



## DETECTION AND ENUMERATION OF FOODBORNE PATHOGENS IN GENOISE CAKES

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### ABSTRACT

**Aim:** The present study was undertaken with the aim to detect and enumerate foodborne pathogens in Genoise cake, a particularly high-risk bakery product that is most preferred and consumed by Mauritians. **Methods:** A consumer survey was carried out to determine the type of bakery products most consumed by Mauritians and the buying point mostly attended by Mauritians to purchase bakery products. A total of 50 Genoise cakes were collected from bakeries from 5 selected regions in Mauritius. The samples were then examined for microbial contamination post storage at room temperature and 4°C for 1 hour. A two-way ANOVA was carried out to statistically analyse the data. **Results:** *S.aureus*, *B.cereus*, yeasts and moulds were present in all the samples while *Salmonella* was not detected in any of the samples. Coliforms were detected in 96% of the samples. 88% and 84% of the samples contained *L.monocytogenes* and *E.coli* respectively and 76% of samples contained *Campylobacter* while *C.perfringens* was present in 62% of the samples. The microbial load for most of the samples was marginal to the recommended level with samples from the North and Central regions having unsatisfactory TVC levels. The 2 storage temperatures were also found to have significant effect on the growth of *S.aureus* and *B.cereus* while no differences were found in the growth of coliforms and *E.coli* at the 2 different temperatures. **Conclusion:** The findings indicate that Genoise cakes are potentially high-risk bakery products and that the 5 selected bakeries do not follow and/or apply proper hygienic practices.

### KEY WORDS

Bakery products, foodborne pathogens, Genoise cakes, microbial spoilage

### 1. INTRODUCTION

Bakery products form part of the processed food category and include bread, cream-filled pastries and cakes and other savoury and sweet products. Consumer demand for bakery products is increasing because of their convenience that helps to keep up with daily hectic routines [1].

Despite being an important part of food expenditure, bakery products can be unsafe to public health. During production and distribution, bakery products are often subjected to chemical, physical and microbiological spoilage, the latter being of utmost concern. If stored under favourable conditions, bakery products,

especially those with high moisture content, can harbour a plethora of pathogenic bacteria, yeasts and moulds and hence, often serve as food vehicles that transfer pathogenic organisms such as *Salmonella*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus* spp. to consumers (Pubs.sciepub.com).

Freshly baked products are usually sterile but they are contaminated as soon as they are passed through the series of steps involved in production, processing, handling, distribution and packaging [1]. Cream-filled bakery products are often contaminated by the addition of toppings or fillings such as fresh cream or cold custard, post baking. Coliforms, *Escherichia coli*,

*Staphylococcus aureus* and *Salmonella* are usually the predominating contaminants in cream-filled bakery products [2]. Other foodborne pathogens include *Listeria monocytogenes*, *Campylobacter* spp., *Bacillus cereus*, moulds and yeasts.

Given the high level of consumption of bakery products and the high-risk of intoxication associated with the consumption of these products, this study was undertaken with a primary aim to detect and enumerate foodborne pathogens in a particular bakery product.

## 2. MATERIALS AND METHODS

### 2.1: Consumer survey

A consumer survey was carried out to determine the type of bakery products most consumed by Mauritians and to obtain an idea about the buying point mostly attended by Mauritians to purchase bakery products. The survey was carried out among a population of 50 people and targeted Mauritians of above 15 years of age living in all 5 regions across the island (i.e. Northern, Southern, Eastern, Western and Central regions). The Software Package for the Social Science (SPSS) was used to collate and analyse the responses.

### 2.2: Microbiological analysis

#### 2.2.1: Sample selection and collection

The samples were collected from buying points found in the 5 regions of Mauritius. The samples and buying points were selected based upon the responses obtained from the survey. 10 samples were collected from each region, of which 5 were stored at room temperature for 1 hour and the remaining 5 at 4°C for 1 hour.

#### 2.2.2: Sample processing

25g of each sample was weighed and placed in a sterile stomacher bag. The content was blended in 225 ml of Maximum Recovery Diluent (MRD) to produce a homogenous mother sample (MS). 1ml of the MS was serially diluted in 9ml of MRD to obtain a 10-fold dilution. The MS was diluted to a highest dilution of  $10^{-6}$ .

### 2.2.3: Detection of foodborne pathogens

#### 2.2.3.1: Detection of *Salmonella*

1ml from the MS was inoculated in Selenite cystine broth. The tubes were incubated at 37°C for 24 hours. A loopful from the enrichment broth was streaked onto XLD agar. The plates were incubated at 37°C for 24 hours.

