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Effect of Javanese turmeric (*Curcuma xanthorrhiza* Roxb.) extract on natural microflora of oyster mushroom *(Pleurotus sajur-caju)* and its sensory acceptability

[1]Lew, K. F., [1]Goh, G. L., [1]Son, R., [1,2*]Rukayadi, Y.

[1]Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
[2]Laboratory of Natural Products, Institute of Bioscience (IBS), Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

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Abstract

The effects of methanolic extract of Javanese turmeric (*Curcuma xanthorrhiza* Roxb.) at different level of concentrations on the inactivation of *Bacillus cereus, Escherichia coli, Pseudomonas* spp. and *Staphylococcus aureus* in oyster mushroom *(Pleurotus sajor-caju)* were investigated. This study was conducted principally for the achievement on the best combination between the susceptibility of *C. xanthorrhiza* extract on natural microflora and foodborne pathogenic bacteria with the sensory acceptability of the soaked oyster mushroom. Three different concentrations (g/ml), 0.05%, 0.50% and 5.00%, of *C. xanthorrhiza* extract prepared with dilution method were designed as sanitizing agent in treating the oyster mushroom at 5 minutes and 10 minutes. There was significance reduction in the survival of microbial load between the untreated fresh oyster mushroom and those soaked with 0.05%, 0.50% and 5.00% rhizome extract ($P < 0.05$). The relative best combination between antimicrobial ability and sensory acceptability can be achieved with 0.05% rhizome extract where it showed a significant bacterial population reduction ($P<0.05$) of 0.81, 0.73 and 5.54 $\log_{10}$ CFU/g for total plate count, *B. cereus* and *Pseudomonas* spp., as well as a higher mean scores for the tested sensory. The results showed that *C. xanthorrhiza* extract can be developed as natural sanitizer for food materials.

Introduction

Mushrooms have high popularity nowadays due to its high nutritional value. Oyster mushroom with the scientific name, *Pleurotus sajor-caju*, is best known medically for their cardiovascular and cholesterol-controlling benefits as it contains mevinolin and related compounds that limit the cholesterol biosynthesis (Kamal *et al*., 2009). Oyster mushroom has gained an increase in commercial cultivation in many nations particularly in South-East Asia including India (Biswas *et al.*, 2012).

After harvesting, fresh oyster mushroom may diminish in quality in terms of microbiological contamination during transportation from farm to table. Based on food handling practice in households and food services, tap water is generally used to serve as either the washing or soaking agent. However, the tap water cannot completely remove both the pathogenic bacteria and the naturally occurring bacteria (Brackett, 1992). A variety of sanitizers have been used to serve the purpose of reducing microbial population in fresh produce. Chlorine-based sanitizers have been the most commonly used chemical disinfectants, such as sodium hypochlorite (Zhang and Farber, 1996; Cao *et al*., 2009), gaseous chlorine dioxide treatment (Camp *et al.*, 2009). Others interventions of washing technologies are for instance, strong acid electrolyzed water (SAEW) (Wullaert, 1997), neutral electrolyzed oxidizing water (EOW) (Camp *et al*., 2009), peroxyacetic acid (PAA) (Camp *et al*., 2009), organic acids (Adams *et al*., 1989; Kim *et al*., 2000), and ozone (Nagashima and Kamoi, 1997; Yuk *et al*., 2007). Some of these sanitizers possess specific limitation in usage and the practical considerations in their use have to be considered.

Recently, there is an interest in the use of natural products such as plants extract to eliminate microorganism contamination on food or food materials. Javanese turmeric or "temulawak" (*Curcuma xanthorrhiza* Roxb.) extract is used as the natural sanitizer agent for antimicrobial activity against potential pathogenic bacteria. *C. xanthorrhiza* belongs to the family Zingiberaceae and the rhizome extract contains active phytochemical constituents with xanthorrhizol (64.38%) as the main compounds (Mary Helen *et al*., 2012). Xanthorrhizol

*Corresponding author.
Email: *yaya_rukayadi@upm.edu.my*
Tel: +603-3-8946-8519; Fax: +603-8942-3552

isolated from the methanolic rhizome extract of *C. xanthorrhiza* shows potent antibacterial activity against a wide spectrum of Gram-positive and negative bacterial pathogens (Hwang *et al.*, 2000). Moreover, *C. xanthorrhiza* extract has antibacterial activity against foodborne pathogens (Lee *et al.*, 2008), anticandidal and antifungal (Rukayadi *et al*., 2006; Rukayadi and Hwang, 2007a,b). Therefore, the objective of this study is to determine the effects of methanolic extract of Javanese turmeric at different level of concentrations on the inactivation of *Bacillus cereus, Escherichia coli, Pseudomonas* spp. and *Staphylococcus aureus* in oyster mushroom.

