Heliyon 8 (2022) e09357

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Production of biomaterials from seafood waste for application as vegetable wash disinfectant

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ARTICLE INFO

Keywords:
Biomaterial
Seafood waste
Crassostrea belcheri
Chitosan
Disinfectant
Vegetable

ABSTRACT

The production of seafood waste was studied by analyzing calcium oxide from the shells of tropical oyster and chitosan from the shells of white shrimp to use as a vegetable wash disinfectant. The preparations used were: natural oyster shell powder (NOSP), calcined tropical oyster shell powder in a programmable furnace for 2 h at 700 °C (OSP_{700}), 800 °C (OSP_{800}) and 900 °C (OSP_{900}) including white shrimp shell chitosan (CS). The physical properties of all biomaterials were analyzed using Thermogravimetric analysis, X–ray diffraction and Fourier–transformed infrared spectrometry. The results showed that NOSP and OSP_{700} were calcite calcium carbonate crystal, but OSP_{800} and OSP_{900} were transformed to calcium oxide and calcium hydroxide. The amino group found in the chitin from white shrimp shell was deacetylated to chitosan. By investigating the qualitative antibacterial activity of OSP_{900} and CS, the inhibition zone of OSP_{900} against *E. coli* was higher than that of CS ($p < 0.05$); however, the inhibition zone of CS against *S. aureus* was higher than that of OSP_{900} ($p < 0.05$). In addition, OSP_{900} had significantly higher quantitative antibacterial activity against *E. coli* than *S. aureus*. The MIC of OSP_{900} against *E. coli* and *S. aureus* for 15 min were 2.5 and 5 mg/mL, respectively; furthermore, the MBC of OSP_{900} against *E. coli* and *S. aureus* were 5 and 10 mg/mL, respectively. However, the inhibitory activity of CS against *S. aureus* was higher than against *E. coli* with MIC and MBC values of 5 and 10 mg/mL, respectively, for 15 min. When testing the biomaterials, OSP_{900} and CS, to inhibit the bacteria on kale and lettuce, 2.5 mg/mL of OSP_{900} for a vegetable–washing time of 15 min had the highest *E. coli* inhibition for both vegetables, while 2.5 mg/mL of CS for the same washing time had the highest *S. aureus* inhibition for both vegetables. Therefore, this research indicated that biomaterials prepared from tropical oyster shell and white shrimp shell wastes could be used as effective wash disinfectants to eliminate contaminated bacteria on vegetables.

1. Introduction

Nowadays, the waste generated by seafood consumption has raised a lot of environmental concerns. In 2020, a Thai survey estimated oyster and white shrimp cultivation to be about 27,730,000 kg and 132,000 kg, respectively (Fisheries Statistics of Thailand, 2021). The rising consumption rate has resulted in the accumulation of shell wastes on the coast (Martins et al., 2017) and shrimp shells in large quantities, causing environmental pollution problems (Huang et al., 2020; Islam and Peñarubia, 2021). However, such wastes can be utilized because they contain crucial components such as calcium carbonate ($CaCO_3$) in tropical oyster shells and deacetylated chitin from shrimp shells. According to Alidoust et al. (2015), when heated or incinerated at a high temperatures of 700 °C and above, calcium carbonate is converted to calcium oxide (CaO). When dissolved in water or exposed to moisture, this changes to calcium hydroxide ($Ca(OH)_2$) that has the ability to inhibit bacteria (Stuart et al., 2012). Yeesang et al. (2016) found that chitosan has the ability to inhibit pathogens as well.

Fresh vegetables are essential to our daily diet since the consumption of vegetables keeps people healthy as the vegetables are rich in nutrients that are important to the body (Denis et al., 2016). At the same time, green leafy vegetables and raw vegetables are often contaminated with human pathogenic bacteria (Rosberg et al., 2021), including the Gram–negative bacterium *Escherichia coli* (*E. coli*), which can cause severe infection resulting in diarrhea, severe bloody diarrhea, hemorrhagic colitis and kidney failure (Abdissa et al., 2017), and the Gram–positive

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https://doi.org/10.1016/j.heliyon.2022.e09357
Received 22 November 2021; Received in revised form 26 February 2022; Accepted 27 April 2022

bacterium *Staphylococcus aureus* (*S. aureus*), which causes skin and joint infections as well as food poisoning (Sultana and Bishayi, 2020; Rodriguez–Garcia et al., 2020). The contamination of bacteria from cultivating, harvesting, cleaning or packaging can be removed by using bactericides in the washing process (Rosberg et al., 2021).

Therefore, this research investigated biomaterials from tropical oyster shells and white shrimp shells produced as waste from seafood consumption for their ability to inhibit the Gram–negative bacterium *Escherichia coli* ATCC25923, the Gram–positive bacterium *Staphylococcus aureus* ATCC25922 and pathogenic bacteria isolated from vegetable surfaces. Biomaterials are also applied as a vegetable washing agent to reduce bacterial contamination on vegetables before consumption and to promote safety of food consumption.

2. Materials and methods

2.1. Preparation and characterization of tropical oyster shell powder

Samples of shell waste from the tropical oyster (*Crassostrea belcheri* (Sowerby, 1871)) were collected from Tha Thong subdistrict, Kanchanadit district, Surat Thani province, Thailand. The samples were soaked in sodium hydroxide (NaOH) at a concentration of 10% for 1 h, dried, ground and sifted through an automatic sieve (250 μm mesh size) and dried at 60 °C for 24 h to acquire natural oyster shell powder (NOSP) (Tsou et al., 2018). The oyster shell powder was heated to 700 °C (OSP_{700}), 800 °C (OSP_{800}) and 900 °C (OSP_{900}) in a high temperature furnace (FSMF–270HT, JSR, Korea) for 2 h. The resulting products (NOSP, OSP_{700}, OSP_{800} and OSP_{900}) were investigated to determine the nature of the physical change and to calculate the percentage weight change based on Eq. (1) (Dangkanid, 1995):

$$\text{Weight loss}\ (\%) = \frac{\textit{Initial weight} - \textit{Final weight}}{\textit{Initial weight}} \times 100 \quad (1)$$

Their thermal properties were studied using Thermogravimetric analysis (TGA; Pyris Diamond, PerkinElmer, USA), the crystal structure was investigated using X–ray diffraction (XRD; D8 Advance, Bruker AXS, Germany), and chemical functional groups were explored using Fourier transformed infrared spectrometry (FTIR; FTIR–4100, JASCO, Spain).

2.2. Preparation and characterization of chitosan from white shrimp shells

Samples of waste from shrimp shells (*Litopenaeus vannamei* Boone) were collected from a local fish market, Ang Sila subdistrict, Mueang district, Chonburi province. The shrimp shells were separated, dried at 60 °C for 3 h, ground and passed through an automatic sieve (70–850 μm mesh size). Then, they were demineralized (Ali et al., 2018) using a 1:20 ratio of 2 M hydrochloric acid (HCl) solution for 2 h, rinsed with distilled water to neutral pH and dried at 60 °C for 3 h. The samples were deproteinated using a 1:20 ratio of 2 M sodium hydroxide (NaOH) solution at 55 °C for 2 h, rinsed with distilled water to neutral pH and dried at 60 °C for 3 h to obtain chitin. In addition, they were decolorated using a 1:20 ratio of 95% (v/v) ethanol, stirred for 5 min, rinsed with distilled water to neutral pH and dried at 60 °C for 3 h (Tungse et al., 2016). They were deacetylated in a 1:20 ratio of 50% NaOH (w/v) for 4 h, rinsed with distilled water to neutral pH and dried at 60 °C for 3 h to obtain chitosan (Ali et al., 2018). The chemical functional groups of chitosan from shrimp shells were studied using an infrared technique (FTIR) (FTIR–4100, JASCO, Spain).

2.3. Preparation of solutions of biological materials

Preparation of the oyster shell powder solution: The oyster shell powder heated at 900 °C (OSP_{900}) was dissolved in sterile distilled water.

Preparation of the chitosan solution: The chitosan powder (CS) was dissolved in 1% (v/v) acetic acid (CH_3COOH) at 60 °C and stirred until the mixture was homogeneous. Then, the pH was neutralized with NaOH at a concentration of 2M to study its ability to inhibit bacteria (Etemadi et al., 2021).

2.4. Bacterial preparation

The bacteria used in the experiment (*Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC25923) were obtained from the Department of Medical Sciences Culture Collection. They were cultured in nutrient broth (NB), incubated at 37 °C for 24 h, inoculated into 10 mL of 0.85% (w/v) sterile NaCl and adjusted to a bacterial density of approximately 1 × 10^8 colony forming units per milliliter (CFU/mL) using McFarland standard No. 0.5 (Roy et al., 2013).

