

Microbiological and physico-chemical changes in lotus seeds as influenced by ultraviolet radiation

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Abstract

Lotus seeds are sensitively perishable and browning. However, few publications have emphasised on the application of UV-C treatment. The purpose of this work was to assess the potential effect of UV-C irradiation on the physico-chemical and microbiological quality of lotus seeds during storage at 4°C for 8 days. The UV-C exposure times of 5 mins and 10 mins were evaluated. The results showed that the total viable count of 10 mins-UV-C treated lotus seeds met the standard quality of Thai Industrial Standards Institute (TISI) crispy lotus seeds (TCPS 490-2547) ($\leq 3 \log \text{CFU/g}$) although the yeast and mould of all treatments were not affected by UV-C radiation ($> 1 \log \text{CFU/g}$). Moreover, the reduction level of browning degree was not affected by UV-C, whereas the accumulation of phenolic content and the delay of product softening were found in the UV-C treated sample for 10 mins when compared with the control treatment. Consequently, the UV-C treated for 10 mins could be used as a promising approach to control the growth of total bacterial count in lotus seed product during storage at 4°C.

1. Introduction

Lotus seeds (*Nelumbo nucifera*) have been used as functional food and medicine for a long time due to being a rich source of bioactive compounds like phenolics, antioxidants, minerals, proteins, and fatty acids. In the present, many people are concerned about their health which is correlated to reducing the high risk of chronic diseases (Yen *et al.*, 2005; Moro *et al.*, 2013; Arooj *et al.*, 2021). Food industries have been interested in its nutraceutical properties and as a source of functional food products. The global production of lotus seeds indicates that it is widely cultivated in Asian countries such as Japan, India, and China, while internationally, its price is about USD 200/1000 seeds (Arooj *et al.*, 2021).

Lotus seeds are sensitively perishable and senescent because of postharvest, chilled storage and transportation. The enzymatic browning could induce low quality leading to a loss of income as has been emphasised by many studies. In addition, microbial spoilage causes a change in physico-chemical and microbiological quality of the seeds during vacuum-packaged storage (Huang *et al.*, 2018; Luo *et al.*, 2020).

Ultraviolet (UV-C) irradiation can be used as a potential approach to decrease microorganisms in foods.

This technique is a non-ionising region of the electromagnetic spectrum in the range of 200-280 nm and has the benefits of antimicrobial activity and the reduction of browning reaction. Implementation of UV-C could be used to control the quality of fruits, vegetables, and cereals (de Souza Pedrosa *et al.*, 2021; Loconsole and Santamaria; 2021; Roy *et al.*, 2021; Ruiz-Hernández *et al.*, 2021). According to Lei *et al.* (2018), UV-C treatment inhibited the reaction of enzymatic browning of *Agaricus bisporus*, whilst its gene transcription levels of polyphenol oxidase genes were affected. Wang *et al.* (2019) suggested that UV-C treatment could inhibit the ability of the browning degree of fresh-cut lotus roots, which was attributed to lower activities of browning enzymes. Furthermore, Ferreira *et al.* (2020) presented that the UV-C demonstrated potential in eliminating the spore of microbial spoilage, *Alicyclobacillus acidoterrestris*, in orange juice.

Although UV-C could help prevent the quality loss in wheat, rice, and maize (Srivastava and Mishra, 2021), few publications have accentuated the application of UV-C treatment to the physico-chemical and microbiological qualities of lotus seeds. Therefore, this work aimed to evaluate the potential effect of UV-C radiation on the quality of lotus seeds during storage at 4°C under vacuum packed condition. This investigation

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might provide a useful approach for further implementation of UV-C in cereal postharvest, chilled storage and transportation.

