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FULL PAPER

Evaluation of biofilm-forming abilities of *Listeria monocytogenes* (ATCC 19115) and efficacy of different washing methods for removal of biofilm on apple

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Abstract

Fresh produce can be contaminated at any stage along the food supply chain. In this study, apple was chosen to determine the time course of biofilm formation by *Listeria monocytogenes* (ATCC 19115), as well as to compare the efficacy of different household washing methods such as scrubbing with hands under running tap water, soaking with and without commercial vegetable wash with different treatment times in removing the biofilm formation by *L. monocytogenes* on apple surface. The biofilm formation was quantified using crystal violet assay and the result showed that *L. monocytogenes* took 18 hrs to form matured biofilm on apple surface. Besides, scrubbing apples with hands under running tap water for 30 s and 60 s were the most effective method which significantly removed ($P<0.05$) biofilm formed on the apple surface with approximately 5.93 log reduction. Soaking apples with vegetable wash for 5 mins and 10 mins were also found to be significantly effective ($P<0.05$) in reducing *L. monocytogenes* biofilm. Since *L. monocytogenes* can form matured biofilm on fresh produce, therefore efficient washing step is important before consuming fresh produce to lower the risk of foodborne illness.

1. Introduction

Foodborne illness is a disease caused by consuming food or water contaminated with pathogens including viruses, bacteria, parasites and other microorganisms (Feltes *et al*., 2017). *Listeria monocytogenes* is a Gram-positive, rod-shaped, motile and facultative anaerobic bacterium (Liu, 2008). It is a pathogenic bacterium that can be found in ready-to-eat food, cold-stored food, dairy products, and processed food. This pathogen will cause listeriosis which is a dangerous infection resulting in a high mortality rate of 20% to 30%, and more than 90% of patients with listeriosis are hospitalized (United States Food and Drug Administration, 2020). The elderly, pregnant woman, infants, and toddlers are the vulnerable groups that are susceptible to listeriosis (Kuan *et al*., 2013). Listeriosis can lead to serious infection which can spread to the brain causing encephalitis and meningitis. Pregnant women with listeriosis can have a miscarriage or premature birth (Castellazzi *et al*., 2018).

In the past two decades, the reported listeriosis

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outbreak associated with fresh produce such as lettuce, celery, and cantaloupe melon has been increased (Botticella *et al*., 2013). *L. monocytogenes* can be found in soil, so fresh produces may be contaminated at their source (Kuan *et al*., 2017b). Contamination also can occur during harvesting and processing. Many studies have been done showing that post-harvest washing is not able to remove bacteria on fresh produce efficiently and there are chances of cross-contamination along the food supply chain (Murray *et al*., 2017). Moreover, biofilm forms by *L. monocytogenes* pose a severe threat to the safety of ready-to-eat fresh produce as they can survive and grow at low temperatures. Biofilm is defined as an association of microorganisms that are attached to living or non-living surfaces by embedding in a self-produced matrix of extracellular polymeric substances (Jamal *et al*., 2015). Biofilm can be formed in food processing environments such as the processing equipment which can lead to serious impacts on the health of consumers because cross-contamination of products by foodborne pathogens can occur during the processing (Galié *et al*., 2018).

Nowadays, many people are more health-conscious and leading to a healthy diet by increasing the consumption of fruits and vegetables as eating more fresh produce can lower the risk of chronic disease and improve the digestive system (Slavin and Lloyd, 2012). Increased demand for fresh produce is found to be associated with the increased number of human infections and foodborne outbreaks in recent years because fresh produce serves as a reservoir for pathogens (Al-Kharousi *et al*., 2016; Kuan *et al*., 2017b). Washing fresh produce not only helps remove dirt and residual pesticides but also helps remove bacteria. The common washing methods used by consumers are washing under running tap water, soaking in a basin, rubbing with hands, scrubbing with a brush, soaking in diluted vinegar or dish detergent (Li-Cohen and Bruhn, 2002). Also, a study by Kilonzo-Nthenge *et al.* (2006) showed commercial cleaning solutions such as vegetable wash was able to reduce *Listeria innocua* on tomatoes. An efficient washing step is believed to be able to reduce the microbial load on fresh produce (Rodrigues *et al*., 2014). Some factors such as washing method, washing time, washing force and adherence of microorganisms on the surface will affect the efficiency of washing treatment (Kuan *et al*., 2017a). The present study aimed to determine the time course of *L. monocytogenes* biofilm formation on the apple surface and investigate the efficiency of different household washing methods in removing *L. monocytogenes* on artificially contaminated apples.

