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Evaluation of Antimicrobial Activity of Allyl Isothiocyanate (AITC) Adsorbed in Oyster Shell on Food-borne Bacteria

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요 약

굴 패각은 한국 남쪽의 해안의 바다 양식 폐기물로써 처리문제로 대두되고 있다. 폐기물인 굴 패각을 실용화하기 위해서, 현지 회사에서 구입한 소성된 굴 패각(COS)에 AITC (allyl isothiocyanate)를 흡착시킨 후 식품 감염 질병을 일으키는 박테리아에 대해 성장억제능력을 시험하였다. COS 분말은 *Escherichia coli*, *Staphylococcus aureus*, *Salmonella thyphimurium* 균에 대해 1% 정도의 농도에서 세균 발육 억제 효과를 3에서 5 log 10 CFU/mL로 나타냄으로써 세균 발육 억제 효과를 보였으며, 순수 AITC의 MIC 결과는 *Escherichia coli*, *Staphylococcus aureus*, *Salmonella thyphimurium*에 대해 각각 1 mg/mL, 0.8 mg/mL 0.7 mg/mL을 나타내었다. 소성된 굴 패각은 소성과정에서 기공이 생성되어 225 mg/g의 AITC를 흡착하였고, FTIR 결과로 COS에 AITC가 흡착이 되었음을 확인하였다. 입자의 특성은 매우 미세한 입자 크기 및 높은 선상 표면을 나타내었다. AITC가 흡착된 소성된 굴 패각(ACOS)은 1% 농도에서 완전히 세균 세포를 억제함에 따라, ACOS는 COS보다 더 나은 항균활성을 나타냄을 확인하였으며, 이는 박테리아에 대해 AITC와 소성된 굴 패각의 상승효과가 있음을 나타내었다.

주제어 : 소성, 굴, Allyl isothiocyanate (AITC), 항균

Abstract : Oyster shells are a waste product from mariculture that creates a major disposal problem in coastal regions of southeast Korea. To make practical use of unused oyster shells, calcined oyster shell (COS) collected from a local company was allowed to adsorb AITC (allyl isothiocyanate), and then tested the powder's ability to inhibit the growth of some potential food borne disease-causing bacteria. COS powder showed bacteriostatic effect that inhibited cell growth of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* from 3 to 5 log10 CFU/mL at concentrations around 1%. The MIC of pure AITC was found as 1 mg/mL, 0.8 mg/mL and 0.7 mg/mL for *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium*, respectively. The calcined powder adsorbed about 225 mg of AITC per gram of shell, indicating porous material was created by calcination. FTIR data confirmed the adsorption of AITC by COS. Characterization of particle data showed very fine particle size and highly convoluted surface. AITC adsorbed calcined oyster shell (ACOS) completely inhibited bacterial cell at 1% concentration. ACOS showed better antibacterial effect than COS, indicating synergistic effect of AITC and calcined oyster shell powder on bacteria.

Keywords : Calcination, Oyster, Allyl isothiocyanate (AITC), Antimicrobial

1. Introduction

Shellfish cultivation is an expanding economic activity worldwide as well as one of the major industrial sectors in coastal cities in South Korea. It is the second most important group of marine aquaculture products in Korea accounting 391060 metric tons in 2006[1]. Oyster is considered as the most common shellfish that contributes 71% of total shellfish produced in Korea[2]. However, intensive shellfish production generates a large amount of waste that accounts for hundreds of thousands of tons a year[3].

At present, in oyster shell-harvesting districts in Korea, large amounts of shells are piled up near the seaside, which creates several serious problems such as the emission of offensive odors and soil pollution from heavy metals contained in the viscera, considered as an environmental hazard. Therefore, recycling of waste oyster shell has arisen as an imminent issue. The ideal solution would be to convert the waste oyster shells to a product that is both beneficial and economically viable, and eliminates environmental problem. Oyster produces 60% waste materials

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of its total weight depending on different collection seasons. Several scientists tried to explore alternative uses of this waste material. Some authors reported the use of oyster shell waste as a substituent for aggregates in construction materials[4-6] or cement clinker[7]. Kwon et al.[3] applied oyster shell materials to solve problems of water eutrophication by removing phosphate from waste water.

