A Challenge testing study of custard and cream products with Bacillus cereus, Coagulase positive Staphylococci and Escherichia coli at ambient temperatures.

ABSTRACT

The objective of this project was to determine the growth of three microorganisms, Bacillus cereus, Coagulase positive Staphylococci and Escherichia coli in a variety of custard and cream based products at ambient temperatures over an eight hour time period. Various intrinsic factors such as water activity and pH were monitored to determine if they played a role in reducing or sustaining bacterial growth. This study found that over a four hour time period 13.0% of samples were found to sustain the growth of either Bacillus cereus, Coagulase positive Staphylococci or Escherichia coli and that over an eight hour time period 34.8% of samples sustained bacterial growth.

Introduction

Challenge testing "to the food microbiologist is the most direct evidence of product safety and stability." (2) This study focused on the ability of a product to sustain or inhibit the growth of various inoculated microorganisms under a defined set of conditions, the results of which can provide an overall picture of product safety.

When held at temperatures above 5°C or at less than 60°C the potential for bacterial growth may be substantially increased. The Food Safety Standard 3.2.2 (3) will permit potentially hazardous foods to be stored on display at a temperature other than at or below 5°C (or at 60°C and above) for a period of two to four hours. This study focused on both custard and cream filled products stored out of temperature control over an eight hour time period. Literature has shown that these products in their nature and through their processing have the ability to become contaminated and sustain potential pathogens. (1) A number of food borne outbreaks have been associated with the consumption of custard and cream filled products. (1) Three microorganisms were used in this study, Coagulase Positive Staphylococci a gram-positive bacterium, Escherichia coli a gram negative rod and Bacillus cereus a gram positive spore former. These bacteria were chosen for the challenge test as they represent a range of bacterial populations, may be inherent in the raw ingredients or could be introduced into these products via poor handling and manufacturing practices.
Materials and methods

Sampling

A variety of both custard and cream filled products were purchased commercially by Environmental Health Officers from within the Western Metropolitan Region. These products were sampled in a hygienic manner and transported to the Laboratory at room temperature to avoid condensation and possible water activity fluctuations. Upon arrival at the Laboratory, sample details were documented and each sample given a unique Laboratory number.

Preparation and addition of Inoculum

Fresh working broth cultures were inoculated onto selective and non-selective agars and incubated at 37°C for 24 hours. The purity of these organisms was verified prior to inoculation into the challenge products. The three bacterial strains used in the inoculation were DSS16 Coagulase positive Staphylococci (ATCC 11632) DSS2 Bacillus cereus (ATCC 10876) and DSS7 Escherichia coli (ATCC11775). Cotton swabs were used to transfer culture from selective agar into 10mls of sterile water which was made up to 1 MacFarland (using BioMerieux Mac Farland Standards) Then 0.1 ml of the solution was added and thoroughly mixed into the cream or custard portion of each product. One sample from each set of products was left uninoculated and used as a control.

Testing Regime

Sixteen samples of each product type were provided for testing and were inoculated as follows; five samples were inoculated with Coagulase positive Staphylococci, Bacillus cereus and Escherichia coli respectively with the sixteenth sample left uninoculated and used as a control. Melton Shire Council provided eight large custard tarts and cream filled sponges that were cut in half to make up a total of six-teen samples. One each of the samples inoculated with Coagulase positive Staphylococci, Bacillus cereus, Escherichia coli and the control were tested initially, at two, four, six and eight hours, for the relevant bacteria, pH and water activity. (where sufficient sample was available) The agars used for the detection of the Coagulase positive Staphylococci, Bacillus cereus and Escherichia coli were Baird Parker Agar, Bacillus Cereus Agar and Eoisin Methylen Blue Agar respectively.

Results

The following graphs represent the levels of each bacterium following inoculation over an eight hour time period for each product analysed. Also contained with each graph are the water activity and pH results obtained.
Graph 1: Graph of inoculated *B. cereus*, *E. coli* and Coagulase positive *Staphylococci* levels in an egg custard tart sample number 0129 (Maribyrnong City Council) over an eight hour time period.

Control: Water activity 0.94, pH 7.19
Sample: Water activity 0.94, pH 7.20
Temperature: 20ºC±1ºC

Observation over 4 hours

*B. cereus* – No significant changes noted over a four hour time period.
*E. coli* – Increase over four hour time period, however not considered significant.
Coagulase positive *Staphylococci* – No significant change noted over a four hour time period.