Materials and Methods

Extract preparation

Oyster mushroom species *(P. sajor-caju)* were purchased from 'Seksyen Penyelidikan dan Agroteknologi, Bangunan Pleurotus, Unit Agroteknologi Taman Pertanian Universiti (TPU), Universiti Putra Malaysia, Serdang, Selangor'. The dried powder of *C. xanthorrhiza* (100 g) was extracted twice with 200 ml of >99.8% (v/v) food grade methanol for 48 h at room temperature. Plant extracts were filtered with Whatman filter paper No. 1 (Whatman International Ltd. Middlesex, England) and concentrated with a rotary vacuum evaporator (Heidolph Instruments, Germany) at 50°C and a speed of 150 RPM, yielding methanol extracts. The yield of the extract was calculated in percentage and the extract was dissolved in sterile deionized water (DIW) to prepare three different concentrations. The extraction method used in this work was according to Rukayadi *et al.* (2008) with slight modification.

Preparation of treatment solutions

Sterile deionized water (DIW) was purchased from Megamal Pharmacy Sdn. Bhd., Penang with the brand name of "Water for Injections B. P." (B. Braun Medical Industries Sdn. Bhd., Penang, Malaysia). Methanolic extract of *C. xanthorrhiza* was dissolved in 10% of DMSO and followed by dilutions to make solutions of 5%, 0.5% and 0.05% (g/ml) for the soaking in terms of sanitizing purpose of the grey oyster mushroom samples.

Experimental procedure

Oyster mushrooms were cut into small pieces of 3 x 1 cm and mixed thoroughly to enhance homogeneity of natural background microflora that might present originally in the sample. The homogenate mushrooms were divided into 11 parts stored at 4°C and used within 24 hours. The grey oyster mushroom samples of 10 g each were immersed in 50 ml of filtered tap water, DIW, different concentrations (g/ml) of 0.05%, 0.5%, 5% extract solutions at room temperature (23 ± 2°C) for exposure time for 5 minutes. A sample was prepared without soaking with any solution to be held as the positive control.

Microbial analysis

All treated samples were aseptically removed using a flame-sterilized knife together with a clamp and drained on absorbent paper for drying. This methodology was adapted to the work of Ding *et al.* (2011). For the detection of the specified pathogenic microorganisms, each 10 g of mushroom samples was weighed and diluted into the stomacher bag (BAGLIGHT, BagSystem, Interscience, France) with 90 ml of 0.85% saline solution for *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Pseudomonas* spp. The mixture was homogenized using a stomacher machine (BagMixer 400-P, Interscience, France) for 2 minutes at 250 RPM. After homogenization, the enrichment was serially diluted with 1 ml of the enrichment into 9 ml 0.85 % saline solution to make up 10^{-2} to 10^{-5} dilutions. Plate count agar (PCA), Polymyxin-Mannitol-Egg Yolk-Phenol Red Agar (PMYPA), Baird-Parker (BP) agar, ChromoCult Coliform agar and Pseudomonas CFC Selective Agar were used.

Sensory evaluation of acceptability

The visual sensory session was held for the evaluation of five treated grey oyster mushroom samples, corresponding to the five different treatments with water and sanitizing solutions (except the positive control samples) within the specific treatment period at 5 minutes that show gradual decrease in microbial load. The oyster mushrooms were prepared with water steaming for 15 minutes to be ready for sensory testing. The test was conducted based on the 9-point hedonic scale for visual inspection acceptance testing where consumers assessed in terms of colour acceptability (1-extremely disliked colour; 9-extremely liked colour), odour acceptability (1-extremely disliked odour; 9-extremely liked odour) and texture acceptability (1-extremely disliked texture; 9-extremely liked texture). This sensory evaluation method was adopted from the previous study conducted by Brasil *et al*. (2012), on visual sensory inspection with modification.

