

MICROBIOLOGICAL QUALITY OF READY-TO-EAT MEAT BASED FOOD AVAILABLE IN TEMPORARY FOOD OUTLETS IN GALL FACE GREEN, COLOMBO, SRI LANKA

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ABSTRACT

Ready-to-eat (RTE) food are increasingly demanding with the busy life style of the urban population, as well as result in significant number of foodborne illnesses per year, intensifying the emerging concern over the microbiological quality. This study analyzes the microbiological quality of 22 food samples of RTE meat based products, purchased in 2014 February to November from temporary food stalls at Colombo Gall face green. The RTE food were categorized according to the major food ingredient and were analyzed for Total viable count, Total coliforms, Faecal coliforms, *Escherichia coli*, *Salmonella* and *Staphylococcus aureus*. The incidence of both total coliforms, faecal coliforms of RTE meat based food was 63% and *E. coli*, *S. aureus* and *Salmonella* were detected in 59%, 32% and 4.5%, respectively. Among the analyzed food samples, 68% contained total viable counts exceeding 10^5 CFU/g and were of unacceptable quality for consumption in accordance with the Sri Lanka Standards (SLS). RTE food served with fresh vegetables including noodles, kebab, ham had higher total viable counts exceeding 8 log CFU/g and also detected total coliforms, faecal coliforms and *Escherichia coli* ($0.6 - \geq 3.04$ log CFU/g). Noodles were found to have the highest total viable count (7.21- 8.64 log CFU/g), and were significantly different ($0.05 < P$) from shawarma and devilled products. *S. aureus* was found only in shawarma and ham. *Bacillus* spp. were identified as the predominant bacteria present in the analyzed food products. This study indicate that the analyzed RTE food offer a higher risk for the consumers. Further, it emphasizes the importance of enforcing strict regulations and training on food handling at the selling points to ensure the microbiological quality of the RTE food.

Key words; Ready-to-eat, microbiological quality

Introduction

Ready-to-eat (RTE) food refers to an assortment of food and beverages for direct human consumption without the need of further effective processing or preparation. These food are prepared/sold at a range of dining-out establishments varying from hawker centers by the road sides to restaurants. However, as in other developing countries, RTE food sold in Sri Lankan street markets are highly demanding. They provide relatively cheap food which are easily accessible and convenient to the needs of urban inhabitants (Fellows and Hilmi., 2011). Further, it is a good source of income for food vendors and contributes significantly to their household incomes (Adjrah *et al.*, 2013).

On the other hand, RTE food are also identified as a commonest source of foodborne disease outbreaks (Estrada-Garcia *et al.*, 2004). Studies revealed that each year foodborne microbial pathogens cause illnesses, which affect 6 to 80 million persons in United States, causing 9,000 deaths (CDC, 2010). Mainly *Campylobacter*, *Salmonella* Typhi, Non typhoidal *Salmonella enterica*, and enteropathogenic *Escherichia coli* (EPEC) are known to cause devastating diarrheal diseases. Although Sri Lanka has a poor outbreak surveillance system, it has also reported over 1000 food poisoning cases in 2013 and also the health services delivery here, reports diarrheal diseases as the 5th leading cause of hospitalizations and the numbers fluctuate between 676 to 961 hospitalizations per 100,000 population over the past 20 years (Gunasekara, 2013; Ministry of Health, 2008). Not only the direct health consequences, foodborne illnesses affect the productivity and economic output of the country while imposing a substantial stress on health care system (Nazni and Jaganathan., 2014). Therefore, the microbiological quality and safety of RTE food has become an emerging health concern all around the world.

Nutritious food such as meat provide the favorable intrinsic condition to support the colonization of contaminating pathogenic and spoilage microorganisms (Clarence, *et al.*, 2009). A study in United States identified that animal products as a major vehicle of foodborne pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogens*, *Camphylobacter jejuni*, *Clostridium perfringens*, *Salmonella* spp., and *Staphylococcus aureus* (Clarence *et al.*, 2009). Therefore they are usually considered as high risk food and careful hygienic handling is needed throughout its operation.

