



## Evaluation of the bacteriological quality of fourth range products in the commune of Douala 3, Cameroon: Sanitary risks and public health implications

Gerard Kaptue Wambo<sup>1</sup>, Honorine Ntangmo Tsafack\*<sup>1</sup>, Arielle Marie Pierre Kammegne<sup>1</sup>, Laura Ladouce Yangem<sup>1</sup>, Jules Vales Teikeu Teoussi<sup>1</sup>, Godfroy Rostant Pokam Djoko<sup>1</sup>, Joseph Lubala Amani<sup>1</sup>, Teh Exodus Akwa<sup>1</sup>, Franck Rubean Wamba<sup>2</sup>, Emile Temgoua<sup>3</sup>

<sup>1</sup>Research Unit of Biology and Applied Ecology, Department of Animal Biology, Faculty of Science, University of Dschang, Cameroon.

<sup>2</sup>Department of Forestry, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon.

<sup>3</sup>Research Unit of Soil Analysis and Environmental Chemistry, Department of Soil Sciences, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon.

ARTICLE INFO	ABSTRACT
<p><i>Article history:</i> Received 23.06.2024 Received in revised form 17.09.2024 Accepted 23.09.2024</p> <p><i>Keywords:</i> Fourth range products; Bacteriological quality; Consumers; Health risk; Waterborne disease</p>	<p>Rapid urbanization in Sub-Saharan Africa has led to the expansion of the informal sector, particularly in the sale of fourth-range food products, as in Douala. This situation, without adequate regulation, exposes consumers to health risks, notably water-borne diseases due to precarious hygiene conditions. This study assesses the bacteriological quality of fourth-range products in the Douala 3 commune in order to identify sanitary risks and analyze the implications in public health. A total of 112 samples, including apples, oranges, African eggplants, sweet peas, carrots, papayas and pineapples, were collected from vendors and transported in refrigerated containers to the laboratory. Bacteriological analysis was carried out by plating the samples on selective media. Results showed that, except for apples and some papayas, most samples were contaminated, exceeding EU thresholds (0 cfu/g). Oranges were highly contaminated with fecal coliforms (<math>4.75 \times 10^3</math> cfu/g), but lesser with <i>Vibrio</i> spp. (1.4 cfu/g) and <i>Salmonella</i> spp. (5.33 cfu/g). Contamination with <i>Vibrio</i> spp was higher in carrot (603.80 cfu/g). Papayas and pineapples showed high concentrations of fecal streptococci (<math>5.37 \times 10^4</math> cfu/g and <math>4.74 \times 10^4</math> cfu/g, respectively). Sweet peas were the most contaminated, with high levels of <i>Escherichia coli</i> (<math>2.93 \times 10^6</math> cfu/g), <i>Salmonella</i> spp. (3120 cfu/g), <i>Shigella</i> spp. (<math>6.63 \times 10^7</math> cfu/g) and fecal coliforms (<math>5.38 \times 10^7</math> cfu/g). These results underline the urgent need to improve hygiene conditions and strengthen regulation of the informal food sector. Control and awareness-raising measures are essential to protect public health.</p>

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### 1. Introduction

African cities are undergoing a process of rapid

urbanization, accompanied by strong demographic growth, which is encouraging the expansion of informal sectors, particularly in the food sector. This phenomenon is the result of a desire to make urban life

\*Corresponding author. Tel.: +237699740686  
E-mail address: tsafackhonorine@yahoo.fr



more accessible to city dwellers, whose cost of living remains relatively low. The informal food sector, defined as all activities involving the production and sale of ready-to-eat food, often prepared or sold on the streets, is growing rapidly in these urban areas (1). Among the products concerned, fruit and vegetables are frequently prepared in unhygienic conditions, posing a potential risk to public health.

Fruits and vegetables are highly recommended in large-scale diets such as the Mediterranean diet, at a dose of around 100g/day (2, 3). This is because they are rich in vitamins and phenolic compounds and can be used to combat cardiovascular disease, avitaminosis, obesity prevention, trace element deficiencies in an individual and colon cancer (4). Culinary processes and practices disintegrate their properties, hence the need to resort to less processed plant products such as fourth range products, which are raw plant products ready for consumption after simple post-harvest processing such as washing, grating and cutting (5, 6, 7). These products are therefore heavily consumed in Sub-Saharan Africa and this is materialized by an increase in the trade rate of fruits and vegetables of 10.3% between 2002 and 2017 (8).

