Value Chain Hygiene Practices and Microbial Contamination of Street and Market Vended Ready-to-Eat grasshopper, Ruspolia differens in Uganda: Implications for food safety and public health

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Abstract

Background

Food safety is a major public health issue, particularly in developing countries. Ready-to-eat street-vended foods contribute to daily dietary life, but with elevated food safety burden. Here, hygiene and food safety practices as well as the microbial contamination in Uganda's edible grasshopper value chain was evaluated.

Methods

This was a cross-sectional mixed methods study with both qualitative and quantitative approaches. A face-to-face administered questionnaire and observational checklists were used to collect data. A total of 29 households (Kampala; 12 and Masaka; 17) participated, and grasshopper samples collected along the value chain. Indicator pathogens were analysed using standard microbiological methods.

Results

Sociodemographic characteristics reveal that two-thirds of households in Kampala and 53% in Masaka were female headed. In Kampala and Masaka, respectively, 50% and 12% of households had earth or sand floors. All households in Kampala were one or two-roomed dwellings with no separate room as a kitchen, and shared a toilet compared to 43%, 65% and 53%, respectively, in Masaka. 83% households in Kampala and 56% in Masaka obtained drinking water from public taps. Handwashing was inadequate and none of the actors was observed to wash their hands after taking a break or handling waste. Only 8.3% respondents had raised racks for drying utensils. For vendors, wearing protective clothing was not common, with only 28.5% in Kampala and 30.8% in Masaka wearing an apron. Containers for vending grasshoppers were largely uncovered and the utensils (spoon or cup) for measuring the grasshoppers were left mainly uncovered during the observation period. Indicator organisms, *E. coli* and *Salmonella typhimurium*, were detected. *E. coli* was the most common contaminant, but with lower levels in Masaka compared to Kampala. *Salmonella typhimurium* was mainly a burden in Kampala.

Conclusions

Our findings demonstrate that there are enormous contributors to poor hygiene and sanitation along the value chain. The existence of pathogenic bacteria such as *E. coli* in ready-to-eat foods imply that their consumption pose a health risk. There is an urgent need to create awareness among the actors,through regular trainings on food safety and personal hygiene practices in food handling and inspection to prevent foodborne disease outbreaks.

Background

Street foods are ready-to-eat foods and beverages, processed or fresh that are sold at stationary locations or by mobile vendors in streets and open places as opposed to stores and licensed establishments [1]. These foods contribute significantly to the diet of many, and their consumption is an essential contributor to the dietary intake of mostly urban and peri-urban populations in developing countries [2]. Street foods are responsible for daily energy intake ranging from 13–50% and 13–40% in adults and children, respectively [2]. Street foods present several advantages to the urban poor as they are convenient, cheap, and easily accessible and a source of income for many who otherwise would not have livelihoods. Unfortunately, the street food trade in many countries is largely unregulated, associated with poor hygiene and safety issues, and is thus considered a public health threat [3].

Concerns related to water and sanitation facilities, as well as the knowledge and expertise of street food handlers, are rising [4]. For example, in an urban area in Brazil, 95% of street vendors did not wash their hands between food and money transactions and restroom breaks, and 100% did not have access to a water supply [5]. Relatedly, a study conducted in Cape Town, South Africa, showed that 77% of vendors handled food and money simultaneously, and soap was not available at 86% of stalls [6]. Literature, mainly from Africa, indicates that major life-threatening pathogens such as *Escherichia coli* (*E. coli*), *Salmonella typhimurium (S. Typhimurium)* and *Staphylococcus aureus* (*S. aureus*) are prevalent in street foods [7]. A study in Uganda showed that *S. aureus* was present in about 85% of beef and chicken samples and in 75% of goat meat samples, while *E. coli* was present in up to 50% of meat samples hawked at markets along highways [8]. Similarly, a study by Mugampoza et al. [9] found *E. coli* levels in 100% and 60%, respectively of all studied street foods sold around Nakawa and Naguru Parishes within Kampala City.

Edible insects are a potential food security solution because of their nutritive value and low environmental impact [10]. However, the presence of pathogenic microorganisms commonly associated with edible insects and their products is of a great concern [11]. This issue could be related to how insects are handled, processed and/or packaged [12]. A review of articles published between 2000 and 2019 on a variety of edible insects, both raw and cooked, showed complex ecosystems, with marked variations in microbial load and diversity including mesophilic aerobes, bacterial endospores, *Enterobacteriaceae*, lactic acid bacteria, psychrotrophic aerobes, and fungi, and potentially harmful species (i.e.. pathogenic, mycotoxigenic, and spoilage microbes) [13].

In Uganda, the long-horned edible grasshopper, *Ruspolia differens* (Serville) (Orthoptera: Tettigoniidae), locally known in Luganda as ‘nsenene’ is a common street-vended food and is considered a delicacy [14]. During the swarming seasons of April-May and November-December each year [15], they are hawked on the streets of major cities and towns around the Lake Victoria basin, including Kampala and Masaka Cities. Prior to hawking on streets, primary “processors”, usually low-income slum dwellers, pluck off the appendages (wings and legs), and at times fry the grasshoppers ahead of distribution to market vendors and hawkers who sell them, raw or fried, on the city streets and in the suburbs [15]. Previous studies of raw and fried edible grasshoppers in Uganda and Tanzania reported high microbial counts and potential pathogens, including *Campylobacter* and *Staphylococcus* [16, 17], making edible grasshoppers a potential source of foodborne diseases. Despite its increased recognition as a food resource and a major protein source [10], the edible insect value chain still has policy loopholes regarding harvesting, transportation, trading and processing [18], leading to potential contamination [3], thus creating opportunity for disease outbreaks caused by the microbial and chemical food contaminants. For public health purposes, it is therefore important to understand how each actor in the edible grasshopper value chain potentially contributes to the contamination of processed grasshoppers, especially those marketed as ready-to-eat (RTE). However, the information on the microbial safety and quality of ready-to-eat street-vended edible insect products sold in Ugandan cities is limited. Therefore, the present study was designed to document and evaluate the hygiene practices of key actors in the value chain of RTE edible grasshoppers in cities of Kampala and Masaka. Specifically, we set out to: (i) document the hygiene practices along the RTE grasshopper value chain, including the personnel involved and (ii) determine the level of microbial contamination in the RTE grasshoppers at different stages of the value chain. The findings of this study have the potential to inform public health policy, and to strengthen regulation of market- and street-vended RTE edible grasshoppers as well as other street-vended foods.