Generally raw meat contaminating microorganisms are mesophilic and psychrotrophic. Although, adequate cooking can drastically reduce initial numbers of microorganisms including pathogens or sublethally injured bacteria, spores and some thermophilic bacteria such as Micrococci, Bacilli, Enterococci, and Lactobacilli can survive. This surviving microbial population contributes to total microbial flora of the end product, qualitatively and quantitatively (Knipe *et al.*, 2013). However, the post cook handling practices, food ingredients, condition and the duration of the food storage at selling points can significantly contribute in the presence of pathogenic and spoilage microorganisms in RTE food (Khairuzzaman *et al.*, 2014).

In most RTE food operations, processed ingredients such as meat, buns are prepared at a central supply facility and then shipped to individual food stalls where they are reheated or assembled in a short amount of time (Felgo and Sakyi., 2012). However, the

physical environments in which these stalls are located, typically lack proper infrastructure, such as clean running water, sanitation facilities, and solid waste management etc. (Khairuzzaman *et al.*, 2014). Since many vendors are congregated in overcrowded areas, there are scarce facilities for liquid drainage or garbage disposal. This result in improper waste discarding to nearby gutters, streets which provide excellent habitats for rodents, breeding points for flies and microorganisms. Therefore RTE food kept in open stalls are at a higher risk of contaminations by pathogenic and spoilage bacteria (Cuprasitru, *et al.*, 2011). In addition, using unsanitized food contact surfaces (chopping boards, knives etc.) in food preparation and improper segregation of raw ingredients from finished food can result in cross contaminations with pathogenic and spoilage bacteria (Rane., 2011). Further, ambient storage condition given for extended periods support the elevation of microbial numbers to unacceptable levels leading to low quality food with potential health hazards.

According to WHO (1989), food handling personnel play a very important role in ensuring food safety through-out the chain of food processing, storage and preparation. Generally, the relevant authorities of the country carry out the physical and medical screening of potential food vendors especially for communicable diseases prior to issuing certificates which allow their trade. However, this is not the common practice with so many food vendors around the world due to the lack of food safety knowledge or their role in transmitting pathogens into the food (Mensah *et al.*, 2002). A study conducted in Egypt revealed that improperly washed hands of food handlers are a major source of staphylococci and faecal coliforms in RTE food (Fawzi *et al.*, 2009).

Therefore, a proper microbiological analysis of these high risk food are paramount to ensure their fitness and safety for consumption. Accordingly, the present study is carried out to analyse the microbiological quality and safety of the selected RTE meat based food sold at a popular highly urbanized area in Sri Lanka which is rather questionable, in consumers' point of view. Furthermore the study intends to develop the consumer awareness and outline the current quality status of RTE food market in metropolitan areas of Sri Lanka.

METHODOLOGY

Study area selection

The selected study area Gall Face green is a sea side, free urban park located in Colombo city, Sri Lanka which has reported to bare the highest urban population and urban growth rate in the Island. It is a delightful location for many locals and attracts thousands of visitors especially in the evenings. Gall Face green has a large patronage by vendors, shoppers, hawkers. However, this area lacks sanitary facilities or potable water supply, and there is a significant rise of dust and dirt due to sea wind and sea water drops.

Sample Selection

A total of 22 RTE food samples comprising chicken, beef and mutton were purchased randomly from temporary food vending stalls at Gall Face. Sampling was done during late hours (6.00 pm to 9.00 pm) when the demand is high. Samples were stored in sterile containers in cooler boxes between 0-4°C and transported to the laboratory within 20 minutes of sampling. All samples were examined within 24 hours of sampling and stored between 0-4°C prior to analysis.

