Songklanakarin J. Sci. Technol.
46 (3), 270–278, May – Jun. 2024

http://www.sjst.psu.ac.th

Original Article

Minimization of hazard risk from fresh-cut galangals using natural biosurfactant fermented from sour cherry

Priyada Sittisart[1], Piyawan Gasaluck[2], Janjira Pinyo[2], Patcharawan Sittisart[3], and Thitikorn Mahidsanan[4*]

[1] *Department of Agricultural Technology, Faculty of Science and Arts, Burapha University, Chanthaburi Campus, Tha Mai, Chanthaburi, 22170 Thailand*

[2] *School of Food Technology, Institute of Agricultural Technology, Suranaree University of Technology, Mueang, Nakhon Ratchasima, 30000 Thailand*

[3] *Division of Environmental Science, Faculty of Liberal Arts and Science, Sisaket Rajabhat University, Mueang, Si Sa Ket, 33000 Thailand*

[4] *Department of Food Science and Technology, Faculty of Agricultural Innovation and Technology, Rajamangala University of Technology Isan, Mueang, Nakhon Ratchasima, 30000 Thailand*

Received: 15 September 2023; Revised: 21 January 2024; Accepted: 19 March 2024

Abstract

This investigation aimed to assess the effects of washing fresh galangal rhizomes on their quality and safety using natural based non-ionic biosurfactant (BSF, 50-200 mg L^{-1}) produced from waste sour cherries fermentation. Chlorine-free water and 8 mg L^{-1} chlorine dioxide were used as control sanitizer treatments. The efficiency of removing *S. rolfsii* mycelium, dimethoate, and lead (Pb) was tested. The results revealed that 200 mg L^{-1} BSF reduced the membrane fluidity, and damaged vegetative hypha of *S. rolfsii*. Due to removing pesticide and heavy metals, 200 mg L^{-1} BSF was found to be the most effective in controlling dimethoate and Pb levels during storage at 4 °C for 7 days, which met the standard of maximum residue level (MRL). It might be concluded that BSF could remove all hazards from contaminated fresh galangals. The washing procedure prototype in this study could be practically used as a sanitation standard operating procedure (SSOP) in food safety management in fresh-cut industry.

Keywords: galangal, biosurfactant, sour cherry, *Sclerotium rolfsii*, dimethoate, lead, food safety management

1. Introduction

Fresh-cut produce is prevalently obtained from leafy vegetables, tropical fruits, herbs and spices. However, microbial outbreaks still remain (Mritunjay & Kumar, 2015) due to improper post-harvest handling and washing steps (Murray, Wu, Shi, Jun Xue, & Warriner 2017). Fresh-cut products have been linked to numerous pesticides and heavy metals in different regions of the world (Ahmed, Siddique, Rahman, Bari, & Ferdousi, 2019). According to the 2017 FDA food code (2013), galangals (*Alpinia galanga* (L.) Willd.) have a high risk of pathogens and filth. Galangal extracts have been used in food and pharmaceutical formulations due to their antimicrobial properties (Oonmetta-aree, Suzuki, Gasaluck, & Eumkeb, 2006). However, the rhizome of edible galangal is contaminated with a soilborne pathogen, *Sclerotium rolfsii* (Xie, Huang, & Vallad, 2014), which possesses a special hazardous structure called sclerotia

*Corresponding author
Email address: thitikorn_mahi@hotmail.com; thitikorn.ma@rmuti.ac.th

(mycelium clump). Meanwhile, organophosphate pesticides, especially dimethoate, as well as lead (Pb), persist and have adverse effects in soil layer and irrigation (Lin, Tsai, Wu, Yeh, & Saalia, 2006; Thummajitsakul *et al.*, 2018).

Rinsing and soaking steps are considered as critical control points (CCPs) in fresh-cut produce processing for food safety management based on SSOP (Tzamalis, Panagiotakos, & Drosinos, 2016). The International Commission on Microbiological Specifications for Foods (ICMSF) proposed a preventative action for managing hazard levels in food products, which must rely on the significant concept of food safety objective (FSO) (Augustin & Guillier, 2018). For this reason, aqueous chlorine dioxide (ClO_2) has been recognized as a sanitizer in washing steps (Malka & Park, 2022). The ClO_2 is permitted by the FDA according to the food safety policy of Food safety management (ISO 22000). Unfortunately, owing to safety and efficacy concerns, the use of chlorine for the sterilization of fresh-cut produce has been banned in many countries, such as Belgium, Switzerland, and Netherlands (Deng, Mujumdar, Pan, Vidyarthi, Xu, Zielinska, & Xiao, 2020).

Biosurfactants (BSF) have been applied as an alternative natural-based sanitizer in potato washing in the production process to decrease microbial load (Mule & Bhathena, 2012). BSFs are FDA-approved wetting agents and solubilizers, and are eco-friendly with low acute mammalian toxicity (Nitschke & Costa, 2007). Additionally, low-cost renewable substrates for natural microbial fermentation, such as orange peelings and banana waste, have been reported (George & Jayachandran, 2009). The BSF-substances mentioned above are mostly amphiphilic compounds containing both hydrophobic and hydrophilic moieties owing to BSF fermentation (Nitschke & Costa, 2007). Carbon substrates from agricultural waste have been optimally metabolized by predominant microorganisms, especially *Bacillus subtilis* and *Lactobacillus plantarum* (Sharma, Soni, Kaur, & Kaur, 2014; Sittisart & Gasaluck, 2022). Therefore, it is possible to apply these as sanitizing agents for washing fresh produce.

