

ASSESSMENT OF PATHOGENICITY AND BIOCHEMICAL CHARACTERIZATION OF *LISTERIA* SPP. IN READY-TO-EAT BAKERY PRODUCTS

Aimen Shabbir¹, Ponam Gill², Sharmeen Arif³, Adila Naseem⁴, Aiman Athar⁵,
Muhammad Akhtar⁶, Tayyaba Alvi⁷, Irza Saif⁸

^{1,8} Department Food Science and Technology, NFC-Institute of Engineering and Technology, Multan.

^{2,3} National Institute of Food Science and Technology, (NIFSAT) University of Agriculture Faisalabad.

⁴ Department of Food Nutrition and Home sciences Ghazi University Dera Ghazi Khan.

⁵ Punjab Food Authority, Nanakana Sahib.

⁶ Department of Food and Nutritional Sciences, University of Central Punjab, Lahore.

⁷ Department of Nutrition and Dietetics Green International University Lahore.

¹aimenshabbir111@gmail.com, ⁶muhammadakhtar@ucp.edu.pk

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Corresponding Author: *

Aimen Shabbir

Muhammad Akhtar

Abstract

In this modern era people are very busy in their professional life and lack time to cook food on their own. Now they are inclined to consume RTE foods to save time. Bakery foods like pizza, sandwich, and pastry are very popular in people but these must be safe to consume. Safety and quality of foods is affected by the environment in which these food products are prepared. This study was designed to evaluate the prevalence of *Listeria* in most commonly consumed bakery products in Faisalabad city. Food samples for presence of *Listeria* were tested by adopting spread plate method and biochemical confirmation was done by Gram staining, Methyl red, Oxidase, Catalase, Citrate, Indole and Hydrogen sulphide tests. The study concluded that sandwich, pastry and pizza samples collected from local and branded bakeries of Faisalabad city were found to be contaminated with *Listeria spp.* *Listeria* count was higher in local bakeries (7.2×10^5 CFU/g) as compared to the branded bakeries (2.4×10^4 CFU/g). The ultimate results highlight the need to introduce awareness and intervention programs to adopt food safety in Pakistan.

Introduction

Food is a nutritious substance, comprising of vital nutrients. Due to unsafe food there has been increase in foodborne diseases. According to published data, about 30% people fall sick annually due to unhealthy food (WHO, 2015). WHO reported that alone in USA 325,000 persons are hospitalized and about 5 thousand

people die each year due to foodborne illnesses. The higher incidence of foodborne health issues, particularly in western countries, has routed to an accumulated considerations regarding food safety worldwide and has been related to food handlers with poor personal hygiene. About seventy-six million foodborne disease cases are recorded in U.S annually (Tonder *et al.*, 2007). Center for

Disease Control and Prevention (CDC) has directed a current review describing that 31 most commonly found foodborne pathogens are causing the 9.4 million foodborne diseases (Scallan *et al.*, 2011; Devleess chauwer *et al.*, 2018). WHO proclaims that food related diseases and outbreaks have truly harmed the health and economic aspect of numerous developed and developing nations and stays to be a continuous risk (WHO, 2015). It is revealed that the highest rate of hospitalized people 90%, was due to foodborne diseases with life threatening symptoms (Jemmi and Stephan, 2006; Beloglazova *et al.*, 2019).

Some industrialists do not adopt the food safety standards in their processing and production unit while customers lack the knowledge about food safety that how to deal with food safety in their daily routine. In several developing countries, foodborne illnesses outbreak from microorganisms, *E.coli* impose a considerable burden on health care systems and may markedly cut back the economic productivity of the countries. (Oranusi *et al.*, 2013). Ready to eat food is turning into priority for modern time population. Because of limitation of people's time, consumers are step by step looking forward to food catering industries and people usually depend on such facilities are bigger than ever before (Soriano *et al.*, 2000; Deng *et al.*, 2020). Bakery foods have turned into a notably increasingly products in now-a-days life. In developed countries bakery industry produce different products relatively at larger scale. Different bakery products like cream pies, pastries, filled and unfilled pastries have also been responsible in different foodborne diseases because of high moisture content of these bakery products and thus it is related to serious food safety issues. Many American consumers experience some kind of food-borne diseases and among these diseases some are deadly. Common symptoms of food poisoning are abdominal pain, nausea, vomiting, diarrhea and cramps and thus it affects the whole people in world with the foodborne illnesses (Madrigal *et al.*, 2020).

