Dissipation Dynamic And Processing Factor Of Myclobutanil In Tomato

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Abstract

The effect of different processing factors on myclobutanil levels during canned tomato paste production was investigated. A validated residue analysis method was developed to identify myclobutanil by using QuEChERS extraction and UPLC-MS/MS analysis. To eliminate the matrix effect, a matrix-matched standard calibration curve was applied for accurate quantification. Myclobutanil miscible oil was sprayed under field conditions, and then tomato fruits were collected. The dissipation of myclobutanil in tomato fruits followed a first-order dynamic equation with a half-life of 2.88 days. The result was similar to that in previous reports. The half-lives of myclobutanil in plants were short. The results of processing experiment indicated that the processing factors were all less than 1, showing that the processing procedure significantly decreased the residual level of myclobutanil in canned tomato paste in comparison with the raw agricultural commodity. Washing, peeling, and homogenization played a critical role in reducing pesticide residues.

1 Introduction

Tomato (*Lycopersicon esculentum*) belongs to eggplant fruit vegetables, which is rich in organic acids, cellulose, vitamins and carotenoids, and has the functions of promoting digestion, clearing heat, detoxification, cosmetology and so on. Moreover, the medicinal value of tomato in preventing cancer and cardiovascular and cerebrovascular diseases has long been recorded (Bhowmik et al. 2012). According to data provided by Food and Agriculture Organization of the United Nations (FAO), tomato production of China in 2020 was about 583,599 hg/ha (<https://www.fao.org/faostat/en/#compare>). Diseases and pests may be responsible for the decrease in yield (Clark et al. 1999), especially tomato late blight, virus disease, bacterial wilt, cotton bollworm and aphids (Nowicki et al. 2012), resulting in a yield reduction of about 30% (Jones et al. 2014). Therefore, pesticides are frequently applied to tomatoes during planting (Jankowska et al. 2016). According to reports, tomato ranks among the top ten of the most pesticide-contaminated vegetables and fruits (Raluca et al. 2018). Authorities and regulators such as: CAC (Codex Alimentarius Commission), EU (European Union), USEPA (United States Environmental Protection Agency), and relevant bodies in China, Canada, Japan, and elsewhere have established maximum residue limits (MRLs) for pesticides for raw agricultural commodities.

Myclobutanil (Fig. 1), (RS)-2-(4-chlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl) hexanenitrile, is a systemic and foliar fungicide, which belongs to the triazole fungicide family (Authority 2009). It inhibits ergosterol biosynthesis and has good control effect on ascomycetes and basidiomycetes (Allen et al. 2006). However, myclobutanil is classified as a moderately toxic class II compound due to its effects on antioxidant enzyme genes and malondialdehyde levels in animal. It is reported that myclobutanil persists in the environment and accumulates along the food chain, which may threaten human health and environmental pollution (Sun et al. 2014). In recent years, there are many reports about the residues of myclobutanil in agricultural products and environmental samples, including the pretreatment method (Deng et al. 2010), the dissipation dynamic (Hwang & Kim 2013, Wang et al. 2012), the level of

myclobutanil residues in the actual samples(Abdallah et al. 2021, Lou et al. 2008) which has caused public concern.

Food processing is transforming raw agricultural products into other food forms for consumption by humans or animals(Kaushik et al. 2009, Minmin et al. 2015). The household and industrial processing, such as washing, peeling, juicing, blanching, drying, pickling and boiling, can significantly decrease or concentrate the level of pesticide residues, or even transform pesticides to more toxic metabolites(Aguilera et al. 2014), which has attracted many scholars' discussion. Kong et al found that processing can effectively remove difenoconazole residues in tomatoes, in which washing and peeling can reduce the residue level by 99%(Kong et al. 2012). Wu et al. reported that processing of washing, juicing and cooking can effectively remove pesticide residues on the surface of kumquats, cucumbers and spinach(Wu et al. 2019). The PF was ranged from 0.09 to 0.7. Saber et al. showed that the residual amount of hexythiazox on strawberry surface decreased from 0.261 mg/kg to 0.084 mg/kg after cleaning(Saber et al. 2016). It was also reported that the residue of pyrimidone in cabbage decreased by 40.2% after cleaning(Gong et al. 2019). Fresh tomatoes can be used as fruits and are usually eaten directly after washing, but the changes of myclobutanil residues on the surface have not been well reported.

The aim of our study was designed to evaluate the fate and magnitude of myclobutanil in tomato under field conditions, to investigate the dissipation of myclobutanil in tomato during processing and examine various processing factors for myclobutanil. The study provides information about food safety and health risk evaluation of myclobutanil.

