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Effect of processing on herbicide residues and metabolite formation during traditional Chinese tofu production

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ABSTRACT

The fates of clomazone, fomesafen, and quizalofop-p-ethyl and its metabolite quizalofop (acid) in soybean samples during tofu processing were systematically assessed. Residues were determined using ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) after each processing step, including washing, soaking, grinding and filtering, cooking, coagulating, and squeezing. The pesticide distribution at each step of the process was studied, and pesticide processing factors were calculated. Differences in the pesticide residue levels were found at each processing step. Changes in pesticide residues in the tofu products was closely related to their physicochemical properties such as octanol-water partition coefficient (Kow), water solubility, and vapor pressure. The results showed that soaking prominently decreased fomesafen residues by 72.0%, as indicated by its high water-solubility and low log Kow. Grinding and filtering reduced pesticide residues by 88.8%–94.8%, mainly due to dilution or okara separation. The processing factors were generally < 1 for each step and for the entire process, except those for cooking, coagulating, and squeezing. These results demonstrated that the overall process could significantly reduce clomazone, fomesafen, quizalofop-p-ethyl, quizalofop (acid) residues during tofu processing.

1. Introduction

Tofu is a nutritious traditional Chinese vegetarian food with an increasing worldwide demand (Nikolić et al., 2017). China, with its time-honored tofu diet culture, is the largest tofu producer and consumer. Tofu is also favored by the West for its healthy nutritional properties. Traditional tofu is commonly divided into marinated, gypsum, and home-made vinegar tofu. During the manufacturing process, tofu is prepared by coagulating hot soymilk with various coagulants, followed by molding and squeezing of the coagulated curd to remove the whey (Lim, DeMan, & DeMan, 1990). Soybean, the main ingredient of tofu, is one of the most widely cultivated crops globally (Pizzutti et al., 2007). Regions where soybeans are grown usually suffer from heavy weed infestation, which affects the soybean crop. Hence, large amounts of herbicides are extensively used during soybean cultivation and certain herbicides are relatively stable in the surrounding environment (Springer, Aprile, & Lista, 2014). Excessive herbicide residues might be present in soybeans, and most pesticides are toxic to living organisms.

Clomazone, fomesafen, and quizalofop-p-ethyl are widely used commercial formulations for weed control alone or in combination during soybean crop cultivation in China and other areas (Kyongjin & Jiye, 2020; Zhu, Qi, Cao, Mu, Yang, & Wang, 2016). Clomazone is an isoxazolidinone herbicide, which is selectively used for pre-emergence control of various grasses and broadleaf weeds (EFSA, 2011). A

Abbreviations: UPLC-MS/MS, ultra-performance liquid chromatography coupled with tandem mass spectrometry; Kow, octanol-water partition coefficient; PFs, processing factors; HPLC, high-performance liquid chromatography; Sw, water solubility

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previous study showed that clomazone mediates its toxicity through inhibition of AChE and catalase activity, thereby posing a risk to human health (Santi et al., 2011). Fomesafen is a post-emergence selective herbicide widely applied for the early control of annual broadleaf weeds that grow in soybean and rubber estate fields (Sikkema, Shropshire, & Soltani, 2009). Long-term high-doses of fomesafen inhibit the protoporphyrinogen oxidase enzyme, resulting in porphyrin accumulation in the liver of mice (Krijt, Pšenák, Vokurka, Chlumská, & Fakan, 2003). In addition, fomesafen could damage the reproductive system of freshwater snails (Dong et al., 2019).

Quizalofop-p-ethyl is an aryloxy-phenoxy propionate herbicide that exhibits low toxicity to the skin and mildly affects the eye for human beings, according to WHO and EPA reports; It acts as an endocrine-disrupting chemical in zebrafish (Zhu et al., 2016). Quizalofop (acid) is the primary metabolite of quizalofop-p-ethyl produced by hydrolysis in plants, exhibiting higher toxicity than the parent compound in acute toxicity assays performed on earthworms (Liang et al., 2014). Application of quizalofop-p-ethyl has been banned by the European Union since several years (Zhu et al., 2016). However, the use of quizalofop-p-ethyl is widespread in several other parts of the world, especially China, owing to its efficiency as an herbicide and evident cost-efficiency. As these compounds are applied to soybean fields, pesticide residues would remain in soybean plants or seeds (Kyongjin & Jiye, 2020; Aksoy, Deveci, kızılırmak, & Akdeniz, 2013). After the pesticide residues in soybeans are processed, a certain concentration of pesticides may still be retained in the processed products, such as tofu (Miyahara and Saito, 1994), and hence the product will be potentially toxic. Therefore, to ensure food safety, it is necessary to investigate the presence of herbicidal residues in soybean and perform a comprehensive risk assessment.

Previous studies have shown that a large number of food processing technologies might lead to an increase in contaminants in the processed product compared to the raw material. These processing steps include drying (Kaushik, Satya, & Naik, 2009), cheese squeezing (Duan, Cheng, Bi, & Xu, 2017), and crude soybean oil production (Zhao, Ge, Liu, & Jiang, 2014). Moreover, pesticide residues could be degraded, concentrated, or converted to toxic metabolites during food processing (Han et al., 2013; Huan, Xu, Jiang, Chen, & Luo, 2015; Kong et al., 2012; Zhao, Liu, Wu, Xue, & Hou, 2016). Food processing, such as tofu production (Miyahara and Saito, 1994), soybean oil production, and processing of vegetable products (Kaushik et al., 2009), affects the physicochemical properties of pesticides such as water–octanol partition coefficient (Kow), volatility, water solubility (Sw), thermal degradation, vapor pressure (Martin et al., 2013), and causes complex changes in the biological properties of raw materials, which have a noteworthy impact on pesticide residue behavior (Kaushik et al., 2009).