2.5. Testing antibacterial ability of biomaterials using disc diffusion method

The disc diffusion method was used to test for bacterial inhibition (Meesub and Buachard, 2018). Samples (each 0.1 mL) of prepared *E. coli* and *S. aureus* solutions were spread evenly on the surface of Mueller Hinton Agar (MHA) using the spread plate method. Then, 6 mm paper discs were immersed in the OSP_{900} and CS solutions at concentrations of 800 mg/mL and 5 mg/mL, respectively, and compared with 1 mg/mL amoxicillin as a positive control and with sterile distilled water and 1% (v/v) acetic acid as negative controls. The disc specimens were placed on MHA medium and incubated at 37 °C for 24 h. The diameter of the inhibition zone was measured (mean of four measurements) and calculated using Eq. (2) (Kositchaiyong et al., 2010):

$$R_a = \frac{D_c - D_s}{2} \quad (2)$$

where R_a is the inhibition zone (mm), D_c is the diameter of the clear zone (mm) and D_s is the diameter of the paper disc (mm).

2.6. Investigating minimum concentration of biomaterials to inhibit bacterial growth (minimum inhibitory concentration: MIC)

The quantitative determination of the MIC of biomaterials to inhibit *E. coli* and *S. aureus* was investigated on plate count agar (PCA) using the colony counting technique adapted from the ASTM E2149–01 standard method (American Society for Testing and Materials, 2001). Two–fold serial dilutions of OSP_{900} and CS solutions were performed with sterile distilled water and 1% (v/v) acetic acid respectively to gain the final concentrations of 0, 1.25, 2.5, 5 and 10 mg/mL. Then, samples (0.1 mL) of bacterial cell suspensions at approximately 1 × 10^6 CFU/mL were transferred to all flasks of diluted samples. Amoxicillin (1 mg/mL) was used as a positive control, while sterile distilled water and 1% acetic acid were used as negative controls (Meesub and Buachard, 2018). All the sample flasks were shaken at 170 rpm for 0, 5, 15 and 30 min. Then, 0.1 mL of each sample was spread on the PCA surface using the spread plate technique and incubated at 37 °C for 24 h. The procedure was repeated four times. After the incubation time, the bacterial colonies were counted and the bacterial concentration was expressed in colony forming units per milliliter (CFU/mL) (Chammanee et al., 2009). The percentage of bacterial inhibition was calculated using Eq. (3), and the *in vitro* MIC value was recorded as the lowest concentration of each biomaterial sample that inhibited the visible growth of bacteria after overnight incubation:

$$\text{Antibacterial activity}\ (\%) = \frac{C - D}{C} \times 100 \quad (3)$$

where C is the number of bacteria surviving after testing (no biomaterials treated), and D is the number of bacteria surviving after testing (biomaterials treated).

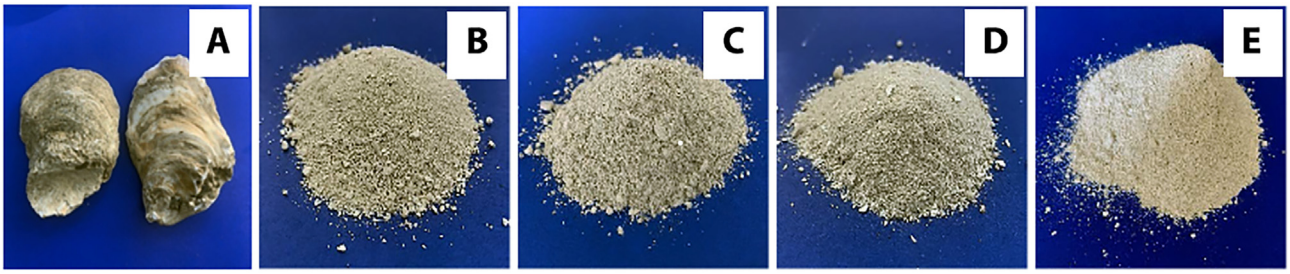

Figure 1. Physical characteristics of *Crassostrea belcheri* (A) oyster shell, (B) natural oyster shell powder (NOSP), (C) oyster shell powder calcined at 700 °C (OSP_{700}), (D) oyster shell powder calcined at 800 °C (OSP_{800}) and (E) oyster shell powder calcined at 900 °C (OSP_{900}).

2.7. Determining lowest concentration of biomaterials that can thoroughly kill tested bacteria (minimal bactericidal concentration: MBC)

The agar dilution technique was used for the determination of the MBC of the biomaterials against *E. coli* and *S. aureus* (Rungseephanurat et al., 2016). After investigating the MIC, 0.1 mL of biomaterial samples that had no visible growth of *E. coli* and *S. aureus* was spread on the PCA surface using the spread plate technique. All sample plates were incubated at 37 °C for 24 h. Each experiment was repeated four times independently. The MBC value was recorded as the mg/mL of the lowest concentration of each biomaterial sample that had no visible bacterial growth.

2.8. Testing antibacterial ability of biomaterials on vegetables

Samples of kale (*Brassica alboglabra* Bailey) and lettuce (*Lactuca sativa* L.) were cut into 3 × 3 cm pieces, and 1 g of the vegetable pieces was soaked in 5 mg/mL OSP_{900} solution from *Saccostrea cucullata* (Born, 1778) (Tongwanichiniyom et al., 2021) for 3 min, following washing with sterile distilled water. A sample (1 g) of vegetable pieces was added to 500 μL of 1 × 10^6 CFU/mL bacterial suspension and shaken for 1 min (Paomephan et al., 2018). The contaminated pieces were transferred in 0, 1.25, 2.5, 5 and 10 mg/mL of the solutions of OSP_{900} from *Crassostrea belcheri* and of CS for 0, 5, 15 and 30 min, respectively. A sample (0.1 mL) of each treatment was pipetted and spread evenly on the PCA surface using the spread plate technique; all plates were incubated at 37 °C for 24 h. Each treatment was performed four times independently. The visible colonies in CFU/mL were recorded, and the antibacterial ability was calculated using Eq. (3).

2.9. Investigating the inhibition ability on bacteria isolated from vegetable surfaces

10 g of kale and lettuce pieces were transferred into sterile plastic bags containing 100 mL of Butterfield's phosphate buffered water (0.6 mM KH_2PO_4, pH 7.2). The samples were rubbed and shaken for 3 min to extract the microbes on the vegetable surfaces. The 0.1 mL of cell suspensions were spread on nutrient agar (NA) and incubated in a range of 30–37 °C for 24 h, then each single bacterial colony was streaked on the NA plate for purification, and on differential and selective media following the Bergey's manual of determinative bacteriology (Bergey and Holt, 1994). Gram staining and biochemical tests were performed for identification and confirmation of bacterial isolates. A phytopathogenic bacterium, *Erwinia carotovara carotovara*, was identified by Plant Protection Research and Development Office, Department of Agriculture, Thailand.

The bacteria, i.e., *Salmonella* Typhimurium, *Enterobacter* sp., *Pseudomonas* sp. and *Erwinia carotovara carotovara*, isolated from the vegetable surfaces were tested with biomaterials using the disc diffusion method. 1 mg/mL of ciprofloxacin was used as a positive control, and sterile distilled water and 1% (v/v) of acetic acid were used as negative controls. The NA containing each bacterium was incubated at optimal temperatures. The inhibition zone (in mm) was measured and calculated using Eq. (2).

The minimum concentration of biomaterials that can inhibit the bacterial growth (MIC) were performed by using colony count technique on the PCA containing the diluted OSP_{900} in sterile distilled water and CS in 1% (v/v) acetic acid at the concentrations of 0, 1.25 and 2.5 mg/mL. Cell suspension at 1 × 10^6 CFU/mL was shaken for 0, 5 and 15 min, and then spread on the PCA plate containing biomaterials. The colonies was counted in unit of CFU/mL (Chammanee et al., 2009), and the antibacterial activity was calculated using Eq. (3). *In vitro* MIC value was recorded as the lowest concentration of each biomaterial sample that inhibited the visible growth of bacteria after overnight incubation (Rungseephanurat et al., 2016).

To test the inhibition ability of biomaterials on kale and lettuce, the vegetables were soaked in 5 mg/mL of OSP_{900} for 3 min, and then wash with sterile distilled water to eliminate the microbes. A volume of 500 μL in each isolated bacterium at 1 × 10^6 CFU/mL was added in 1 g of vegetable, and then shaken for 1 min (Paomephan et al., 2018). The vegetable pieces containing bacteria was soaked in OSP_{900} and CS at 0, 1.25 and 2.5 mg/mL for 0, 5 and 15 min. The colonies (in CFU/mL) were counted and calculated the inhibition activity using Eq. (3).

2.10. Statistical analysis

The antibacterial ability of OSP_{900} and CS at the different concentrations and washing times against *E. coli* and *S. aureus* as well as pathogenic bacteria isolated from vegetable surfaces was analyzed using two–way ANOVA. Mean differences were compared using Duncan's new multiple range test (DMRT) in the SPSS 26.0 statistical package at the 0.05 significance level. Values were presented as mean ± standard deviation (SD).