#### 2.2.3.2: Detection of *Listeria monocytogenes*

1ml from the MS was inoculated in Fraser broth. The tubes were incubated at 37°C for 24 hours. A loopful from the enrichment broth was streaked onto PALCAM agar. The plates were incubated at 37°C for 24 hours under micro-aerophilic conditions.

#### 2.2.3.3: Detection of *Clostridium perfringens*

1ml from the MS was inoculated in Cooked Meat medium. The tubes were incubated at 37°C for 24 hours. A loopful from the enrichment broth was streaked onto blood agar. The plates were anaerobically incubated at 37°C for 24 hours.

#### 2.2.3.4: Detection of *Campylobacter*

1ml from the MS was inoculated in Bolton broth. The tubes were incubated at 37°C for 24 hours. A loopful from the enrichment broth was streaked onto *Campylobacter* blood-free selective agar. The plates were incubated at 37°C for 24 hours in a microaerobic gaseous atmosphere.

#### 2.2.3.5: Detection of yeast and moulds

A loopful from the MS was streaked onto Sabouraud Dextrose Agar and the plates were incubated at 37°C for 24 hours.

### 2.2.4: Enumeration of foodborne pathogens

#### 2.2.4.1: Total viable count

The pour plate method was used to perform the Total Viable Count. The colony count was determined according to International Organization for Standardization (ISO) standard methods for the enumeration of microorganisms (ISO 4833:1991):

$$N = \sum c / [(n_1 + 0.1n_2) * d]$$

N: cfu/g

$\sum c$ : sum of all colonies counted ranging between 15-300 colonies

$n_1$  and  $n_2$ : number of plates retained for 1<sup>st</sup> and 2<sup>nd</sup> dilution respectively

d: dilution corresponding to the 1<sup>st</sup> dilution counted

#### 2.2.4.2: Miles and Misra method

A sterile calibrated pipette was used to place 0.02ml drops from the serial dilution factors  $10^{-5}$  and  $10^{-6}$  on the selective media Baird Parker and Brilliance *Bacillus cereus* agar. The colony count was determined according to the Miles and Misra method:

$$N = n / [0.02 * d]$$

N: cfu/g

n: number of colonies counted

d: dilution factor used

### 2.2.4.3: Most Probable Number

**Presumptive test:** 1ml samples of the dilutions  $10^{-1}$ ,  $10^{-2}$  and  $10^{-3}$  were inoculated in triplicate Brilliant Green Bile broth tubes. The tubes were examined for turbidity and gas formation after incubation at 44°C for 48 hours. The results were interpreted according to the MPN table adapted from [9].

**Confirmative test:** A loopful from positive tubes for total coliforms from the presumptive test was inoculated in test tubes containing Tryptone water. After a 24 hour incubation at 44°C, 2-3 drops of Kovac's Indole reagent were added to confirm the presence of *E.coli*. The results were interpreted according to the MPN table adapted from [9].

**Completed test:** A loopful from the Indole positive tubes was inoculated on Sorbitol MacConkey agar and the plates were incubated at 35°C for 24 hours.

### 2.2.5: Confirmatory tests

#### 2.2.5.1: Biochemical tests

##### 2.2.5.1.1: Catalase test

A drop of  $H_2O_2$  was added to a small amount of fresh isolates for each pathogen. Bubbling was observed for catalase-positive pathogens.

##### 2.2.5.1.2: Oxidase test

The colony to be tested was smeared on a filter paper soaked with tetramethyl-p-phenylenediamine dihydrochloride. A colour change to deep blue or purple was observed for oxidase-positive pathogens.

##### 2.2.5.1.3: Coagulase test

A drop of rabbit plasma was added to an emulsified smeared of fresh isolate of *S.aureus*. Agglutination was observed for coagulase-positive *S.aureus*.

### 2.2.5.2: Staining methods

#### 2.2.5.2.1: Gram staining

Gram staining was performed to confirm the presence of Gram-negative and Gram-positive organisms. Microscopic observations were carried out at magnifications 40x, 100x and 400x.

#### 2.2.5.2.2: Endospore staining

Endospore staining was performed to confirm the presence of the spore-forming organisms *B.cereus* and *C.perfringens*. Microscopic observations were carried out at magnifications 40x, 100x and 400x.

#### 2.2.5.2.3: Lactophenol Cotton Blue staining

LPCB was performed to confirm the presence of yeasts and moulds. Microscopic observations were carried out at magnifications 40x, 100x and 400x.