Materials And Methods

Study site

The study was carried out in the cities of Kampala and Masaka districts located in central Uganda (Fig. 1). These sites were purposively selected because both are areas where edible grasshoppers are in very high demand and are widely consumed. Masaka is considered the traditional source of edible grasshopper and is the leading supplier for Kampala City. Katanga and Kisenyi in Kampala city and Nyendo-Ssenyange in Masaka city were purposively selected because they have a high concentration of edible grasshopper businesses. Also, Nyendo-Ssenyange is the main grasshopper harvesting hub [19]. Coincidentally, these areas are also characterized largely by informal settlements (slums). Most dwellers are low-income earners who shift trade to grasshopper harvesting and processing during the main swarming seasons in April-May and November-December each year.

Study Design And Data Collection Tools

This was a cross-sectional mixed methods study with both qualitative and quantitative approaches. Data was collected by trained field assistants in November and December 2021 during the season of grasshopper harvest. Questionaire (adapted from UBOS [20]) and observation check lists adapted from Hill et al. [6], were used to collect food hygiene practices and sanitation status of the actors (vendors and processors). The questionnaire was pre-tested in Nateete, a city suburb to the west of Kampala, with similar social, economic and demographic dynamics to the sampled areas and appropriate modifications made. Questions asked included the socio-demographic characteristics of the household heads (e.g., age, native language and housing infrastructure, food handling practices and food safety knowledge of the vendors, and access to clean water supply and other sanitary facilities). The observation checklists focused on assessing the hygienic practices of food handlers, waste handling and disposal, food handling practices and sanitary facilities, including toilet and hand washing facilities. The hygienic practices were evaluated as either yes or no, then expressed in proportions.

Participant Enrolment

Participation in the study was open to all active actors (processors and vendors) in the selected areas. All enrolment procedures were conducted at the study site by the study team. Prior to participant enrolment, the study investigators held meetings with the area Local Councils and the leadership of the grasshopper actors, the “Basenene”-a derivation from the local name of the edible grasshopper, “nsenene”, in each study area to confirm their interest in the study, establish contact persons, and obtain the number of the actors in each locality. Given the informal nature of this trade, vendor and processor lists were not readily available with the area leadership nor the “Basenene”. Therefore, snowballing sampling was used to locate all the vending and processor household heads.

Data Collection

For Masaka, all HHs (17; M1-M17)involved in grasshopper processing were included in the study. However, for Kampala, due to the irregular season in November-December 2021 and the intermittent supply of grasshoppers to Kampala (https://ugandaradionetwork.net/story/grasshopper-trappers-feel-the-pinch-as-season-enters-18-days-without-catch), the selected study areas were visited till late December to interview and survey from all participating 12 HHs (K1-K12). All HHs visited were interviewed and grasshoppers were sampled as follows: fresh, with wings and legs (Sample 1); “dressed” grasshoppers with wings and legs plucked off (Sample 2) and fried RTE grasshoppers (Sample 3). Then, individual hawkers/vendors supplied with RTE grasshoppers from the interviewed households were followed up and observed using a checklist between 12:00–14:00 hours, the peak time for RTE grasshopper sales. Each vendor was closely observed for approximately two hours to note behaviours before and during active trade. A sample (sample 4) of RTE grasshoppers was then purchased from each of the followed up hawker/vendor. Precisely, indicator pathogenic microorganisms of poor hygiene, such as *E. coli* and *S. typhimurium* were tracked at each stage of the value-addition process. For households K08 and K10, obtaining samples for the final stage from the vending points was impossible. Street vendors are reported to be at risk of arrest by city authorities, who usually confiscate their merchandise [21]. To circumvent this, vendors opt to hawk the grasshoppers in bars at night. Households K09 and K11 in Kampala reported exporting their processed grasshoppers to distant places like the UK and USA, as reported by [22]. Therefore, we could only obtain relevant data up to stage 2. Households M13, M14 and M15 indicated a specialization in trading only in live “winged and de-winged” grasshoppers (stage 1 and 2 samples), and only fried the leftover “unsold” ones later in the night (the would-be sample 3), for selling at a later date, ostensibly at a higher price. Therefore, the data collectors (who followed up with the HHs the following day) could only obtain samples 1, 2 (on day 1) and 4 (on day 2) from the indicated HHs, while for HHs M16 and M17, reported removing appendages in the wee hours, thus the grasshoppers had already undergone the first stage of processing by the time the data collectors arrived at the HHs. All samples were immediately packaged into sterile Whirl-Pak bags (Nasco, USA) and placed in a cold box with ice packs. Samples from Kampala were immediately transported to the Microbiology laboratory, Department of Biochemistry, Makerere University on the collection day. In contrast, samples from Masaka, about 140 km away from Kampala, were frozen and later transported to the same laboratory (Microbiology laboratory) in a portable freezer before being transferred to -20°C until analysis.

Assessment Of Microbial Contamination Along The Value Chain

Ten gram (10g) portions of each grasshopper sample (i.e., 27 sample 1, 29 sample 2, 24 sample 3, and 25 sample 4) were suspended in 100 mL of distilled water in conical flasks and agitated gently for 1 hour to enable suspension of any microbes present. The remaining suspension was decanted off, centrifuged at 5000 rpm for 10 min and the pellet stored at – 80°C. Thereafter, aliquots of 1 mL of each sample were pipetted into sterile microfuge tubes and labelled according to the household number and sampling site for further analysis of culturable microorganisms. To determine the presence of potential pathogens in the samples, aliquots of 100 µL from each suspension were serially diluted up to 10^3. Subsequently, 100 µL of both 10^2 and 10^3 dilutions was plated onto MSA agar media (composed in g/L of mannitol [10 g], beef extract [1 g], tryptone [5 g], sodium chloride [75 g], phenol red [0.025 g] and Agar [15 g]) and XLD (HiMedia Laboratories Pvt. Ltd, Mumbai, India). Another aliquot of 100 µL was diluted to approximately 10 mL and filtered through a membrane filter. Each filter was transferred to the *E. coli*-chromogenic medium (Condalab Conda S.A, Madrid, Spain). The plates were then incubated at 37 ^{o}C for 48 hours. *E. coli* was identified by colonies turning blue on the *E. coli*-chromogenic medium, while presumptive *S. typhimurium* appeared red with a black center on XLD. Other colonies on XLD which also appeared red, possibly *Shigella* and other genera, did not have black centers and were grouped as other red colonies (Fig. 2). It was also noted that various other types of bacteria were able to grow on XLD, producing yellow colonies. Additionally, various isolates were observed to grow at high salt concentrations and fermented mannitol, thus lowering the overall pH of the medium, signified by the phenol-red indicator turning yellow. Although MSA is primarily selective for *S. aureus*, other microbes were identified based on their colony morphologies, so the microorganisms that grew on MSA agar were generally grouped as mannitol fermenters. Representative isolates that could be cultured on both XLD and MSA were purified by repeated streaking on the same media and cultured in nutrient broth, then stored as glycerol stocks at -25 ^{o}C for further phylogenetic analyses.