The high level of contamination in fourth range produce can be attributed mainly to inadequate hygiene practices during preparation and handling. These unsanitary conditions expose consumers to significant health risks, including fecal-borne diseases, which can be transmitted through the consumption of contaminated fruit and vegetables. These products can be contaminated by pathogens present in the water, soil or preparation surfaces, often soiled by human faeces. The faeces of infected individuals, whether used as

fertilizer in agriculture or present in the retail environment, are potential vectors for pathogen transmission.

Water-borne diseases, contracted through the ingestion of water or food contaminated by human faeces, represent a major threat to public health. Among these pathologies, cholera, caused by *Vibrio cholerae*, is of particular concern, with WHO estimating around 3 million clinical cases and over 95,000 deaths annually, with over 50% of cases reported in Africa (9). Typhoid fever, caused by *Salmonella typhi*, affects nearly 9 million people every year, causing around 11,000 deaths (10). In addition, common parasitoses such as ascariasis, caused by *Ascaris lumbricoides*, affect more than a quarter of the world's population, with prevalence rates reaching up to 80% in some regions (11). Amoebiasis, caused by *Entamoeba histolytica*, affects around 10% of the world's population, a significant proportion of whom remain asymptomatic (12).

Cameroon is not exempted from this problem, as evident by the recurrent epidemics that have affected public health since 1974, notably cholera. In 2022, 26 new cases of cholera were reported, accompanied by 502 cases of dehydrating diarrhea in children under five, resulting in 90 deaths. Meanwhile, 193 cases of bloody diarrhea, associated with 32 deaths, and 9,671 cases of typhoid fever, causing 344 deaths, were reported (13). Previous studies, such as that by Jura et al. (14), have shown that poor cooking practices and inadequate hygiene conditions during food preparation can promote the transmission of waterborne diseases. These poor practices include inadequate hygiene in food preparation and handling,

which is a major risk factor for the spread of these diseases. Studies carried out by Joko Tamoufe (15), in Bafoussam on fourth range products had revealed a high level of bacteriological contamination of these foods sold along the street to users, exposing them to a contraction of water-borne diseases ranking second only to malaria as a health threat in Cameroon.

Douala, as the economic capital of Cameroon, is faced with numerous problems of insalubrity, which favors the spread and persistence of water-borne diseases. Faced with this situation, several solutions have been implemented in the past, mainly focused on improving the supply of drinking water (16). However, the transmission of these diseases can also result from the consumption of fourth-range products, often marketed in deplorably unhygienic conditions. Unfortunately, in a city as unhealthy and densely populated as Douala, there is a significant gap in the available data concerning the bacteriological quality of fourth range food products sold in markets.

The aim of this article is therefore to assess the bacteriological quality of fourth range products marketed in the commune of Douala 3, and to identify the health risks associated with their consumption. By highlighting the microbiological contamination of these products, this research aims to raise awareness of food safety and public health issues. The results obtained will provide recommendations for improving hygiene practices in the informal food sector, thereby contributing to consumer protection and the fight against water-borne diseases in a rapidly expanding urban context. This study is of crucial importance for the formulation of public health policies aimed at reducing the risks associated with the consumption of

fourth range products in sub-Saharan Africa, while promoting access to safe, nutritious food.

## **2. Materials and Methods**

### **2.1. Study and sampling site**

This study was carried out in the city of Douala precisely in the Douala 3 markets (PK14, PK 10, Ndogpassi, Dakar, and Non glacé). This choice was guided by the reproachable level of insalubrity and population density in the city (17). The commune of Douala 3 belongs to the six communes that make up the urban community of Douala, Cameroon's economic city. It is located between (3°94'1253"N, 4°12'0657"N). It is bounded to the north by the commune of Douala 5 and the Nkam department, to the south and east by the Dibamba river, and to the west by the communes of Douala 1 and Douala 2. This district commune is the most densely populated in the city of Douala, with an estimated population of 646,347. It consists of 326,550 men and 319,797 women. Its average density is 38.6 inhabitants/Km<sup>2</sup> (17). However, population densities in excess of 300 inhabitants/Km<sup>2</sup> are recorded in neighborhoods such as CCC, Oyack, Madagascar and Autres. In 2017, this population was estimated at around 900,000 inhabitants (17).

### **2.2. Sample collection**

A total of 112 fourth range products (raw plant products ready for consumption after simple post-harvest processing such as washing, grating and cutting) was sampled for this study. This included 15 sweet peas, 15 peeled oranges, 15 eggplants, 10 carrots, 15 peeled pineapple slices, 15 watermelon slices, 13 apples and 14 peeled papaya slices. These samples were purchased, bagged and labelled, then placed in a

refrigerated cabinet and sent to the laboratory for bacteriological analysis.

