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Microbiological quality of fresh vegetables and fruits collected from markets in Dschang, Cameroon

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ABSTRACT

We investigated the bacteriological quality of fruits and vegetables sold in three major markets in Dschang locality, Cameroon, the hygiene and preservation practices of vendors and market gardeners. Bacterial contamination was high on all samples collected in these markets. Mean aerobic bacteria counts ranged from $1x10^7$ to $4.18x10^8$ cfu/g. Bacterial species were isolated from vegetables and namely: *Staphylococcus aureus* (100 to $1.15x10^4$ cfu/g); *Salmonella* spp (1.35 to $3.35x10^4$ cfu/g); *Shigella* spp (1.27 to 2.76 $x10^3$ cfu/g); *Escherichia coli* (1.43 $x10^4$ to 4.56 $x10^4$ cfu/g), with varying rates depending on types of vegetables and markets. Carrots and lettuce were the most infected vegetables without significant difference in the degree of contamination between them. The levels of vegetables contamination by *Escherichia coli* and *Salmonella* spp were highly significant. Market gardeners (95%) use organic fertilizers for vegetables production and mostly hen's droppings without no microbiological analyses. Most of them (80%) produce in the dregs and use the river water for irrigation. Well water (49.8% of vendors) is used mainly for washing of vegetables by traders because it is free and availabile in the markets. Hygiene and vegetable preservation practices of vendors were poor and could aggravate contamination. There is thus an urgent need to sensitize vendors on good hygiene and preservation of vegetables, and to apply good agricultural practices during production.

Keywords: Food safety, Fresh fruit, Raw Vegetables, Bacteriological quality, Hygiene practices

1. INTRODUCTION

Hygienic fresh vegetables and fruits are important components of the human diet and there is a strong relationship between health and consumption of fresh vegetable and fruit [1]. Raw vegetables and fruits provide proteins, carbonhydrates, vitamins and minerals which are essential for human health and well being. These commodities are convenient food for contemporary lifestyles. Various types of fruits and vegetables eaten raw are also very popular due to their attributes.

In many countries, including Cameroon, consumption rates of raw fruits and vegetable is increasing day by day. The recent increase in awareness of the health benefits of vegetables has accounted in increased consumption. Insufficient consumption of fruit and vegetable contributes to poor health and also increases the risk of noncommunicable diseases [2]. Consumption of vegetables as part of a diet can help with weight loss [3] reducing the risk of obesity and a risk factor for noncommunicable diseases. Because of their health benefits, WHO and FAO, 2003 launched a global initiative to promote the consumption of fruits and vegetables [4]. Fresh product may be contaminated with different kind of microorganisms [2-5]. Microbial contamination can occur during any step of the farm-

to-table. This contamination can arise from environmental, animal, human sources and technological applications. Microorganisms could spread not only by direct contact, but also through air and water [6-7].

Fruits and vegetables eaten raw are consumed without enough heating process, and therefore the possibility of food poisoning and food-borne infections always exists [8]. The number of reported food-borne outbreaks associated with raw fruits and vegetables, has increased in the last years. Identified outbreaks etiology is primarily of bacteriological origin [9]. However, most of farmers do not still have enough information about hygienic production and good agricultural practices. There are documented outbreaks of human infections associated with the consumption of raw vegetables [10]. Trade has led to the geographic spread of these pathogens [11]. Recent years have witnessed an increase in the frequency of these infections occurrence. Studies in developed and developing countries have demonstrated the potential of raw vegetables to transmit pathogens [7, 12, 13–14].

Vegetables production in Cameroon was estimated at 450 million tonnes in 2006 [15] dominated by Western Cameroon for tomato and leafy vegetables, the far North for onion [16]. Data on microbial quality of vegetables in Cameroon is scarce despite the fact that it has witnessed an increase in vegetable consumption and cultivation. In addition, cultivation has ensured food security, thus also become an income generating activity [17, 18].

Despite this, this activity is experiencing difficulties in bringing all the guarantees to the population in particular in terms of health, which remains a major problem [19]. On the other hand, the search for profit and profitability is a concern for farmers to the detriment of the quality of foodstuffs present in the Cameroonian markets. According to the National Plant Protection Organisation, between 2015 and 2017, 159 intercepts were recorded and notified to Cameroon because of the presence of harmful organisms (moulds, bacteria, pesticide residues, fruit flies) on fruits and vegetables to health and to the environment.

Recent studies in Cameroon have reported the use of contaminated water for irrigation of vegetables [20, 21–22]. Thus, there is an urgent need to evaluate microbiological safety of fruits and vegetables sold in some localities of Cameroon. The present survey was intended to assess the microbiological quality of marketed fresh vegetables and fruits in Dschang (Menoua Division, Cameroon). The hygiene and preservation practices of vendors were also investigated.

