LHAAC

LOCAL HEALTH AUTHORITIES ANALYTICAL COMMITTEE

COORDINATED SAMPLING PROJECT 24 -

Microbial Safety of Sushi and Rice Paper Rolls

Conducted November 2018 to January 2019 with Local Governments across Western Australia

Local Health Authorities Analytical Committee

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Acknowledgements

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Executive Summary

This Coordinated Sampling Project (CSP) involved analysis of the microbiological quality of sushi and rice paper rolls sold throughout Western Australia (WA). This was the first microbiologically based CSP for the Local Health Authorities Analytical Committee (LHAAC). It is important that consumers in WA have access to safe food which is defined to be satisfactory in accordance with the Food Standards Australia and New Zealand (FSANZ)'s Microbiological Guidelines (FSANZ, 2018a).

The LHAAC worked with Western Australian Local Government Authorities (LGA) to execute this project. WA Environmental Health Officers submitted samples for assessment to Agrifood Technology (AT) or Analytical Reference Laboratory (ARL), the two appointed analysts to LHAAC, from November 2018 through to January 2019.

At the end of the sampling period, a total of 382 samples of sushi and rice paper rolls were submitted to the analysts. The food samples were tested for Standard Plate Count, *Bacillus cereus* (*B. cereus*), *Escherichia coli* (*E. coli*), coagulase-positive *Staphylococci*, *Salmonella* species (spp.) and *Listeria monocytogenes* (*L. monocytogenes*). An additional test for the presence of *Vibrio Parahaemolyticus* (*V. Parahaemolyticus*) was conducted on any sample which contained raw seafood.

The majority of the products had satisfactory microbial quality in accordance with the FSANZ's (2018a) Microbiological Guidelines for ready-to-eat (RTE) foods. Marginal, unsatisfactory and potentially hazardous results were detected as follows:

- 2 detections of *L. monocytogenes* at potentially hazardous levels.
- 4 detections of *B. cereus* at unsatisfactory levels and 20 detections at marginal levels.
- 6 detections of *E. coli* at marginal levels.
- 2 detections of coagulase-positive *Staphylococci* at marginal levels.
- 73 detections of marginal microbial quality for the Standard Plate Count and
  31 detections of unsatisfactory microbial quality levels.

An optional survey filled out by the sampling officers determined that approximately 86.6% of the sushi rice was acidified and 32.4% of food businesses verified this process by checking the pH of the rice.

LGAs participated in follow up action on inconsistent products in their locality to ensure continued consistency with FSANZ guidelines.

Contents

List of Abbreviations

AT	Agrifood Technology
ARL	Analytical Reference Laboratory
CDC	Centres for Disease Control and Prevention
CSP	Coordinated Sampling Project
FSANZ	Food Standards Australia and New Zealand
FSC	Australia New Zealand Food Standards Code
LGA	Local Government Authorities
LHAAC	Local Health Authorities Analytical Committee
NATA	National Association of Testing Authorities
NSW	New South Wales
RTE	Ready-to-eat
Spp.	Species
WA	Western Australia
WHO	World Health Organisation

1.0 Introduction

1.1 Sushi and Rice Paper Rolls

This CSP focused on the microbial safety of sushi and rice paper rolls sold in WA. Sushi is a traditional Japanese dish which consists of cooked rice served with a topping. The main types of sushi sold in Australia include Nigiri (rice topped with seafood or egg) and Maki or Nori Rolls (rice and ingredients such as fish, chicken, meat, egg and vegetables wrapped in seaweed) (NSW Food Authority, 2008). Sushi rice is often acidified with vinegar. Rice paper rolls (gỏi cuốn) are a Vietnamese dish of rice paper wrapped around a range of ingredients such as prawn, chicken, duck, tofu, rice noodles and fresh vegetables or herbs (Department of Health State of Victoria, 2015). Sushi and rice paper rolls are both categorised as potentially hazardous food due to their characteristics that "support the growth of pathogenic microorganisms or the production of toxins" (Food Standards Australia and New Zealand [FSANZ], 2016, p. 148).

1.2 Bacterial Contamination Risk and Foodborne Illness

Sushi and rice paper rolls are RTE foods that require extensive handling to produce. Both products do not generally undergo a cooking or other pathogen reduction step prior to sale. The microbiological quality of food can be impacted by temperature control, food handler hygiene and food quality (Hoel, Jakobsen, & Vadstein, 2017). There is a risk of bacteria transferring to food ingredients at any stage of processing including transport, storage and at the point of sale. Raw seafood, a common ingredient used in sushi, carries additional risk for bacterial contamination from the water it was sourced from (Centre for Food Safety, 2014).