2. Materials and methods

2.1 Preparation of inoculums

A 0.1 mL of *L. monocytogenes* (ATCC 19115) pure culture was inoculated into a universal bottle with 10 mL of Tryptic Soy Broth (TSB; Merck, Darmstadt, Germany) and the mixture was incubated in a shaking incubator (Infors Thermotron, Switzerland) at 120 rpm, 37℃ for 24 hrs. Revived *L. monocytogenes* was streaked on PALCAM agar (Merck, Darmstadt, Germany) and incubated at 30℃ for 48 hrs. After incubation, one of the colonies from PALCAM agar was inoculated into a universal bottle containing 10 mL of TSB and incubated in a shaking incubator at 120 rpm, 37℃ for 24 hrs. One millilitre of the overnight cultures was transferred to a 1.5 mL Eppendorf tube and centrifuged at 13400×*g* for 5 mins. The bacterial pellets were resuspended in 0.85% (w/v) saline solution (NaCl, Merck, Darmstadt, Germany). The absorbance of the bacteria suspension was adjusted to 0.393 at 600 nm using a UV-visible spectrophotometer (GENESYS 10S; Thermo Scientific, Malaysia) which was about 1.4×10^9 CFU/mL.

2.2 Biofilm formation on the test surface

Fresh Royal Gala apples (*Malus domestica* 'Gala') were purchased from a hypermarket in Kampar, Perak in February 2019. All the apples were placed in the refrigerator (4±1℃) before experimenting. The apple surfaces were sliced into the same sizes of 4×4 cm by using a sterile knife and placed onto sterile Petri dishes. The cut surfaces were put under UV light in a horizontal laminar flow cabinet (with UV light intensity of 36000 μJ/cm^2) for 15 mins to inhibit the growth of native microflora. Then, 1 mL of *L. monocytogenes* suspension was inoculated onto the surfaces of the apples and the surfaces were placed in an incubator at 30℃ for 0, 3, 6, 9, 12, 18 and 24 hrs to allow the attachment and biofilm formation of *L. monocytogenes*.

2.3 Quantification of biofilm

To quantify the biofilm formation on the apple surface, a crystal violet assay adapted from Tang *et al*. (2012) was performed with some modifications. After the particular incubation time, the apple surface was rinsed three times with 1 mL of distilled water to remove unattached *L. monocytogenes* cells. After air dried, 1 mL of 0.1% (w/v) crystal violet was used to stain the attached cells at room temperature (25±5℃) for 30 mins. After that, the staining solution was removed, and the surface was rinsed three times with 1 mL of distilled water. The apple surface was allowed to air-dry. Upon drying, the crystal violet bounded to the biofilm formed on the apple surface was solubilised using 1 mL of 95% (v/v) ethanol (Sigma-Aldrich Co. USA) for 30 mins. The

solution was collected into a cuvette and the absorbance was measured at 600 nm using a UV-visible spectrophotometer to determine the crystal violet concentration. The experiment was performed in triplicate.

2.4 Inoculation procedure

After determining the time course of biofilm formation by *L. monocytogenes* on the apple surface which was 18 hrs, the experiment was proceeded to determine the efficacy of household washing methods to remove the matured biofilm formed on the apple surface. All the apples were undergone UV treatment in a biological safety cabinet with UV light intensity of 36000 μJ/cm^2 for 30 mins (15 mins for each side). It was assumed that the native microflora was inactivated after being treated with UV light. Then, the surfaces of apples were inoculated with 2 mL of revived *L. monocytogenes* (approximately 1.4×10^9 CFU/mL) and incubated in an incubator at 30℃ for 18 hrs.

2.5 Removal of Listeria monocytogenes biofilm on apple by different washing methods

The washing methods applied in this study were mimicked the common household washing practices in Malaysia. The artificially contaminated apples were subjected to seven treatments (Table 1). The apples were being air-dried for 5 mins before subjected to microbiological analysis.

Table 1. Different washing methods that applied to remove *L. monocytogenes* biofilm on apple.

