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Processing factors of triadimefon and triadimenol in barley brewing based on response surface methodology

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ABSTRACT

Numerous studies have focused on the effects of food-processing techniques on pesticide residues. However, it remains a challenge to ensure high-quality processing and effective pesticide removal from foods. Triadimefon (TF) is a broad-spectrum fungicide that is widely used in barley crop, and its residues are detected in its products, including beer. In this study, we investigated the dissipation kinetics of TF during fermentation mediated by two different yeast strains, *Saccharomyces cerevisiae* IAPPST 1401 (Y1) and CICC 1202 (Y2), and found that Y2 promoted the degradation of TF. Response surface methodology was used to optimize fermentation process variables, in order to achieve the maximum removal rate of TF and the minimum production of its corresponding metabolite, triadimenol (TN). Processing factors (PFs) were also evaluated during the optimized brewing process and were close to 1 for TF during the malting, milling, boiling, and cooling steps, but not in mashing and fermentation that were 0.19 and 0.13, respectively. TF degraded to TN during brewing, and a PF value of >1 for TN was also observed in malting and fermentation. Our analysis concluded that beers produced using the yeasts and brewing methods we investigated are safe for human consumption.

1. Introduction

Triadimefon (TF) is a registered broad-spectrum systemic sterol biosynthesis-inhibiting fungicide, which is extensively used to treat diseases and protect yield in barley (Rawlinson, Muthyalu, & Cayley, 1982). In plants and fungi, TF is enzymatically transformed by the reduction of a carbonyl group to its corresponding alcohol, triadimenol (TN), which is also registered as a systemic fungicide with a greater pesticidal activity than that of TF (Liang et al., 2013). Previous studies have shown that TF and TN exhibit clear teratogenic effects and are harmful to the mammalian central nervous system (Menegola, Broccia, Di Renzo, Prati, & Giavini, 2000). When fungicides are appropriately utilized, they do not cause any health- or environment-related problems. However, safety recommendations are not always followed, and undesirable residues remain in barley and found in its products, including beer (Inoue, Nagatomi, Suga, Uyama, & Mochizuki, 2011; Navarro, Vela, & Navarro, 2011).

Beer is one of the world's most widely consumed alcoholic beverages, which commercially produced by the controlled fermentation of wort, a liquid rich in sugars, nitrogenous compounds, sulfur compounds, and trace elements extracted from malted barley. Fermentation is the heart of brewing, in which yeast is the most important ingredient. During fermentation, a series of complex biochemical reactions occurs, producing both toxins and nutrients. As reported in previous studies, if barley contains pesticide residues, these may also be present in beer (Bajwa & Sandhu, 2014; Hengel & Shibamoto, 2002; Navarro, Perez, Navarro, Mena, & Vela, 2006). Therefore, the brewing industry needs to strictly manage the risks caused by applied pesticides for ensuring consumer safety. The transfer of pesticides into beer depends on the used process and physiochemical properties of pesticides such as the water–octanol partition coefficient (K_{ow}), solubility, and volatility (Holland, Hamilton, Ohlin, & Skidmore, 1994; Kaushik, Satya, & Naik, 2009; Regueiro, Lopez-Fernandez, Rial-Otero, Cancho-Grande, & Simal-Gandara, 2015). Navarro, Vela, Perez, and

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Navarro (2011) reported that flutriafol and cyproconazole (log K_{ow} of 2.3 and 3.1, respectively) remain in the beer after fermentation, while significantly high amounts of tebuconazole, epoxiconazole, and diniconazole (log K_{ow} of 3.4–4.3) are eliminated. Hence, it is essential to investigate the fate of pesticide residues during fermentation to help the brewing industry.

In China, similar to many other countries worldwide, an increasing number of studies use the processing factors (PFs) of pesticides during food processing for the assessment of dietary risk (Aguilera, Valverde, Camacho, Boulaid, & García-Fuentes, 2014; Boon et al., 2015; Kong et al., 2012). However, no method can simultaneously ensure the quality of processing and effectively assess the removal efficiency of pesticide residues. In addition, PF studies often fail to obtain accurate conclusions (Li et al., 2015). Hence, a statistical optimization strategy using response surface methodology (RSM) for food processing was developed to accurately track pesticide PFs. The combination of PF analysis and RSM allows pesticide residues to be monitored and controlled during food processing and strategies to be developed to enhance food safety.

This study aimed to: 1) investigate the effects of commercial brewing process (malting, milling, mashing, boiling, cooling, and fermentation) on TF and TN residues, 2) enhance the understanding on the effects of brewing process on PFs, and 3) develop an RSM approach to optimize the removal efficiency of TF and TN residues. Furthermore, beer samples of commercial brands were analyzed for TF and TN residues and evaluated by dietary exposure assessments.

2. Materials and methods

2.1. Chemicals and reagents

TF and TN analytical standards (purity $\geq$ 98.0%) were acquired by Dr. Ehrenstorfer GmbH (Augsburg, Germany); commercial TF 20% emulsifiable concentrate (EC) by Jiangsu Sword Agrochemicals Co., Ltd. (Jiangsu, China); primary secondary amine (PSA) by Agela Technologies (Tianjin, China); analytical grade acetonitrile, hydrochloric acid (HCl), anhydrous magnesium sulfate ($MgSO_4$), sodium chloride (NaCl), and sodium acetate (NaAc) by Beijing Chemical Reagents (Beijing, China); spectrophotometric grade acetonitrile and acetone by Honeywell International Inc. (New Jersey, USA); and ultra-pure deionized water for Arium comfort I ultra-pure water system by Sartorius A.G. (Gottingen, Germany).