Food that is contaminated with pathogenic microorganisms can cause the consumer to suffer from foodborne illness. Bacteria that are commonly responsible for causing foodborne illness include *Salmonella, Campylobacter, E. coli* and *Listeria* (NSW Food Authority, 2018). From 2008 to 2017, the three microorganisms most commonly associated with microbial food recalls in Australia were *L. monocytogenes*, *Salmonella* and *E. coli* (FSANZ, 2018c). Foodborne illness has previously been linked to sushi consumption (Muscolino et al., 2014). The symptoms of foodborne illness are dependent on the causative microorganism, but common symptoms include vomiting, diarrhoea, or abdominal cramps (NSW Food Authority, 2018).

1.3 Temperature Control

As per Standard 3.2.2 of the Australia New Zealand Food Standards Code (FSC), potentially hazardous food (sushi and rice paper rolls) must be stored under temperature control which can be achieved by storing food below 5° Celsius or above 60° Celsius (FSANZ, 2018b). Sushi businesses have previously reported that they store their sushi out of this safe temperature zone due to concerns that refrigeration ruins the quality of rice (NSW Food Authority, 2008).

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 (FSANZ, 2018b). Food businesses often achieve this requirement with the application of the 2 hour/4 hour rule (Appendix B), a procedure that requires documented procedures to ensure that potentially hazardous food is safe while stored out of temperature control for a limited time (NSW Food Authority, 2008). Another method commonly used in the sushi industry is the acidification of rice with the addition of vinegar to discourage the growth of bacteria (Department of Health State of Victoria, 2014). The food business must store the rice below 15° Celsius and conduct and document pH checks to ensure the rice and vinegar mix is pH 4.0 and the end sushi product is pH 4.5 (Department of Health State of Victoria, 2014).

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 by each LGA was determined by each local government in consideration of their sampling allowance and other activity planned or anticipated for the financial year.

The minimum sample size for submission to the analyst was 200 grams. It was requested that the purchased samples be submitted to the laboratory on the day of collection from the food premises. Additional instructions requested that the use-by or best-before date and manufacturing date was recorded on the sample submission form, to assist with the interpretation of *Listeria* results. It was advised that the internal temperature of the food sample must be recorded on the sample submission form as well as on the optional survey form on manufacturing processes. The optional survey form (Appendix C) was completed voluntarily at the discretion of the LGA and emailed directly to the LHAAC.

Samples of RTE sushi and rice paper rolls were submitted to AT or ARL between November 2018 and January 2019. AT and ARL conducted microbial analysis of the food utilising National Association of Testing Authorities (NATA) accredited methods (Appendix A). All sushi and rice paper roll samples were analysed in the laboratory for:

a) Standard Plate Count
b) B. cereus
c) E. coli
d) Coagulase-positive *Staphylococci*
e) Salmonella
f) Listeria spp. / (*L. monocytogenes*)
g) V. Parahaemolyticus (Advised only if the product contained raw seafood).

LGAs were notified and were requested to review the results by assessing them against the FSANZ's microbiology guidelines (Appendix D) (FSANZ, 2018b). LGAs completed the appropriate follow-up action for products with results defined as marginal, unsatisfactory or potentially hazardous.

3.0 Results

Forty LGAs submitted a total of 382 samples. Of the samples submitted, approximately 84.8% were sushi and 15.2% were rice paper rolls.

3.1 Microbiological Results

Overall the microbiological quality of the sushi was predominantly categorised as satisfactory compared against the FSANZ's (2018a) Microbiological Guidelines (Table 1).

Table 1. Microbiological results categorised by the FSANZ's Microbiological Guidelines (2018a).

