LHAAC

LOCAL HEALTH AUTHORITIES ANALYTICAL COMMITTEE

COORDINATED SAMPLING PROJECT 31 -

MICROBIAL SAFETY OF BANH MI PRODUCT

Conducted March to April 2021 with Local Government's across Western Australia

Local Health Authorities Analytical Committee

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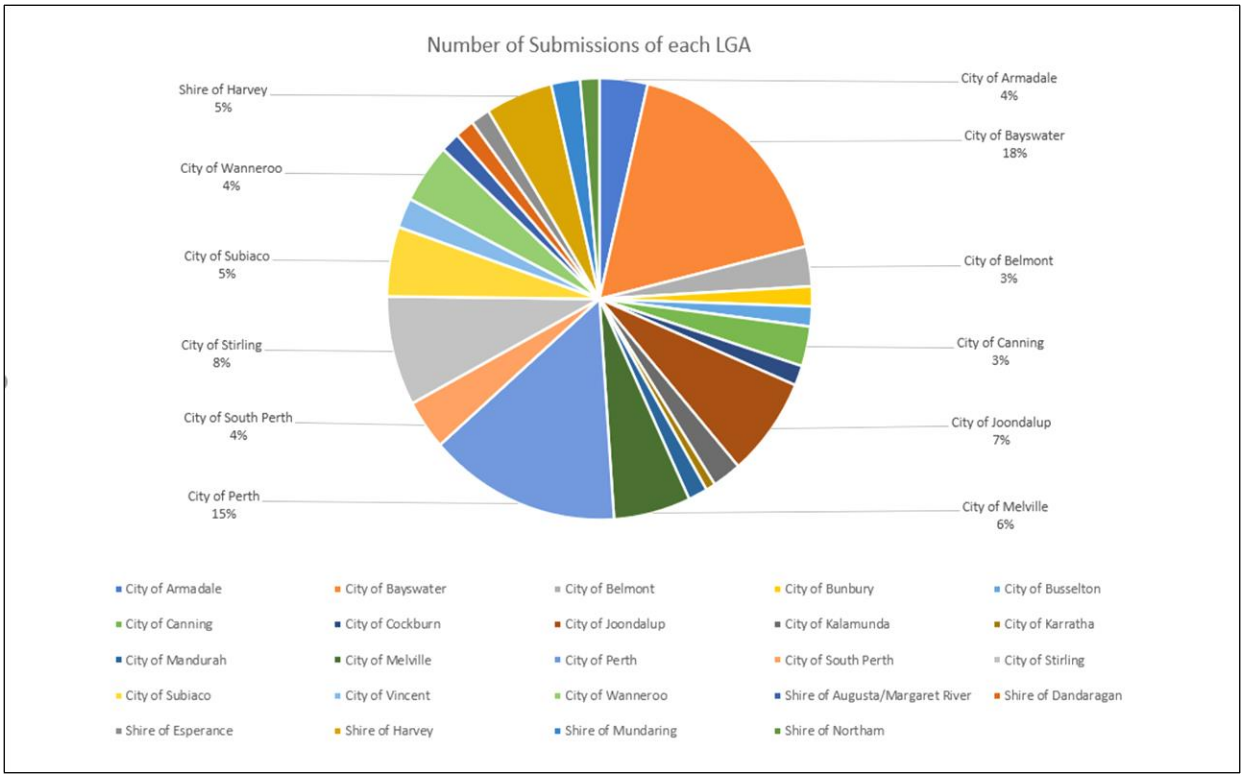


Executive Summary

The first microbiologically based LHAAC Coordinated Sampling Project (CSP) in 2021 will focus on Banh Mi product. In this survey the aim is to assess the microbiological quality of these products as they are sold to the public from specialist food businesses and general retail outlets.

Western Australian (WA) Environmental Health Officers (EHO) submitted samples for assessment to Agrifood Technology (AT) or Eurofins I Analytical Reference Laboratory (EARL) in March to April 2021. At the end of the sampling period, 24 Local Government Authorities (LGA) had submitted a total of 137 food samples to the laboratory for analysis. Food samples were tested for the presence of Escherichia coli (E. coli), Coagulase-positive Staphylococci, Bacillus Cereus (B. Cereus), Salmonella species (spp.), and Listeria Monocytogenes (L. Monocytogenes), Vibrio parahaemolyticus (V. parahaemolyticus), and Campylobacter spp.