Increase in demand of bakery food items additionally will raises the chance of foodborne

illnesses towards the consumers due to usage of prepared products like sandwiches, pastries and pizza. Bakery products like cream-filled pastries and breads can be contaminated by the *Salmonella*, *Staphylococcus aureus*, *Escherichia coli*, *Campylobacter*, yeasts and molds, these are major microbes responsible for foodborne illnesses in humans (Gill *et al.*, 2020). Pastries and cakes provide standard environment for these certain microorganisms that show higher growth in refrigerated temperature like *Listeria* (Gutie´ rez *et al.*, 2012; Lambrechts *et al.*, 2014). Dairy and dairy-based products like cakes and desserts containing cream or milk are suitable supplement media for high load of microorganisms. Food handlers, retailers, staff and customers do not adapt the food safety and food hygiene standards might cause food contamination (Sami *et al.*, 2013). The study was designed to evaluate the prevalence of *Listeria* in most commonly consumed bakery products in Faisalabad city.

Materials and Methods

Procuration of samples and chemicals

Sixteen popular bakeries, eight local and eight branded were selected situated in different locations of Faisalabad city. From these bakeries three items were selected i.e. sandwich, pastry and pizza. According to sampling plan, samples were collected under standard conditions using polyethylene bags. Peptone water and particular agar media for *Listeria* were purchased of Oxide (UK). The microbial analysis and biochemical confirmation of this study was done in Food Microbiology Laboratory of National Institute of Food Science and Technology, University of agriculture Faisalabad.

Sample preparation

To enumerate the *Listeria* from particular samples (sandwich, pastry, and pizza), 25g of each sample was mixed with 25mL peptone water in separate sterilized stomacher bag. Conditions of stomacher were set at 200 rpm for two minutes, to detach *Listeria* spp, (Harakeh *et al.*, 2005).

Microbial examination

Listeria selective agar was used to isolate *Listeria monocytogenes* by adaptation of spread plate method as described by Prescott *et al.*, (2002) and Cheesbrough, (2002). After incubation at 37°C colonies were counted with the help of colony counter ranging 30 to 300 CFU/g. Final results were calculated by following formula.

$$\text{CFU/g} = \frac{\text{Average number of colonies from duplicate plate}}{\text{Volume plated or dilution}}$$

$$\text{Dilution Factor} = \frac{\text{Sample volume}}{\text{Diluents volume} + \text{sample volume}}$$

Biochemical testing

Gram staining, Methyl red, Oxidase, Catalase, Citrate, Indole, and Hydrogen sulphide tests were performed to identify the bacterium (Gill *et al.*, 2020)

Statistical Analysis

All analysis were performed in triplicates and the figures were reported as means. Significant difference among treatments was evaluated through analysis of variance (ANOVA) under completely randomized design (CRD) according to the method described by Campos *et al.* (2015). For the analysis of likert scale IBM SPSS Statistics 22 was used.

Results and discussion

Prevalence and comparison of isolation of *Listeria* in sandwiches from local and branded bakeries of Faisalabad city

It is concluded that the prevalence ratio was 16/24 in local bakeries whereas in branded bakeries it was 13/24 and 11 samples were found free of *Listeria*. These are presented in table 1.