The main purpose of post-harvest washing is to prevent cross-contamination. Challenges in validating wash processes were indicated by a working group that was established to define validation procedures (Murray *et al.*, 2017). A challenge test based on artificial inoculation of fresh produce with surrogate microorganisms could be generally implemented (Beuchat, Harris, Ward, & Kajs, 2001; Busta *et al.*, 2003; Chalmers *et al.*, 2022). Truchado, Gómez-Galindo, Gil, and Allende (2023) recently demonstrated sodium hypochlorite and chlorine dioxide as sanitizers on testing artificially contaminated leafy greens for the growth potential of *L. monocytogenes* and *Escherichia coli* O157:H7.

So far, very few studies have reported on natural-based BSF for effects on the reduction of hazard risks in galangals. With this background, the aim of this study was to ascertain the efficacy of natural-based sanitizer (non-ionic biosurfactant; BSF) produced from waste sour cherries fermentation (Gasaluck, 2011; Sittisart, Mahidsanan, & Gasaluck, 2016). The results demonstrated the minimization of *S. rolfsii*, dimethoate, and Pb under artificial contamination. The experiment was aligned with the existing applicable regulations and it achieved a good practice prototype of fresh-produce washing procedure.

2. Materials and Methods

2.1 Galangal preparation

Galangal was obtained from a local market (Suranakhon market, Nakhon Ratchasima province, Thailand). One kilogram of galangal was rinsed with tap water for 2 min at 30 °C.

2.2 *S. rolfsii* mycelium preparation

S. rolfsii DOAC 2312 was obtained from the Plant Protection Research and Development Office, Department of Agriculture, Thailand. It was cultured on potato dextrose agar (PDA) with 10% (v/v) tartaric acid at 25 ° C, stored at 4 ° C and sub-cultured monthly. *S. rolfsii* was cultured on PDA plate then incubated at 25°C for 7 days. A 5 mm diameter agar piece with mycelium was moved into a flask containing 50 mL PDB and cultured in a shaking incubator at 125 rpm and 25 °C for 7 days. The mycelia were collected on Whatman filter paper No.1. Twenty grams of wet mycelium was used for further tests (Kishore, Pande, Rao, & Podile, 2005).

2.3 *S. rolfsii* mycelium challenge test

Twenty grams of dry weight basis mycelium was suspended into 3 L of 0.85% (w/v) sodium chloride solution. One kilogram of galangal was dipped into that suspension for 20 sec and incubated at 25 °C for 72 h.

2.4 Dimethoate and Pb challenge test

One kilogram of galangal was dipped separately into 3 L of the 0.2 mg kg^{-1} dimethoate solution for 2 min. For the Pb-treated samples,1 kg galangal was dipped into 3 L of 1.0 mg kg^{-1} lead (II) acetate trihydrate in distilled water for 2 min (Beyer & Biziuk, 2008). The solution was allowed to absorb into the galangal before drying for 60 min at room temperature, and then transfering to the washing process.

2.5 BSF (sanitizing agent) washing treatment

The production of BSF was performed as in our previous experiment (Gasaluck, 2011; Sittisart, Mahidsanan, & Gasaluck, 2016). The effectiveness of BSF washing was evaluated (James, Ngarmsak, & Rolle, 2010). The impurities in galangal samples were rinsed out with water for 2 min before being soaking in sanitizing agents as follows: chlorine-free water (CFW), 8 mg L^{-1} aqueous chlorine dioxide (ClO_2), or BSF at 50, 100, 150 or 200 mg L^{-1} for 5 min. The samples were drained aseptically for 30 min, then packed in perforated plastic bags (LLDPE) and stored at 25 °C and 4 °C. The population of mold was monitored on days 0 and 7, and quantitative analyses of the reduction of pesticide and heavy metals in galangal were performed after washing.

2.6 Analysis of microbial risk level in galangals

All samples were subjected to serial dilutions with 0.1% (w/v) sterile peptone water. Aliquots of 0.1 mL samples were then spread on PDA and incubated in darkness at 25 °C for 72 h (USFDA 2001). Microbial risk level was calculated

according to the food safety objective (FSO) value by the following equation (Cole, 2004).

$$H0 - \sum R + \sum I \leq FSO$$

Here FSO is defined as the food safety objective, H0 is the initial level of the hazard, ∑R is the total (cumulative) reduction of the hazard and ∑I (total cumulative) is the increase of the hazard.

2.7 Analysis of membrane fluidity

The change in membrane dynamics was carried out by DPH fluorescence probe according to Mahidsanan, Gasaluck, and Eumkeb (2017). Various concentrations of aqueous BSF (50-200 mg L^{-1}) were tested with *S. rolfsii* mycelium.

2.8 Analysis of morphological changes in sclerotia by scanning electron microscopy (SEM)

S. rolfsii was cultured on PDA at 25 ℃ for 14 days, then washed with 0.05 mM PBS. Sclerotia were added into a 125 mL flask containing 50 mL of PDB and treated with a series of sanitizing agents, followed by shaking incubation (125 r.p.m.) at 25 ℃ for 3 days. All samples were washed twice with 0.05 mM PBS, then fixed with 2 % (w/v) glutaraldehyde for 18 h. The sclerotia were subjected to dehydration with an increasing concentration of ethanol (0 - 100 % v/v) and dried at 50 °C overnight (Ordóñez-Valencia *et al.*, 2015). The dehydrated sclerotia were placed on gold-coated staff base, with untreated sclerotia serving as a control in this experiment. The morphological changes were observed under scanning electron microscope (SEM; JEOL JSM-6010lV).