2.2. Field trials

Field experiments were conducted at Sheyang Farm, located in Yancheng, Jiangsu Province, China, using the barley (*Hordeum vulgare* L.) variety 'Yangnong 3.' Barley plants were tested and determined to be free of TF and TN before the application of target pesticides. In order to ensure adequate pesticide deposition, TF 20% EC was applied twice 7 d apart at 5-fold the recommended dosage (3000 g active ingredient ha^{-1}). Barley samples of 100 kg were collected 3 d after the last pesticide treatment, placed in polyethylene bags, and transported to the laboratory.

2.3. In vitro assays for studying TF behavior

Two yeast strains were used to investigate the effects of fermentation on fungicide behavior: *Saccharomyces cerevisiae* IAPPST 1401 (Y1), which was isolated from beer in the lab; and *S. cerevisiae* CICC 1202 (Y2), commercially available active dry yeast. These two yeast strains were first cultured in fresh YPD medium (1% yeast extract, 2% peptone, and 2% glucose), grown to an optical density of 1 at 600 nm (OD_{600}), and then placed to TF-enriched YPD liquid broth. TF-enriched YPD liquid broth without yeast was used as a control. The inoculum was added into Erlenmeyer flasks and incubated in an orbital shaker (180 rpm, 30 °C). Three groups from each experiment were aseptically collected at 0, 3, 6, and 12 h and 1, 2, 3, 5, and 12 d after inoculation. Fermentation was performed in triplicate at a TF concentration of 5.0 mg L^{-1}.

2.4. Brewing process

A 20-kg barley sample was placed in a steep vat of water for about 40 h. Then, barley was spread out on the floor of a germination room and rootlets began to form 3 d later. After germination, green malt was dried on metal racks in a kiln house at 50 °C. Then, malted barley was milled using a malt mill machine (Gongda Machine Co., Ltd., Shandong, China). Milled barley was heated into 50 L of water at 53 °C for 70 min, and then in a large cooking vessel, namely 'mash tun,' at around 66 °C for 80 min. In the mash tun, grain and water created a cereal mash, in which the starch was transformed into sugar. Spent grains were filtered, and the wort was boiled at 140 °C for 90 min to sterilize and concentrate. During this stage, certain types of hops and carrageenan were added at different times during boiling to adjust bitterness or aroma and help with preservation. Then, the wort was quickly transferred from the brew kettle through a device to filter hops and then onto a heat exchanger for cooling. The cooled wort was saturated with air, which is essential for the growth of yeast, and transferred to a fermentation tank before adding yeast. The wort transformed to beer some days later.

2.5. Analysis of TF and TN residues

2.5.1. Extraction and purification of barley samples

A 5-g homogenized sample was placed into a 50-mL PTFE centrifuge tube with 3 mL of pure water and shaken for 1 min. Then, 20 mL acetonitrile was added, and the mixture was placed on a Geno/Grinder mechanical shaker (SPEX SamplePrep, Metuchen, NJ, USA) for 3 min at 1200 strokes min^{-1}. A total of 2 g anhydrous $MgSO_4$ and 1 g NaCl were added and vortexed with an XW-80A Vortex (Kyova-Kirin, Tokyo, Japan) at full speed for 3 min, and then the tube was centrifuged with a TG16-WS centrifuge (Xiangyi Centrifuge, Hunan, China) for 5 min at 2077 $\times$ *g*. Then, 8 mL of the upper layer (acetonitrile) was collected in a round-bottom flask and concentrated almost to dryness using a rotary evaporator (Yarong Technologies, Shanghai, China) at 35 °C. The residue was reconstituted in 2 mL acetonitrile and transferred to a 2.5-mL centrifuge tube containing 50 mg PSA and 150 mg anhydrous $MgSO_4$. The mixture was vortexed at full speed for 1 min and briefly centrifuged. Subsequently, 1 mL of the upper layer (acetonitrile) was filtered with a 0.22-mm syringe filter for liquid chromatography-mass spectrometry (HPLC-MS/MS) analysis.

2.5.2. Extraction and purification of beer samples

Beer samples of 10 mL each were weighed into a 50-mL PTFE centrifuge tube. Then, 600 μL of 1 M HCl was added to adjust the pH to 2.0. Next, 10 mL acetonitrile was added and the mixture was placed on a Geno/Grinder mechanical shaker for 3 min at 1200 strokes min^{-1}. A total of 4 g of anhydrous $MgSO_4$, 1 g NaCl, and 1 g NaAc were added and vortexed with a XW-80A Vortex at full speed for 3 min. The tube was then centrifuged with a TG16-WS centrifuge for 5 min at 2077 $\times$ *g*. Then, 1.5 mL of the upper layer (acetonitrile) was transferred to a 2.5-mL centrifuge tube containing 50 mg PSA and 150 mg anhydrous $MgSO_4$. The mixture was vortexed at full speed for 1 min and briefly centrifuged. Finally, 1 mL of the upper layer (acetonitrile) was filtered with a 0.22-mm syringe filter for HPLC-MS analysis.