The main component of oyster shell is calcium carbonate ($CaCO_3$) that is converted to calcium oxide (CaO) by heat treatment, which exhibits antibacterial activity[3]. In fact, there are several reports that shell powder heated to over 700 ℃ exhibited bactericidal activity[8,9]. Oyster shell powder was also found to be applied in preparing noodles, fried chicken, sardine ball [10] and kimchi[11] for quality improvement or extension of shelf life.

In recent years, inorganic antimicrobial agents have attracted great interests for controlling of micro-organisms[12]. Sawai and Yoshikawa[13] studied the antifungal activity of metallic oxide powders (MgO, CaO and ZnO) against *Candida albicans* NBRC1060. The results showed that MgO and CaO powders exhibited antimicrobial activities against all fungi examined in their study and showed minimum difference between types of fungi. Oyster shells are mainly composed of calcium carbonate. This gives us a clue that oyster shells may have antimicrobial activities.

Allyl isothiocyanate (AITC) is one of the most common isothiocyanates that are found in cruciferous plants either in the free form or as glucosinolates. AITC from natural sources is permitted for use as a food preservative in Japan, and as a GRAS flavoring agent in the US[14]. In addition to these applications, it was found that AITC was applied in the treatment of human prostate cancers since it acts as a cancer chemopreventive[15]. Although several studies reported that AITC was effective against various foodborne pathogens such as *Escherichia coli* O157:H7, *L. monocytogenes*, *S. typhimurium*, *Bacillus cereus, Staphylococcus aureus* and *Campylobacter jejuni*, *in vitro*[16-18] or in meat products including ground beef, fresh chicken, fermented sausage and Westphalian ham[19], its high volatility, strong pungency limit its application in food systems[20]. Several materials such as alginate beads, cyclodextrin, maize, and mesoporous silica were used to adsorb AITC and its subsequent release to control microorganisms.

AITC cannot be used along in food system as an antimicrobial compound due to its' high volatile nature. Raw oyster shells are expected to produce porous materials at high temperature. The porous materials will allow adsorbing volatile AITC in its porous structures. The COS will be used as a carrier for the controlled release of AITC from the COS when it will be applied as antimicrobial agent. The synergistic effect of AITC and calcined oyster shell will affect on bacteria. Therefore, the aim of the present study was to investigate the antibacterial activity of calcined oyster shell (COS) and AITC adsorbed calcined oyster shell (ACOS).

2. Material and Methods

2.1. Materials

COS was collected from a Korean local company, A-sung Fine Chemistry, Ulsan, Korea. AITC (>93% GC purity) were purchased from Sigma-Aldrich, Germany (Cat. no. W203410). All other reagents used were of analytical grade.

2.2. Adsorption of AITC in calcined oyster shell

COS loading with AITC was achieved via vapor adsorption by placing calcined shell in a long glass-made column (length 21 cm, diameter 2.8 cm) which was set on a AITC containing round flask. In this experiment, 4 gram of COS was taken in the column. One mL of AITC was taken in the flask. The flask was heated at 40 ℃ on a water bath. The opening of the column was sealed with aluminum foil with a tiny pore. The amount of AITC adsorbed by the calcined shell was determined by monitoring sample weight increase with time.

2.3. Characterization of particle

The particle was characterized by scanning electron microscopy and particle size analyzer.

2.3.1. Scanning electron microscopy (SEM)

An SEM equipped with energy dispersive X-ray microanalysis (Cat: JSM-6490LV, JEOL Ltd., Japan) was used to image the physical features of the oyster shell. Dried sample of oyster powder was attached to double-sided tape on the sample stubs, and then coated via gold sputter under reduced pressure. Image was obtained with an electron accelerating voltage of 10 kV.

2.3.2. Particle size analysis (PSA)

The size distributions of the COS were measured by particle size analyzer (LS 13320, Beckman Coulier, USA). A size distribution curve was plotted from the analysis.

2.4. Fourier-transform infrared measurements (FTIR)

FTIR measurements were made to detect any chemical interactions between AITC and calcined oyster shell. A series of transmission spectra were obtained as: (a) calcined oyster shell, (b) calcined oyster shell loaded with AITC via vapour adsorption, (c) standard AITC. The spectrometer used was a Jasco V-670 (Jasco International Co. Ltd., Tokyo, Japan); spectra were meas-

ured over the range of 3,600-800 cm^{-1}, with a resolution of 4 cm^{-1}.