2. MATERIALS AND METHODS

2.1 Study area and study design

The study was conducted in three big market in Dschang, Western region, subdivision of Menoua. The town of Dschang is located in the agro-ecological zone of the Western Highlands at 5 ° 26.617 North latitude, and 10 ° 04.814 ' East longitude. The climate is Equatorial type with an average elevation of 1400 m. This climate is characterized by two seasons: a rainy season that takes from mid-March to mid-November and a dry season that runs from mid-November to mid-March. The annual rainfall is around 1959 mm. The monthly temperatures are between 22 °C and 36 °C with an average of 28 °C [23].

2.2 Sample collection and processing

Sixty samples made of tomato, carrot, lettuce, parsley and white cabbage, were purchased from randomly selected vendors in three major markets of Dschang. One sample of each vegetable was collected per week per site for 04 weeks. Each sample was placed in a sterile polythene bag and transported to the laboratory at temperature range 4–6 °C. Twenty-five grams of sample was immersed in 225 ml of physiological water for 30 min, vigorously agitated and waste water used for analyses.

2.3 Enumeration of aerobic bacteria and Staphylococcus

Aerobic bacterial count was determined by statistical process control method where the colony forming units (CFUg-1 /CFUml-1) of the food samples were determined. A spread plate of each dilution was done on Plate Count Agar plates and incubated at 37 °C for 24h. After incubation, the plates were analyzed and CFUs were counted for appropriate plates.

2.4 Isolation and identification of bacteria

The following media were used: Plate Count Agar, Trypton Agar, MacConkey agar, *Salmonella-Shigella* agar and Eosin Blue Methylene agar. Agar plates were inoculated in duplicate with 100 µl of sample by the spread plate technique. Prior to inoculation of *Salmonella–Shigella* agar, samples were pre-enriched overnight in selenite F broth (Becton, Dickinson & Company) at 37 °C. Plates were incubated at 37 °C for 24 h. The growth in the medium was depicted as turbidity in the broth. Aloopful from broth was streaked on nutrient agar, eosin methylene blue agar, MacConkey agar, Mannitol salt agar plates were incubated at 35-37°C for 24h and pure cultures of isolated bacteria were obtained. Microscopic examination and biochemical characterisation of the isolates was carried out as per the standard laboratory protocol [24, 25] and the isolates were identified using Bergey's manual of determinative bacteriology, (9th edn).

2.5 Evaluation of hygiene and preservation practices of vendors

A questionnaire was administered to 100 vegetables vendors randomly selected from the three markets, and from whom the samples were obtained. Data was also collected by visual inspection.

2.6 Statistical Analysis

The mean values obtained from the microbiological evaluation of fruits and vegetables were analysed by independent samples *t*-test and to determine any statistically significant difference ($P<0.05$) among all commodities means by one-way analysis of variance (ANOVA) followed by post hoc Tukey's test using SPSS 21.0 software.

3. RESULTS AND DISCUSSION

3.1 Bacterial contamination of vegetable samples from the different markets

Table 1 below shows the bacterial contamination rates of vegetable samples from market B, Foto market and bus station market in the locality of Dschang.

Table 1 : Level of bacterial contamination of vegetables from the different markets.

		% of sample contamination by bacteria			
Samples	**Markets**	*E. coli*	*S. aureus*	*Salmonella*	*Shigella*
Lettuce	B	75	50	50	25
	Station	50	25	100	70
Tomato	B	50	15	20	05
	Foto	15	05	25	50
	Station	25	20	15	25
Carrot	B	100	100	100	100
	Foto	100	100	100	100
	Station	100	100	100	100
White cabbage	B	100	25	100	100
	Foto	20	25	100	100
	Station	100	25	100	100
Persil	B	50	20	55	20
	Foto	25	50	25	15
	Station	15	50	50	25

From these, it appears that all vegetable samples analyzed are contaminated with at least one microorganism. Carrots samples have a contamination rate of 100% regardless of the market. In fact, the samples of carrots taken from market B, Foto market and the market of bus station were all contaminated by the 4 studied germs (*E. coli*, *Salmonella* spp, *Shigella* spp, *Staphylococcus aureus*). Tomatoes collected from the three localities have contamination rates ranging from 5 to 50%. It observed that these tomato samples appear to be less contaminated than other fruit vegetables. The parsley samples collected from the three markets show contamination rates ranging from 15 to 50%. Concerning white Cabbage, samples in markets B and the bus station market are 100% contaminated with *E. coli*, *Salmonella* spp and *Shigella* spp. On the other hand, the presence of *S. aureus* was noted at 25%. As for the Lettue samples, 100% of the samples taken from the bus station market were infected with the 4 germs. It is clearly established that all samples analysed are contaminated by certain microorganisms, sometimes making them unfit for direct consumption. It would be important to assess the microbial load and investigate the causes of this contamination.