Sample Analysis

For the microbiological analysis, 10.00 g of each sample was weighed in to sterile stomacher bag and homogenized using a stomacher Bag Mixer™ following the addition of 90.0 mL of 1% buffered peptone water. Thereafter a dilution series (10^{-1} – 10^{-7}) in 1% buffered peptone water was prepared from food homogenate. Enumeration of total viable count (TVC) was done as per Sri Lanka Standard- SLS 516-1-Sec, 1: 2013 and Plate count agar (LAB149) was used. Total Coliforms, Fecal Coliforms and *E.coli* enumeration was done by Most Probable Number (MPN) 03 tube method as per SLS 516-3-Sec, 1: 2013. Presumptive, confirmation and completed tests were done in MacConkey broth (Oxoid CM0505), Brilliant Green Lactose Bile broth (BGLB-CM0031) and Eosine Methylene Blue Agar (Oxoid CM0069) respectively. Indole and methyl red tests were carried out for confirmation of *E.coli*. *Staphylococcus aureus* enumeration was done as per the SLS 516-6-Sec, 1: 2013), where Baird Parker Agar (Oxoid CM0275) was used. Typical colonies were identified by their distinct morphological properties i.e. dark grey and black convex colonies surrounding a clear zones. Thereafter, selected colonies were subcultured on Brain Heart Infusion Agar (Oxoid CM1136) and slide coagulase test was performed with human plasma for confirmation of pathogenicity. The method used for detecting *Salmonella spp* is as per SLS 516-5: 2013. 25.00 g of the sample is homogenized in stomacher Bag Mixer™ and pre-enrichment was carried out in buffered peptone water for 18 hours. Then the selective enrichment was done in Rappaport Vassiliadis broth (Hi media MH1491) and Muller Kaufmann tetrathionate broth (Oxoid CM0343) for 24 hours. Enriched cultures were streaked on Brilliant Green Agar (Hi media MU016) and Xylose Lysine Deoxycholate (XLD) Agar (Hi media M031). The colonies were selected based on morphological characteristics (Pink colonies on BGA and Black centered pink to red colonies on XLD Agar) and subcultured on Nutrient agar (Oxoid CM3). The isolates were confirmed by morphological (Gram's staining, Motility, Endospore staining) biochemical (Catalase test, Oxidase test, Indole test, Citrate Utilisation test, MR-VP test, Urease test, Lysine decarboxylase test, Triple Sugar Iron Agar test) and serological tests (Oxoid DR 1108A test kit).

Identification of predominant bacteria

Representative bacterial colonies from the Plate Count Agar plates were picked and transferred into Nutrient Agar plates (Oxoid CM3) and stored at 4°C prior to use. The isolates were identified based on morphological and biochemical characteristics; Gram's reaction, Motility, presence of Endospores, Catalase and Oxidase enzymes, production of Indole, Citrate Utilisation, MR-VP reaction, presence of Urease, Nitrate reduction, Gelatin/Casein/Starch hydrolysis, Lysine decarboxylation and Sugar Fermentation (Cowan, 1989).

Statistical Analysis

Statistical analysis was accomplished using MINITAB version 14. Statistical significance was set at $p < 0.05$. The significance of any perceived differences was determined by analysis of independent samples. The data were also analyzed using One Way ANOVA to determine statistical difference of microbial loads between all samples under study.

RESULTS

The microbiological analysis of RTE meat based food products are presented in Table 1a and Table 1b. Regarding the distribution of microbial populations, almost all the analyzed samples were found to have a total viable count higher than 10^3 CFU/g while 68% of the samples exceeded 10^5 CFU/g (Table 1a). *S. aureus* was detected in 32% of the analyzed food samples ranging between 2.50 and 4.51 log CFU/g and one sample detected *Salmonella* in 25g of the food (Table 1a). The highest microbial count of total coliforms and *E. coli* exceeds 10^3 MPN/g. Among the analyzed food samples, Faecal coliforms and *E. coli* were detected in 63% and 59% of the samples respectively (Table 1b).

The percentages of various samples which did not satisfy the microbiological guidelines imposed by the Sri Lanka Standards Institution are presented in figure 1. The highest rate of noncompliance (68%) is with the total viable counts. Total coliforms, Faecal coliforms and *E. coli* are the second highest non complying microbiological parameters with the standard. Percentage of noncompliance with the standard is 32% for *S. aureus* and much lower for *Salmonella* (5%).

Table 2 present the distribution of microbial counts in RTE meat based food samples depending on the type of meat based food product, i.e., noodles, devil, shawarma, ham and kebab. All the noodles samples are found to have a very high total viable count in the range of 7.21 to 8.64 log CFU/g and it is significantly different ($p < 0.05$) from devil and shawarma. *S. aureus* were detected only in shawarma and ham samples, and the counts vary between 2.51 and 4.48 log CFU/g. Devil and Shawarma are free of coliforms while almost all the noodles, ham and kebab detected high numbers of total coliforms, faecal coliforms and *E. coli*. Again the number of total coliforms, faecal coliforms and *E. coli* in noodles were significantly different ($p < 0.05$). Further, *Salmonella* was found to be on 25g of one of the Ham products.

