

Assessment of the Microbiological Quality of Salads in the North Central Region of Morocco

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ABSTRACT: Monitoring the safety and microbiological quality of food is an integral part of national development programs. It can protect consumer health and prevent the occurrence of food borne infections. In this context, the present study is the first one performed in Fez region, on evaluation of the microbiological quality of raw, cooked and fruits salads. In this regard, 224 samples were studied during three years (The January 2011 to December 2013). The total aerobic mesophilic flora, total coliforms, fecal coliforms, *E.coli*, *Staphylococcus aureus*, Sulphite-Reducing Anaerobic and *Salmonella spp* were counted. The results obtained showed that the 86 samples were classified as unsatisfactory microbiological quality according to Moroccan guidelines. A high number of salads of all categories, which were classified as unsatisfactory, were contaminated by *E. coli*. A variation of unsatisfactory microbiological quality salad was detected between a category and another. The difference between the categories was significant ($P=0.024$). The Results obtained revealed that the different categories of salads were contaminated by the total aerobic mesophilic flora, total coliform and *E. coli*. By against, only raw salads were charged by Sulphite-reducing anaerobic bacteria and *Salmonella spp*. The *Staphylococcus aureus* was detected both in raw and cooked salads. In order to prevent contamination and bacterial growth, good hygiene practices must be implemented by producers who must ensure that the hygiene, environmental and conditions (not clear) are controlled and maintained.

KEYWORDS: Assessment, Salads, Microbiological quality, sanitary risk, Fez, Morocco.

1 INTRODUCTION

Fruits and vegetables are essential components of the human diet because they provide essential nutrients, such as vitamins, minerals and fibers. There is considerable evidence of the health and nutritional benefits associated to their regular consumption [1]; [2].

Their insufficient intake is recognized as one of the biggest factors contributing to the increase in chronic non communicable diseases spread all over the world, causing 2.7 million deaths annually. These facts have led the World Health Organization (WHO) and many health authorities in various countries, to stimulate the intake of 400g of vegetables and fruits, equivalent to 5 portions by day [3].

Salad vegetables are consumed in large quantities all over the world [4]. The majority of them enter the home or restaurant with little or no prior processing beyond trimming and removal of gross contamination. Thus, the fruits and salads vegetables consumption was considered to be an important source of food borne sicknesses in cases where they are contaminated [5].

The removal of any potentially hazardous microorganisms from the vegetables is therefore dependent on the washing and sanitizing technics employed in the home or restaurant. A basic washing procedure includes several variable parameters, such as the water temperature, immersion time, use of surfactants and biocidal agents, etc [5].

The microbiological quality and safety of minimally processed vegetables has been considered in the last two decades due to their bigger association with food borne disease outbreaks [6]. The Relevant studies have been conducted in several countries to assess bacterial levels in salads [4]; [7]; [8]; [9]; [6]; [3]; [10] whereas, to our knowledge, no studies were conducted in our area to assess the hygienic quality of salads.

The purpose of this study is to establish the microbiological safety of salad vegetables served in restaurants, in order to estimate the food products risk consumed in Fez city. The results are interesting for both local and national policies in the field of food safety products.

2 MATERIALS AND METHODS

2.1 STUDY AREA

This survey was conducted in Fez city, located in the north central region of Morocco. Its area is 20 318 km² and its inhabitants' number is about 1 573 055.

2.2 SAMPLES

A total 224 vegetable sample were collected in diverse restaurants distributed in different places in Fez city. These samples were divided in 3 categories: raw salads (n = 178), cooked salads (n = 23) and fruits salads (n = 23). Raw salads were most taken and analyzed since they are more prepared and consumed than other salads. The samples included different varieties: lettuces, tomato, beets, pepper, cucumber, carrot, artichoke, choux, as well as cooked pasta, rice, zucchini and olives. These sampling was conducted by health environment technicians. All collected samples weighed at least 100 g and were "in use" at the time of sampling. Aseptic technic was used to transfer the sample into a sterile plastic container, using single use sterile utensils. Samples were collected and transported promptly to the laboratory in ice boxes and kept under refrigeration until tested. All samples were analyzed within 24h after time of sampling. Details of each sample, such as the manufacturer, packing date, country of origin and retailer, were recorded systematically.

2.3 PLACE AND TIME OF THE STUDY

This work took place over a period of 3 years (the January 2011 to December 2013) and the distribution of samples in time is as follows: 94 in 2011, 63 in 2012 and 67 in 2013. Microbiological analyzes of samples and tests identifications germs were carried out at the Regional Diagnostic Laboratory of Epidemiological and Environmental Health of Fez city (RDLEEH).

2.4 MICROBIOLOGICAL ANALYSIS

In this study, 25 grams of each sample were placed inside a sterile plastic bag with 225 ml of 0.1% buffered peptone water. Total Aerobic Mesophilic Flora, Total Coliforms (TC), Faecal Coliforms (FC), *E.coli* and Sulphite-Reducing Anaerobic bacteria (SRA) were enumerated respectively by the following methods (NM: ISO 4833 (2007) ; NM: ISO 4832 (2007) ; NM: 08.0.124 (2004) ; NM: 08.0.125 (2004)). The *Staphylococcus aureus* and *Salmonella* spp, were searched respectively by (NM 08.0.112 (2009) ; NM ISO 6579 (2007)).