Observation over 8 hours

*B. cereus* – Levels decreased over an eight hour time period.
*E. coli* – Significant increase in bacterial levels over an eight hour time period.
Coagulase positive *Staphylococci* – No significant change noted over an eight hour time period

Controls

Levels of test organisms in control samples:

*B. cereus* <100 cfu/g
Coagulase positive *Staphylococci* <100 cfu/g
*E. coli* <100 cfu/g
Graph 2: Graph of inoculated *B. cereus*, *E. coli* and Coagulase positive *Staphylococci* levels in an egg custard tart sample number 0130 (Maribynong City Council) over an eight hour time period.

Control: Water activity 0.94, pH 6.52  
Sample: Water activity 0.94, pH 6.50  
Temperature: 20°C±1°C

**Observation over 4 hours**

*B. cereus* – Levels fluctuating over a four hour time period.  
*E.coli* - No significant change noted over a four hour time period.  
Coagulase positive *Staphylococci* – No significant change noted over a four hour time period.

**Observation over 8 hours**

*B. cereus* – Levels fluctuating over an eight hour time period.  
*E.coli* - Significant increase in bacterial levels over an eight hour time period.  
Coagulase positive *Staphylococci* – No significant change noted over an eight hour time period.

**Controls**

Levels of test organisms in control samples:

*B. cereus* <100 cfu/g  
Coagulase positive *Staphylococci* <100 cfu/g  
*E. coli* <100 cfu/g
Graph 3: Graph of inoculated *B. cereus*, *E. coli* and Coagulase positive *Staphylococci* levels in a custard tart sample number 0131 (Maribyrnong City Council) over an eight hour time period.

Control: Water activity 0.94, pH 6.38  
Sample: Water activity 0.94, pH 6.40  
Temperature: 20°C±1°C

Observation over 4 hours

*B. cereus* – Levels decreased over a four hour time period.  
*E. coli* - No significant change noted over a four hour time period.  
Coagulase positive *Staphylococci* – No significant change noted over a four hour time period.

Observation over 8 hours

*B. cereus* – Levels decreased over an eight hour time period.  
*E. coli* - Increase observed , however not considered significant.  
Coagulase positive *Staphylococci* – No significant change noted over an eight hour time period.

Controls

Levels of test organisms in control samples:

*B.cereus* <100cfu/g  
Coagulase positive *Staphylococci* <100 cfu/g  
*E.coli* <100cfu/g
Graph 4: Graph of inoculated *B. cereus*, *E. coli* and Coagulase positive *Staphylococci* levels in a custard danish sample number 0015 (Maribyrnong City Council) over an eight hour time period.

Control: Water activity 0.94, pH 6.68
Sample: Water activity 0.94, pH 6.70
Temperature: 20°C±1°C

**Observation over 4 hours**

*B. cereus* – No significant change noted over a four hour time period.
*E. coli* - No significant change noted over a four hour time period.
Coagulase positive *Staphylococci* – No significant change noted over a four hour time period.

**Observation over 8 hours**

*B. cereus* – No significant change noted over a six hour time period.
*E. coli* - Levels fluctuating over an eight hour time period.
Coagulase positive *Staphylococci* – No significant change noted over an eight hour time period.

**Controls**

Levels of test organisms in control samples:

*B.cereus* <100 cfu/g
Coagulase positive *Staphylococci* <100 cfu/g
*E.coli* <100 cfu/g
Graph 5: Graph of inoculated *B. cereus*, *E. coli* and Coagulase positive *Staphylococci* levels in a custard donut sample number 409 (Brimbank City Council) over an eight hour time period.

Control: Water activity 0.93, pH 6.42  
Sample: Water activity 0.93, pH 6.40  
Temperature: 20ºC±1ºC

Observation over 4 hours

*B. cereus* - Levels fluctuating over a four hour time period.  
*E. coli* – Increase observed, however not considered significant.  
Coagulase positive *Staphylococci* - No significant change observed over a four hour time period.

Observation over 8 hours

*B. cereus* - Levels fluctuating over an eight hour time period.  
*E. coli* – Levels fluctuating over an eight hour time period.  
Coagulase positive *Staphylococci* - Increase observed, however not considered significant.

Controls

Levels of test organisms in control samples:

*B. cereus* <100cfu/g  
Coagulase positive *Staphylococci* <100 cfu/g  
*E. coli* <100cfu/g
Graph 6: Graph of inoculated *B.cereus*, *E.coli* and Coagulase positive *Staphylococci* levels in a custard bun sample number 473 (Brimbank City Council) over an eight hour time period.