3.2 Microbial charges of isolated bacteria

The different charges of isolated bacteria are recorded in table 2.

Table 2: Microbial charges of different vegetables (cfu/g) depending on the market.

Germs	**Different markets**			**Acceptable Level**	**Interpretation (AFNOR, 2001)**
	B	**Foto**	**Station**		
Tomato					
E. coli	185	133.4	121.5	10^3 - 10^4	A
S. aureus	91.3	11.33.	1.11	10^2 - 10^4	A
Salmonella	+++	+	+	0	U
Shigella	+++	+	+	0	U
FMAT	1 .5x10^8	1,21.10^8	1x10^8	10^7 - 10^8	U
Carrot					
E. coli	3.67x10^4	2.54 x10^4	2.52 x10^4	10^3 - 10^4	U
S. aureus	1.27x10^3	2.45x10^3	1.97x10^3	10^2 - 10^4	M
Salmonella	++++	++++	++++	0	U
Shigella	++++	++++	++++	0	U

FMAT	$2x10^8$	$1.025x10^8$	$1.02x10^8$	10^7 - 10^8	U
White Cabbage					
E. coli	$1.15x10^2$	$1.05x10^2$	$1.10x10^2$	10^3 - 10^4	A
S. aureus	$1x10^2$	$1.03x10^2$	$1.01x10^2$	10^2 - 10^4	A
Salmonella	+++	+++	+++	0	U
Shigella	+++	+++	+++	0	U
FMAT	$2.60x10^7$	$1.62x10^8$	$1.30x10^8$	10^7 - 10^8	U
Parsley					
E. coli	$2.06x10^4$	$2.15x10^4$	$2.33x10^4$	10^3 - 10^4	U
S. aureus	$1.25x10^3$	$1.13x10^3$	$1.01x10^3$	10^2 - 10^4	M
Salmonella	++++	++++	++++	0	U
Shigella	++++	++++	++++	0	U
FMAT	$4.18 x10^8$	$2.96x10^8$	$2.79x10^8$	10^7 - 10^8	U
Lettuce					
E. coli	$5.4 x10^4$	/	$2.6 x10^4$	10^3 - 10^4	U
S. aureus	$1.04 x10^3$	/	$1.15x10^4$	10^2 - 10^4	M
Salmonella	++++	/	++++	0	U
Shigella	++	/	++++	0	U
FMAT	$2.0 x10^8$		$2 x10^8$	10^7 - 10^8	U

NB. A : Acceptable ; M : Mediocre U : unacceptable ; + : less ; ++ : abundant ; +++ : more abundant

Aerobic bacteria counts were high, ranging from 1×10^7 to 4.18×10^8 cfu/g. Considering all the work, parsiley had the highest mean count (4.18×10^8 cfu/g) for B market compared to others vegetables. ANOVA test showed a significant difference in counts between vegetables ($P = 0.00$). Concerning *E. coli,* mean count was highest in lettuce followed by carrot ($4.56 \pm 1.07 \times 10^4$) and tomato ($14.33 \pm 3.21 \times 10^3$). *Salmonella* spp and *Shigella* spp were found in all the vegetables except parsley that still had considerable contamination rates by *E. coli* and *S. aureus*

According to markets, market B presented more contaminated samples than other markets. In this market the cabbages are more contaminated followed by the lettuce and then the tomatoes. Mean counts were significantly higher in white cabbage ($P < 0.05$). From these results recorded in this table, we noted that bacterial loads exceeded their limits of acceptability, so these fruit vegetables are unacceptable for direct consumption. The presence of these bacteria raises questions in view of their involvement in food borne outbreaks infections and other diseases.

3.3 Evaluation of hygiene and preservation practices of vendors

In general more females (80%) participated in the survey. The majority of participants (60.7%) had no formal education, and did not cultivate the crops (85%). Hand washing with soap was not practiced. None of the vendors transported vegetables to the market or sold them under controlled temperature conditions.

3.4 Sale modes of vegetables in markets

As a result of the observations, 45% of sellers have the vegetables on the counters; 31.2% of sellers have the vegetables on the ground; 14.2% in basins; 6.5% in wheelbarrow and 3.1% in bags. Counter sellers are the most represented (fig 1).

