EVALUATION OF BACTERIAL CONTAMINATION IN SELECTED INSTANT NOODLES BRANDS COMMERCIALLY AVAILABLE IN SRI LANKA

M.B.M.F. Jumana[1*], T.S.C. Sawja[1], E.A.M. Edirisinghe[1], M.N.F. Nasna[1], W.M.D.M. Gunasekara[1], U.L.R.M. de Silva[1] and S.S.M. Hettiarachchi[2]

[1]Department of Biomedical Science, KIU, Sri Lanka

[2]Department of Medical Laboratary Sciences, The Open University of Sri Lanka, Sri Lanka

Instant noodles have become a popular meal option in Sri Lanka. Although several studies done worldwide have observed the presence of significant amounts of pathogenic bacteria in them, this type of a study has not been done in Sri Lanka. Hence, the current study aims to find the bacterial contamination in instant noodles that are commercially available in Sri Lanka to evaluate the bacterial load and to identify the organisms up to the genus level.

Quantitative and qualitative analyses of 23 products from 8 different instant noodles brands were carried out with a dilution series for raw, cooked without added seasonings, and cooked with added seasonings samples from each product followed by colony count using both pour plate and spread plate methods to avoid limitations of pour plating. The isolates were cultured in enriched and selective media (MacConkey Agar, Mannitol Salt Agar) according to the result of Gram staining and relevant biochemical tests were carried out for further identification.

All samples had a heavy mixed growth on Nutrient Agar for colony enumeration with colony counts exceeding 3 x 10^3 CFU/g. Colony counts were comparatively low in most of the cooked instant noodle samples and extremely high in instant noodles samples cooked with added seasonings. A noticeable difference in the growth of organisms in pour plate and spread plate was observed with a higher growth in spread plates than pour plates indicating a high presence of aerobic bacteria. The highest colony count was observed in KCH3-BS sample with added seasonings (2.1 x 10^4 CFU/g).

Gram positive cocci and diplococci species were found to dominate 84% of the samples (58 of 69 samples) and Gram-negative bacilli were found in the rest. *Staphylococcus aureus*, Coagulase negative *Staphylococci*, *Streptococcus spp*. were identified following relevant biochemical tests. *E. coli* was observed only in one sample (KCH3-BS).

Instant noodles consumed in Sri Lanka have a high bacterial contamination predominantly by Gram positive bacteria. The seasonings provided with the instant noodles seem to have a higher amount of bacterial contamination. However, thorough cooking of the products can minimize the bacterial load present in the product thus, minimizing the potential risks associated with it.

Keywords: Bacteriological investigation, instant noodles, microbial quality, pour plate method, spread plate method

[*]Corresponding Author: b6b6790@kiu.ac.lk

EVALUATION OF BACTERIAL CONTAMINATION IN SELECTED INSTANT NOODLES BRANDS COMMERCIALLY AVAILABLE IN SRI LANKA

M.B.M.F. Jumana[1*], T.S.C. Sawja[1], E.A.M. Edirisinghe[1], M.N.F. Nasna[1], W.M.D.M. Gunasekara[1], U.L.R.M. de Silva[1] and S.S.M. Hettiarachchi[2]

[1]Department of Biomedical Science, KIU, Sri Lanka

[2]Department of Medical Laboratary Sciences, The Open University of Sri Lanka, Sri Lanka

INTRODUCTION

Many fast-food products have flooded the market, with instant noodles emerging as one of the most popular items (Okafor, 2014). Machines are used to produce most of the instant noodle types in the present world. The basic process related to all machine made noodles includes; dough mixing, sheet forming, compounding, sheeting/reduction, and cutting. (Bin, 2008). The cleaning and disinfecting procedures are not routinely done in manufacturing, which facilitates the growth of microbes such as bacteria and fungi within the residuals that remained in the machine with the previous manufacturing cycles. (Okafor & Omodamiro, 2006). Studies have shown that there is a high bacterial growth – including both Gram-positive and Gram-negative bacteria – with a prominent bacterial growth in the seasoning packet available inside. (Lipsa, 2020). Isolates *of Bacillus spp., Staphylococcus spp., Pseudomonas spp.,* and *Escherichia coli* have been identified in all the instant noodles samples (Okafor, 2014; Okunye, 2022; Tony, 2003).