2. Materials and methods

2.1 Sample preparation and UV-C treatment

Lotus pods were purchased from Suranakhon market in Nakhon Ratchasima province, Thailand. The pods were then packed in polypropylene plastic bags in the presence of ice and transported to the Department of Food Science and Technology, Faculty of Agricultural Innovation and Technology, Rajamangala University of Technology Isan within 30 mins. A part of lotus seeds (cotyledon with membrane) was separated from the pericarp and washed in 1% (w/v) NaCl for 5 mins. Each lotus seed was dried for 2 mins and packed in vacuum sterile bag. Each sample was approximately 100 g. Each treatment was exposed to UV-C lamp (Philips TUV 30W/G30 T8) at a distance of 68 cm above the produce tray for 0, 5 and 10 mins at ambient temperature. All physico-chemical and microbiological parameters were then monitored for 0, 2, 4, 6, and 8 days during storage at 4°C.

2.2 Measurement of moisture content and water activity

To analyse the moisture content, one gram of each sample was measured by moisture analyser (Sartorius, MA160). Meanwhile, the water activity was analysed using a_w analyser (Lab Master-aw neo).

2.3 Measurement of colours and texture profiles

The colour values of each treatment were measured by Chroma meter CR-410 (Konica minolta, Japan). The colour parameters, including L^* (brightness), a^* (redness/greenness), and b^* (yellowness/blueness), were observed. Meanwhile, the texture profiles were analysed by using a texture analyser (CT3 10K, BROOKFIELD, USA). The texture parameters, including hardness, resilience, stringiness length, and fracturability were recorded.

2.4 Browning degree analysis

The browning degree of each treatment was analysed with the procedure described by Lei *et al.* (2018). Approximately 2 g of lotus seeds was homogenised in 5 mL of (95%) ethyl alcohol solution and centrifuged at 8000 rpm for 10 mins at 4°C. The absorbance of the supernatant was measured at 410 nm on a spectrophotometer.

2.5 Total phenolic content analysis

The total phenolic content in each treatment was

measured as described by Singleton *et al.* (1999). A 1.0 mL aliquot of Folin-Ciocalteu (10-fold diluted solution) was mixed with sample (0.5 mL) and left for 6 min at room temperature. After inoculating with 2.0 mL sodium carbonate (200 g/1000 mL), the mixture was incubated at room temperature for 60 min in the dark. The absorbance was then measured at 760 nm. Finally, the content of total phenols in each sample was expressed by mg of gallic acid equivalents (GAE) per gram of sample.

2.6 Microbiological analysis

A total of 25 g of each treatment was diluted in 225 mL sterile 0.85 % (w/v) NaCl solution. Serial dilutions and spread plate method were done. The total viable count was determined on plate count agar incubated at 37°C for 24 hrs. The total count of yeasts and moulds was determined on potato dextrose agar incubated at 30°C for 72 hrs. The microbial number was expressed as log CFU/g (FDA-BAM, 2001).

2.7 Statistical analysis

Physico-chemical parameters were analysed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). A value of $P < 0.05$ was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics 23.0 software.

3. Results

Figures 1A and 1B display the physical parameters, including moisture content and a_w of each treatment, respectively. On day 8, the moisture content of all treatments increased ($P < 0.05$), which was approximately 51.26-51.34. Meanwhile, UV-C treated lotus seeds had the highest a_w , accounting for 1.00 on days 6-8. The colour changes in L^* , a^* and b^* are revealed in Figures 2A-C, respectively. On day 2, a decrease in L^* , a^* and b^* was found in UV-C treated samples and control treatment ($P < 0.05$). In addition, on days 2-6, the increase in a^* and b^* was detected in the UV-C treated sample for 10 mins when compared with the control treatment ($P < 0.05$). A change in colour values might have been related to the surface appearance of UV-C treated lotus seeds as shown in Figure 3. The texture parameters, including hardness, resilience, stringiness length, and fracturability are illustrated in Figures 4A-C, respectively. At the end of storage, no significant difference ($P > 0.05$) was observed in resilience and stringiness length in all treatments, while the hardness and fracturability of the UV-C 10 mins treated lotus seeds had increased when compared with other treatments ($P < 0.05$). As shown in Figures 5A and B, the UV-C 10 mins treated lotus seeds had a higher browning