2.5. Test Bacteria

The test were performed against one Gram-positive and two Gram-negative food borne bacteria. Gram-positive: *Staphylococcus aureus* KCCM 11335, Gram-negative: *Escherichia coli* ATCC 25922, and *Salmonella typhimurium* KCCM 11862. Mueller-Hinton agar (Cat.: 70191, Sigma Aldrich, USA) and tryptone soya broth (Cat.: CM 0129, Oxoid Ltd., Hampshire, England) were used for this test. All bacterial strains were purchased from the Korean Culture Center of Micro-organisms (KCCM), Republic of Korea.

2.6. Preparation of Bacteria

From a pure 20-24 hour bacterial culture, 4-5 isolated colonies were taken and sub-cultured to a tube with 3 mL tryptone soya broth. It was incubated at 35 ℃ in a shaking incubator until it reaches or exceeds the turbidity of 0.5 MacFarland standard (corresponds to approximately 1.5×10^8 CFU/mL). The inoculum was standardized based on optical density (OD600 of 0.08-0.1) using a spectrophotometer (UV mini-1240, Shimadzu, Kyoto, Japan). Within 15 minutes of adjusting the inoculum to the 0.5 McFarland turbidity standards, it was diluted so that the final concentration achieves at 1.5×10^8 CFU/mL.

2.7. Determination of MIC of AITC

The minimal inhibitory concentration (MIC) of an antimicrobial agent is the lowest (i.e. minimal) concentration of the antimicrobial agent that inhibits a given bacterial isolate from multiplying and producing visible growth in the test system. Broth macro-dilution method was used for testing MIC. Different serial dilutions of standards AITC was prepared of which 1 mL of each dilution was added to each tube. Within 15 minutes after the inoculum was standardized, as described previously, 1 mL of the adjusted inoculum was added to each tube containing the antimicrobial agent. Each tube was mixed and incubated at 35 ℃ for 20 hours in an incubator. The MIC was determined as the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in the tubes as detected by the unaided eye.

2.8. Antibacterial assay

The antibacterial activity was evaluated by broth macro-dilution method counting CFU/mL. Different dilutions of calcined shell and AITC adsorbed calcined shell were prepared by mixing with sterile water. One mL of each dilution was added to each tube. Within 15 minutes after the inoculum was standardized, as described previously, 1 mL of the adjusted inoculum was added to each tube containing the antimicrobial agent. Each tube was mixed and incubated at 35 ℃ for 20 hours in a shaking incubator. After incubation, colony counting method was applied to know the number of viable bacteria. For that, 10 μL was poured on agar plate from each serial dilution. The plates were incubated at 35 ℃ for 16 to 20 hours. Colony forming unit (CFU/mL) was calculated by counting number of colony on agar plates.

2.9. Statistical analysis

All tests and analyses were repeated at least three times. The results are expressed as means±SD. A one way analysis of variance (ANOVA) and Duncan test were used for multiple comparisons using the SPSS program (IBM SPSS Statistics 20). Values were considered to differ significantly if the *P* value was less than 0.05.

3. Results and Discussion

3.1. Adsorption of AITC in calcined oyster shell

Adsorption of AITC by COS was increased with time (Figure 1). After reaching saturation level, adsorbing 225 mg/g after 312 h, it continued stable. Calcined oyster shell adsorbed AITC vapor, which indicates porous material was created upon heat treatment in oyster shell.

3.2. Characterization of particle

From the result of SEM, we found that the surface of the calcined oyster was highly convoluted (Figure 2). The rough and convoluted surface indicates good decomposition during calcination[3]. A distribution curve was obtained from the PSA data (Figure 3). The particle was found very fine consisting 32 μm mean size, suggesting wide surface area.

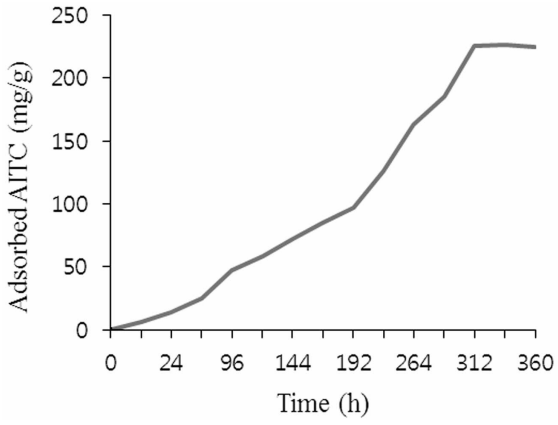

Figure 1. Adsorption of AITC in calcined oyster shell.