Test results were assessed by the LGA against Food Standards Australia and New Zealand's (FSANZ) Compendium of Microbiological Criteria for Food. The results were categorised as satisfactory, marginal, unsatisfactory or potentially hazardous. Where necessary, further investigation or action by the appropriate LGA was undertaken.

E. coli test results indicated 96.4% of samples to be within the satisfactory range, while marginal levels were reported in 1.5% of samples and unsatisfactory levels were reported in 2.2%. Coagulase-positive Staphylococci test results indicated 94.2% of samples to be within the satisfactory range, while marginal levels were reported in 5.1% of samples and unsatisfactory level were reported in 0.7% of samples. B. Cereus test results indicated 90.5% of samples to be within the satisfactory range, while marginal levels were reported in 8.8% of samples, and unsatisfactory levels were recorded in 0.7% of samples. L. monocytogenes, Salmonella spp., Campylobacter spp. as well as Vibrio Parahaemolyticus, test results indicated 100% of samples to be within the satisfactory range.

Contents

Abbreviations

AT	**Agrifood Technology**
CSP	**Coordinated Sampling Project**
EARL	**Eurofins Analytical Reference Laboratory**
EHO	**Environmental Health Officer**
FSANZ	**Food Standards Australia and New Zealand**
FSC	**Food Standards Code**
LGA	**Local Government Authority**
LHAAC	**Local Health Authorities Analytical Committee**
NATA	**National Association of Testing Authorities**
RTE	**Ready-to-eat**
SPC	**Standard plate count**
Spp.	**Species**
VPR	**Vietnamese Pork Roll**
WA	**Western Australia**

1.0 Introduction

1.1 Background

As a microbiologically based Coordinated Sampling Project (CSP) conducted by the Local Health Authorities Analytical Committee (LHAAC), this project involved the microbial analysis of Banh Mi products. In this survey the aim was to assess the microbiological quality of these products as they are sold to the public from specialist food businesses and general retail outlets.

Traditional Vietnamese Pork Rolls (VPR) are made with pork, but VPRs fall into the general category of Banh Mi which can include various versions of a bread roll filled with ham, chicken, pork, beef, brawn etc. Other ingredients including pate, vegetables, salad, spices, mayo, and pickles. They can also be made in rice paper as an alternative to a bread roll.

These products can involve extensive handling and pose an increased food safety risk as some ingredients are not cooked or do not have a pathogen reduction step.

1.2 Standards

Microbiological guidelines can be used by regulatory agencies to check that food for sale is safe and suitable and the food handling controls, and hygienic practices of food business are adequate. The microbial quality of food products analysed for this project was assessed using FSANZ's Compendium of Microbiological Criteria for Food [1].

1.3 Bacterial Contamination Risk and Foodborne Illness

The production of Banh Mi products requires extensive handling prior to sale. The microbiological quality of food can be impacted by temperature control, food handler hygiene and food quality [2]. There is a risk of bacteria transferring to food ingredients at any stage including transport, processing, storage, and at the point of sale. Food that is contaminated with pathogenic microorganisms can cause the consumer to suffer from foodborne illnesses. Bacteria that are commonly responsible for causing foodborne illnesses include E. coli, Salmonella spp., L. monocytogenes, and Campylobacter spp. [3]. In fact, the three microorganisms most

commonly associated with microbial food recalls in Australia between 2008 and 2017 were E. coli, Salmonella spp., and L. monocytogenes [4].

1.4 Temperature Control

Banh Mi products are not expected to undergo further cooking or processing prior to consumption. These types of meals would generally be considered as potentially hazardous foods as they usually need to be kept under temperature control to minimise the growth of pathogenic microorganisms that may be present in the food, or to prevent the formation of toxins in the food.

