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## Sous-vide pasteurisation of eggs: a simple method to produce eggs free from *Salmonella enterica* serovar Typhimurium contamination

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# Method for reducing salmonellosis linked to eggs in Australia (including sous-vide pasteurisation)

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#### Salmonellosis

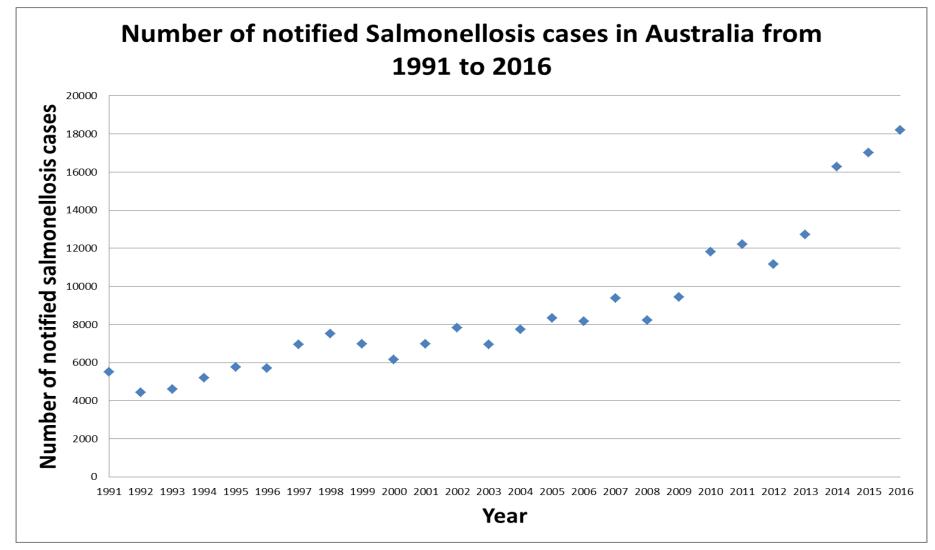
- Worldwide, Salmonella is one of the most prevalent causes of foodborne illness (Galis et al. 2013)
- Globally, it is estimated that the annual incidence of foodborne salmonellosis ranges from 200 million to 1.3 billion cases (Coburn et al. 2006)



#### Symptoms

- Nausea
- Vomiting
- Abdominal cramps
- Diarrhoea
- Fever
- Chills
- Headache
- Blood in the stool



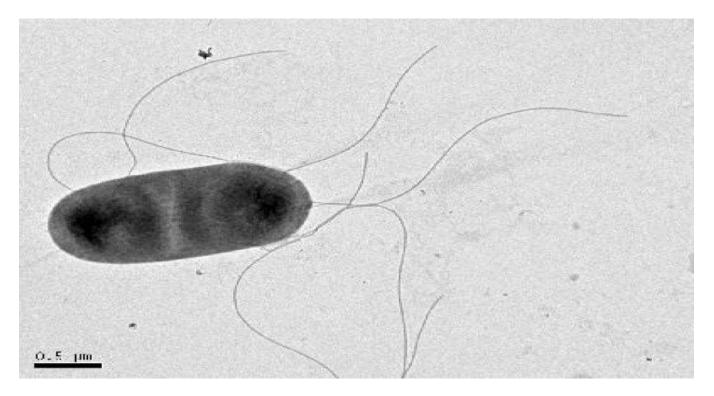


Department of Health (2017), Australia's National Notifiable Diseases Surveillance System (NNDSS). Canberra, Commonwealth of Australia. [online] available: <a href="http://www9.health.gov.au/cda/source/cda-index.cfm">http://www9.health.gov.au/cda/source/cda-index.cfm</a>



#### Foodborne outbreak data

• In 2014 *Salmonella* was identified as the cause of 67% of foodborne outbreaks in Australia (OzFoodNet Working Group 2017)



Salmonella under the Microscope



## NSW 2014 Salmonellosis outbreak data

Setting prepared	Number affected	Number hospitalised	Responsible vehicle	Setting prepared	Number affected	Number hospitalised	Responsible vehicle
Aged care facility	6	2	Unknown	Restaurant	13	0	Unknown
Bakery	13	0	Raw egg mayonnaise	Restaurant	4	0	Burger
Bakery	33	7	Raw egg mayonnaise	Restaurant	38	6	Unknown
Bakery	24	9	Vietnamese rolls	Restaurant	35	0	Pre-prepared meals
Bakery	26	3	Vietnamese rolls with raw egg	Restaurant	19	4	Unknown
Dakery	20	5	butter	ter Restaurant	4	1	Unknown
Bakery	10	7	Multiple foods	Restaurant	4	0	Unknown
Cruise/airline	3	1	Unknown	Restaurant	20	5	Cross-contaminated chocolate milk
Fair/festival/mobile service	4	1	Unknown	Restaurant	4	0	Unknown
Other	16	4	Unknown	Restaurant	7	1	Multiple foods
Other	8	2	Raw egg mayonnaise	Restaurant	9	1	Unknown
Private residence	13	0	Tiramisu with raw egg	Restaurant	2	0	Raw egg Caesar salad dressing
				Takeaway	26	0	Chocolate mousse cake made with lightly cooked eggs
				Takeaway	11	2	Raw egg salad dressing