Similarly, chicken is also indispensable part of sandwich which can be potential source of *Listeria*. Results were also reported by Capita *et al.*, (2001) who concluded that *Listeria* may be present in raw meat and chicken carcasses. He observed that chicken contaminated with *Listeria* is used in making sandwiches. Additionally, the study of Crépet, *et al.*, (2007) also discussed that *Listeria* and its species are present in unprocessed and unwashed vegetables and salads. Similarly, Abadias *et al.*, (2008) also provided the same results that high moisture content of salads provides the favorable media for the growth and multiplication of these bacteria which strengthen the present findings. It shows that results are non-significant ($p > 0.05$) regarding comparison of enumeration of local and branded bakery sandwiches. In the Table 1 comparative enumeration of local and branded bakeries has been shown and results described that local bakeries have high count of CFU/g as compared to the branded bakeries. Local bakeries enumeration results indicated that highest count was 1.7×10^5 CFU/g and lowest count value was 1.0×10^4 CFU/g whereas for branded bakeries concerned enumeration findings depicted that highest count in branded bakeries was 1.07×10^6 CFU/g while the lowest count detected 5.6×10^3 . So, it is revealed that samples collected from the local and branded bakeries have shown the non-significant results and count ranges from 10^3 - 10^6 CFU/g. The results show that sandwich being sold in local and branded bakeries is contaminated with *Listeria* and its species. It is concluded that there was non-significant difference of count results in local and branded bakeries

Table 1. Enumeration of *Listeria* in sandwich samples from local and branded bakeries

Sr.#	Local bakeries (CFU/g)	Branded bakeries (CFU/g)
1.	1.7×10^5	2.4×10^4
2.	8.3×10^4	ND**
3.	1.5×10^5	9.3×10^5
4.	8.2×10^4	1.07×10^6

5.	1.0×10^4	1.6×10^4
6.	6.8×10^4	5.6×10^3
7.	8.0×10^4	2.6×10^4
8.	7.2×10^5	2.4×10^4

*CFU = Colony forming unit

Sr. #.	Local bakeries (CFU/g)	Branded bakeries(CFU/g)
1.	1.0×10^5	4.7×10^5
2.	8.4×10^3	2.1×10^5
3.	1.1×10^5	ND
4.	1.1×10^5	2.5×10^5
5.	9.0×10^3	2.4×10^5
6.	1.3×10^5	2.8×10^5
7.	1.2×10^4	2.8×10^5
8.	1.1×10^4	4.3×10^4

**ND = Not detected

*CFU = Colony forming unit

**ND = Not detected

Prevalence and comparison of isolation of *Listeria* in pastries from branded and local bakeries of Faisalabad city

The results of local bakeries presented the prevalence ratio 20/24 which make the prevalence of 80%. In case of branded bakeries, the prevalence ratio was 14/24 which results in 58% prevalence of *Listeria*. Local bakeries show

higher prevalence of *Listeria* than branded bakeries due to the poor sanitization, unhygienic and inadequate handling and processing of pastries. The highest count in the enumeration of local bakeries was 1.3×10^5 CFU/g and lowest count value was 8.4×10^3 CFU/g. Enumeration results of branded bakeries indicated that highest value was 4.7×10^5 CFU/g while the lowest count detected was 4.3×10^4

Prevalence and Comparison of isolation of *Listeria* in pizza from local and branded bakeries of Faisalabad

The prevalence of pizza samples brought from local and branded bakeries was checked as presented in Table 3. The prevalence of *Listeria* in local bakeries was 58% but in case of branded bakeries, the prevalence ratio was 11/24 and 13 samples have been found free of contamination of *Listeria*, which shows the 45 % prevalence.