The processing factors (PFs: the ratio of residual concentration in processed food and the raw food material) for tofu production are unclear (BfR, 2018). PFs can help in evaluating the dietary intake of pesticides present in processed products (Amvrazi & Albanis, 2008). Furthermore, due to the increase in the residue concentration caused by the processing steps, PFs can also be adapted to recommend maximum residue limits (MRLs) for processed food materials (González-Rodríguez, Rial-Otero, Cancho-Grande, Gonzalez-Barreiro, & Simal-Gándara, 2011). It is essential to elaborate on the PFs of several pesticides during tofu production from soybeans. Therefore, in order to guarantee the safety of food for consumers, continuous monitoring of the behavior of clomazone, fomesafen, quizalofop-p-ethyl, and its metabolite quizalofop (acid) is necessary during soybean processing and in soybean products.

In order to investigate the distribution of the four aforementioned compounds during tofu processing and most importantly, to investigate the PFs of tofu processing applicable to the established MRLs, we first performed simultaneous analysis of the four compounds in fat-containing soybean samples using UPLC-MS/MS combined with the modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) method (Zhao et al., 2016), a rapid and effective extraction procedure. In the present study, the factors responsible for the change in pesticide residue levels during tofu processing was analyzed by taking into account the physicochemical properties of pesticides. Meanwhile, the data regarding residue changes in tofu products would provide further insights into the evaluation of chronic dietary risk using the risk quotients (RQs) method based on Chinese dietary habits. The findings of this study contribute to the study of pesticide behavior during the processing of the traditional Chinese food tofu.

2. Material and methods

2.1. Material

Analytical standards of quizalofop-p-ethyl, fomesafen, clomazone, and quizalofop (acid) (purity ≥99.0%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), and the commercial compounds (emulsifiable concentrates) were obtained from 20% clomazone (Suzhou Fumeishi Plant Protection Agent Co., Ltd., Suzhou, China), 25% fomesafen (Shandong Binnong Technology Co., Ltd., Shandong, China), and 10% quizalofop-p-ethyl (Shandong Kaifengyuan Biotechnology Co., Ltd., Shandong, China). HPLC-grade acetonitrile, and analytical-grade anhydrous magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl) were obtained from Beijing Chemical and Reagent (Beijing, China). Ultra-pure water was produced using a Millipore purification system (Millipore, Bedford, MA, USA). Octadecylsilane (C18, 50 μm) was obtained from Agela Technologies (Tianjin, China). LC-MS grade formic acid was acquired from Thermo Fisher (Shanghai, China). Standard stock solutions (1000 mg/L) for the four compounds were individually prepared in acetonitrile and diluted into a mixed standard solution of 10 mg/L. Diluted solutions were stored in a freezer at −20 °C in the dark.

2.2. Soybean treatment

For the treatment of soybeans, we referred to treatment procedures from previous studies (Zhao et al., 2016). Soybeans (1 kg) were soaked in the aqueous solution of three commercial pesticides (clomazone, fomesafen, and quizalofop-p-ethyl) for 6 h in a sealed plastic drum (5 L). The soybeans were stirred with a glass rod every 0.5 h to ensure uniform absorption of pesticides. Thereafter, the soybeans were allowed to air dry naturally at room temperature (25 °C) for 72 h in order to restore their original state. The treated soybean samples were stored in a freezer at −20 °C, for further use as raw material for tofu processing.

2.3. Tofu preparation

Marinated, gypsum, and home-made tofu were coagulated using magnesium chloride, calcium sulfate, and white vinegar, respectively. Generally, tofu processing was performed through six consecutive steps (Dan, Liantao, Dian, Hua, & Ping, 2017), as shown in Supplementary Fig. 1.

Process 1. Washing. Soybean samples (500 g) were washed with 1000 mL of water for 3 min.

Process 2. Soaking. The washed samples were soaked in water for 8 h.

Process 3. Grinding and Filtering. An ALLJ-B12k1 semi-automatic juice extractor (Guangdong Bear Electrical Co., Ltd., China) was used to process soybeans (300 g) with water (1.6 L) in order to obtain raw soymilk. The soymilk was then filtered through two layers of gauze.

Process 4. Boiling. The filtrate (raw soymilk) was heated at 100 °C for 10 min.

Process 5. Coagulating. The cooked soymilk was coagulated using an aqueous coagulant (magnesium chloride, calcium sulfate, and white vinegar) solution for 10 min, at 80 °C, respectively.

Process 6. Squeezing. After coagulating, the tofu pudding obtained

from process 5 was poured into a box with gauze and holes and pressed with 1 kg weights for 10 min.

2.4. LC-MS/MS analysis

Chromatographic separation was performed using an Agilent 1290 UPLC system (Agilent Technologies, Wilmington, DE, USA) equipped with a binary pump and degasser. The analytical column was packed with a reversed-phase C18 column (2.1 mm × 100 mm, 1.8 μm) (Agilent Zorbax Eclipse Plus). The mobile phase comprised of A: water with 0.1% formic, B: acetonitrile. The flow rate was 0.3 mL/min, and the gradient elution was: 0–0.5 min 80%–80% A, 0.5–1.0 min 80%–50% A, 1.0–4.0 min 50%–50% A, 4.0–7.0 min 50%–0% A, 7.0–8.0 min 0%–0% A, 8.0–8.01 min 0%–80% A, and 8.01–9 min 80%–80% A. Thereafter, the column was balanced for 0.99 min before the next injection. The column was maintained at 40 °C, and the injection volume was 5 μL. The determination was carried out with an Agilent 6495A triple quadrupole mass spectrometer (Agilent Technologies, Wilmington, DE, USA) using the dynamic multiple reaction monitoring mode (DMRM). The typical optimized MS detection parameter settings are shown in Supplementary Table 1.