Takeaway

11

1



Vietnamese rolls raw egg

#### QLD 2014 Salmonellosis outbreak data

Setting prepared	Number affected	Number hospitalised	Responsible vehicle
Bakery	8	0	Various bakery products (custard buns)
Community	12	3	Bakery products (various)
Institution	57	4	Unknown
Institution	10	3	Unknown
Restaurant	11	2	Unknown
Restaurant	12	Unknown	Chocolate mousse
Restaurant	10	Unknown	Deep fried ice cream
Restaurant	6	0	Chocolate mousse
Restaurant	9	2	Unknown
Restaurant	12	1	Eggs Benedict with potato rosti
Restaurant	3	2	Suspected egg and lettuce sandwiches
Restaurant	10	1	Suspected raw egg sauce
Restaurant	18	3	Unknown
Unknown	5	0	Unknown



#### SA 2014 Salmonellosis outbreak data

Setting prepared	Number affected	Number hospitalised	Responsible vehicle
Bakery	12	3	Raw egg butter on Vietnamese roll
Fair/festival	8	0	Unknown
Private residence	14	3	Raw egg tiramisu
Private residence	18	1	Pork spit roast
Restaurant	10	2	Unknown
Restaurant	11	4	Raw egg aioli
Restaurant	7	0	Unknown
Restaurant	8	1	Unknown
Restaurant	12	3	Suspected eggs
Restaurant	4	0	Unknown
Restaurant	4	2	Raw egg aioli
Restaurant	33	5	Suspected raw egg contamination of pesto
School	5	1	Chicken burger
Takeaway	17	2	Raw egg mayonnaise on Vietnamese roll
Takeaway	5	2	Unknown



## VIC 2014 Salmonellosis outbreak data

Setting prepared	Number affected	Number hospitalised	Responsible vehicle	Setting prepared	Number affected	Number hospitalised	Responsible vehicle
Aged care facility	14	0	Suspect chicken patties	Restaurant	19	3	Mixed foods including rice paper rolls
Bakery	10	5	Chicken liver pâté	Restaurant	45	Unknown	Probable food handler
Bakery	27	3	Unknown	Restaurant	21	Unknown	Raw egg aioli/mayonnaise
Bakery	24	1	Raw egg chocolate mousse	Restaurant	94	17	Unknown
Camp	4	0	Lightly cooked eggs and/or hollandaise sauce	Restaurant	2	0	Lightly cooked eggs and/or hollandaise sauce
Hospital	22	1	Unknown	Restaurant	2	1	Raw egg aioli
Private residence	13	1	Tiramisu	Restaurant	3	1	Undercooked eggs
Private residence	4	1	Uncooked brownie batter	Restaurant	6	0	Undercooked eggs
Private residence	6	5	Raw egg chocolate mousse	Restaurant	15	26	Raw egg mayonnaise
Private residence	2	0	Raw egg chocolate mousse	Restaurant	13	1	Suspect raw egg aioli
Private residence	12	3	Unknown	Restaurant	242	5	Lightly cooked eggs
Private residence	6	3	Suspect undercooked eggs in pasta dish	Restaurant	3	2	Raw egg aioli
Private residence	3	0	Raw egg tiramisu	Restaurant	14	5	Undercooked eggs in hollandaise sauce
Private residence	2	1	Unknown	School	10	0	Beef appetiser or frittata



#### WA, NT and ACT 2014 Salmonellosis outbreak data

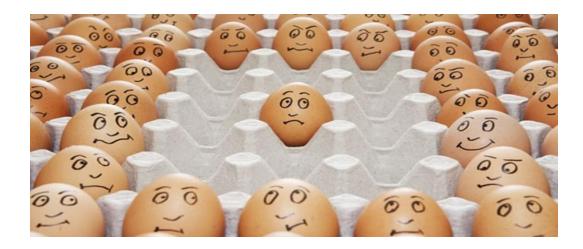
Western Australia						
Setting prepared	Number affected	Number hospitalised	Responsible vehicle			
Commercial caterer	6	0	Nasi-Lemak			
Private residence	10	1	Unknown			
Private residence	3	0	Multiple foods			
Private residence	3	0	Unknown			
Restaurant	4	1	Slow cooked pork hock			
Restaurant	2	0	Unknown			
Restaurant	5	0	Lamb shanks or salad			
Unknown	6	0	Unknown			
		Northern Te	erritory			
Camp	30	0	Cordial			
Restaurant	9	3	Hollandaise sauce containing raw eggs			
Private residence	3	0	Turkey			
		Australian Capit	al Territory			
Private residence	3	1	Eggnog			
Private residence	2	2	Milkshake containing raw egg			