Organism	Microbiological Quality			
	Satisfactory	**Marginal**	**Unsatisfactory**	**Potentially Hazardous**
Listeria monocytogenes	380 (99.5%)	N/A	N/A	2 (0.5%)
Bacillus Cereus	358 (93.7%)	20 (5.2%)	4 (1.1%)	0
Escherichia coli	376 (98.4%)	6 (1.6%)	0	0
Coagulase-positive *Staphylococci*	380 (99.5%)	2 (0.5%)	0	0
Vibrio Parahaemolyticus	185 (100%)	0	0	0
Salmonella spp.	382 (100%)	N/A	N/A	0
Standard Plate Count	278 (72.8%)	73 (19.1%)	31 (8.1%)	N/A

Figure 1. represents the test results for each microbial contaminant and provides a visual distinction between levels of contamination. The samples categorised with marginal microbiological quality were due to the presence of *B. cereus* (20 samples), *E. coli* (6 samples) and coagulase-positive *Staphylococci* (2 samples). Unsatisfactory microbiological quality was observed in four samples with the presence of *B. cereus.* Two samples were categorised potentially hazardous with the detection of *L. monocytogenes.* The standard plate count ranged from satisfactory (n = 278), marginal (n = 73) to unsatisfactory (n = 31), in accordance with FSANZ's Microbiological Guidelines.

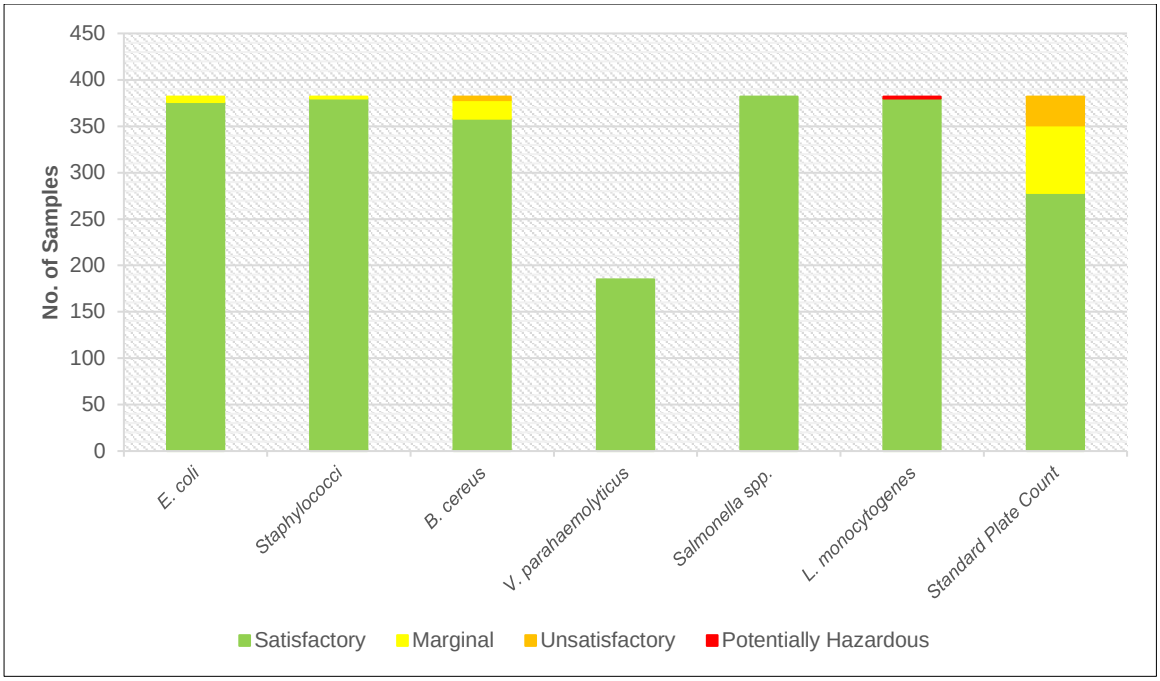

Figure 1. Test results for each microbial contaminant and distinction between levels of contamination

3.2 Acidification and Temperature

Additional information was recorded for some samples. An optional survey about manufacturing processes was completed for 100 products. Survey responses demonstrated that 71 of 82 (86.6%) sushi products contained acidified rice. Approximately 58.5% (n = 48) of the food businesses did not measure the pH of their rice for the products assessed following acidification procedures.

The internal temperature of 207 products was checked at the time of purchase. A minor portion (6.8%) of the sushi and rice paper rolls measured an internal temperature of, or below, 5° Celsius. The majority (93.2%) of the food contained a core temperature which exceeded 5° Celsius at the time of purchase (Figure 2).

