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Enterobacteriaceae contamination in locally packaged roasted cashew kernels from the Coast and Morogoro regions, Tanzania

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Keywords

Enterobacteriaceae;
Pathogenic bacteria;
Food safety;
Food hygiene;
Roasted cashew kernels

Abstract

The increased sales of locally packaged roasted cashew kernels (RCK) in Tanzania reflect growing consumer demand for healthy, convenient snacks. Despite this popularity and frequently reported cases of gastrointestinal illness, the microbial safety of these plastic-bagged products is not well-documented, posing a potential public health concern. To address this, a study was performed to evaluate packaging hygiene, package integrity, and microbial contamination of randomly selected packages of RCK (small: n=99; medium: n=87; large: n=118) from vendors in Coast and Morogoro regions, Tanzania. Package integrity was assessed using a vacuum decay leak tester while microbial contamination on surfaces including RCK, packers' hands, and unused packaging bags was determined by a culture-based method, with presumptive isolates biochemically confirmed. While no Enterobacteriaceae were detected on the unused packaging bags, RCK samples contained them at levels ranging from 1 to 192 cfu/g. Out of 304 samples, 14.8% (45) were inadequately sealed and had significantly higher concentrations of Enterobacteriaceae compared to well-sealed samples ($P < 0.05$). Bacterial pathogens isolated from the RCK included *Klebsiella pneumonia* (65.2%), *Escherichia coli* (11.6%), *Proteus vulgaris* (11.6%), *Enterobacter aerogenes* (4.95%), *Salmonella typhi* (3.31%), *Enterobacter cloacae* (1.65%), *Salmonella typhimurium* (0.83%), and *Salmonella enteritidis* (0.83%). These findings indicate that RCK may be an emerging vehicle for the transmission of human pathogens. Furthermore, Enterobacteriaceae counts in the RCK strongly correlated ($r = 0.67$, $P < 0.05$) with the concentration found on the hands of RCK packers, suggesting that the packaging process contributed to the contamination. This study highlights inadequate hand hygiene during packaging and insufficient sealing as major factors compromising the microbiological quality and safety of locally packaged RCK by small-scale industries in Tanzania's Coast and Morogoro regions. We recommend using these data to establish a quantitative risk assessment for human pathogens associated with RCK consumption and to devise effective interventions for managing these microbial risks.

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Introduction

Cashew kernels play a crucial role in global nutrition (Rico et al. 2016, Makinde et al. 2020, Khalili et al. 2022), disease management (Rico et al. 2016, Cordaro et al. 2020, Khalili et al. 2022), and global economic growth (Dendena and Corsi 2014, Boafo and Lyons 2019, Boafo et al. 2019). This has led to the expansion of cashew cultivation to more than 5,300,000 hectares worldwide (FAO 2013). While cashew nuts were traditionally exported from developing nations (Azam-Ali and Judge 2001, Catarino et al. 2015), there is now a trend towards domestic processing (Lawal et al. 2011). This has resulted in the emergence of numerous small-scale industries in various parts of the developing world, including Tanzania, the world's second-highest producer of cashews after Côte d'Ivoire. These small-scale industries grade raw cashew kernels, roast them, package the roasted cashew kernels, and hermetically seal the packages before supplying them to vendors, shops, and supermarkets for retail sale (Lawal et al. 2011, Dendena and Corsi 2014).

Small-scale industries spread cashew kernels on a stainless steel wire mesh tray and roast them in a hot air oven at 140 °C for 30 minutes to make RCK. The RCK are then cooled at room temperature before packaging. Although roasting effectively removes microorganisms from cashew kernels (Kurian and Raghavan 2020), it is unclear if small-scale industries can maintain the sanitation and safety of roasted cashew kernels up to the point of consumption as required by the Codex International Code of Hygienic Practice for tree nuts (CAC 1972). Roasted cashew kernels are rich in proteins, fats, vitamins and minerals, and have low water content, making them highly susceptible to contamination by various microorganisms (Adetunji et al. 2018). Previous studies have demonstrated that pathogenic microorganisms can survive on the surface of roasted cashew kernels (Little et al. 2010, Zhan et al. 2017, Louvau and Harris 2023). However, these studies have focused on developed countries in temperate regions with strict food laws and regulations. The microbiological safety of roasted cashew kernels produced by small-scale industries in tropical countries of the developing world has been inadequately investigated, despite the increasing production of locally packaged roasted cashew kernels (Azam-Ali and Judge 2001) and frequent outbreaks of foodborne diseases (Oluwafemi et al. 2009, Louvau and Harris 2023).