2.9 Analysis of pesticide residues in galangals

Twenty-five grams of galangal were extracted with 100 mL of a mixture (acetone, dichloromethane and sodium chloride at the ratio of 5:4:1) at 100 ℃ for 4 h, and homogenized at 11,000 rpm for 2 min. Fifteen grams of anhydrous sodium sulfate was added into the supernatant, shaken for 1 min, filtrated through cotton and eluted with acetone. The pesticide residue was detected by a chromatography-flame photometric detector (Agilent Gas Chromatography, Column: DB 1701 J&W 0.25mm x 250 µm x 30 m), model 6890N (Parveen & Masud, 2002).

2.10 Analysis of Pb residues in galangals

Half a gram of galangal dried at 450 ℃ was digested in polytetrafluoroethylene and incubated at 95 ℃ in a water bath for 2 h. The volume was adjusted to 25 mL with deionized water, then the samples were filtered with Whatman paper No. 42. Then 20 µL of filtrates and of the standard solution were each injected to Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The gas flow rate used was 0.9 min L^{-1} with a makeup gas rate of 0.2 min L^{-1}, the applied power was 1500 W, and the monitored plasma zone was 7 mm above the upper load coil (Zhong, Ren, & Zhao, 2016).

2.11 Functional characterization of washing agents on dimethoate and Pb minimization by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectrometry

The vibration frequency changes in dimethoate and Pb in BSF were determined by ATR-FTIR (Bruker, Karlsruhe, Germany). For 5 min, 0.2 mg L^{-1} dimethoate and 1.0 mg L^{-1} Pb solution were alternately subjected to aqueous 8 mg L^{-1} ClO_2 and 50-200 mg L^{-1} BSF. The vibration frequency was displayed under the functional group and recorded in a controlled chamber at 25 ℃. Each spectrum was collected over the wave number range 4000-400 cm^{-1} by averaging 32 scans at 4 cm^{-1} resolution. The spectral data were analyzed by OPUS 7.0 software.

2.12 Statistical analysis

The quantitative experiments were performed in triplicates, and the results are presented as the mean ± standard deviation (SD). The significance of differences between treatments at all times was determined by one-way ANOVA and Duncan's multiple range test for $P < 0.05$, using SPSS software (Version 17.0, Chicago, IL, USA).

3. Results and Discussion

The effectiveness of BSF washing was assessed according to FSO. The reduction of hazard risks (*S. rolfsii*, dimethoate, and Pb) met MRL standard requirement guideline of food safety management system, as demonstrated below.

3.1 The effectiveness of BSF washing in *S. rolfsii* reduction

Table 1 shows that after washing with varying BSF concentrations, the ∑R of galangal were in conformity with FSO standard requirement (< 4.0 log CFU g^{-1}, Kenneth, 2013). Mule and Bhathena (2012) reported that BSF 10% (v/v) induced spore lysis of *Aspergillus parasiticum* NCIM 898 in potato tubers. However, contact times are also needed (Kumar, Dubey, Tiwari, Tripathi, & Sinha, 2007). Based on food safety management system of the fresh-cut production line (Tzamalis *et al.*, 2016), washing procedure should be effective in minimizing risks (CP/CCP standardized MRL requirement). Chlorine-based compounds are often selected to control microbial hazards in food during washing, but some pathogens have developed resistance (Gu *et al.* 2020). Other disinfectants might be able to reduce sanitizer-resistant microbiome. The results of this study showed that soaking with 50-200 mg L^{-1} BSF for 5 min had the potential to control risk factors during the washing step that aligned with FSO. However, to make sure one might consider observations during storage, after washing, and in the results a lower ΣR means a higher safety level. This suggests that soaking with 100-200 mg L-1 BSF for 5 min was able to reduce *S. rolfsii* risk in this product during storage at 4 °C.

Table 1. Microbial quality of perforated plastic packaged galangal after washing and storage at 4 °C and 25° C for 7 days

Washing agent (mg L^{-1})	log CFU g^{-1}								
	After washing			Storage at 4 °C			Storage at 25 °C		
	Initial	Reduction	∑R	Initial	Reduction	∑R	Initial	Reduction	∑R
Control	6.08	0.00	8.16	7.46	-1.38	11.62	9.51	-3.43	13.67
CFW	5.33	0.75	7.41	6.07	0.01	9.48	9.13	-3.05	12.54
ClO_2 8	0.00	6.08	2.08	5.44	0.64	1.44	6.67	-0.59	2.67
BSF 50	0.00	6.08	2.08	4.90	1.18	0.90	6.69	-0.61	2.69
BSF 100	0.00	6.08	2.08	3.73	2.35	-0.27	4.90	1.18	0.90
BSF 150	0.00	6.08	2.08	3.44	2.64	-0.56	4.53	1.55	0.53
BSF 200	0.00	6.08	2.08	3.40	2.68	-0.60	4.40	1.68	0.40

The equation H0 - ∑R + ∑I ≤ FSO; the hazard (*S. rolfsii* viability) ≤ 4 log CFU g^{-1}, H0 = the initial level of the hazard, ∑R is the total (cumulative) reduction of the hazard, and ∑I is the increase of the hazard. A lower ∑R indicates a higher safety level.

3.2 The effect of BSF on *S. rolfsii* membrane fluidity

Figure 1 shows a significant increase in the fluorescence intensity in the mycelium treated with 50-200 mg L^{-1}BSF and with 8 mg L^{-1} ClO_2 compared to that of control ($P < 0.05$). In addition, the samples treated with 150 and 200 mg L^{-1} BSF had higher fluorescence intensity than with ClO_2 treatment. This might have been due to the electrostatic and hydrophobic chain of nonionic BSF interacting with adjacent phospholipid bilayer via the wall of chitin, β-1,6-glucan, β-1,3-glucan and mannoprotein of the cell membrane (Fatma, Panda, & Beg, 2016). Moreover, the molecular hydro phobicity, adsorption ability and electron density of a polyether and/or polyhydroxyl are the main key factors influencing the antifungal activity of BSF. Once the membrane became misshapen, it dispersed the probe via hydrocarbon tail region of phospholipid bilayer because of high fluorescence intensity (Fesel & Zuccaro, 2016; Sung & Lee, 2010).