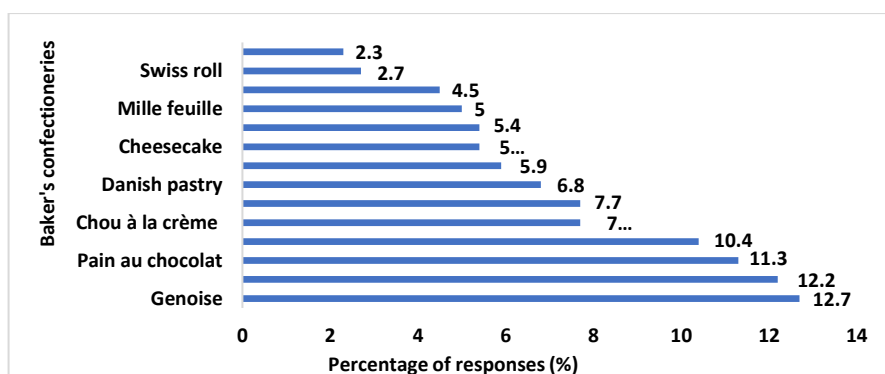
### 2.3: Statistical analysis

The Minitab® 18 Statistical Software was used to carry out a two-way analysis of variance (ANOVA) at a 5% significance level to determine any significant differences in the bacterial count and TVC of the different regions at 4°C and room temperature.

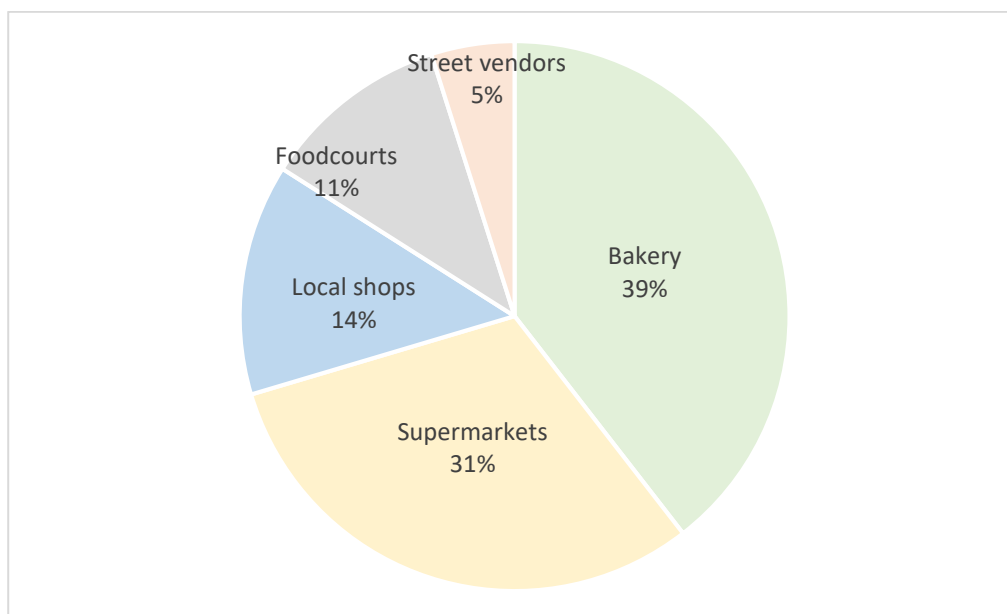
## 3. RESULTS AND DISCUSSION

### 3.1: Consumer survey

In Mauritius, a variety of baker's confectioneries are available for sale. Baker's confectioneries are often considered as high-risk products since they contain ingredients like cream and raw eggs which have previously been implicated in foodborne outbreaks. From the consumer survey, it was determined that Genoise was the bakery product mostly preferred and consumed by the respondents (Figure 1) while bakery was the retail point selected by respondents as the most attended to purchase bakery products (Figure 2).



**Figure 1 shows a list of some baker's confectioneries sold in Mauritius and their respective level of preference and consumption by the respondents:** Genoise 12.7%; Banana tart 12.2%; Pain au chocolat 11.3%; Puits d'amour 10.4%; Chou à la crème 7.7%; Eclairs 7.7%; Danish pastry 6.8%; Pains aux raisins 5.9%; Cheesecake 5.4%; Doughnuts 5.4%; Mille feuille 5%; Cupcakes 4.5%; Swiss roll 2.7%; Macarons 2.3%.