Washing method	Treatment
Scrubbing with hands under running tap water (flow rate*: 2 L/min)	10 s
Scrubbing with hands under running tap water (flow rate: 2 L/min)	30 s
Scrubbing with hands under running tap water (flow rate: 2 L/min)	60 s
Soaking without commercial vegetable wash# followed by rinsing with 150 mL of tap water	10 mins
Soaking with commercial vegetable wash followed by rinsing with 150 mL of tap water	1 min
Soaking with commercial vegetable wash followed by rinsing with 150 mL of tap water	5 mins
Soaking with commercial vegetable wash followed by rinsing with 150 mL of tap water	10 mins

*The flow rate of running tap water was determined by the volume of tap water collected within a specified period of time using a measuring jug and a stopwatch.

One capful of commercial vegetable wash per gallon (3.79 L) of water. This vegetable wash is made with deionized water, vegetable-derived non-ionic surfactant, citrus fragrance, botanical extracts (cucumber, lemon, orange, ginger, cassia), potassium sorbate and citric acid.

2.6 Microbiological analysis

After the artificially contaminated apple was subjected to washing treatment, the skin of the apple was peeled off using a sterile knife. The peel was weighed and added into a stomacher bag, 0.85% (w/v) NaCl was then added in a 1:10 dilution. The sample was homogenised for 5 min using the stomacher machine (Interscience BagMixer 400 P, France). Serial dilutions were prepared in the same diluents and 0.1 mL of each dilution was spread plated onto PALCAM agar followed by incubation at 30°C for 48 hrs. The steps were repeated for the positive control (without treated with any washing treatment) and negative control (without inoculation with bacterial culture). All the results were expressed as colony-forming units per gram (CFU/g).

2.7 Data analysis

For the evaluation of biofilm-forming abilities, the experiment and absorbance measurement were performed in triplicate. Also, all the washing simulation experiments were conducted in triplicate. The microbiological counts were expressed as $\log_{10}$ CFU/g. The log reduction was calculated to determine the efficiency of different washing methods using the formula:

Log reduction = $\log_{10}$ CFU/g of positive control (without treatment) – $\log_{10}$ CFU/g with washing treatment

For statistical analysis, one-way analysis of variance (ANOVA) (IBM Statistical Package for the Social Science (SPSS) software, version 25.0) was used to determine the differences between the washing methods and treatment times in reducing the *L. monocytogenes* biofilm on apple at $P<0.05$ level of significance.

3. Results

3.1 Biofilm-forming ability

The biofilm formation by *L. monocytogenes* on the apple surface was observed over 24 hrs. The OD value increased from 0 hr to 18 hrs and decreased after 18 hrs (Figure 1). There was a significant increase ($P<0.05$) in OD value (0.469 to 1.468) from 0 h to 3 hrs. After 3 hrs, the OD value increased gradually to 2.094 at 18 hrs and eventually dropped to 1.859 at 24 hrs.

3.2 Decontamination of Listeria monocytogenes by washing methods

Table 2 shows the efficacy of different washing methods in removing *L. monocytogenes* biofilm on the apple surface. Based on the results, washing apple by scrubbing with hands under running tap water for 30 s

Table 2. Mean concentration (log_{10} CFU/g) and log reduction of *L. monocytogenes* on the apple surfaces after being treated with different washing methods.

	Treatment time	Mean concentration (log_{10} CFU/g)	Log reduction
Positive control	-	5.93±0.42	-
Type of washing methods			
Scrubbing under running tap water (flow rate: 2 L/min)	10 s	4.72±0.56	1.21±0.17[b]
Scrubbing under running tap water (flow rate: 2 L/min)	30 s	TFTC	~5.93
Scrubbing under running tap water (flow rate: 2 L/min)	60 s	TFTC	~5.93
Soaking without commercial vegetable wash	10 mins	4.39±0.69	1.54±0.36[b]
Soaking with commercial vegetable wash	1 min	4.23±0.63	1.70±0.22[b]
Soaking with commercial vegetable wash	5 mins	3.42±0.30	2.51±0.18[a]
Soaking with commercial vegetable wash	10 mins	3.54±0.07	2.39±0.36[a]

TFTC: indicates that the colony formed was too few to count (less than 25 colonies). Values are as mean±standard deviation (log_{10} CFU/g) of triplicates. Values with different superscript in the same column are significantly different ($P<0.05$).