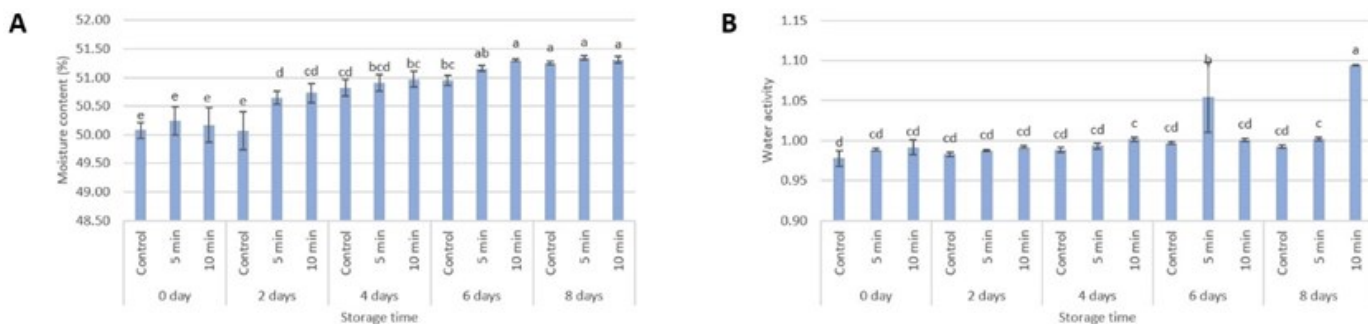


Figure 1. Effect of UV-C treatment on moisture content (A) and water activity (B) of lotus seeds during storage at 4°C. Bars with different notations are statistically significantly different (P<0.05) according to DMRT.