Data analysis

Sociodemographic and household characteristics from the processor HHs and data from the observation checklists (HHs and street vendors) were entered into MS Excel, cleaned and then imported to Statistical Package for Social Scientists (SPSS version 23, SPSS Inc., Chicago, IL, USA) for analysis. Frequencies and proportions (%) of sociodemographic and household characteristics and checklist variables by study site were obtained using cross-tabulation. Data from observation checklists was further transformed into a hygiene practices score whereby each positive practice was scored 1, and negative practice scored 0. An environment and equipment hygiene score was similarly created. Mean scores were calculated for processors and for vendors separately. Scores for individual processors and vendors above and below the group means were categorized as good and inadequate hygiene practices. Process hygiene criteria for minced meat [16], were used for the first and second processing stages (Samples 1 and 2, respectively), whereby the guideline lower limit for *E. coli* was 50 colony-forming units (cfu)/g and the upper limit 500 cfu/g. Uganda National Bureau of Standards (UNBS) Edible Insects Standard-2020 (US 2146:2020), was used for processing the third processing stage (Sample 3) and for the vendors (Sample 4), whereby *E.coli, Staphylococcus spp.* and *Salmonella spp.* should be absent.

Results And Discussion

Socio-demographic profile of the Households

A total of 29 households participated in the study, i.e., 12 in Kampala and 17 in Masaka (Table 1). Majority of respondents were female (67% in Kampala and 53% in Masaka) and were household heads (83% in Kampala and 71% in Masaka), implying that most of the HHS involved in the grasshopper processing are female-headed, though a study by [15] found 86% and 14% male and female, respectively, in grasshopper business in Uganda. However, a different study [23] also found that 64% of HHs were female-headed in Banda, a low-income, densely populated (slum) suburb of Kampala City. Notably, while Kampala and Masaka are in the Central Region of Uganda with Luganda as their native language, none of the respondents in Kampala reported Luganda as their native language, compared to 14/17 (82%) of the respondents in Masaka. This indicates that the participant HHs in Kampala originated from outside Buganda, which is typical of Kampala's low-income, densely-populated informal settlements (slums) where impoverished migrants from rural areas first settle. Indeed, a study exploring the livelihoods of the urban poor found that only 14% of respondents were born in Kampala [24]. Compared to Masaka, a higher proportion of participating households in Kampala had earth or sand floors (50% vs 12%); one or two-roomed dwellings (100% vs 41%); no room dedicated as a kitchen (100% vs 65%) and shared a toilet (100% vs 53%) further reflecting the lower income status of the participating Kampala households. A study by Mukiibi [25] in Kampala, showed that increased housing demand, rising land prices and growing urban poverty in the city had reduced low-income earners’ accessibility to decent shelter. Most households obtained drinking water from public taps/standing pipes (83% in Kampala and 56% in Masaka), as also highlighted by Ssemugabo et al. [26]. However, in Masaka, some HHs also obtained their drinking water from public boreholes (11%) and protected wells/springs (6%).

Table 1
Sociodemographic and household characteristics of edible grasshopper processors in Kampala and Masaka cities, central Uganda

Variable		Kampala (12)	Masaka (17)	All (29)
Age	Mean (SD)	37.2	34.8	39.0
	Min, Max	25, 49	17, 66	17, 66
Respondent	Females, number (%)	8 (67)	9 (53)	17 (59)
	Head of household	10 (83.3)	12 (70.6)	22 (75.9)
	Spouse	2 (16.7)	3 (17.6)	5 (17.2)
	Child	0	2 (11.8)	2 (6.9)
Native language	Runyakitara	11 (91.7)	3 (17.6)	14 (48.3)
	Lugbara	1 (8.3)	0	1 (3.4)
	Luganda	0	14 (82.4)	14 (48.3)
HH members	1,2	3 (25)	3 (17.6)	6 (21.7)
	3 to 5	7 (58.3)	4 (23.5)	11 (37.9)
	6 plus	2 (16.7)	10 (58.8)	12 (41.4)
Roof	Iron sheets	12 (100)	13 (76.5)	25 (89.3)
	Cement slab	0	3 (17.6)	3 (10.7)
	Other	0	1 (5.9)	1 (3.6)
Walls	Unburnt clay bricks with cement plastering	1 (8.3)	1 (5.9)	2 (6.9)
	Cement blocks	3 (25)	0	3 (10.3)
	Other	8 (66.7)	16 (94.1)	24 (82.8)
Floor	Earth or sand	6 (50)	2 (11.8)	8 (27.6)
	Cement	6 (50)	13 (76.5)	19 (65.5)
	Bricks	0	1 (5.9)	1 (3.4)

[1]Total for Msk is 18 because one HH has two sources. [2]Total for Msk is 18 because one HH uses two types of toilet

Variable		Kampala (12)	Masaka (17)	All (29)
	Other	0	1 (5.9)	1 (3.4)
Rooms	1, 2	12 (100)	7 (41.1)	19 (65.5)
	3 plus	0	10 (58.8)	10 (34.5)
Bedrooms	1	12 (100)	6 (35.2)	18 (62.1)
	2 plus	0	11 (64.7)	11 (37.9)
Kitchen	Yes	0	6 (35.3)	6 (20.7)
	No	12 (100)	11 (64.7)	23 (79.3)
Lighting	Electricity National Grid	11 (91.7)	15 (88.2)	26 (89.7)
	Electricity Solar	0	2 (11.8)	2 (6.9)
	Other	1 (8.3)	0	1 (3.4)
Cooking	Charcoal	12 (100)	16 (94.1)	28 (96.6)
	Firewood	0	1 (5.9)	1 (3.4)
Drinking water[1]	Piped into dwelling	0	2 (11.1)	2 (6.7)
	Piped to yard/plot	1 (8.3)	1 (5.6)	2 (6.7)
	Public tap/standing pipe	10 (83.3)	10 (55.6)	20 (66.7)
	Bottled water	1 (8.3)	1 (5.6)	2 (6.7)
	Borehole in yard/plot	0	1 (5.6)	1 (3.3)
	Public borehole	0	2 (11.1)	2 (6.7)
	Protected public well/spring	0	1 (5.6)	1 (3.3)
Toilet[2]	Pit latrine - Covered - No slab	1 (8.3)	5 (27.8)	6 (20.0)
	Pit latrine - Covered - with slab	1 (8.3)	12 (66.7)	13 (43.3)
	Flush or pour flush toilet	10 (83.3)	1 (5.6)	11 (36.7)

[1]Total for Msk is 18 because one HH has two sources. [2]Total for Msk is 18 because one HH uses two types of toilet