When considering the food safety level in Sri Lanka, thousands of people are reported to be in danger owing to the consumption of food contaminated with biological, chemical, or physical hazards (Wanniarachchi et al., 2022). Food-borne pathogens such as *Salmonella spp., Bacillus cereus* and *S. aureus* have been isolated from food-borne outbreaks over the years 2012 to 2015 (Pathirage, et al., 2016). However, in Sri Lanka, no studies have been done to evaluate the microbial quality of instant noodles which are available in the Sri Lankan market. Therefore, the present study focuses on evaluating the microbiological quality of several instant noodles brands that are commercially available in Sri Lanka.

METHODOLOGY

Collection and preparation of samples

Three packets from each of 07 different brands and two packets from one brand (total 23 packets) of instant noodles available in Sri Lankan markets were randomly purchased and 1 g of each instant noodles was weighed using an analytical scale on a sterile watch glass. Three samples (raw noodles without seasonings, boiled noodles without seasonings, boiled noodles with seasonings) were prepared from each instant noodles packet in three sterile test tubes (a total of 69 samples). The test tubes were properly labelled, and 9 mL of sterile distilled water was added into all three test tubes. Two test tubes (boiled without seasonings and boiled with seasonings) were heated until the water boiled. Then, 1 g of instant noodles was added into all the test tubes and 0.1-0.2 g of the seasonings was added only to the "boiled with seasonings" test tube. Noodles added to the two test tubes for boiled noodles were cooked for 2-5 minutes (according to the manufacturer's instructions) and the tube with raw noodles without seasonings was left undisturbed for 2-5 minutes at room temperature.

Preparation of the dilution series

One milliliter from each sample test tube prepared above was transferred to 9 mL of sterile distilled water and was serially diluted up to three times.

Enumeration of bacteria in instant noodles

For every dilution concentration, both spread plate and pour plate methods were used to determine the bacterial load of each sample. Both plating techniques were used as the restriction of oxygen in the depth of agar media in pour plates may prevent or minimize the growth of obligate aerobes (Roberts & Greenwood, 2003), as well as due to other limitations in pour plates such as difficulty in colony counting, and heat sensitivity. Hence, an appropriate volume (1 mL for pour plate and 0.1 mL for spread plate) of the sample from each dilution series was transferred into Nutrient Agar plates for bacterial enumeration and incubated aerobically at 37˚ C up to 24 – 48 hours. Positive controls (*Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 8739) and negative controls (sterile distilled water) were used during the procedure. After incubation, the average number of colonies was taken by counting the visible predominant colonies from duplicated plates and dividing from the number of plates (two), and the colony count in CFU/g was obtained using the formula:

$$\text{CFU/g} = (\text{number of colonies counted} \times \text{dilution factor}) / \text{volume plated}$$

Isolation and presumptive identification of bacteria

Colonies from the Nutrient Agar were subjected to Gram staining, and Gram-positive colonies were sub-cultured on Mannitol Salt Agar for the identification of more clinically significant *Staphylococcus aureus*, and Gram-negative colonies were sub-cultured on MacConkey Agar for the differentiation of lactose fermenters and non-lactose fermenters, and the plates were incubated aerobically at 37˚ C up to 24 – 48 hours. After incubation, the sub-cultured colonies were again subjected to Gram staining to determine the suitable biochemical test methods.

For Gram-positive cocci, Catalase, and Coagulase (tube and slide) tests were conducted and for Gram-negative bacilli, Indole, and sugar fermentation tests (Kligler's Iron Agar - KIA) were carried out.

