

Full Length Research Paper

Microbiological quality of raw vegetables and ready to eat products sold in Abidjan (Côte d'Ivoire) markets

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Vegetables are usually consumed raw. This implied best hygienic conditions from the harvest to the processing because of the gastro-enteritis that they could provoke. This study was conducted with the aim to appreciate microbiological quality of raw tomatoes, endives and ready-to-eat products sold in markets. Samples were taken randomly in two markets of Abidjan. A microbiological analysis was done in order to identify and enumerate faecal coliforms, *Escherichia coli*, *Enterococcus* and *Pseudomonas*. A decontamination treatment based on washing samples with running water and sodium hypochlorite solution 1° chlorymetric was also applied to tomatoes and endives. The results indicated that, for tomatoes and endives, the average load was $1.5 \cdot 10^4$ CFU/g of *Enterococcus*, $1.3 \cdot 10^3$ CFU/g of *Pseudomonas* and $1.7 \cdot 10^2$ CFU/g of faecal coliforms. In ready-to-eat products, the load was $9.3 \cdot 10^1$ CFU/g for *Enterococcus*, $1.03 \cdot 10^1$ CFU/g for *Pseudomonas* and $9.9 \cdot 10^1$ CFU/g for faecal coliforms. The disinfection with a sodium hypochlorite solution 1° chlorymetric reduced *Enterococcus* and faecal coliforms load to 98% and *Pseudomonas* load to 97% as compared to the washing with running water in which *Enterococcus* was only reduced to 80%, faecal coliforms to 78% and *Pseudomonas* to 73%. *Escherichia coli* were isolated in 28 samples as follow: 15 stumps from endives (54%), 10 stumps from tomatoes (36%) and 3 stumps from ready-to-eat products (10%). Results showed that before consumption, vegetables need to be washed, cleaned and disinfected. This will avoid sanitary hazard.

Key words: Hygienic quality, raw vegetables, ready-to-eat products, disinfection, germs.

INTRODUCTION

Vegetables have beneficial effects on health and diseases prevention (Remesy et al., 1998). Their consumption is encouraged in many countries by governmental agencies to protect against a range of illnesses such as cancers

and cardiovascular diseases. They are important components of a healthy and balanced diet because of their nutrients content such as vitamins, minerals and dietary fiber (Koffi-Nevry et al., 2012).

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Vegetables are consumed in the raw or cooked form. In the raw state, they can be sold entirely or after minimal processing which consist of peeling, cutting and slicing the fresh product in order to increase their functionality (Siddiqui et al., 2011). This second presentation constitutes the ready-to-eat products. However, vegetables must be treated cautiously while processing because of microbial flora and pathogen germs contamination which can represent a serious health risk (Cardamone et al., 2015).

In Côte d'Ivoire, these vegetables are not always cultivated under the best hygienic conditions. Sallou (2001) showed that farmers often used partially decomposed organic manure and polluted water for their cultures. Moreover, according to Sackou et al. (2006), 75% of lettuce sold in Abidjan markets did not meet the microbiological criteria set up for vegetables and, most of the time, the immediate surroundings of sales points of vegetables are real sources of contamination (Koffi-Névy et al., 2012).

In Côte d'Ivoire, several consumers (44%) did not disinfect vegetables before eaten (Sackou et al., 2006) and unfortunately, ready-to-eat products are sometimes handled in such conditions by sellers or kitchen personnel. Seow et al. (2012) have showed that among several vegetables and fruits, raw and ready-to eat lettuce are more contaminated with high level of mesophilic bacteria and coliforms. Vegetables need to be well washed and disinfected before been processed. According to Alvaro et al. (2009), the most common method to reduce the microbial activity of fruits and vegetables is the disinfection of washing water by chlorination. Lorougnon (1996) and Sorianoa et al. (2000) have also demonstrated that disinfection by chlorination reduced microbial load around 99%.

This study was led firstly, with the aim to evaluate the microbiological quality of tomatoes and endives, two vegetables highly consumed by Ivorian population, and ready-to-eat salads. Secondly, to appreciate the effect of decontamination treatment by simple washing with clean water or washing with sodium hypochlorite 1° chlorymetric on *Escherichia coli*, *Enterococcus*, *Pseudomonas* and coliforms load in these vegetables.