Variable		Kampala (12)	Masaka (17)	All (29)
Toilet shared	Yes	12 (100)	9 (52.9)	21 (72.4)
	No	0	8 (47.1)	8 (27.6)
Toilet handwash facility	Yes	8 (66.7)	15 (88.2)	23 (79.3)
	No	4 (33.3)	2 (11.8)	6 (20.7)
Handwash place observed	No specific place for handwashing	11 (91.7)	17 (100)	28 (96.6)
	Handwashing place observed	1 (8.3)	0	1 (3.4)

[1]Total for Msk is 18 because one HH has two sources. [2]Total for Msk is 18 because one HH uses two types of toilet

Hygiene And Food Safety Practices Of Street Food Vendors And Processors

Tables 2A–C provide data on the hygiene and food safety practices at the grasshopper processing household level and of the street vendors. Overall, at the household level, handwashing was inadequate: Only five out of all 29 participating HHs in Kampala and Masaka were observed to wash their hands after each processing stage; none were observed to wash their hands after taking a rest or handling waste; only five out of all 29 HHs had a handwashing facility with running/flowing water and only two out of all 29 HHs had a handwashing facility with soap. Regarding environment and equipment hygiene (Table 2B), raised racks for drying utensils were rare across both Kampala and Masaka (three out of all 29 HHs). Furthermore, four of the 12 HHs in Kampala and 12 of the 17 HHs in Masaka placed grasshoppers on the ground during the plucking of wings (first stage of processing). Notably, compared to 10 out of 17 HHs in Masaka, only two of the 12 HHs in Kampala had a rubbish dump more than 15 m away from the processing area, characteristic of the low-income, densely populated informal settlements of Kampala. The impediments resulting in poor sanitation in poor urban communities had earlier been reported [26, 27].

Table 2
Hygiene practices of edible grasshopper processors and vendors in Masaka and Kampala cities, Uganda A) Personal hygiene practices

	Kampala (N = 12)				**Masaka (N = 17)**				**All (N = 29)**			
	Yes		No		Yes		No		Yes		No	
Part A: Personal Hygiene Practices	n	%	n	%	n	%	n	%	n	%	n	%
Clothes are clean	10	83.3	2	16.7	13	76.5	4	23.5	23	79.3	6	20.7
Wearing a hairnet or head-cloth	3	25.0	9	75.0	4	23.5	13	76.5	7	24.1	22	75.9
Fingernails short and clean	10	83.3	2	16.7	13	76.5	4	23.5	23	79.3	6	20.7
Does not touch nose, mouth, hair and skin	9	75.0	3	25.0	13	76.5	4	23.5	22	75.9	7	24.1
Does not lick fingers	10	83.3	2	16.7	13	76.5	4	23.5	23	79.3	6	20.7
Does not touch pimples or sores	10	83.3	2	16.7	14	82.4	3	17.6	24	82.8	5	17.2
Does not cough onto the grasshoppers	10	83.3	2	16.7	11	64.7	6	35.3	21	72.4	8	27.6
Not wearing rings on fingers	12	100.0	0	0.0	15	88.2	2	11.8	27	93.1	2	6.9
Does not have wounds or cuts on hands	12	100.0	0	0.0	13	76.5	4	23.5	25	86.2	4	13.8
Wound on the hand is covered with a plaster	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Does not have flu-like symptoms	10	88.9	2	11.1	15	88.2	2	11.8	25	86.2	4	13.8
Hands washed after each processing stage	2	16.7	10	83.3	3	17.6	14	82.4	5	17.24	24	82.8
Hands washed after a break	0	0.0	12	100.0	0	0.0	17	100.0	0	0.00	29	100.0

	Kampala (N = 12)				Masaka (N = 17)				All (N = 29)			
Hands washed after handling waste	0	0.0	12	100.0	0	0.0	17	100.0	0	0.00	29	100.0
Hands washed after cleaning duties	0	0.0	12	100.0	3	17.6	14	82.4	3	10.34	26	89.7
Hand-wash place has running/flowing water	2	16.7	10	83.3	3	17.6	14	82.4	5	17.24	24	82.8
Hand-wash place has soap	0	0.0	9	100.0	2	11.8	15	88.2	2	6.90	24	93.1

B) Hh Environment And Equipment Hygiene

	Kampala (N = 12)				Masaka (N = 17)				All (N = 29)			
	Yes		No		Yes		No		Yes		No	
Processsing area and equipment hygiene	n	%	n	%	n	%	n	%	n	%	n	%
Utensils are clean	12	100.0	0	0.0	15	88.2	2	11.8	27	91.7	2	8.3
Wash place for utensils is clean	7	58.3	5	41.7	10	58.8	7	41.2	17	58.3	12	41.7
Drying rack/katandaro available	2	16.7	10	83.3	1	5.9	16	94.1	3	8.3	26	91.7
Drying rack used to dry utensils	2	16.7	10	83.3	3	17.6	14	82.4	5	16.7	24	83.3
Grasshoppers are not placed on the ground	8	66.7	4	33.3	5	29.4	12	70.6	13	45.8	16	54.2
No domestic animals roaming around	9	75.0	3	25.0	13	76.5	2	23.5	22	75	7	25
No stagnant water nearby	10	83.3	2	16.7	17	100.0	0	0.0	27	95.8	2	4.2
Solid waste is safely disposed off	8	66.7	4	33.3	16	94.1	1	5.9	24	75	5	25
Wastewater is safely disposed off	9	75.0	3	25.0	12	70.6	5	29.4	21	70.8	8	29.2
Latrines are at least 15 m from the processing area	10	83.3	2	16.7	15	88.2	2	11.8	25	87.5	4	12.5
The rubbish dump is at least 15 m away	2	16.7	10	83.3	10	58.8	7	41.2	12	37.5	17	62.5
Not many flies hovering around (more than 10)	4	33.3	8	66.7	9	52.9	8	47.1	13	41.7	16	58.3
Fried grasshoppers are in a covered container	10	83.3	2	16.7	8	47.1	9	52.9	18	58.3	11	41.7
Rubbish eg left over food not scattered around	9	75.0	3	25.0	12	70.6	5	29.4	21	70.8	8	29.2
Fried grasshoppers are not put in dirty containers	9	75.0	3	25.0	13	76.5	4	23.5	22	75	7	25
Separate containers used (raw and fried)	9	75.0	3	25.0	16	94.1	1	5.9	25	87.5	4	12.5

	Kampala (N = 12)				Masaka (N = 17)				All (N = 29)			
Children are not playing around	4	33.3	8	66.7	14	82.4	3	17.6	18	62.5	11	37.5
Grasshoppers are washed before frying	12	100.0	0	0.0	17	100.0	0	0.0	29	100	0	0