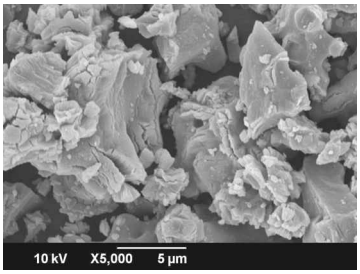

Figure 2. Image of the calcined oyster shells surface by scanning electron micrograph.

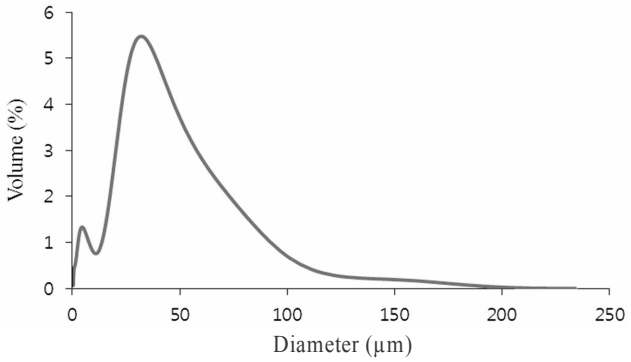

Figure 3. Particle size distribution of calcined oyster shell.

3.3. Fourier-transform infrared measurements

The presence of AITC on the calcined oyster shell powder was confirmed by FTIR. The IR spectrum shown in Figure 4 (a) represents calcined oyster shell, while spectrum (b) represents the calcined oyster shell filled with AITC. The absorbance bands at 2,164 and 2,086 cm^{-1} were attributed to stretching vibrations of isothiocyanate (−N = C = S) and the bands at 986 and 921 cm^{-1} indicated AITC stretching frequencies from (= C − CH, − CH = CH2)[17,21,22]. These bands are also shown in the spectrum (b) confirming the presence of AITC in calcined oyster shell. The subtle shifts in the AITC peaks to lower energy in the adsorbed phase confirm liquid-like structure within the adsorbed phase in the pore network.

3.4. Minimum Inhibitory Concentration (MIC) of AITC

The lowest (i.e. minimal) concentration of the antimicrobial agent that inhibits a given bacterial isolate from multiplying and producing visible growth in the test system was referred to as minimal inhibitory concentration (MIC). According to MIC test, *Salmonella typhimurium* was the most sensitive bacteria inhibited by AITC at 0.7 mg/mL (Table 1). Other bacteria were also inhibited by AITC at reasonably low concentration. The minimum inhibitory concentration of pure AITC at vapor phase was reported as 34-110 ng/mL for some bacteria by Isshiki et al.[23]. The difference arrived due to method of experiment and strains of bacteria studied.

3.5. Antibacterial test

The antibacterial activity of calcined oyster shell (COS) and AITC adsorbed calcined oyster shell (ACOS) were found by estimating colony forming unit (CFU/mL). The results were shown in Table 2, Figure 5-7. *E. coli*, *S. aureus* and *S. typhimurium*

Table 1. Minimum Inhibitory Concentration (MIC) of standard AITC for different bacteria

Bacteria	Standard AITC
E. coli	1.0 ± 0.00 mg/mL
S. aureus	0.8 ± 0.00 mg/mL
S. typhimurium	0.7 ± 0.00 mg/mL