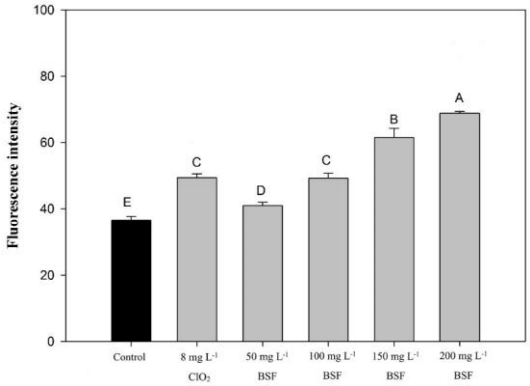

Figure 1. DPH fluorescence intensity at various concentrations of natural based BSF and aqueous chlorine dioxide. Different letters among the washing agents indicate significant fluorescence intensity differences ($P < 0.05$).

3.3 The effect of BSF on sclerotia morphological characteristics

As seen in Figure 2, the sclerotia diameter (mm) decreased with BSF concentration tested at 50, 100, 150 and 200 mg L^{-1} (0.92, 0.83, 0.74 and 0.62 mm, respectively). A damaged hypha wall appeared at 8 mg L^{-1} ClO_2 treatment (Figure 2b) compared to that of untreated sclerotia cells (Figure 2a), which corresponded to 150 and 200 mg L^{-1} BSF (Figure 2e, and 2f, respectively). As a result, 150 and 200 mg L^{-1} BSF affected the sclerotia structure by damaging the outer membrane and vegetative hypha (Ordóñez-Valencia *et al.*, 2015). Moreover, Figures 2E, 2e, and 2F, 2f show the damage in mycelium treated with 150-200 mg L^{-1} BSF, which were smaller than when using ClO_2. It should be noted that BSF may denature the lipid-protein interface of integral proteins, putting the central medulla of normal hyphae (vegetative hypha) at risk of disruption (Blum & Rodríguez-Kábana, 2004; Wu, Lu, Zhong, Schwarz, Chen, & Rao, 2019).

3.4 The effectiveness of BSF washing on dimethoate and Pb reduction

As seen in Table 2, a high reduction (%) in dimethoate and Pb was found in 150 and 200 mg L^{-1} BSF based on MRL (EC Regulation No 1097/2005 and CODEX, 2009, respectively). In this study, soaking in 150 and 200 mg L^{-1} BSF for 5 min was the most effective control measure in achieving the MRL of dimethoate and Pb.

3.5 Functional characterization of washing agents on dimethoate and Pb minimization

Table 3 demonstrates the ATR-FTIR wavenumber relevant to C-N-H functional group interaction with 200 mg L^{-1} BSF, which was at approximately 2,000-2,100 cm^{-1}. The band of 1,200-1,400 cm^{-1} reflected the aliphatic chain of BSF, which interacted with the C-N-H stretching and was clarified by the aliphatic BSF structure, which enhanced solubility on the left of methyl group, while methoxy group migrated to the N atom to water OH group (Fleming *et al.*, 2012; Wattanaphon, Kerdsin, Thammacharoen, Sangvanich, & Vangnai, 2008). This might be interpreted so that the mode of BSF action is to form a BSF-pesticide micellar complex, which interacts by hydrogen bonds with water molecules, resulting in a better solubilization of pesticides (López-Prieto, Moldes, Cruz, & Perez Cid, 2020). A lipopeptide biosurfactant obtained from *Bacillus amyloliquefaciens* C11 increased the solubility of chlorpyrifos, iprodione and atrazine