2 Materials And Methods

2.1 Chemicals and reagents

Myclobutanil standard material was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Myclobutanil formulation was supplied by the Yifan Biotechnology group (Zhejiang, China). Methanol (LC-MS grade) was obtained from Merck KGaA (Darmstadt, Germany). Acetonitrile and formic acid (HPLC grade) were purchased from Thermo Fisher Scientific(Shanghai, China). Sodium chloride(analytical grade) purchased from Beijing Reagent Company (Beijing, China). Water was purchased from Watson's (Guangzhou, China). WondaPak QuEChERS dispersive tubes (2 mL) were purchased from SHIMADZU-GL (Shanghai, China).

2.2 Field experiments and sampling

Field trials were conducted in Jinzhong City, Shanxi Province, China. Two separate field experiments were performed to investigate the residual behavior of myclobutanil in tomato under field conditions and during processing.

In order to reveal the dissipation kinetics of myclobutanil in tomato fruits, myclobutanil miscible oil (10%) was applied as a spray in a single dosage as recommended. The test plot measured 15 m², each experiment was performed in triplicate, and a control experiment was conducted. Tomato fruit samples were collected at 2 h (0 d), 1 d, 3 d, 5d, 7 d, 10 d, 14 d, and 21 d after application. The collected tomato samples were transported to the laboratory as soon as possible and were homogenized and stored in a freezer at below – 18°C until analysis.

In the second field experiment: to obtain sufficient residual concentration for processing studies, myclobutanil was sprayed three times at double dose as recommended and the application interval was 7d. Two days after the last treatment, about 10 kg of tomatoes were collected for processing.

2.3 Processing treatments

Part of the raw tomato samples were analysed without processing treatment, some of the raw tomatoes were peeled, and the remainder sample was processed to tomato paste. Canned tomato paste production procedures generally involve five steps: washing, peeling, homogenization, simmering, and canning. The processing steps and sampling points are shown in Fig. 2. In processing, the whole tomato, washed whole tomato, washed tomato skin, tomato pulp, the tomato puree, tomato juice, tomato seeds, tomato paste, and canned tomato paste were collected and assayed to demonstrate the variation of residue levels and processing factors as pertaining to myclobutanil.

2.4 Extraction and cleaning of samples

Representative samples of 5 g of homogenized material, 5 g of sodium chloride, and 10 mL of acetonitrile were added to a 50-mL centrifuge tube. The samples were mixed using an advanced multi-tube vortexer for 5 min at 2,500 rpm, and then centrifuged for 5 min at 8,000 rpm. 1 mL of the supernatant was transferred to a QuEChERS dispersive tube containing 25 mg of Primary Secondary Amine (PSA) and 25 mg of C18, vortexed for 2 min at 2,500 rpm, and subsequently centrifuged for 2 min at 2,500 rpm. 0.5 mL of the upper layer was placed into a 2-mL tube, to which we added 0.5 mL water, mixed it, then filtered the material through 0.22-µm filters for UPLC-MS/MS analysis.

2.5 Instrument analysis

Chromatographic separation analysis of myclobutanil was conducted on a Waters Acquity UPLC I—class binary solvent manager (Waters, USA), which was equipped with a BEH C₁₈ column (50 mm×2.1 mm, 1.7µm) (Waters, USA). The column temperature was 40°C, the sample volume injected was 5 µL and the flow rate was 0.3 mL/min. The mobile phase consisted of phase A: water with 0.1% formic acid and 4mM ammonium acetate, and phase B: methanol with 0.1% formic acid and 4mM ammonium acetate. The gradient elution was: 0 ~ 1 min 50% A phase, 1.1 ~ 4.0 min 5% A phase, 4.1 ~ 5.0 min 50% A phase.

Waters Xevo TQ-S MicroMS/MS (Waters, USA) was used for mass spectrometric analysis. The ESI (electrospray ionisation) source was used, which operated in positive ionisation mode. MS analyses were performed using the MRM (multiple reaction monitoring) mode. The source voltage was 3000 V and the

desolvation temperature was 400°C. The desolvation gas flow was 800 L/h and source temperature was 150°C. The quantitative and qualitative ions of myclobutanil were m/z 288.9/69.9 and 288.9/124.9, and collision energies were 24 V and 38 V, respectively. The cone voltage for myclobutanil was 36 V.