3. Results and discussion

3.1. Characterization of tropical oyster shell powder

The study of the physical characteristics of the tropical oyster shells (Figure 1, Table 1) showed that the outer shell was a brownish white

Table 1. Physical characteristics and percentage weight loss of NOSP, OSP_{700}, OSP_{800} and OSP_{900}.

Sample	Physical characteristic			
	Color	Softness	pH	Weight loss (%)
NOSP	Brownish white	Hard	9.54	–
OSP_{700}	Dark gray	Brittle	11.72	4.61
OSP_{800}	Dark gray	Brittle	12.47	20.65
OSP_{900}	Grayish white	Brittle	12.56	73.69

NOSP = natural oyster shell powder, OSP_{700} = oyster shell powder calcined at 700 °C, OSP_{800} = oyster shell powder calcined at 800 °C and OSP_{900} = oyster shell powder calcined at 900 °C.

color with a hard, thick and rough surface (Figure 1A). After crushing, sifting (Figure 1B) and being calcined at 700 °C (OSP_{700}) and 800 °C (OSP_{800}), the characteristics of the shell powder changed to a dark gray color and were brittle, as well as having reduced weights by 4.61% and 20.65%, respectively (Figures 1C, 1D). Calcination at 900 °C (OSP_{900}) changed the powder color to grayish white and it was brittle with a weight loss of 73.69% (Figure 1E). After sintering and being calcined at high temperature, the weight percentage of the shell powder was reduced by at least one–half. In addition, the powder weight after low–temperature calcination was slightly reduced (Boonyuen et al., 2015; Tongwanichiniyom et al., 2021). The weight and color changes of the shell powder were due to the decomposition of calcium carbonate to calcium oxide, with the product from each calcination temperature having different characteristics.

By analyzing the thermal properties using TGA (Figure 2), the NOSP decomposed in the high temperature range 693.23–759.33 °C and the weight loss of NOSP was 43.25% (w/w). This temperature range caused the calcium carbonate ($CaCO_3$) to decompose to calcium oxide (CaO) and carbon dioxide (CO_2). When calcined at high temperature, the crystal morphology of calcite undergoes decomposition to calcium oxide, according to Eq. (4) (Boonyuen et al., 2015).

$$CaCO_3(s) \xrightarrow{\Delta} CaO(s) + CO_2(g) \quad (4)$$

The crystal structure transformation based on XRD (Figure 3) showed that the NOSP and OSP_{700} were mainly composed of calcium carbonate, which is a rhombohedral calcite crystal of the group R–3C. In addition, X–ray diffraction patterns were detected at 2θ angles of 23.0°, 29.4°, 31.5°, 36.0°, 39.4°, 43.2°, 47.4° and 48.5°. Compared to the JCPDS database 05–0586, there was only a slight decrease in the weight loss percentage (Table 1) because the low–temperature calcination only removed the contaminated organic substances and did not change the crystal morphology. The OSP_{800} had a common X–ray diffraction pattern that showed the change from calcium carbonate to calcium oxide. At the higher temperature calcination (OSP_{900}) there was a complete change in the morphology from calcium carbonate to calcium oxide with the X–ray diffraction patterns at 2θ values of 32.2°, 37.5°, 54.0°, 64.2° and 67.5° according to JCPDS database No.78–0649. Then, there was a further conversion to calcium hydroxide with its X–ray diffraction patterns at 2θ values of 28.6°, 34.1°, 47.1° and 50.8° corresponding to the JCPDS database No.76–0570, which differed from the result of Boonyuen et al. (2015). The calcium carbonate of natural shells, such as from mussels, spotted babylon and cockles is composed of aragonite crystal which is an orthorhombic morphology. When calcined at higher than 500 °C, the calcium carbonate is transformed into calcite, which is rhombohedral.

The functional group of the four shell powder biomaterials NOSP (without calcination), OSP_{700}, OSP_{800} and OSP_{900} were investigated using FTIR in the 400–4000 cm^{-1} wavenumber range (Figure 4). Apparent peaks were identical in both NOSP and OSP_{700}. The functional group of $CaCO_3$ for tropical oyster shell powder was at wavenumbers 713 and 875 cm^{-1}. The peak at 713 cm^{-1} was the C–O functional group of the carbonate ion (CO_3^{2-}) in a stretching vibration mode of $CaCO_3$ that indicated the calcite morphology, and the stretching vibration mode of the C=O functional group from the combustion process of CO_3^{2-} and CO_2 from the environment showed at the peak at 1419 cm^{-1} (Lin et al., 2020; Benni et al., 2021). Furthermore, a small peak was found at wavenumber 1797 cm^{-1}, indicating the C=O stretching of CO_3^{2-} and the peak at the 2515 cm^{-1} represented the O–H functional group of the stretching vibration mode of HCO_3^- (Chang et al., 2019). The peak at 2873 cm^{-1} was the stretching mode of the C–H functional group of shell organic matter (Areeprasert et al., 2014). When calcined at 800 °C, OSP_{800} began to decompose from calcium carbonate to calcium oxide (CaO) and calcium hydroxide ($Ca(OH)_2$) as indicated by the oscillation peak of the Ca=O functional group was evident at 523 cm^{-1} and the stretching oscillation mode of the O–H functional group peak of $Ca(OH)_2$ was observed at 3618 cm^{-1} (Choudhary et al., 2015; Huh et al., 2016). The stretching oscillation modes of Ca=O and an O–H functional group peaks of $Ca(OH)_2$ were found in OSP_{900}. There was no C–H peak from the decomposition of organic substances. The results of TGA were consistent with the XRD analysis; however, CaO, which is moisture sensitive, undergoes hydrolysis and converts to $Ca(OH)_2$, according to Eq. (5) (Mustakimah et al., 2012; Oikawa et al., 2000):

$$CaO\ (s) + H_2O\ (l) \rightleftharpoons Ca\ (OH)_2\ (s) + 15.2\ kcal \quad (5)$$

3.2. Characterization of chitosan from white shrimp shells

The physical characteristics of the white shrimp shells used for chitosan extraction (Figure 5) showed that the shells of fresh shrimp were soft, smooth and had shiny surface (Figure 5A). After drying, the shells were hard and brittle (Figure 5B), and 70–850 μm ground powder of the shrimp shells had a mixed color of white and light orange (Figure 5C). When 40 g of shrimp shell powder was demineralized using 2 M HCl

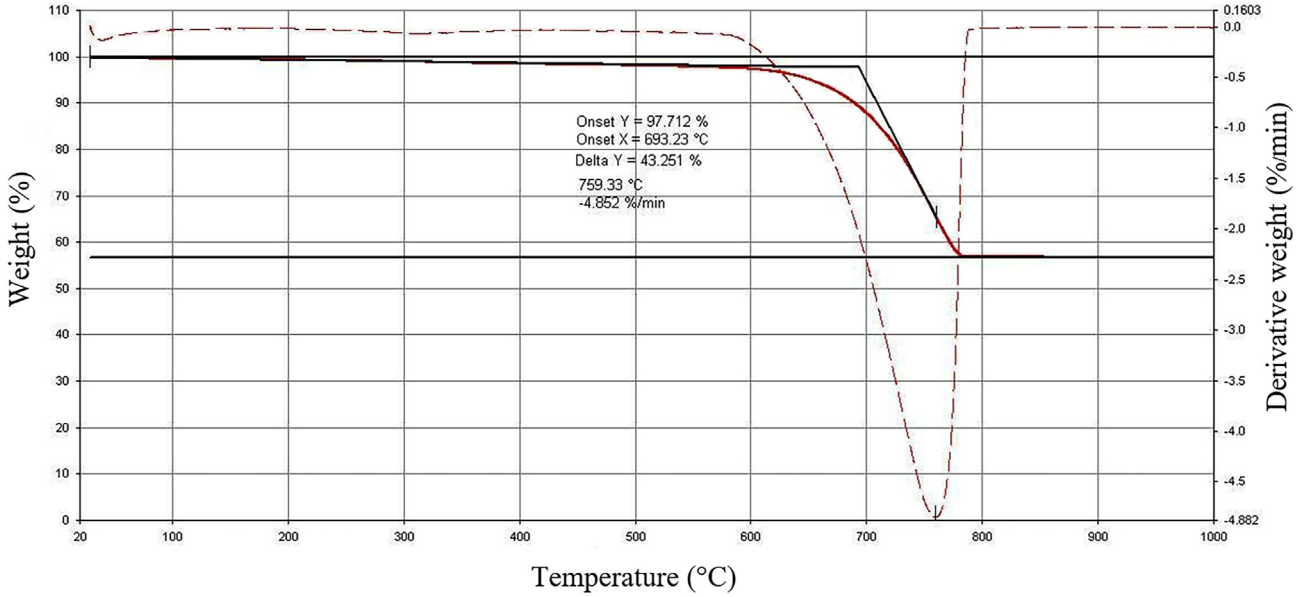

Figure 2. Thermogravimetric curves of NOSP.

followed with 2 M NaOH for deproteination, the powder changed to grayish white (Figure 5D) and yellowish white of chitin (Figure 5E), respectively. The yield of cream white chitin powder was 16.32 g or 59.20% (w/w) after decolorization with 95% (v/v) ethanol (Figure 5F, Table 2). When the chitin powder was deacetylated with 50% (w/v) NaOH, 4.40 g or 73.04% (w/w) white chitosan were obtained (Figure 5G, Table 2).