2.5. Calculation of processing factors

The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) evaluated food processing data on residue behavior in those cases where obvious residues occur in plants or plant products, which are further processed into food (FAO, & WHO, 2006). According to the impact on residue concentrations and the disposition of the residues in the different processed commodities, PFs are calculated as follows:

$$PF = \frac{\text{residues in processed food (mg / kg)}}{\text{residues in raw materials (mg / kg)}}$$

PF < 1 (reduction factor) demonstrates the dissipation of the residue in the processed material. In contrast, PF > 1 (concentration factor) demonstrates the enrichment of the residue in the processed material (BfR, 2018).

2.6. Statistical analysis

All reported values are means ± standard deviation (SD) of five replicates, and significant differences in data were statistically evaluated by paired-samples T-test using SPSS base 17.0 software.

3. Results and discussion

3.1. Method validation

According to the extraction and purification procedure highlighted in Supplementary Paragraph 1 and the method performances in Supplementary Paragraph 2, the linearity was evaluated by preparing six matrix-matched calibration standards (5, 10, 20, 100, 500, and 1000 μg/L) for clomazone, fomesafen, quizalofop-p-ethyl, and quizalofop (acid) in acetonitrile, soybean, soymilk, and tofu matrix, respectively. Outstanding linearity was observed for all the four compounds ($R^2 \geq 0.9991$ in each sample) (Table 1).

In order to avoid signal suppression or enhancement, matrix-matched calibrations were used to compensate for matrix effects (Han et al., 2013). The results showed no prominent enhancement or suppression effects for soybean and soymilk within 10% of the slope ratio of 1.0 (0.91–1.05). The matrix suppression effect was observed for tofu with slope ratios lower than 0.9 (0.76–0.87) for quizalofop-p-ethyl and fomesafen (Table 1).

As shown in Table 2, the mean recovery for clomazone, fomesafen, quizalofop-p-ethyl, and quizalofop (acid) was within the range of 100.0%–118.5%, 101.6%–116.7%, 70.8%–118.9%, and 87.4%–116.2%, respectively, with RSDs (n = 5) lower than 19.4% at different concentration levels, which were within the expected range for residue analysis. Fig. 1 shows the chromatograms of 10 μg/kg spiked soybean, soymilk, and tofu samples. The reproducibility of the recovery results, as demonstrated by RSDs, validated that the method adequately met the requirements for pesticide analysis in this study (OECD, 2008).

The LODs and LOQs for four substances in soybean, soymilk, and tofu samples ranged from 0.001 to 0.100 μg/kg and from 0.003 to 0.500 μg/kg, respectively (Table 1).

3.2. Effect of individual tofu processing step on pesticide residue dissipation

3.2.1. Washing

In this study, the treated soybean samples were washed with water for 2 min. The initial concentrations of clomazone, fomesafen, quizalofop-p-ethyl, and quizalofop (acid) were 10.818 mg/kg, 7.349 mg/kg, 4.995 mg/kg, and 8.529 mg/kg, respectively in the treated soybean samples (Table 3). The results show that quizalofop (acid) might be present in quizalofop-p-ethyl commercial formulations or formed during the soaking treatment with the mixed aqueous solution of the three commercial pesticides. Compared to the concentrations of the four compounds in soybeans, 18.0% of clomazone, 16.5% of fomesafen, 37.9% of quizalofop-p-ethyl, and 16.7% of quizalofop (acid) were removed by washing (Table 3 and Fig. 2). A significant difference was found between the analyte residues of unwashed and washed soybean samples ($p < 0.01$) (Table 3). The difference in the average reductions of the four compounds might be related to their physicochemical properties such as log Kow and Sw, as shown in Supplementary Table 2. More than 80% of malathion, chlorpyrifos, dichlorvos, and captan in soybeans were removed by washing (Miyahara and Saito, 1994). In fact, washing is a primary processing step used in tofu preparation. Various studies have found that pesticides with a low log Kow and high water solubility are easily removed through washing (Huan et al., 2015; Kong et al., 2012; Kaushik et al., 2009; González-Rodríguez et al., 2011). However, compared to the other three compounds, quizalofop-p-ethyl showed an opposite result with the highest reduction after washing despite its high log Kow (4.61) and low Sw (0.61 mg/L) values, as shown in Supplementary Table 2. The results for quizalofop-p-ethyl could be attributed to its accumulation from the soybean surface during the pre-treatment step of soaking with commercial pesticides, which allows more residues to be dislodged through washing.