The inspection of the vegetable sales environment reveals an unsanitary sales area. In fact, the vegetable counters are close to the garbage cans, others without counters spread the vegetables on the ground or on a bag near the mud (see pictures).

As for the conservation of unsold vegetables, they are sometimes kept in the open air covered with tarpaulins and near the waste (see picture of vegetables conservation at market B), or left in the backyard of their houses.

3.5 Methods of washing vegetables

In Dschang town markets, the wells water is used by 49.8% of sellers, rain water by 20.55% of sellers and 15% use the water of faucet for the cleaning of vegetables (figure 2). Similarly, 9.45% of these sellers use drilling water; 3.53% spring water and finally 1.67% use river water.

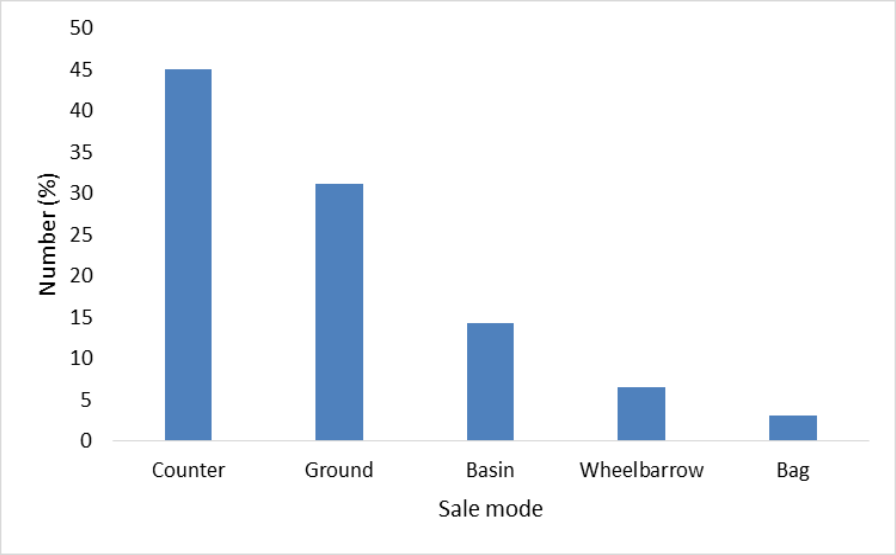

Figure 1: Different modes of arrangement of vegetables in market

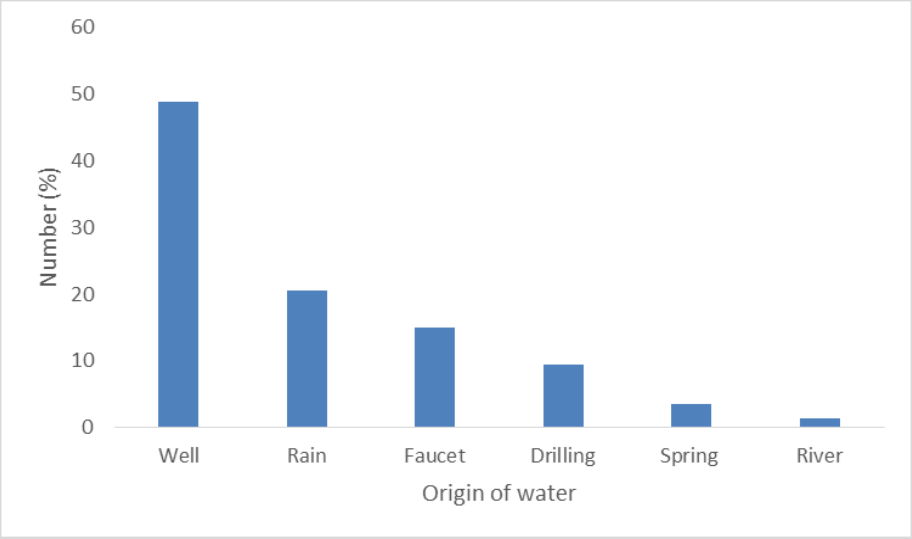

Figure 2 : Origins of water used by sellers to wash vegetables

Vegetables sold on a bag on the ground

Selling vegetables close to a trash can

Vegetables stored at the market

Our surveys of market gardeners have shown that in field, they use organic fertilizers (95% of market gardeners) for vegetables production of and mostly hen's droppings. Indeed, these are substances that are incorporated on the ground to improve physical, chemical and biological properties. The work done by some workers [26] in Brazil on the microbiological analysis of 130 vegetable samples of which 65 vegetables had been grown using fowl droppings and the other 65 using the chemical fertilizers showed that the vegetables that had been treated with organic fertilizers were more contaminated with a high load of total aerobic mesophilic flora, *Escherichia coli* (fecal coliforms) and *Salmonella* spp. They validated the hypothesis that fowl droppings would host pathogenic bacteria to consumers. Moreover, [1] confirms this hypothesis. In fact, it has isolated from poultry intestines the pathogenic bacteria to consumers. So this could explain the presence of bacteria on the analyzed vegetables. We have also noted that market gardeners mainly produce in the dregs and use the river water for irrigation (80%).