Statistical analysis

Means of bacterial populations ($\log_{10}$ CFU/g) from each treatment were calculated from two replications of each experiment. Data were analyzed

employing MINITAB for the analysis of variance (ANOVA), one-way, unstacked, where Tukey's test was used to determine the significance of difference (P = 0.05) between different treatments.

For sensory evaluation, one-way analysis of variance (ANOVA) was applied to determine if there is significant difference among the means for each sample or treatment by using MINITAB with P value <0.05 indicates the significant effect of treatment. If there is significant difference, statistical analysis was made by Tukey's Test in MINITAB to determine which means differ from each other.

Results and Discussion

Based on the primary focus of this research in reducing the microbial load on fresh oyster mushroom, where the natural extract of *C. xanthorrhiza* was served as a protocol in sanitizing the fresh produce, an extraction was conducted from this rhizome. The yield from methanolic extraction of 100 g dried C. *xanthorrhiza* rhizomes powder is 15.39%.

For microbial analysis, the number of natural microflora, *Pseudomonas* spp., and *S. aureus* were 7.91 ± 0.014, 7.76 ± 0.028, and 5.54 ± 0.085 $\log_{10}$ CFU/g, respectively. In this study, there was no *B. cereus* and *E. coli* could be found in oyster mushrooms. The initial bacteria load of oyster mushroom in this study was high as it exceeds 10^7 CFU/g. All of these falls to the reason that mushrooms have soft tissues that are easily damaged and irregular surfaces with microenvironments that protect microbes, which make them difficult to clean (Doyle, 2005).

The effects of filtered tap water and 0.00%, 0.05%, 0.50% and 5.00% of *C. xanthorrhiza* extract on the natural microflora, *Pseudomonas* spp., and *B. cereus* of fresh harvested oyster mushroom were presented in Figure 1, 2 and 3. After treated with sanitizing solutions with the rhizome extract at room temperature for 5 minutes, the total bacteria count on oyster mushroom were 7.10, 6.33 and 5.25 for 0.05%, 0.50% and 5.00% of extract respectively. In terms of significant difference, the maximum 2.66 $\log_{10}$ CFU/g reduction and minimum 0.81 $\log_{10}$ CFU/g of total bacterial count in oyster mushroom was observed by 5.00% and 0.05% *C. xanthorrhiza* extract exposure respectively and with significance difference in log reductions observed by 0.50% extract (P <0.05) under similar treatment conditions. This study shows that increase by 10% in the extract concentration used yield approximately one log reduction in the bacterial counts. On the contrary, immersing in filtered tap water and DIW (0.00% extract) only reduced the cell count by 0.12 and 0.17 $\log_{10}$ CFU/g

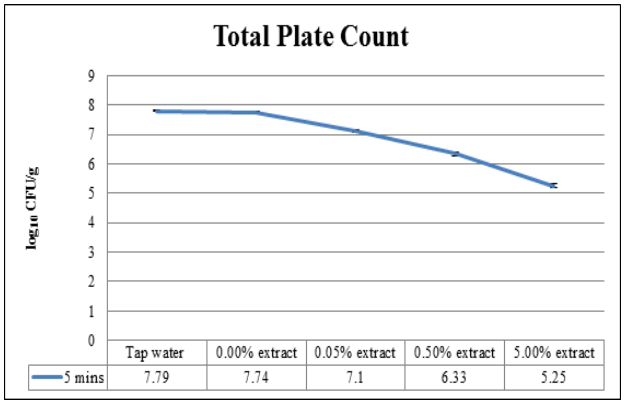

Figure 1. Log_{10} reductions of TPC present on fresh grey oyster mushroom slices treated for 5 minutes at the room temperature (23 ± 2°C).

