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

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Abstract

This investigation aimed to assess the effects of washing fresh galangal rhizomes on their quality and safety using natural based non-ionic biosurfactant (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 (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, solubilizers, eco-friendly and have 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 post-harvest washing process 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 that sodium hypochlorite and chlorine dioxide were used as a sanitizer for testing the growth potential of artificially contaminated leafy greens with *L. monocytogenes and Escherichia coli* O157:H7.

So far, very few studies have reported on the effect of natural-based BSF 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 ºC.

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 ºC, stored at 4 ºC and sub-cultured monthly. *S. rolfsii* was cultured on PDA plate then incubated at 25ºC for 7 days. A 5 mm diameter agar piece with mycelium was moved into a flask containing PDB 50 mL and cultured in a shaking incubator at 125 r.p.m. at 25 ºC for 7 days. The mycelium was collected through 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 ^{o}C for 72 h.

2.4 Dimethoate and Pb challenge test

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

2.5 BSF (sanitizing agent) washing treatment

The production of BSF was obtained from 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 soaked in sanitizing agents as follows: chlorine-free water (CFW), 8 mg L^{-1} aqueous chlorine dioxide (ClO_2), BSF 50, 100, 150 and 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 ^{o}C and 4 ^{o}C. The population of mold was monitored on days 0 and 7, and a quantitative analysis 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 made from serial dilutions with 0.1% (w/v) sterile peptone water. Aliquots of 0.1 mL samples were then spread on PDA and incubated in a dark condition 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).

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

Where FSO is defined as the food safety objective, H0 is the initial level of the hazard, $\sum R$ is the total (cumulative) reduction of the hazard and $\sum I$ (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 BSF aqueous (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 °C for 14 days, then washed with 0.05 mM PBS. Sclerotia was 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 °C 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 samples (100 °C, 4 h) were extracted with 100 mL of a mixture (acetone, dichloromethane and sodium chloride at the ratio of 5:4:1, respectively) and homogenized at 11,000 r.p.m. for 2 min. Fifteen grams of sodium sulfate anhydrous 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 dried galangal samples (450 °C) were digested in polytetrafluoroethylene and incubated at 95 °C 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. Each of 20 µL filtrates and the standard solution were 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)

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 °C. Each spectrum was collected at the wave number 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 were presented as the mean ± standard deviation (SD). The differences between treatments at all times $P < 0.05$ were determined by one-way ANOVA and Duncan's multiple range test 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 on *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

209 microbial hazards in food during washing, but some pathogens have developed resistance (Gu et
210 al. 2020). Other disinfectants might be able to reduce sanitizer-resistant microbiome. The results
211 of this study showed that soaking with 50-200 mg L^{-1} BSF for 5 min had the potential to control
212 risk factors during the washing step that aligned with FSO. However, for ensure might consider
213 during storage, after washing, the results indicated that a lower ΣR means a higher safety level.
214 This suggests that soaking with 100-200 mg L-1 BSF for 5 min was able to reduce *S. rolfsii* risk
215 in this product during storage at 4 ^{o}C.
216

217 **3.2 The effect of BSF on *S. rolfsii* membrane fluidity**

218 Figure 1 shows a significant increase in the fluorescence intensity in the mycelium treated
219 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,
220 the samples treated with 150 and 200 mg L^{-1} BSF had higher fluorescence intensity than ClO_2.
221 This might have been due to the electrostatic and hydrophobic chain of nonionic BSF interactions
222 with adjacent phospholipid bilayer via the wall of chitin, β-1,6-glucan, β-1,3-glucan and
223 mannoprotein of the cell membrane (Fatma, Panda, & Beg, 2016). Moreover, the molecular
224 hydrophobicity, adsorption ability and electron density of a polyether and/or polyhydroxyl are the
225 main key factors influencing the antifungal activity of BSF. Once the membrane became
226 misshapen, it dispersed the probe via hydrocarbon tail region of phospholipid bilayer because of
227 high fluorescence intensity (Fesel & Zuccaro, 2016; Sung & Lee, 2010).