The production and sale of food in WA must comply with the requirements of the Australia New Zealand Food Standards Code (FSC). As per Standard 3.2.2 of the FSC, potentially hazardous food must be stored under temperature control which can be achieved by either refrigeration to below 5° Celsius or heating to above 60° Celsius [5]. In accordance with the FSC, a food business may maintain the food out of temperature control if it can be demonstrated that the alternative temperature does not adversely affect the microbiological safety of the food [5]. Food businesses often achieve this requirement with the application of the 2 hour – 4-hour rule (Appendix B), a process that requires documented procedures to ensure that potentially hazardous food is safe while stored out of temperature control for a limited time [6] [7].

2.0 Methodology

Sampling instructions were supplied to WA LGAs. Both metropolitan and non-metropolitan LGAs were encouraged to participate in this CSP if suitable products were available in their locality. The number of samples to be collected was determined by each LGA in consideration of their sampling allowance and other activity planned or anticipated for the financial year.

Samples of Banh Mi products from across WA were submitted to either AT or Eurofins I ARL, the two appointed analysts to the LHAAC in March to April 2021. The minimum sample size for submission to the analysts was 200 grams. Each laboratory conducted microbial analysis of the samples utilising National Association of Testing Authorities (NATA) accredited methods (Appendix A). All of the food samples (137) were tested for the presence of E. coli, Coagulase-positive Staphylococci, B. cereus, Salmonella spp., and L. monocytogenes. Additionally, 41 samples were tested for Campylobacter spp. and 8 samples were tested for V. parahaemolyticus.

Upon completion, LGAs were requested to review the results by assessing them against the FSANZ's Compendium of Microbiological Criteria [1]. Recommended follow-up actions were provided to each LGA within the sampling instructions.

3.0 Result

By the end of the sampling period, 24 LGAs had submitted a total of 137 food samples of varying types to the laboratories for analysis. Overall, a total of 708 tests were carried out. All 137 samples were tested for the presence of E. coli, Coagulase-positive Staphylococci, B. cereus, Salmonella spp., and L. monocytogenes. Additionally, 41 samples were tested for Campylobacter spp. and 8 samples were tested for V. parahaemolyticus. All test results were compared against the FSANZ's Compendium of Microbiological Criteria (Table 1)

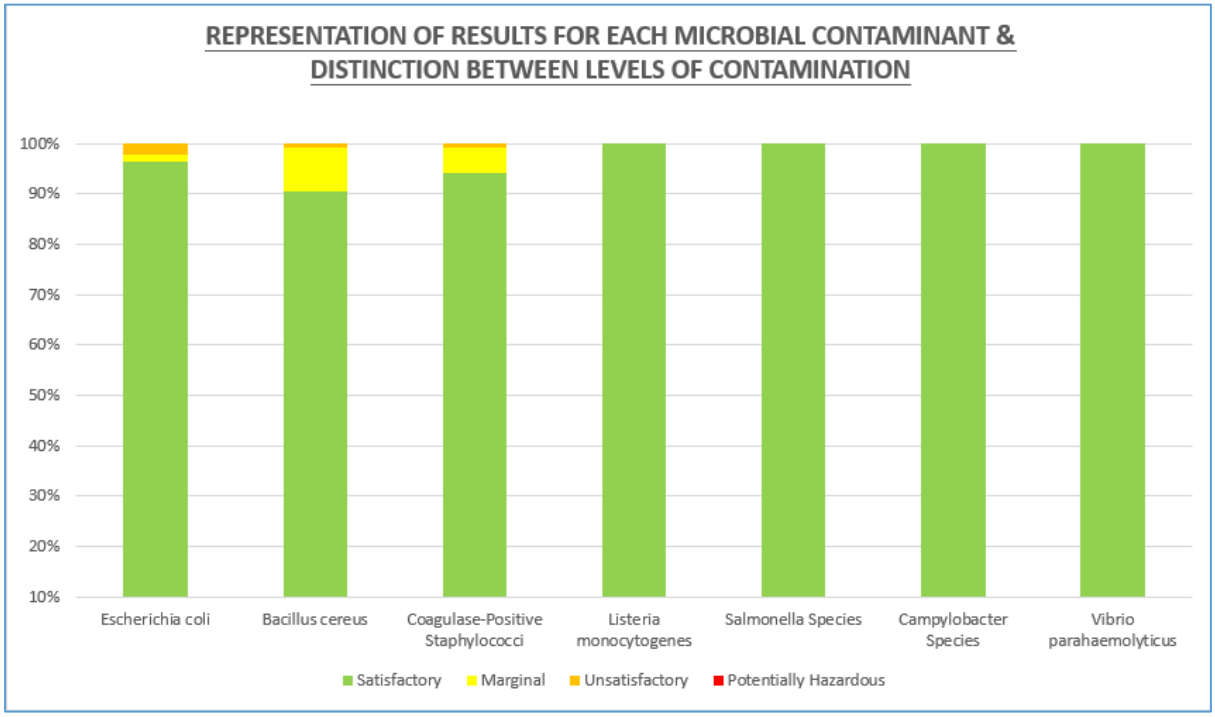
Table 1. Guideline levels for determining the microbiological quality of RTE foods 2018 [1].