In small-scale industries in tropical developing countries, RCK packaging is often done by individuals without proper knowledge (Rahmat et al. 2016), leading to potential microbial contamination. Unhygienic packaging and inadequate sealing processes can expose RCK to hand microflora, food-borne pathogens and moisture, increasing the risk of intoxication or poisoning (Cutter 2002, Giwa et al. 2021). Limited scientific data exist on the microbiological safety of locally packed RCK, despite frequent foodborne outbreaks (Mutua et al. 2021). This research gap makes it challenging to comprehend the specific factors contributing to microbial contamination in RCK and predict foodborne disease outbreaks in low-income countries (Fellows and Hilmi 2011). The aim of this study was to estimate levels of microbial contamination, identify prevalent pathogens, and understand primary factors contributing to RCK contamination. The outcome of this study is expected to address data deficiencies in the risk assessment of microbial pathogens from consuming RCK in Tanzania.

In this study, 304 samples of the locally packed RCK sold by retailers at three bus stops along the highway connecting Dar es Salaam and Morogoro regions in Tanzania were investigated. A new integrated approach included assessing package integrity, measuring levels of Enterobacteriaceae (in RCK, unused packaging bags, and on the hands of RCK packers during packaging) and determining the prevalence of microbial pathogens in RCK samples was applied.

Materials and Methods

Study area and RCK sampling

Samples of the locally packed RCK were randomly purchased from retailers at three bus stops along the highway connecting Dar es Salaam and Morogoro regions in Tanzania, between October 2016 and November 2017. The three bus stops are: Kibaha, Chalinze, and Msamvu along the highway (192 km) connecting Dar es Salaam and Morogoro. This highway was chosen because it serves a large number of travelers, crosses a region where cashew kernels are produced and processed, and attracts many RCK vendors. Before sampling, approval from the vendors was obtained and discussed the facilities responsible for packing the RCK, the types of RCK packages they sell, the number of packages sold per day, and the price of each package. A total of 304 RCK samples were collected from vendors, labeled appropriately and placed in a cool box at 4 ^{o}C before being transported to the laboratory at Sokoine University of Agriculture for analysis within 6 to 8 hours of collection.

Testing the integrity of the roasted cashew kernel packages

The integrity of the RCK packages were assessed using the sterile vacuum decay system (Wilco AG, Switzerland), following the method outlined by Sivaramakrishna et al. (2007). It is worth noting that the vacuum decay leak tester was chosen because it provides a non-destructive means of testing both rigid and flexible packages. The RCK package was placed in the vacuum chamber, causing it to expand due to the pressure differential. Any leaks in the RCK package would result in the release of air into the evacuated chamber, indicating a faulty package. The percentage of faulty RCK packages was calculated based on the total samples tested.

Bacteriological analysis of roasted cashew kernels

Before conducting the microbiological analysis, RCK samples were inspected for worms and larval forms of insects using a light microscope in a sterile environment (Ayeh-Kumi 2014). The samples were then placed in separate sterile Whirl-Pak® (Nasco, Fort Atkinson, WI) bags with 100 mL of sterile distilled water. The RCK samples were left in the water for 1 minute and then vigorously shaken for 2 minutes to release bacteria. Aliquots (10 mL) of water from each sample were filtered through 0.45-µm-pore-size and 47-mm-diameter