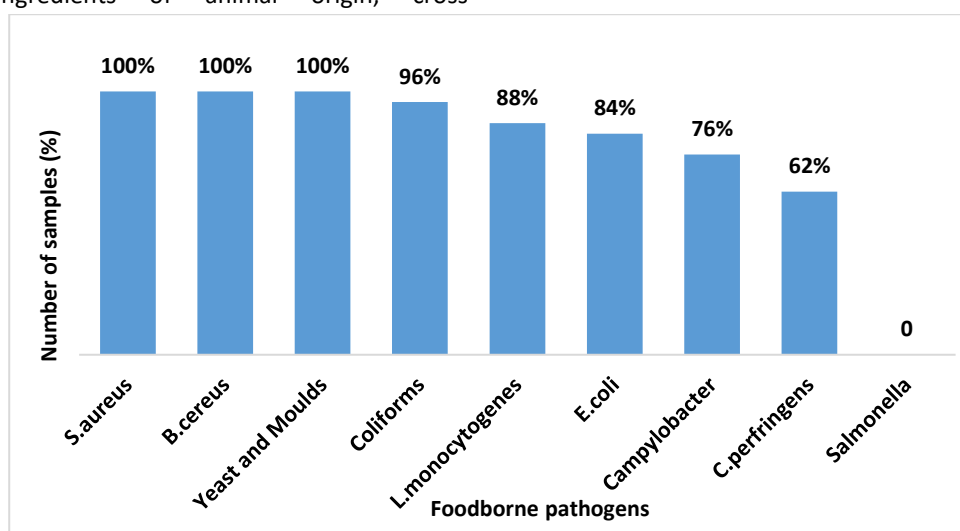
**Figure 2 shows the various retail points in Mauritius where bakery products are put on sale.** Bakery was selected as the most attended to purchase bakery products by 39% of the respondents, followed by supermarkets (31%), local shops (14%), foodcourts (11%) and street vendors (5%).

### 3.2: Microbiological analysis

The microbiological analysis (Figure 3) revealed the presence of *S.aureus*, *B.cereus* and yeasts and moulds in all the samples while *Salmonella* was not detected in any of the samples. Other foodborne pathogens that were detected include coliforms, *L.monocytogenes*, *E.coli*, *Campylobacter* and *C.perfringens*. The presence of the foodborne pathogens detected in the Genoise cakes may have been introduced into the cream either through ingredients of animal origin, cross-

contamination via kitchen equipment or inadequate hygienic practices by food handlers [4].

The presence of *S.aureus* in the samples is a clear indication of unhygienic practices by the bakeries. As reported by [5], contamination of edibles by *S.aureus* occurs mostly via food handlers. Since the pathogen forms part of the human flora, it could have been introduced through unwashed hands or from superficial wounds on the skin of the food vendors [6].



**Figure 3 shows the results of the microbiological analysis.** *S.aureus*, *B.cereus*, yeast and moulds were detected in all the samples (100%); Coliforms were detected in 96% of the samples; *L.monocytogenes* was detected in 88% of the samples; *E.coli* was detected in 84% of the samples; *Campylobacter* was detected in 76% of the samples; *C.perfringens* was detected in 62% of the samples; *Salmonella* was not detected in any of the samples.

The presence of *B.cereus* is attributed to its ubiquity in the environment. Due to its ability to produce spores that are resistant to various stresses, *B.cereus* is easily spread to eggs, milk and RTE foods [7]. The detection of *B.cereus* in Genoise cakes can be associated with the use of eggs which are incorporated in large quantities during preparation as leavening agents. *B.cereus* can then escape the baking process due to its ability to produce thermotolerant spores [7].

The presence of yeasts and moulds in the samples is an indication of post baking contamination since fungal spores cannot resist the thermal process during baking [8, 9]. Since yeasts and moulds have an affinity for products with high sugar content, their presence in the Genoise cakes is not surprising. Moreover, [9] stated that air conditioning facilitates the movement of fungal spores from the bakery flooring, further promoting contamination and spoilage of bakery products.

Contamination of RTE foods by *L.monocytogenes* usually occur post processing [10]. The high incidence of *L.monocytogenes* in the Genoise cakes may be explained by the fact that *L.monocytogenes* has the ability to persist in cooking premises even after sanitation due to biofilm formation. Therefore, the pathogen can easily contaminate the cream filling and topping which is added post baking and which does not receive any heat treatment. Another factor is refrigeration. Despite refrigeration is the sole means to mitigate bacterial growth and multiplication in cream-filled pastries, *L.monocytogenes* can easily multiply in refrigerated foods due to its ability to grow at temperatures as low as 0°C.