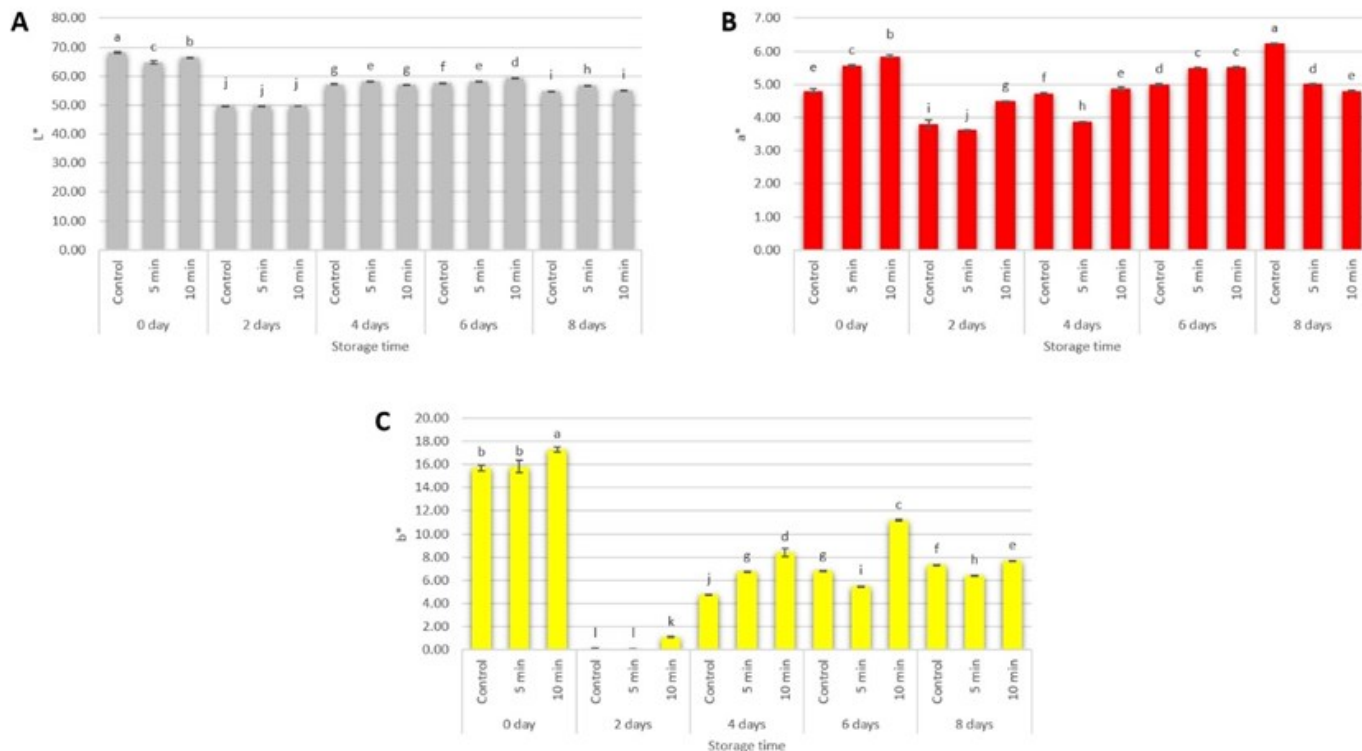


Figure 2. Effect of UV-C treatment on L* (A), a* (B), and b* (C) of lotus seeds during storage at 4°C. Bars with different notations are statistically significantly different (P<0.05) according to DMRT.



Figure 3. Effect of UV-C treatment on surface appearance of lotus seeds during storage at 4°C.

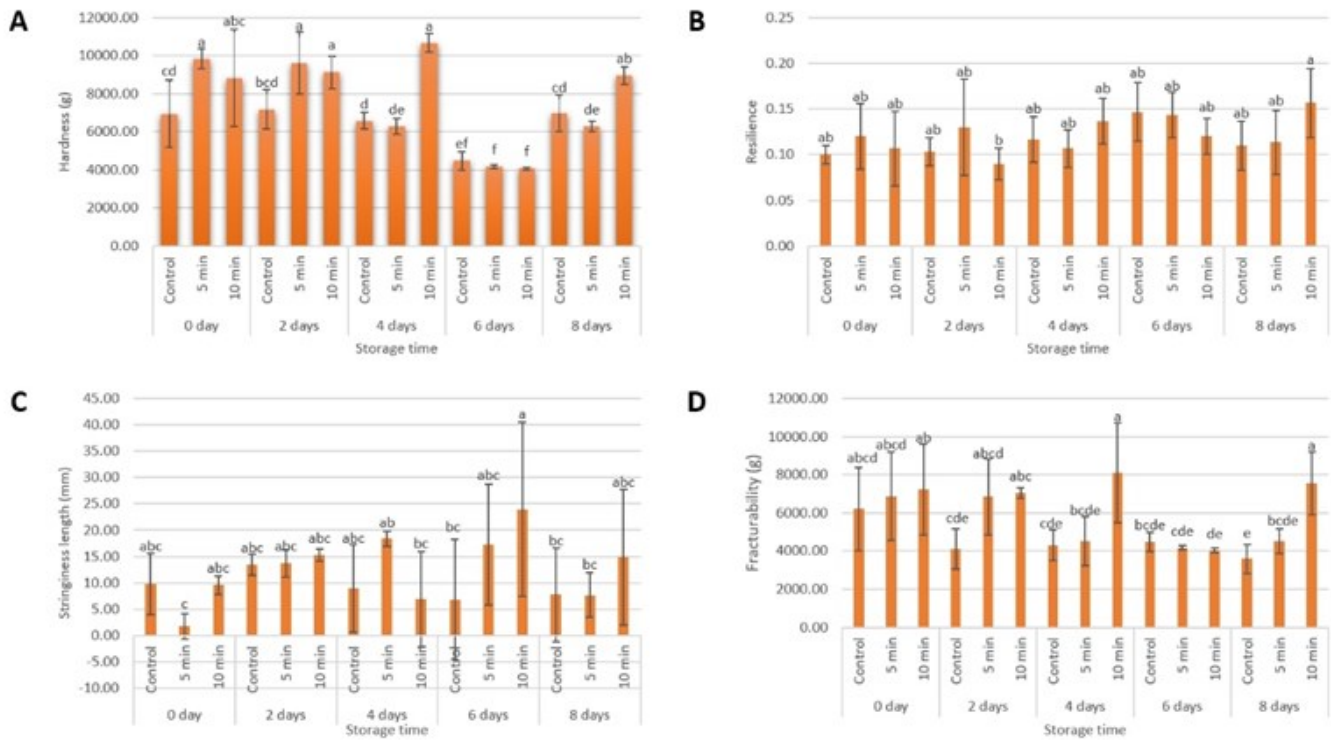


Figure 4. Effect of UV-C treatment on hardness (A), resilience (B), stringiness length (C), and fracturability (D) of lotus seeds during storage at 4°C. Bars with different notations are statistically significantly different ($P < 0.05$) according to DMRT.