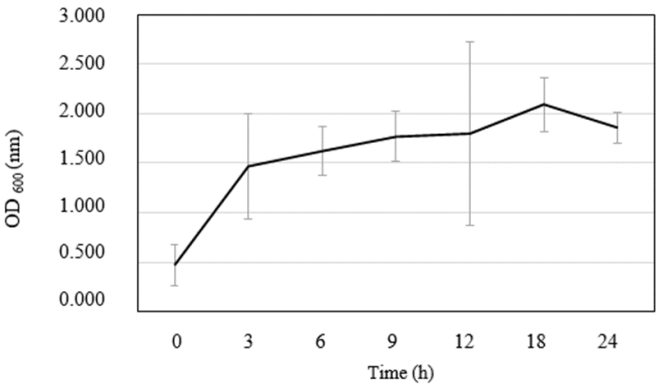

Figure 1. Mean values of biofilm formation by *L. monocytogenes* on the apple surface from 0 hr to 24 hrs were represented by OD_{600}. Each error bar shows the standard error of the mean for triplicate measurements.

and 60 s were found to be the most effective ways to eliminate *L. monocytogenes* biofilm on the apple surface because *L. monocytogenes* biofilm was almost completely removed from the apple surface as the colonies detected on PALCAM agar were too few to count. The log reduction for both methods was approximately 5.93 log CFU/g. Besides, soaking with vegetable wash for 5 mins and 10 mins were significantly effective ($P<0.05$) compared to soaking without the use of vegetable wash for 10 mins. Washing apple by soaking with vegetable wash for 5 mins and 10 mins was able to achieve 2.39 and 2.51 log reduction, respectively, while washing apple by soaking with vegetable wash for 1 min only able to achieve 1.70 log reduction. Findings also showed that there was a significant difference ($P<0.05$) for soaking with vegetable wash for 5 mins and 10 mins compared to soaking with vegetable wash for 1 min. Overall, scrubbing artificially contaminated apples under running tap water for 10 s had the smallest reduction which was only 1.21 log reduction.

4. Discussion

In this study, findings showed that *L. monocytogenes* started to attach and form biofilm on the apple surface at 0 hr. Bacterial cells can adhere to a surface and started to form biofilm within minutes of exposure, and this is the first stage of biofilm formation which is adhesion (Donlan, 2002). The low OD value at 0 hr is mainly due to the bacteria are newly inoculated onto a surface, and time is needed to fit themselves in the new environment and look for suitable sites for adhesion (Pui *et al*., 2011). The OD value increased from 0 hr to 18 hrs, this indicated that when the incubation time increased, the number of bacterial cells adhered to the apple surface also increased. During the incubation time, the bacterial cells have more time to contact the apple surface. Increasing incubation time allowed the bacterial cells to adhere firmly and started to form biofilm on the apple surface due to the interaction forces formed. Ukuku and Fett (2002) found that increasing incubation time will increase the attachment strength of *Salmonella enterica* serovar Typhi on the fresh produce surface. Tang *et al*. (2012) also reported similar findings that the attachment strength of *S. enterica* ser. Typhi on the cucumber and mango surfaces increased when the contact time increased.

The OD value at 18 hrs showed the peak value which indicated the highest number of attached cells on the apple surface. This showed that the biofilm had reached the maturation stage (Jamal *et al*., 2015). At 24 hrs, the OD value started to decrease as the planktonic bacterial cells started to detach from the biofilm after the maturation stage (Jamal *et al*., 2015). The time for biofilm formation varies for different bacteria, some need a few hours or days even weeks (Jessen and Lammert, 2003). Tang *et al*. (2012) reported that the formation time of mature biofilm on cucumber, mango, and guava by *S.* Typhi was 12 hrs while *L. monocytogenes* needed about 18 h to form mature biofilm on the apple surface

as shown in this study. Also, Mittal *et al*. (2009) found that the mature biofilm formation time of *Pseudomonas aeruginosa* on the surface of indwelling catheters took 5 to 7 days.

Several ways can be conducted to quantify the biofilm formed on surfaces. In this study, crystal violet assay was used as it is a convenient method to quantify biofilm formation (Pui *et al*., 2011; Tang *et al*., 2012). This method involves rinsing, staining, destaining the bacterial cells using crystal violet, and determining the OD of the stained cells spectrophotometrically (Wu *et al*., 2019). This method is simple and cheap to quantify biofilm formation by bacteria. Hence, crystal violet assay is often used to study different types of bacteria that grow under different environmental conditions, and this assay can provide reproducible results (Pui *et al*., 2011). Crystal violet assay is simple to perform, convenient and cost-effective because specialized equipment needs not be purchased and the dye is also cheap (Wilson *et al*., 2017).