(OzFoodNet Working Group 2017)

## Salmonellosis and eggs

- Eggs and raw egg products identified as one of the most common sources of salmonellosis (Howard et al 2012 and OzFoodNet Working Group 2011).
- Between 2001 and 2011 there was a significant increase in the proportion of foodborne Salmonella outbreaks linked to eggs (p < 0.001) (Moffatt et al 2016)</li>





#### **Australia vs Global perspective**



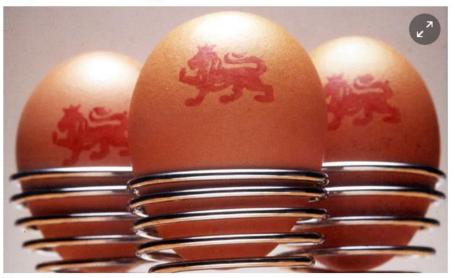
Pregnant women, infants and elderly people told it is now safe for them to eat runny or even raw British eggs



< 2152

Rebecca Smithers Consumer affairs correspondent

Wednesday 11 October 2017 10.01 AEDT





🕖 The advice applies to British eggs that bear the red lion symbol. Photograph: PA



## S. Typhimurium vs S. Enteritidis

Globally, the primary causative agent for salmonellosis is *Salmonella enterica* serovar Enteritidis (SE) but in Australia it is *Salmonella enterica* serovar Typhimurium (ST) (Moffatt et al 2016)

#### **Direct contamination**

Internal contamination of eggs/chicks with *Salmonella* occurs in the ovaries during egg formation (SE)

#### **Indirect contamination**

Contamination of environmental sources with *Salmonella* has the potential to infect other hens and cause both egg shell contamination and internal egg contamination through penetration of the egg shell post laying (both ST and SE)

Vaccination of flocks in the UK







### Salmonellosis and eggs in Australia

In Australia we can not currently guarantee that eggs are free from Salmonella contamination – this is why post production control measures are so important (Whiley and Ross 2015)

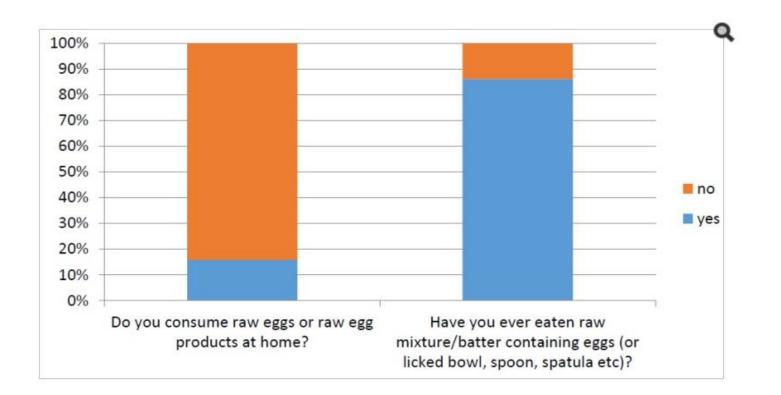




## Food handlers and risk perception

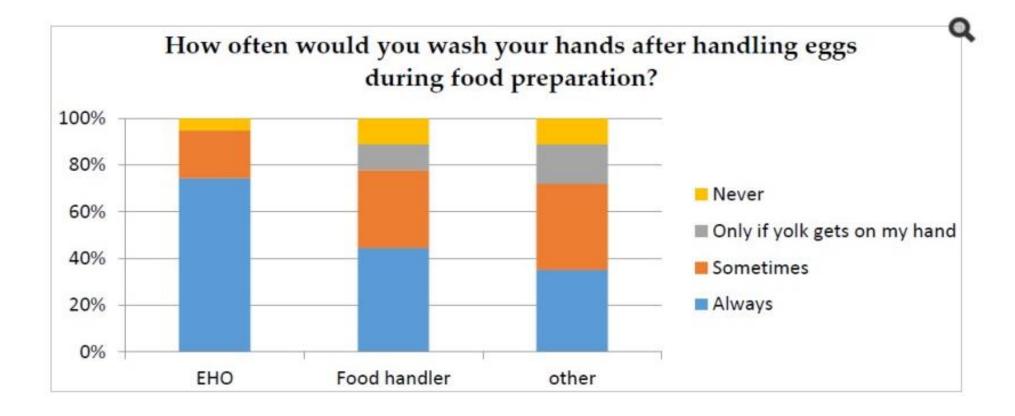
- Online survey promoted from different EH and farmers markets fb pages
- 294 participants promoted on social media
- Consumer preference
  - free range eggs 68.6%
  - keep poultry 10.7%
  - do not have any preference 7.1%
  - buy cage eggs 6.8%
  - barn eggs 4.0%
  - organic eggs 2.1%
  - pasteurised eggs 0.7%
- The overwhelming majority of participants (91%) reported they stored their eggs in the fridge