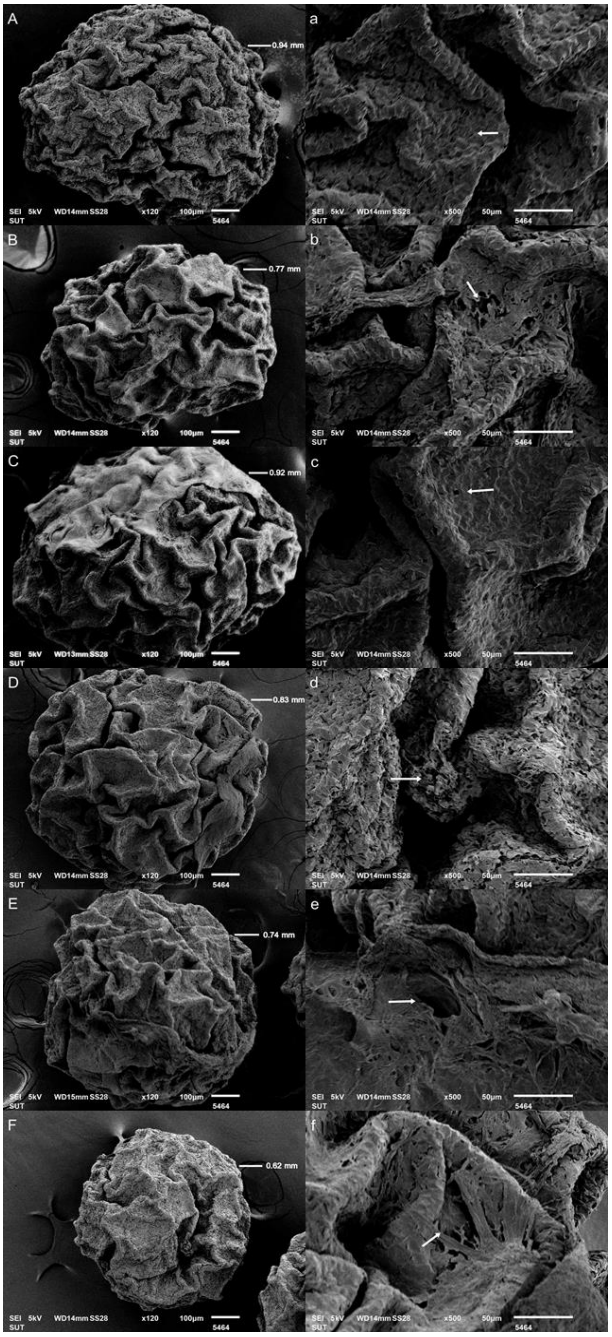

Figure 2. SEM images of morphological characteristics of mature sclerotia. Left panel: magnified image x120 (capital letters). Right panel: magnified image x500 (small letters). Untreated sclerotia (A, a), 8 mg L^{-1} ClO_2 treated (B, b), 50 mg L^{-1} BSF treated (C, c), 100 mg L^{-1} BSF treated (D, d), 150 mg L^{-1} BSF treated (E, e) and 200 mg L^{-1} BSF treated (F, f).

in water, indicating that it improved the bioavailability of pesticides and, consequently, the efficiency of bioremediation processes (Schalchli *et al.*, 2023). Furthermore, the possible characteristics of microbial strains as BSF producers could accelerate pesticide biodegradability (Pacwa-Płociniczak, Płaza, Piotrowska-Seget, & Cameotra, 2011).

As shown in Table 4, no peak for Pb^{2+} vibration (670-660 cm^{-1}) appeared at 150 and 200 mg L^{-1} BSF. This could be attributed to the removal of Pb^{2+} cations. The mechanism of Pb (II) adsorption on BSF might have been due to physical adsorption and complexation (BSF micelles) with functional groups and chemical reactions at the complex surface via binding with the hydrogen atom of water, which then would be separated (Pacwa-Płociniczak, *et al.*, 2011). According to da Rocha Junior *et al.* (2019), crude and isolated BSF could remove heavy metals from contaminated sand. They found that the removal rate for Zn and Cu ranged from 30 to 80%, while the optimum removal rate for Pb was approximately 15%. Surfactins with two -COO− groups can remove heavy metals by binding them through electrostatic interactions at pH levels higher than their pKa (Yu *et al.*, 2023). Sharma, Rekhi, and Debnath (2022) confirmed that surfactin extracted from *Staphylococcus sciuri subsp. rodentium* strain SE I could be used as an alternative biosorbent and function as a bioremediation agent. The application of BSFs in heavy metals bioremediation is a promising approach because of their ecofriendly nature and biodegradability. Several microorganisms have been investigated for BSF production (da Silva, da Silva, de Lima e Silva, Sarubbo, & de Luna, 2023). However, the potential of a surfactant varies by heavy metal. The removal of heavy metals depends on the category of BSF, its concentration, its interactions with additive substances (acids and bases) and the characteristics of materials (Ochoa-Loza, Noordman, Jannsen, Brusseau, & Maier, 2007).

4. Conclusions

BSF was produced from waste sour cherries by spontaneous fermentation, and the non-ionic surfactant could be used as a sanitizer for controlling the soilborne pathogen *S. rolfsii*, and dimethoate and Pb risks in minimally processed (fresh-cut) galangals to the acceptable standard level. The prototype of rinsing galangals with water for 2 min followed by soaking them with 200 mg L^{-1} BSF for 5 min could be a feasible SSOP-based practice in food safety management, especially in controlling *S. rolfsii*, dimethoate, and Pb.

Acknowledgements

This work was supported by Suranaree University of Technology (SUT 3-305-53-12-33). We also thank Miss Martha Maloi Eromine for editing the English language.

References

Additives, F. (2009). Codex general standard for contaminants and toxins in food and feed (Codex stan 193-1995). Rome, Italy: Food and Agriculture Organization of the United Nations.

Ahmed, S., Siddique, M. A., Rahman, M., Bari, M. L., & Ferdousi, S. (2019). A study on the prevalence of heavy metals, pesticides, and microbial contaminants and antibiotics resistance pathogens in raw salad vegetables sold in Dhaka, Bangladesh. *Heliyon*, *5*(2), e01205.

Table 2. Reduced levels of dimethoate and lead (Pb) after washing with chlorine dioxide (ClO_2) and various BSF concentrations

Washing agents (mg L^{-1})	Dimethoate*		Lead (Pb)*	
	Residue	Reduction	Residue	Reduction
	ppm	(%)	ppm	(%)
No-wash (control)	0.023±0.000[a]	0	0.57±0.04[a]	0
CFW	0.023±0.000[a]	0	0.29±0.07[b]	48.54
ClO_2 8	0.009±0.000[c]	60.87	0.23±0.05[cd]	65.50
BSF 50	0.011±0.000[b]	47.97	0.26±0.04[bc]	53.80
BSF 100	0.010±0.002[b]	50.87	0.20±0.03[bcd]	60.24
BSF 150	0.009±0.000[c]	60.29	0.19±0.04[cd]	66.08
BSF 200	0.009±0.000[c]	60.72	0.16±0.03[d]	71.35

* The standard MRL requirements of dimethoate and Pb were 0.2 and 1.0 mg L^{-1}, respectively. All values in the same column were significantly different as determined by the Duncan´s multiple range test ($P< 0.05$).