When studying the chemical functional groups using FTIR of chitin, chitosan and commercial chitosan biomaterials from shrimp shells (Figure 6), the infrared spectra of chitin showed that the main functional group was the acetyl group (–$NHCOCH_3$) of the amide molecule. There were peaks representing –NH stretching, C=O stretching, –NH bending and C–N stretching at wavenumbers 3255 cm^{-1} (Liu et al., 2020), 1652 cm^{-1}, 1617 cm^{-1} and 1373 cm^{-1}, respectively. After deacetylation to transform the chitin into chitosan, the functional group of second carbon in some monomers of the chitin molecules had been changed to the amino group (–NH_2). There was an increase in –NH stretching of the amine molecules at wavenumber 3392 cm^{-1}. The infrared spectra of the structures were similar for the chitosan extracted from shrimp shells and commercial chitosan, which implied that the extracted chitosan had a molecular structure similar to the commercial chitosan.

3.3. Antibacterial ability of biomaterials using disc diffusion method

The qualitative study of the ability of biomaterials to inhibit the growth of *E. coli* and *S. aureus* based on the disc diffusion method (Table 3), indicated that OSP_{900} and CS were able to inhibit both bacterial species. OSP_{900} was the more effective at inhibition against *E. coli*

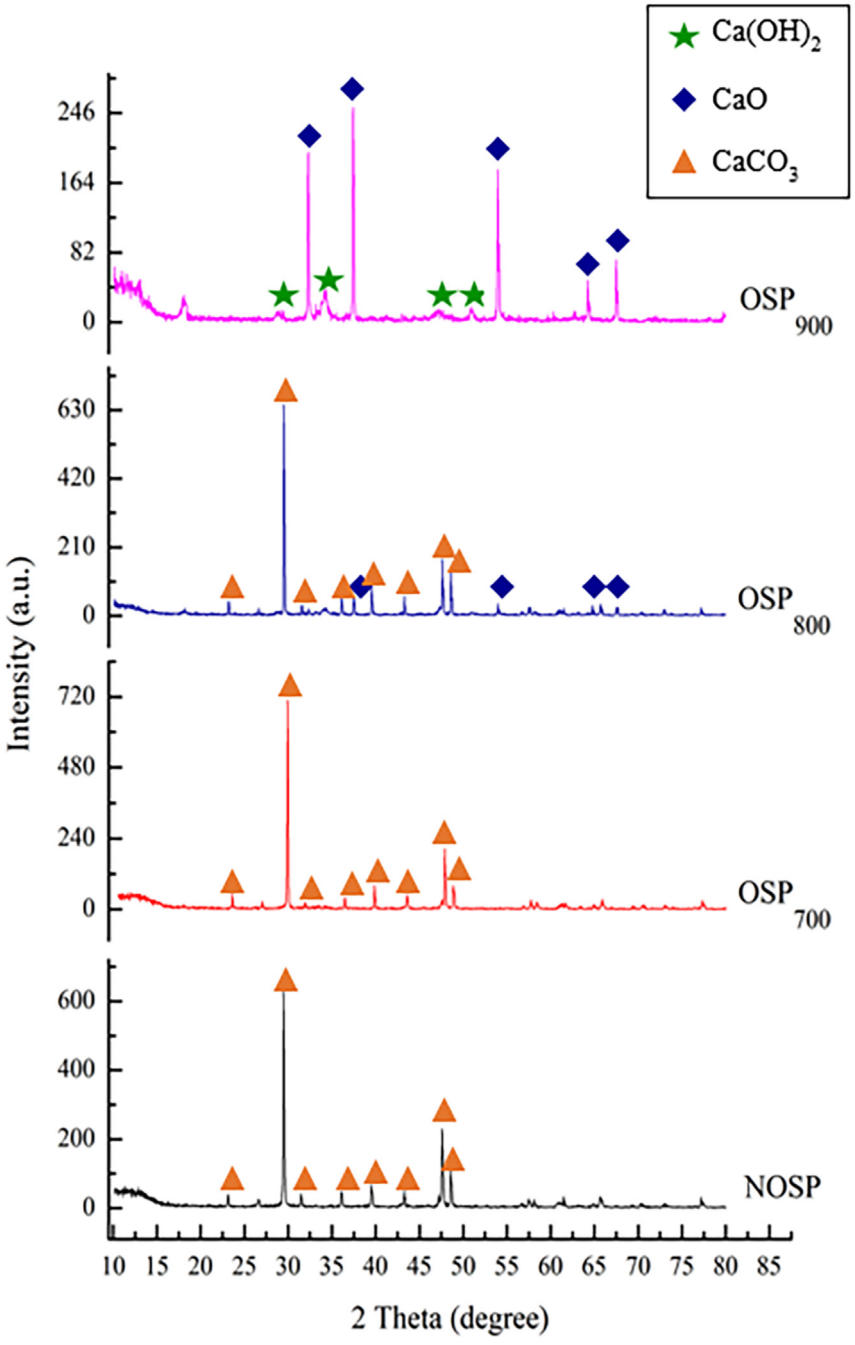

Figure 3. X–ray diffraction patterns of natural oyster shell powder (NOSP), oyster shell powder calcined at 700 °C (OSP_{700}), oyster shell powder calcined at 800 °C (OSP_{800}) and oyster shell powder calcined at 900 °C (OSP_{900}).

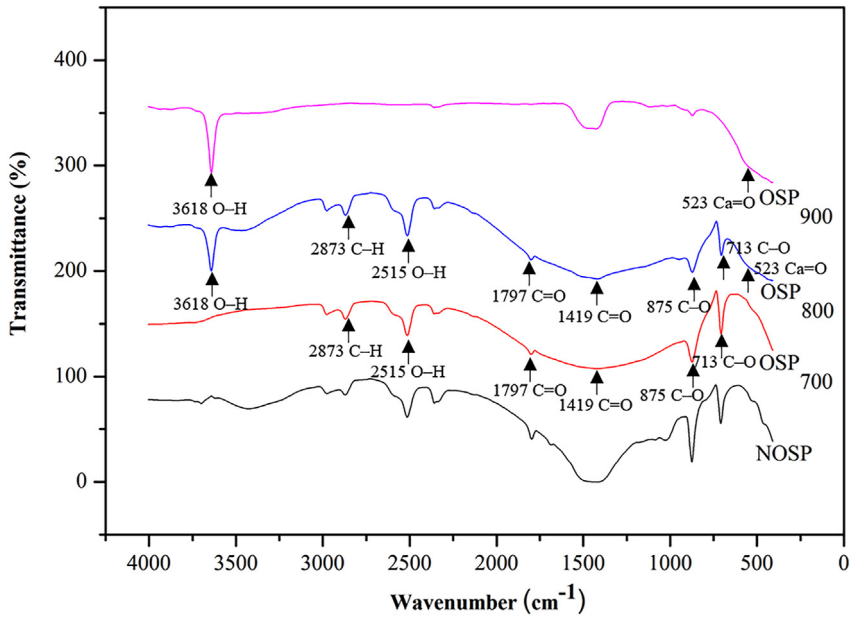

Figure 4. FTIR spectra of natural oyster shell powder (NOSP), oyster shell powder calcined at 700 °C (OSP_{700}), oyster shell powder calcined at 800 °C (OSP_{800}) and oyster shell powder calcined at 900 °C (OSP_{900}).