In the washing simulation study, the results showed that soaking the apple with commercial vegetable wash for 5 mins and 10 mins achieved higher log reduction than other methods except for scrubbing with hands under running tap water for 30 s and 60 s. Soaking with vegetable wash for 5 mins and 10 mins was significantly effective ($P<0.05$) in reducing *L. monocytogenes* biofilm on apple surface compared to soaking without the use of vegetable wash for 10 mins. This indicated that vegetable wash is effective to remove bacterial cells on the apple surface. Kilonzo-Nthenge *et al*. (2006) also showed a similar result, they found that soaking with vegetable wash had a greater log reduction of bacteria on the tomato surface compared to other methods. In this study, findings showed that prolonging the treatment time of soaking with vegetable wash from 1 min to 5 mins can remove more bacteria on the apple surface but there was no significant difference ($P>0.05$) between soaking with vegetable wash for 5 mins and 10 mins. Kuan *et al*. (2017a) also showed that increasing the time of soaking from 5 to 10 mins was no significant difference ($P>0.05$) in reducing bacteria on lettuce.

Scrubbing apples with hands under running tap water for 30 s and 60 s were the most efficient method to remove *L. monocytogenes* biofilm on the apple surface with approximately 5.93 log reduction. Surprisingly, almost all the *L. monocytogenes* were completely removed from the artificially contaminated apple surface after being subjected to these washing treatments. Kuan *et al*. (2017a) also found that scrubbing lettuce under running tap water for 60 s achieved 2.44 log reduction, which was the most effective method in removing bacteria among all the washing methods. Findings from Kilonzo-Nthenge *et al*. (2006) and Kuan *et al*. (2017a) also suggested that the efficacy of the scrubbing method may be affected by the scrubbing force, different types of fresh produce, attachment and detachment ability of bacteria on the fresh produce. A study by O'Beirne *et al*. (2014) found that some bacteria attached firmly to the fresh produce surface was more difficult to remove during the decontamination step. However, there is still uncertainty on the principle of adhesion ability of different foodborne pathogens including *L. monocytogenes*, *E. coli*, and *Salmonella* on the fresh produce surfaces (Brandl, 2006; O'Beirne *et al*., 2014). Previous studies by Jeter and Matthysse (2005) and Xicohtencatl-Cortes *et al*. (2009) reported that the ability of different foodborne pathogens in facilitating bacteria attachment on fresh produce surface by flagella and curli fibres. Gorski *et al*. (2003) found that different strains of *L. monocytogenes* can adhere to radish depended on the temperature and flagellar motility. The attachment ability increased with the temperature increased from 10℃ to 30℃ but decreased at 37℃. It is because the production and motility of flagellin were repressed (Gorski *et al*., 2003). Although scrubbing apples with hands under running tap water for more than 30 s efficiently removed *L. monocytogenes* biofilms on the apple surface, it still poses a substantial risk to public health. A low infectious dose of *L. monocytogenes* can contribute to severe invasive illness which is able to cause listeriosis among susceptible populations such as pregnant women, the elderly and infants (Kuan *et al*., 2013).

5. Conclusion

In this study, findings indicated that *L. monocytogenes* can form matured biofilm on the apple surface after 18 hrs. The adhesion ability of bacteria cells may affect by surface roughness, disinfection ability, and wettability. Household washing methods applied in this study were able to reduce the number of bacteria on the apple surface. It was showed that scrubbing under running tap water for at least 30 s is the most effective method to remove *L. monocytogenes* biofilm on the apple surface compared to soaking methods. This suggested that scrubbing or external force is needed in removing bacteria especially the biofilm which is attached firmly to the fresh produce surface. Meanwhile, not all the washing methods were effective in removing the bacteria on the apple surface. Therefore, hygiene practices are very important to prevent the formation of mature biofilm on fresh produce. An efficient washing step is also necessary before consuming fresh produce to lower the risk of foodborne illness.

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Conflict of interest

The authors declare no conflict of interest.

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References

Al-Kharousi, Z.S., Guizani, N., Al-Sadi, A.M., Al-Bulushi, I.M. and Shaharoona, B. (2016). Hiding in fresh fruits and vegetables: Opportunistic pathogens may cross geographical barriers. *International Journal of Microbiology*, 2016(1), 1–14. https://doi.org/10.1155/2016/4292417.