MATERIALS AND METHODS

Sampling sites

Samples have been collected in the principal market of Adjamé, the most famous market of Abidjan city. This market received food and vegetables coming from different part of the country. The second market chosen is the market of Cocody. The neighbourhood of Cocody is considered to be the rich part of the city. So, one market is used by all kind of social categories of populations and another one is supposed to be used by the wealthy persons.

Sampling procedure

Vegetables were sampled as follow: 50 raw tomatoes (*Lipopersicum*

esculentum var *Lima*), 50 raw endives (*Cichorium endivia* var *latifolia*), 50 ready-to-eat product consisted of onions, tomatoes and endives, all cut up and seasoned with oil and salt.

In this study, sampling of the different products was made by the same method. Two sellers were randomly chosen by market and by vegetables. So, 2 women were chosen for the tomatoes, 2 for ready to eat products and 2 others for endives. They participated in the study. Sampling was done 3 times per week during 4 weeks. One sample by kind of vegetable was taken randomly each time and packed in plastic bag by the seller. Before going to the laboratory for the analysis, samples were placed in an isothermal box equipped with ice packs.

Microbiological analysis

For the microbial analyses, *Escherichia coli* was only researched (presence or absence) on, while the others (faecal coliforms, *Enterococcus* and *Pseudomonas*) were enumerated. Coliforms, *E. coli* and *Enterococcus* are faecal contamination indicator germs, while *Pseudomonas* is vegetables deterioration indicator germ. A portion of vegetable (10.0 g) was taken into plastic sterile bags with 90 ml of plugged water. The mixture was blended in a Stomacher. Decimal dilutions were made by adding 1 ml of the first solution to 9 ml of a solution of salt trypton. *Enterococcus* was counted on Bile Esculin Agar (BEA) (BIORAD®) after incubation 24 h at 37°C (NF T 90-416, 1985). *Pseudomonas* grows on Cetrimid agar (BIOMERIEUX®) and was counted after incubation for 48 h at 30°C (NF V 04-504, 1995). For species identification, two check stumps were used: *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853. Faecal Coliforms were counted on violet red bile lactose agar (VRBL) (BIORAD®) after incubation for 24 h at 44°C (V 08-010, 1982; Le Minor et al, 1989). *E. coli* were detected by selecting five characteristic colonies of faecal coliforms (AFNOR, 1999; Le Minor et al., 1989). Isolation was made on methylene blue eosin agar (EMB) (BIOMERIEUX®). The check stump of *E. coli* gave by Pasteur Institute of Côte d'Ivoire was identified and confirmed by API 20^F gallery.

Microbiological criteria

The bacteriological criteria used for faecal coliforms in vegetables and ready-to-eat products was $m = 10^2$ CFU/g, $M = 10^3$ CFU/g. The bacteriological criteria for *Enterococcus*, *Pseudomonas* were determined from the numeration criteria and was $m = 1.5 \cdot 10^3$ CFU/g, $M = 1.5 \cdot 10^3$ CFU/g for *Enterococcus* and $m = 30$ CFU/g, $M = 3 \cdot 10^2$ CFU/g for *Pseudomonas*. Concerning *E. coli*, the criteria was absence or presence after research on characteristic colonies of faecal coliforms (Table 1). "m" is the value below which the product is considered to have a very good and satisfactory quality, so it could be used. "M" is the value above which the quality of the product is considered to be bad, and it have to be rejected. The values between "m" and "M" characterize products qualities as acceptable.

Treatment method

Only tomatoes and endives were subjected to decontamination treatment because they were sold entirely and not cut up like the ready-to-eat products. Three different treatments were used and consisted of: no washing samples, washing with running water by soaking samples during 10 min and washing with a sodium hypochlorite solution 1° chlorymetric by soaking also the samples during 10 min.

Table 1. Microbiological criteria for vegetable and ready to eat.

Microorganisms	Microbiological criteria	Microbiological quality
<i>Enterococcus</i>	<1.5×10 ² cfu/g	Satisfactory
	1.5×10 ² to 1.5×10 ³ cfu/g	Acceptable
	>1.5×10 ³ cfu/g	Unsatisfactory
	<30 cfu/g	Satisfactory
<i>Pseudomonas</i>	30 to 3×10 ² cfu/g	Acceptable
	>3×10 ² cfu/g	Unsatisfactory
	<10 ² cfu/g	Satisfactory
Faecal Coliform	10 ² to 10 ³ cfu/g	Acceptable
	>10 ³ cfu/g	Unsatisfactory
<i>Escherichia Coli</i>	Absence	Satisfactory
	Presence	Acceptable

Table 2. Total repartition of isolated species in the samples from 2 markets of Abidjan.