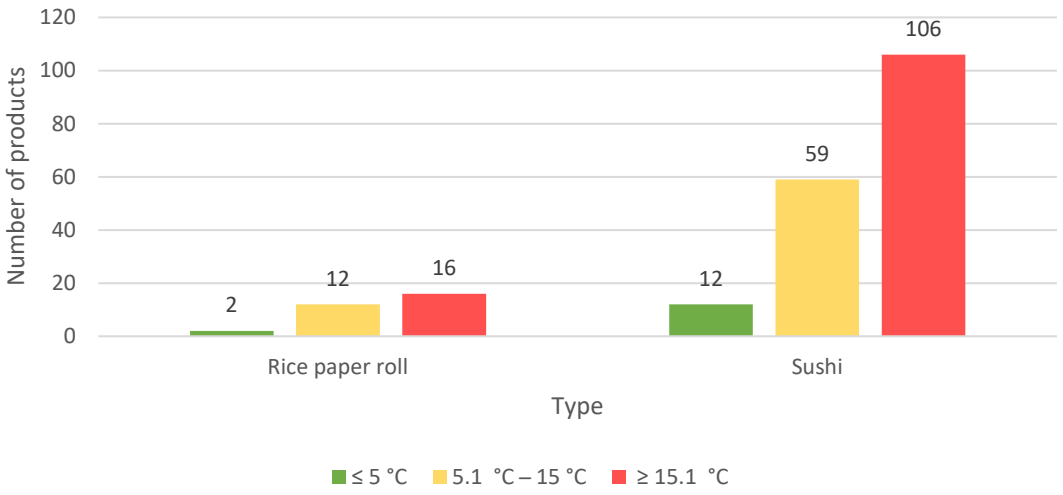

Figure 2. The internal temperature of the sushi and rice paper roll products (n = 207) at the time of purchase.

4.0 Discussion

4.1 *Listeria monocytogenes*

L. monocytogenes is a bacterium responsible for causing a foodborne disease named listeriosis (World Health Organisation [WHO], 2018a). Non-invasive listeriosis can affect otherwise healthy individuals with symptoms including headache, muscle pain, fever and diarrhoea (WHO, 2018a). Invasive listeriosis is a serious threat to high risk population groups including pregnant women, the immunocompromised, children and the elderly (FSANZ, 2013b). Symptoms of invasive listeriosis can include septicaemia and bacterial meningitis, which are capable of causing premature death (WHO, 2018a). RTE foods are at higher risk of *Listeria* contamination since there is no final cooking step and *L. monocytogenes* is killed by cooking (FSANZ, 2013b). Sushi is often considered to have a high risk of *L. monocytogenes* contamination and it is suggested that women should avoid eating this food type during pregnancy (FSANZ, 2018a).

This study detected the presence of *L. monocytogenes* in two sushi samples (0.5%) which were both salmon sushi products. Previous foodborne outbreaks of listeriosis have been linked to fish products (FSANZ, 2013b). As per the Compendium of Microbiological Criteria for Food, the presence of *Listeria* spp. in RTE foods is an indication of “inadequate processing or cross contamination from the environment” (FSANZ, 2018a, p.64). The result from this study is similar to previous studies; in Australia the NSW Food Authority (2008) detected *L. monocytogenes* in 3% of 850 sushi samples, a study in Wales isolated *L. monocytogenes* from 2% of 50 sushi samples (FSANZ, 2013b), and 1.2% of 250 sushi samples in Germany contained detectable levels of *L. monocytogenes* (Atanassova,, Reich, & Klein, 2008). Continued education on safe food handling procedures will assist to prevent the spread of *L. monocytogenes* in food businesses (WHO, 2018a).

4.2 *Bacillus cereus*

B. cereus is a bacterium that is found in nature and is commonly detected in soil. *B. cereus* is usually found in raw ingredients and is commonly associated with rice dishes (FSANZ, 2018a). *B. cereus* illness is often related to improper cooling of food and temperature abuse (FSANZ, 2013a). Two types of foodborne illness are caused by *B. cereus* and they are characterised by either vomiting or diarrhoea (FSANZ, 2013a).

This CSP found *B. cereus* was detected at marginal levels in 20 samples and unsatisfactory levels in 4 samples, in accordance with the FSANZ's Microbiological Guidelines (2018a). The four samples categorised with unsatisfactory levels of *B. cereus* were all sushi samples. The optional survey on the rice acidification procedures was not completed for these four sushi samples. The standards outlined by FSANZ (2018a) state that unsatisfactory and potentially hazardous levels of *B. cereus* are likely a result of the inadequate acidification of rice since the growth of *B. cereus* can be controlled when food is adequately acidified to below pH 4.6 (FSANZ, 2018a).