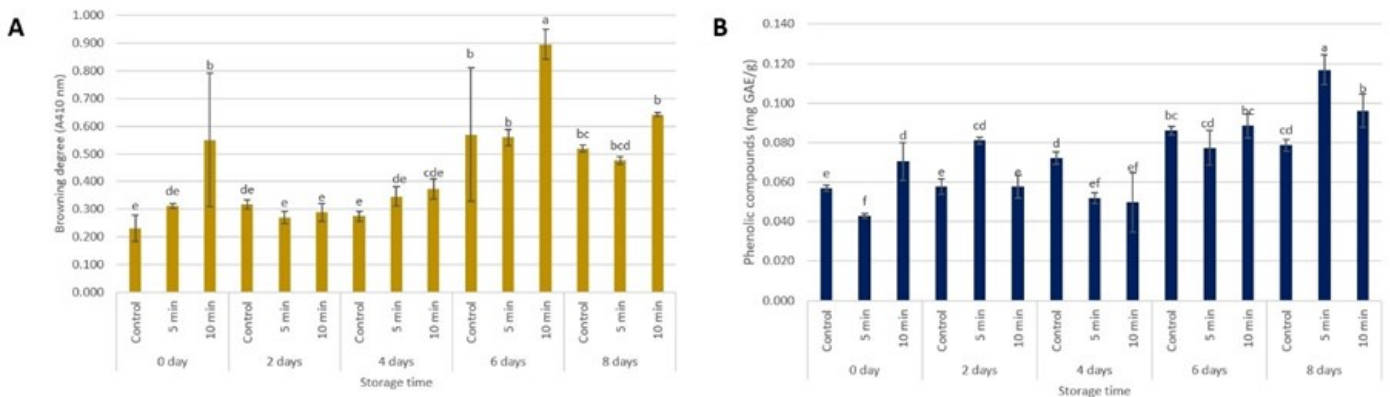


Figure 5. Effect of UV-C treatment on browning degree (A) and total phenolics (B) of lotus seeds during storage at 4°C. Bars with different notations are statistically significantly different ($P < 0.05$) according to DMRT.

Table 1. Microbial quality of UV-C treated lotus seeds and untreated UV-C lotus seeds (control) during storage at 4°C for 8 days.

Microbiological parameters	Groups	Storage time (Days)				
		0	2	4	6	8
Total viable count (log CFU/g)	Control	3.15±0.21	4.30±0.43	<3	4.00±0.00	3.50±0.71
	UV-C 5 mins	<2	3.39±0.55	<3	3.15±0.21	3.15±0.21
	UV-C 10 mins	2.30±0.00	3.00±0.00	<3	1.50±2.12	1.50±2.12
Yeast and mould count (log CFU/g)	Control	2.76±0.40	4.00±1.41	3.74±1.04	4.97±0.02	5.10±0.00
	UV-C 5 mins	2.85±0.21	3.39±0.12	3.74±1.04	4.72±0.01	4.97±0.02
	UV-C 10 mins	2.45±0.64	3.15±0.21	1.50±2.12	4.48±0.01	4.93±0.01

The microbiological criteria were compared to TISI (2013) crispy lotus seeds (TCPS 490-2547). It was specified that the total viable count, and yeast and mould count are approximately ≤ 3 and < 1 log CFU/g, respectively.

index on days 0 and 6, and the higher phenolic content on days 0 and 8 when compared with the control treatment ($P < 0.05$).

Table 1 reveals the results of the microbiological quality of each treatment. The total viable count of the UV-C 10 mins treated lotus seeds met the TISI (2013) crispy lotus seeds (TCPS 490-2547) ($\leq 3 \log \text{CFU/g}$). Contrastingly, the UV-C 5 mins treated lotus seeds and control treatment were noted to have a higher level of total viable count on days 6 and 8 ($> 3 \log \text{CFU/g}$), which did not meet the standard microbiological quality. In addition, the viable count of yeast and mould found in all treatments during storage did not meet the standard quality of crispy lotus seeds ($> 1 \log \text{CFU/g}$).