Control: Water activity 0.93, pH 6.44  
Sample: Water activity 0.93, pH 6.40  
Temperature: 20°C±1°C

Observation over 4 hours

*B. cereus* - Levels fluctuating over a four hour time period.  
*E.coli* – No significant change noted over a four hour time period.  
Coagulase positive *Staphylococci* – No significant change noted over a four hour time period.

Observation over 8 hours

*B. cereus* - Levels fluctuating over an eight hour time period.  
*E.coli* – No significant change noted over an eight hour time period.  
Coagulase positive *Staphylococci* – No significant change noted over an eight hour time period.

Controls

Levels of test organisms in control samples:

*B.cereus* <100 cfu/g  
Coagulase positive *Staphylococci* <100 cfu/g  
*E.coli* <100 cfu/g
Graph 7: Graph of inoculated *B.cereus*, *E.coli* and Coagulase positive *Staphylococci* levels in a jam and mocha cream stick sample number 457 (Brimbank City Council) over an eight hour time period.

Control: Water activity 0.93, pH 4.32
Sample: Water activity 0.93, pH 4.36
Temperature: 20°C±1°C

Observation over 4 hours

*B. cereus* - Bacterial levels decreased over a four hour time period.
*E.coli* - Levels fluctuating over a four hour time period.
Coagulase positive *Staphylococci* – Increase over four hour time period, however not considered significant.

Observation over 8 hours

*B. cereus* - Decrease in levels over an eight hour time period.
*E.coli* - No significant change noted over an eight hour period.
Coagulase positive *Staphylococci* – Levels fluctuating over an eight hour time period.

Controls

Levels of test organisms in control samples:

*B.cereus* <100cfu/g
Coagulase positive *Staphylococci* <100 cfu/g
*E.coli* <100cfu/g
Graph 8: Graph of inoculated *B. cereus*, *E. coli* and Coagulase positive *Staphylococci* levels in a profiteroles sample number 441 (Brimbank City Council) over an eight hour time period.

Control: Water activity 0.93, pH 4.19  
Sample: Water activity 0.93, pH 4.20  
Temperature: 20°C±1°C

![Graph of inoculated *B. cereus*, *E. coli* and Coagulase positive *Staphylococci* levels](image)

**Observation over 4 hours**

*B. cereus* - No significant change noted over a four hour time period.  
*E. coli* - No significant change noted over a four hour time period.  
Coagulase positive *Staphylococci* - No significant change noted over a four hour time period.

**Observation over 8 hours**

*B. cereus* - No significant change noted over an eight hour time period.  
*E. coli* - No significant change noted over an eight hour time period.  
Coagulase positive *Staphylococci* - No significant change noted over an eight hour time period.

**Controls**

Levels of test organisms in control samples:

*B. cereus* <100 cfu/g  
Coagulase positive *Staphylococci* <100 cfu/g  
*E. coli* <100 cfu/g
Graph 9: Graph of inoculated *B. cereus*, *E. coli* and Coagulase positive *Staphylococci* levels in a cream filled sponge sample number 425 (Brimbank City Council) over an eight hour time period.

Control: Water activity 0.90, pH 6.54  
Sample: Water activity 0.90, pH 6.13  
Temperature: 20°C±1°C

**Observation over 4 hours**

*B. cereus* - No significant change noted over a four hour time period.  
*E. coli* - Levels fluctuating over a four hour time period.  
Coagulase positive *Staphylococci* - Levels fluctuating over a four hour time period.

**Observation over 8 hours**

*B. cereus* - No significant change noted over an eight hour time period.  
*E. coli* - Levels fluctuating over an eight hour time period.  
Coagulase positive *Staphylococci* - Levels fluctuating over an eight hour time period.

**Controls**

Levels of test organisms in control samples:

*B. cereus* <100 cfu/g  
Coagulase positive *Staphylococci* <100 cfu/g  
*E. coli* <100 cfu/g
Graph 10: Graph of inoculated *B. cereus*, *E. coli* and Coagulase positive *Staphylococci* levels in a Choc filled imitation cream sponge sample number 630 (Moonee Valley City Council) over an eight hour time period.