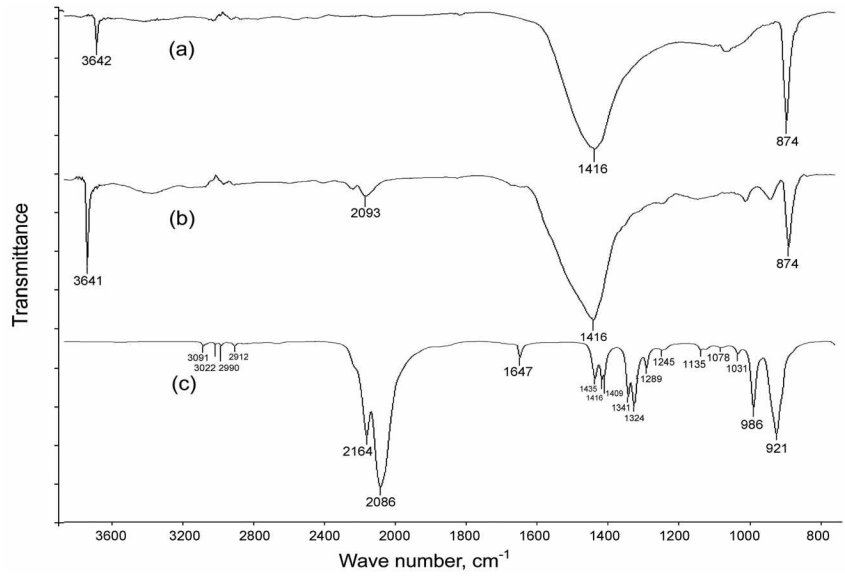

Figure 4. FTIR spectra of (a) calcined oyster shell (b) calcined oyster shell loaded with AITC and (c) standard AITC.

Table 2. Antibacterial test of calcined oyster shell and AITC absorbed calcined oyster shell

Bacteria	CFU/mL						
	Control	Calcined oyster shell (COS)			AITC absorbed calcined shell (ACOS)		
		0.1%	0.5%	1.0%	0.1%	0.5%	1.0%
E. coli	$3.2\times10^{8}\pm3.6\times10^{7a}$	$1.5\times10^{8}\pm2.6\times10^{7b}$	$2.8\times10^{6}\pm3.6\times10^{5c}$	$1.2\times10^{3}\pm1.0\times10^{2c}$	$4.1\times10^{7}\pm4.5\times10^{6c}$	$3.2\times10^{5}\pm3.6\times10^{4c}$	0^{c}
S. aureus	$2.1\times10^{7}\pm2.6\times10^{6b}$	$2.5\times10^{7}\pm2.6\times10^{6a}$	$2.4\times10^{6}\pm2.6\times10^{5d}$	$1.6\times10^{3}\pm2.0\times10^{2d}$	$2.2\times10^{7}\pm3.0\times10^{6c}$	$5.2\times10^{3}\pm4.5\times10^{2d}$	0^{d}
S. typhimurium	$4.4\times10^{7}\pm5.5\times10^{6a}$	$2.5\times10^{7}\pm3.2\times10^{6b}$	$1.5\times10^{7}\pm1.7\times10^{5c}$	$3.5\times10^{4}\pm4.5\times10^{3d}$	$1.8\times10^{7}\pm3.0\times10^{6c}$	$2.6\times10^{4}\pm4.0\times10^{3d}$	0^{d}

Means±SD (n-3). Different small letters in each row indicate significant differences ($P < 0.05$)

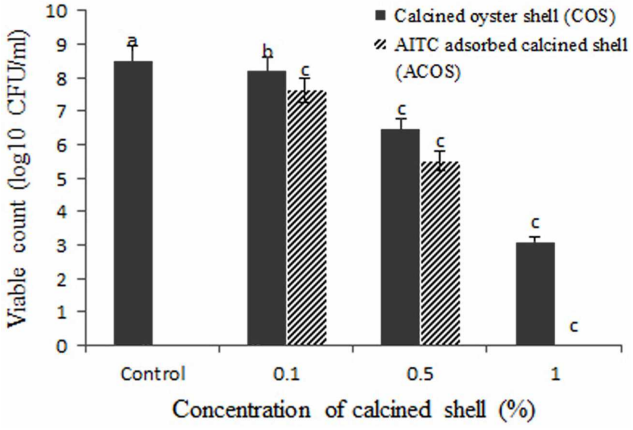

Figure 5. Effect of AITC adsorbed oyster shell on *E. coli.* (Means ± SD (n-3). Different small letters in each column bar indicate significant differences ($P < 0.05$)).

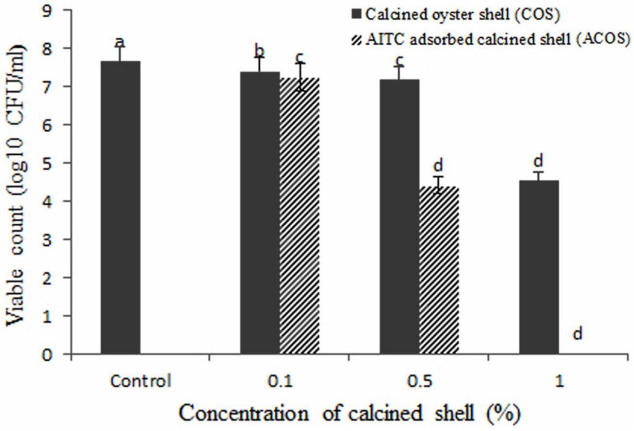

Figure 7. Effect of AITC adsorbed oyster shell on *S. typhimurium.* (Means±SD (n-3). Different small letters in each column bar indicate significant differences ($P < 0.05$)).