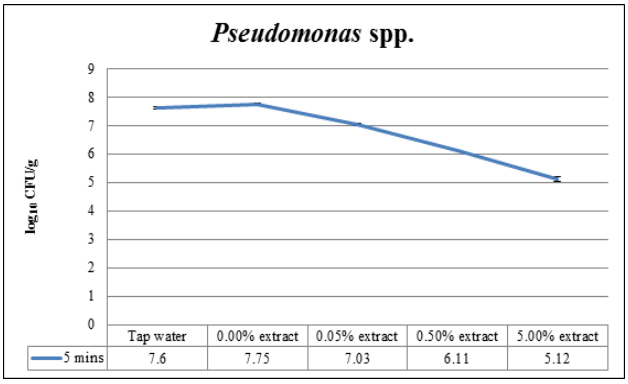

Figure 2. Log_{10} reductions of *Pseudomonas* spp. present on fresh grey oyster mushroom slices treated for 5 minutes at the room temperature (23 ± 2°C).

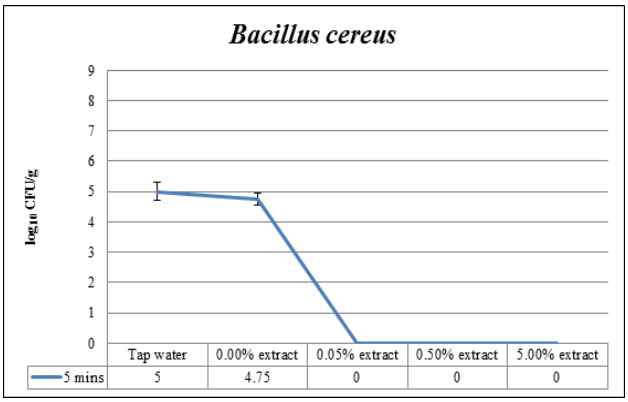

Figure 3. Log_{10} reductions of *B. cereus* present on fresh grey oyster mushroom slices treated for 5 minutes at the room temperature (23 ± 2°C).

respectively and with no significance difference (P >0.05). It was observed that the relative influence in terms of inactivation was: filtered tap water ≈ DIW < 0.05% < 0.50% < 5.00% of *C. xanthorrhiza* extract. The result indicates that the germicidal efficacy of *C. xanthorrhiza* extract on naturally occurring microflora on the oyster mushroom was stronger than that of filtered tap water and DIW used in this study, while 5.00% give the maximum significant germicidal efficacy among the three variable concentrations. Evidence has shown that xanthorrhizol isolated from the methanol extract *C. xanthorrhiza* showed potent

Table 1. Visual sensory evaluation on *C. xanthorrhiza* methanolic extract sanitized grey oyster mushroom

Attributes	Mean ± standard deviation				
	Sample A	Sample B	Sample C	Sample D	Sample E
Colour	6.90±1.553[a]	6.50±1.762[a]	6.20±1.795[a]	4.15±1.663[b]	2.90±1.447[b]
Odour	5.70±1.895[a]	5.65±1.785[a]	5.20±2.308[ab]	4.80±1.765[ab]	3.50±2.236[b]
Texture	6.70±1.031[a]	6.15±1.496[ab]	6.15±1.631[ab]	4.90±1.447[b]	4.90±1.804[b]

Mean values ± standard deviation with different lowercase letters in the same row have significance different (P<0.05). Sample A- Filtered tap water treatment; Sample B- 0.00% of *C. xanthorrhiza* extract in DIW treatment; Sample C- 0.05% of *C. xanthorrhiza* extract in DIW treatment; Sample D- 0.50% of *C. xanthorrhiza* extract in DIW treatment; Sample E- 5.00% of *C. xanthorrhiza* extract in DIW treatment

antibacterial activity (Hwang *et al*., 2000).