Table 3. Functional characterization of the washing agents; actions of chlorine dioxide (ClO_2) and various BSF concentrations on dimethoate minimization

Functional group	Frequency range	The wavenumber interaction between sanitizers and dimethoate (cm^{-1})					
		Untreated (control)	ClO_2 8 mg L^{-1}	BSF 50 mg L^{-1}	BSF 100 mg L^{-1}	BSF 150 mg L^{-1}	BSF 200 mg L^{-1}
OH Stretch	~3700	-	3779.30	3781.10	3783.67	3784.01	3779.29
N-H stretching	3300-3400	3319.31	3330.90	3318.74	3319.84	3318.99	3318.69
CO_2 formation, C-O bond broad	~2300	2360.20	2345.32	2345.64	2360.29	2360.25	2353.41
		2349.30			2349.26	2349.29	
		2325.41			2325.32	2325.43	
C-N-H stretching	2000-2100	2073.50	2047.69	2047.47	2047.85	2047.96	-
P-O-CH_3 and/or CO-N stretching	1600-1700	1636.02	1635.95	1636.09	1636.02	1636.16	1636.00
C=C stretching	~1500	-	1541.51	1541.94	-	-	1541.53
			1507.22				1507.24
-CH3 stretching	1470-1430	-	1457.37				1457.33
P=S	690-650	671.16	674.82	674.83	671.08	671.14	674.88
		664.36		667.14	664.22		
O-P-O bending	415-500	449.14	442.20	446.67	471.94	440.76	432.42

Table 4. Functional characterization of the washing agents; actions of chlorine dioxide (ClO_2) and various BSF concentrations on Pb minimization

Functional group	Frequency range	The wavenumber interaction between sanitizers and dimethoate (cm^{-1})					
		Untreated (control)	ClO_2 8 mg L^{-1}	BSF 50 mg L^{-1}	BSF 100 mg L^{-1}	BSF 150 mg L^{-1}	BSF 200 mg L^{-1}
water OH Stretch	~3700	3783.82	3779.28	3784.06	3780.73	3780.09	3783.50
alcohol OH stretch	3400-3200	3319.38	3319.66	3319.96	3319.26	3319.40	3330.87
CO_2 formation, C-O bond broad	~2300	2349.30	2351.86	2360.21	2349.29	2359.89	2350.44
		2325.38		2349.29	2325.37		
				2325.41			
C=O conjugate with carboxyl group or –(C=O)-OH	~1600	1636.04	1635.98	1636.09	1636.10	1636.03	1636.05
C=C stretching	~1500		1541.51	-		-	-
			1507.22				
-CH3, methyl group	1470-1430		1457.36	-		-	-
Pb^{2+} vibration	670-660	671.11	-	671.12	671.12	-	-
		664.27					
PO_4 bending	415-500	468.76	442.23	446.53	442.48	450.65	472.11

Augustin, J. C., & Guillier, L. (2018). Quantitative approaches for microbial risk management in the vegetable industry: case-studies of application of food safety objectives and other risk metrics in the vegetable industry. *Quantitative Methods for Food Safety and Quality in the Vegetable Industry*, 175-192.

Beuchat, L. R., Harris, L. J., Ward, T. E., & Kajs, T. M. (2001). Development of a proposed standard method for assessing the efficacy of fresh produce sanitizers. *Journal of Food Protection*, *64*(8), 1103-1109.

Beyer, A., & Biziuk, M. (2008). Methods for determining pesticides and polychlorinated biphenyls in food samples—problems and challenges. *Critical Reviews in Food Science and Nutrition*, *48*(10), 888-904.

Blum, L. E., & Rodríguez-Kábana, R. (2004). Effect of organic amendments on sclerotial germination, mycelial growth, and Sclerotium rolfsii-induced diseases. *Fitopatologia Brasileira*, *29*, 66-74.

Busta, F. F., Suslow, T. V., Parish, M. E., Beuchat, L. R., Farber, J. N., Garrett, E. H., & Harris, L. J. (2003). The use of indicators and surrogate microorganisms for the evaluation of pathogens in fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety*, *2*, 179-185.

Chalmers, R. M., Katzer, F., La Carbona, S., Lalle, M., Razakandrainibe, R., Robertson, L. J., . . . Mayer-Scholl, A. (2022). A guide to standardise artificial contamination procedures with protozoan parasite oocysts or cysts during method evaluation, using Cryptosporidium and leafy greens as models. *Food Control*, *134*, 108678.

Cole, M. (2004). Food safety objectives-Concept and current status. *Mitteilungen aus Lebensmitteluntersuchung and Hygiene, 95*, 13-20.

da Rocha Junior, R. B., Meira, H. M., Almeida, D. G., Rufino, R. D., Luna, J. M., Santos, V. A., & Sarubbo, L. A. (2019). Application of a low-cost biosurfactant in heavy metal remediation processes. *Biodegradation*, *30*, 215-233.

da Silva, R. R., da Silva, Y. A., de Lima e Silva, T. A., Sarubbo, L. A., & de Luna, J. M. (2023). Biosurfactants as an eco-friendly technology in heavy metal remediation. *Advancements in Biosurfactants Research* (pp. 225-235). Cham, Italy: Springer.

Deng, L. Z., Mujumdar, A. S., Pan, Z., Vidyarthi, S. K., Xu, J., Zielinska, M., & Xiao, H. W. (2020). Emerging chemical and physical disinfection technologies of fruits and vegetables: a comprehensive review. *Critical Reviews in Food Science and Nutrition*, *60*(15), 2481-2508.