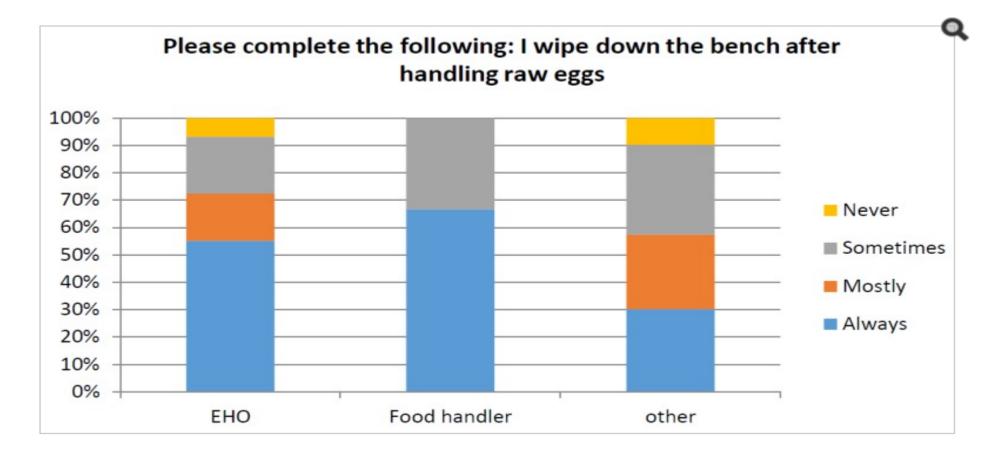
**Figure 1.** The percentages of participant responses for the questions "Do you consume raw eggs or raw egg products at home?" and "Have you ever eaten raw mixture/batter containing eggs (or licked bowl, spoon, spatula, etc.)?"





**Figure 2.** Demonstrates the percentage of responses for the question "How often would you wash your hands after handling eggs during food preparation?" for respondent who identified their profession as an "Environmental Health Officer (EHO)", Food handler and all other professions.





**Figure 3.** Demonstrates the percentage of responses for the question "Please complete the following: I wipe down the bench after handling raw eggs?" for respondent who identified their profession as an "Environmental Health Officer (EHO)", Food handler and all other professions.



#### **Storage temperature**

- The Australia New Zealand Food Standards Code (Standard 2.2.2—Eggs and egg products) states that although it is recommended that eggs are stored chilled, there is no legislative requirement as Australian intact eggs are unlikely to be internally infected with *Salmonella* (FSANZ 2016)
- These guidelines do not take into consideration the potential for *S*. Typhimurium to penetrate through the eggshell resulting in internal contamination (Whiley et al 2016, Gole et al 2014).

#### • Egg washing!!









#### **Storage temperature**

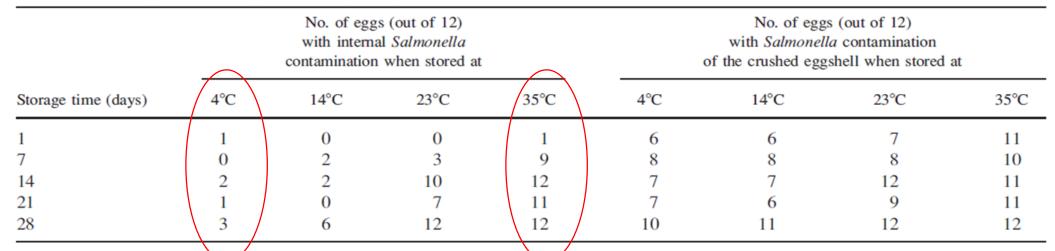


- Outside of egg shell, crushed egg shell, internal egg contents (yolk and albumin)
- Storage temps = 4°C, 14°C, 23°C and 35°C
- Days 1, 7, 14, 21, and 28