3.2.2. Soaking

Soybeans could absorb sufficient amounts of water during soaking, facilitating effective crushing (Han et al., 2016). The results of the present study showed that soaking caused a residual reduction in clomazone, fomesafen, quizalofop-p-ethyl, and quizalofop (acid) levels by 38.9%, 72.0%, 61.5%, and 13.1%, respectively (Table 3 and Fig. 2). Soaking has a significant effect on fomesafen residues in soybean. Fomesafen exhibited the highest reduction of > 70% ($p < 0.01$); the reduction was approximately 7.7%, 52.4%, 5.4%, and 29.9% for clomazone, fomesafen, quizalofop-p-ethyl, and quizalofop (acid), respectively, distributed in the soaking water (Table 3 and Fig. 2). The highest decrease in fomesafen could be explained by its high Sw (50 mg/L) and low log Kow (−1.2) values. Thus, the hydrophilic pesticide transfer behavior might be closely related to their physicochemical properties such as Sw and log Kow (Kaushik et al., 2009; Han et al., 2016; Timme and Walz-Tylla, 2003).

3.2.3. Grinding and filtering

Grinding and filtering facilitates homogenization of soymilk and its separation from okara. As presented in Table 3 and Fig. 2, compared to the levels of clomazone, fomesafen, quizalofop-p-ethyl, and quizalofop (acid) in soaked soybeans, their residues in raw soymilk decreased by 89.3%, 94.4%, 94.8%, and 88.8%, respectively, after grinding and

Table 1
Linear range (μg/L), Regression equation, Calibration curve coefficients (R^2), LODs (μg/kg), LOQs (μg/kg) and Matrix effects (ME), for the four compounds in solvent, soybean, soymilk and tofu.

Pesticides	Calibration (matrix)	Linear range	Regression equation	R^2	LOD	LOQ	ME
Clomazone	Solvent	5–1000	y = 6645.7x + 17582.0	0.9995			
	Soybean	5–1000	y = 6398.1x + 80674.9	0.9995	0.001	0.003	0.96
	Soymilk	5–1000	y = 6704.5x + 82810.0	0.9997	0.001	0.003	1.01
	Tofu	5–1000	y = 6523.6x + 177764.9	0.9991	0.001	0.003	0.98
Fomesafen	Solvent	5–1000	y = 110.3x + 337.1	0.9991			
	Soybean	5–1000	y = 100.2x + 558.2	0.9996	0.050	0.145	0.91
	Soymilk	5–1000	y = 104.0x + 599.8	0.9991	0.100	0.350	0.94
	Tofu	5–1000	y = 95.5x + 556.9	0.9991	0.060	0.200	0.87
quizalofop-p-ethyl	Solvent	5–1000	y = 2735.8x-1569.8	0.9996			
	Soybean	5–1000	y = 2618.0x + 13312.7	0.9995	0.030	0.100	0.96
	Soymilk	5–1000	y = 2686.1x + 5654.5	0.9999	0.010	0.050	0.98
	Tofu	5–1000	y = 2081.1x + 120755.1	0.9995	0.001	0.003	0.76
quizalofop (acid)	Solvent	5–1000	y = 511.4x-3763.4	0.9998			
	Soybean	5–1000	y = 516.5x + 423.6	0.9999	0.025	0.080	1.01
	Soymilk	5–1000	y = 535.8x + 141.1	0.9999	0.100	0.500	1.05
	Tofu	5–1000	y = 470.7x + 689.9	0.9999	0.007	0.025	0.92

filtering ($p < 0.001$). The addition of water led to the dilution of pesticide residues (FAO, & WHO, 2006), while the decrease in levels of the four analytes could be attributed to the dislodgment of okara (Kaushik et al., 2009). In addition, the residues only remain in okara at 24.8% for clomazone, 14.4% for fomesafen, 32.3% for quizalofop-p-ethyl, and 23.7% for quizalofop (acid). These results were consistent with previous studies in which pesticide residues were removed during apple juice processing within the range of 57%–100% (Martin et al., 2013). Abou-Arab (1999) reported that juicing caused reductions of pesticide residues in tomatoes, ranging from 22.4% to 27.3%. Furthermore, comparison of log Kow of three compounds showed that log Kow of quizalofop-p-ethyl (4.61) is significantly higher than those of clomazone (2.58) and fomesafen (−1.2) (Supplementary Table 2). Lipophilic pesticides with a high log Kow (e.g., log Kow > 3) are reportedly easier to concentrate in an oily matrix (OECD, 2008). Quizalofop-p-ethyl is more likely to accumulate in okara than in other substances, thereby allowing more residues to be dislodged during grinding and filtering. Therefore, the highest residues of quizalofop-p-ethyl in okara might be due to its high log Kow (4.61).

3.2.4. Boiling

Boiling is an essential step for processing raw food material by using heat. The cooking process, to some extent, increased residues by 1.7% for clomazone, 9.9% for fomesafen ($p < 0.05$), 14.5% for quizalofop-p-ethyl ($p < 0.05$), and 8.9% for quizalofop (acid) ($p < 0.05$) compared to those in raw soymilk (Table 3 and Fig. 2). The increase of the four analytes residues during boiling might be due to water loss in open systems (Keikotlhaile, Spanoghe, & Steurbaut, 2010). Simultaneously, quizalofop-p-ethyl might also undergo thermal degradation and be converted into its metabolite quizalofop (acid) during cooking. In addition, pesticide physicochemical properties of these pesticides might be another reason for this behavior (Kaushik et al., 2009). Comparison of the vapor pressure of three compounds (Supplementary Table 2) showed that the vapor pressure of clomazone (27 MPa) is greater than that of fomesafen (4.0×10^{-3} MPa) and quizalofop-p-ethyl (1.1×10^{-4} MPa). Thus, the lowest increase ratio (1.7%) of clomazone might be due to its high vapor pressure, resulting in a high temperature that facilitates its volatilization or degradation (Han et al., 2016; Holland, Hamilton, Ohlin, & Skidmore, 1994). Similar results were reported by Miyahara and Saito (1994) and Han et al. (2016) who observed that dichlorvos is readily vaporized by heating due to its high vapor pressure (2.1×10^{3} MPa).