Species	Tomatoes		Endive		Ready to eat products		Total	
<i>Enterococcus faecalis</i>	7	47	30	50	10	34	47	131
<i>Enterococcus</i> (Others species)	40		20		24		84	
<i>Pseudomonas aeruginosa</i>	4	11	14	50	8	24	26	85
<i>Pseudomonas</i> (Others species)	7		36		16		59	
Faecal coliforms	34		42		8		84	
<i>Escherichia coli</i>	10		15		3		28	

Statistical analysis

A statistical study was made using STATISTICA 7.1. A Friedman Anova and Khi-2 tests were made ($\alpha = 5\%$) to discriminate the treatments.

RESULTS

Microbiological repartition of germs in tomatoes, endives and ready-to-eat products

In all analysed samples (tomatoes, endives and ready-to-eat products), 131 (87%) revealed the presence of *Enterococcus*, 85 (57 %) this of *Pseudomonas*, 84 (56 %) that of faecal coliforms and *E. coli* was isolated in 28 samples (19 %) (Table 2).

The percentage of *Enterococcus* is higher than that of *Pseudomonas* and faecal coliforms. The most important loads of microorganisms were in endives. In this product, *Enterococcus* and *Pseudomonas* were isolated in all samples, faecal coliforms in 42 samples and *E. coli* in 15 samples. In tomatoes, *Enterococcus* was isolated in 47 samples while only 11 samples contain *Pseudomonas*. In ready-to-eat products, the prevalence of germs is lower

than that of tomatoes and endives. *Enterococcus* was counted in 34 samples, *Pseudomonas* in 24 samples, faecal coliforms in 8 samples and *E. coli* in 15 samples.

At the species level, *E. faecalis* was isolated in 7 samples of tomatoes, 30 endives and 10 ready-to-eat products. *P. aeruginosa* was isolated in 4 samples of tomatoes, 14 endives and 8 ready-to-eat products. The repartition of *E. coli* stumps in the samples was as follow: 15 stumps from endives (54%), 10 stumps from tomatoes (36%) and 3 stumps from ready-to-eat products (10%) (Figure 1). Others species of *Enterococcus* and *Pseudomonas* were present on the samples (Table 2).

Microbiological quality of samples

The numeration revealed that the load of *Enterococcus* is higher than that of others microorganisms. There was 1.4. 10³ CFU/g of *Pseudomonas* in endives and faecal coliforms were in lower number (Table 3).

According to microbiological criteria presented in the Table 1, considering *Enterococcus*, 50% of tomatoes, 90% of endives and 18% of ready-to-eat products have an unsatisfactory microbiological quality. For

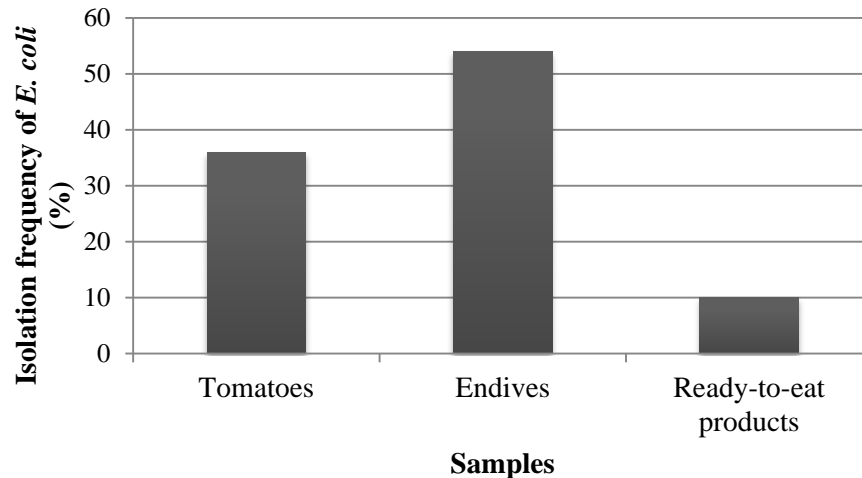


Figure 1. Repartition of *E. coli* in vegetables.

Table 3. Average load of germs in samples of 2 markets of Abidjan.