Whatman cellulose nitrate membrane filters (Sartorius, Vienna, Austria) fitted on the membrane filtration unit (Byamukama et al. 2005). For the enumeration of Enterobacteriaceae, the filters were transferred to violet red bile glucose agar (VRBG, Difco, USA) and incubated at 37 ºC for 24 hours (ISO 21528:2). The colonies of Enterobacteriaceae were then counted and expressed as colony forming units per gram (cfu/g) of RCK. Furthermore, the hygienic condition of RCK packer's hands (n = 20) in the visited small-scale industry was investigated by requesting them to wash their bare hands in Whirl-Pak® bags with 500 mL sterile distilled water without soap, as described previously by Pickering et al. (2010). Afterward, 10 mL aliquots of the water were drawn from each of the Whirl-Pak® bags and analyzed for Enterobacteriaceae counts using a similar procedure as for the RCK samples. To understand the relationship between RCK packer's hands and the microbiological quality of the RCK, twenty (20) packs of RCK packed by the workers before washing their hands in the Whirl-Pak® bags were analyzed for Enterobacteriaceae counts using the method described previously. On the other hand, ten (10) polyethylene bags were randomly selected from those prepared by the small-scale industry for RCK packaging. Their sterility was tested by swabbing a 10 cm² area (2 cm × 5 cm) on the inside of each bag with sterile cotton swabs (Keeratipibul et al. 2017). The swabs were separately transferred to 10 mL sterile distilled water before being vigorously shaken to release bacterial cells into the water. The entire volume (10 mL) of the water sample was analyzed for Enterobacteriaceae counts using the previously described method for the Enterobacteriaceae analysis. It should be noted that sterile water was used in this study to aid recovery of Enterobacteriaceae from the samples as it has been shown to have minimal effect on the outcome of the analysis (Byamukama et al. 2005, Mushi et al. 2010, Mushi et al. 2012). The presence of Enterobacteriaceae was measured in colony forming units per two hands (cfu/2 hands) and per area (cfu/cm^2) for the tested RCK packers' hands and polythene bag surfaces, respectively.

To analyze the safety of the RCK, well-isolated colonies were obtained from VRBG agar (Difco, USA) plates and sub-cultured on nutrient agar (Merck, Germany) for purity. The nutrient agar plates were incubated aerobically at 37 ºC for 18 hours (Byamukama et al. 2005). The pure colonies were identified using the API 20E biochemical technique (bioMe´rieux SA, Marcy l'Etoile, France) following the manufacturer's instructions. Additionally, the *Escherichia coli* ATCC 25922 control strain was used per the manufacturer's instructions. In short, a well-isolated colony from the nutrient agar plate was mixed with 5 mL of sterile 0.85% sodium chloride and adjusted to match the 0.5 McFarland standards (Budiarso et al. 2023). The standardized bacterial suspension was carefully distributed into test strip tubes to avoid bubbles. Anaerobic conditions were created with sterile mineral oil, and the strips were placed in a humid environment and incubated for 24 hours at 37 ºC. Data interpretation was done using API 20E database (V4.1) with apiwebTM identification software. The occurrence of each species was quantified by dividing the number of individuals in a given species by the total number of individuals in all bacterial species in the RCK category, then multiplying by 100, and expressing it as a percentage. Additionally, RCK control samples (n = 10) were obtained from a small-scale industry immediately after roasting and analyzed in the same way as the samples collected from the bus stops. Isolates demonstrating typical *Salmonella* reactions on API 20E were first streaked onto nutrient agar for isolation and then identified and subtyped using somatic and flagellar serotyping antisera (Schrader et al. 2008).

Data analysis

Microsoft Excel 2010 (Redmond, USA) was used for the management of raw data, creation of infographics, and calculation of median values of Enterobacteriaceae in various sizes of RCK packages, as well as in well-sealed and inadequately sealed packages. Additionally, SPSS version 23 (SPSS Inc., Chicago) was used to compute the Spearman rank correlation of Enterobacteriaceae counts between RCK packers' hands and RCK surfaces, and to determine statistical differences in pack weights and Enterobacteriaceae counts among RCK categories using the Kruskal-Wallis test. Furthermore, the Mann-Whitney U test was employed to assess the difference between well-sealed and inadequately sealed packages in terms of Enterobacteriaceae counts. The significance value was established at a probability (P) level of < 0.05.