RESULTS AND DISCUSSION

The highest bacterial colony count in raw instant noodles samples was observed in RGP3-R sample with a colony count of 10260 cfu/g, and the lowest bacterial colony count in raw instant noodles samples was observed in MMC-R sample with a count of 420 cfu/g. Gram-positive bacteria were found to dominate all the observed raw instant noodles samples, with *Staphylococcus* spp. present in most of them.

In boiled instant noodles samples, the lowest bacterial count was observed in BDS-B (63 cfu/g). Gram positive bacteria were observed in 87% (20 out of 23) of the boiled samples, and were suspected to have predominantly *Staphylococcus* spp.

Boiled instant noodles samples with added seasonings were observed to have the highest colony count out of all samples. The highest bacterial count was recorded in KCH3-BS sample with a colony count of 2.1×10^4 cfu/g and the lowest count was recorded in BDS-BS sample with a count of 94 cfu/g.

A significantly higher level of Gram-positive bacteria was observed in the samples than Gram-negative bacteria ($p < 0.01$). Out of all 69 samples observed, 58 samples (84%) contained Gram-positive cocci and diplococci while 11 samples (16%) had Gram-negative bacilli. 12 samples (17.4%) had both Gram-positive and Gram-negative bacteria present in the samples.

Boiled instant noodles samples with the added seasonings were observed to have the highest bacterial load (2.1×10^4 cfu/g) and the boiled instant noodles samples without added seasonings were observed

to have the lowest bacterial count (63 cfu/g).

Table 1: Colony counts of raw (R), boiled without seasonings (B), and boiled with seasonings (BS) instant noodles samples.

Raw Samples	CFU/g	Boiled Samples	CFU/g	Boiled with Seasonings Samples	CFU/g
PRS-R	>3x 10^3	PRS-B	126	PRS-BS	>3x 10^3
PRT-R	1160	PRT-B	>3x 10^3	PRT-BS	>3x 10^3
PRK-R	>3x 10^3	PRK-B	82	PRK-BS	>3x 10^3
MGD-R	>3x 10^3	MGD-B	>3x 10^3	MGD-BS	>3x 10^3
MGC-R	>3x 10^3	MGC-B	>3x 10^3	MGC-BS	470
MGK-R	1490	MGK-B	300-600	MGK-BS	>3x 10^3
SYO-R	1880	SYO-B	102	SYO-BS	>3x 10^3
SYP-R	>3x 10^3	SYP-B	>3x 10^3	SYP-BS	>3x 10^3
SYB-R	>3x 10^3	SYB-B	>3x 10^3	SYB-BS	>3x 10^3
MMC-R	420	MMC-B	300	MMC-BS	>3x 10^3
MMV-R	780	MMV-B	< 300	MMV-BS	460
RGP1-R	>3x 10^3	RGP1-B	> 3000	RGP1-BS	>3x 10^3
RGP2-R	>3x 10^3	RGP2-B	> 3000	RGP2-BS	>3x 10^3
RGP3-R	10260	RGP3-B	8670	RGP3-BS	8670
SHP1-R	>3x 10^3	SHP1-B	176	SHP1-BS	>3x 10^3
SHP2-R	>3x 10^3	SHP2-B	129	SHP2-BS	>3x 10^3
SHP3-R	3800	SHP3-B	10710	SHP3-BS	12360
BDS-R	>3x 10^3	BDS-B	63	BDS-BS	94
BDC-R	>3x 10^3	BDC-B	193	BDC-BS	104
BDS4-R	777	BDS4-B	>9000	BDS4-BS	>9000
KCH1-R	>3x 10^3	KCH1-B	267	KCH1-BS	288
KCH2-R	>3x 10^3	KCH2-B	390	KCH2-BS	2400
KCH3-R	9330	KCH3-B	2740	KCH3-BS	21000

The microbial load obtained from the noodles varies from brand to brand as well as the sample type of each brand. In addition, the number of CFU was higher in most but slightly lower in some samples (Okunye, 2022). An unexpectedly higher colony count was observed in some boiled without seasonings samples than the raw samples in a few products. This may be due to the activation of the bacterial spores into vegetative cells at sublethal temperatures provided for a short time (Juan, et al., 2022).