(Whiley et al 2016)

TABLE 1. Number of artificially contaminated, commercially washed free range eggs with Salmonella contamination of the internal egg or the crushed eggshell after different storage temperatures and times



 Chi-squared analysis demonstrated that the contamination of eggs at different storage temperatures was significantly different (P < 0.05).</li>

#### Take home message

Park et al (2015) showed greater survival (but not growth) of both *S*. Enteritidis, *S*. Typhimurium) in faeces when stored at low temperatures BUT

Salmonella penetration and internal contamination poses greatest public health risk therefore I recommend that you store at 4°C





# Influence of temperature on *S.* Typhimurium acid tolerance

#### Salmonella and raw egg mayonnaise – Australian Guidelines

- Adjust pH using lemon juice or vinegar so it is at or below 4.6
- Immediately refrigerate and store <5°C
- Make in and store in small batches





	Issue 27C - October 2013
	COMMUNICATION
	Food Safety and Nutrition Branch
	To: Food Businesses
	Title: Preparation of Raw Egg Products
1	Restaurants, cafes, bakeries, caterers and manufacturers that make raw egg products need to be aware of the associated risks of preparing raw egg products and ensure safe food handling practices are maintained at all times.
	Businesses should try to use alternatives to raw eggs in foods which are not cooked. Alternatives include commercially produced dressings and sauces, or pasteurised egg pulp.
	If businesses continue to use raw eggs in foods, then food safety controls must be adopted. There are many controls that can be implemented to help minimise the risk of harmful bacteria being in foods.
	Many food poisoning outbreaks have been associated with foods containing raw or partially cooked eggs, including:
:	Sauces and dressings such as mayonnaise, aioli, hollandaise and tartare sauces. Desserts made without a cooking step such as tiramisu, cheesecake (cold-set), meringue, mousse, custard (cold-set), fried ice-cream batter, some ice cream and gelati
	<ul> <li>made in-house.</li> <li>Drinks such as protein drinks containing raw egg.</li> </ul>
	Safer Alternatives Raw egg products made with pasteurised egg pulp are safer than those made from raw eggs as it eliminates the associated egg handling risks. Pasteurisation destroys harmful bacteria such as Salmonella. For foods that traditionally contain raw or lightly cooked eggs (e.g. mayonnaise, hollandaise sauce, mousse), use commercially prepared foods instead of making these foods from raw eggs.
	<ul> <li>Use pasteurised egg pulp instead of raw eggs as an ingredient in foods that traditionally contain raw or lightly cooked eggs.</li> </ul>
	Food Safety Tips Below are recommendations on how to prepare and store raw eggs products safely.
	<ol> <li>Process         <ul> <li>Ensure you use a standard recipe and process. It may be useful to have your proces and recipe checked by an expert food technologist.</li> <li>Use ingredients such as lemon juice or vinegar to acidify the raw egg product to a pi- value at or below 4.6, Acidifying raw egg products and storing them under temperature control is crucial for the safety of these products. This should be done</li> </ul> </li> </ol>

## What does the literature say?

- The effectiveness of pH as a control mechanism is influenced by many factors including:
  - The type of acid used, with the effectiveness of lemon juice compared with vinegar highly debated
  - Storage temperature
  - Its ability to develop acid tolerance
  - Fat content
  - NaCl content
  - Garlic content
  - Plant essential oils (PEOs) from mint, cinnamon, cardamom and clove (Keerthirathne et al 2016)



# Effect of pH on the <u>growth</u> of *Salmonella* in different stages of their growth curve

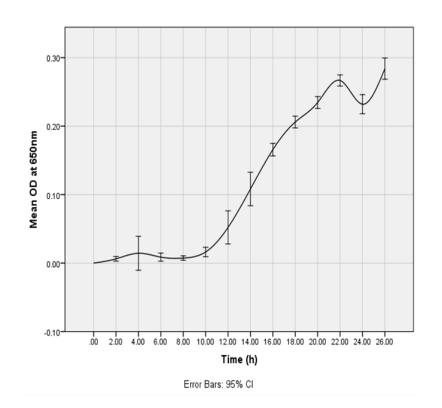


Fig. The growth curve of Salmonella Typhimurium ATCC 53647 in Nutrient Broth pH 7.0 at 37 °C





2hr, 12hr, 24hr and 48hr *S*. Typhimurium growth inoculum tested

#### Results

There was a significant bactericidal effect of pH 4.2, 4.4 and 4.6 on no *S*. Typhimurium growth was observed regardless of the inoculums growth stage