3.2.5. Coagulating

Coagulation facilitates the separation of the supernatant from the tofu pudding. As shown in Table 3 and Fig. 2, the residues of the four compounds in the pudding (vinegar) increased by 108.6%, 134.0%, 183.1%, and 89.9% compared to those in cooked soymilk. Compared to the concentrations of the four compounds in cooked soymilk, the residues of the four compounds increased by 156.3%, 131.1%, 180.3%, and 124.6% in the pudding (brine), and by 167.9%, 134.9%, 193.0%, and 133.6% in the pudding (gypsum), respectively. Duan et al. (2017) reported that the pesticide level increased by 1.94–4.96 fold during the cheese-curding process. However, Miyahara and Saito (1994) reported that the coagulation processes reduce pesticide residues in tofu, which might be related to the differences between the processing techniques.

Table 2
Recoveries and relative standard deviations (RSD %) of the four compounds in soybean and tofu products at different fortification levels (n = 5).

Pesticides	Fortification (mg/kg)	Mean recoveries (%)											
		Soybean	RSD (%)	Okara	RSD (%)	Washed water	RSD (%)	Raw soymilk	RSD (%)	Tofu pudding	RSD (%)	Tofu	RSD (%)
Clomazone	1	102.6	2.6	106.4	2.8	103.1	1.1	107.8	1.2	110.2	2.7	102.9	0.8
	0.1	101.4	1.8	102.2	1.7	102.2	1.1	100.0	3.5	105.2	0.7	107.0	1.1
	0.01	118.5	0.4	104.3	3.6	110.9	2.0	103.8	1.7	112.0	0.8	112.3	0.5
Fomesafen	1	116.6	4.8	114.4	2.7	114.3	0.7	114.8	1.0	116.2	0.7	115.6	2.7
	0.1	101.6	1.0	102.0	2.9	102.8	0.4	102.2	3.6	108.6	2.3	104.2	1.4
	0.01	115.8	2.0	108.5	5.1	116.7	1.3	108.1	3.5	115.0	1.4	108.0	1.5
Quizalofop-p-ethyl	1	92.4	4.6	86.6	4.6	118.9	2.8	102.1	2.2	109.4	3.9	100.9	5.1
	0.1	89.0	9.0	72.5	11.1	116.0	8.0	89.0	2.7	83.2	8.3	97.0	4.4
	0.01	100.2	15.3	67.4	12.2	98.6	19.4	92.9	0.6	95.3	3.1	102.6	3.2
Quizalofop (acid)	1	87.4	1.5	99.8	2.6	108.3	1.0	106.2	2.2	109.5	2.2	104.9	0.2
	0.1	90.0	3.0	100.8	3.2	106.6	1.6	94.2	2.0	100.2	1.7	100.2	0.8
	0.01	100.4	0.3	102.8	3.4	116.2	1.7	107.0	0.7	112.4	1.4	111.6	1.8