The morphological and biochemical analysis of the predominant colonies on Plate Count Agar revealed that, most of the isolates are Gram positive and *Bacillus spp.* are the predominant.

Table 1a Microbiological Analysis of RTE meat based food products.

Microbiological Parameter	Range of microbial count (log CFU/g)	Percentage of samples in the following range (%)						Incidence %
		ND	<10 ²	10 ² to <10 ³	10 ³ to <10 ⁴	10 ⁴ to <10 ⁵	≤ 10 ⁵	
Total viable count	3.05 – 8.59	0	0	0	4.5	27	68	100
Pathogens								
<i>S. aureus</i>	2.50-4.51	15	0	9	9	14	0	32
<i>Salmonella</i>	Detected in 25 g	21	N/A	N/A	N/A	N/A	N/A	4.5

Table 1b Microbiological Analysis of RTE meat based food products

Microbiological Parameter	Range of microbial count (log MPN/g)	Percentage of samples in the following range (%)					Incidence %
		ND	< 3	3 to <10 ²	10 ² to <10 ³	≤ 10 ³	
Total coliforms	1.36- >3.04	36	0	18	18	27	63
Faecal coliforms	0.60- >3.04	36	0	23	18	23	63
<i>Escherichia coli</i>	0.60->3.04	41	0	18	14	23	59

ND – Not detected

N/A – Not Acceptable

Incidence % indicates the presence of positive samples for given microbiological parameter

Figure 1: Percentage of RTE meat based foods that did not comply with the microbiological standards imposed by Sri Lanka Standards Institution regarding TVC (Total Viable Count), TC (Total Coliforms), FC (Faecal coliforms), EC (*Escherichia coli*), SA (*Staphylococcus aureus*) and SAL (*Salmonella*).

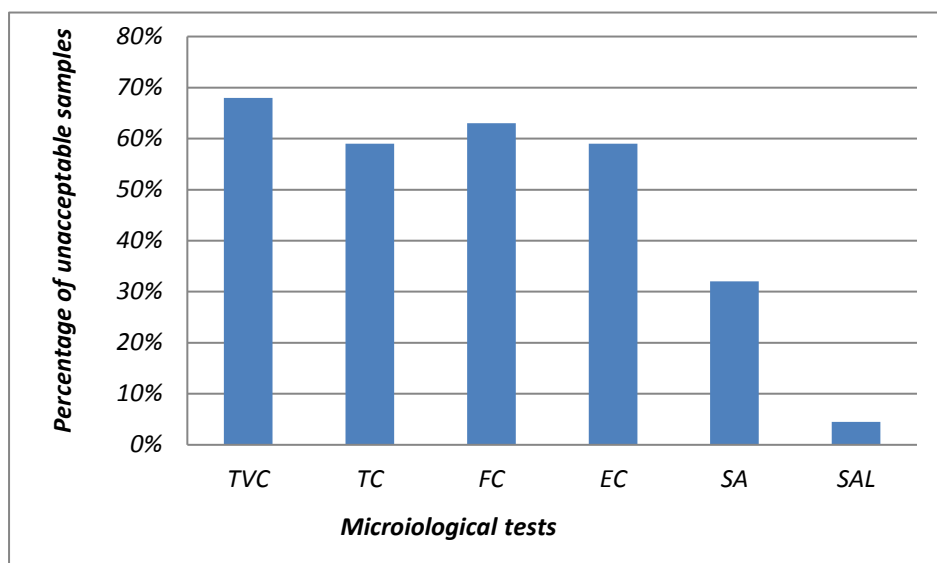


Table 2. Detection range of various Total viable count (TVC), total coliforms, faecal coliforms, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* in RTE meat based food depending on the type of food product.