228 **3.3 The effect of BSF on Sclerotia morphological characteristics**

229 As seen in Figure 2, the sclerotia diameter (mm) decreased with varying BSF
230 concentrations of 50, 100, 150 and 200 mg L^{-1} (0.92, 0.83, 0.74 and 0.62 mm, respectively). A
231 damaged hypha wall appeared at 8 mg L^{-1} ClO_2 treatment (Fig 2b.) compared to that of untreated

232 sclerotia cells (Fig 2a.), which corresponded to 150 and 200 mg L^{-1} BSF (Fig 2e., and 2f,
233 respectively). As a result, 150 and 200 mg L^{-1} BSF affected the sclerotia structure by damaging
234 the outer membrane and vegetative hypha (Ordóñez-Valencia et al., 2015). Moreover, Figures 2E,
235 2e. and 2F, 2f. show the damage in mycelium treated with 150-200 mg L^{-1} BSF, which were
236 smaller than that of ClO_2. It should be noted that BSF may denature the lipid-protein interface of
237 integral proteins, putting the central medulla of normal hyphae (vegetative hypha) at risk of
238 disruption (Blum & Rodríguez-Kábana, 2004; Wu, Lu, Zhong, Schwarz, Chen, & Rao, 2019).
239
240 **3.4 The effectiveness of BSF washing on dimethoate and Pb reduction**
241 As seen in Table 2, a high reduction (%) in dimethoate and Pb was found in 150 and 200
242 mg L^{-1} BSF based on MRL (EC Regulation No 1097/2005 and CODEX, 2009, respectively). In
243 this study, soaking in 150 and 200 mg L^{-1} BSF for 5 min was the most effective control measure
244 in achieving the MRL of dimethoate and Pb.
245
246 **3.5 Functional characterization of washing agents on dimethoate and Pb minimization**
247 Table 3 demonstrates the ATR-FTIR wavenumber relevant to C-N-H functional group
248 interaction with 200 mg L^{-1} BSF, which was approximately 2000-2100 cm^{-1}. The band of 1200-
249 1400 cm^{-1} reflected the aliphatic chain of BSF, which interacted with the C-N-H stretching and
250 was clarified by the aliphatic BSF structure, which enhanced solubility on the left of methyl group,
251 while methoxy group migrated to the N atom to water OH group (Fleming et al., 2012;
252 Wattanaphon, Kerdsin, Thammacharoen, Sangvanich, & Vangnai, 2008). It might be interpreted
253 that the mode of BSF action is accorded to the formation of BSF-pesticide micelles complex,
254 which interact with the hydrogen bonds of water molecules, resulting in a better solubilization

of pesticides (López-Prieto, Moldes, Cruz, & Perez Cid, 2020). Likely, lipopeptide biosurfactant obtained from *Bacillus amyloliquefaciens* C11 increased the solubility of chlorpyrifos, iprodione and atrazine 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 its eco-friendly 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 surfactant varies with different heavy metals. The removal of heavy metals depends on the category of BSF, its

277 concentration, its interaction with additive substances (acids and bases) and the characteristics of
278 materials (Ochoa-Loza, Noordman, Jannsen, Brusseau, & Maier, 2007).
279

280 ## 4. Conclusion

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

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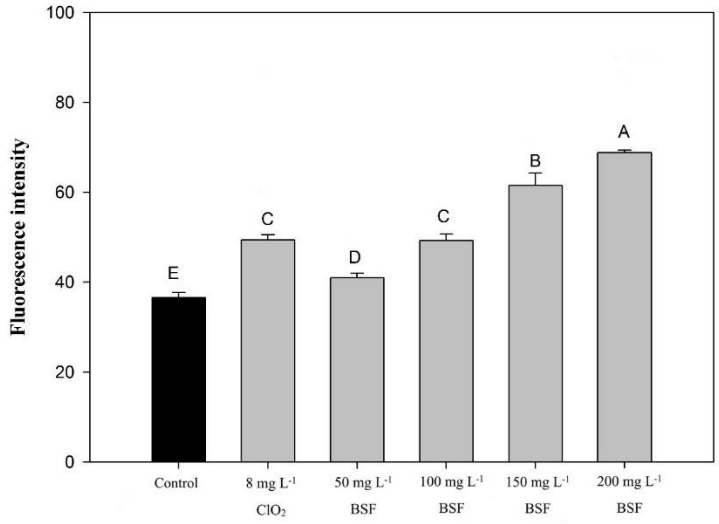

Figure 1. DPH fluorescence intensity at various concentrations of natural based BSF and aqueous chlorine dioxide. Different letters among the washing agents indicate significant fluorescence intensity differences ($P < 0.05$).