### 2.3. Characterization of the preparation and sales area for fourth range products

The characterization of preparation and sales areas for fourth range products was carried out by direct observation of the sites concerned, paying particular attention to the following elements: the location of product preparation areas, the conditions under which these products are displayed for sale, and the immediate environment of preparation points, in particular factors of insalubrity or potential contamination. This approach has enabled us to assess hygiene conditions and identify the risks associated with handling and marketing these food products.

### 2.4. Bacteriological characterization of fourth range products

Bacteriological analyses were carried out under aseptic conditions using standard techniques in accordance with the protocol of Amoah et al. (18), to detect the presence of *Escherichia coli*, *Salmonella* spp, *Shigella* spp, *Vibrio* spp, as well as faecal coliforms and faecal streptococci. For this purpose, 25 g of fresh fruit or vegetable were sampled, with the exception of sweet peas, for which 25 g were ground in a sterile mortar, then transferred to a sterile beaker containing 180 mL distilled water. After homogenization, the sample obtained constituted the stock solution from which a series of successive dilutions were made from  $10^0$  to  $10^6$ . A 0.1 mL aliquot of each dilution was then inoculated onto agar media and incubated under pathogen-specific conditions. For *Salmonella* spp. and *Shigella* spp., inoculation was done on *Salmonella-Shigella* agar and incubated at 37°C for 24 h. Faecal coliforms and

*Escherichia coli* were tested on specific media, including lactose with triphenyl tetrazolium chloride (TTC) and tergitol 7, as well as Endo-agar, incubated at 44°C for 24 h. Faecal streptococci were tested on Slanetz-Bartlett agar, incubated at 37°C for 48 h. *Vibrio* spp were identified on TCBS agar, incubated at 37°C for 24 h.

The identification of the various bacteria was based on colors of the colonies formed and gas production during culture. *Salmonella* forms black colonies with the production of hydrogen sulfide gas. *Shigella* forms transparent colonies and does not produce gas. For *E. coli*, a metallic burgundy colony is formed. Faecal coliforms are burgundy pink, while faecal streptococci form a distinguishing red colored colony Amoah et al. (18).

Quantification of colony forming units (cfu) was carried out by direct counting on agar media, using the standard method.

The parameter to be assessed was bacterial load, based on the following formula (19):

$$\text{Bacterial load (N)} = \frac{\sum c}{V(n_1 + 0,1n_2)d}$$

N: the number of cfu per gram of initial product;  $\sum(c)$ : the number of colonies counted; V: the volume of solution deposited;  $n_1$  the number of dishes considered at the first dilution retained;  $n_2$  the number of dishes considered at the second dilution retained; d the dilution factor.

### 2.5. Statistical data analysis

Data obtained in this study were entered into Microsoft Excel 2016, then transferred to SPSS version 2010 for statistical analysis. The normality of the distributions was assessed using the Shapiro-Wilk test.

Comparisons between different levels of contamination were made using the Kruskal-Wallis test. For pairwise comparisons, a Wilcoxon post-test was used. Statistical tests were performed with a significance level set at  $\alpha = 0.05$ , and a  $p < 0.05$  was considered significant.

### 3. Results

#### 3.1. Distribution of fourth range fruit and vegetable samples obtained in this study

Table 1 shows the distribution of fourth range fruits and vegetables according to their frequency, revealing interesting trends in consumer preferences. Oranges, sweet peas, eggplants and watermelons stand out as the most distributed products, each accounting for 13.4% of distribution, underlining their acceptability and availability on the market. Carrots, on the other hand, are the least frequently distributed, accounting for only 8.9%, which could indicate a lower demand for this vegetable compared to the others. Pineapples, apples and papayas also had a significant distribution, with 12.5% each, contributing to a diversity appreciated by consumers.

Preparation and display area for fourth range products  
Fourth range products are sold in open-air markets; Fig. 1 (image 5 and image 1). It has been observed that vendors do not use food gloves when handling products; Fig. 1 (image 2). In addition, some items are displayed directly on the ground; Fig. 1 (image 6), and product preparation takes place near garbage dumps; Fig. 1 (image 4) and stagnant water bodies (image 5).

#### Bacteriological quality of fourth range products

##### Bacterial loads on fourth range fruits

Table 2 provides a summary of bacterial levels for the different fruits analyzed. Interpretation of the table

shows the average bacterial count  $\pm$  standard deviation (cfu/g) for the different fruits analyzed, according to the pathogens: *Escherichia coli* (*E. coli*), fecal coliforms, *Salmonella* spp, *Shigella* spp, fecal streptococci and *Vibrio* spp. P values were used to determine the statistical differences observed between fruits for each parameter.