Botticella, G., Russo, P., Capozzi, V., Amodio, M.L., Massa, S., Spano, G. and Beneduce, L. (2013). *Listeria monocytogenes*, biofilm formation and fresh cut produce. In Méndez-Vilas, A. (Ed.). Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education, p. 114–123. Badajoz: Formatex.

Brandl, M.T. (2006). Fitness of human enteric pathogens on plants and implications for food safety. *Annual Review of Phytopathology*, 44, 367–392. https://doi.org/10.1146/annurev.phyto.44.070505.143359.

Castellazzi, M.L., Marchisio, P. and Bosis, S. (2018). *Listeria monocytogenes* meningitis in immunocompetent and healthy children: a case report and a review of the literature. *Italian Journal of Pediatrics*, 44, 152. https://doi.org/10.1186/s13052-018-0595-5.

Donlan, R.M. (2002). Biofilms: microbial life on surfaces. *Emerging Infectious Diseases*, 8(9), 881–890. https://dx.doi.org/10.3201/eid0809.020063.

Feltes, C.M.M., Arisseto-Bragotto, A.P. and Block, J.M. (2017). Food quality, food-borne diseases, and food safety in the Brazilian food industry. *Food Quality and Safety*, 1(1), 13–27. https://doi.org/10.1093/fqsafe/fyx003.

Galié, S., García-Gutiérrez, C., Miguélez, E.M., Villar, C.J. and Lombó, F. (2018). Biofilms in the food industry: Health aspects and control methods. *Frontiers in Microbiology*, 9, 898. https://doi.org/10.3389/fmicb.2018.00898.

Gorski, L., Palumbo, J.D. and Mandrell, R.E. (2003). Attachment of *Listeria monocytogenes* to radish tissue is dependent upon temperature and flagellar motility. *Applied and Environmental Microbiology*, 69(1), 258–266. https://doi.org/10.1128/AEM.69.1.258-266.2003.

Jamal, M., Tasneem, U., Hussain, T. and Andleeb, S. (2015). Bacterial biofilm: its composition, formation and role in human infections. *Research and Reviews: Journal of Microbiology and Biotechnology*, 4(3), 1–14. https://www.rroij.com/open-access/bacterial-biofilm-its-composition-formation-and-role-in-human-infections.php?aid=61426.

Jessen, B. and Lammert, L. (2003). Biofilm and disinfection in meat processing plants. *International Biodeterioration and Biodegradation*, 51(4), 265–269. https://doi.org/10.1016/S0964-8305(03)00046-5.

Jeter, C. and Matthysse, A.G. (2005). Characterization of the binding of diarrheagenic strains of *E. coli* to plant surfaces and the role of curli in the interaction of the bacteria with alfalfa sprouts. *Molecular Plant-Microbe Interactions*, 18(11), 1235–1242. https://doi.org/10.1094/MPMI-18-1235.

Kilonzo-Nthenge, A., Chen, F.C. and Godwin, S.L. (2006). Efficacy of home washing methods in controlling surface microbial contamination on fresh produce. *Journal of Food Protection*, 69(2), 330–334. https://doi.org/10.4315/0362-028X-69.2.330.

Kuan, C.H., Lim, L.W.K., Ting, T.W., Rukayadi, Y., Ahmad, S.H., WanMohamed Radzi, C.W.J., Thung, T.Y., Ramzi, O.B., Chang, W.S., Loo, Y.Y., Kuan, C.S., Yeo, S.K. and Radu, S. (2017a). Simulation of decontamination and transmission of *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes* during handling of raw vegetables in domestic kitchens. *Food Control*, 80, 395–400. https://doi.org/10.1016/j.foodcont.2017.05.029.

Kuan, C.H., Rukayadi, Y., Ahmad, S.H., Wan Mohamed Radzi, C.W.J., Thung, T.Y., Premarathne, J.M.K.J.K., Chang, W.S., Loo, Y.Y., Tan, C.W., Ramzi, O.B., Mohd Fadzil, S.N., Kuan, C.S., Yeo, S.K., Nishibuchi, M. and Radu, S. (2017b). Comparison of the microbiological quality and safety between conventional and organic vegetables sold in Malaysia. *Frontiers in Microbiology*, 8, 1433. https://doi.org/10.3389/fmicb.2017.01433.