European Community. (2009). Regulation EC No1097/2009 of the European Parliament and of the Council as regards maximum residue levels for dimethoate, ethephon, fenamiphos, fenarimol, methamidophos, methomyl, omethoate, oxydemeton-methyl, procymidone, thiodicarb and vinclozolin in or on certain products of 16 November 2009. *Official Journal of the European Union L, 301*, 1-17.

Fatma, N., Panda, M., & Beg, M. (2016). Ester-bonded cationic gemini surfactants: Assessment of their cytotoxicity and antimicrobial activity. *Journal of Molecular Liquids*, *222*, 390-394.

Fesel, P. H., & Zuccaro, A. (2016). β-glucan: Crucial component of the fungal cell wall and elusive MAMP in plants. *Fungal Genetics and Biology*, *90*, 53-60.

Food and Drug Administration. (2013). *Draft risk profile: Pathogens and filth in spices.* Montgomery, MD: Center for Food Safety and Applied Nutrition, Food and Drug Administration, U.S. Department of Health and Human Services.

Fleming, G. D., Celis, F., Aracena, A., Campos-Vallette, M., Aliaga, A. E., & Koch, R. (2012). Vibrational and scaled quantum chemical study of O, O-dimethyl S-methylcarbamoylmethyl phosphorodithioate, dimethoate. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *89*, 222-230.

Gasaluck P. (2011). *Biosurfactant from sour cherry (Prunus cerasus L.) conventional Fermentation*. Nakhon Ratchasima, Thailand: Suranaree University of Technology.

George, S., & Jayachandran, K. (2009). Analysis of rhamnolipid biosurfactants produced through submerged fermentation using orange fruit peelings as sole carbon source. *Applied Biochemistry and Biotechnology*, *158*, 694-705.

Gu, G., Ottesen, A., Bolten, S., Luo, Y., Rideout, S., & Nou, X. (2020). Microbiome convergence following sanitizer treatment and identification of sanitizer resistant species from spinach and lettuce rinse water. *International Journal of Food Microbiology*, *318*, 108458.

James, J. B., Ngarmsak, T., & Rolle, R. S. (2010). Processing of fresh-cut tropical fruits and vegetables: A technical guide. *RAP Publication No. 2010/16*. Bangkok, Thailand: Foood and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific.

Kenneth, Y. H. (2013). Revised guidelines for the assessment of microbiological quality of processed foods. *FDA Circular No. 2013-10*. Montgomery, MD: Food and Drug Administration.

Kishore, G. K., Pande, S., Rao, J. N., & Podile, A. R. (2005). Pseudomonas aeruginosa inhibits the plant cell wall degrading enzymes of Sclerotium rolfsii and reduces the severity of groundnut stem rot. *European Journal of Plant Pathology*, *113*, 315-320.

Kumar, R., Dubey, N. K., Tiwari, O. P., Tripathi, Y. B., & Sinha, K. K. (2007). Evaluation of some essential oils as botanical fungitoxicants for the protection of stored food commodities from fungal infestation. *Journal of the Science of Food and Agriculture*, *87*(9), 1737-1742.

Lee, W.-N., Huang, C.-H., & Zhu, G. (2019). Analytical methods for conventional and emerging disinfection by-products in fresh-cut produce. *Food Chemistry*, *291*, 30-37.

Lin, C. S., Tsai, P. J., Wu, C., Yeh, J. Y., & Saalia, F. K. (2006). Evaluation of electrolysed water as an agent for reducing methamidophos and dimethoate concentrations in vegetables. *International Journal of Food Science and Technology*, *41*(9), 1099-1104.

López-Prieto, A., Moldes, A. B., Cruz, J. M., & Perez Cid, B. (2020). Towards more ecofriendly pesticides: Use of biosurfactants obtained from the corn milling industry as solubilizing agent of copper oxychloride. *Journal of Surfactants and Detergents*, *23*(6), 1055-1066.

Mahidsanan, T., Gasaluck, P., & Eumkeb, G. (2017). A novel soybean flour as a cryoprotectant in freeze-dried Bacillus subtilis SB-MYP-1. *LWT*, *77*, 152-159.

Malka, S. K., & Park, M. H. (2022). Fresh produce safety and quality: chlorine dioxide's role. *Frontiers in Plant Science*, *12*, 775629.

Mritunjay, S. K., & Kumar, V. (2015). Potential hazards of microbial contamination associated with raw eaten salad vegetables and fresh produces. *Middle-East Journal of Scientific Research*, *23*(4), 741-749.

Mule, A. D., & Bhathena, Z. P. (2012). Control of Aspergillus parasiticum NCIM 898 infection in potato tubers using biosurfactant. *Asian Journal of Experimental Sciences*, *26*(1), 27-38.

Murray, K., Wu, F., Shi, J., Jun Xue, S., & Warriner, K. (2017). Challenges in the microbiological food safety of fresh produce: Limitations of post-harvest washing and the need for alternative interventions. *Food Quality and Safety*, *1*(4), 289-301.

Nitschke, M., & Costa, S. G. V. A. O. (2007). Biosurfactants in food industry. *Trends in Food Science and Technology*, *18*(5), 252-259.

Ochoa-Loza, F. J., Noordman, W. H., Jannsen, D. B., Brusseau, M. L., & Maier, R. M. (2007). Effect of clays, metal oxides, and organic matter on rhamnolipid biosurfactant sorption by soil. *Chemosphere*, *66*(9), 1634-1642.