Control: Water activity 0.89, pH 6.52  
Sample: Water activity 0.89, pH 6.50  
Temperature: 20ºC±1ºC

Observation over 4 hours

*B. cereus* - No significant change noted over a four hour time period.  
*E. coli* - No significant change noted over a four hour time period.  
Coagulase positive *Staphylococci* - No significant change noted over a four hour time period.

Observation over 8 hours

*B. cereus* - No significant change noted over an eight hour time period.  
*E. coli* - No significant change noted over an eight hour time period.  
Coagulase positive *Staphylococci* - No significant change noted over an eight hour time period.

Controls

Levels of test organisms in control samples:

*B. cereus* <100 cfu/g  
Coagulase positive *Staphylococci* <100 cfu/g  
*E. coli* <100 cfu/g
Graph 11: Graph of inoculated *B. cereus*, *E. coli* and Coagulase positive *Staphylococci* levels in a vanilla slice sample number 631 (Moonee Valley City Council) over an eight hour time period.

Control: Water activity 0.93, pH 7.22  
Sample: Water activity 0.92, pH 7.16  
Temperature: 20°C±1°C

**Observation over 4 hours**

*B. cereus* - Levels fluctuating over a four hour time period.  
*E. coli* - Levels fluctuating over a four hour time period.  
Coagulase positive *Staphylococci* - No significant change noted over a four hour time period.

**Observation over 8 hours**

*B. cereus* - Levels fluctuating over an eight hour time period.  
*E. coli* - Levels fluctuating over an eight hour time period.  
Coagulase positive *Staphylococci* - No significant change noted over an eight hour time period.

**Controls**

Levels of test organisms in control samples:  

*B. cereus* <100cfu/g  
Coagulase positive *Staphylococci* detected in the sample  
*E. coli* detected in the sample
Graph 12: Graph of inoculated *B. cereus*, *E. coli* and Coagulase positive *Staphylococci* levels in a vanilla slice sample number 632 (Moonee Valley City Council) over an eight hour time period.

Control: Water activity 0.95, pH 6.17  
Sample: Water activity 0.95, pH 6.28  
Temperature: 20°C±1°C

Observation over 4 hours

*B. cereus* - No significant change noted over a four hour time period.  
*E. coli* - Levels fluctuating over a four hour time period.  
Coagulase positive *Staphylococci* - Levels fluctuating over a four hour time period.

Observation over 8 hours

*B. cereus* - Ten fold increase over an 8 hour time period from 18000 cfu/g to 150,000 cfu/g.  
*E. coli* - Levels fluctuating over an eight hour time period.  
Coagulase positive *Staphylococci* - Levels fluctuating over an eight hour time period.

Controls

Levels of test organisms in control samples:

*B. cereus* <100cfu/g  
Coagulase positive *Staphylococci* <100 cfu/g  
*E. coli* <100cfu/g
Graph 13: Graph of inoculated *B. cereus*, *E. coli* and Coagulase positive *Staphylococci* levels in a cream filled sponge sample number 633 (Moonee Valley City Council) over an eight hour time period.

Control: Water activity 0.93, pH 7.15  
Sample: Water activity 0.92, pH 7.10  
Temperature: 20°C±1°C

Observation over 4 hours

*B. cereus* - Ten fold increase over a four hour time period from 1700 cfu/g to 15,000 cfu/g.  
*E. coli* - Levels fluctuating over four hour time period.  
Coagulase positive *Staphylococci* - Levels fluctuating over a four hour time period.

Observation over 8 hours

*B. cereus* - Significant increase over an eight hour time period from 1700 cfu/g to 17,000 cfu/g.  
*E. coli* - Levels fluctuating over an eight hour time period.  
Coagulase positive *Staphylococci* - Levels fluctuating over an eight hour time period.

Controls

Levels of test organisms in control samples:

*B. cereus* <100cfu/g  
Coagulase positive *Staphylococci* <100 cfu/g  
*E. coli* <100cfu/g
Graph 14: Graph of inoculated *B. cereus*, *E. coli* and Coagulase positive *Staphylococci* levels in a custard tart sample number 694 (Melton City Council) over an eight hour time period.

Control: Water activity 0.92, pH 6.15
Sample: Water activity 0.92, pH 6.20
Temperature: 20ºC±1ºC

**Melton Laboratory Job number 694**

**Observation over 4 hours**

*B. cereus* - Levels fluctuating over a four hour time period.
*E. coli* - Levels fluctuating over a four hour time period.
Coagulase positive *Staphylococci* - No significant change noted over a four hour time period.