C) Rte Grasshopper Vendors

	Kampala (N = 7)				Masaka (N = 13)				All (N = 20)			
	Yes		No		Yes		No		Yes		No	
Practices	n	%	n	%	n	%	n	%	n	%	n	%
Clothes are clean	5	71.4	2	28.6	13	100.0	0	0.0	18	90.0	2	10.0
Wearing apron or lesu or coat	2	28.6	5	71.4	4	30.8	9	69.2	6	30.0	14	70.0
Wearing a hairnet or head-cloth	1	14.3	6	85.7	1	7.7	12	92.3	2	10.0	18	90.0
Fingernails short and clean	5	71.4	2	28.6	12	92.3	1	7.7	17	85.0	3	15.0
Not touching nose, hair and skin during work	6	85.7	1	14.3	13	100.0	0	0.0	19	95.0	1	5.0
Does not lick fingers	7	100.0	0	0.0	12	92.3	1	7.7	19	95.0	1	5.0
Does not touch pimples or sores	7	100.0	0	0.0	13	100.0	0	0.0	20	100.0	0	0.0
Does not cough or sneeze	7	100.0	0	0.0	13	100.0	0	0.0	20	100.0	0	0.0
Does not have wounds or cuts on hands	7	100.0	0	0.0	13	100.0	0	0.0	20	100.0	0	0.0
Doesn not have flu-like symptoms	7	100.0	0	0.0	12	92.3	1	7.7	19	95.0	1	5.0
No stagnant water nearby	6	85.7	1	14.3	13	100.0	0	0.0	19	95.0	1	5.0
Latrine is at least 15 m away from vendor	7	100.0	0	0.0	11	84.6	2	15.4	18	90.0	2	10.0
Rubbish dump is at least 15 m from vendor	6	85.7	1	14.3	9	69.2	4	30.8	15	75.0	5	25.0
Not many flies (≥ 10) hovering around	6	85.7	1	14.3	10	76.9	3	23.1	16	80.0	4	20.0
Fried grasshoppers are in covered containers	5	71.4	2	28.6	5	38.5	8	61.5	10	50.0	10	50.0
No rubbish around the vending point	5	71.4	2	28.6	9	69.2	4	30.8	14	70.0	6	30.0
No bare hand touching the grasshoppers	7	100.0	0	0.0	11	84.6	2	15.4	18	90.0	2	10.0

	Kampala (N = 7)				Masaka (N = 13)				All (N = 20)			
Cover to grasshopper measuring utensils	3	42.9	4	57.1	2	15.4	11	84.6	5	25.0	15	75.0
Hand-washing point nearby	0	0.0	7	100.0	3	23.1	10	76.9	3	15.0	17	85.0

All HHs in Kampala and Masaka were observed to wash the grasshoppers before frying them. However, this may not necessarily have been motivated by hygiene and food safety, given that processors use ash or sand to counter the oiliness of the grasshoppers while plucking off the legs and wings. The main aim of washing the grasshoppers is to enhance the mouthfeel by removing the ash or sand. The use of protective clothing amongst vendors was not common: only two of the seven vendors observed in Kampala, and four out of 13 in Masaka wore an apron, while one in each of Kampala and Masaka wore a hairnet or head covering. Largely, RTE grasshoppers were not vended in closed containers (two out of seven vendors in Kampala and eight out of 13 vendors in Masaka). The utensils (spoon or cup) used to measure the grasshoppers was not covered for over half (four out of seven) vendors in Kampala and almost all (11 out of 13) vendors in Masaka. All vendors in Kampala did not have a hand-washing facility nearby, while only three out of 13 in Masaka did. Regardless of the country or product sold, African street vendors appear to share common unhygienic practices. For example, a study in Ethiopia found that 88.6% of vendors did not wear aprons and 95% had their hair uncovered during cooking. In comparison, all the vendors (100%) surveyed handled money with bare hands while serving food [28]. These drivers of poor sanitation were also observed in the current study (Tables 2A-C).

Microbial Contamination Along The Value Chain

The level of hygiene among actors in the grasshopper value chain is critical for the safety of the final consumers. Poor hygiene practices among the processors and/ or vendors along the value chain can introduce potential pathogens into the grasshoppers. The results of microbial contaimination along the grasshopper value chain are summarized in Fig. 2.

A total of 105 edible grasshopper samples (27 sample 1, 29 sample 2, 24 sample 3 and 25 sample 4) were analyzed for the presence of bacterial pathogens. The results of this study revealed that unprocessed grasshoppers in both Kampala and Masaka were contaminated with indicator microbes (Fig. 2). Overall, 85.2%, 40.7%, 48.2% and 96.3% of the unprocessed grasshoppers were contaminated with *E. coli, S. typhimurium*, XLD fermenters and mannitol fermenters, respectively. The existence of these indicator organisms in the raw, unprocessed grasshoppers suggests that some of the contamination may come from outside the value chain actors. Grasshoppers could potentially get contaminated from where they originate, given that they are thought to swarm to the point of harvest from “unknown sources”, but a study by Opoke et al. [29] suggests a local origin from which they aggregate and swarm upon maturity. Previous studies by Ssepuuya et al. [16] and Labu et al. [30] show that grasshoppers surveyed at the point of harvest already had high levels of contamination with potential human pathogens.

Contamination levels for all studied indicator organisms were reduced with processing (Fig. 1). This agrees with the findings of Labu et al. [30], who observed that bacterial and fungal counts in processed grasshoppers were generally lower than in freshly harvested (unprocessed) raw insects. Heat processing (frying) would be expected to eliminate any contaminating microorganisms in the grasshoppers. Indeed, this was a general observation, except for the observed re-contamination in households K08 and K10 in Kampala and M02, M03 and M08 in Masaka (Fig. 2). Food re-contamination is largely associated with poor hygiene practices, such as not washing hands after handling waste or after taking a break [31]. In this study, handwashing was inadequate at the household level, with only five (17.2%) out of all 29 participating HHs in Kampala and Masaka carrying out hand washing after each processing stage (simultaneously handling both cooked and raw grasshoppers), and none were observed to wash their hands after taking a break or handling waste.

Contamination with *Salmonella typhimurium* was mainly a burden among Kampala grasshopper processors compared to those in Masaka (Fig. 2). The major reservoirs of *Salmonella* are food animals, such as poultry, pigs and cows; however, humans, especially infected food handlers, and contaminated environments are also reservoirs of *Salmonella* [32]. Because the grasshoppers in this study were wild-harvested, the presence of *S. typhimurium* suggests infected processors and/or contaminated environments in the Kampala processing HHs. This is supported by the HH characteristics data, as already discussed, exemplified by Kampala's low-income, densely-populated informal settlements (slums).