	Microbiological Quality (CFU per gram)			
	Satisfactory	Marginal	Unsatisfactory	Potentially Hazardous
Standard Plate Count [a]				
Category 1	$< 10^3$	10^3 - $<10^5$	$\geq 10^5$	
Category 2a	$< 10^4$	10^4 - $<10^6$	$\geq 10^6$	
Category 2b	$< 10^4$	10^4 - $<10^7$	$\geq 10^7$	
Category 3	$< 10^5$	10^5 - $<10^7$	$\geq 10^7$	
Category 4	$< 10^6$	10^6 - $<10^7$	$\geq 10^7$	
Category 5	N/A	N/A	N/A	
Indicators				
Escherichia coli	<3	3 - $<10^2$	$>10^2$	
Pathogens				
Staphylococcus aureus and other coagulase +ve staphylococci	$<10^2$	10^2 - $<10^3$	$10^3 - \leq 10^4$	$>10^4$
Clostridium Perfringens	$<10^2$	$10^2 - <10^3$	$10^3 - \leq 10^5$	$>10^5$
Bacillus cereus and other pathogenic Bacillus spp	$<10^2$	$10^2 - <10^3$	$10^3 - \leq 10^5$	$>10^5$
Vibrio Parahaemolyticus	<3	$<3 - 10^2$	10^2 - 10^4	$>10^4$
Campylobacter spp	Not detected in 25g			Detected in 25g
Salmonella spp	Not detected in 25g			Detected in 25g
Listeria monocytogenes 1. (RTE food in which growth of L. monocytogenes can occur)	Not detected in 25g			Detected in 25g
Listeria monocytogenes 2. (RTE food in which growth of L. monocytogenes will not occur)	Not detected in 25g / Dectected but $\leq 10^2$ if a listericidal process has not been applied.	Dectected but $\leq 10^2$ if a listericidal process has been applied.		$>10^2$

Upon analysis, E. coli test results indicated 96.4% (n = 132) of samples to be within the satisfactory range, while marginal levels were reported in 1.5% (n = 2) of samples and unsatisfactory levels were reported in 2.2% (n=3). Coagulase-positive Staphylococci test results indicated 94.2% (n = 129) of samples to be within the satisfactory range, while marginal level was reported in 5.1% (n =7) and unsatisfactory level was reported in 0.7% (n=1) of samples. B. Cereus test results indicated 90.5% (n = 124) of samples to be within the satisfactory range, while marginal levels were reported in 8.8% (n = 12) of samples, and unsatisfactory levels were recorded in 0.7 % (n = 1) of samples. L. monocytogenes as well as Salmonella spp. test results indicated 100% (n = 137) of samples to be within the satisfactory range. Campylobacter Sp. test results indicated 100% (n = 41)

of samples to be within the satisfactory range. V. Parahaemolyticus test results also indicated 100% (n = 8) of samples to be within the satisfactory range.

Figure 2. Represents the test results for each microbial contaminant and provides a visual distinction between levels.



When compared against the FSANZ's Compendium of Microbiological Criteria levels for determining the microbiological quality of Banh Mi products, 96.5% of all test results indicated that the sample was within the satisfactory range, 2.9% indicated marginal levels of microbial contamination, 0.68% indicated unsatisfactory levels of microbial contamination, and no case of potentially hazardous levels of microbial contamination (Table 2).