The presence of coliforms and *E.coli* in foods is an evidence of inappropriate hygienic practices among handlers. Coliforms indicate the possibility of fecal contamination and the presence of *E.coli* represents secondary contamination [6]. They may have been introduced into the Genoise cakes through contaminated kitchen equipment such as piping bags or contaminated kitchen surfaces. Refrigeration of left-overs and putting them back on sale the following day also promotes the rapid multiplication of *E.coli*.

The presence of *C.perfringens* and *Campylobacter* in the samples may be a result of cross-contamination via the use of kitchen equipment or raw ingredients such as meat or poultry [11]. Since *C.perfringens* is a spore-forming bacterium, it can resist heating and can multiply

during cooling [12]. Though *Campylobacter* is susceptible to heating, it can still contaminate the Genoise cakes via the cream topping and filling which is not heat-treated and added post baking.

*Salmonella* was not detected in any of the samples. A study by [13] reported the absence of *Salmonella* in cream-filled pastries; a result that correlates with that of this study. *Salmonella* is the most reported microbiological food safety hazard in Mauritius (l'express.mu). After a *Salmonella* outbreak in 2016, a control scheme was set up by the Ministry of Agroindustry to mitigate the prevalence of the pathogen (defimedia.info). Hence, this explains the absence of *Salmonella* in the Genoise cakes.

### 3.3: Statistical analysis

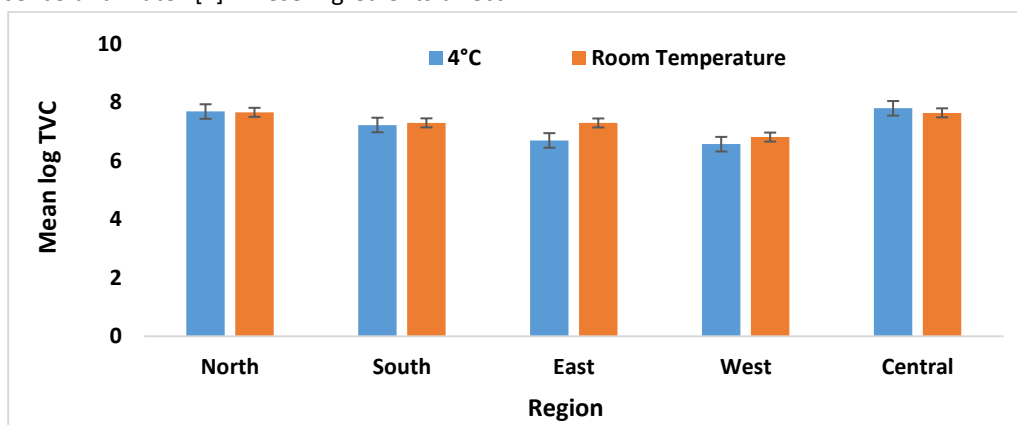
#### 3.3.1: Comparing differences in the means of the total viable count of the 5 selected regions at 4°C and room temperature

From the two-way ANOVA, it was deduced that storage temperatures had no significant effect on the TVC since p-value was greater than 0.05. Though refrigeration at 5°C or below is recommended to prevent microbial growth in cream-filled pastries, spoilage bacteria can still grow at a slow rate [14]. This is because refrigeration slows down bacterial regulatory mechanisms that control enzyme activity when compared to freezing that completely prevents bacterial growth (Shelflifeadvice.com). Hence, this explains why no significant difference was observed in the TVC at room temperature and 4°C.

Significant differences were observed in the TVC of 5 regions (p-value < 0.05). From Figure 4, it can be deduced that samples from the Central region had the highest TVC while those collected from the West had the lowest TVC. The TVC were classified as satisfactory, borderline and unsatisfactory (Table 1) according to [10]. 70% and 80% of the samples collected from the North and Central regions respectively had unsatisfactory TVC levels while samples collected from the remaining regions had TVC marginal to the recommended level. The difference observed in the TVC can be explained by the fact that part of the samples may have been freshly prepared, hence a low TVC while those with a high TVC may be left-overs that have been put for re-sale. Some bakery firms store left-overs for resale to avoid economic loss. As reported by [4], improperly storing cream-filled pastries that have been

prepared a day or more before serving greatly contributes to microbial spoilage. Moreover, some foodborne pathogens like *L.monocytogenes*, *E.coli* and *C.perfringens* can resist and multiply during refrigeration. There are intrinsic and extrinsic factors that affect the growth of microorganisms in foods. Cream fillings used in pastries contain ingredients like eggs, shortening and emulsifying agents, sugar, milk, vanilla essence and water [4]. These ingredients affect

the  $a_w$  of the product. Given the amount of sugar added, cream fillings have a low  $a_w$  that is unsuitable to support the growth of microorganisms. However, cakes having layers of cream, as in the case of Genoise, were found to favour microbial growth due to the formation of localized areas of high  $a_w$  caused by the movement of water from the cake to the interface of the cake and the cream filling [4].