Type of food product	No. of Samples	Range of microbial count (log CFU/g)			Range of microbial count (log MPN/g)		
		TVC	<i>S. aureus</i>	<i>Salmonella</i>	Total coliforms	Faecal coliforms	<i>E. coli</i>
Noodles	4	7.21- 8.64	0	absent	2.38- >3.04	2.38- >3.04	2.38- >3.04
Devil	4	4.36- 5.50	0	absent	0	0	0
Shawarma	4	4.15- 4.48	3.40- 4.00	absent	0	0	0
Ham	7	6.42- 8.53	2.51- 4.48	detected	0.95- >3.04	0.60 ->3.04	0.60->3.04
Kebab	3	4.34- 8.53	0	absent	2.66- >3.04	2.66->3.04	2.66->3.04

DISCUSSION

It is a basic right of consumers to access a good quality and safe food product (CAA, 2010). Although the number of food vending sites are mushrooming today, many violate consumer rights by selling low quality, hazardous food. According to Colombo Municipal Council there are about 990 food outlets reside in Colombo district, where only 678 have obtained the trade license and the rest are temporary food outlets located in areas which operate under low infrastructure facilities. In addition, food vendors have not obtained a training on safe food handling nor the medical screening for the specific infections. However, these food vending sites attract a large number of consumers per day as they provide more economic, attractive and delicious RTE food. In fact, RTE food are not given an effective treatment prior to consumption, thus most probably contribute in transmitting foodborne pathogens to the consumer. Therefore it is paramount to ensure the microbiological quality and safety at the point of sale and the current study was conducted to determine the level of quality and safety of selected highly demanding meat based RTE food.

According to Sri Lanka standards (SLS), Total viable count (TVC) of RTE meat based food, should not exceed 10^5 CFU/g and total coliform count should be below 10 MPN/g. In addition, Faecal coliforms, *E.coli* and foodborne pathogens such as *Salmonella*, *Staphylococcus aureus* should not detect in RTE food at all. However, the study detected high numbers of Aerobic plate counts, indicator organisms (Total coliforms, Faecal coliforms, *Escherichia coli*) and *Staphylococcus aureus* in majority of the analyzed samples implying a potential public health hazard. Further, detecting *Salmonella* in one of the analyzed samples offer a significant health risk in consumption of these food.

Presence of high TVC in RTE food indicate poor storage condition, use of low quality ingredients and poor food handling practices (FSANZ, 2009). It is not surprising to detect unacceptable level of TVC (Figure 1) in the present study, because the cooked food were stored at open air ambient storage, therefore can contaminate through flies and dust laden microorganisms. Among the analyzed food, Noodles were reported to bear the highest TVC, which could be attributed to the use of low quality raw material. However, similar studies conducted on sliced meat and meat products (Gillespie *et al.*, 2000; Soriano *et al.* 2000) and RTE food at Restaurants and Hawker Centers in Malaysia (Yee and Ngoh, 2002) were also reported high TVC similar to current study. Further, the study agree with findings of Saadia and Hassanein as the food containing raw vegetables such as noodles, ham and kebab (Table 2) showed high TVC (exceeding 8 log CFU) than all component cooked food such as devil and shawarma.

Although cooking can reduce the number of vegetative microbial cells, *Bacillus* spp. are more likely to survive the heating process, by the formation of heat resistant spores. Therefore *Bacillus* spp are frequently recovered from cooked food products. However, it is also known as a major secondary contaminant of the food during handling and storage (Fang *et al.*, 2002). A study in Ghana found that RTE food sold in open air street stalls has the highest frequency of contaminations by *Bacillus* spp (Feglo & Sakyi, 2012), similarly this study isolated *Bacillus* spp. in almost all the samples (Table 3). Further, ingredients such as condiments, flour can be identified as another important sources of *Bacillus* in RTE food (Umoh and Odobab., 1999).

Coliforms and *E. coli* were the second most prevalent contaminants detected in the present analysis. It is found that poor food handling practices at point of sale can significantly contribute in the prevalence of coliforms and *E.coli* in RTE food (Kwiri, *et al.*, 2014). Further, detection of *E. coli* indicate a recent faecal contamination through poor food handling and sanitation practices of food vendors and also indicate the possibility of contaminating enteric pathogens (Carrasco, *et al.*, 2012; Wei, *et al.*, 2006). A survey conducted in Southern Taiwan reported higher incidences of coliforms and *E.coli* in RTE food sold at temporary stalls, rather than at super markets (Wei, *et al.*, 2006). Unlike the supermarkets, food vendors at public areas operate under poor hygienic conditions as they lack potable water supply or sanitary facilities or enough space for waste disposal (Rayza, *et al.*, 2016). Therefore, many street food vendors are reluctant to wash hands between food and money transactions and, the food utensils are usually washed with water in large basins enabling cross contaminations between food and utensils (Cuprasitrit, *et al.*, 2011). On the other hand, presence of coliforms in cooked food ensure the inadequate processing during food preparation (FSANZ, 2009). In line with WHO, cooking at temperatures above 70°C for at least an hour, destroys *E. coli*, thus presence of *E. coli* in cooked food is a clear indication of incomplete heating (WHO, 1996). However, in the present study, only Ham, kebab and Noodles products were contaminated with coliforms, faecal coliforms and *E. coli*, but Devils and Shawarma were free of coliforms (Table 2). This might be due to the addition of raw vegetables which were improperly peeled or cleaned, in the post cook handling of the RTE food products (Beuchat, 2002).