4. Discussion

The moisture content and a_w are significant factors in controlling microbial growth during food storage (Chirife *et al.*, 1996). In the present investigation, an increase in moisture content in all treatments was found at the end of storage. This phenomenon is related to the results of Bakhtavar *et al.* (2019), who reported that seed moisture content was higher during storage. It might be interpreted that the polyethylene bags provided free access to water vapours (Hussein *et al.*, 2011) and the seed products absorbed the water vapours, leading to an increase in moisture content and microbial growth index. Meanwhile, a_w of all treatments was approximately 1.0 which was relevant to the intrinsic factor for the growth of mesophilic microorganisms (Chirife *et al.*, 1996; Doyle *et al.*, 2020).

The colour parameter is a basic sensory characteristic for evaluating consumer acceptability. The UV-C 10 mins treated samples exhibited higher a^* and b^* than other treatments on days 2-6, showed the highest browning index on day 6, and displayed the highest total phenolic content on day 8. A colour change in lotus seeds according to the picture was in accordance with the value of the browning degree. Previous investigations confirmed that browning in food products could be increased by UV-C exposure. Although the activity of polyphenol oxidase was inactivated by UV-C, the browned appearance could occur during storage at 4°C (Lei *et al.*, 2018; Wang *et al.*, 2019). Regarding phenolic content, the UV-C stress enhanced the accumulation of nutraceutical-relevant phenolic metabolites due to the activation of the phenolic biosynthesis pathway (Ouhibi *et al.*, 2014; Hernandez-Aguilar *et al.*, 2021). According to texture analysis, hardness and fracturability were considered based on their increase in the treatment with UV-C for 10 mins at the end of storage (day 8). Basically, fracturability is the force at the first peak in the time force curve during texture profile analysis. It

correlates with the sensorial hardness (Kaaber *et al.*, 2001). Likewise, Abdipour *et al.* (2019) demonstrated that the application of UV-C could delay fruit softening.

Considering the microbiological quality, the treatment with UV-C for 10 mins could control the level of total bacterial count ($\leq 3 \log \text{CFU/g}$), which was up to the standard of food safety of TISI (2013) crispy lotus seeds (TCPS 490-2547). The mode of UV-C action disrupts the nucleic acid (RNA/DNA) in bacterial cells, induces cross-linking of pyrimidine nucleotide bases and alters the metabolism, leading to cell death (Singh *et al.*, 2021). However, the count of yeast and mould did not meet the standard TISI (2013) crispy lotus seeds (TCPS 490-2547) ($> 1 \log \text{CFU/g}$). This might be interpreted that the cell wall of fungal spores had a relative tolerance to the UV-C exposure time based on the UV tolerance levels of yeasts and moulds (Wong *et al.*, 2019).

5. Conclusion

Among the results, the UV-C irradiation for 10 mins is a promising approach to control the growth of total bacterial count in lotus seed product during storage at 4°C for 8 days, which was up to the standard of Thai community product standard of crispy lotus seeds. In contrast, the growth of yeast and mould was not inhibited by UV-C. Meanwhile, the UV-C could enhance the accumulation of phenolic content and delay lotus seed softening. However, the reduction of browning degree was not affected by UV-C. Further studies are required to extend the UV-C exposure times and to evaluate the sensorial attributes in lotus seed products.

Conflict of interest

The authors declare no conflict of interest.

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References

- Abdipour, M., Hosseinfarahi, M. and Naseri, N. (2019). Combination method of UV-B and UV-C prevents post-harvest decay and improves organoleptic quality of peach fruit. *Scientia Horticulturae*, 256, 108564. <https://doi.org/10.1016/j.scienta.2019.108564>
- Arooj, M., Imran, S., Inam-ur-Raheem, M., Rajoka, M.S.R., Sameen, A., Siddique, R., Sahar, A., Tariq, S., Riaz, A., Hussain, A., Siddeeg, A., Aadil, R.M.