**Figure 4 shows the results for the Two-way ANOVA for the comparisons of the mean differences between the TVC of the 5 regions at 4°C and room temperature.** Significant differences were observed in the TVC of 5 regions (p-value < 0.05); storage temperatures had no significant effect (p-value > 0.05).

**Table 1: Classification of the TVC from the 5 regions**

Region	Satisfactory	Borderline	Unsatisfactory
North	0%	30%	70%
South	0%	80%	20%
East	0%	100%	0%
West	0%	100%	0%
Central	0%	20%	80%

### 3.3.2: Comparing differences in the means of the bacterial count of *S.aureus* at 4°C and room temperature

Significant differences were noted in the means of the bacterial count since p-value was less than 0.05. It was also deduced that storage temperatures had a significant effect on the bacterial count. As shown in Figure 5, the bacterial counts were higher at room temperature. The high bacterial count of *S.aureus* in the samples at the 2 storage temperatures is due to the cream portion which is conducive for the growth of

*S.aureus* [4]. Bacterial multiplication must have further occurred during transportation from the bakery to the laboratory since the conditions were suitable for toxin production and growth. Even though the bacterial loads recorded at 4°C were lower than those at room temperature, they were still above the recommended level, which according to [15] should not exceed  $10^7$  staphylococci per g of food. These results can be attributed to the production of staphylococcal enterotoxins which are resistant to refrigeration.



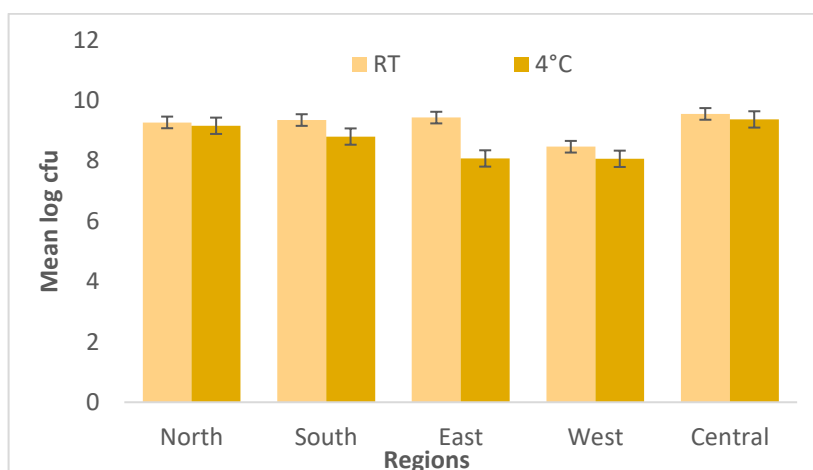


Figure 5 shows the results for the Two-way ANOVA for the comparisons of the means of the bacterial count of *S.aureus* among the 5 regions at 4°C and room temperature (RT). Significant differences were noted in the means of the bacterial count; storage temperatures had a significant effect on the bacterial count; bacterial counts were higher at room temperature (RT).