## Effect of pH and nutrient content on S. Typhimurium growth

- Effect of pH (4.2, 4.4 4.6 and 7 ) on *Salmonella* Typhimurium growth in broth with both low (peptone water) and high (nutrient both) nutrient content at 37°C (optimum growth temperature)
- No growth observed within one week except in pH 7 control
- Tested two different S. Typhimurium strains



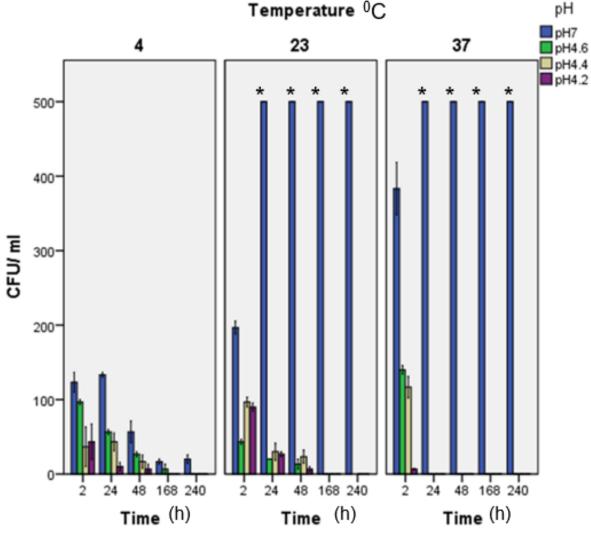




#### <u>Survival</u> of *Salmonella* under different pH and temperature conditions

pH 4.2 killed all the salmonella within 48 hrs at all storage temperatures

When pH <4.6 storing at higher temperature cause S. Typhimurium to die more quickly BUT If pH is not low enough (pH 7) then 4°C is best and higher temperatures actually promote growth



Error Bars: +/- 1 SE

Figure 3: S. Typhimurium survival over time in peptone water adjusted to pH 7, 4.6, 4.4 and 4.2 and incubated at 4°C, 23°C and 37°C. \*Indicates confluent growth which was reported as 500 CFU/mL. The limit of detection was 10 CFU/mL.



# Survival of S. Typhimurium over time at different pH and temperature conditions

- Still testing different variables (difference in nutrient concentrations, different strains, lemon juice vs vinegar)
- Potential for acid tolerance?

#### Take home messages...

- You need to be confirming the pH of your raw egg mayonnaise
- pH 4.2 killed all the salmonella within 48 hrs
- 4°C protected S. Typhimurium from pH
  - This has implication for cross contamination / importance of sanitation etc







## **Pasteurised eggs**

 Pasteurised eggs are great for vulnerable populations and for high risk foods BUT chefs don't like them as they don't think the quality is as good















## **Pasteurised eggs**

 NSW Food Authority, 'Food safety guidelines for the preparation of raw egg products' – recommends the use of sous-vide to 'pasteurise eggs' – reference a cooking book

Strain	Concentration	In/Out	Temperature	Time	Reference
Stram	CFU/ml	in your	٥C	Time	Reference
SE	Overnight culture	In	57	25 min	(Hou et al. <i>,</i> 1996)
-	-	-	57	75 min	(NSW Health department 2016)
SE	4 x 10 <sup>5</sup> - 4 x 10 <sup>7</sup>	In	56	20 min	(Stadelman et al., 1996)
ST	10 <sup>8</sup> (only a 2 log reduction was achieved)	In	57	15 min	(Shenga et al., 2010)
SE	8·5 log cfu in 50 ml	In	58	50 min - 57.5 min	(Schuman et al., 1997)
SE	10 <sup>9</sup>	Out	Boiling water	5s	(Gast, 1993)
SE	10 <sup>8</sup>	Out	Boiling water	3s	(Himathongkham et al., 1999)

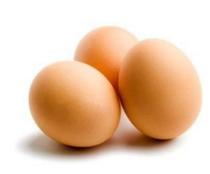
Experimentally-infected hens - average 220 CFU per egg (Schuman et al 1997)



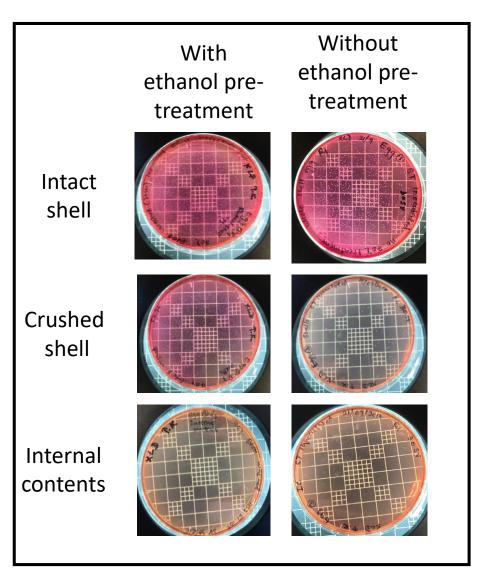
(Keerthirathne et al 2016)

Eggs

- Washed , Caged, Multi aged hens, Weight ~ 700g
- Chilled until it was brought to the lab and stored in the fridge until use
- Artificially contaminate with *S.* Typhimurium (with or without ethanol pre-treatment?)