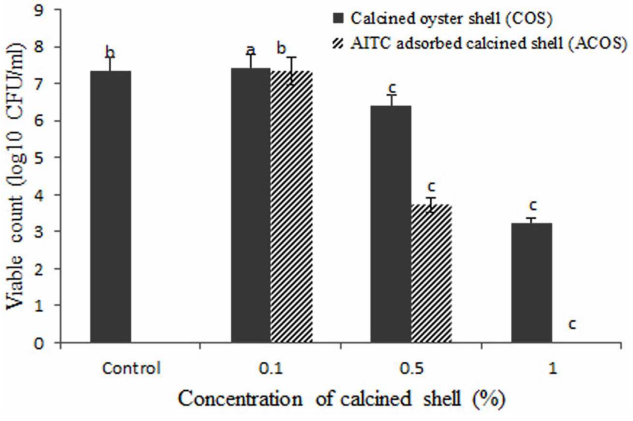

Figure 6. Effect of AITC adsorbed oyster shell on *S. aureus.* (Means±SD (n-3). Different small letters in each column bar indicate significant differences ($P < 0.05$)).

were restricted to 10^{3}-10^{4} CFU/mL by COS at 1% from its initial content 10^{8}-10^{7} CFU/mL whereas, completely destroyed by ACOS at 1%. Although 1% of COS and ACOS did not show significant differences ($P < 0.05$), ACOS killed all bacteria. This may have significant advantage when ACOS is used for long term preservation of food materials. Several reports showed that shell powder heated to over 700 ℃ exhibited antibacterial activity[8,9]. Oyster shell powder was found to prepare noodles, fried chicken, sardine ball, tafu and kimchi for quality improvement or extension of shelf life[10,11,24]. Li et al.[25] explored the antibacterial activity of mussel shell waste against *E. coli* and *S. aureus* which supports the result of present study. The raw oyster shell contains calcium carbonate ($CaCO_3$) as the main component. This $CaCO_3$ is converted to calcium oxide (CaO) by heat treatment, which exhibits antibacterial activity[3]. Oikawa et al.[26] reported that the antibacterial activity was due to the strong alkalinity of aqueous solutions of this calcined calcium preparation.

AITC has been reported to have strong antimicrobial activity against several food poisoning bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas fragi*, and *Pseudomonas aeruginosa*[27-29]). AITC act on bacteria by destroying cell membrane and thus leakage of cellular metabolites [30]. AITC has been shown to have antimicrobial activity against some pathogenic bacteria, yeasts, or molds and is permitted for use as a food preservative, but use of AITC in food systems is limited due to its strong odor[31,32]. Thus, reduction of the odor of AITC is necessary for food applications. When AITC was adsorbed by calcined oyster shell, antimicrobial activity of that shell increased due to combined effect of AITC and calcined

shell. This synergistic effect could allow less concentration of AITC to be lethal against bacteria so that it can be used as food preservatives with minimum odor. AITC adsorbed calcined shell (ACOS) showed more antibacterial activity than calcined shell at all concentration against all three bacteria (Figure 5-7).

4. Conclusions

To reclaim large amount of waste materials produced from oyster and to explore a new antimicrobial material, calcined oyster shell loaded with AITC was evaluated. The present study has demonstrated that a waste product-oyster shells-can be transformed into an effective antibacterial material. AITC adsorbed by the calcined oyster shell enhanced the antibacterial activity against three food borne bacteria. The antibacterial activity of oyster shell was involved with the calcium oxide that was converted from calcium carbonate by heat treatment. AITC adsorbed by the calcined shell enhanced the antibacterial activity of the powder. Porous material was created by heat treatment of oyster shell. The prepared material could be explored as antimicrobial agent applied in food and other industries that facilitate the alternative use of waste produced from oyster shell.