From the results, the untreated sample of oyster mushroom carried the initial loads of *Pseudomonas* spp. in 7.76 $\log_{10}$ CFU/g. The predominant bacterial species found from plant-based fresh produces are *Pseudomonas* spp., which can reach levels of 7.3 - 8.4 $\log_{10}$ CFU/g (Chikthimmah and Beelman, 2006). Soaking slices of oyster mushroom in filtered tap water reduced bacterial counts by 0.16 and 0.54 $\log_{10}$ CFU/g, while soaking in DIW reduced the cell counts by 0.01 and 0.79 $\log_{10}$ CFU/g for *Pseudomonas* spp. and *B. cereus* respectively under exposure time of 5 minutes. For statistical analysis, there was no significant difference in the sterilizing ability between filtered tap water and DIW. Meanwhile, the *Pseudomonas* spp. cell counts were reduced by 2.64 $\log_{10}$ CFU/g when treated with 5.00% of methanolic extract for 5 minutes, which was the greatest compare to the others two lower concentration, 0.73 and 1.65 $\log_{10}$ CFU/g on 0.05% and 0.50% extract, respectively. The three variations in concentrations of rhizome extract treatment showed significance difference at P <0.05 on oyster mushroom naturally presence *Pseudomonas* spp. log reduction. This showed that *C. xanthorrhiza* extract was effective towards *Pseudomonas* spp. inhabited on oyster mushroom. As proven by research study, *Pseudomonas aeruginosa*, a species within the family of *Pseudomonas* spp., displayed the inhibition zone of 9 mm against the rhizome extract of *C. xanthorrhiza* (Mary Helen *et al*., 2012). On the other hands, treating the oyster mushroom slices with methanolic rhizome extract for 5 minutes possess satisfactory outcome where there are absence of presumptive *B. cereus* colonies which gives a total of zero cell count from 0.05% to 5.00% of extract used.

Among the four foodborne pathogens selected in the present study, *Pseudomonas* spp. survive better than *B. cereus* when treated with different concentrations of methanolic extract of *C. xanthorrhiza.* This may due to the naturally high load of *Pseudomonas* spp. on oyster mushroom as *Pseudomonas* and *Penicillium* species were most frequently isolated from the air of oyster mushroom cultivation facility (Chun *et al.*, 2012). From the study work performed by Lee *et al.* (2008), xanthorrhizol isolated from *C. xanthorrhiza* possess satisfactory antibacterial activity with the MIC and MBC of 8- and 16 µg/ml respectively against *B. cereus*. In addition to their work, the bactericidal study revealed by xanthorrhizol treatment at 4x MIC reduced the viable cells count of *B. cereus* by at least 6 to 8 log in 4 hours (Lee *et al*., 2008). Apart from this, this study has showed that Gram-positive *S. aureus* and Gram-negative *E. coli* were not inhabited on oyster mushroom due to the absence of cell count.

Twenty untrained panellists have been evaluated visual sensory on the effect of *C. xanthorrhiza* extract on oyster mushroom. Colour, odour and texture are the parameters of visual sensory evaluation. The results of the visual sensory evaluation of *C. xanthorrhiza* extract on oyster mushroom were presented in Table 1. The differences in sensory appearance for the treated oyster mushroom at different levels of concentration were shown in Fig. 4. Among those sanitizing treatment solutions, the relative best combination between antimicrobial ability and sensory acceptability can be achieved with 0.05% treated oyster mushroom where it showed a significant bacterial population reduction (P <0.05) of 0.81, 0.73 and 5.54 $\log_{10}$ CFU/g for TPC, *Pseudomonas* spp. and *B. cereus* respectively as well as a higher mean scores for the tested sensory attributes in contrast with the oyster mushroom treated with 0.50% and 5.00% extract in terms of sensory acceptability hedonic test. Although the antimicrobial efficacy at 0.05% is not as ideal as 0.50% or 5.00% of the extract, exposure to 0.05% of