4.0 Discussion

4.1 Escherichia coli

E. coli is a bacterium that is naturally found in human and animal intestines [9]. It is often spread to food via the faecal-oral route and can cause an infection when ingested [9]. As such, their presence in ready-to-eat foods (fully cooked or those containing raw fruits or vegetables) can be an indication of poor hygiene and sanitation or inadequate heat treatment [8]. The presence of E. coli can suggest faecal contamination and microbiological tests are often conducted to provide a reference in order to evaluate the hygienic quality of food [10].

The results from this CSP indicated that 132 of 137 samples (96.4%) which were tested for the presence of E. coli fell within satisfactory levels when compared against the FSANZ's Compendium of Microbiological Criteria [1]. 2 samples indicated marginal levels (1.5%) and 3 samples (2.2%) reported in unsatisfactory level.

The detection of E. coli in foods is not a direct indication that the food is unsafe rather it is an indication of potential problems involving the preparing and handling of foods. However, widespread detection in several samples may indicate poor hygienic practices in the food production environment [1].

4.2 Coagulase-positive Staphylococci

Staphylococcus is a genus of bacteria which can be further categorised by its ability to produce coagulase [11]. Coagulase-positive species are generally considered potentially pathogenic to humans [11]. Staphylococcus aureus (S. aureus) is a Coagulase-positive species that can cause food poisoning [12]. Some humans naturally carry S. aureus on their skin and in their nose. S. aureus can transmit to food as a result of poor food handling practices and temperature abuse can result in its multiplication [12].

The results from this CSP indicated that 129 of 137 samples (94.2%) which were tested for the presence of coagulase-positive Staphylococcus fell within satisfactory levels when compared against the FSANZ's Compendium of Microbiological Criteria [1]. 7 samples indicated marginal levels (5.1%) and 1 sample (0.7%)

reported an unsatisfactory level. These results may indicate hygiene and handling controls could be improved by the food manufacturers.

[1].

4.3 Bacillus cereus

B. cereus is a bacterium that is found in nature and is commonly detected in soil. It is usually found in raw ingredients and is commonly associated with rice dishes [1]. B. cereus illness is often related to improper cooling of food and temperature abuse [13]. There are two main types of foodborne illness that are caused by the bacterium, one is characterised by vomiting or nausea while the other causes diarrhoea [13].

Bacillus thuringiensis and the other 7 species of spore forming Gram positive bacteria, including B. cereus, B. cytotoxicus, B. anthracis, B. pseudomycoides, B. weihenstephanensis, B. toyonensis, and B. mycoidesare, are primarily detected in soil. [14] Because these bacteria are highly similar in genotype and phenotype, the bacteria are classified as B. cereus group in taxonomy. [14] B. cereus and B. thuringiensis are highly detectable in foods since they are observed in raw materials from agricultural soil during cultivation and distribution. [14] In addition, these two bacteria are usually not discriminated in clinical diagnostics. [14] In particular, B. thuringiensis was used as a pesticide in the cultivation of certain crops and it is well-known as microbial insecticides that have been used to reduce the amount of chemical pesticides. [14]

The results from this CSP indicated that 124 of 137 samples (90.5%) which were tested for the presence of B. Cereus fell within satisfactory levels fell within satisfactory levels when compared against the FSANZ's Compendium of Microbiological Criteria [1]. 12 samples indicated marginal levels (8.8%) and 1 sample (0.7%) reported in unsatisfactory level. These results showed that the time and temperature control during cooling and storage may not be achieved appropriately. [1] However, an assessment was not undertaken by LHAAC on the individual types of food being tested or whether any b.cereus results could have been detected of b.thuringenises which can be presented in foods with salad type ingredients due to the bacteria being used as a biological pesticide and in organic farming.

4.4 Campylobacter spp.

Campylobacter is a species of bacteria that is found within the gastrointestinal system and faecal matter of animals and is most commonly in or on raw poultry [15]. A condition known as Campylobacteriosis is caused by ingesting undercooked or Campylobacter spp. contaminated meat, particularly chicken, which infects the digestive tract of humans [15]. Campylobacteriosis is considered to be the most common bacterial cause of human gastroenteritis worldwide, accounting for around half of all reported gastrointestinal infections in WA [15] [16].