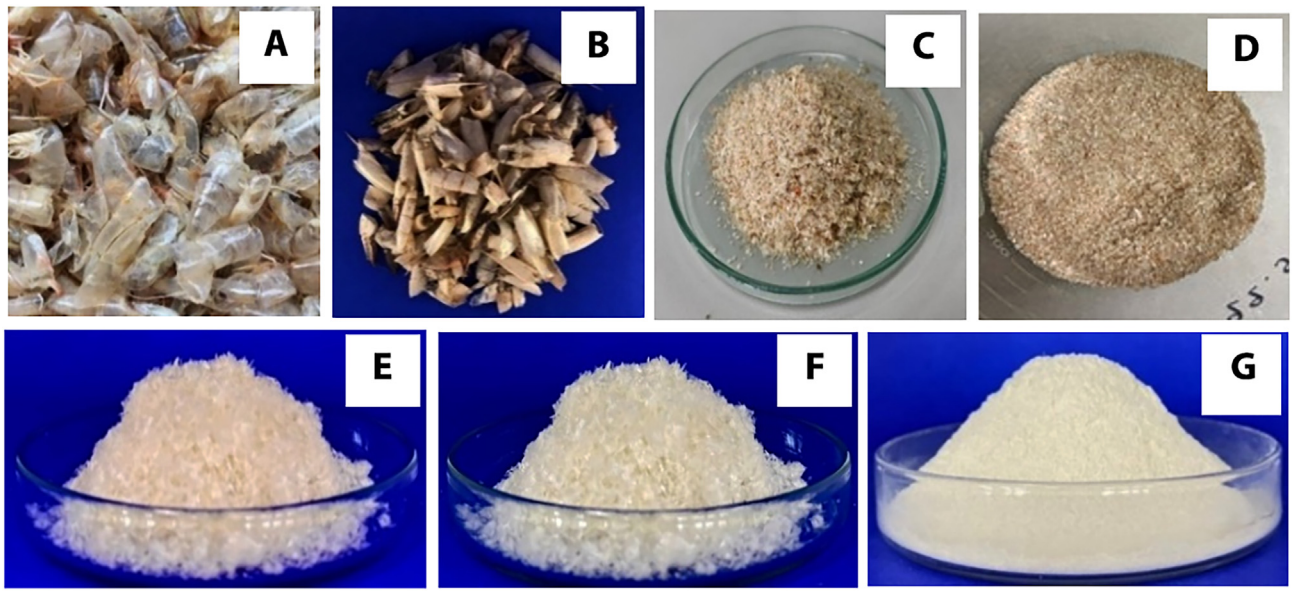

Figure 5. Physical characteristics of (A) shrimp shell, (B) dried shrimp shell, (C) shrimp shell powder, (D) shrimp shell powder demineralization, (E) chitin, (F) chitin decolorization and (G) chitosan (CS).

than *S. aureus* ($p < 0.05$), with inhibition diameter means of 12.50 ± 1.73 and 7.50 ± 0.58 mm, respectively. The inhibition of CS against *S. aureus* was higher than that against *E. coli* ($p < 0.05$), with inhibition diameter means of 10.25 ± 0.50 and 7.25 ± 0.50 mm, respectively, because the CaO structure of OSP_{900} converts to $Ca(OH)_2$ when exposed to moisture. The Ca=O group of CaO dissociates to the superoxide ion (O_2^-), while the hydroxyl group (–OH) dissociates to the hydroxyl ion (OH^-), which has a strong reaction with peptidoglycan and causes damage to the bacterial cell membrane (Mohammadi et al., 2012). This can damage the bacterial cell wall and destroy the genetic material of bacteria (Siqueira and Lopes, 1999).

Table 2. Percentage weight loss of chitin and chitosan from shrimp shell.

Shrimp shell (g)	Chitin		Chitosan	
	Weight (g)	Weight loss (%)	Weight (g)	Weight loss (%)
40.00	16.32	59.20	4.40	73.04

3.4. Minimum concentration of biomaterials to inhibit bacterial growth (minimum inhibitory concentration: MIC)

Based on the quantitative determination of the inhibition of *E. coli* and *S. aureus* growth by the biomaterials OSP_{900} and CS at all concentrations (Table 4), increasing the concentration and the incubation time increased the inhibition of both bacteria ($p < 0.05$). OSP_{900} inhibited *E. coli* and *S. aureus* by 100.00 ± 0.00% at the lowest concentrations of 2.5 and 5 mg/mL for 15 min, respectively. OSP_{900} from *Saccostrea cucullata* had the best inhibition of both *E. coli* and *S. aureus* at

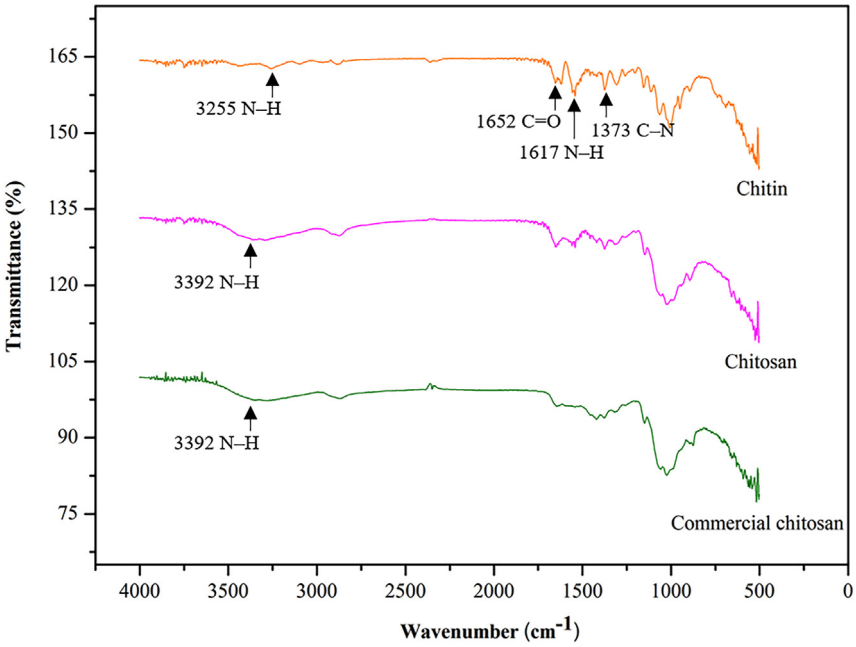

Figure 6. FTIR spectra of commercial chitosan, chitosan and chitin.

concentrations of 0.5% (w/v) and 1.0% (w/v), respectively, for 30 min (Tongwanichiniyom et al., 2021), which was less effective than for OSP_{900} from *Crassostrea belcheri*. Comparing the inhibition of antibacterial ability of both bacteria, OSP_{900} was able to inhibit *E. coli* better than *S. aureus* since the cell wall of Gram–negative bacteria is thinner and less complex than for Gram–positive bacteria, thus more easily causing damage (Brown et al., 2020). In addition, the inhibitory mechanism involved Ca^{2+} due to the positively charged ambient conditions so that the Ca^{2+} can bind to cardiolipin, causing changes in cellular metabolism which affect the integrity of the cell wall and causes bacterial cell wall damage (Sadeghi et al., 2019). The inhibition percentage of 5 mg/mL CS for 30 min against *E.coli* was 98.60 ± 1.25%; however, 5 mg/mL CS for 5 min against *S. aureus* resulted in complete inhibition (100.00 ± 0.00%). Comparing the inhibition ability for both bacteria, CS was more effective against *S. aureus* than against *E. coli* because CS has an amino functional group ($–NH_2$) and a hydroxyl functional group (–OH) from the acetyl group ($NH–CO–CH_3$) removal process at the second carbon. The amino group ($–NH_2$) accepts a proton (H^+) becomes NH_3^+, and this results in a positively charged condition on the chain that can interact with the bacterial cell wall (Chaisrikhwun et al., 2017). Thus, the positively charged CS can bind better to Gram–positive bacteria and interact with intracellular chemicals, resulting in *S. aureus* being inhibited and unable to grow (Duan et al., 2019). Chitosan also has the ability to bind to negatively charged phosphate groups in the cell wall using electrostatic interactions. This results in damage to the cell structure by allowing more substances to enter the cell and cause damage and death of the bacterial cell (Wang et al., 2021).

3.5. Lowest concentration of biomaterial ability that kills both test bacteria (minimal bactericidal concentration; MBC)

The minimum concentrations of OSP_{900} to entirely eliminate *E. coli* and *S. aureus* were 5 and 10 mg/mL, respectively, for 15 min (Table 5). The lowest concentration of CS to eliminate all *S. aureus* was 10 mg/mL for 15 min, but it was unable to eliminate all the *E. coli* because CS is positively charged and so it can bind to Gram–positive bacteria better than to Gram–negative bacteria. The main antibacterial mechanism of chitosan is associated with its positively charged amino groups (Ceron et al., 2021); these rupture cell membranes and leak cytoplasmic compounds resulting in cell death (Rashki et al., 2021).

3.6. Ability of biomaterials to inhibit bacteria on vegetables

On kale, OSP_{900} inhibited *E. coli* and *S. aureus* by 100.00 ± 0.00% at the same minimum concentration of 2.5 mg/mL for 5 and 30 min, respectively (Table 6). *E. coli* was inhibited at 98.15 ± 0.48% by 10 mg/mL CS for 15 min, and *S. aureus* was inhibited at 99.68 ± 0.65% by 2.5 mg/mL CS for 30 min.

On lettuce, OSP_{900} inhibited *E. coli* and *S. aureus* 100.00 ± 0.00% at the lowest concentrations of 2.5 and 10 mg/mL, respectively at 30 min (Table 7). In addition, OSP_{900} inhibited *E. coli* better than *S. aureus* since the Gram–negative bacterial cell wall has an outer membrane that is thinner and less complex than that of Gram–positive bacteria (Brown et al., 2020). This allows OSP_{900}, which has a high base value, to destroy the cell wall, enter the cell and ruin the genetic material in bacteria. For CS, *E. coli* was inhibited at 98.13 ± 0.78% at 2.5 mg/mL and *S. aureus* was inhibited at 98.38 ± 0.59% at 1.25 mg/mL for the same 30 min test period. Thus, CS inhibited *S. aureus* better than *E. coli* because the cell wall of Gram–negative bacteria contains hydrophilic lipopolysaccharides which are more resistant to inhibition from CS than Gram–positive bacteria whose cell wall is composed of peptidoglycans and teichoic acid (Rashki et al., 2021). It was also found that by reducing the viscosity of CS, its ability to inhibit Gram–positive bacteria was increased because the shorter CS chain can interact better with bacterial cells (Benhabiles et al., 2012).