Results showed that *E. coli* contamination rates varied significantly between fruits, with a statistically significant difference observed ( $p = 0.003$ ). Oranges showed a relatively low level ( $1.23 \times 10^3 \pm 1.93 \times 10^3$  cfu/g) of *E. coli* contamination. However, the other fruits such as pineapple ( $4.03 \times 10^3 \pm 1.06 \times 10^4$  cfu/g) and papaya ( $6.48 \times 10^3 \pm 2.13 \times 10^4$  cfu/g), had higher contamination levels. Apples, on the other hand, had contamination of *E. coli* at very low levels ( $21.53 \pm 77.65$  cfu/g).

For fecal coliforms, significant differences were observed between fruits ( $P < 0.001$ ), with particularly high levels in pineapple ( $6.84 \times 10^4 \pm 5.33 \times 10^4$  cfu/g) and watermelon ( $4.56 \times 10^4 \pm 7.44 \times 10^4$  cfu/g). Oranges and papaya also show relatively high levels, at  $4.75 \times 10^3 \pm 4.86 \times 10^3$  cfu/g and  $5.42 \times 10^3 \pm 6.09 \times 10^4$  cfu/g respectively. French apples show much lower levels ( $1.51 \times 10^3 \pm 1.25 \times 10^3$  cfu/g), suggesting less contamination by fecal coliforms.

No statistically significant difference was found for *Salmonella* spp ( $p = 0.185$ ) between fruits. Contamination levels remained low for all fruits, with zero presence in apples and papaya. Oranges and pineapple had levels of  $5.3 \pm 20.65$  cfu/g and  $2.86 \times 10^2 \pm 6.74 \times 10^2$  cfu/g respectively, indicating low levels of *Salmonella* spp contamination in these fruits.

For *Shigella* spp, significant differences were observed ( $p < 0.001$ ), with higher contamination levels in papaya ( $4.41 \times 10^4 \pm 5.046 \times 10^3$  cfu/g) and pineapple ( $1.74 \times 10^4 \pm 2.09 \times 10^4$  cfu/g). Oranges showed a lower rate of  $1.04 \times 10^3 \pm 1.43 \times 10^3$  cfu/g. Apples also showed a high rate of *Shigella* contamination ( $1.79 \times 10^4 \pm 2.77 \times 10^3$  cfu/g). Fecal streptococci levels varied significantly between fruits ( $p < 0.001$ ), with particularly high levels in pineapple ( $4.74 \times 10^4 \pm 5.29 \times 10^4$  cfu/g) and papaya ( $5.37 \times 10^4 \pm 6.75 \times 10^4$  cfu/g).

Oranges and watermelon showed moderate contamination levels ( $3.32 \times 10^3 \pm 3.33 \times 10^3$  cfu/g and  $7.4 \times 10^3 \pm 1.09 \times 10^4$  cfu/g respectively), while apples had a low level ( $448 \pm 545$  cfu/g).

*Vibrio* spp levels showed no statistically significant difference between fruits ( $p = 0.631$ ). The contamination levels were generally low, at  $1.4 \pm 5.15$  cfu/g for oranges and  $1 \pm 1$  cfu/g for watermelon. Pineapple and papaya showed slightly higher levels ( $2.71 \pm 9.87$  cfu/g and  $72 \pm 267.1$  cfu/g respectively).

Bacterial loads on fourth range vegetables

Table 3 shows the average bacterial count for the fourth range vegetables analyzed. The results show notable variability between vegetables for each microbiological parameter. Concerning *E. coli*, sweet peas showed the highest contamination with an average rate of  $2.93 \times 10^6 \pm 1.08 \times 10^7$  cfu/g, followed by carrots with  $3.068 \times 10^3 \pm 5.68 \times 10^3$  cfu/g. African eggplants had a much lower rate ( $186.66 \pm 722.95$  cfu/g), although this difference was not statistically significant ( $p = 0.15$ ).