	<i>Enterococcus</i> (CFU/g)	<i>Pseudomonas</i> (CFU/g)	Faecal coliforms (CFU/g)
Tomatoes	$1.82 \times 10^4 \pm 3.68 \times 10^4$	$6.63 \times 10^1 \pm 2.48 \times 10^2$	$1.05 \times 10^2 \pm 1.75 \times 10^2$
Endives	$1.33 \times 10^4 \pm 1.58 \times 10^4$	$1.63 \times 10^3 \pm 2.93 \times 10^3$	$2.12 \times 10^2 \pm 2.96 \times 10^2$
Ready-to-eat products	$6.29 \times 10^2 \pm 3.68 \times 10^4$	$4.99 \times 10^1 \pm 8.53 \times 10^1$	$1.58 \times 10^1 \pm 4.99 \times 10^1$

Table 4. Microbiological quality of vegetables and ready-to-eat products of 2 markets of Abidjan.

Microorganisms	Microbiological quality	Tomatoes (%)	Endives (%)	Ready-to-eat products (%)
<i>Enterococcus</i>	Satisfactory	22	0	50
	Acceptable	28	10	32
	Unsatisfactory	50	90	18
<i>Pseudomonas</i>	Satisfactory	84	6	66
	Acceptable	10	34	30
	Unsatisfactory	6	60	4
Faecal coliforms	Satisfactory	74	56	94
	Acceptable	26	44	6
	Unsatisfactory	0	0	0
<i>E. coli</i>	Satisfactory	80	70	94
	Acceptable	20	30	6

Pseudomonas, 6% of tomatoes, 60% of endives and 4% of ready-to-eat products have an unsatisfactory microbiological quality. The ready to eat products had in percentage the most samples with satisfactory microbiological quality. This good quality is observed in 50% of samples for *Enterococcus*, 66% for *Pseudomonas*, 94% for faecal coliforms and *E. coli*. Samples of endives

have an unsatisfactory microbiological quality (Table 4).

Effect of decontamination treatment

Washing methods applied on tomatoes and endives have reduced significantly, the rate of germs when compared

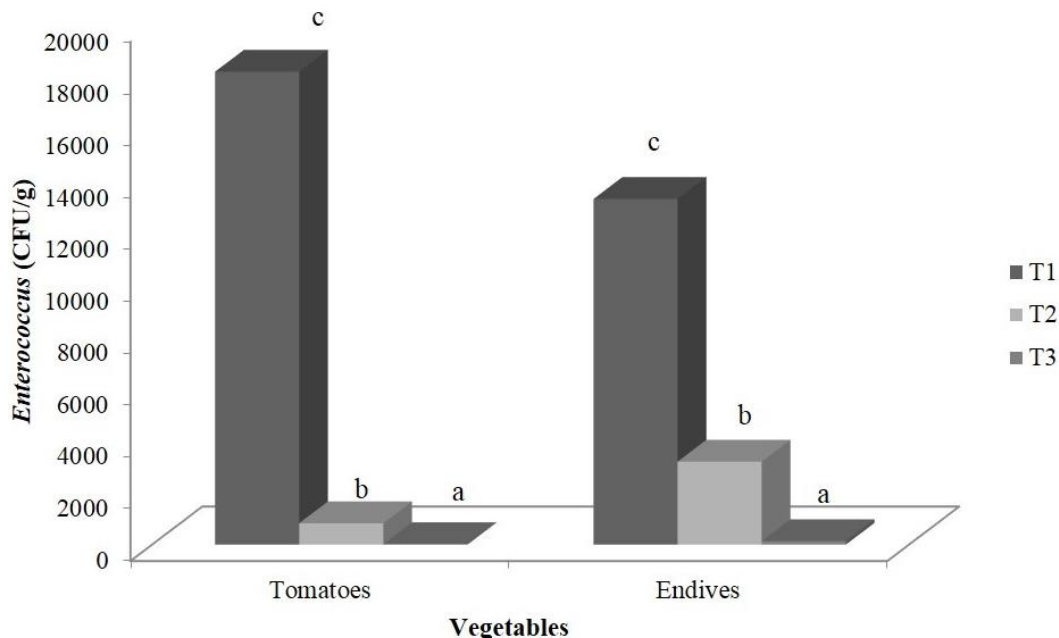


Figure 2. Variation of *Enterococcus* load according to treatments. T1: No washing; T2: Washing with running water by soaking sample during 10 min. T3: Washing with a 1° Chl sodium hypochlorite solution by soaking samples during 10 min. Letters indicate significant difference between the treatments for each vegetable (χ^2 test, $p \leq 0.05$).