A previous Australian study analysed sushi samples (n = 851) and found potentially hazardous levels of *B. cereus* in six samples and unsatisfactory levels in two samples (NSW Food Authority, 2008). Similar to the results of this CSP, a study in the State of Victoria did not detect *B. cereus* in six rice paper roll products (Department of Health State of Victoria, 2015).

4.3 *Escherichia coli*

E. coli is a bacterium that is naturally found in human and animal intestines (Centres for Disease Control and Prevention [CDC], 2019). *E. coli* is often spread to food via the faecal-oral route and can cause an infection when ingested (CDC, 2019). The presence of *E. coli* in food is a test often conducted to provide a reference to evaluate the hygienic quality of food since its presence suggests faecal contamination (Centre for Food Safety, 2014).

The majority (98.4%) of analysed samples in this CSP were deemed satisfactory with the FSANZ's Microbiological Guidelines. Six samples (1.6%), consisting of five sushi samples and one rice paper roll sample, had *E. coli* detected at marginal levels in accordance with the FSANZ's Microbiological Guidelines. A previous Australian study analysed 851 sushi samples and found the presence of *E. coli* at unsatisfactory levels in six samples and marginal levels in 34 samples (NSW Food Authority, 2008). An Australian study based in Victoria found 13% of 139 rice paper rolls contained unsatisfactory levels of *E. coli* and 6% contained marginal levels (Department of Health State of Victoria, 2015).

4.4 Coagulase-positive *Staphylococci*

Staphylococcus is a genus of bacteria which can be further categorised by its ability to produce coagulase (Whitman, 2015). Coagulase-positive species are generally considered potentially pathogenic to humans (Whitman, 2015). *Staphylococcus aureus* (*S. aureus*) is a Coagulase-positive species that can cause food poisoning (CDC, 2018). 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 (CDC, 2018).

In accordance with the FSANZ's Microbiological Guidelines, two samples contained coagulase-positive *Staphylococci* at marginal levels. This result suggests that hygiene and handling controls are not being implemented appropriately at the food premises where they were produced (FSANZ, 2018a). Similarly, a previous Australian study found 2 samples of sushi contained unsatisfactory levels of coagulase-positive *Staphylococci* (NSW Food Authority, 2008). Another Australian study analysed 139 samples of rice paper rolls sold in the State of Victoria for coagulase-positive *Staphylococci* and detected unsatisfactory levels in two samples and potentially hazardous levels in one (Department of Health State of Victoria, 2015).

4.5 *Salmonella*

Salmonella bacteria causes a disease called salmonellosis which is characterised by abdominal pain, diarrhoea and occasionally vomiting (WHO, 2018b). *Salmonella* bacteria 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 (WHO, 2018b).

In this CSP, no *Salmonella* spp. were detected in the sushi and rice paper roll samples (n = 382). This result is similar to a previous Australian study which did not detect *Salmonella* spp. in 850 sushi samples (NSW Food Authority, 2008). Similarly, in Hong Kong, the Centre for Food Safety (2014) did not detect *Salmonella* spp. in 197 samples of sushi and sashimi.

4.6 *Vibrio Parahaemolyticus*

V. Parahaemolyticus is a bacterium found naturally in salt water (South Australia Health, 2019). *V. Parahaemolyticus* can cause gastroenteritis when consumed and is most often associated with raw or undercooked shellfish, fish or crustaceans (FSANZ, 2018a). Accordingly, in this CSP laboratory tests for *V. Parahaemolyticus* were only conducted on products containing raw seafood. *V. Parahaemolyticus* was not detected in all the 185 samples analysed.

The result from this CSP is similar to a previous Australian study which determined that 313 sushi samples contained acceptable levels of *V. Parahaemolyticus* (NSW Food Authority, 2008). Global studies have found similar results; 100% of sushi samples (n = 202) sold in Germany were free from *Vibrio* and 99% of sushi and sashimi sold in Hong Kong contained satisfactory levels of *V. Parahaemolyticus* (Atanassova, Reich, & Klein, 2008; Centre for Food Safety, 2014).