3.7. Applications of inhibition ability on bacterial isolated from vegetable surfaces

Three human pathogenic species, *Salmonella* Typhimurium, *Enterobacter* sp. and *Pseudomonas* sp., and a soft and stem rot bacterium, *Erwinia*

Table 3. Means of inhibition zone diameter of bacteria *Escherichia coli* and *Staphylococcus aureus*.

Biomaterial	Inhibition zone (mean ± SD, mm)	
	Escherichia coli	*Staphylococcus aureus*
OSP_{900}	12.50 ± 1.73^b	7.50 ± 0.58^a
CS	7.25 ± 0.50^a	10.25 ± 0.50^b

Diameter of paper disc equals 6 mm. Means with the different lowercase superscript letters in same column are significantly different ($p < 0.05$).

Table 4. Minimal inhibitory concentration (MIC) of oyster shell powder calcined at 900 °C (OSP_{900}) and chitosan (CS) against *E. coli* and *S. aureus*.

Concentration (mg/mL)	Time (min)	Antibacterial activity (mean ± SD, %)			
		OSP_{900}		CS	
		E. coli	*S. aureus*	*E. coli*	*S. aureus*
0	0	28.38 ± 2.04	25.72 ± 0.72	21.90 ± 1.96	26.14 ± 1.20
1.25	5	$98.95 \pm 0.35^{B,b}$	$88.87 \pm 2.97^{C,b}$	$55.10 \pm 2.12^{B,b}$	$97.27 \pm 0.75^{C,c}$
	15	$99.80 \pm 0.00^{B,a}$	$98.53 \pm 0.41^{C,a}$	$73.40 \pm 1.70^{B,a}$	$98.00 \pm 0.57^{C,b}$
	30	$99.47 \pm 0.29^{B,ab}$	$99.60 \pm 0.20^{C,a}$	$66.40 \pm 3.11^{B,a}$	$99.75 \pm 0.35^{C,a}$
2.5	5	$99.97 \pm 0.06^{A,b}$	$96.20 \pm 0.26^{B,b}$	$56.97 \pm 0.75^{B,b}$	$98.10 \pm 0.26^{B,c}$
	15	$100.00 \pm 0.00^{A,a}$	$99.67 \pm 0.21^{B,a}$	$64.13 \pm 4.44^{B,a}$	$99.23 \pm 0.06^{B,b}$
	30	$100.00 \pm 0.00^{A,ab}$	$99.93 \pm 0.12^{B,a}$	$66.80 \pm 2.85^{B,a}$	$99.70 \pm 0.10^{B,a}$
5	5	$100.00 \pm 0.00^{A,b}$	$98.33 \pm 0.29^{AB,b}$	$85.50 \pm 2.83^{A,b}$	$100.00 \pm 0.00^{A,c}$
	15	$100.00 \pm 0.00^{A,a}$	$100.00 \pm 0.00^{AB,a}$	$93.70 \pm 0.20^{A,a}$	$100.00 \pm 0.00^{A,b}$
	30	$100.00 \pm 0.00^{A,ab}$	$100.00 \pm 0.00^{AB,a}$	$98.60 \pm 1.25^{A,a}$	$100.00 \pm 0.00^{A,a}$
10	5	$99.90 \pm 0.10^{A,b}$	$99.03 \pm 0.35^{A,b}$	$80.53 \pm 3.01^{A,b}$	$99.70 \pm 0.30^{A,c}$
	15	$100.00 \pm 0.00^{A,a}$	$100.00 \pm 0.00^{A,a}$	$95.47 \pm 4.48^{A,a}$	$100.00 \pm 0.00^{A,b}$
	30	$100.00 \pm 0.00^{A,ab}$	$100.00 \pm 0.00^{A,a}$	$98.27 \pm 1.60^{A,a}$	$100.00 \pm 0.00^{A,a}$

Different uppercase superscripts for concentrations within same column indicate significant differences ($p < 0.05$). Different lowercase superscripts for times within same column indicate significant differences ($p < 0.05$).

Table 5. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of oyster shell powder calcined at 900 °C (OSP_{900}) and chitosan (CS).

Biomaterial	*E. coli*		*S. aureus*	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
OSP_{900}	2.5	5	5	10
CS	-	-	5	10

carotovara carotovara, were isolated from vegetable surfaces before washing with sterile distilled water. When investigating OSP_{900} and CS against four species isolated from vegetable surfaces using disc diffusion method (Table 8), the inhibition zone between OSP_{900} and CS were no difference ($p \geq 0.05$). When comparing among bacterial species, OSP_{900} gave no difference to inhibit growth of all species ($p \geq 0.05$), but CS gave the best inhibition against *E. carotovara* ($p < 0.05$).

Based on the quantitative determination of the inhibition of vegetable-isolated bacterial growth by biomaterials OSP_{900} and CS (Table 9), OSP_{900} were able to inhibit all four pathogenic species at 100.00 ± 0.00% at the lowest concentration of 2.5 mg/mL for 15 min. The calcination of oyster shell powder at 900 °C can change the $CaCO_3$ into CaO thoroughly; therefore, the CaO powder slurries exhibited bactericidal action on Gram–negative bacteria such as *E. coli*, *S.* Typhimurium, *P. aeruginosa* (Sawai et al., 1995, 1997; Roy et al., 2013) including a phytopathogenic bacterium, *E. cavotovora*. The CS at concentration of 2.5 mg/mL for 15 min can also inhibit Gram–negative bacteria, *S.* Typhimurium, *Enterobacter* sp., *Pseudomonas* sp. and *E. carotovora* at 92.79 ± 1.82%, 89.65 ± 1.43%, 87.73 ± 2.91% and 96.30 ± 1.07%, respectively. This is because chitosan have antimicrobial action mode to be electrostatic communication between positive–-charged amino acid of glucosamine and negative–charged molecules of bacterial cell membrane. This interaction leads the modification of cell membrane permeability and affects cellular osmotic balance. Thus, intracellular substances are leaked until cell death (Mohammadi et al., 2016; Chandrasekaran et al., 2020).

The MIC of OSP_{900} and CS against all four species and MBC of the bacterial models, *E. coli* and *S. aureus*, were considered. The concentration of OSP_{900} and CS of 1.25 and 2.5 mg/mL for 0, 5 and 15 min were selected to investigate antibacterial activity on kale and lettuce. On kale

Table 6. Amount of antibacterial activity (*Escherichia coli* and *Staphylococcus aureus*) on kale using oyster shell powder calcined at 900 °C (OSP_{900}) and chitosan (CS).

Concentration (mg/mL)	Time (min)	Antibacterial activity (mean ± SD, %)			
		OSP_{900}		CS	
		E. coli	*S. aureus*	*E. coli*	*S. aureus*
0	0	29.68 ± 1.20	23.00 ± 3.25	26.50 ± 2.66	20.23 ± 2.22
1.25	5	$99.00 \pm 1.18^{A,a}$	$94.23 \pm 4.79^{A,a}$	$95.60 \pm 3.77^{B,a}$	$90.70 \pm 2.37^{A,a}$
	15	$99.53 \pm 0.95^{A,a}$	$98.75 \pm 0.88^{A,b}$	$96.18 \pm 1.97^{B,b}$	$95.78 \pm 2.43^{A,b}$
	30	$99.73 \pm 0.55^{A,a}$	$100.00 \pm 0.00^{A,b}$	$96.88 \pm 1.61^{B,a}$	$94.73 \pm 3.93^{A,b}$
2.5	5	$100.00 \pm 0.00^{B,a}$	$97.20 \pm 1.02^{A,a}$	$93.50 \pm 1.83^{A,a}$	$97.98 \pm 0.57^{B,a}$
	15	$100.00 \pm 0.00^{B,a}$	$99.08 \pm 1.08^{A,b}$	$93.80 \pm 0.00^{A,b}$	$98.73 \pm 0.90^{B,b}$
	30	$100.00 \pm 0.00^{B,a}$	$100.00 \pm 0.00^{A,b}$	$91.67 \pm 0.99^{A,a}$	$99.68 \pm 0.65^{B,b}$
5	5	$100.00 \pm 0.00^{B,a}$	$98.93 \pm 1.26^{B,a}$	$93.83 \pm 1.33^{A,a}$	$97.03 \pm 1.13^{B,a}$
	15	$100.00 \pm 0.00^{B,a}$	$100.00 \pm 0.00^{B,b}$	$94.65 \pm 4.88^{A,b}$	$97.70 \pm 1.16^{B,b}$
	30	$100.00 \pm 0.00^{B,a}$	$100.00 \pm 0.00^{B,b}$	$92.45 \pm 2.76^{A,a}$	$98.20 \pm 0.43^{B,b}$
10	5	$100.00 \pm 0.00^{B,a}$	$100.00 \pm 0.00^{B,a}$	$85.00 \pm 1.73^{A,a}$	$97.78 \pm 0.28^{B,a}$
	15	$100.00 \pm 0.00^{B,a}$	$100.00 \pm 0.00^{B,b}$	$98.15 \pm 0.48^{A,b}$	$98.20 \pm 0.61^{B,b}$
	30	$100.00 \pm 0.00^{B,a}$	$100.00 \pm 0.00^{B,b}$	$92.55 \pm 1.41^{A,a}$	$98.55 \pm 0.40^{B,b}$

Different uppercase superscripts of concentrations within same column indicate significant differences ($p < 0.05$). Different lowercase superscripts of times within same column indicate significant differences ($p < 0.05$).