**Observation over 8 hours**

*B. cereus* - Levels fluctuating over an eight hour time period.
*E. coli* - Levels fluctuating over an eight hour time period.
Coagulase positive *Staphylococci* - No significant change noted over an eight hour time period.

**Controls**

Levels of test organisms in control samples:

*B. cereus* <100 cfu/g
Coagulase positive *Staphylococci* <100 cfu/g
*E. coli* <100 cfu/g
Graph 15: Graph of inoculated B.cereus, E.coli and Coagulase positive Staphylococci levels in a large custard tart sample number 695 (Melton City Council) over an eight hour time period.

Control: Water activity 0.92, pH 6.15  
Sample: Water activity 0.92, pH 6.10  
Temperature: 16±1°C

Observation over 4 hours

B. cereus – No significant change noted over a four hour time period.  
E.coli - No significant change noted over a four hour time period.  
Coagulase positive Staphylococci - No significant change noted over a four hour time period.

Observation over 8 hours

B. cereus – No significant change noted over an eight hour time period.  
E.coli - No significant change noted over an eight hour time period.  
Coagulase positive Staphylococci - Fluctuating levels over an eight hour time period.

Controls

Levels of test organisms in control samples:

B.cereus <100cfu/g  
Coagulase positive Staphylococci <100 cfu/g  
E.coli <100cfu/g
Graph 16: Graph of inoculated *B.cereus*, *E.coli* and Coagulase positive *Staphylococci* levels in a large mooch cream filled sponges sample number 696 (Melton City Council) over an eight hour time period.

Control: Water activity ~0.92, pH 5.42  
Sample: Water activity ~0.92, pH 5.40  
Temperature: 16±1°C

Observation over 4 hours

*B. cereus* - No significant change noted over a four hour time period.  
*E.coli* - Fluctuating levels over a four hour time period.  
Coagulase positive *Staphylococci* - No significant change noted over a four hour time period.

Observation over 8 hours

*B. cereus* - No significant change noted over an eight hour period.  
*E.coli* - Fluctuating levels over an eight hour time period.  
Coagulase positive *Staphylococci* - No significant change noted over an eight hour time period.

Controls

Levels of test organisms in control samples:

*B.cereus* <100 cfu/g  
Coagulase positive *Staphylococci* <100 cfu/g  
*E.coli* <100 cfu/g
Graph 17: Graph of inoculated *B.cereus*, *E.coli* and Coagulase positive *Staphylococci* levels in a custard tart sample number (Melbourne City Council) over an eight hour time period.

Control: Water activity 0.92, pH 6.30
Sample: Water activity 0.92, pH 6.22
Temperature: 10°C±1°C

### Observation over 4 hours

*B. cereus* – Significant increase in bacterial levels observed over a four hour time period from 1200 cfu/g to 15000 cfu/g over a 4 hour time period.

*E.coli* - No significant change noted over a four hour time period.

Coagulase positive *Staphylococci* - No significant change noted over a four hour period

### Observation over 8 hours

*B. cereus* – Significant increase in bacterial levels was observed over an eight hour time period.

*E.coli* - Significant increase in bacterial observed Ten fold increase over an 8 hour time period from 121000 to >2.5 million cfu/g.

Coagulase positive *Staphylococci* - No significant change noted over an eight hour time period.

### Controls

Levels of test organisms in control samples:

*B.cereus* <100 cfu/g
Coagulase positive *Staphylococci* <100 cfu/g
*E.coli* <100 cfu/g
Graph 18: Graph of inoculated *B. cereus*, *E. coli* and Coagulase positive *Staphylococci* levels in a vanilla slice sample number 726 (Melbourne City Council) over an eight hour time period.

Control: Water activity 0.92, pH 6.68  
Sample: Water activity 0.92, ~pH 6.68  
Temperature: 10°C±1°C

**Observation over 4 hours**

*B. cereus* – Increase in bacterial levels was observed, however not considered significant.  
*E. coli* – Significant increase in bacterial levels were observed over a four hour time period.  
Coagulase positive *Staphylococci* - Increase in bacterial levels was observed, however not considered significant.

**Observation over 8 hours**

*B. cereus* - Although fluctuating levels were observed over an eight hour time period, a significant bacterial increase was observed.  
*E. coli* – Increase in bacterial levels was observed, however not considered significant.  
Coagulase positive *Staphylococci* - Increase in bacterial levels was observed, however not considered significant.