Enumeration of local and branded bakeries is significant ($p \leq 0.05$) according to analysis of variance. Table 4 depicted that higher microbial load in local bakeries was 3.17×10^7 CFU/g and lower enumeration value was 1.46×10^6 CFU/g. As compared to the branded bakeries highest

count was 3.7×10^3 CFU/g and lowest count detected was 3.86×10^2 CFU/g. It is concluded that the local bakeries have higher microbial load comparatively with the branded bakeries as presented in Figure 4.6.5. A pizza ingredient such as cheese carries a microbial load due to the water contents or dairy. Cordano *et al.* (2001) and EFSA, (2009) described in their reports that *Listeria* also have been a part of cheese and cheese containing products. Cheese provides better environment to *Listeria* to grow as it contains the dairy proportion and water content which is suitable environment for *Listeria* results are in similarity with the study of Garedew *et al.*, (2015) who isolated *Listeria monocytogenes* from raw meat,

minced beef, pizza and fish. So, my study results also in agreement with him because of *Listeria*

load have been found in local and branded pizza.

Table 3. Enumeration of *Listeria* in pizza samples from local and branded bakeries

Sr.#	Local bakeries (CFU/g)	Branded bakeries (CFU/g)
1.	3.92×10 ⁶	3.86×10 ²
2.	5.54×10 ⁶	2.3×10 ³
3.	2.54×10 ⁶	ND
4.	3.64×10 ⁶	2.0×10 ³
5.	9.71×10 ⁶	1.3×10 ³
6.	1.46×10 ⁶	ND
7.	3.17×10 ⁷	3.7×10 ³
8.	5.24×10 ⁶	2.9×10 ³

Biochemical confirmation of *Listeria* and its species

Biochemical tests are used to identify bacteria because bacteria are very much different from each other. All bacteria have specific physical characters which differentiate them from each

other. Biochemical tests are used to identify *Listeria* and its various species in this study. These tests are gram staining, methyl red test, indole test, citrate test, catalase test, hydrogen sulphite test and oxidase test.

Table 3. Biochemical confirmation for *Listeria* and its various species

Sr. #	Biochemical tests	<i>Listeria</i>	<i>Listeria Monocytogenes</i>	<i>Listeria Ivanovii</i>	<i>Listeria Innocua</i>
1	Gram Staining test	+	+	+	+
2	Oxidase test	-	-	-	-
3	Methyl Red test	+	+	+	+
4	Indole test	-	-	-	-
5	Citrate test	-	-	-	-
6	Hydrogen Sulphide test	-	-	-	-
7	Catalase test	+	+	+	+

Safety status of (*Listeria monocytogenes* standards) of all samples of local and branded bakeries of Faisalabad city

Public Health Laboratory Service, (2000) described the safety standards of *Listeria* as satisfactory (< 10¹ CFU/g), acceptable (10¹ - ≤ 10² CFU/g) and unsatisfactory (> 10² CFU/g). Health Protection Agency, (2007) also provided

the same guidelines for the safety standards about *Listeria*. The enumeration values of local and branded pizza have been provided in the Table 4 according to safety standards of *Listeria*. Table 4 provided the unsatisfactory results regarding the safety status of local and branded bakeries.

Table 4. Safety level of *Listeria* isolated from sandwich from local and branded bakeries

Sr.#	Satisfactory range (< 10 ¹ CFU/g)		Acceptable range (10 ¹ - ≤ 10 ² CFU/g)		Unsatisfactory range (> 10 ² CFU/g)		Frequency (n)
	L	B	L	B	L	B	
1	-	-	-	-	1.7×10 ⁵	2.4×10 ⁴	3
2	-	-	-	-	8.3×10 ⁴	ND	3
3	-	-	-	-	1.5×10 ⁵	9.3×10 ⁵	3
4	-	-	-	-	8.2×10 ⁴	1.07×10 ⁶	3
5	-	-	-	-	1.0×10 ⁴	1.6×10 ⁴	3
6	-	-	-	-	6.8×10 ⁴	5.6×10 ³	3
7	-	-	-	-	8.0×10 ⁴	2.6×10 ⁴	3
8	-	-	-	-	7.2×10 ⁵	2.4×10 ⁴	3