It is also noteworthy that *Staphylococcus aureus*, one of the key mannitol fermenters (Fig. 2), is a usual constituent of the microbiota of the body, frequently found in the upper respiratory tract and on the skin in about 30% of humans [33]. However, it can also become an opportunistic pathogen and is a common cause of skin infections, respiratory infections, and food poisoning [34]. The presence of mannitol fermenters in the stage 3 (fried) and stage 4 (vended) grasshopper samples potentially indicates that the RTE have been contaminated by the food handlers themselves. Hand hygiene is key in the prevention of *Staphylococcus* infections, and RTE foods are especially risky if contaminated with *S. aureus*. As observed in this study, hand hygiene was poor, making RTE grasshoppers a hazard for *S. aureus* infection. *Salmonella typhimurium*, *S. aureus* and other enteric pathogens associated with man are highly vulnerable to destruction by heat treatment and nearly all sanitizing agents. The fact that grasshoppers, like other RTE foods are usually consumed without further heating indicates that the consumers have a lot of confidence in the processors [35]. However, based on the findings of this study, this confidence is misplaced especially for the RTE grasshoppers vended in Kampala. As observed in this study (Table. 2), the surveyed areas lacked good access to clean water, adequate sanitation and proper waste management, thus raising the transmission possibility through contaminated water, utensils and environments during the preparation of RTE foods. Therefore, contamination and recontamination at all levels along the value chain should be avoided.

Generally, it was recognized that samples from Masaka were less contaminated by all classes of indicator organisms compared to those in Kampala (Fig. 3). The reason for this is unclear but could be related to poor housing infrastructure and general sanitation in Kampala slums compared to Masaka, which had relatively better housing and sanitation infrastructures. A related study by Labu et al. [30] carried out in both localities, found the mean bacterial counts in *R. differens* samples from Masaka (season 1 (April-May) were significantly higher than those in samples from Kampala. However, there was no significant difference in season two (November-December), but microbial species were most diverse in wild, freshly harvested samples.

Conclusion

Our study findings demonstrate that there are enormous contributors to poor hygiene and sanitation along the value chain, resulting in possible contamination and re-contamination with potential pathogens. The existence of pathogenic bacteria such as *E. coli*, *S. typhimurium*, and *S. aureus* in readty-to-eat foods imply that consumption these foods are a health risk for consumers. There is an urgent need for government and the public health department to create awareness among street and market vendors and consumers through regular trainings on food safety and personal hygiene practices in food handing and inspection to prevent foodborne disease outbreaks. Furthermore, since the grasshopper business generates income for participating households, it is recommended that Uganda National Bureau of Standards regulates the grasshopper value-chain business. Finally, government should provide basic social services/infrastructure, e.g., water, sanitation facilities to improve the working conditions of street vendors, including RTE grasshopper vendors.

Declarations

Ethics approval and consent to participate

All methods were performed in accordance with the relevant guidelines and regulations of the Declaration of Helsinki. The study protocol and informed consent documents were reviewed and approved by the Makerere University School of Social Sciences Research and Ethics Committee (No. MAKSSREC09.21.496) and registered with Uganda National Council for Science and Technology (No. HS1869ES). Participants provided written informed consent for their interviews.

Consent for publication

Not applicable

Availability of data and materials

The data sets generated and or analyzed during the current study are not publicly available. However, they can be accessed from the corresponding author upon reasonable request.

Competing interest

None declared.

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Author contribution

KR secured the funding. KR, JH, JN, JM, GMM and RKB participated in planning the experiments, data collection, data analysis and preparation of the manuscript draft.

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References

1. WHO. Regional consultation on safe street foods. WHO Regional Office for South-East Asia; 2012.
2. Steyn NP, Mchiza Z, Hill J, Davids YD, Venter I, Hinrichsen E, et al. Review Article Nutritional contribution of street foods to the diet of people in developing countries: a systematic review. 2013;17:1363–74.
3. FAO. Looking at edible insects from a food safety perspective. Challenges and opportunities for the sector. 2021.
4. Imathiu S. Street Vended Foods: Potential for Improving Food and Nutrition Security or a Risk Factor for Foodborne Diseases in Developing Countries? Curr Rendsearch Nutr a Food Sci. 2017;05:55–65.
5. Cortese, Dal RM, Boro M, Feldman C, Barletto S. Food safety and hygiene practices of vendors during the chain of street food production in Florianopolis , Brazil: A cross-sectional study. 2016;62:178–86.
6. Hill J, Mchiza Z, Puoane T, Steyn NP, Hill J, Mchiza Z, et al. Food sold by street-food vendors in Cape Town and surrounding areas: a focus on food and nutrition knowledge as well as practices related to food preparation of street-food vendors. 2018;0248.
7. Paudyal N, Anihouvi V, Hounhouigan J, Ignatius M, Sekwati-monang B, Amoa-awua W, et al. International Journal of Food Microbiology Prevalence of foodborne pathogens in food from selected African countries – A meta-analysis. Int J Food Microbiol. 2017;249:35–43.
8. Bagumire A, Karumuna R. Bacterial contamination of ready-to-eat meats vended in highway markets in Uganda. 2017;11 June:160–70.
9. Mugampoza D, Byarugaba GWB, Nyonyintono A, Nakitto P. Occurrence of Escherichia coli and Salmonella spp. in street-vended foods and general hygienic and trading practices in Nakawa Division, Uganda. Am J Food Nutr. 2013;3:167–75.
10. van Huis A. Edible insects contributing to food security? 2015;:1–9.
11. Garofalo C, Osimani A, Milanović V, Taccari M, Cardinali F, Aquilanti L, et al. The microbiota of marketed processed edible insects as revealed by high-throughput sequencing. Food Microbiol. 2017;62:15–22.
12. Niassy S, Omuse ER, Roos N, Halloran A, Eilenberg J, Egonyu JP, et al. Safety, regulatory and environmental issues related to breeding and international trade of edible insects in Africa. Rev Sci Tech l'OIE. 2022;41:117–31.
13. Garofalo C, Milanović V, Cardinali F, Aquilanti L, Clementi F, Osimani A. Current knowledge on the microbiota of edible insects intended for human consumption: A state-of-the-art review. Food Res Int. 2019;125:108527.
14. Okia CA, Odongo W, Nzabamwita P, Ndimubandi J, Nalika N, Nyeko P. Local knowledge and practices on use and management of edible insects in Lake Victoria basin , East Africa. 2017;3:83–93.
15. Odongo W, Okia CA, Nalika N, Nzabamwita PH, Ndimubandi J, Nyeko P. Marketing of edible insects in Lake Victoria basin: the case of Uganda and Burundi. J Insects as Food Feed. 2018;1:1–9.
16. Ssepuuya G, Wynants E, Verreth C, Crauwels S, Lievens B, Claes J, et al. Microbial characterisation of the edible grasshopper Ruspolia differens in raw condition after wild-harvesting in Uganda. Food Microbiol. 2019;77:106–17. doi:10.1016/j.fm.2018.09.005.