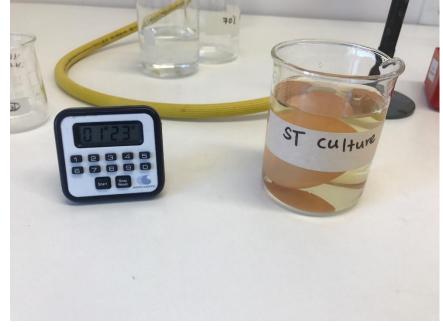


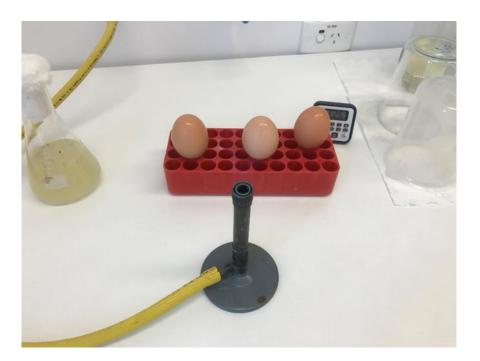


#### Artificial inoculation of ST on to the Egg shell

Immerse the egg in the ST culture 10<sup>5</sup> (90 sec)

Airdry









#### 57°C

Time (30sec, 1 min, 2min, 3min)

Two S. Typhimurium strains

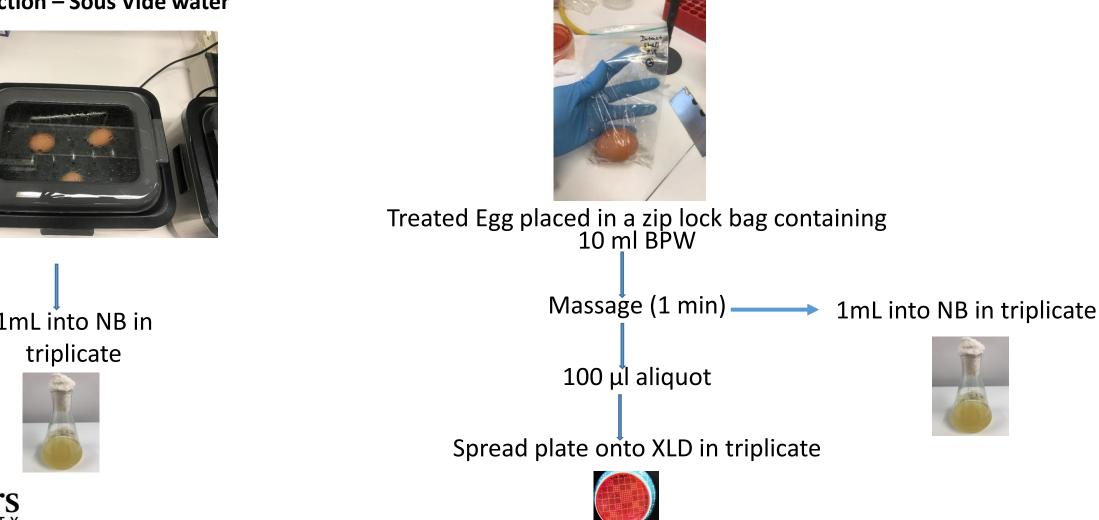
Three egg replicates

Experiments conducted in triplicate

Whole egg shell, crushed egg shell, internal contents and sous-vide water was tested