**Controls**

Levels of test organisms in control samples:

*B. cereus* <100cfu/g  
Coagulase positive *Staphylococci* <100 cfu/g  
*E. coli* <100cfu/g
Graph 19: Graph of inoculated *B.cereus*, *E.coli* and Coagulase positive *Staphylococci* levels in a custard tart sample number 727 (Melbourne City Council) over an eight hour time period.

Control: Water activity 0.92, pH 5.49  
Sample: Water activity 0.92, pH 5.42  
Temperature: 10°C ± 1°C

Observation over 4 hours

*B. cereus* – No significant change noted over a four hour period  
*E.coli* - No significant change noted over a four hour period  
Coagulase positive *Staphylococci* - No significant change noted over a four hour period

Observation over 8 hours

*B. cereus* – No significant change noted over an eight hour period  
*E.coli* - Increase in bacterila levels observed however not considered significant increase  
Coagulase positive *Staphylococci* - No significant change noted over an eight hour period

Controls

Levels of test organisms in control samples:

*B.cereus* <100cfu/g  
Coagulase positive *Staphylococci* <100 cfu/g  
*E.coli* <100cfu/g
Graph 20: Graph of inoculated *B. cereus*, *E. coli* and Coagulase positive *Staphylococci* levels in a vanilla slice sample number 728 (Melbourne City Council) over an eight hour time period.  

Control: Water activity 0.92, pH 6.42  
Sample: Water activity 0.92, pH 6.42  
Temperature: -10°C +/- 1°C  

Observation over 4 hours  

*B. cereus* – No significant change noted over a four hour period  
*E. coli* – Increase in bacterial levels observed however not considered significant  
Coagulase positive *Staphylococci* - No significant change noted over a four hour period  

Observation over 8 hours  

*B. cereus* – No significant change noted over a 8 hour period  
*E. coli* – Slight increase, however not a ten fold increase  
Coagulase positive *Staphylococci* - Slight increase, however not a ten fold increase  

Controls  

Levels of test organisms in control samples:  

*B. cereus* <100cfu/g  
Coagulase positive *Staphylococci* <100 cfu/g  
*E. coli* <100cfu/g
Graph 21: Graph of inoculated *B. cereus*, *E. coli* and Coagulase positive *Staphylococci* levels in a custard tart sample number 214/2001 (City of Greater Geelong) over an eight hour time period.

Control: Water activity 0.92, pH 6.59
Sample: Water activity 0.92, pH 6.53
Temperature: 20ºC±1ºC

**Observation over 4 hours**

*B. cereus* - - No significant change noted over a four hour time period
*E. coli* - Fluctuating levels over a four hour time period.
Coagulase positive *Staphylococci* - No significant change noted over a four hour time period

**Observation over 8 hours**

*B. cereus* - No significant change noted over an eight hour time period
*E. coli* - Fluctuating levels over an eight hour time period.
Coagulase positive *Staphylococci* - No significant change noted over an eight hour time period

**Controls**

Levels of test organisms in control samples:

*B. cereus* <100cfu/g
Coagulase positive *Staphylococci* <100 cfu/g
*E. coli* <100cfu/g
Graph 22: Graph of inoculated *B.cereus*, *E.coli* and Coagulase positive *Staphylococci* levels in a custard tart sample number 215/2001 (City of Greater Geelong) over an eight hour time period.

Control: Water activity 0.93, pH 6.41
Sample: Water activity 0.93, pH 6.42
Temperature: 20°C±1°C

Observation over 4 hours

*B. cereus* - No significant change noted over a four hour time period.
*E.coli* - No significant change noted over a four hour time period.
Coagulase positive *Staphylococci* - No significant change noted over a four hour time period.

Observation over 8 hours

*B. cereus* - No significant change noted over an eight hour time period.
*E.coli* - No significant change noted over an eight hour time period
Coagulase positive *Staphylococci* - Significant increase in bacterial levels over an eight hour time period.

Controls

Levels of test organisms in control samples:

*B.cereus* <100cfu/g
Coagulase positive *Staphylococci* <100 cfu/g
*E.coli* <100cfu/g
Graph 23: Graph of inoculated *B.cereus*, *E.coli* and Coagulase positive *Staphylococci* levels in a custard tart sample number 216/2001 (City of Greater Geelong) over an eight hour time period.

Control: Water activity 0.92, pH 6.59
Sample: Water activity 0.92, pH 6.40
Temperature: 20°C±1°C

**Observation over 4 hours**

*B. cereus* - No significant change over a four hour time period.
*E.coli* - Increase in bacterial levels noted, however not considered significant.
Coagulase positive *Staphylococci* - No significant change over a 4 hour time period.