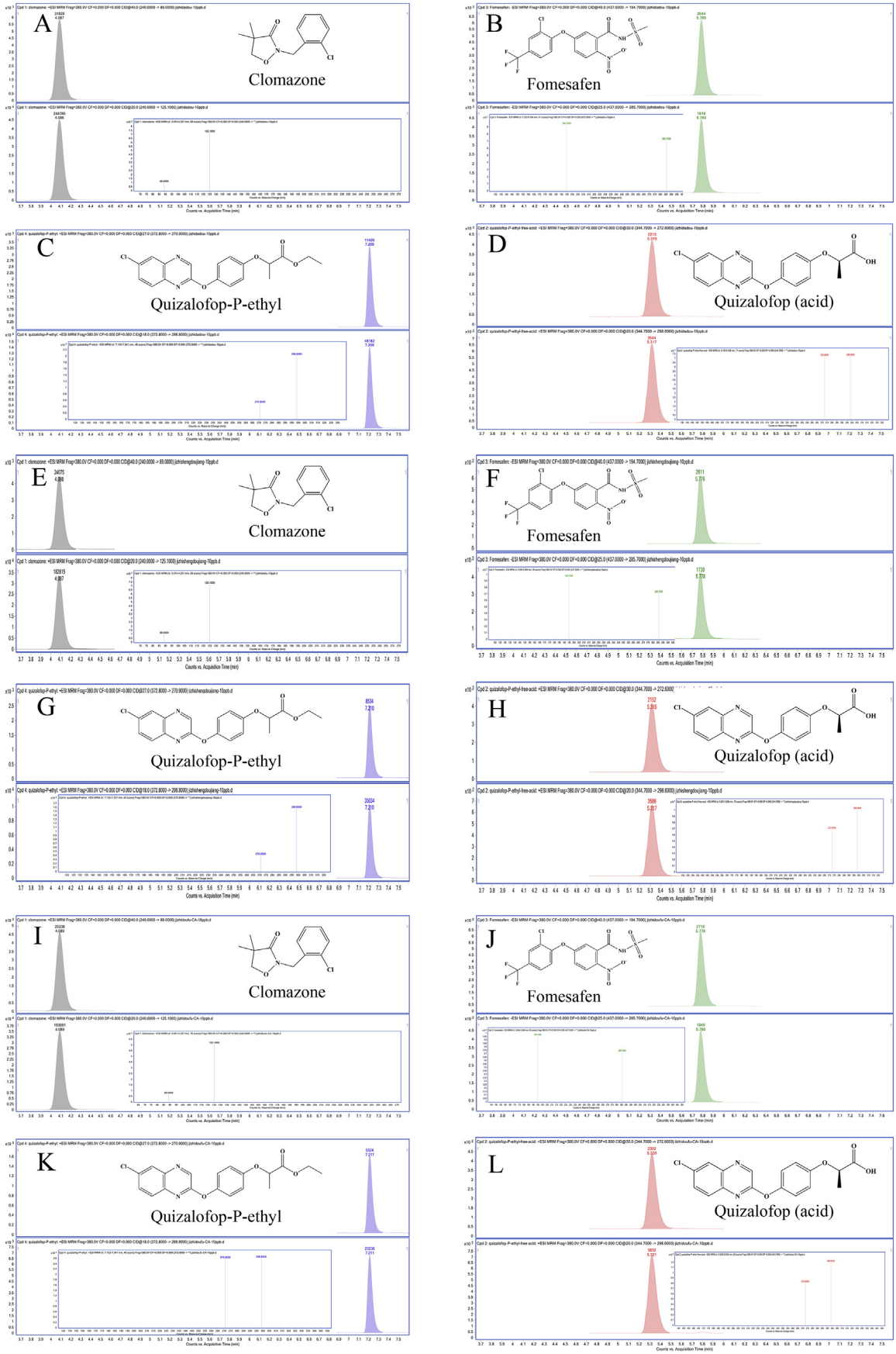

Fig. 1. UPLC-MS/MS chromatograms and mass spectra of the (A–D) soybeans spiked at 10 μg/kg, (E–H) raw soymilk spiked at 10 μg/kg, and (I–L) tofu spiked at 10 μg/kg.

Table 3
The residues of the four compounds in tofu products (mg/kg) during tofu processing (n = 3).

Processing steps	Processed commodity	Clomazone (mean ± SD)	Fomesafen (mean ± SD)	Quizalofop-p-ethyl (mean ± SD)	Quizalofop (acid) (mean ± SD)
	Soybeans	10.818 ± 0.45	7.349 ± 0.18	4.995 ± 0.03	8.529 ± 0.18
Washing	Washed soybeans	8.873 ^{a**} ± 0.35	6.137 ^{a**} ± 0.47	3.099 ^{a**} ± 0.19	7.101 ^{a**} ± 0.06
	Washed water	0.437 ^{b***} ± 0.05	0.627 ^{b***} ± 0.27	0.418 ^{b***} ± 0.12	0.384 ^{b***} ± 0.04
Soaking	Soaked soybeans	5.426 ^{a**} ± 0.01	1.720 ^{a**} ± 0.12	1.195 ^{a**} ± 0.08	6.172 ^{a*} ± 0.03
	Soaking water	0.687 ^{b***} ± 0.04	3.216 ^{b**} ± 0.21	0.169 ^{b***} ± 0.02	2.120 ^{b***} ± 0.01
Grinding and filtering	Raw soymilk	0.582 ^{a***} ± 0.03	0.097 ^{a***} ± 0.01	0.062 ^{a***} ± 0.01	0.692 ^{a***} ± 0.03
	Okara	1.346 ^{b***} ± 0.01	0.248 ^{b**} ± 0.03	0.386 ^{b***} ± 0.03	1.463 ^{b***} ± 0.05
Cooking	Cooked soymilk	0.592 ± 0.03	0.106 * ± 0.01	0.071 * ± 0.01	0.753 * ± 0.04
Coagulating	Tofu pudding (Vinegar)	1.235 ^{a*} ± 0.20	0.248 ^{a**} ± 0.02	0.201 ^{a*} ± 0.04	1.430 ^{a*} ± 0.26
	Tofu supernatant (Vinegar)	0.250 ^{b**} ± 0.00	0.051 ^{b**} ± 0.00	0.003 ^{b**} ± 0.00	0.446 ^{b**} ± 0.00
Squeezing	Tofu (Vinegar)	3.176 ** ± 0.14	0.607 ** ± 0.07	0.574 * ± 0.08	3.198 * ± 0.23
Coagulating	Tofu pudding (Brine)	1.517 ^{a*} ± 0.26	0.245 ^{a*} ± 0.06	0.199 ^{a*} ± 0.04	1.691 ^{a*} ± 0.32
	Tofu supernatant (Brine)	0.198 ^{b**} ± 0.00	0.041 ^{b**} ± 0.00	0.001 ^{b**} ± 0.00	0.362 ^{b**} ± 0.00
Squeezing	Tofu (Brine)	3.896 *** ± 0.26	0.663 *** ± 0.05	0.643 *** ± 0.04	3.625 *** ± 0.22
Coagulating	Tofu pudding (Gypsum)	1.586 ^{a**} ± 0.24	0.249 ^{a*} ± 0.06	0.208 ^{a*} ± 0.04	1.759 ^{a**} ± 0.28
	Tofu supernatant (Gypsum)	0.246 ^{b**} ± 0.00	0.061 ^{b*} ± 0.00	0.002 ^{b**} ± 0.00	0.514 ^{b**} ± 0.01
Squeezing	Tofu (Gypsum)	2.473 ** ± 0.16	0.425 * ± 0.03	0.459 ** ± 0.03	2.401 * ± 0.13

Note: * Indicates a significant difference of the residues of the four compounds in tofu product of the step versus the previous step (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). The different letters indicate a significant difference ($P < 0.05$) between the effects of same processing step, while the same letter indicates no significant difference observed.