**Detection – Whole shell egg/egg shell surface** 

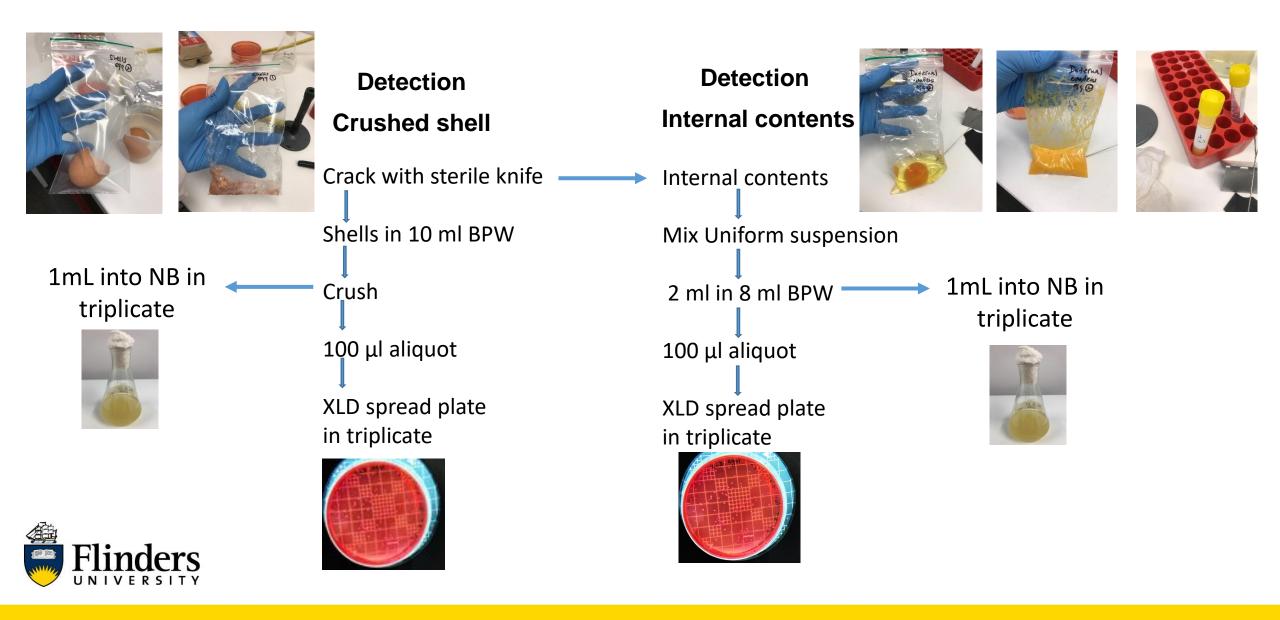


**Detection – Sous Vide water** 



1mL into NB in





## Sous-vide pasteurisation of eggs – initial results

#### Control

23°C water Sous-Vide pasteurisation (whole and crushed egg remain contaminated)

#### Sous-Vide water

At 57°C all samples are negative at all time points

#### **Plate results**

Comparable with broth results – but large variation between eggs – will need lots or replicates and bootstrapping to be able to examine statistical significance



Positive (green) and negative (red) broth cultures after Sous Vide pasteurisation at 57°C (1 experiment and 1 strain)



Egg quality parameter	Control eggs				Pasteurised eggs (57°C for 3 min)			
	1	2	3	Average	1	2	3	Average
Weight of the egg (g)	56.5	56.6	60.7	58.0	57.7	62.1	60.6	60.1
Weight of egg after pasteurisation (g)					57.7	62.1	60.6	60.1
Weight loss					0.02	0.02	0.02	0.02
Height of the egg white(mm)	6.4	6.7	7.3	6.8	6.2	7.3	7.6	7.0
Haugh unit	80.8	82.8	85.3	83.0	79.1	84.9	87.2	83.7
Albumen pH	9.01	9.07	9.03	9.0	9.1	9.1	8.9	9.0
OD at 600 nm against water / Thermocoagulation	0.01	0.01	0.02	0.01	0.02	0.03	0.06	0.04
Height of the yolk (mm)	22.9	16.5	21.8	20.4	16.9	18.7	15.1	17.0
Width of the yolk (mm)	42	43	45	43	44	50	44	46
Yolk index (Height of the yolk/ Width of the yolk)	0.5	0.4	0.5	0.5	0.4	0.4	0.3	0.4
Foam stability (mL)	27	30	43	33.3	27	30	30	29

#### Haugh Unit: industry measure of egg protein quality

Haugh Unit rating	Quality of eggs
90 and above	Excellent
80 - 89	Very good
70 – 79	Acceptable
65 - 69	Fair
60 - 64	Consumer resistance point
55 – 59	Poor
50 and below	Unacceptable



#### Next step

- Chefs acceptance (ethics granted will commence in the next couple of months)
  - Blind control asked to make mayonnaise using provided eggs and then rate the quality of the egg

- Washed vs unwashed
- Heat tolerance?





## Take home messages

- Different serovar of Salmonella enterica contaminating Australian eggs compared to the rest of the world
- Australians generally don't consider eggs as a risk when cooking in the home
- Store eggs in the fridge to prevent S. Typhimurium internal contamination
- Raw egg mayonnaise: adjusted pH to <4.6 then storing at 23°C and above is best BUT if pH not low enough then refrigeration is best and the higher temperatures will actually promote growth
- Sous-vide pasteurisation initial results look promising







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## Thank you

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