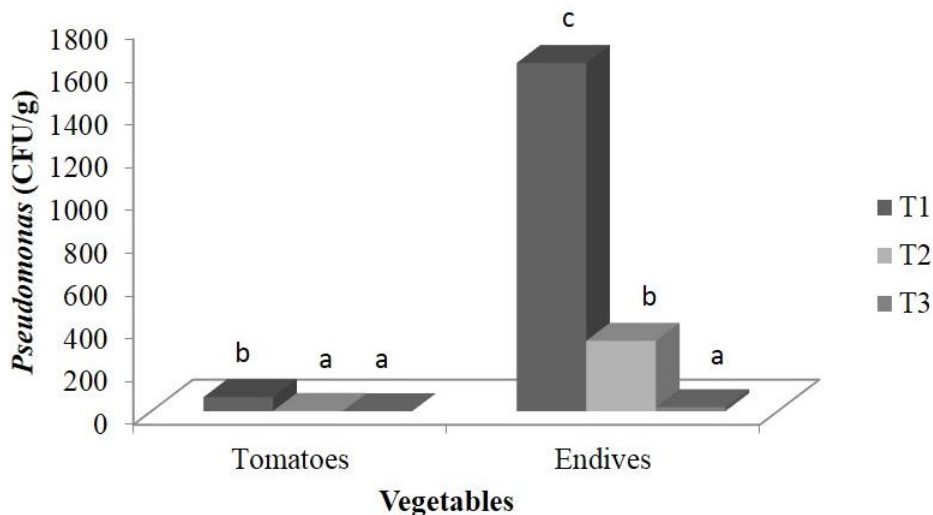


Figure 3. Variation of *Pseudomonas* load according to treatments. T1: No washing. T2: Washing with running water by soaking sample during 10 min. T3: Washing with a 1° Chl sodium hypochlorite solution by soaking samples during 10 min. Letters indicate significant difference between the treatments for each vegetable (χ^2 test, $p \leq 0.05$).

with that of no washing samples (Figures 2, 3 and 4).

All treatments differed significantly ($p < 0.05$) for each microorganism except in tomatoes where there is any statistical difference between washing with running water and washing with sodium hypochlorite water 1° chlorimetric

for *Pseudomonas* load. The percentage of reduction was 80% for *Enterococcus*, 78% for faecal coliforms and 73% for *Pseudomonas* with the washing with running water and 98% for *Enterococcus* and faecal coliforms and 97% for *Pseudomonas* with the sodium hypochlorite water 1°

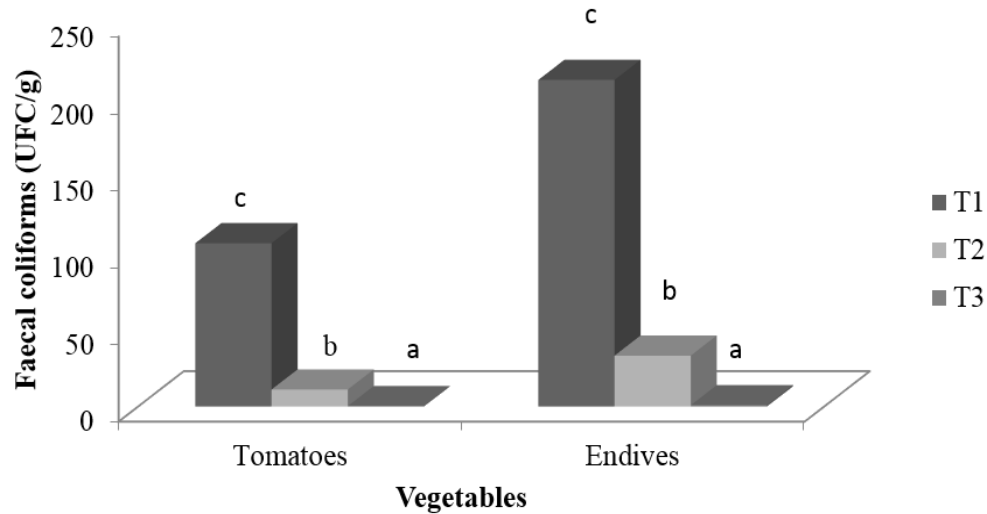


Figure 4. Variation of faecal coliforms load according to treatments. T1: No washing. T2: Washing with running water by soaking sample during 10 min. T3: Washing with a 1° Chl sodium hypochlorite solution by soaking samples during 10 min. Letters indicate significant difference between the treatments for each vegetable (χ^2 test, $p \leq 0.05$).