- and Aadil, R.M. (2021). Lotus seeds (*Nelumbinis semen*) as an emerging therapeutic seed: A comprehensive review. *Food Science and Nutrition*, 9(7), 3971-3987. <https://doi.org/10.1002/fsn3.2313>
- Bakhtavar, M.A., Afzal, I. and Basra, S.M.A. (2019). Moisture adsorption isotherms and quality of seeds stored in conventional packaging materials and hermetic Super Bag. *PLoS ONE*, 14(2), e0207569. <https://doi.org/10.1371/journal.pone.0207569>
- Chirife, J., del Pilar Buera, M. and Labuza, T.P. (1996). Water activity, water glass dynamics, and the control of microbiological growth in foods. *Critical Reviews in Food Science and Nutrition*, 36(5), 465-513. <https://doi.org/10.1080/10408399609527736>
- de Souza Pedrosa, G.T., Pimentel, T.C., Gavahian, M., de Medeiros, L.L., Pagán, R. and Magnani, M. (2021). The combined effect of essential oils and emerging technologies on food safety and quality. *LWT*, 147, 111593. <https://doi.org/10.1016/j.lwt.2021.111593>
- Doyle, M.P., Diez-Gonzalez, F. and Hill, C. (Eds.). (2020). *Food microbiology: fundamentals and frontiers*. United Kingdom: John Wiley and Sons. <https://doi.org/10.1128/9781555819972>
- FDA-BAM. (2001). In FDA's bacteriological analytical manual. Retrieved March 10, 2021, from Website: <https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam>
- Ferreira, T.V., Mizuta, A.G., de Menezes, J.L., Dutra, T.V., Bonin, E., Castro, J.C., Szczerepa, M.M.D.A., Pilau, E.J., Nakamura, C.V., Mikcha, J.M.G. and de Abreu Filho, B.A. (2020). Effect of ultraviolet treatment (UV-C) combined with nisin on industrialized orange juice in *Alicyclobacillus acidoterrestris* spores. *LWT*, 133, 109911. <https://doi.org/10.1016/j.lwt.2020.109911>
- Hernandez-Aguilar, C., Dominguez-Pacheco, A., Tenango, M.P., Valderrama-Bravo, C., Hernández, M.S., Cruz-Orea, A. and Ordonez-Miranda, J. (2021). Characterization of bean seeds, germination, and phenolic compounds of seedlings by UV-C radiation. *Journal of Plant Growth Regulation*, 40 (2), 642-655. <https://doi.org/10.1007/s00344-020-10125-0>
- Huang, Z., Ge, S., Lin, S., Chen, N., Liu, W., Qin, S., Wu, W., Zheng, B., Zeng, S. and Huang, X. (2018). An environmental study on bacterial diversity in spoilage lotus seeds and identification of its predominant spoilage bacteria. *Ekoloji*, 27(106), 1945-1954.
- Hussein, A.A., Salim, R.D. and Sultan, A.A. (2011). Water absorption and mechanical properties of high-density polyethylene/egg shell composite. *Journal of Basrah Researches (Sciences)*, 37(3A/15), 36-42.
- Kaaber, L., Bråthen, E., Martinsen, B.K. and Shomer, I. (2001). The effect of storage conditions on chemical content of raw potatoes and texture of cooked potatoes. *Potato Research*, 44(2), 153-163. <https://doi.org/10.1007/BF02410102>
- Lei, J., Li, B., Zhang, N., Yan, R., Guan, W., Brennan, C. S., Brennan, C.S., Gao, H. and Peng, B. (2018). Effects of UV-C treatment on browning and the expression of polyphenol oxidase (PPO) genes in different tissues of *Agaricus bisporus* during cold storage. *Postharvest Biology and Technology*, 139, 99-105. <https://doi.org/10.1016/j.postharvbio.2017.11.022>
- Loconsole, D. and Santamaria, P. (2021). UV lighting in horticulture: A sustainable tool for improving production quality and food safety. *Horticulturae*, 7 (1), 9. <https://doi.org/10.3390/horticulturae7010009>
- Luo, S., Hu, H., Wang, Y., Zhou, H., Zhang, Y., Zhang, L. and Li, P. (2020). The role of melatonin in alleviating the postharvest browning of lotus seeds through energy metabolism and membrane lipid metabolism. *Postharvest Biology and Technology*, 167, 111243. <https://doi.org/10.1016/j.postharvbio.2020.111243>
- Moro, C.F., Yonekura, M., Kouzuma, Y., Agrawal, G.K. and Rakwal, R. (2013). Lotus—a source of food and medicine: current status and future perspectives in context of the seed proteomics. *International Journal of Life Sciences*, 7(1), 6394. <https://doi.org/10.3126/ijls.v7i1.6394>
- Ouhibi, C., Attia, H., Rebah, F., Msilini, N., Chebbi, M., Aarouf, J., Urban, L. and Lachaal, M. (2014). Salt stress mitigation by seed priming with UV-C in lettuce plants: Growth, antioxidant activity and phenolic compounds. *Plant Physiology and Biochemistry*, 83, 126-133. <https://doi.org/10.1016/j.plaphy.2014.07.019>
- Roy, P.K., Mizan, M.F.R., Hossain, M.I., Han, N., Nahar, S., Ashrafudoulla, M., Tushik, S.H., Shim, W.B., Kim, Y.M. and Ha, S.D. (2021). Elimination of *Vibrio parahaemolyticus* biofilms on crab and shrimp surfaces using ultraviolet C irradiation coupled with sodium hypochlorite and slightly acidic electrolyzed water. *Food Control*, 128, 108179. <https://doi.org/10.1016/j.foodcont.2021.108179>
- Ruiz-Hernández, K., Ramírez-Rojas, N.Z., Meza-Plaza, E.F., García-Mosqueda, C., Jauregui-Vázquez, D., Rojas-Laguna, R. and Sosa-Morales, M.E. (2021). UV-C treatments against *Salmonella* Typhimurium ATCC 14028 in inoculated peanuts and