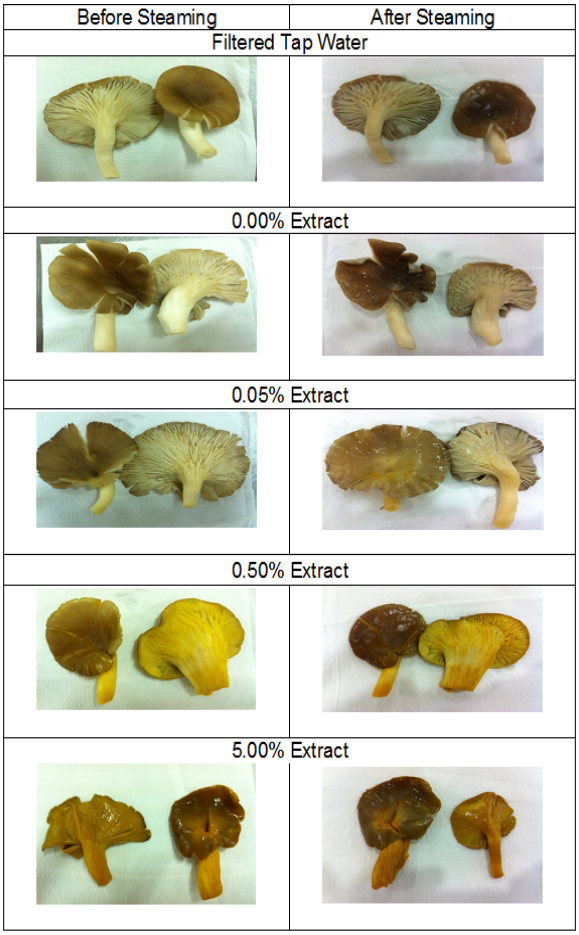

Figure 4. Sensory appearance of treated samples before and after steaming

extract has showed a significant reduction, and proper cooking after sanitizing can help to further reduce the foodborne risk. In terms of sensory evaluation statistical analysis, sample treated with 0.05% extract was chosen as the best combination because visual sensory test provides no significant difference ($P > 0.05$) in terms of colour, odour and texture attributes between Sample C (0.05% of *C. xanthorrhiza* extract in DIW treatment) and Sample A (normal practice of using filtered tap water treatment). These shows that Sample C, the treated sample with 0.05% of rhizome extract was as acceptable as the sample not treated with rhizome extract, which is the Sample A that treat with filtered tap water. On the opposite sides, as shown in Table 1, there were significant differences ($P < 0.05$) among Sample A with Sample E for all three visual sensory attributes; and Sample A has significant difference to Sample D in terms of colour and texture except odour.

Conclusion

The combination of antimicrobial efficacy and sensory acceptability was obtained at the sanitization solution made of 0.05% food grade methanolic *C. xanthorrhiza* extract. The urgency of identifying natural sanitizer with extracted active compounds from plants or herbs, which carries less toxic anti-microbial properties have been achieved by the utilization of Javanese turmeric *(C. xanthorrhiza)* extracted by food grade methanol. Hence, this finding suggests that methanolic extract of Javanese turmeric can be effectively used as a natural sanitizer for fresh produce washing to prevent the overgrowth of microorganisms includes foodborne pathogens. In the present studies, only grey oyster mushroom was used as the sanitized fresh produce sample, yet in the future for the further research, others types of fresh commodities can be used to perform the research to add value on the non-chemically synthesized solutions of *C. xanthorrhiza* extract.

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References

Adams, M. R., Hartley, A. D. and Cox, L. J. 1989. Factors affecting the efficacy of washing procedures used in the production of prepared salads. Food Microbiology 6 (2): 69-77.

Biswas, S., Datta, M. and Ngachan, S. V. 2012. Introduction. Mushrooms a manual for cultivation. New Delhi: PHI Learning Private Limited.

Brackett, R. E. 1992. Shelf stability and safety of fresh produce as influenced by sanitation and disinfection. Journal of Food Protection 55(10): 808-814.

Brasil, I. M., Gomes, C., Puerta-Gomez, A., Castell-Perez, M. E. and Moreira, R. G. 2012. Polysaccharide-based multilayered antimicrobial edible coating enhances quality of fresh-cut papaya. LWT-Food Science and Technology 47(1): 39-45.

Camp, J. V., Vandekinderen, I., Meulenaer, B. D., Veramme, K., Bernaert, N., Denon, Q., Ragaert, P. and Devlieghere, F. 2009. Moderate and high doses of sodium hypochlorite, neutral electrolyzed oxidizing water, peroxyacetic acid, and gaseous chlorine dioxide did not affect the nutritional and sensory qualities of fresh-cut iceberg lettuce (*Lactuca sativa* var. *capitata* L.) after washing. Journal of Agricultural and Food Chemistry 57(10): 4195-4203.

Cao, W., Zhu, Z. W., Shi, Z. X., Wang, C. Y. and Li, B. M. 2009. Efficiency of slightly acidic electrolyzed water for inactivation of *Salmonella enteritidis* and its contaminated shell eggs. International Journal of Food Microbiology 130(2): 88-93.