Table 5. Percentage of microorganisms reduction in all samples.

Germes	Washing with running water	Washing with a sodium hypochlorite solution 1° chl
<i>Enterococcus</i>	80	98
<i>Pseudomonas</i>	73	97
Faecal Coliforms	78	98

chlorimetric (Table 5).

DISCUSSION

The percentage of *Enterococcus* is more important than that of *Pseudomonas* and faecal coliforms. This could be due to the large range of temperature (10 and 45°C) and pH (4 to 9) in which *Enterococcus* is able to grow. It seems to be an advantage for this microorganism (Rollins and Joseph, 2000; Nar et al., 1991). They may be on the vegetables before the harvest but they can also be brought by seller's manipulations. *Pseudomonas* is present in all the samples of endives which constitute a good habitat for microorganism. Indeed, leafy vegetables (like endives) have some large and rough surfaces which are in contact with the ground and the irrigation water facilitating the accumulation of dirt and the adhesion of bacteria (Abadias et al., 2008). Certain species such as *Pseudomonas cichorii* and *Pseudomonas cepacia* can impair the commercial quality of leafy vegetables by inducing some brown or black blemish (Chaux and Foury, 1994). *E. faecalis* is responsible of 90% of infections induced by the *Enterococci* and it was an important life-

threatening nosocomial infection (Rollins and Joseph, 2000). *E. coli* used to be more isolated in endives than in tomatoes and ready-to-eat products. This is in phase with Bohaychuk et al. (2009) who have revealed a load of 0.48 log CFU/g in minimally processed vegetables and a load of 2 log CFU/g in raw vegetables.

P. aeruginosa is an opportunistic pathogen of humans responsible of most of the nosocomial infections (Todar, 2002). According to Kominos et al. (1972), a patient consuming an average portion of tomato salad might ingest as many 5.10^3 CFU of *P. aeruginosa*. The detection of faecal coliforms is low in ready-to-eat products and can be explained by the fact that vegetables are thoroughly washed. This result is confirmed by Pingulkar et al. (2001). In their study, Faecal coliforms were absent in ready-to-eat salad samples and *E. coli* was detected in relatively low numbers in batches of some prepared salad vegetables (Brocklehurst et al., 1987).

Tomatoes and endives sold in the markets did not fit to the bacteriological criteria. Most of them have an acceptable or an unsatisfactory microbiological quality. The first cause is the use of partially decomposed organic manure and polluted water on the crops. So, environment

germs are seen on vegetables. A second cause is the bad conditions of harvesting, transportation, sale in market and processing (Sallou, 2001; Adjrah et al., 2011). In fact, markets have an unhealthy environment and sellers usually wash and soak the roots of endives in water in which germs could increase. In ready-to-eat products the contamination was made from the cooking to the selling. They were prepared at markets and although they were thoroughly washed, vegetables could be contaminated by the hands and the utensils which are not disinfected. So, besides the disinfection of food, the kitchen environment must be clean because it can also serve as a reservoir of large numbers of micro-organisms (Kagan et al., 2002). Sodium hypochlorite water 1° chlorimetric has an efficient action on germs. The efficient action was also mentioned by Lorougnon (1996) who revealed 100% of reduction.

Conclusion

The microbiological analysis of raw tomatoes, endives and ready-to-eat products sold in the market revealed a high level of contamination by *Enterococcus*, *Pseudomonas* and faecal coliforms. Most of samples have an unsatisfactory microbiological quality. The treatment applied on vegetables reduced the microorganisms. The best one was the decontamination with sodium hypochlorite solution 1° chlorimetric because it reduces the presence of germs to 97% at least. The hygienic quality of ready-to-eat vegetables is satisfying because *E. coli* were detected only in 3 samples. However, all sanitary risk due to the consumption of these vegetables must be avoided. So vegetables need to be cleaned, washed and disinfected.

Conflict of interest

The authors declare that there is no conflict of interest.

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