The results from this CSP indicated that 41 of 41 samples (100%) which were tested for the presence of B. Cereus fell within satisfactory levels when compared against the FSANZ's Compendium of Microbiological Criteria [1]. This result suggests that the food outlets who supplied these samples have taken care in both the preparation and in the cooking process of the RTE meals which contained poultry.

4.5 Salmonella spp.

Salmonella spp. are bacteria which are known to cause a disease called Salmonellosis which is characterised by abdominal pain, diarrhoea and occasionally vomiting [17]. Salmonella spp. can transmit from animals to contaminate food of animal origin (such as eggs, meat or dairy) or it can be transmitted by humans through the faecal-oral route [17].

The results from this CSP indicated that 137 of 137 samples (100%) which were tested for the presence of Salmonella spp. fell within satisfactory levels when compared against the FSANZ's Compendium of Microbiological Criteria [1]. This result suggests that the food outlets who supplied these samples have taken care in both the preparation and in the cooking process of the RTE meals.

4.6 Listeria monocytogenes

L. monocytogenes is a bacterium responsible for causing a foodborne disease named Listeriosis [18]. Non-invasive Listeriosis can affect otherwise healthy individuals with symptoms including headache, muscle pain, fever and diarrhoea [18]. Invasive Listeriosis is a serious threat to high-risk population groups including

pregnant women, the immunocompromised, children and the elderly [19]. Symptoms of invasive Listeriosis can include septicaemia and bacterial meningitis, with symptoms capable of causing premature death [18].

The results from this CSP indicated that 137 of 137 samples (100%) which were tested for the presence of L. monocytogenes fell within satisfactory levels when compared against the FSANZ's Compendium of Microbiological Criteria [1]. This result suggests that the food outlets who supplied these samples have taken care in both the preparation and in the cooking process of the RTE meals.

4.7 Vibrio Parahaemolyticus

V. parahaemolyticus is a bacterium found naturally in salt water [1]. It can cause gastroenteritis when consumed and is most often associated with raw or undercooked shellfish, fish or crustaceans [1]. Accordingly, this CSP only tested for the presence of V. parahaemolyticus in products containing raw seafood.

The results from this CSP indicated that 8 of 8 samples (100%) which were tested for the presence of V. parahaemolyticus fell within satisfactory levels when compared against the FSANZ's Compendium of Microbiological Criteria. This result suggests that the food outlets who supplied these samples have taken care in the preparation of the RTE meals which contained raw seafood.

5.0 Conclusion

This CSP looked at the microbiological quality of Banh Mi products. All the samples (n=137) were tested for the presence of E. coli, Coagulase-positive Staphylococci, B, cereus, Salmonella spp., L. monocytogenes. Moreover, 41 samples were tested for Campylobacter spp. and 8 samples were tested for V. Parahaemolyticus.

L. monocytogenes, Salmonella spp., Campylobacter spp. and V. Parahaemolyticus test results indicated 100% of samples (n=137) to be within the satisfactory range. 96.4% of food samples (n=132) were tested for the presence of E. coli fell within satisfactory levels. 94.2% of food samples (n=129) were tested for the presence of Coagulase-positive Staphylococci. 90.5% of food samples (n=124) were tested for the presence of B. cereus. Most of results are within expected microbiological levels and present no food safety concern.

However, 21 food samples (2.9%) reported in marginal level and 5 (0.68%) reported in unsatisfactory level which reflected food handling controls may not be implemented appropriately at the food premises where the affected samples were produced. Food poisoning including nausea, vomiting and diarrhoea can be caused by eating food contaminated with some bacteria (e.g., Coagulase-positive Staphylococci and E. coli) [12]. Healthy people rarely become seriously ill from food poisoning, but the disease can be fatal to unborn babies, newborns and people with weakened immune systems [20]. Where there are marginal or unsatisfactory microbiological levels, a further action is required to ensure effective food handling controls. This can include investigation into temperature and time profiles used for cooking and storage of cooked foods and assessing the quality of high-risk raw ingredients.