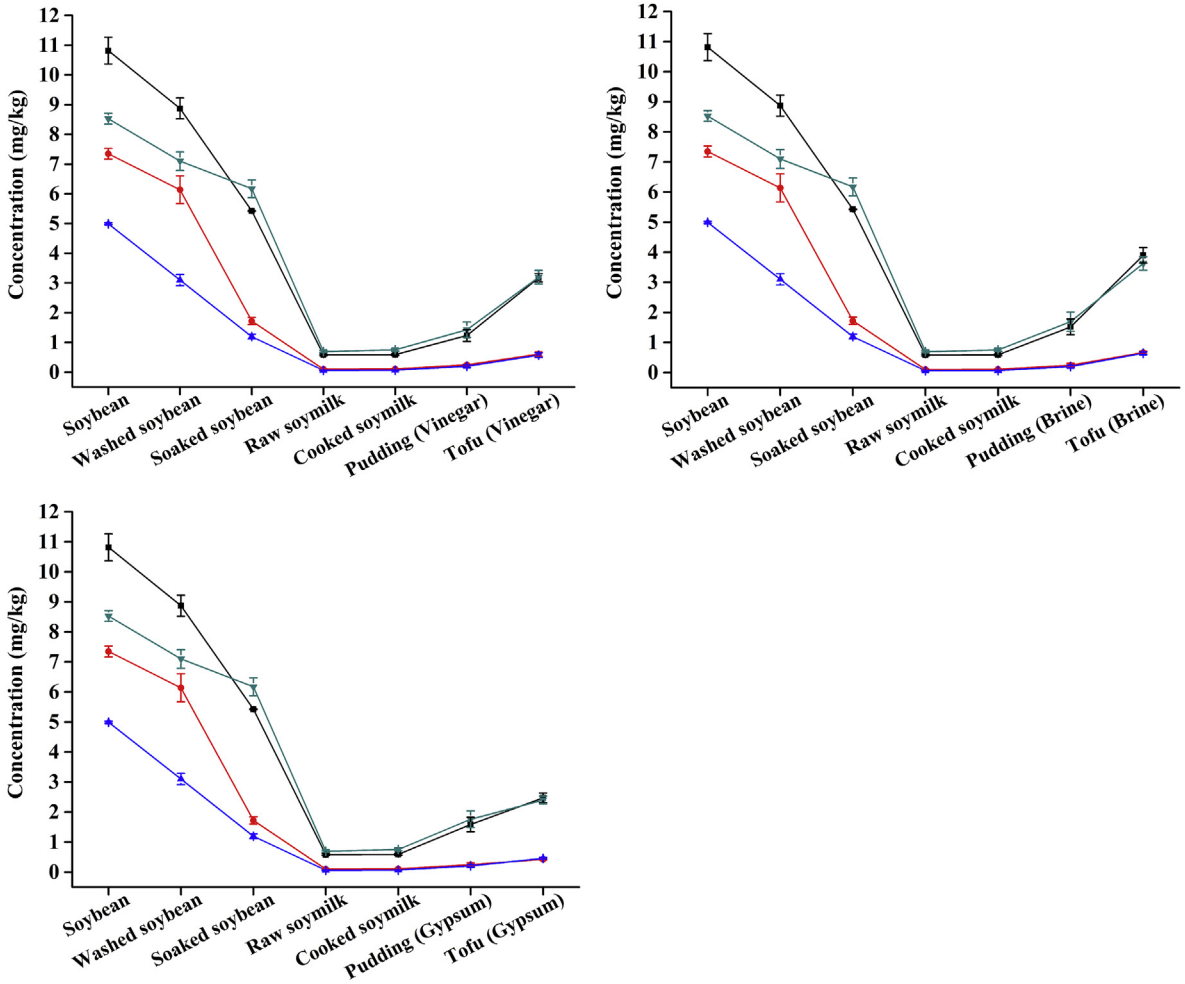

Fig. 2. Trend showing the content of the four compounds in each product during tofu (tofu (vinegar); tofu (brine); tofu (gypsum)) processing. Black line: clomazone; Green line: fomesafen; Red line: quizalofop-P-ethyl; Blue line: quizalofop (acid). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

In the present study, quizalofop-p-ethyl exhibited the highest increase, possibly owing to its higher log Kow (4.61). Moreover, some pesticide residues were still distributed in the supernatant after the coagulating step. It was observed that 42.3% clomazone, 47.8% fomesafen, 3.5% quizalofop-p-ethyl, and 59.2% quizalofop (acid) of the total pesticide residues of cooked soymilk were present in the vinegar supernatant. In the other two types of supernatants, these values were: 33.4%, 38.7%, 1.4%, and 48.1% in the case of brine, and 41.6%, 57.5%, 2.8%, and 68.3% in the case of gypsum (Table 3 and Fig. 2). The amount of pesticide residues in the supernatant removed by coagulating was > 96% for quizalofop-p-ethyl, which could also be attributed to its high log Kow value, indicating that quizalofop-p-ethyl preferentially accumulates in the lipophilic part rather than in water (Duan et al., 2017; OECD, 2008).