2.6 Method validation

A series of myclobutanil working standard solutions (0.001, 0.005, 0.01, 0.05, and 0.1 µg/mL) were prepared by diluting the stock standard solution with acetonitrile, the curves in solvent and tomato matrix were prepared, respectively. Recovery evaluation was conducted at three spiked concentration levels (0.01, 0.1, and 0.5 mg/kg) in three blank matrices (tomato, tomato juice, and tomato seeds). Each experiment was replicated five times. The limits of detection and quantification (LOD and LOQ) were defined as the concentrations that produced signal-to-noise (S/N) ratios of 3 and 10, respectively.

2.7 Data analysis

The degradation kinetics of myclobutanil in tomato followed the first-order kinetics equation: $C_t = C_0 e^{-kt}$, and the half-life ($t_{1/2}$) was calculated from the k value, using the following equation: $t_{1/2} = \ln 2/k$. C_t and C_0 represent the residual level of myclobutanil (mg/kg) at time t and the initial concentration of myclobutanil (mg/kg). k is the first-order rate constant (Jankowska et al. 2016) [5].

According to OECD guideline for the testing of chemicals, Test No. 508: Magnitude of the Pesticide Residues in Processed Commodities (OECD 2008) and the agricultural industry standard of China (NY/T 3095—2017): Guideline for the testing of pesticide residues in processed agricultural commodities, the processing factor is equal to residues in processed agricultural commodities (mg/kg) divided by residues in raw agricultural commodities (mg/kg). When the processing factor is greater than 1, it indicates a concentration increase in processed agricultural commodities during processing, whereas a decrease in the residue concentration is expressed by a factor of less than 1 (Hassan et al. 2019, Jing et al. 2017).

3 Results And Discussion

3.1 Method validation and matrix effect

Under optimised instrument conditions, the residual method showed reliable performances in terms of linearity, recovery, and precision. The average recoveries and relative standard deviations (RSDs) of myclobutanil in three matrixes are displayed in Table 1. The average recoveries in three matrices varied from 82–102% with the RSD ranging from 1.2–9.1%. The LOD and LOQ were 0.0003 mg/kg and 0.001 mg/kg, respectively. A good linear relationship was obtained in the range from 0.001 µg/mL to 0.1 µg/mL. The regression equation of solvent calibration curves and tomato matrix calibration curves were $y = 21070552x + 11173$ and $y = 11116881x + 7501$, and the regression coefficients were 0.9999 and 0.9996, respectively.

Table 1
Average recoveries and RSD of myclobutanil in tomato, tomato juice, and tomato seeds ($n = 5$).

Matrix	Spiked levels (mg/kg)	Average recoveries (%)	RSD (%)
Tomato	0.01	96	1.5
	0.1	92	1.9
	0.5	93	2.1
Tomato juice	0.01	97	1.8
	0.1	96	1.8
	0.5	94	2.5
Tomato seed	0.01	102	9.1
	0.1	94	1.2
	0.5	82	6.7

Matrix effect (ion suppression or ion enhancement) results from ionisation competition between co-eluting components and target compounds. It is critical to ascertain the matrix effect in developing an analytical method, especially in LC-MS/MS analysis with an electrospray ionisation source (Chambers et al. 2007, Huang et al. 2013). In the present study, the ratio of the slope of the calibration curves was used to assess the matrix effect (Matuszewski et al. 2003, Suchi et al.). The matrix effect was -47% , suggesting strong ion suppression (Kmellár et al. 2008). Thus, a matrix-matched standard calibration curve was applied for accurate quantification.

3.2 The dissipation of myclobutanil in tomato under field conditions

The magnitude of myclobutanil in tomato fruits was determined using the validated method. The dissipation curve of myclobutanil in tomato was obtained by plotting sampling time *versus* residual levels of myclobutanil (Fig. 3). The dissipation of myclobutanil in tomato followed the first-order dynamic equation. The corresponding regression equation was $y = 0.047e^{-0.24x}$ with a regression coefficient of 0.9347.

As shown in Fig. 3, the residual concentration of myclobutanil decreased with time. The initial deposition concentration of myclobutanil in tomato was 0.055 mg/kg, and the concentration was 0.002 mg/kg after 14 days, then the concentration decreased to below the LOQ on the 21st day. The half-life of myclobutanil in tomato was 2.88 d. The result was similar to that in previous reports. The half-lives of myclobutanil in plants were short with values range from 2.2 d to 3.4 d in foliar spraying mode (Liu et al. 2009).

3.3 The dissipation of myclobutanil in tomato during processing

The risk assessment of residual pesticides is usually performed on the foundation of raw agricultural commodities. The effects of processing modes on pesticide residues in agricultural products have rarely been considered. The residual level and nature of pesticides in food may vary during food processing procedures(Kaushik et al. 2009). Therefore, the influences of different treatments on the level and fate of pesticide residues in food should be considered, as they are crucial for assessing dietary intake.