Oonmetta-aree, J., Suzuki, T., Gasaluck, P., & Eumkeb, G. (2006). Antimicrobial properties and action of galangal (Alpinia galanga Linn.) on Staphylococcus aureus. *LWT-Food Science and Technology*, *39*(10), 1214-1220.

Ordóñez-Valencia, C., Ferrera-Cerrato, R., Quintanar-Zúñiga, R. E., Flores-Ortiz, C. M., Guzmán, G. J. M., Alarcón, A., . . . García-Barradas, O. (2015). Morphological development of sclerotia by Sclerotinia sclerotiorum: A view from light and scanning electron microscopy. *Annals of Microbiology*, *65*(2), 765-770.

Pacwa-Płociniczak, M., Płaza, G. A., Piotrowska-Seget, Z., & Cameotra, S. S. (2011). Environmental applications of biosurfactants: recent advances. *International Journal of Molecular Sciences*, *12*(1), 633-654.

Parveen, Z., & Masud, S. Z. (2002). Fungicide residues in apple and citrus fruits after post harvest treatment. *Biological Sciences-PJSIR*, *45*(4), 246-249.

Schalchli, H., Lamilla, C., Rubilar, O., Briceño, G., Gallardo, F., Durán, N., . . . Diez, M. C. (2023). Production and characterization of a biosurfactant produced by Bacillus amyloliquefaciens C11 for enhancing the solubility of pesticides. *Journal of Environmental Chemical Engineering*, *11*(6), 111572.

Sharma, P., Rekhi, P., & Debnath, M. (2022). Removal of heavy metal by biosurfactant producing novel halophilic staphylococcus sciuri subsp. rodentium strain SE I isolated from Sambhar Salt Lake. *ChemistrySelect*, *7*(37), e202202970.

Sharma, A., Soni, J., Kaur, G., & Kaur, J. (2014). A study on biosurfactant production in Lactobacillus and Bacillus sp. *Int. J. Curr. Microbiol. App. Sci*, *3*(11), 723-733.

Sittisart, P., & Gasaluck, P. (2022). Biosurfactant production by Lactobacillus plantarum MGL-8 from mango waste. *Journal of Applied Microbiology*, *132*(4), 2883-2893.

Sittisart P, Mahidsanan T., & Gasaluck P. (2016). Decontamination and quality safety control of chili (*Capsicum annuum* L.) after post-harvest using washing process with biosurfactant. *Agricultural Science Journal*, *47*(3), (Suppl.): 43-46.

Sung, W. S., & Lee, D. G. (2010). Antifungal action of chlorogenic acid against pathogenic fungi, mediated by membrane disruption. *Pure and applied chemistry*, *82*(1), 219-226.

Thummajitsakul, S., Subsinsungnern, R., Treerassapanich, N., Kunsanprasit, N., Puttirat, L., Kroeksakul, P., & Silprasit, K. (2018). Pesticide and heavy metal contamination: Potential health risks of some vegetables and fruits from a local market and family farm in Ongkharak district of Nakhon Nayok province, Thailand. *Pertanika Journal of Tropical Agricultural Science*, *41*(3), 987-1001.

Truchado, P., Gómez-Galindo, M., Gil, M. I., & Allende, A. (2023). Cross-contamination of Escherichia coli O157: H7 and Listeria monocytogenes in the viable but non-culturable (VBNC) state during washing of leafy greens and the revival during shelf-life. *Food Microbiology*, *109*, 104155.

Tzamalis, P. G., Panagiotakos, D. B., & Drosinos, E. H. (2016). A 'best practice score'for the assessment of food quality and safety management systems in fresh-cut produce sector. *Food Control*, *63*,179-186.

U.S. Food and Drug Administration. (2001). Bacteriological analytical manual, chapter 18, yeasts, molds and mycotoxins. Retrieved from https://www.fda.gov/food/laboratory-methods-food/bam-yeasts-molds-and-mycotoxins.

Wattanaphon, H. T., Kerdsin, A., Thammacharoen, C., Sangvanich, P., & Vangnai, A. S. (2008). A biosurfactant from Burkholderia cenocepacia BSP3 and its enhancement of pesticide solubilization. *Journal of Applied Microbiology*, *105*(2), 416-423.

Wu, D., Lu, J., Zhong, S., Schwarz, P., Chen, B., & Rao, J. (2019). Influence of nonionic and ionic surfactants on the antifungal and mycotoxin inhibitory efficacy of cinnamon oil nanoemulsions. *Food and Function*, *10*(5), 2817-2827.

Xie, C., Huang, C. H., & Vallad, G. E. (2014). Mycelial compatibility and pathogenic diversity among Sclerotium rolfsii isolates in the southern United States. *Plant Disease*, *98*(12), 1685-1694.

Yu, F., Du, Y., Guo, M., Zhao, M., Luo, L., Cui, T., . . . Long, X. (2023). Application of biosurfactant surfactin for the removal of heavy metals from contaminated water and soil via a micellar-enhanced ultrafiltration process. *Separation and Purification Technology, 327*, 124947.

Zhong, W. S., Ren, T., & Zhao, L. J. (2016). Determination of Pb (Lead), Cd (Cadmium), Cr (Chromium), Cu (Copper), and Ni (Nickel) in Chinese tea with high-resolution continuum source graphite furnace atomic absorption spectrometry. *Journal of Food and Drug analysis*, *24*(1), 46-55.

Ziyaina, M., Rajab, A., Alkhweldi, K., Algami, W., Al-Toumi, O., & Rasco, B.)2014(. Lead and cadmium residue determination in spices available in Tripoli City markets)Libya(. *African Journal of Biochemistry Research*, *8*)7(, 137-140.