves, black tea and green tea) were shattered homogenously in a blender. 1.5 g of tea samples, except the dry samples, were extracted by ultrasonication for 10 min in 15 mL 20% acetone in *n*-hexane. The dry tea was firstly soaked in 1.5 mL deionized water for 30 min. 1 g $MgSO_4$ was added into tea samples and vortexed for 30 s. After centrifugation for 5 min at 6000 rpm, 10 mL upper organic phase was evaporated to nearly dryness at 35 °C under vacuum. The extract was re-dissolved in 2.5% acetone in *n*-hexane for the clean-up. As for tea brew, AQ in 25 mL tea infusion was partitioned into 25 mL acetonitrile with the help of 6 g NaCl. The supernatant organic liquid from the liquid–liquid extraction was concentrated, and re-dissolved in 2.5% acetone in *n*-hexane.

Florisil was conditioned by heating at 650 °C for 4 h and deactivated by adding 7% water (w/w). A glass column, 10 cm × 0.8 cm I.D., was filled from the bottom with glass wool, 2 g florisil between the Na_2SO_4 layers. The column was prewashed with 10 mL 2.5% acetone in *n*-hexane. After the re-dissolved solutions were loaded, AQ was eluted with 20 mL 2.5% acetone in *n*-hexane. The dry residue was finally reconstituted in 1 mL 2.5% acetone in *n*-hexane including 0.04 mg/L d8-AQ and filtered through a 0.22 μm pore membrane filter for GC–MS/MS analysis.

2.5. GC–MS/MS analysis

AQ analysis was performed on a Varian 450 gas chromatograph equipped with Varian 300 tandem mass detector (Varian, Walnut Creek, CA, USA). The system was operated by MS WorkStation version 6.9.3 software. Chromatographic separation was conducted with a Varian Factor Four capillary column VF-5ms (30 m × 0.25 mm × 0.25 μm). The oven temperature program was: 80 °C, held 1 min; ramped 20 °C/min to 260 °C as final temperature and held for 5 min. Helium was used as carrier gas at a constant flow rate of 1.0 mL/min. Argon was used as collision gas. The ion source, manifold and transfer line temperatures were set at 210 °C, 40 °C and 280 °C, respectively. The electron energy was 70 eV. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode for target analysis. The injection volume was set at 1.0 μL.

2.6. Statistical analysis

The dissipation of AQ in tea samples was evaluated using nonlinear regression with Microsoft Excel software. The degradation dynamic followed the first-order equation $C_t = C_0 e^{-kt}$, where C_t is the concentration of AQ residue (mg/kg) at time (t), C_0 is the initial concentration of AQ (mg/kg), and k is the rate constant (day^{-1}). Half-life ($t_{1/2}$) is calculated from the Hoskins formula: $t_{1/2} = ln2/k$, which expresses the time after 50% of the deposited AQ has degraded.

Processing factor (*PF*) was applied to estimate the change of AQ during tea processing. $PF = R_l/R_f$, where R_f is the concentration of AQ residue before the imposed procedure, R_l is the concentration of AQ residue after the imposed procedure. The PF indicates reduction ($PF < 1$) or elevation ($PF > 1$) of the AQ residue in the processing.

Infusion factor (IF) indicates the residue transfer from dry tea to tea brew. $IF = R_i/R_d$, where R_i is the concentration of AQ residue in tea brew, R_d is the level of AQ residue in dry tea.

3. Results and discussion

3.1. MS/MS mechanism of AQ

From the total ion chromatogram (TIC), $[M]+\cdot$ at m/z 208 with high abundance was chosen as the precursor ion, then the precursor ion was fragmented by adding collision energy to obtain the product ion. Fig. 1 shows the product ion mass spectra of AQ when the collision energy was set at 5V. In electron ionization (EI) source, the loss of 28 mass unit was observed, corresponding to elimination of [CO] from m/z 208, and attribution to form the fragments of $[M\text{-}CO]+\cdot$m/z 180 and $[M\text{-}2CO]+\cdot$m/z 152. The fragmentation mechanism of AQ was similar to 1,2-naphthaquinones (Oliver & Rashman, 1968), and the decomposition processes of consecutive loss of [CO] were conclusive. For quantification of AQ, the effect of collision energy (CE, 5–40 V) on intensity of ion