Chikthimmah, N. and Beelman, R. B. 2006. Microbial spoilage of fresh mushrooms. In Sapers, G. M., Gorny, J. R. and Yousef, A. E. (Eds). Microbiology of fruits and vegetables, p. 135-158. Boca Raton: CRC Press

Taylor and Francis Group.

Chun, S. C., Ahn, Y. N., Khan, S. M., Chung, I. M., Won, H. Y., Jhune, C. S. and Park, Y. J. 2012. The microbial population in the air of cultivation facility of oyster mushrooms. Journal of Microbiology 50(6): 1053-1057.

Ding, T., Rahman, S. M. E. and Oh, D-H. 2011. Inhibitory effects of low concentration electrolyzed water and other sanitizers against foodborne pathogens on oyster mushroom. Food Control 22(2): 318-322.

Doyle, M. E. 2005. Food antimicrobials, cleaners, and sanitizers. A review of the scientific literature. Food Research Institute, University of Wisconsin. Downloaded from *http://fri.wisc.edu/docs/pdf/Antimicrob_Clean_Sanit_05.pdf*.

Hwang, J. K., Shim, J. S. and Pyun, Y. R. 2000. Antibacterial activity of xanthorrhizol from *Curcuma xanthorrhiza* against oral pathogens. Fitoterapia 71(3): 321-323.

Mary Helen, P. A., Susheela Gomathy, K., Jayasree, S., Nizzy, A. M., Rajagopal, B. and Jeeva, S. 2012. Phytochemical characterization and antimicrobial activity of *Curcuma xanthorrhiza* Roxb. Asian Pacific Journal of Tropical Biomedicine 2(2): S637-S640.

Kamal, S., Pandey, J., Ghignone, S. and Varma, A. 2009. Mushroom biology and biotechnology an overview. In Chauhan, A. K. and Varma, A. (Eds). A textbook of molecular biotechnology. (3rd Eds), p. 573-628. India: I. K. International Publishing House Pvt. Ltd.

Kim, C., Hung, Y. C. and Brackett, R. E. 2000. Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of foodborne pathogens. International Journal of Food Microbiology 61(2): 199-207.

Lee, Y. L., Shim, J. S., Rukayadi, Y. and Hwang, J. K. 2008. Antibacterial activity of xanthorrhizol isolated from *Curcuma xanthorrhiza* Roxb. against foodborne pathogens. Journal of Food Protection 71(9): 1926-1930.

Nagashima, T. and Kamoi, I. 1997. Sterilization and preservation of vegetables by ozonated water treatment. Food Preservation Science 23(3): 127-131.

Rukayadi, Y. and Hwang, J. K. 2007a. *In vitro* antimycotic activity of xanthorrhizol isolated from *Curcuma xanthorrhiza* Roxb. against opportunistic filamentous fungi. Phytotherapy Research 21(5): 434-438.

Rukayadi, Y. and Hwang, J. K. 2007b. *In vitro* anti-Malassezia activity of xanthorrhizol isolated from *Curcuma xanthorrhiza* Roxb. Letters in Applied Microbiology 44(2): 126-130.

Rukayadi, Y., Shim, J. S. and Hwang, J. K. 2008. Screening of Thai medicinal plants for anticandidal activity. Mycoses 51(4): 308-312.

Rukayadi, Y., Yong, D. and Hwang, J. K. 2006. *In vitro* anticandidal activity of xanthorrhizol isolated from *Curcuma xanthorrhiza* Roxb. Journal of Antimicrobial Chemotherapy 57(6): 1231-1234.

Wullaert, R. A. 1997. Electrolysis ionization technology in the United States. Report of the 4th Annual Functional Water Symposium. Tokyo, Japan.

Yuk, H. G., Yoo, M. Y., Yoon, J. W., Marshall, D. L. and Oh, D. H. 2007. Effect of combined ozone and organic acid treatment for control of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on enoki mushroom. Food Control 18(5): 548-553.

Zhang, S. and Farber, J. M. 1996. The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. Food Microbiology 13(4): 311-321.