Results

Characteristics of roasted cashew kernel samples

A total of 304 RCK samples were collected from vendors, which were categorized by weight as small (35 ± 3.9 g, n = 99), medium (63 ± 4.0 g, n = 87), and large (153 ± 14 g, n = 118) based on their weight. The RCK sales ranged from 56 to 140 kg per day, equivalent to 1.5 to 4.5 million Tanzanian Shilling (TZS) per day. Chalinze bus stop (Coast region) had the highest average number of vendors and total weight of RCK, while Msamvu bus stop (Morogoro region) had the lowest average number of vendors and total weight of RCK. The small RCK packages priced at TZS 1000/= were found to be more popular than the medium-sized ones at TZS 2000/= and the large packages at TZS 5,000/=. Figure 1 illustrates different sizes of RCK packages available from vendors in the studied sites. The differences in weight between the small, medium, and large RCK packages were statistically significant ($P < 0.05$). The packages were heat-sealed and contained a mix of half and whole roasted kernels (Figure 1). Surprisingly, 45 (14.8%) RCK packages were inadequately sealed or had ruptures. These inadequately sealed packages included 33 small, 5 medium, and 7 large RCK packages. Microscopic examination did not find any worm or insect larvae in the RCK. Additionally, the RCK packages did not meet labeling standards for ready-to-eat foods, as they lacked storage conditions, nutritional contents, and shelf life information.

Figure 1: Images displaying small (A), medium (B) and large (C) RCK packages for sale by vendors in Coast (Kibaha, Chalinze) and Morogoro (Msamvu) regions in Tanzania

Microbiological quality and safety of roasted cashew kernels

The bacteriological data for RCK sampled from vendors at bus stops between October 2016 and July 2017 are presented in Figure 2. Enterobacteriaceae were continuously detected throughout the study, regardless of package size or point of sale. The counts of Enterobacteriaceae in RCK varied widely, with a median of 63 cfu/g and a range of 1 to 192 cfu/g. In contrast, Enterobacteriaceae were not detected in the unused polythene bags or on the surfaces of RCK immediately after roasting but were found in higher numbers (20 to 400 cfu/2 hands) on the hands of the RCK packers compared to the surface of the RCK (1 to 192 cfu/g). There was a significant correlation in Enterobacteriaceae counts (Spearman's rho = 0.67, $P < 0.05$) between RCK surfaces and the hands of RCK packers. Additionally, there was a notable difference in Enterobacteriaceae counts (Kruskal-Wallis test, $P < 0.05$) among categories of RCK packages. Small RCK packages were the most contaminated with Enterobacteriaceae (median: 70 cfu/g) compared to the medium (median: 7 cfu/g) and large (median: 8 cfu/g) RCK categories (Figure 2). It is also important to note that inadequately sealed or ruptured packages had higher Enterobacteriaceae counts (median: 119 cfu/g) compared to well-sealed packages (median: 26 cfu/g), and this difference was statistically significant ($P < 0.05$; Figure 3). Furthermore, Tanzania Bureau of Standards (TZS 122/ISO 6579-1, TZS 730/ISO 16649-1) require that food products like RCK meant for human consumption must be devoid of Enterobacteriaceae such as *E. coli* and *Salmonella spp*. None of the RCK samples examined in this study met this standard, highlighting serious safety issues concerning RCK, especially considering its widespread consumption in Tanzania.