Similarly, [27] conducted studies on the physico-chemical quality of river water and well water used for irrigation and vegetable washing in Dschang town. Water samples were carried out on 5 wells in the various local markets and 11 river water samples from the different dregs. Bacteriological analysis showed an abundance of total coliforms ($5x10^8$ CFU/100ml); fecal coliforms ($9.\ 2x10^5$ - $3.\ 2x10^6$ CFU/100ml); *Escherichia coli* ($4.\ 1x10^6$ – $5.\ 3x10^7$ CFU /100ml); *Salmonella* spp ($3.1x10^6$ – $6.3x10^6$ CFU /100ml) and fecal Streptococci. Therefore, the presence of these germs in river waters and well waters can only contribute to infect our vegetables and these result would explain the high cases of gastro-intestinal infections in Dschang city [27]. The risk being increased, because of the consumption of these raw vegetables in crudity. As far as markets concerned, the sale near the trash cans would be favorable to the contamination of vegetables through insects (flies) which can be vectors of microbes. Thus, according to [29], markets are associated with the spread of certain emerging diseases due to the unsatisfactory hygiene practices of sellers. Well water (49.8% of sellers) are used mainly for washing of vegetables by traders because of their free and their availability in the markets. But according to WHO, these water do not meet the global standards of potability. Moreover, the work carried out Mfoundi at Yaounde on the bacteriological quality of the well waters revealed the presence of bacteria such as *Salmonella* spp, *Escherichia coli*, *Shigella* spp, Streptococci, faecal coliforms, *Vibrio* spp [30]. According to [31] well water is of a more defective microbial quality than that of drilling and public distribution waters. This would explain the presence of pathogenic bacteria isolated from vegetables. Samples had high aerobic bacterial counts. Uzeh et al. [32] reported high levels of bacteria in raw vegetables. This could be explained by the favourable climatic conditions of the tropics to the vegetative growth of microbial cells. Their prolonged stay at room temperature as well as their storage in clusters in the shops and markets of the Dschang city will promote their proliferation. Foods are regarded as harmful when the bacterial load is high even if the bacteria are not known to be harmful [33].

Four bacterial germs were isolated from vegetables namely: *Staphylococcus aureus*; *Salmonella* spp; *Shigella* spp; *Escherichia coli*. However, a variation of contamination rates depending on the types of vegetables was observed. Indeed, there was no significant difference in the degree of contamination of carrots and lettuce, they were the most infected. This could be explained by the fact that they are in direct contact with the soil which is a reservoir of

microorganisms. These can be found up on the plants after irrigation by splashing with an increased risk of contamination of the whole plant.

The levels of vegetables contamination by *Escherichia coli* and *Salmonella* spp were highly significant. Our result is consistent with the results obtained by [34], whose isolated large amounts of *Salmonella* spp and *E. coli* on carrots and lettuce produced in Dschang. [35] isolated *E. coli*, *Salmonella* and *Shigelles* in large proportions on vegetables sold in supermarkets in Turkey. Recent studies in Cameroon [21, 22] reported that these vegetables are irrigated with fecally polluted water. Six bacteria species were isolated with *Staphylococcus aureus* predominating. Their high number would be an indicator of fecal contamination due to the use of faeces (hen droppings, pig manure) as fertilisers during vegetable production by market gardeners. Market B presented the largest bacterial load. Indeed, this market presents a lack of potable water supply and has garbage cans in every corner and selling close to garbage cans constitute a risk of significant contamination through insects.

4. CONCLUSION

Considering the public health, fresh fruits and vegetables are common sources at various microorganisms and also pathogenic bacteria. Our study showed that the health risk factors identified in the field were the use of untreated organic fertilizers and river water for irrigation. In the markets we have noticed the sale of vegetables close to garbage cans, the unsatisfactory storage conditions and the use of contaminated water for the washing of vegetables. In view of these results there is an urgent need to sensitize vendors on good hygiene and preservation of vegetables, and the public on proper washing and sanitization of vegetables prior to consumption. It is also essential to apply good agricultural practices GAP(s) as well as good manufacturing practices (GMP(s)) during production.

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