Although *S. aureus* is an opportunistic pathogen present on the skin and respiratory tract of healthy individuals, it is of considerable importance as a foodborne pathogen due to the recurrent foodborne intoxications. *S. aureus* produce a heat stable enterotoxin when the food are stored at slightly elevated temperatures (Rayza, *et al.*, 2016; Wei, *et al.*, 2006). Current study isolated *S. aureus* from shawarma and ham (Table 2). Their presence evidence extensive manual handling and ambient food storage condition (Garcia *et al.*, 1986; Fang *et al.*, 2002; Snyder, 1998). Although, hot food and chilled food storage is recommended to be higher than 60°C and less than 4°C respectively, vendors lack storage facilities at the selling points or during transportation. Therefore RTE food such as meat products which require considerable handling are possible to be incriminated with Staphylococcal food poisoning (Saadia and Easa., 2010).

Previous studies on *Salmonella* in RTE food demonstrate that their occurrence is relatively low (2.0%) in RTE food when compared to raw food (Yee & Ngoh, 2002). This is because the thermal processing and holding temperatures are somewhat effective in controlling *Salmonella* in RTE food. However, *Salmonella* contaminations can occur through poor personnel hygiene and the use of minimally produced contaminated ingredients. In line with CDC (Center for Disease control and prevention), *Salmonella* is one of the most common source of serious foodborne illnesses and certain strains are of significant importance due to the emerging resistance to common antibiotics (Jay, Davos *et al.*, 2003). Food, of meat and poultry origin were found to have relatively higher isolation rate for *Salmonella* and a study conducted in Cross river State- Nigeria recovered *Salmonella* spp. in 14.2% of the analyzed samples (Odey, *et al.*, 2013; Yee & Ngoh, 2002).

In summary, the present study emphasize that the analyzed food implies a potential health hazard to consumers. Therefore stress the need of food safety precautions by establishing proper control points through Food Safety Management Systems such as HACCP, GMPs, SSOPs and GHPs (Wei, *et al.*, 2006). Further, development of policies and standards would be paramount to ensure the food safety at food vending venues. Such regulations may include strongly enforced requirements for licensure and public provision of training courses on food handling practices. It is important to continuously inform food vendors on their impact on transmitting foodborne pathogens. On the other hand, street food safety is far more to retrieve, unless the proper infrastructure facilities are provided at the vending locations to offer a high quality, safe product in consumer hands.

CONCLUSION

Previous study conducted in Malaysia reported 56% RTE food samples having TVC above 10⁶ CFU/g, *S. aureus* counts up to 4 log CFU/g and *E.coli* of 3 log MPN/g and Mashak reported *Salmonella* in 4% of RTE food products. Therefore, they have concluded the analyzed RTE food has implied a public health hazard (Mashak, *et al.*, 2015; Yee & Ngoh, 2002). Similarly, the present findings conclude that the microbiological quality of the analyzed RTE meat based food sold at Gall Face Green, Colombo are unsatisfactory for human consumption and insist the importance of educating street food vendors regarding the

importance of hygienic practices to reduce the potential risk of foodborne pathogens in RTE food products. Further, providing proper holding temperatures at the food storage and avoiding cross contaminations through the use of sanitized food contact surfaces and proper waste discard policies are paramount to safeguard microbiological quality of these food. In addition, this study stipulates RTE food served with raw vegetables are at higher risk of contaminating with pathogenic microorganisms than food that were completely heat treated. Therefore, ensuring the quality of the minimally processing ingredients is of prime importance for a microbiologically safe end product. These can be practiced and maintained through the implementation of effective Food Safety Management systems at the food vending sites that should become mandatory by the laws and regulations, yet to be enforced.

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