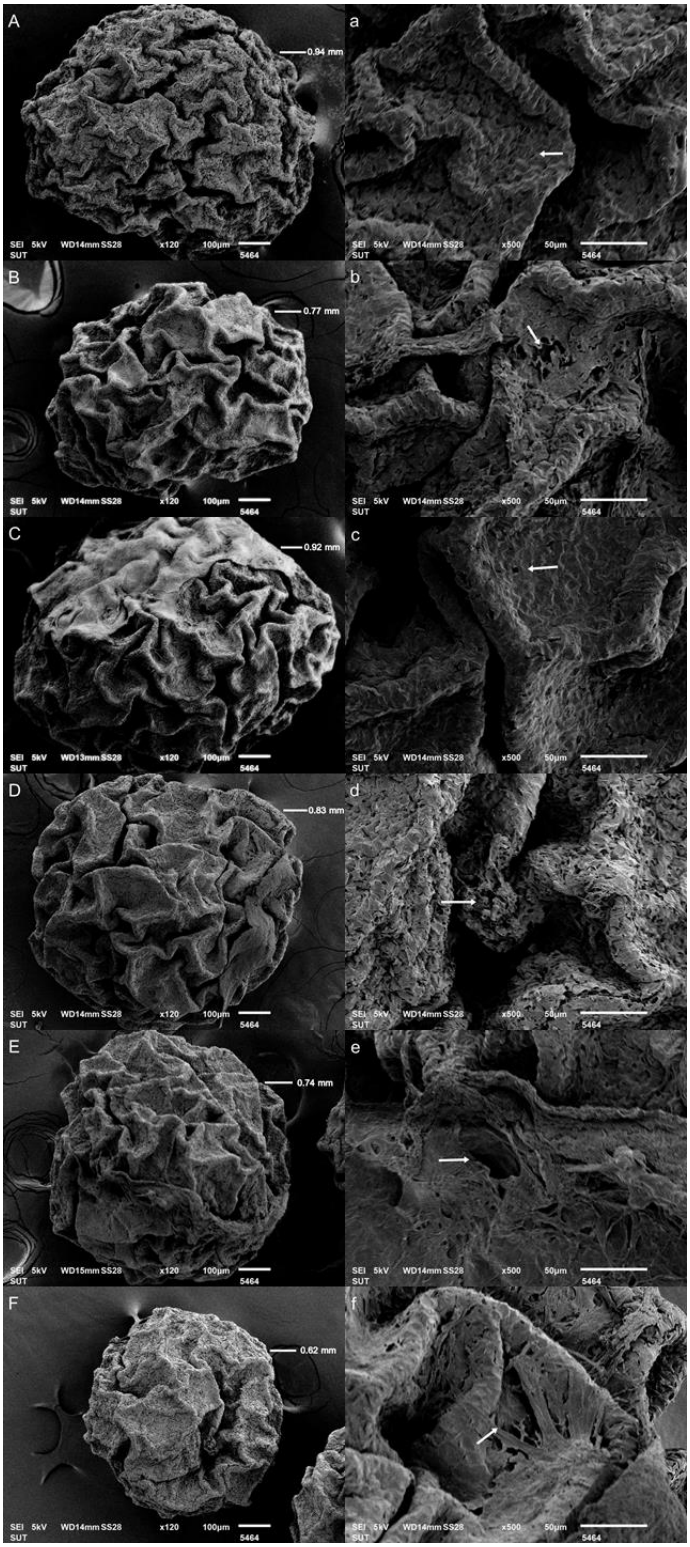

Figure 2. SEM images of morphological characteristics of mature sclerotia. Left panel: magnified image x120 (capital letters). Right panel: magnified image x500 (small letters). Untreated sclerotia

(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).

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

Washing agents (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.

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

Washing agents	**Dimethoate***		**Lead (Pb)***	
(mg L^{-1})	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

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

491 **Table 3.** Functional characterization of the washing agents; chlorine dioxide (ClO_2) and various

492 BSF concentrations action 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 2349.30 2325.41	2345.32	2345.64	2360.29 2349.26 2325.32	2360.25 2349.29 2325.43	2353.41
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 1507.22	1541.94	-	-	1541.53 1507.24
-CH3 stretching	1470-1430	-	1457.37				1457.33
P=S	690-650	671.16 664.36	674.82	674.83 667.14	671.08 664.22	671.14	674.88
O-P-O bending	415-500	449.14	442.20	446.67	471.94	440.76	432.42

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499 **Table 4.** Functional characterization of the washing agents; chlorine dioxide (ClO_2) and various

500 BSF concentrations action on Pb minimization.

Functional group	Frequency range	The wavenumber interaction between sanitizers and Pb (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 2325.38	2351.86	2360.21 2349.29 2325.41	2349.29 2325.37	2359.89	2350.44
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	671.11 664.27	-	671.12	671.12	-	-
PO_4 bending	415-500	468.76	442.23	446.53	442.48	450.65	472.11

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