Overall, the results demonstrated that the vast majority (96.5%) of test results were within satisfactory levels of microbiological quality when assessed against FSANZ's Compendium of Microbiological Criteria for Food [1]. This reflects well on the WA food industry, who demonstrated they provide a good quality of Banh Mi products for dine in or takeaway consumers in WA. This high level of service is supported by rigorous food safety measures practiced by both Local Government environmental health staff and Department of Health officers.

6.0 References

[1] Food Standards Australia and New Zealand. (2018). Compendium of Microbiological Criteria for Food. Retrieved from http://www.foodstandards.gov.au/publications/Documents/Compedium%20of%20Microbiological%20Criteria/Compendium_revised-Sep%202018.pdf

[2] S. Hoel, A. N. Jakobsen and O. Vadstein, "Effects of storage temperature on bacterial growth rates and community structure in fresh retail sushi," Journal of Applied Microbiology, vol. 123, no. 3, pp. 698-709, 2017.

[3] NSW Food Authority, "Food poisoning," 2018. [Online]. Available: http://www.foodauthority.nsw.gov.au/fp/food-poisoning.

[4] Food Standards Australia New Zealand, "Food recall statistics," 2019. [Online]. Available: http://www.foodstandards.gov.au/industry/foodrecalls/recallstats/Pages/default.aspx.

[5] Food Standards Australia New Zealand, "Food Standards Code," 2019. [Online]. Available: http://www.foodstandards.gov.au/code/Pages/default.aspx.

[6] NSW Food Authority, "Report on food handling practices and microbiological quality of sushi in Australia," 2008. [Online]. Available: http://www.foodstandards.gov.au/publications/documents/Microbiological-quality-of-sushi-in-Australia-survey.pdf.

[7] SA Health, "2 Hour/4 Hour Rule Explained," 2009. [Online]. Available: https://www.sahealth.sa.gov.au/wps/wcm/connect/3dd213804376220b92dcdfc9302c1003/2+hour+4+hour+Rule+%28poster% 29.pdf?MOD=AJPERES&CACHEID=ROOTWORKSPACE3dd213804376220b92dcdfc9302c1003-mwMFSAr.

[8] NSW Food Authority, ''Microbiological quality guide for ready-to-eat foods,'' (2009). [Online]. Available: https://Microbiological quality guide for ready-to-eat foods

[9] Centers for Disease Control and Prevention, “E. coli (Escherichia coli),” 2019. [Online]. Available: https://www.cdc.gov/ecoli/index.html.

[10] Centre for Food Safety, “Microbiological quality of sushi and sashimi in Hong Kong (2014),” 2015. [Online]. Available: https://www.cfs.gov.hk/english/programme/programme_rafs/files/programme_rafs_fm_01_23_Report_e.pdf.

[11] W. B. Whitman, Bergey's manual of systematics of archaea and bacteria, Hoboken, NJ: Wiley, 2015.

[12] Centers for Disease Control and Prevention, “Staphylococcal (Staph) Food Poisoning,” 2018. [Online]. Available: https://www.cdc.gov/foodsafety/diseases/staphylococcal.html.

[13] Food Standards Australia New Zealand, “Bacillus cereus,” 2013. [Online]. Available: https://www.foodstandards.gov.au/publications/Documents/Bacillus%20cereus.pdf.

[14] Wei Shuai, Chelliah Ramachandran, Park Byung-Jae, Kim Se-Hun, Forghani Fereidoun, Cho Min Seok, Park Dong-Suk, Jin Yong-Guo and Oh Deog-Hwan, ‘’Differentiation of Bacillus thuringiensis From Bacilluscereus Group Using a Unique Marker Based on Real-Time PCR,’’ 2019. Frontiers in Microbiology Available: https://www.frontiersin.org/article/10.3389/fmicb.2019.00883

[15] Department of Health, “Campylobacter infection,” 2019. [Online]. Available: https://healthywa.wa.gov.au/Articles/A_E/Campylobacter-infection.

[16] World Health Organization, “Campylobacter,” 2018. [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/campylobacter.

[17] World Health Organization, “Salmonella (non-typhoidal),” 2018. [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/salmonella-(non-typhoidal).

[18] World Health Organization, “Listeriosis,” 2018. [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/listeriosis.