Instant noodles belong to 'dried and instant products requiring reconstitution' and according to the Food Act No. 26 of 1980, Sri Lanka, the limit for aerobic plate count per gram is 5 x 10^4 (Food Regulation, 2020). In the present study, even though the highest colony count per gram obtained was 2.1 x 10^4, it is important to remember that only the predominant colonies were counted and reported. Additionally, as shown in Table 1, more than 3 x 10^3 CFU/g of colony count was observed in samples where Gram-negative bacteria including *E. coli* was observed. The presence of such high levels of pathogenic bacteria and the fact that these bacteria were also viable in Boiled instant noodles samples (with or without seasonings) is a concerning factor. Therefore, strict protocols must be implemented to prevent possible food-related outbreaks in Sri Lanka due to instant noodles given their popularity.

Instant noodles are prepared by steam cooking at very high temperatures and pressure levels and the exit temperature of these products varies from 152 to 170 °C. Heat activation at a sublethal temperature is widely applied to promote *Bacillus* species spore germination (Dhital, 2010). Normally, heat damage accumulates at 70°C and 80°C treatment leads to significant killing of spores of bacteria but 60°C -70 °C does not affect *Bacillus* growth, whereas exposure to 75°C-80°C at longer heat treatment times (150, 240, and 300 min) decreases the yield of growing cells (Dhital, 2010). If any error occurs in any step of the process, bacterial spores can survive and can go into the final product.

Boiled with seasoning noodles samples showed the highest bacterial growth than raw noodles samples which indicates that the seasonings packets contain high bacterial contamination as well as that bacteria in the seasoning packets may be viable at the temperature of 100 °C.

Despite the high microbial counts obtained for some of the samples in this study, it is important to note that these samples did not show any visible signs of spoilage. Thus, outward appearance may not be a good criterion for judging the microbial quality of noodles.

Statistical Analysis

The data obtained in the experiments were statistically evaluated using analysis of variance (ANOVA) and the results with $p < 0.05$ were considered as statistically significant.

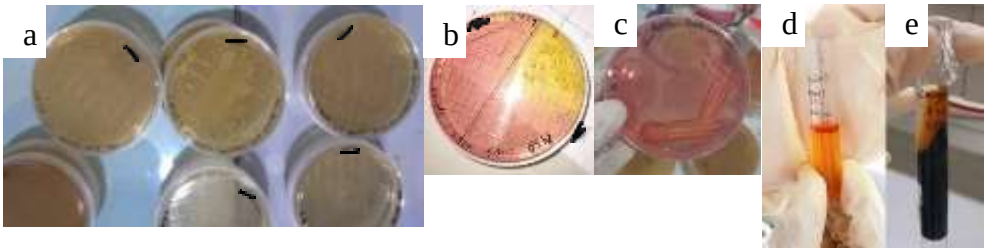

Figure 1: Culture plates and tubes showing significant microbial growth. (a) Spread plates, b) S. aureus positive MSA plate, c) Lactose fermenting MacConkey plate, d) Indole positive tube, e) KIA tube showing lactose fermentative, H_2S producing, gas positive organism)

CONCLUSIONS/RECOMMENDATIONS

Instant noodles consumed in Sri Lanka have high bacterial contamination predominantly by Gram-positive cocci than Gram-negative bacilli. The seasonings provided with the instant noodles have a higher amount of bacterial contamination. Deep cooking of the products can minimize the bacterial load present in order to minimize the potential risk associated.