17. Ng’ang’a, J., Imathiu, S., Fombong, F., Ayieko, M., Vanden Broeck, J., & Kinyuru J. Microbial quality of edible grasshoppers Ruspolia differens (Orthoptera: Tettigoniidae): From wild harvesting to fork in the Kagera Region, Tanzania. , 39(1), e12. J Food Saf. 2019.
18. Bang Alok and Courchamp Franck. Industrial rearing of edible insects could be a major source of new biological invasions. Ecol Lett. 2020.
19. Agea, J. G., Biryomumaisho, D., Buyinza, M., & Nabanoga GN (2008). Commercialization of Ruspolia nitidula (nsenene grasshoppers) in Central Uganda. African J Food, Agric Nutr Dev. 2008;8:319–32.
20. Uganda Bureau of Statistics (UBOS) and ICF. 2018. Uganda Demographic and Health Survey 2016. Kampala, Uganda and Rockville, Maryland, USA: UBOS and ICF. 2018.
21. Kalema S. Street Vending and the Economic Development of Kampala City. 2019.
22. Mmari MW, Kinyuru JN, Laswai HS, Okoth JK. Traditions, beliefs and indigenous technologies in connection with the edible longhorn grasshopper Ruspolia differens (Serville 1838) in Tanzania. J Ethnobiol Ethnomed. 2017;13:1–11.
23. Isingoma BE, Kwesiga S. Microbiological analysis of domestic water sources in Banda slum of Kampala, Uganda. J Water Sanit Hyg Dev. 2021;11:676–86.
24. Dimanin P. Exploring livelihoods of the urban poor in household contextual analysis. 2012; December.
25. Mukiibi S. The Effect of Urbanisation on the Housing Conditions of the Urban Poor in Kampala, Uganda. Second Int Conf Adv Eng Technol. 2012;3:37–42. https://www.mak.ac.ug/documents/Makfiles/aet2011/Mukiibi.pdf.
26. Ssemugabo C, Wafula ST, Ndejjo R, Osuret J, Musoke D. Characteristics of sanitation and hygiene facilities in a slum community in Kampala , Uganda. Int Health. 2021;13 March:13–21.
27. Musoke D, Ndejjo R, Halage AA, Kasasa S, Ssempebwa JC, Carpenter DO. Drinking Water Supply , Sanitation , and Hygiene Promotion Interventions in Two Slum Communities in Central Uganda. J Environ Public Health. 2018;:1–9.
28. Eliku T. Hygienic and Sanitary Practices of Street Food Vendors in the City of Addis Ababa , Ethiopia. 2016; march 2015:32–8.
29. Opoke R, Malinga GM, Rutaro K, Nyeko P, Roininen H, Valtonen A. Seasonal pattern in population dynamics and host plant use of non - swarming Ruspolia differens Serville (Orthoptera : Tettigoniidae). J Appl Entomol. 2018; December:1–9.
30. Labu, S., Subramanian, S., Khamis, F. M., Akite, P., Kasangaki, P., Chemurot, M., ... & Egonyu JP (2021). Microbial contaminants in wild harvested and traded edible long-horned grasshopper , Ruspolia differens (Orthoptera : Tettigoniidae) in Uganda Microbial contaminants in wild harvested and traded edible long-horned grasshopper , Ruspolia differens (Orth. J Insects as Food Feed. 2021; May.
31. Zenbaba D, Sahiledengle B, Nugusu F, Beressa G, Desta F. Food hygiene practices and determinants among food handlers in Ethiopia : a systematic review and meta - analysis. Trop Med Health. 2022;6. doi:10.1186/s41182-022-00423-6.
32. Afema JA, Sischo WM. Salmonella in Wild Birds Utilizing Protected and Human Impacted Habitats, Uganda. Ecohealth. 2016;13:558–69.
33. Kumpitsch C, Koskinen K, Schöpf V, Moissl-eichinger C. The microbiome of the upper respiratory tract in health and disease. BMC Biol. 2019;:1–20.

34. Pal M, Kerorsa GB, Marami LM, Kandi V. Epidemiology , Pathogenicity , Animal Infections , Antibiotic Resistance , Public Health Significance , and Economic Impact of Staphylococcus Aureus : A Comprehensive Review. Am J Public Heal Res. 2020;8:14–21.
35. Ehuwa O, Jaiswal AK, Jaiswal S. Salmonella, food safety and food handling practices. Foods. 2021;10.