[19] Food Standards Australia New Zealand, “Listeria monocytogenes,” 2013. [Online]. Available: https://www.foodstandards.gov.au/publications/Documents/Listeria%20monocytogenes.pdf.

[20] The department of Health. (2010). 8 Food poisoning and contamination, Available: https://www1.health.gov.au/internet/publications/publishing.nsf/Content/ohp-enhealth-manual-atsi-cnt-l~ohp-enhealth-manual-atsi-cnt-l-ch3~ohp-enhealth-manual-atsi-cnt-l-ch3.8

[21] NSW Food Authority. (2021). Food poisoning. Retrieved fromhttps://www.foodauthority.nsw.gov.au/consumer/food-poisoning

Appendix A

Further Information

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Appendix B

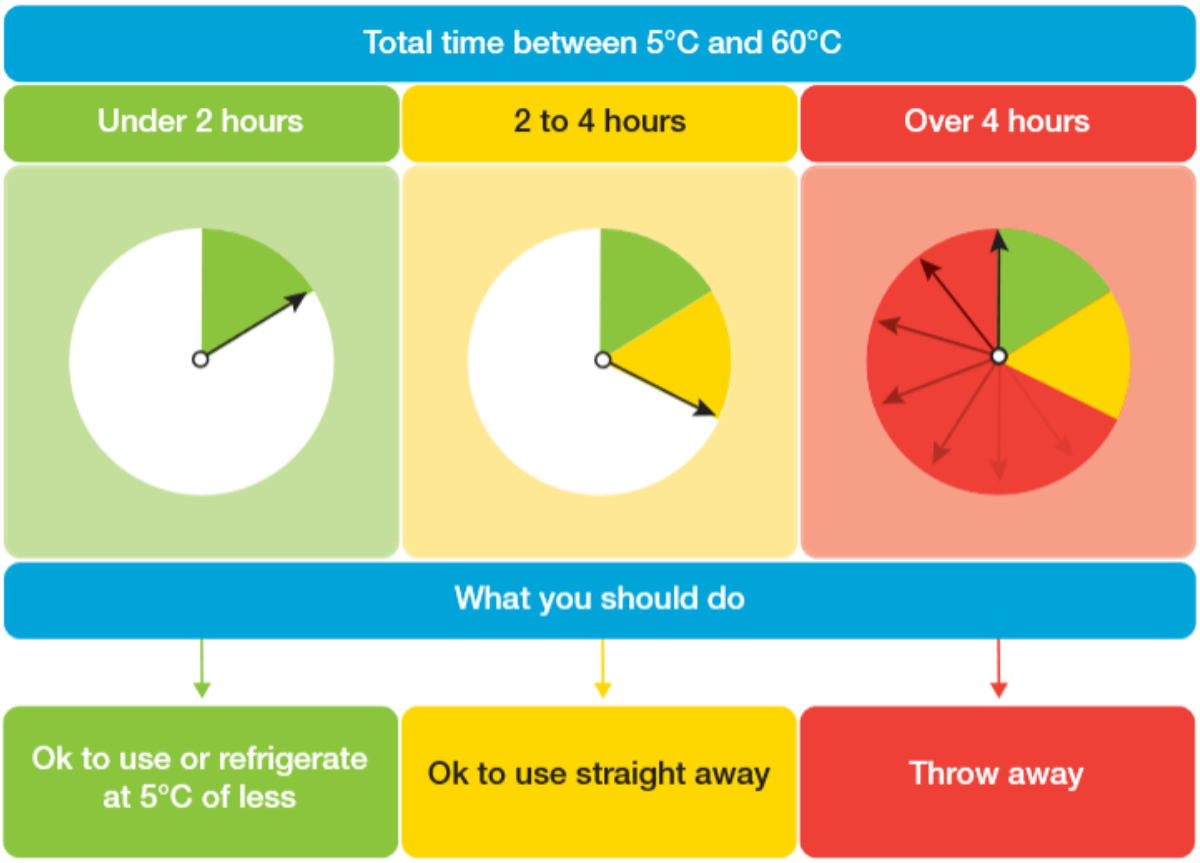

Figure 3. A visual representation of the 2 hour – 4 hour rule [7]