*CFU = Colony forming unit**ND = Not detected

Results of CFU/g of local and branded bakeries have shown that all samples collected from local bakeries is highly contaminated by *Listeria* so safety status of pizza samples collected from local bakeries is unsatisfactory whereas in branded bakeries low count of bacteria revealed that six bakeries fall in unsatisfactory range of bacteria and two were in acceptable range. So, it is concluded that pizza collected from branded bakeries is healthy and safe to eat as

compared to the local bakeries because local bakeries use unsterilized equipment and their food surfaces where food prepared. Knives, cutting boards and other equipment contaminate the particular food product. Cross contamination occurs due use of same equipment, cutting boards and cutlery for preparation and cutting of different food products prepared at different temperature.

Table 5. Safety level of *Listeria* isolated from pastry from local and branded bakeries

Sr.#	Satisfactory range (< 10 ¹ CFU/g)		Acceptable range (10 ¹ - ≤ 10 ² CFU/g)		Unsatisfactory range (> 10 ² CFU/g)		Frequency (n)
	L	B	L	B	L	B	
1	-	-	-	-	1.0×10 ⁵	4.7×10 ⁵	3
2	-	-	-	-	8.4×10 ³	2.1×10 ⁵	3
3	-	-	-	-	1.1×10 ⁵	ND	3
4	-	-	-	-	1.1×10 ⁵	2.5×10 ⁵	3
5	-	-	-	-	9.0×10 ³	2.4×10 ⁵	3
6	-	-	-	-	1.3×10 ⁵	2.8×10 ⁵	3
7	-	-	-	-	1.2×10 ⁴	2.8×10 ⁵	3
8	-	-	-	-	1.1×10 ⁴	4.3×10 ⁴	3

*CFU = Colony forming unit

**ND = Not detected

Table 6. Safety level of *Listeria* isolated from pizza from local and branded bakeries

Sr.#	Satisfactory range (< 10 ¹ CFU/g)		Acceptable range (10 ¹ - ≤ 10 ² CFU/g)		Unsatisfactory range (> 10 ² CFU/g)		Frequency (n)
	L	B	L	B	L	B	
1	—	—	—	3.86×10 ²	3.92×10 ⁶	—	3
2	—	—	—	—	5.54×10 ⁶	2.3×10 ³	3
3	—	—	—	—	2.54×10 ⁶	ND	3
4	—	—	—	—	3.64×10 ⁶	2.0×10 ³	3
5	—	—	—	—	9.71×10 ⁶	1.3×10 ³	3
6	—	—	—	—	1.46×10 ⁶	ND	3
7	—	—	—	—	3.17×10 ⁷	3.7×10 ³	3
8	—	—	—	—	5.24×10 ⁶	2.9×10 ³	3

*CFU = Colony forming unit

**ND = Not detected

Conclusion and recommendations

This study concludes that the sandwich, pastry and pizza from various local and branded bakeries of Faisalabad city have been found to be contaminated with *Listeria* and its species. This study showed that microbial safety of the prepared/ready to eat food offered by bakeries is questionable and needs to be improved. Other safety assessments showed that the overall hygienic environment and the behavior of food handlers from branded bakeries are more satisfactory as compared to the local bakeries. Therefore, this study provides results for microbiological safety of the foods (sandwich, pastry, and pizza) sold in various local and branded bakeries of Faisalabad. The results described that the prevalence of *Listeria monocytogenes* in sandwich and stated that chicken is basic ingredient of sandwich which provides suitable environment for the growth of *Listeria*. The *Listeria* survives at low temperature therefore pastries are contaminated with *Listeria* which provides a proof for prevalence of this pathogen as samples are stored at low temperature. The result of my findings shows that pizza, sandwich and pastries being sold in local and branded bakeries is highly contaminated with *Listeria* and its species, due to many of reasons like unhygienic equipment, utensils, poor practice and unsterilized surfaces where food have been prepared.

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