Figures

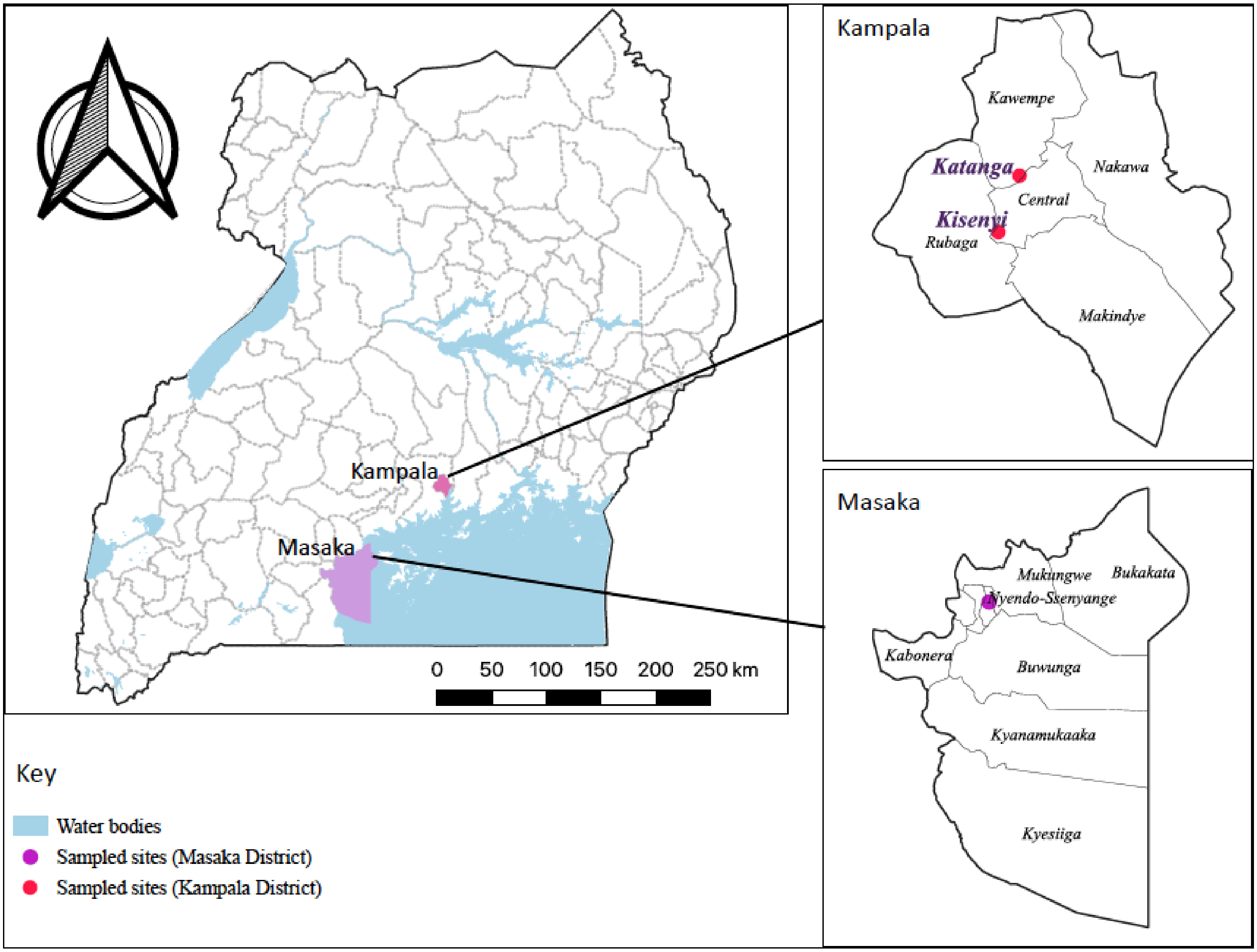

Figure 1

Map showing the location of the study areas in Kampala and Masaka districts, Uganda.

Figure 2

Heatmap showing indicator microorganisms (log cfu/100g) at the different stages of the value chain. K01-K12 and M01-M17 represent grasshopper processing households in Kampala and Masaka, respectively. 1)-unprocessed grasshoppers; 2)-de-legged and de-winged; 3)-fried at the HH; 4)-vendor RTE grasshoppers. White cells: no samples. Mannitol: organisms able to grow on MSA medium and ferment mannitol; XLD: other red colonies able to grow on XLD, including *Shigella*. For households K08 and K10, it was not possible to obtain samples from the vending points. Households K09 and K11 reported exporting the grasshoppers. Households M13, M14 and M15 dealt in live grasshoppers and frying the leftovers, while HHs M16 and M17, the grasshoppers had already undergone the first stage of processing by the time the data collectors arrived at the HHs.

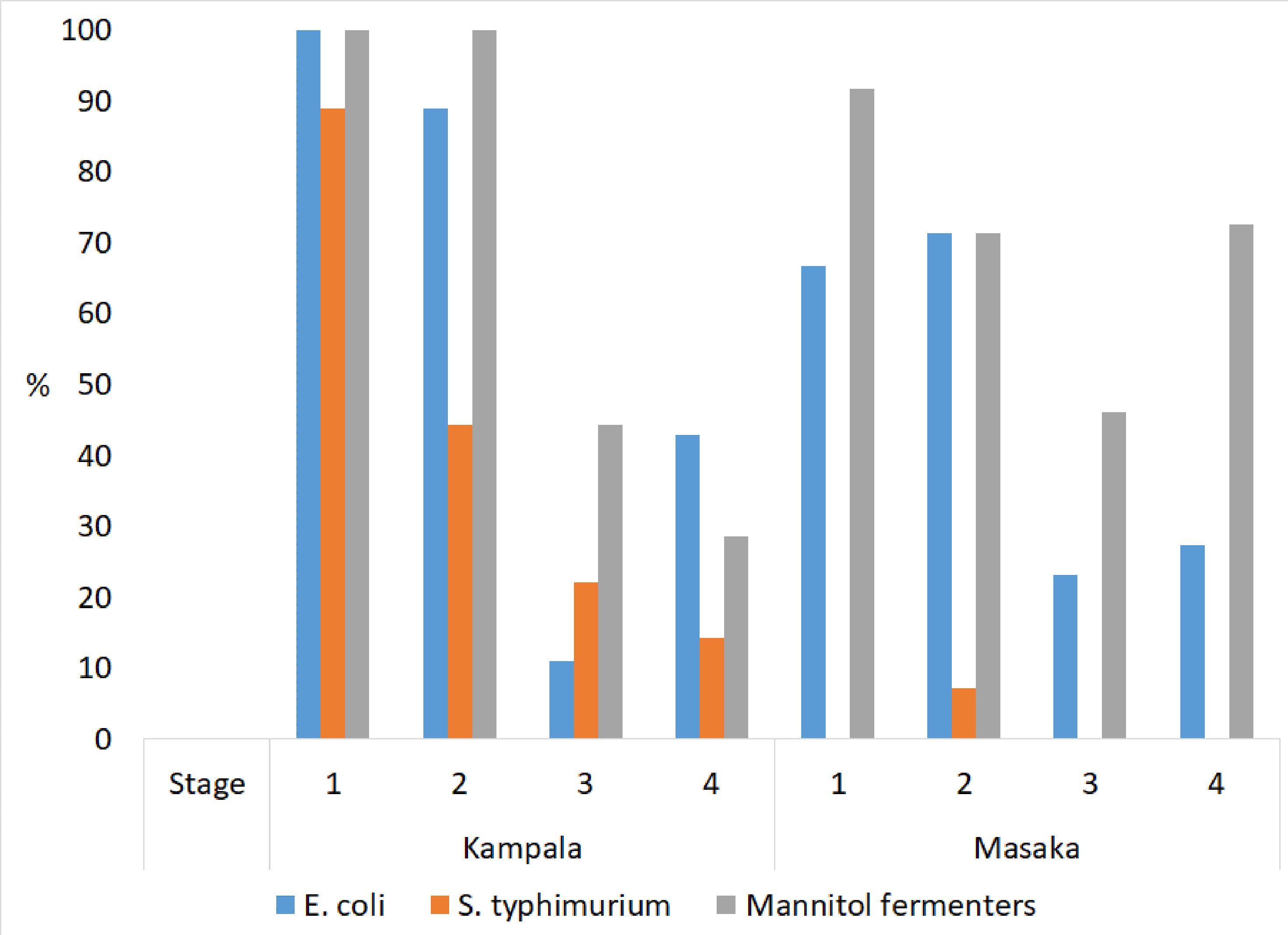

Figure 3

Percentage of households and vendors with grasshoppers categorized as hazardous by indicator organism along the value chain. Stage 1-unprocessed grasshoppers; 2-de-legged and de-winged; 3-fried grasshoppers at the HH; 4-vendor RTE grasshoppers. Masaka Stage 1 = 12 samples, Stage 2 = 14 samples, stage 3 = 13 samples; for Stages 1 and 2: Hazardous = >500 cfu/g. For Stages 3 and 4, hazardous = presence of the indicator organisms.