For fecal coliforms, significant differences were observed ( $p = 0.01$ ), particularly in sweet peas

( $5.38 \times 10^7 \pm 9.91 \times 10^7$  cfu/g) and African eggplants ( $4.04 \times 10^4 \pm 4.60 \times 10^4$  cfu/g), while carrots showed moderate contamination ( $9.58 \times 10^4 \pm 7.05 \times 10^4$  cfu/g). With regard to *Salmonella* spp, no statistically significant differences were observed ( $p = 0.219$ ). Sweet peas had the highest level ( $3120 \pm 1.09 \times 10^4$  cfu/g), followed by carrots ( $320 \pm 1.01 \times 10^3$  cfu/g) and African eggplant ( $1.04 \times 10^3 \pm 3.81 \times 10^3$  cfu/g).

*Shigella* spp levels showed a significant difference ( $p < 0.001$ ), with particularly high levels in sweet peas ( $6.63 \times 10^7 \pm 1.52 \times 10^8$  cfu/g), followed by African eggplant ( $4.30 \times 10^4 \pm 5.88 \times 10^4$  cfu/g) and carrots ( $4.77 \times 10^3 \pm 8.89 \times 10^4$  cfu/g).

For fecal streptococci, no significant difference was found ( $p = 0.629$ ), with sweet peas having the highest level ( $2.39 \times 10^4 \pm 2.64 \times 10^4$  cfu/g), followed by carrots ( $1.33 \times 10^4 \pm 2.54 \times 10^4$  cfu/g) and African eggplant ( $2.69 \times 10^4 \pm 3.72 \times 10^4$  cfu/g).

Finally, for *Vibrio* spp, significant differences were observed ( $p < 0.001$ ), with carrots showing the highest levels ( $603.80 \pm 566.34$  cfu/g), while sweet peas and African eggplants showed much lower levels ( $8.07 \pm 30.96$  cfu/g and  $84.60 \pm 327.37$  cfu/g respectively).

Table 1. Fourth-range fruit and vegetables by frequency distribution.

Fruit and vegetables	Frequency (N)	Percentage (%)	Valid Percentage (%)	Cumulative percentage (%)
Oranges	15	13.4	13.4	13.4
Carrot	10	8.9	8.9	22.3
Pineapple	14	12.5	12.5	34.8
Sweet peas	15	13.4	13.4	48.2
Aubergine	15	13.4	13.4	61.6
Watermelon	15	13.4	13.4	75.9
Apple	14	12.5	12.5	87.5
Papaya	14	12.5	12.5	100
Total	112	100	100	

Table 2. Bacterial count according to fruit analyzed.