3.2.6. Squeezing

The final step of tofu production is squeezing the pudding into a mold. As shown in Table 3 and Fig. 2, after squeezing, the residues of the four compounds in tofu (vinegar) increased by 157.2%, 144.8%, 185.6% and 123.6%, respectively, compared to those in the pudding. For other tofu types, the increases were 156.8%, 170.6%, 223.1%, and 114.4% in the case of tofu (brine), and 55.9%, 70.7%, 120.7%, and 36.5% in the case of tofu (gypsum), respectively. Abou-Arab (1997) reported that pressing increased DDT residues in cheese, due to its lipid solubility. The pesticide residues in tofu were once again concentrated by squeezing, which might be the result of water loss. In the present study, the concentrations of the four compounds increased to different degrees during squeezing. The difference in the average increase among the four substances could be explained by the differences in their log Kow values. Therefore, the quizalofop-p-ethyl in the three tofu types showed the highest increase, which could be attributed to its higher log Kow (4.61) value. The level of increase observed for the four compounds in the three different tofu types was inconsistent during the squeezing process. This might be related to the different water-holding capacities of the different coagulants used in this study. The lowest increase in the residues of the four compounds was observed in gypsum tofu due to its superior water-holding capacity during the pressing process.

3.3. PFs

Table 4 shows the PFs of washing, soaking, and grinding and filtering for the four compounds, ranging from 0.62 to 0.84, 0.28 to 0.87, and 0.04 to 0.11, respectively. All these values were < 1, indicating that these processes facilitated pesticide dilution and thereby decreased exposure risk. The PFs of grinding and filtering for the four compounds were notably lower than those of the other processes. The main reason is water dilution and okara dislodgment. Table 4 showed that the PFs of cooking for the four compounds were 1.02, 1.09, 1.15, and 1.09, respectively. Heating resulted in the evaporation of water and a slight increase in the concentration of the four compounds. During coagulating and squeezing, the PFs of the four compounds ranged from 1.90 to 2.93 and 1.36 to 3.23, respectively, demonstrating that the two steps led to an obvious accumulation of the four analytes. After the coagulating step, the squeezing procedure also showed similar effects on the pesticide levels concentrated during coagulating. The efficiency of the entire fomesafen and quizalofop-p-ethyl pesticide residue elimination process is shown in detail in Table 3. The decrease ranged from 87% to 94%. The PFs of the overall process for the four compounds in three tofu types were within the range of 0.06–0.43, indicating that the entire process could cause a pesticide residue reduction during tofu production (Table 4).

4. Conclusion

In this study, changes in residual clomazone, fomesafen, quizalofop-p-ethyl, and quizalofop (acid) during tofu production were investigated. The concentration of residues of these four compounds significantly reduced in tofu after processing. The dissipation of pesticide residues was impacted by the processing steps to varying degrees. The transfer behavior of the pesticides could be attributed to their physicochemical properties such as Sw, log Kow, and vapor pressure. The PF of soybean samples after washing, soaking, grinding, and filtering was generally less than 1, while the PF after cooking, coagulating, and squeezing was larger than 1. Grinding and filtering were the most efficient processing steps to reduce the residues of the four compounds by 89.3%–94.8%. During the whole process, the PF of the four compounds was less than 0.43. The entire process showed an obvious efficiency in the elimination of fomesafen and quizalofop-p-ethyl residues. The findings of this study could provide additional information for expanding our knowledge about pesticides and pollutants in tofu products.

Table 4
Processing factors (PFs) of the four compounds in tofu processing.

Processing steps	Products	Processing factors			
		Clomazone	Fomesafen	Quizalofop-p-ethyl	Quizalofop (acid)
Washing	Washed soybeans	0.82	0.84	0.62	0.83
	Washed Water	0.05	0.10	0.13	0.05
Soaking	Soaked soybeans	0.61	0.28	0.39	0.87
	Soaking Water	0.13	1.87	0.14	0.34
Grinding and filtering	Raw soymilk	0.11	0.06	0.05	0.11
	Okara	0.25	0.14	0.32	0.24
Cooking	Cooked soymilk	1.02	1.09	1.15	1.09
Coagulating	Tofu pudding (Vinegar)	2.09	2.34	2.83	1.90
	Tofu supernatant (Vinegar)	0.42	0.48	0.04	0.59
Squeezing	Tofu (Vinegar)	2.57	2.45	2.86	2.24
	Tofu (Vinegar)[a]	0.29	0.08	0.11	0.37
Coagulating	Tofu pudding (Brine)	2.56	2.31	2.80	2.25
	Tofu supernatant (Brine)	0.33	0.39	0.01	0.48
Squeezing	Tofu (Brine)	2.57	2.71	3.23	2.14
	Tofu (Brine)[a]	0.36	0.09	0.13	0.43
Coagulating	Tofu pudding (Gypsum)	2.68	2.35	2.93	2.34
	Tofu supernatant (Gypsum)	0.42	0.58	0.03	0.68
Squeezing	Tofu (Gypsum)	1.56	1.71	2.21	1.36
	Tofu (Gypsum)[a]	0.23	0.06	0.09	0.28

[a] The PFs of the whole tofu process.

CRediT authorship contribution statement

Jia Zhang: Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **Min-Min Li:** Conceptualization, Methodology, Validation, Formal analysis, Writing - review & editing, Funding acquisition. **Rui Zhang:** Investigation, Data curation. **Nuo Jin:** Investigation, Validation, Methodology. **Rui Quan:** Conceptualization, Software, Validation. **De-Yong Chen:** Investigation, Data curation. **Frédéric Francis:** Writing - review & editing. **Feng-Zhong Wang:** Resources, Supervision, Project administration, Funding acquisition. **Zhi-Qiang Kong:** Methodology, Software, Formal analysis, Data curation, Writing - review & editing, Funding acquisition. **Bei Fan:** Resources, Supervision, Project administration, Funding acquisition, Writing - review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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