**Observation over 8 hours**

*B. cereus* - No significant change over an eight hour time period.
*E.coli* - No significant change over an eight hour time period.
Coagulase positive *Staphylococci* - No significant change over an eight hour time period.

**Controls**

Levels of test organisms in control samples:

*B.cereus* <100 cfu/g
Coagulase positive *Staphylococci* <100 cfu/g
*E.coli* <100 cfu/g
Result-Summary

In total 23 custard and cream filled products were analysed using three different bacteria. Table 1 is a representation of the product types and numbers of each analysed.

**Table 1 : Product type and quantity challenged**

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>NUMBERS ANALYSED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Custard Tarts</td>
<td>8</td>
</tr>
<tr>
<td>Mock Cream Filled</td>
<td>5</td>
</tr>
<tr>
<td>Vanilla Slices</td>
<td>4</td>
</tr>
<tr>
<td>Egg Custard Tarts</td>
<td>2</td>
</tr>
<tr>
<td>Custard Danish</td>
<td>1</td>
</tr>
<tr>
<td>Custard Bun</td>
<td>1</td>
</tr>
<tr>
<td>Custard Donut</td>
<td>1</td>
</tr>
<tr>
<td>Profiteroles</td>
<td>1</td>
</tr>
</tbody>
</table>

Total number of samples 23
Custard filled 18
Mock Cream filled 5

In total 18 custard filled and 5 mock cream filled products were analysed.

For the purpose of data analysis, the products analysed have been divided into cream and custard filled categories.

**Cream Filled Products**

A smaller proportion of cream filled products were analysed, compared to custard filled, however one in five samples (20%) were found to support the growth of one challenge organism.(Refer to Table 2)

**Table 2 - Table of Cream Filled Product with a 10 fold increase – Bacillus cereus**

<table>
<thead>
<tr>
<th>Product</th>
<th>Initial</th>
<th>Results Over Time</th>
<th>Aw</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>2hr</td>
<td>4hr</td>
<td>8hr</td>
</tr>
<tr>
<td>Mock cream filled sponge</td>
<td>1700 cfu/g</td>
<td>10 fold increase</td>
<td>10 fold increase</td>
<td>10 fold increase</td>
</tr>
<tr>
<td>(Graph 13)</td>
<td>16000 cfu/g</td>
<td>16000 cfu/g</td>
<td>15000 cfu/g</td>
<td>17000 cfu/g</td>
</tr>
</tbody>
</table>

The neutral pH detected for this sample may have contributed to the ability of *Bacillus cereus* to multiply.
The four remaining cream filled samples did not show increased growth over the 2hr, 4hr and 8hr time period. This was possibly due to their low pH of 4.32 to 5.42, or reduced water activity of 0.89 or 0.90.

**Custard Filled Products**

A total of six custard filled samples or 33.33% were found to support the growth of one or more of the challenge organisms, as detailed in Tables 3, 4 and 5.

**Table 3 – Table of Custard Filled Products with a 10 fold increase in E. coli**

<table>
<thead>
<tr>
<th>Product</th>
<th>Results Over Time</th>
<th>Aw</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>2hr</td>
<td>4hr</td>
</tr>
<tr>
<td>Egg Custard Tart (Graph 1)</td>
<td>54000cfu/g</td>
<td>No significant (33000cfu/g)</td>
<td>Slight Increase</td>
</tr>
<tr>
<td>Egg Custard Tart (Graph 2)</td>
<td>46000cfu/g</td>
<td>No significant 52000cfu/g</td>
<td>Not significant 98000cfu/g</td>
</tr>
<tr>
<td>Custard Tart (Graph 17)</td>
<td>120000cfu/g</td>
<td>Fluctuating 57000cfu/g</td>
<td>Not significant 170000cfu/g</td>
</tr>
<tr>
<td>Vanilla Slice (Graph 18)</td>
<td>35000cfu/g</td>
<td>No significant 190000cfu/g</td>
<td>10 fold increase 400000cfu/g</td>
</tr>
</tbody>
</table>

The high water activity and a reasonably neutral pH may contribute to the ability of E. coli to multiply in these products.

Literature has shown that for E. coli the growth limits are a water activity of 0.95 and pH optimum of 6.7 (range minimum pH 4.4 maximum pH 9.0) (1).