Five general processing procedures wereinvestigated, and samples collected. The residual concentrations of myclobutanil in tomato samples are listed in Table 2. In most commercial and household processing procedures, washing is the first step(Li et al. 2012). In this study, tomato fruit samples were rinsed under running water for 1 min. After washing, the residual level of myclobutanil in the whole tomato was decreased by 33% and the concentration was 0.067 mg/kg. Tomato samples were peeled after washing and the tomato skin and tomato pulp samples were collected. As shown in Table 2, myclobutanil was mainly present in the tomato skin. The residual concentration of myclobutanil in tomato pulp further indicated that peeling was an effective approach to remove pesticide residues. Similar to the previous reports, washing and peeling significantly reduced the pesticide residue in tomato(Reiler et al. 2015).

Table 2
The residual concentrations of myclobutanil in the tomato samples

Sample	Concentration (mg/kg)
Raw tomato	0.100
Washed whole tomato	0.067
Tomato skin	0.395
Tomato pulp	0.023
Tomato puree	0.013
Tomato juice	0.009
Tomato seeds	0.027
Tomato paste	0.044
Canned tomato paste	0.041

After peeling, the tomato fruit was chopped into four parts. To make the sample representative, the diagonal parts were collected, then tomato juice and tomato seeds were removed, the remaining tomato pulp homogenised using a knife-mill Grindomix GM300 (Retsch, Germany). As shown in Fig. 4A, after

homogenisation, the myclobutanil load was reduced by 87.3%. It was notable that myclobutanil was detected both in tomato juice and tomato seeds. Pesticides were not discovered in tomato juice and tomato seeds in previous research[31]. This might relate to the mode of action of myclobutanil.

Then, the tomato puree was simmered at 80–90°C for 30 minutes to remove the excess water and form a thick tomato paste. During simmering, the residual concentration of myclobutanil tended to increase, this may have been caused by water loss during simmering. However, the residual level of myclobutanil still decreased during the process.

The tomato paste was sterilised and canned at 121°C. Compared with tomato paste, the amount of myclobutanil in canned tomato paste was reduced by 6.8%. Previous studies report that sterilisation eliminated or reduced pesticide residues in tomato(Kontou et al. 2004). Figure 4A shows that the removal rate of myclobutanil reached 59.3% over the entire processing procedure.

3.4 Processing factors

Processing factors are important tools in risk assessment, which refine the exposure assessments for consumers regarding pesticide residues in processed food. Figure 4B illustrates the processing factors of myclobutanil during processing. The values of washing, peeling, homogenisation, simmering, and canning steps were 0.67, 0.23, 0.13, 0.44, and 0.41, respectively. After peeling and homogenisation, the minimum processing factor was obtained, showing that these steps played an essential role in reducing pesticide residues. The increase in processing factor indicated that water loss led to a significant enrichment of myclobutanil in the tomato samples during simmering. The processing factors were all below 1, indicating that the processing decreased pesticide residues in tomato and perhaps reduced the exposure risk for consumers compared to the consumption of the raw agricultural commodity.

4 Conclusion

In the study, a viable residue analysis approach was established and successfully used to identify the residual magnitude of myclobutanil in tomato. The degradation of myclobutanil in tomato under field conditions followed the first-order kinetic equation with a half-life of 2.88 d. The variation of residual level of myclobutanil in tomato during processing was investigated. The concentration of myclobutanil decreased from 0.1 mg/kg to 0.041 mg/kg. Washing, peeling, and homogenisation played an essential role in reducing pesticide residues. The residual concentration of myclobutanil increased because of water loss during simmering. The processing factors ranged from 0.13 to 0.67, indicating that the processing significantly reduced the residual level of myclobutanil in canned tomato paste compared to that in the raw agriculture commodity.

Declarations

Author Contributions

Q.Y.L.; Experimental design, Data analysis, Software, Writing–original draft preparation. J.L.C.; Writing–original draft preparation. L.J.D.; Methodology, Writing–review and editing, L.C.Y.; Field experiment, Sample collection. R.P.C.; Field experiment, Sample preparation. Q.S.; Experimental supervision, Writing–review and editing.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Informed Consent

Not applicable.

Availability of data and material

Not applicable.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable.

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Figures

Figure 1

Chemical structure of myclobutanil

Figure 2

Processing of tomato.

Figure 3

The dissipation dynamic of myclobutanil in tomato.

Figure 4

The variation of residual levels (A) and processing factors (B) of myclobutanil during processing