The study shows that there is a concerning level of bacterial contamination in instant noodles including *Staphylococcus aureus*, Coagulase negative *Staphylococci spp.*, and *E. coli* among other unidentified pathogens. Therefore, the results from this study can be used in more modified research including agar culture media for the detection of fastidious organisms, additional biochemical tests for further species identification as well as to observe the antibiograms of the isolated species. Further, the present study was solely focused on detecting bacterial contamination, therefore, future studies could be carried out to find the fungal contaminations of instant noodle products.

REFERENCES

Bin, X. F. (2008). Asian noodles: History, classification, raw materials, and processing. *Food Research International*, 41(9), 888-902. https://doi.org/10.1016/j.foodres.2007.11.007

Dhital , S. (2010). Formation of resistant starch during processing and storage of instant noodles. *International Journal of Food Properties*, 13(3), 454-463. https://doi.org/10.1080/10942910802627091

Fang, T. J., Wei, Q.-K., Liao, C.-W., Hung, M.-J., & Wang, T.-H. (2003). Microbiological quality of 18 C ready-to-eat food products sold in Taiwan. *International Journal of Food Microbiology*, 80(3), 241-250. https://doi.org/10.1016/S0168-1605(02)00172-1

Food Regulation (Food Act No. 26 of 1980), (2018). Minister of Health Nutrition and Indigenous Medicine. https://eohfs.health.gov.lk/food/images/pdf/Draftregulations/Food-Cereal-Pulses Legumes-Regulations--2019.08.15-lkn-41.pdf

Juan, W., Jan P., P. S., Norbert, O. V., Arend, L. V., Peter, S., & Stanley, B. (2022). Heat activation and inactivation of bacterial spores: Is there an overlap? *Applied and Environmental Microbiology*, 4(21). doi:https://doi.org/10.1128/aem.02324-21

Lipsa, F. D., Gafencu, A., Ţîmpău, A., Florea, A.-M., & Ulea, E. (2020). Assessment of bacteria and fungi associated with the instant noodles and accompanying seasoning packets. *In Lucrări Ştiinţifice*, 63(2). https://doi.org/20.500.12811/1094

Okafor A. C., S. T. (2014). Detection of Bacteria Associated with the Spoilage of Ready-To-Eat Instant Noodles. *International Journal of Research*, 1239-1245. https://www.researchgate.net/publication/323512562_detection_of_bacteria_associated_with_the_spoilage_of_ready-to-eat_instant_noodles

Okafor, P. N., & Omodamiro, O. D. (2006). Assessment of chemical phytotoxin and microbial contamination of pasta foods. *American Journal of Food Technology*, 190-195. DOI: 10.3923/ajft.2006.190.195

Okunye, O. L., Idowu, P. A., Okanlawon, B. M., Lawal, T. O., Adejumo, O. E., Adeyemo, O. M., Oyinloye, O. E., & Ojieabu, W. A. (2022). Bacteriological evaluation of instant noodles stored under sub-optimal conditions from selected markets in Sagamu- Ibafo axis of Ogun State, Nigeria. *Journal of Pharmacy & Bioresources*, 19(3), 128–135. https://doi.org/10.4314/jpb.v19i3.1

Pathirage, Jayalal, M., Ganegoda, T., de Silva, G., Wickramasinghe, T., & Jayasekara, S. (2016). Retrospective analysis of food borne outbreaks. *Medical Research Institute*.

Roberts, D., & Greenwood, M. (2003). *Practical Food Microbiology* (3rd ed.). Massachusetts: Blackwell Publishing. https://doi.org/10.1002/9780470757512

Wanniarachchi, P. C., Abeysundara, P. D. A., & Peiris, H. S. (2022). Food safety knowledge, attitude, and practices among school children: a cross-sectional study based in the Colombo educational zone, Sri Lanka. Sri Lanka Journal of Social Sciences, 45(2), 241–253. https://doi.org/10.4038/sljss.v45i2.8586.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Biomedical Science of KIU, Sri Lanka, for providing necessary equipment to conduct the study.