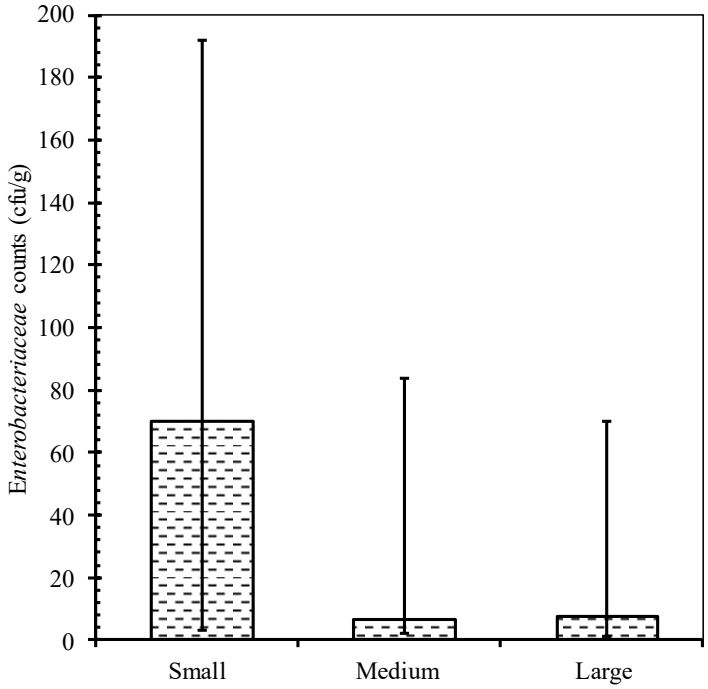

Figure 2: Small roasted cashew kernels packages were significantly contaminated with Enterobacteriaceae than medium and large packages. Bars represent median values while error bars represent minimum and maximum values.

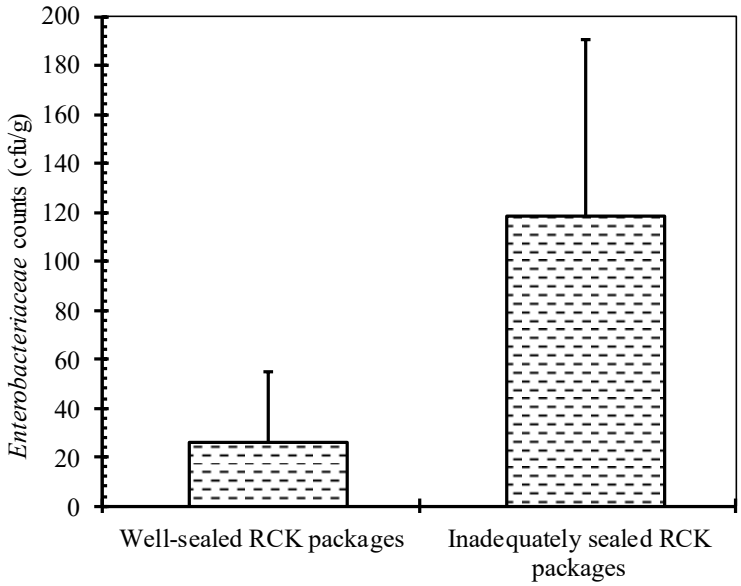

Figure 3: Well-sealed roasted cashew kernels (RCK) packages (n = 45) were characterized with low Enterobacteriaceae counts compared to inadequately sealed RCK packages (n = 262). Bars represent median values while error bars represent maximum values.

On the other hand, characterization and biochemical identification of isolates from the RCK samples revealed six species of Enterobacteriaceae belonging to five genera (see Table 1). *Klebsiella pneumoniae* had the highest frequency of occurrence across the RCK categories with 79 (65.2%) isolates, followed by *Escherichia coli* 14 (11.6%), *Proteus vulgaris* 14 (11.6%), *Enterobacter aerogenes* 6 (4.95%), *Salmonella typhi* 4 (3.31%), *Enterobacter cloacae* 2 (1.65%), *Salmonella typhimurium* 1 (0.83%), and *Salmonella enteritidis* 1 (0.83%). Overall, small packages had a higher prevalence of pathogenic Enterobacteriaceae species 55(45.45%) compared to medium 36 (29.75%) and large 30 (24.79%) packages (Table 1).

Table 1: Frequency of occurrence of bacterial isolates detected from different categories of RCK samples*.

Bacteria isolated	Small package	Medium package	Large package	Frequency	Percentage frequency
Klebsiella pneumoniae	42	17	20	79	65.2
Escherichia coli	7	6	1	14	11.6
Proteus vulgaris	0	8	6	14	11.6
Enterobacter aerogenes	2	3	1	6	4.95
Enterobacter cloaca	0	1	1	2	1.65
Salmonella typhi	3	0	1	4	3.31
Salmonella typhimurium	0	1	0	1	0.83
Salmonella enteritidis	1	0	0	1	0.83
Total	**55**	**36**	**30**	**121**	**100**

*The colour-scaled heatmap represents the number of isolates in every bacterial species against the RCK categories, frequency of isolates and the percentage frequency of isolates. Faint to bold Persian red represents the least number of isolates, faint to bold yellow represents moderate number of isolates and faint to bold green represents the highest number of isolates.