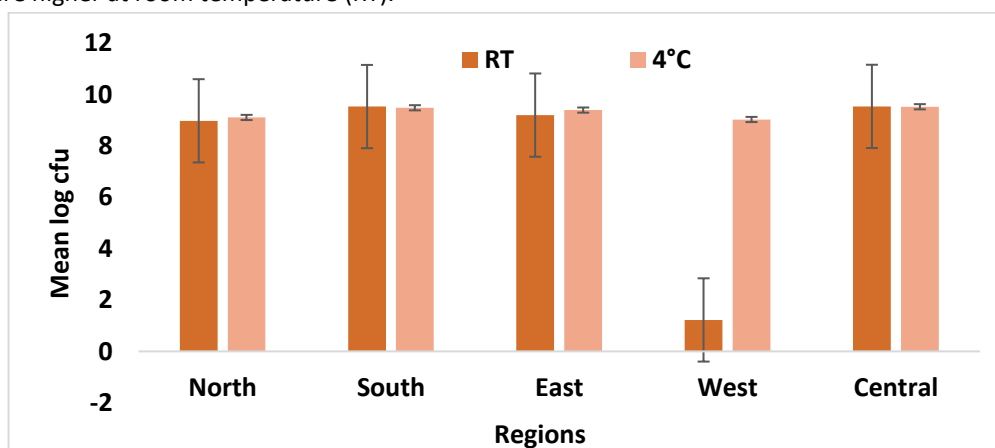


Figure 6 shows the results for the Two-way ANOVA for the comparisons of the means of the bacterial count of *B.cereus* among the 5 regions at 4°C and room temperature (RT). Significant differences were noted in the means of the bacterial count; storage temperatures had a significant effect on the bacterial count; bacterial counts were higher at 4°C.

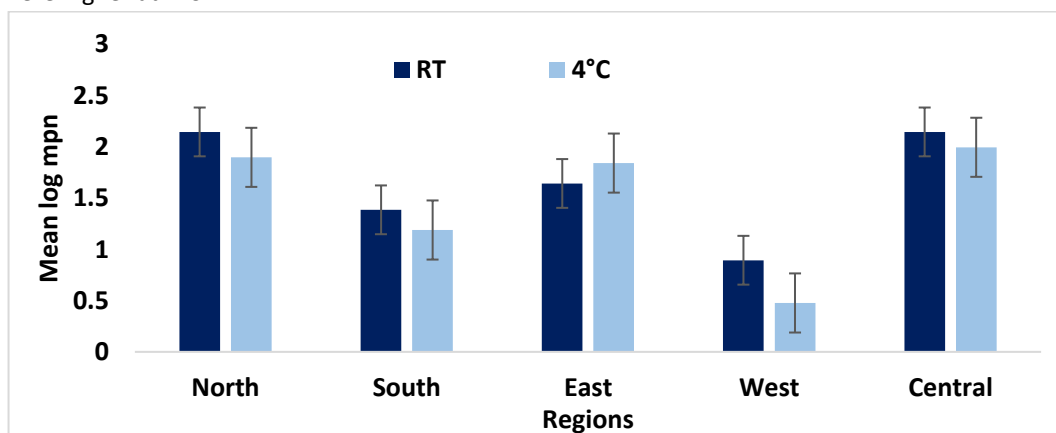
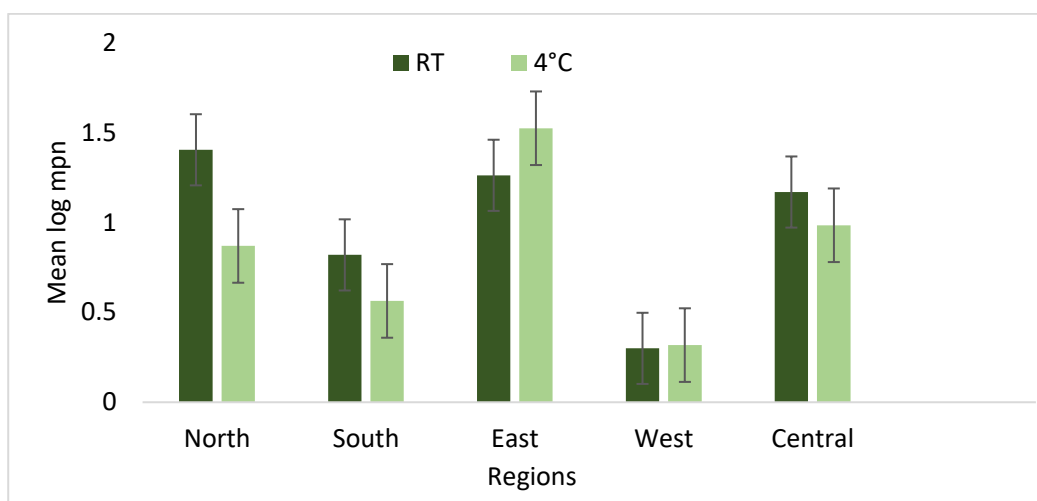


Figure 7 shows the results for the Two-way ANOVA for the comparisons of the mean differences in the MPN of coliforms at 4°C and room temperature (RT). Significant differences were observed in the means of the MPN of coliforms among the 5 regions; storage temperatures had no significant effect.



**Figure 8** shows the results for the Two-way ANOVA for the comparisons of the mean differences in the MPN of *E.coli* at 4°C and room temperature (RT). No significant differences were observed in the means of the MPN among the 5 regions ( $p$ -value > 0.05); storage temperatures had no significant effect.