Fruits	Bacteria count (cfu/g), mean $\pm$ standard error						
		<i>Escherichia coli</i>	Feacal coliforms	<i>Salmonella spp</i>	<i>Shigella spp</i>	Feacal streptococci	<i>Vibrio spp</i>
Oranges	Min	0.00	480.00	0.00	0.00	80.00	0.00
	Max	17.24x10 <sup>3</sup>	1.9 x10 <sup>4</sup>	80.00	5.32 x10 <sup>3</sup>	1.1 x10 <sup>4</sup>	20.00
	Mean	1.23x10 <sup>3</sup> $\pm$ 4.99 x10 <sup>2*</sup>	4.75x10 <sup>3</sup> $\pm$ 1.25 x10 <sup>3*</sup>	5.3 $\pm$ 5.3	1.04x10 <sup>3</sup> $\pm$ 3.7x10 <sup>2</sup>	3.32 x10 <sup>3</sup> $\pm$ 8,6 x10 <sup>2*</sup>	1.4 $\pm$ 1.3
Pineapple	Min	0.00	80.00	0.00	480,00	600.00	0.00
	Max	3.56x10 <sup>4</sup>	1.34 x10 <sup>5</sup>	2.4 x10 <sup>3</sup>	6.92 x10 <sup>4</sup>	1.38 x10 <sup>5</sup>	37.00
	Mean	4,03 x10 <sup>3</sup> $\pm$ 2.84 x10 <sup>3</sup>	6.84 x10 <sup>4</sup> $\pm$ 2.84 x10 <sup>3</sup>	2.86x10 <sup>2</sup> $\pm$ 1.8x10 <sup>2</sup>	1.74 x10 <sup>4</sup> $\pm$ 5.58 x10 <sup>3</sup>	4.74 x10 <sup>4</sup> $\pm$ 1.41 x10 <sup>4</sup>	2.71 $\pm$ 2.63
Water melon	Min	0.00	1.6x10 <sup>3</sup>	0.00	888.00	84.00	0.00
	Max	1.6x10 <sup>4</sup>	2.54 x10 <sup>5</sup>	1.6 x10 <sup>3</sup>	2.01 x10 <sup>5</sup>	4.28 x10 <sup>4</sup>	26
	Mean	1.08 x10 <sup>3</sup> $\pm$ 9.97x10 <sup>2</sup>	4.56 x10 <sup>4</sup> $\pm$ 1.86 x10 <sup>4</sup>	125 $\pm$ 101.44	3.61 x10 <sup>4</sup> $\pm$ 1.61 x10 <sup>4</sup>	7.4 x10 <sup>3</sup> $\pm$ 2.72x10 <sup>4</sup>	1 $\pm$ 1
Apples	Min	0.00	0.00	0.00	80,00	4	0.00
	Max	280.00	4.2 x10 <sup>3</sup>	0.00	11. x10 <sup>4</sup>	2 x10 <sup>3</sup>	0.00
	Mean	21.53 $\pm$ 21.53	1.51x10 <sup>3</sup> $\pm$ 3.48x10 <sup>2</sup>	0 $\pm$ 0	1.79 x10 <sup>4</sup> $\pm$ 1.69 x10 <sup>2*</sup>	448 $\pm$ 151.1*	0 $\pm$ 0
Papaya	Min	0.00	0.00	0.00	0.00	0.00	0.00
	Max	8x10 <sup>4</sup>	2.11 x10 <sup>5</sup>	0.00	1.5 x10 <sup>5</sup>	1.86 x10 <sup>5</sup>	1000
	Mean	6.48x10 <sup>3</sup> $\pm$ 5.69x10 <sup>3</sup>	5.42x10 <sup>3</sup> $\pm$ 1.62x10 <sup>4</sup>	0 $\pm$ 0	4.41 x10 <sup>4</sup> $\pm$ 1.34 x10 <sup>4</sup>	5.37x10 <sup>4</sup> $\pm$ 1.8x10 <sup>4</sup>	72 $\pm$ 71.38
Total	Min	0.00	0.00	0.00	0.00	0.00	0.00
	Max	8x10 <sup>4</sup>	2.54 x10 <sup>5</sup>	2.01 x10 <sup>5</sup>	1.86 x10 <sup>5</sup>	100.00	100.00
	Mean	2.54 x10 <sup>3</sup> $\pm$ 1.25 x10 <sup>3</sup>	3.52 x10 <sup>4</sup> $\pm$ 6.55 x10 <sup>3</sup>	84.44 $\pm$ 42.54	2.05 x10 <sup>4</sup> $\pm$ 4.91 x10 <sup>3</sup>	2.2 x10 <sup>4</sup> $\pm$ 5.14 x10 <sup>3</sup>	14.83 $\pm$ 13.8 8
P-value	0.003	0.00	0.185	0.00	0.00	0.631	

\* p &gt;0.05; Min: minimum; Max: maxim

**Table 3.** Bacterial count for vegetables analysed.

Vegetable		Bacteria count (cfu/g), mean ± standard error					
		<i>Escherichia coli</i>	Feacal coliforms	<i>Salmonella spp</i>	<i>Shigella spp</i>	Feacal streptococci	<i>Vibrio spp</i>
Carrot	Min	00.0	1.24 x10 <sup>4</sup>	0.00	460.00	0.00	0.00
	Max	1.64 x10 <sup>6</sup>	2.4 x10 <sup>5</sup>	3.2 x10 <sup>3</sup>	2.88 x10 <sup>5</sup>	8.44 x10 <sup>4</sup>	1280
	Mean	3.068x10 <sup>3</sup> ±1.79x10 <sup>3</sup>	9.58x10 <sup>4</sup> ±2.22 x10 <sup>4</sup>	320±320	4.77x10 <sup>3</sup> ±2.81x10 <sup>4</sup>	1.33x10 <sup>4</sup> ±8.84 x10 <sup>3</sup>	603.80±179.09*
Sweep peas	Min	0.00	8 x10 <sup>3</sup>	0.00	6.4 x10 <sup>4</sup>	400	0.00
	Max	4.2 x10 <sup>7</sup>	3.52 x10 <sup>8</sup>	4.24 x10 <sup>4</sup>	5.84 x10 <sup>8</sup>	9.72 x10 <sup>4</sup>	120
	Mean	2.93x10 <sup>6</sup> ±2.79x10 <sup>6</sup>	5.38 x10 <sup>7</sup> ±2.55 x10 <sup>7</sup>	3,12 x10 <sup>3</sup> ±2.8x10 <sup>3</sup>	6.63x10 <sup>7</sup> ±3.94x10 <sup>7</sup> *	2.39x10 <sup>4</sup> ±6.82x10 <sup>3</sup>	8.07±7.99
African Aubergine	Min	0.00	0	0.00	0.00	0.00	0.00
	Max	2.8 x10 <sup>3</sup>	1.29 x10 <sup>5</sup>	1.48 x10 <sup>4</sup>	1.72 x10 <sup>5</sup>	1.02 x10 <sup>5</sup>	1268
	Mean	186.66±1,86	4.04x10 <sup>4</sup> ±1.18x10 <sup>4</sup> *	1.04x10 <sup>3</sup> ±9.83x10 <sup>2</sup>	4.30 x10 <sup>4</sup> ±1.51 x10 <sup>4</sup>	2.69 x10 <sup>4</sup> ±9.6x10 <sup>3</sup>	84.60±84.60
Total	Min	0.00	0.00	0.00	0.00	0.00	0.00
	Max	4.2 x10 <sup>7</sup>	3.52 x10 <sup>8</sup>	4.24 x10 <sup>5</sup>	5.84 x10 <sup>8</sup>	1.02 x10 <sup>5</sup>	1268
	Mean	1.10 x10 <sup>6</sup> ± 1.04 x10 <sup>6</sup>	2.02 x10 <sup>7</sup> ± 1.02 x10 <sup>7</sup>	1.6 x10 <sup>3</sup> ± 1.11 x10 <sup>3</sup>	2.49 x10 <sup>7</sup> ± 1.53 x10 <sup>7</sup>	2.24 x10 <sup>4</sup> ± 4.81x10 <sup>3</sup>	185.70± 65,903
P-value		0.15	0.01	0.219	<0.001	0.629	<0.001