Discussion

In recent years, the value addition to cashew kernels has significantly increased in many developing countries due to the growth of the local market and efforts to reduce unemployment and maximize profits. However, there is a lack of data regarding the potential microbiological risks of locally packaged RCK to public health, despite the product's significant growth in the marketplace (Little et al. 2010; FAO 2013) and the burden of foodborne illnesses (Devleesschauwer et al. 2018). This study determines the sanitary condition and microbiological safety of locally packaged RCK by using Enterobacteriaceae (Britton et al. 2021). The study found varying levels of Enterobacteriaceae (Figure 2) in the investigated samples with higher levels of colony counts in small RCK packages than other package categories. This variation may be accounted for by the fact that the majority of the small packages were characterized with ruptures and inadequate sealing compared to the other package categories, which might have exposed the RCK to microbiological contamination (Cutter 2002). This finding was further justified by the fact that there were high levels of Enterobactericeae in the inadequately sealed/ruptured packages than the well-sealed packages (Figure 3). While previous studies have examined coliforms and *Escherichia coli* contamination on RCK in both developed (Little et al. 2009, Eglezos 2010) and developing countries (Kosoko et al. 2014, Adetunji et al. 2018, Noah and Alagamba 2019, Azizkhani et al. 2020), the present investigation is the first to examine levels (Figure 2, 3) and species composition (Table 1) of Enterobacteriaceae on RCK using a unique approach. The detection of Enterobacteriaceae in the ready-to-eat roasted cashew nuts suggests that small scale industries operate under poor hygienic conditions, such as using bare hands for RCK packaging as proven by the positive and significant correlation in Enterobacteriaceae counts between the surface of RCK and bare hands of RCK packers reported herein. This finding was further supported by the fact that no Enterobacteriaceae colony was detected immediately after the roasting process and the inside surfaces of polythene bags prepared for RCK packaging were free from Enterobacteriaceae. This study is the first to report the contribution of the RCK packer's hands to the contamination of locally packed RCK, and it suggests the need for improvement in hygienic quality maintenance of the processing environment, equipment, and workers involved in the production of packaged RCK. Additionally, the establishment of hazard analysis and critical control point programs (Little et al. 2010) is necessary to protect RCK consumers from foodborne illnesses.

The ability of Enterobacteriaceae to significantly discriminate RCK samples exposed to contamination from those which were free from contamination (Figure 3), and the occurrence of pathogenic organisms in the samples (Table 1) where Enterobacteriaceae colonies were continuously detected has proved that Enterobacteriaceae is the reliable bioindicator for poor sanitary practices and presence of pathogenic bacteria. As a result, Enterobacteriaceae may be the promising bioindicator for assessing the hygienic condition of locally packed RCK from the packaging stage to the point of consumption. Given that there are species of Enterobacteriaceae which are faecal in origin and those which are naturally found in the environment such as soil and plants (van Hoek et al. 2015), this bioindicator can also be applied to show the origin of contamination in food industries through identification of the Enterobacteriaceae colonies and classification of Enterobacteriaceae based on their origin in order to understand the nature of contamination so that appropriate quality control measures can be executed.

This study found that pathogenic Enterobacteriaceae species were present in retail packaged RCK samples at a prevalence ranging from 0.83% to 65.2% (Table 1). These pathogenic bacteria included those capable of causing respiratory diseases, such as *Klebsiella pneumoniae* (Bachman et al. 2012), as well as those causing gastrointestinal illnesses, such as *Escherichia coli*, *Salmonella typhi*, *Salmonella typhimurium*, and *Salmonella enteritidis* (Oluwafemi et al. 2009, Zhan et al. 2017, Louvau and Harris 2023). The predominance of *Klebsiella pneumonia* on RCK may be attributed to its superior tolerance to low-moisture conditions compared