- almonds. *Food Engineering Reviews*, 13(3), 706-712. <https://doi.org/10.1007/s12393-020-09272-7>
- Singh, H., Bhardwaj, S. K., Khatri, M., Kim, K.H. and Bhardwaj, N. (2021). UVC radiation for food safety: An emerging technology for the microbial disinfection of food products. *Chemical Engineering Journal*, 417, 128084. <https://doi.org/10.1016/j.cej.2020.128084>
- Singleton, V.L., Orthofer, R. and Lamuela-Raventós, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, 299, 152-178. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)
- Srivastava, S. and Mishra, H.N. (2021). Ecofriendly nonchemical/nonthermal methods for disinfestation and control of pest/fungal infestation during storage of major important cereal grains: A review. *Food Frontiers*, 2(1), 93-105. <https://doi.org/10.1002/fft2.69>
- Thai Industrial Standards Institute (TISI) (2013). Crispy Lotus Seeds (TCPS 490-2547). Retrieved on April 1, 2022 from Website: https://tcps.tisi.go.th/pub%5Ctcps490_47.pdf [In Thai].
- Wang, D., Chen, L., Ma, Y., Zhang, M., Zhao, Y. and Zhao, X. (2019). Effect of UV-C treatment on the quality of fresh-cut lotus (*Nelumbo nucifera* Gaertn.) root. *Food Chemistry*, 278, 659-664. <https://doi.org/10.1016/j.foodchem.2018.11.102>
- Wong, H.J., Mohamad-Fauzi, N., Rizman-Idid, M., Convey, P. and Alias, S.A. (2019). Protective mechanisms and responses of micro-fungi towards ultraviolet-induced cellular damage. *Polar Science*, 20(Part 1), 19-34. <https://doi.org/10.1016/j.polar.2018.10.001>
- Yen, G.C., Duh, P.D. and Su, H.J. (2005). Antioxidant properties of lotus seed and its effect on DNA damage in human lymphocytes. *Food Chemistry*, 89 (3), 379-385. <https://doi.org/10.1016/j.foodchem.2004.02.045>