**Table 4 – Table of Custard Filled Product with a 10 fold increase in Coagulase Positive Staphylococci**

<table>
<thead>
<tr>
<th>Product</th>
<th>Results Over Time</th>
<th>Aw</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>2hr</td>
<td>4hr</td>
</tr>
<tr>
<td>Custard Tart (Graph 22)</td>
<td>100000cfu/g</td>
<td>No significant change 370000cfu/g</td>
<td>No significant change 110000cfu/g</td>
</tr>
</tbody>
</table>

Once again the higher water activity and near neutral pH may have contributed to the growth of Coagulase Positive Staphylococci observed over time in this product.
Growth limits of water activity and pH for Coagulase Positive *Staphylococci* (1) are water activity 0.99 – 0.83 pH minimum 4.2 and maximum 9.3 (optimum 7.0 – 7.5).

### Table 5 – Table of Custard Filled Product with 10 fold increase for *Bacillus cereus*

<table>
<thead>
<tr>
<th>Product</th>
<th>Initial</th>
<th>2hr</th>
<th>4hr</th>
<th>8hr</th>
<th>Aw</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanilla Slice</td>
<td>18000cfu/g</td>
<td>No sig</td>
<td>No sig</td>
<td>10 fold increase</td>
<td>0.95</td>
<td>6.28</td>
</tr>
<tr>
<td>(Graph 12)</td>
<td></td>
<td>change</td>
<td>change</td>
<td>150000cfu/g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Custard Tart</td>
<td>1200cfu/g</td>
<td>No sig</td>
<td>10 fold</td>
<td>10 fold increase</td>
<td>0.92</td>
<td>6.22</td>
</tr>
<tr>
<td>(Graph 17)</td>
<td></td>
<td>change</td>
<td>increase</td>
<td>13000cfu/g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vanilla Slice</td>
<td>800cfu/g</td>
<td>Slight</td>
<td>Slight</td>
<td>10 fold increase</td>
<td>0.92</td>
<td>6.68</td>
</tr>
<tr>
<td>(Graph 18)</td>
<td></td>
<td>increase</td>
<td>increase</td>
<td>7000cfu/g</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The high water activity detected of 0.95 (Graph 12 sample - Vanilla slice) may have contributed to the growth of this organism. Growth limits of water activity and pH for *Bacillus cereus* (1) are 0.92 to 0.95 and 4.3 to 9.3 respectively (optimum pH 6 to 7).

Although not a parameter analysed in this study, sugar content may have been a contributing factor to the growth observed or not observed in certain products.

Those products that have shown fluctuating results may be due to inadequate mixing of the inoculum, which was difficult in some product types or due to pockets of bacterial growth within the food products.

Statistically, this study only provides a glimpse into the potential risk of these products when kept out of temperature control.

Although this study was performed only on those products intended to be held at room temperature further investigation may focus on the growth of these organisms in these products at or below 5°C and subsequently a comparison made.
Recommendations

1. It has been shown that the various intrinsic factors such as water activity and pH may contribute to the ability of these organisms to significantly change in number and as such a reduced pH and water activity is recommended.

2. In a number of products although inoculated bacterial levels remained constant, if gross contamination did occur in practice, the levels in most cases would be maintained over the eight-hour period thus being a potential hazard for the consumer. Therefore if poor manufacturing practices are suspected sampling is recommended as these products could pose a health risk.

3. "The 4hour /two hour rule (Safe Food Australia)
   Any ready-to-eat potentially hazardous food, if it has been at temperatures between 5C and 60C:
   • for a total of less than two hours, must be refrigerated or used immediately
   • for a total of longer than two hours but less than four hours, must be used immediately; or
   • for a total of four hours or longer, must be thrown out." (3)

   Our study found that these products should be:
   - Safe after 2hrs
   - Discarded after 4hrs (as per the 2/4 hrs rule)
   - Extensive verification would be required by proprietors if they want to keep products outside this time frame, as after 8hrs some products in our study did show a ten fold increase in bacterial levels and would be considered unsatisfactory outside of temperature control over this time period
   - However, it must be noted that other samples that had a lower pH and water activity were found to be satisfactory over an eight hour time period.

4. Further investigation is recommended into those products that did sustain bacterial growth as to their safety at ambient temperatures.

Acknowledgments

We would like to thank all participating councils and Food Safety Victoria for their assistance.
References

3. Safe Food Australia, 2001, ANZFA