Kuan, C.H., Wong, W.C., Pui, C.F., Tang, J.Y.H., Nishibuchi, M., Mahyudin, N.A. and Son, R. (2013). Prevalence and quantification of *Listeria monocytogenes* in beef offal at retail level in Selangor, Malaysia. *Brazilian Journal of Microbiology*, 44(4), 1169–1172. https://doi.org/10.1590/S1517-83822014005000002.

Li-Cohen, A.E. and Bruhn, C.M. (2002). Safety of consumer handling of fresh produce from the time of purchase to the plate: a comprehensive consumer

survey. *Journal of Food Protection*, 65(8), 1287–1296. https://doi.org/10.4315/0362-028X-65.8.1287.

Liu, D. (2008). Handbook of *Listeria monocytogenes*. Boca Raton, USA: CRC Press.

Mittal, R., Sharma, S., Chhibber, S. and Harjai, K. (2009). A time course study of production of virulence factors by biofilms of *Pseudomonas aeruginosa*. *American Journal of Biomedical Sciences*, 1(3), 178–187. https://doi.org/10.5099/aj090300178.

Murray, K., Wu, F., Shi, J., Xue, S.J. and Warriner, K. (2017). Challenges in the microbiological food safety of fresh produce: Limitations of post-harvest washing and the need for alternative interventions. *Food Quality and Safety*, 1(4), 289–301. https://doi.org/10.1093/fqsafe/fyx027.

O'Beirne, D., Gleeson, E., Auty, M. and Jordan, K. (2014). Effects of processing and storage variables on penetration and survival of *Escherichia coli* O157:H7 in fresh-cut packaged carrots. *Food Control*, 40(1), 71–77. https://doi.org/10.1016/j.foodcont.2013.11.026.

Pui, C.F., Wong, W.C., Chai, L.C., Lee, H.Y., Tang, J.Y.H., Noorlis, A., Farinazleen, M.G., Cheah, Y.K. and Son, R. (2011). Biofilm formation by *Salmonella* Typhi and *Salmonella* Typhimurium on plastic cutting board and its transfer to dragon fruit. *International Food Research Journal*, 18, 31–38.

Rodrigues, R.Q., Loiko, M.R., Paula, C.M.D., Hessel, C.T., Jacxsens, L., Uyttendaele, M., Bender, R.J. and Tondo, E.C. (2014). Microbiological contamination linked to implementation of good agricultural practices in the production of organic lettuce in Southern Brazil. *Food Control*, 42, 152–164. https://doi.org/10.1016/j.foodcont.2014.01.043.

Slavin, J.L. and Lloyd, B. (2012). Health benefits of fruits and vegetables. *Advances in Nutrition*, 3(4), 506–516. https://doi.org/10.3945/an.112.002154.

Tang, P.L., Pui, C.F., Wong, W.C., Noorlis, A. and Son, R. (2012). Biofilm forming ability and time course study of growth of *Salmonella* typhi on fresh produce surfaces. *International Food Research Journal*, 19(1), 71–76.

United States Food and Drug Administration (USFDA). (2020). Get the facts about *Listeria*! Retrieved on January 1, 2020 from USFDA Website: https://www.fda.gov/animal-veterinary/animal-health-literacy/get-facts-about-listeria

Ukuku, D.O. and Fett, W.F. (2002). Relationship of cell surface charge and hydrophobicity to strength of attachment of bacteria to cantaloupe rind. *Journal of Food Protection*, 65(7), 1093–1099. https://doi.org/10.4315/0362-028X-65.7.1093.

Wilson, C., Lukowicz, R., Merchant, S., Valquier-Flynn, H., Caballero, J., Sandoval, J., Okuom, M., Huber, C., Brooks, T.D., Wilson, E., Clement, B., Wentworth, C.D. and Holmes, A.E. (2017). Quantitative and qualitative assessment methods for biofilm growth: A mini-review. *Research and Reviews: Journal of Engineering and Technology*, 6 (4), 1–25.

Wu, S., Huang, F., Zhang, H. and Lei, L. (2019). *Staphylococcus aureus* biofilm organization modulated by two-component regulatory pathway. *Journal of Orthopaedic Surgery and Research*, 14 (1), 10. https://doi.org/10.1186/s13018-018-1055-z.

Xicohtencatl-Cortes, J., Chacón, E.S., Saldaña, Z., Freer, E. and Girón, J.A. (2009). Interaction of *Escherichia coli* O157:H7 with leafy green produce. *Journal of Food Protection*, 72(7), 1531–1537. https://doi.org/10.4315/0362-028X-72.7.1531.

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