to other members of the Enterobacteriaceae (Centeleghe et al. 2023). This is the first report of contamination by a wide range of pathogenic Enterobacteriaceae in retail RCK in Tanzania. Previous studies have focused on detecting specific pathogen on RCK, rather than multiple pathogens as done in this study. For example, Zhan et al. (2017) found salmonella in 0.55% of the samples collected in the USA, raising concerns about a potential salmonellosis outbreak. *Listeria monocytogenes* was detected in retail packs of ready-to-eat roasted cashew kernels immediately after packing (Taylor and Zhu 2021), and *Cronobacter malonaticus* was detected in ready-to-eat roasted cashew kernels from supermarkets in Poland (Berthold-Pluta et al. 2021). Recent reports from Nigeria (Oluwafemi et al. 2009) and the USA (Louvau and Harris 2023) showed a strong link between the consumption of ready-to-eat roasted cashew kernels and outbreaks of *Escherichia coli* 0157:H7 and Salmonellosis, respectively. These findings suggest that ready-to-eat roasted cashew kernels are an emerging food vehicle for transmitting human pathogenic microorganisms. Given the increasing consumption of ready-to-eat roasted cashew kernels in Tanzania and beyond, the presence of Enterobacteriaceae in RCK is a public health concern. Therefore, the development of evidence-based policies, procedures, and technologies to reduce the risk of RCK contamination is essential in Tanzania.

Microbiological food standards are essential in reducing the risk of food contamination and protecting people from foodborne illnesses (Gilbert et al. 2000). However, this study discovered that small-scale RCK producers in Tanzania are not following these standards as supported by the fact that there was a lack of sanitary measures during packaging and handling, package labels did not include important information such as shelf life, nutritional composition and storage condition, and Enterobacteriaceae were consistently detected in the RCK samples (Figure 2, Table 1). Non-compliance with food quality standards may be attributed to lack of awareness, weak enforcement of regulations by public health authorities, and limited access to best practices (Rahmat et al. 2016). To improve RCK safety and access global markets, local institutions should enforce food safety regulations more rigorously, and the small-scale industries should seek technical assistance from international organizations such as FAO and WHO to improve their safety systems.

The findings of this investigation provide valuable data that could raise awareness among RCK consumers and producers and inform policy makers about improving control measures for RCK safety in Tanzania. However, there were limitations in the enumeration of Enterobacteriaceae. It is possible that viable but non-cultivatable Enterobacteriaceae may have been present on RCK surfaces, including those undetectable by the applied medium due to their extremely low abundance. Additionally, the approach used to detach bacteria from the RCK surface may not have been efficient enough to ensure optimal detection of Enterobacteriaceae colonies, potentially underestimating their presence. Furthermore, clustering of colonies in some VRBG agar plates made it difficult to identify all colonies detected across the samples via biochemical approach, leading to an underestimation of potentially pathogenic species in the RCK samples. Future studies should use the most efficient approach for detecting and identifying Enterobacteriaceae in RCK samples to obtain data reflecting the real microbiological safety of RCK produced by small scale industries in Tanzania.

Conclusion

This study detected Enterobacteriaceae on the surfaces of locally packaged RCK. The results demonstrate that manual packaging and inadequate sealing are key factors contributing to the compromised microbiological quality and safety of these products within Tanzania's small-scale industry sector. However, it is important to note that this study only included three sampling locations and a one-year sampling program, so the findings may not be fully representative of all locally packaged RCK in Tanzania. As a result, further microbiological and epidemiological research is necessary to gain additional understanding of the safety and potential public health impact of RCK. To prevent the spread of bacterial pathogens to humans through contaminated RCK, Tanzania's environmental health officials should educate RCK manufacturers on local and international food safety regulations and good manufacturing practices. Government food agencies must enforce food quality and safety regulations, conduct inspections, and ensure that RCK manufacturers follow good manufacturing practices. RCK packaging industries should create a hazard analysis and critical control points (HACCP) plan to identify potential contamination points in the production process and implement measures to reduce these risks. It is crucial for RCK packaging industries to use appropriate packaging materials that protect against moisture and contaminants and ensure that packaging processes are carried out in hygienic conditions to prevent cross-contamination. Additionally, environmental health officials should train RCK vendors on good hygiene practices to maintain high standards in the RCK vending business and safeguard public health.

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Conflict of interest

The authors have no conflicts of interest to declare

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