4.7 Standard Plate Count

The standard plate count results are interpreted to provide a general assessment of the microbial quality of food (FSANZ, 2018a). This CSP found the Standard Plate Count was detected at marginal levels in 73 samples and unsatisfactory levels in 31 samples, in accordance with the FSANZ's Microbiological Guidelines (2018a). RTE foods often contain components that have not been cooked (raw fish and vegetables) which results in a higher expected standard plate count (FSANZ, 2018a). Marginal and unsatisfactory levels may indicate insufficient hygienic measures during processing and handling (FSANZ, 2018a). This was evident in a study conducted by Yap et al. (2019) who recorded significantly higher Standard Plate Counts in rice and sushi samples which had been prepared using reused gloves or bare hands.

4.8 Temperature

The internal temperature of 207 samples were checked at the time of purchase. Approximately 6.8% (n = 14) were at a temperature at or below 5° Celsius. It is possible that the products with higher temperatures were made prior to the time of purchase which may explain the elevated temperature. There is a second possibility that the products are not being stored under the appropriate temperature control.

A previous Australian study in the State of Victoria found 35% of food premises stored their rice paper rolls at room temperature (Department of Health State of Victoria, 2015). Another Australian study determined that the sushi display cabinet temperatures ranged from 5° Celsius to 19.8° Celsius at 52 food premises (NSW Food Authority, 2008). Sushi must be refrigerated at, or below, 5° Celsius to suppress bacterial growth during the time of the products shelf life. Further education is required in the industry to ensure that rice paper rolls and sushi are being stored under safe temperature control. When the product is being stored at room temperature then alternative procedures must be applied and all staff must be trained on its application. For example, an alternative method is the 2 hour/4 hour rule which requires documentation such as the time the food was removed from refrigeration and the time for disposal.

4.9 pH of rice

The optional survey was completed for 82 sushi products and determined that 86.6% of sushi products contained acidified rice. Data collected found that 32.4% (n = 23) of the proprietors measured the pH of the rice after the acidification procedure was complete to verify the process. Verification of the acidification procedure is important to minimise the risk of hazardous microorganisms growing in rice. A previous Australian study found that six out of 62 food businesses measured the pH of their rice after acidification (NSW Food Authority, 2008). Continued education is required to ensure that food businesses are aware of their requirements to verify the acidification of their rice for every batch made.

5.0 Conclusion

The results of this CSP found that most of the sushi and rice paper rolls were of satisfactory microbiological quality, in accordance with the FSANZ's microbiological guidelines. Marginal microbiological quality was due to the presence of *B. cereus* (n = 20), *E. coli* (n = 6) and coagulase-positive *Staphylococci* (n = 2). Unsatisfactory microbial quality was identified due to the presence of *B. cereus* (n = 4). *L. monocytogenes* was detected in two samples which were deemed potentially hazardous. Standard plate count ranged from satisfactory (n = 278) through marginal (n = 73) to unsatisfactory (n = 31). Proper hygiene techniques, temperature control and promotion of improved record keeping for the acidification of rice and the 2 hour/4 hour rule will continue to improve standards across the industry.

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Appendix A

Raw Data

For further questions or inquiries about raw data contact LHAAC Co-ordinator Trevor Chapman:

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Appendix B

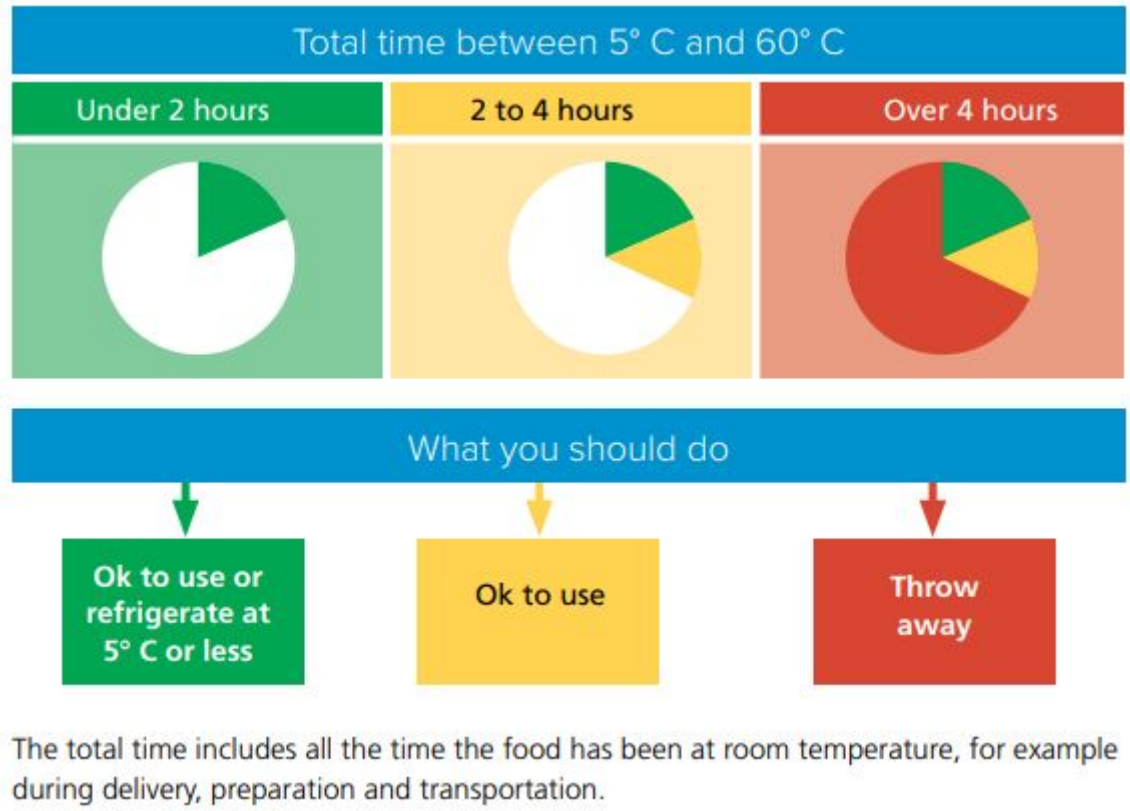

Figure 3. Visual depiction of the 2 Hour/4 Hour rule. Reprinted from *2 Hour/4 Hour Rule Explained* by South Australia Health, 2009, Retrieved from https://www.sahealth.sa.gov.au/wps/wcm/connect/3dd213804376220b92dcdfc9302c1003/2+hour+4+hour+Rule+%28poster%29.pdf?MOD=AJPERES&CACHEID=ROOTWORKSPACE-3dd213804376220b92dcdfc9302c1003-mwMFSAr

Appendix C

Table 1C. Optional Survey on Manufacturing Processes included in the sampling instructions.

Sample Number	Description	Internal Temperature	Sushi Acidified Yes / No	Is pH Checked Yes / No	Is equipment used to sheet rice, roll or cut sushi? Please provide details.

Appendix D

Table 1D. Interpretation guidelines for determining the microbiological quality of RTE foods. Adapted from the FSANZ (2018a).

	Microbiological Quality (CFU per gram)			
	Satisfactory	**Marginal**	**Unsatisfactory**	**Potentially Hazardous**
Standard Plate Count				
Category 4	$<10^6$	$<10^7$	$\geq 10^7$	
Indicators				
Escherichia coli [a]	<3	3 – 100	≥100	[a]
Pathogens				
Coagulase-positive *Staphylococci*	$<10^2$	$10^2 - 10^3$	$10^3 - 10^4$	$\geq 10^4$ SET +ve
Bacillus Cereus	$<10^2$	$<10^2 – 10^3$	$10^3 - 10^4$	$\geq 10^4$
Vibrio Parahaemolyticus [b]	<3	$<3 - 10^2$	$<10^2 - 10^4$	$\geq 10^4$
Salmonella spp.	Not Detected in 25g			Detected
Listeria monocytogenes (RTE food in which growth can occur) [c]	Not Detected in 25g			Detected in 25g
Listeria monocytogenes (RTE food in which growth will not occur) [c]	Not Detected in 25g	Detected but $<10^2$ [d]		$>10^2$ [d]

[a] = Pathogenic strains of *E.coli* should be absent (FSANZ, 2011).

[b] = *V. Parahaemolyticus* should not be present in seafood that has been cooked. For RTE seafood that is raw, a higher satisfactory level may be applied ($<10^2$ cfu/g). The potentially hazardous level of *V. Parahaemolyticus* relates to Kanagawa-positive strains (FSANZ, 2018a).

[c] = Schedule 27 of the FSC specifies microbiological criteria for RTE food on the basis of whether growth of *L. monocytogenes* can occur or will not occur (FSANZ, 2018a).

[d] = The detection of *L. monocytogenes* in RTE foods prepared specifically for 'at risk' population groups (the elderly, immunocompromised and infants) should also be considered as potentially hazardous (FSANZ, 2011).