Table 7. Amount of antibacterial activity (*Escherichia coli* and *Staphylococcus aureus*) on lettuce using oyster shell powder calcined at 900 °C (OSP_{900}) and chitosan (CS).

Concentration (mg/mL)	Time (min)	Antibacterial activity (mean ± SD, %)			
		OSP_{900}		CS	
		E. coli	*S. aureus*	*E. coli*	*S. aureus*
0	0	30.25 ± 1.26	29.20 ± 1.36	22.28 ± 1.77	26.67 ± 2.00
1.25	5	$93.05 \pm 5.73^{A,a}$	$90.68 \pm 0.56^{A,a}$	$94.23 \pm 3.57^{A,a}$	$93.98 \pm 3.25^{A,a}$
	15	$95.00 \pm 1.61^{A,a}$	$94.88 \pm 1.30^{A,b}$	$96.68 \pm 1.59^{A,b}$	$94.03 \pm 3.59^{A,a}$
	30	$96.25 \pm 2.93^{A,b}$	$96.13 \pm 2.30^{A,b}$	$97.95 \pm 0.90^{A,b}$	$98.38 \pm 0.59^{B,b}$
2.5	5	$97.88 \pm 1.44^{B,a}$	$96.38 \pm 0.95^{B,a}$	$93.85 \pm 0.99^{A,a}$	$94.28 \pm 1.60^{A,a}$
	15	$99.33 \pm 1.16^{B,a}$	$97.20 \pm 0.20^{B,b}$	$97.20 \pm 0.56^{A,b}$	$97.32 \pm 0.64^{B,a}$
	30	$100.00 \pm 0.00^{B,b}$	$97.50 \pm 0.00^{B,b}$	$98.13 \pm 0.78^{A,b}$	$98.20 \pm 0.43^{B,b}$
5	5	$97.30 \pm 0.42^{B,a}$	$97.30 \pm 0.16^{C,a}$	$95.07 \pm 2.78^{A,a}$	$96.70 \pm 0.88^{AB,a}$
	15	$97.83 \pm 0.46^{B,a}$	$98.40 \pm 1.17^{C,b}$	$96.95 \pm 0.64^{A,b}$	$97.13 \pm 0.92^{B,a}$
	30	$100.00 \pm 0.00^{B,b}$	$98.73 \pm 0.95^{C,b}$	$97.70 \pm 0.77^{A,b}$	$97.58 \pm 1.03^{B,b}$
10	5	$96.50 \pm 2.80^{B,a}$	$97.28 \pm 1.36^{C,a}$	$94.05 \pm 2.47^{A,a}$	$96.95 \pm 0.33^{AB,a}$
	15	$98.20 \pm 0.32^{B,a}$	$99.53 \pm 0.55^{C,b}$	$93.75 \pm 0.49^{A,b}$	$97.03 \pm 1.65^{B,a}$
	30	$100.00 \pm 0.00^{B,b}$	$100.00 \pm 0.00^{C,b}$	$96.73 \pm 1.51^{A,b}$	$98.15 \pm 1.34^{B,b}$

Different uppercase superscripts of concentrations within same column indicate significant differences ($p < 0.05$). Different lowercase superscripts of times within same column indicate significant differences ($p < 0.05$).

Table 8. Means of inhibition zone diameter of bacteria isolated from vegetable surfaces.

Biomaterial	Inhibition zone (mean ± SD, mm)			
	S. Typhimurium	*Enterobacter* sp.	*Pseudomonas* sp.	*E. carotovara*
OSP_{900}	$9.36 \pm 1.08^{A,a}$	$8.93 \pm 1.14^{A,a}$	$9.14 \pm 2.95^{A,a}$	$10.00 \pm 1.96^{A,a}$
CS	$9.29 \pm 1.33^{A,b}$	$7.93 \pm 0.83^{A,a}$	$8.86 \pm 1.75^{A,ab}$	$9.50 \pm 1.605^{A,b}$

Diameter of paper disc equals 6 mm. Different uppercase superscripts of biomaterials for each bacterial species in same column indicate significant differences ($p < 0.05$). Different lowercase superscripts of bacterial species between biomaterials within same row indicate significant differences ($p < 0.05$).

Table 9. Minimal inhibitory concentration (MIC) of oyster shell powder calcined at 900 °C (OSP_{900}) and chitosan (CS) against bacteria isolated from vegetable surfaces.

Conc. (mg/mL)	Time (min)	Antibacterial activity (mean ± SD, %)							
		OSP_{900}				CS			
		S. Typhimurium	*Enterobacter* sp.	*Pseudomonas* sp.	*E. carotovara*	*S.* Typhimurium	*Enterobacter* sp.	*Pseudomonas* sp.	*E. carotovara*
1.25	0	$91.19 \pm 2.96^{A,a}$	$66.01 \pm 7.30^{A,a}$	$80.17 \pm 11.20^{A,a}$	$93.02 \pm 5.76^{A,a}$	$21.03 \pm 6.03^{A,a}$	$42.22 \pm 16.12^{A,a}$	$42.93 \pm 1.33^{A,a}$	$69.80 \pm 2.44^{A,a}$
	5	$97.50 \pm 2.25^{B,b}$	$95.06 \pm 11.30^{B,b}$	$95.06 \pm 8.55^{C,c}$	$97.27 \pm 4.73^{B,b}$	$58.50 \pm 12.69^{B,b}$	$54.41 \pm 12.69^{B,b}$	$52.61 \pm 6.64^{AB,a}$	$90.41 \pm 2.74^{C,c}$
	15	$99.45 \pm 0.95^{B,b}$	$97.10 \pm 5.02^{B,b}$	$97.53 \pm 4.28^{C,cd}$	$98.91 \pm 1.89^{B,bc}$	$90.96 \pm 0.95^{D,d}$	$70.03 \pm 2.93^{C,cd}$	$62.63 \pm 1.21^{B,b}$	$94.29 \pm 2.63^{D,c}$
2.5	0	$97.68 \pm 1.94^{B,b}$	$80.38 \pm 7.27^{B,b}$	$90.70 \pm 5.18^{B,b}$	$96.24 \pm 4.14^{B,b}$	$29.09 \pm 4.34^{A,a}$	$67.37 \pm 3.76^{C,c}$	$59.09 \pm 4.13^{B,b}$	$77.09 \pm 4.64^{B,b}$
	5	$99.38 \pm 1.07^{B,b}$	$97.83 \pm 3.76^{B,b}$	$98.77 \pm 2.14^{C,cd}$	$99.77 \pm 0.89^{B,c}$	$85.06 \pm 4.68^{C,c}$	$68.45 \pm 3.08^{C,c}$	$73.60 \pm 2.43^{C,c}$	$93.46 \pm 4.62^{D,c}$
	15	$100.00 \pm 0.00^{B,b}$	$100.00 \pm 0.00^{B,b}$	$100.00 \pm 0.00^{C,d}$	$100.00 \pm 0.00^{B,c}$	$92.79 \pm 1.82^{D,e}$	$89.65 \pm 1.43^{D,d}$	$87.73 \pm 2.91^{D,d}$	$96.30 \pm 1.07^{D,c}$

Different uppercase superscripts for concentrations within same column indicate significant differences ($p < 0.05$). Different lowercase superscripts for times within same column indicate significant differences ($p < 0.05$).

Table 10. Amount of antibacterial activity against bacteria isolated from vegetable surfaces on kale using oyster shell powder calcined at 900 °C (OSP_{900}) and chitosan (CS).