\* p &gt;0.05, Min: minimum; Max: maximum

**Figure 1.** Preparation and sales area for fourth range products.



#### 4. Discussion

The sale of fourth range products on the Douala market, particularly in the Douala 3 commune, is taking place in precarious conditions. The roadside sale counters are exposed to an uncontrolled atmosphere. Products are prepared directly on site, often close to garbage dumps and stagnant water. The vendors, who wear neither food gloves nor smocks, do not comply with minimum hygiene standards. What's more, the products are not washed in running water, raising serious concerns about food safety.

It's important to remember that these practices run counter to the recommendations of the 'Codex Alimentarius', which stipulates that preparation points for fourth range products should be buildings constructed of solid materials and equipped with appropriate facilities. These facilities must include a well-planned and sealed floor, allowing adequate circulation of wash water, as well as a drinking water tap to ensure effective washing.

Food safety literature emphasizes the importance of cleanliness and hygiene in food preparation. The conditions observed in Douala, where safety standards are not respected, can lead to product contamination, increasing the risk of food poisoning. Possible explanations for this situation include a lack of awareness of health risks among vendors, insufficient regulatory controls by local authorities, and economic conditions that encourage informal and unregulated sales practices.

The results of the analysis of fourth range products sold in Douala reveal a worrying prevalence of faecal coliforms and *Escherichia coli*, with high levels consistent with the work of Anin et al. (5), thus indicating contamination by faecal matter. This

contamination can contribute to product degradation (20) and implies an increased risk of transmission of pathogenic enterobacteria, which may be associated with the emergence of waterborne diseases. The high presence of these bacteria is probably linked to the location of sales counters, as demonstrated by Isa et al. (21), who show that coliform and *E. coli* levels vary according to market frequentation, often correlated with poor hygiene conditions.

Rapid urbanization and population density in Douala lead to inadequate management of household waste, as pointed out by Clarisse and Mougoue (22). This creates an environment conducive to food contamination, exacerbating public health problems.

With regard to the presence of *Salmonella* spp. in the products, the results differ from those obtained by Anni et al. (5) in Côte d'Ivoire. However, the detection of these pathogenic bacteria on fourth range products highlights their potential to cause diseases such as gastrointestinal salmonellosis and typhoid fever (23). In addition, the samples analyzed also showed high levels of *Shigella* spp. consistent with studies by Ngeugang et al. (24) on food sold in Douala schools, this bacterium being associated with bacillary dysentery (11).

The presence of *Vibrio* spp. in these products is particularly alarming, as these bacteria are responsible for water-borne diseases such as cholera, which has been on the increase in Cameroon's economic capital. This situation is disturbing, especially in light of the epidemiological outbreaks recently reported by the WHO since 2021 (25).

A comparative analysis reveals that fourth-range vegetables are more contaminated than fruit. This can be explained by the processing method, with

vegetables often simply washed and exposed, whereas fruit is washed and peeled, thus reducing the risk of contamination. In addition, vegetables can accumulate bacteria during storage, particularly when stored at room temperature, where their bacterial load can grow rapidly (26).