### 3.3.3: Comparing differences in the means of the bacterial count of *B.cereus* at 4°C and room temperature

Significant differences were noted in the means of the bacterial count since  $p$ -value was less than 0.05 and storage temperatures had a significant effect on the bacterial count since variations were observed in the bacterial load. High levels of *B.cereus* were enumerated at 4°C. The high level of *B.cereus* in the samples at 4°C is attributed to the bacterium ability to grow at temperatures  $\leq 7^\circ\text{C}$  [16]. According to [17], *B.cereus* strains in the food environment are psychrotolerant and extended refrigeration will further promote spores germination and even outgrowth to levels that may be detrimental to consumers.

According to an article by [18], *B.cereus* strains associated with foods are either psychrotrophic or mesophilic. The mesophilic ones have optimal growth at temperatures  $30^\circ\text{C}$ - $40^\circ\text{C}$ . Hence, this explains the detection of low levels of *B.cereus* in Genoise cakes stored at room temperature.

### 3.3.4: Comparing differences in the means of coliforms at 4°C and room temperature

Significant differences were observed in the means of the MPN of coliforms among the 5 regions while storage temperature had no significant effect on the MPN of coliforms since  $p$ -value was greater than 0.05. According to [19], coliforms in dairy products are heat-labile and have a psychrotolerant nature. Their presence in the samples indicate post-baking contamination, i.e. through the addition of the cream and the negligible

differences observed in the means of the MPN at the storage temperature can be attributed to their psychrotolerant nature. The variations observed among the 5 regions clearly demonstrate a lack of hygiene by the retail points in question.

### 3.3.5: Comparing differences in the means of *E.coli* at 4°C and room temperature

No significant differences were determined in the means of the MPN of *E.coli* since  $p$ -value was greater than 0.05 and it was also observed that storage temperature had no significant effect as  $p$ -value was greater than 0.05. The statistically insignificant differences observed in the means of the MPN of *E.coli* at the storage temperatures can be attributed to its ability to grow at temperatures  $4$ - $45^\circ\text{C}$ . The presence of *E.coli* in the samples can be linked to the utilisation of contaminated water and equipment or poor hygiene by the food handlers and once the food is contaminated, refrigeration will not make it safer for consumption [20, 21].

### 3.3.6: Limitations & suggestions for further research

A major issue encountered during this project was the detection of *C. perfringens*. Though the microbiological and biochemical tests corresponded to the characteristics of the organism, microscopic examination for endospores hampered the confirmed identification of the latter. To address this issue, alternative methods based on molecular technologies could be used as diagnostic tools. In the same context, [22] used a multiparametric PCR-based tool to identify spore-forming food contaminants. The analysis



consisted of a biochip assay based on the use of a GeneDisc® cycler automated system, GeneDisc® plate disposable device and real-time PCR for the rapid detection of spore-forming contaminants. An increased detection limit was achieved with the alternative method.

Since the PCR is emerging as a novel diagnostic tool with highly promising results, it would be of utmost interest to implement this molecular technique in further research related to this study to obtain precise results.

#### 4. CONCLUSIONS

The results of the study show that the microbial load for most of the samples from the selected regions are marginal to the recommended level with the North and Central regions as exceptions since the samples had unsatisfactory TVC levels. This observation therefore leads to a conclusion that the 5 selected bakeries do not follow and apply proper hygienic practices, hence putting consumers' health at risk.

Secondly, the data show meagre differences in the microbial load at the storage temperatures. Statistical analyses demonstrate that although significant differences were observed in the growth of *S.aureus* and *B.cereus*, these foodborne pathogens were still present in high levels in the samples. The conclusion that can be drawn from these observations is that once a contaminated product has been purchased, further storing it at room temperature or 4°C will promote bacterial growth and multiplication.

*Salmonella* is the microbiological hazard that is most documented in Mauritius. However, there are no reported cases of food poisoning caused by other foodborne pathogens that were detected in the Genoise cakes during this study. Unreported definitely does not imply that these bacteria are not prevalent in Mauritius. Therefore, food laboratory services should be developed with an aim to keep a track of potential food safety hazards on a continuous basis to develop strategies according to the HACCP systems in preventing foodborne poisoning.

The findings of this study clearly point out that Genoise cakes are potentially high-risk bakery products. Therefore, consumers should take the various food safety hazards into consideration while buying cream-filled pastries as well as other commercially available edibles.

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