Conc. (mg/mL)	Time (min)	Antibacterial activity (mean ± SD, %)							
		OSP_{900}				CS			
		S. Typhimurium	*Enterobacter* sp.	*Pseudomonas* sp.	*E. carotovara*	*S.* Typhimurium	*Enterobacter* sp.	*Pseudomonas* sp.	*E. carotovara*
1.25	0	$86.82 \pm 17.76^{A,a}$	$63.70 \pm 10.48^{A,a}$	$88.97 \pm 4.15^{A,a}$	$59.97 \pm 6.33^{A,a}$	$63.73 \pm 3.46^{A,a}$	$43.23 \pm 13.59^{A,a}$	$60.23 \pm 8.76^{A,a}$	$74.90 \pm 7.28^{A,a}$
	5	$99.51 \pm 0.83^{B,b}$	$98.43 \pm 2.72^{B,b}$	$99.94 \pm 0.11^{B,b}$	$99.57 \pm 0.15^{B,b}$	$79.82 \pm 7.75^{B,b}$	$58.39 \pm 17.32^{B,b}$	$86.37 \pm 2.35^{C,c}$	$81.90 \pm 6.44^{B,b}$
	15	$99.88 \pm 0.21^{B,b}$	$99.41 \pm 1.02^{B,b}$	$99.90 \pm 0.69^{B,b}$	$99.90 \pm 0.17^{B,b}$	$88.43 \pm 4.06^{C,c}$	$90.76 \pm 6.44^{D,d}$	$92.52 \pm 1.35^{D,d}$	$89.15 \pm 5.36^{C,b}$
2.5	0	$99.17 \pm 0.88^{B,b}$	$95.74 \pm 1.84^{B,b}$	$99.58 \pm 0.23^{B,b}$	$69.74 \pm 3.15^{B,b}$	$65.39 \pm 15.74^{A,a}$	$72.29 \pm 5.43^{C,c}$	$79.96 \pm 7.95^{B,b}$	$77.09 \pm 4.64^{AB,a}$
	5	$99.85 \pm 0.25^{B,b}$	$99.72 \pm 0.49^{B,b}$	$99.87 \pm 0.15^{B,b}$	$90.16 \pm 1.73^{B,b}$	$78.27 \pm 14.91^{B,b}$	$90.99 \pm 1.63^{D,d}$	$91.12 \pm 1.63^{D,d}$	$91.12 \pm 3.08^{D,c}$
	15	$100.00 \pm 0.00^{B,b}$	$100.00 \pm 0.00^{B,b}$	$100.00 \pm 0.00^{B,b}$	$100.00 \pm 0.00^{B,b}$	$94.06 \pm 0.56^{D,d}$	$90.32 \pm 3.67^{D,d}$	$94.96 \pm 1.12^{D,d}$	$96.44 \pm 0.97^{D,c}$

Different uppercase superscripts for concentrations within same column indicate significant differences ($p < 0.05$). Different lowercase superscripts for times within same column indicate significant differences ($p < 0.05$).

Table 11. Amount of antibacterial activity against bacteria isolated from vegetable surfaces on lettuce using oyster shell powder calcined at 900 °C (OSP_{900}) and chitosan (CS).

Conc. (mg/mL)	Time (min)	Antibacterial activity (mean ± SD, %)							
		OSP_{900}				CS			
		S. Typhimurium	*Enterobacter* sp.	*Pseudomonas* sp.	*E. carotovara*	*S.* Typhimurium	*Enterobacter* sp.	*Pseudomonas* sp.	*E. carotovara*
1.25	0	$74.24 \pm 7.05^{A,a}$	$96.81 \pm 4.26^{A,a}$	$99.64 \pm 0.18^{A,a}$	$98.72 \pm 0.52^{A,a}$	$32.51 \pm 2.92^{A,a}$	$78.42 \pm 1.16^{A,a}$	$72.62 \pm 4.51^{A,a}$	$54.13 \pm 10.65^{A,a}$
	5	$97.07 \pm 0.89^{B,b}$	$99.07 \pm 0.89^{B,b}$	$99.92 \pm 0.14^{A,a}$	$99.85 \pm 0.23^{A,a}$	$36.23 \pm 11.53^{B,b}$	$85.06 \pm 4.70^{AB,a}$	$81.21 \pm 3.12^{B,b}$	$82.60 \pm 4.13^{B,b}$
	15	$99.42 \pm 0.30^{B,b}$	$99.87 \pm 0.23^{B,b}$	$99.98 \pm 0.35^{A,a}$	$99.91 \pm 0.15^{A,a}$	$87.44 \pm 6.04^{D,d}$	$92.32 \pm 0.97^{B,b}$	$90.02 \pm 0.69^{C,c}$	$89.72 \pm 2.24^{C,c}$
2.5	0	$98.57 \pm 0.50^{B,b}$	$99.58 \pm 0.39^{B,b}$	$99.94 \pm 0.11^{A,a}$	$99.84 \pm 0.28^{A,a}$	$36.74 \pm 2.71^{B,b}$	$82.89 \pm 0.25^{AB,a}$	$87.09 \pm 0.93^{C,c}$	$73.95 \pm 10.60^{B,b}$
	5	$99.83 \pm 0.28^{B,b}$	$99.93 \pm 0.11^{B,b}$	$99.98 \pm 0.34^{A,a}$	$99.95 \pm 0.09^{A,a}$	$68.48 \pm 12.18^{C,c}$	$90.97 \pm 2.07^{B,b}$	$91.35 \pm 1.50^{CD,c}$	$90.19 \pm 3.66^{A,a}$
	15	$100.00 \pm 0.00^{B,b}$	$100.00 \pm 0.00^{B,b}$	$100.00 \pm 0.00^{A,a}$	$100.00 \pm 0.00^{A,a}$	$91.06 \pm 3.64^{E,e}$	$95.60 \pm 0.66^{C,c}$	$94.66 \pm 0.65^{D,c}$	$96.86 \pm 0.87^{D,d}$

Different uppercase superscripts for concentrations within same column indicate significant differences ($p < 0.05$). Different lowercase superscripts for times within same column indicate significant differences ($p < 0.05$).

(Table 10), OSP_{900} at 2.5 mg/mL for 15 min gave the highest antibacterial activity against all four species at 100.00 ± 0.00%. The highest activity of CS on kale was the same as that of OSP_{900}. The CS at 2.5 mg/mL for 15 min inhibited *S.* Typhimurium, *Enterobacter* sp., *Pseudomonas* sp. and *E. carotovora* at 94.06 ± 0.56%, 90.32 ± 3.67%, 94.96 ± 1.12% and 96.44 ± 0.97%, respectively. On lettuce (Table 11), OSP_{900} at 2.5 mg/mL for 15 min gave the highest antibacterial activity against all four species at 100.00 ± 0.00%. The highest activity of CS on lettuce was the same as that of OSP_{900}. The CS at 2.5 mg/mL for 15 min inhibited *S.* Typhimurium, *Enterobacter* sp., *Pseudomonas* sp. and *E. carotovora* at 91.06 ± 3.64%, 95.60 ± 0.66%, 94.66 ± 0.65% and 96.86 ± 0.87%, respectively. Therefore, OSP_{900} can use for eliminating Gram–negative bacteria better than Gram–positive bacteria, and CS can apply to inhibit Gram–positive better than Gram–negative (Benhabiles et al., 2012; Rashki et al., 2021).

4. Conclusion

Biomaterials made from seafood waste by synthesizing CaO from natural oyster shell powder (NOSP) are best decomposed at 900 °C into CaO, and this produced better inhibition against *E. coli* than *S. aureus* with no visible growth at MIC values of 2.5 and 5 mg/mL, respectively, for 15 min OSP_{900} eliminated *E. coli* and *S. aureus* completely at MBC values of 5 and 10 mg/mL, respectively, for 15 min. The deacetylation of chitosan powder to obtain amino and hydroxyl groups in CS had better inhibitory ability against *S. aureus* than *E. coli* at the lowest concentration at 5 mg/mL for 5 min. Based on inhibition of bacteria on kale and lettuce, soaking in 2.5 mg/mL of OSP_{900} for at least 15 min could be recommended for better inhibition of *E. coli*. Soaking in 2.5 mg/mL of CS for 15 min could also be recommended and was more effective at inhibiting *E. coli*. Therefore, these research results indicated the usefulness of biomaterials synthesized from seafood wastes as vegetable washing agents to reduce the amount of contaminated bacteria on the vegetables, particularly Gram–negative pathogenic bacteria and to ensure the safety of fresh vegetable consumption by humans. In addition, OSP_{900} can decrease the deterioration of vegetable from phytopathogenic bacteria such as *Erwinia carotovora carotovora*, thus the OSP_{900} and CS has been used as the biofilm to warp and increase the shelf-life of agricultural products.

Declarations

Author contribution statement

Suree Tongwanichniyom, Somkiat Phornphisutthimas: Conceived and designed experiments; Performed the experiments; Analyzed and interpreted data; Contributed reagents, materials, analysis tools and data; Wrote the paper.

Sunan Kitjaruwankul: Performed the experiments; Analyzed and interpreted data; Contributed reagents, materials, analysis tools and data.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The materials and instruments used in this research project were provided by the Faculty of Science at Sriracha, Kasetsart University, Thailand, and Research Unit on Science, Technology and Environment for Learning and Department of Biology, Faculty of Science, Srinakharinwirot University.

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