Abbreviations

ACOS	AITC Adsorbed Calcined Oyster Shell
AITC	Allyl Isothiocyanate
ATCC	American Type Culture Collection
CFU	Colony Forming Unit
COS	Calcined Oyster Shell
FTIR	Fourier-transform Infrared Measurements
GRAS	Generally Regarded as Safe
KCCM	Korean Culture Center of Micro-organisms
MIC	Minimum Inhibitory Concentration
PSA	Particle Size Analysis
SEM	Scanning Electron Microscopy

References

1. Yoon, G. H., "Aquaculture in Korea," *Aqua. News.*, **34**, 16-17 (2008).
2. Choi, K. S., "Oyster Capture-based Aquaculture in the Republic of Korea," *FAO Fish. Tech. Paper*, **508**, 271-286 (2008).
3. Kwon, H.-B., Lee, C.-W., Jun, B.-S., Weon, S.-Y., and Koopman, B., "Recycling Waste Oyster Shells for Eutrophication Control," *Resour. Conserv. and Recy.*, **41**(1), 75-82 (2004).
4. Yoon, G.-L., Kim, B.-T., Kim, B.-O., and Han, S.-H., "Chemical-mechanical Characteristics of Crushed Oyster-shell," *Waste Manage.*, **23**(9), 825-834 (2003).
5. Yoon, H., Park, S., Lee, K., and Park, J., "Oyster Shell as Substitute for Aggregate in Mortar," *Waste Manage. Res*., **22** (3), 158-170 (2004).
6. Yang, E.-I., Yi, S.-T., and Leem, Y.-M., "Effect of Oyster Shell Substituted for Fine Aggregate on Concrete Characteristics: Part I. Fundamental Properties," *Cement. Concrete. Res.*, **35** (11), 2175-2182 (2005).
7. Cheon, S.-M., and Song, T.-W., "Study on Formation of Cement Clinker from the Mixture of Oyster Shell, Casting Dust and BOF Slag," *J. Korean Ceram. Soc.*, **40**(12), 1235-1240 (2003).
8. Sawai, J., Shiga, H., and Kojima H., "Kinetic Analysis of the Bacterial Action of Heated Scallop-shell Powder," *Int. J. Food. Microbiol*., **71**, 211-218 (2001).
9. Shiga, H., Sawai, J., and Kojima, H., "Utilization of Heated Shell Powder in Biocontrol," *T. Mr. S. Jap.*, **24**, 557-560 (1999).
10. Suhara, H., "Applicaton of Antimicrobial Calcium Agent in Food Products," *Food. Indus. (in Japanese)*, **38**, 32-44 (1195).
11. Choi, Y. M., Whang, J. Y., Kim, J. M., and Suh, H. J., "The Effect of Shell Powder on the Extension of the Shelf Life of Kimchi," *Food Control*., **17**, 695-699 (2006).
12. Nakashima, T., Sakagami, Y., and Matsuo, M., "Antibacterial Effect of Cotton Fabrics Chemically Modified by Metal Salt," *Biocontrol Sci.*, **6**, 9-15 (2001).
13. Sawai, J., and Yoshikawa, T., "Quantitative Evaluation of Antifungal Activity of Mettalic Oxide Powders (MgO, CaO and ZnO) by an Indirect Conductimetric Assay," *J. Appl. Microbiol*., **96**(4), 803-809 (2004).
14. Kim, Y., Ahn, E., and Shin, D., "Extension of Shelf Life by Treatment with Allyl Isothiocyanate in Combination with Acetic Acid on Cooked Rice," *J. Food. Sci.*, **67**(1), 274-279 (2002).
15. Zhang, Y., "Allyl Isothiocyanate as a Cancer Chemopreventive Phytochemical," *Mol. Nutr. Food Res*., **54**(1), 127-135 (2010).
16. Dufour, V., Alazzam, B., Ermel, G., Thepaut, M., Rossero, A., Tresse, O., and Baysse, C., "Antimicrobial Activities of Isothiocyanates Against *Campylobacter Jejuni* Isolates," *Front Cell. Inf. Microbiol.*, **2**, (2012).
17. Siahaan, E. A., Meillisa, A., Woo, H.-C., Lee, C.-W., Han, J.-H., and Chun, B.-S., "Controlled Rlease of Allyl Isothiocyanate from Brown Algae *Laminari Japonica* and Msoporous Slica MCM-41 for Inhibiting Food-borne Bacteria," *Food Sci. Biotech*., **22**(1), 19-24 (2013).
18. Zou, Y., Jung, L. S., Lee, S. H., Kim, S., Cho, Y., and Ahn, J., "Enhanced Antimicrobial Activity of Nisin in Combination with Allyl Isothiocyanate Against Listeria Monocytogenes, Staphylococcus aureus, Salmonella Typhimurium and Shigella Boydii," *Int. J. Food Sci. Tech*., **48**(2), 324-333 (2013).
19. Graumann, G. H., and Holley, R. A., "Survival of *E. coli* O157:H7 during Manufacture of Dry-curved Westphalian Ham Surface-treated with Allyl Isothiocyanate or Hot Mustard Powder," *J. Sci. Food Agril*., **89**(4), 617-624 (2009).