According to European Union regulations (ISO 16649-1 or 2, EC Regulation 2073/2005), a fourth range product is considered wholesome if it complies with specific bacteriological thresholds. The products analyzed in Douala show alarming microbiological levels, far exceeding EU standards for *E. coli*, *Salmonella* spp., *Shigella* spp. and *Vibrio* spp. Indeed, *E. coli* levels vary between 500 and  $7.3 \times 10^4$  times above the standard, and those for *Shigella* spp. and *Vibrio* spp. reach up to  $1.66 \times 10^9$  times the acceptable limits.

These results not only point to the poor microbiological quality of food sold on the streets of Douala, but also to a significant risk of water-borne diseases. Indeed, the probability of contracting diseases such as bacillary dysentery caused by *Shigella* spp. after consumption of these products is alarmingly high, ranging from  $2.61 \times 10^4$  to  $1.6 \times 10^9$  times the acceptable risk.

These findings corroborate earlier studies by Ngueugang et al. (24) on food sold on the streets and in schools in Douala, as well as those by Anin et al. (5) and Kasse et al. (27) in Côte d'Ivoire and Senegal. They underline the urgency of interventions to improve food safety and hygiene in the sale of fourth range products in Douala.

#### **Study limits**

This study has several limitations that need to be taken into account when interpreting the results. Firstly, although 112 samples were collected, these represent

only a fraction of the fourth range products available in the Douala 3 commune, and seasonal and geographical variability in sales conditions could influence the results, making it difficult to generalize. In addition, the bacteriological analyses were carried out in the laboratory, which may not accurately reflect the often unsanitary handling conditions in the field. The analysis methodology, although standardized, may introduce biases during sampling and handling, and the quantification of colony-forming units (cfu) may underestimate or overestimate the actual bacterial load. In addition, visual assessments of hygiene conditions during the sale and preparation of products may lack precision, and environmental factors such as contamination by insects or animals have not been systematically taken into account. Finally, fluctuations in bacterial contamination over time, particularly during busy periods or after climatic events, were not considered, further limiting the scope of the conclusions.

#### **5. Conclusion**

In conclusion, this study reveals a worrying level of contamination in fourth range products marketed in the Douala 3 commune, highlighting the health risks associated with their consumption. The results show that the majority of products sampled far exceed the bacteriological standards set by the European Union, exposing consumers to an increased risk of water-borne diseases. The most alarming results concern sweet peas, which show extremely high levels of *Escherichia coli* and *Shigella* spp. followed by fruits such as papayas and pineapples, which also show worrying levels of contamination. This situation is exacerbated by the

precarious hygiene conditions observed in sales areas, where produce is often exposed to unsanitary environments, close to waste and without adequate protection for handlers. It is imperative that urgent measures are put in place to improve hygiene practices in Douala's informal food sector. This could include hygiene awareness campaigns for vendors, training in food safety standards, and a tightening of regulations concerning the sale of food products. In addition, setting up a quality control and monitoring system for fourth range products is essential to protect public health. Health authorities need to work with informal sector players to establish clear standards and verification mechanisms to ensure the safety of food sold in markets. Finally, this study highlights the importance of further research to assess the evolution of bacteriological quality of food products in the rapidly expanding urban context of Douala and other cities in sub-Saharan Africa. A better understanding of consumption practices and consumer behavior could also contribute to the formulation of effective public health policies, aimed at reducing the risks associated with the consumption of contaminated food products.

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### **Author contributions**

Co-authors' contributions have been organized according to CRediT roles as follows:

Gerard Kaptue Wambo: conceptualization, investigation, writing - original draft. Honorine Ntangmo Tsafack: supervision, validation, writing -

revision and editing. Arielle Marie Pierre Kammegne: data curation, formal analysis. JulesVales Teikeu Teoussi: methodology, resources. Godfroy Rostant Pokam Djoko and Laura Ladouce Yangem: project administration. Gerard Kaptue Wambo and Joseph Lubala Amani: investigation, data collection. Teh Exodus Akwa: data analysis, visualization. Franck Rubeon Wamba and Emile Temgua: validation of analytical methods.

### **Declaration of competing interests**

The authors declare that they have no personal or financial interests to disclose in connection with this research. None of the authors has any financial or personal relationships with other individuals or organizations that could inappropriately influence or bias their work.

### **Data availability**

Data generated and analyzed during this study are available on request from the authors. We encourage transparency and are willing to share data to support future research. Data will be provided subject to requests respecting the ethical and confidentiality conditions established during the study.

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