20. Kim, W.-T., Chung, H., Shin, I.-S., Yan, K. L., and Chung, D., "Characterization of Calcium Alginate and Chitosan-treated Calcium Alginate Gel Beads Entrapping Allyll Isothiocyanate," *Carbohyd. Polym.*, **71**(4), 566-573 (2008).
21. Park, S.-Y., Barton, M., and Pendleton, P., "Controlled Release of Allyl Isothiocyanate for Bacteria Growth Management," *Food Control*, **23**, 478-484 (2012).
22. Siahaan, E. M., Pendleton, P., Woo, H.-C., and Chun, B.-S., "Brown Seaweed (*Saccharina Japonica*) as an Edible Natural Delivery Matrix for Allyl Isothiocyanate Inhibiting Food-borne Bacteria," *Food Chem*., **152**, 11-17 (2014).
23. Isshiki, K., Tokuoka, K., Mori, R., and Chiba, S., "Preliminary Examination of Allyl Isothiocyanate Vapor for Food Preservation," *Biosci. Biotech. Bioch.*, **56**(9), 1476-1477 (1992).
24. Kim, Y. S., Choi, Y. M., Noh, D. O., Cho, S. Y., and Suh, H. J., "The Effect of Oyster Shell Powder on the Extension of the Shelf Life of Tofu," *Food Chem.*, **103**, 155-160 (2007).
25. Li, M., Yao, Z. T., Chen, T., Lou, Z. H., and Xia, M., "The Antibacterial Activity and Mechanism of Mussel Waste Derived Material," *Powder Tech*., **264**, 577-582 (2014).
26. Oikawa, K., Asada, T., Yamamoto, K., Wakabayashi, H., Sasaki, M., Sato, M., and Matsuda, J., "Antibacterial Activity of Calcined Shell Calcium Prepared from Wild Surf Clam," *J. Health Sci*., **46**, 98-103 (2000).
27. Hasegawa, N., Matsumoto, Y., Hoshino, A., and Iwashita, K., "Comparison of Effect of *Wasabia Japonica* and Allyl Isothiocyanate on the Growth of Four Strains of Vibrio Parahaemolyticus in Lean and Fatty Tuna Meat Suspensions," *Int. J. Food Microbiol*., **49**(1-2), 27-34 (1999).
28. Lin, C.-M., Kim, J., Du, W.-X., and Wei, C. I., "Bacterial Activity of Isothiocyanate Against Pathogens on Fresh Produce," *J. Food Prot.*, **63**(1), 25-30 (2000).
29. Shin, I.-S., Han, J.-S., Choi, K.-D., Chung, D.-H., Choi, G.-P., and Ahn, J., "Effect of Isothiocyanates from Horseradish (Armoracia Rusticana) on the Quality and Shelf Life of Tofu," *Food Control*, **21**(8), 1081-1086 (2010).
30. Lin, C. M., Preston, J. F., and Wei, C. I., "Antibacterial Mechanism of Allyl Isothiocyanate," *J. Food Prot.*, **63**(6), 727-734 (2000).
31. Inatsu, Y., Bari, M. L., Kawasaki, S., and Kawamoto, S., "Effectiveness of Some Natural Antimicrobial Compounds in Controlling Pathogen or Spoilage Bacteria in Lightly Fermented Chinese Cabbage," *J. Food Sci*., **70**, 393-397 (2005).
32. Jin, T., and Gurtler, J. B., "Inactivation of Salmonella in Liquid Egg Albumen by Antimicrobial Bottle Coatings Infused with Allyl Isothiocyanate, Nisin and Zinc Oxide Nanoparticles," *J. Appl. Microbiol*., **110**, 704-712 (2011).