The results were expressed as colony-forming units per gram (CFU/g). The parameters, culture media and thresholds were selected as recommended by the Official Bulletin N° 5214 (2004) [11], setting microbiological criteria for raw salads, and Ministry of Health (2007) setting microbiological criteria for cooked salads [12].

2.5 STATISTICAL ANALYSIS

Results expressed as CFU.g⁻¹ were converted to decimal logs and treated by the K_{hi}²-test, depending on variable distribution, to determine whether the levels of contamination of raw, cooked and fruits salads differed significantly (p < 0.05). The EPI-Info version 2003 was used for the statistical treatments.

3 RESULTS

This is the first investigation of this nature performed in our country. It was carried out under the control of the hygienic quality of salads. The number of samples analyzed was 224 divided into 3 categories: raw salads, cooked salads and fruits salads. Their distribution showed that the raw salads category was the most representative (79.48%), followed by the cooked salads and fruit salads (10.26%).

The microbiological quality of vegetables was determined by analysis of *Salmonella spp.* and enumeration of the following microorganisms: total aerobic mesophilic flora, total coliforms, fecal coliforms, *E.coli*, *Staphylococcus aureus* and SRA. Of the 224 vegetable salads sampled, 38.4% (86) were classified of unsatisfactory microbiological quality (Figure 1).

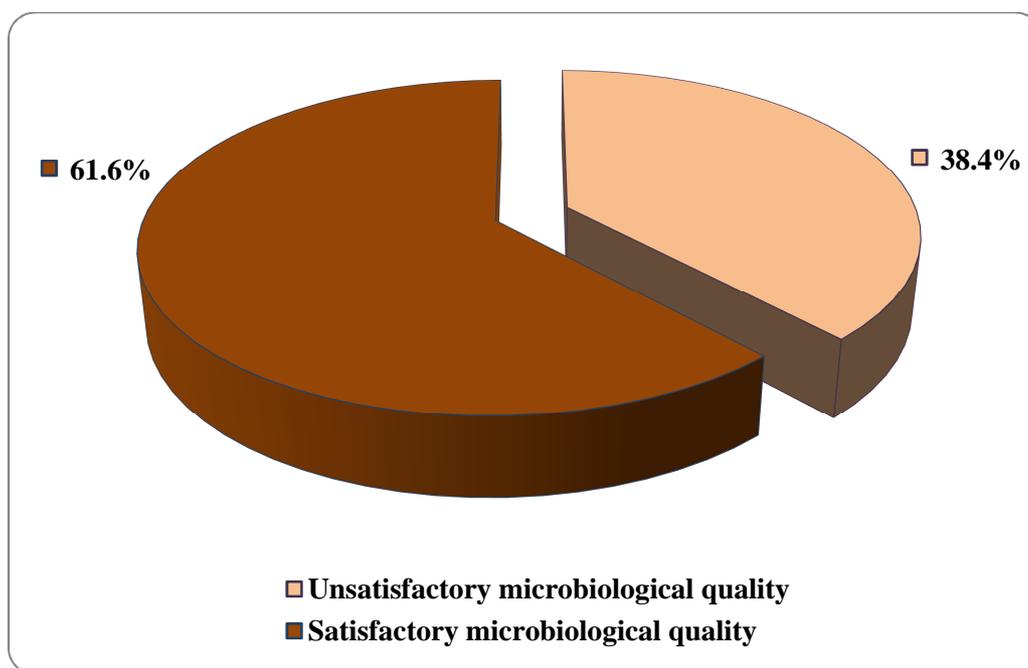


Figure 1 : Microbiological non-compliance of analyzed salads

A variation of unsatisfactory microbiological quality salad category to another was observed (Table 1). It was higher in the case of raw salads (% NC: 42.13%) than in the case of cooked salads (% NC 34.8%) and fruit salads (% NC 13.04%). The difference between the groups was significant (p=0.024).

Table 1 : Unsatisfactory microbiological quality by category salad

Category	Percentage of unsatisfactory microbiological quality		P value
	Calculated by category	Calculated by al samples	
Raw salads	42.13	33.5	0.024
Cooked salads	34.8	3.6	
Fruits salads	13.04	1.3	

The evaluation of the rate of unsatisfactory microbiological quality by year shows that the unsatisfactory microbiological quality salad was 30.9% in 2011, 42.9% in 2012 and 44.8% in 2013 (Table 2). The difference between the groups wasn't significant.

Table 2 : Unsatisfactory microbiological quality by year

Year (number of sample of salads)	Percentage of unsatisfactory microbiological quality		P value
	Calculated by year	Calculated by al samples	
2011 (94)	30.9	12.9	
2012 (63)	42.9	12.1	0.13
2013 (67)	44.8	13.4	

The prevalence of microbial counts in salads indicates that the raw salads were the most contaminated category (Table 3).

Table 3: Prevalence of microbial counts in salads (a Range in CFU/g ; (1) : Raw salads; (2) : Cooked salads ; (3) : Fruits)

Count interval ^(a)	Mesophilic Aerobic Bacteria (%)			Total Coliforms (%)			<i>Escherichia coli</i> (%)			<i>Staphylococcus aureus</i>			Sulphite-Reducing Anaerobic bacteria (SRA)		
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
0-10 ¹	7	1	4	71	12	18	96	12	18	171	22	23	176	23	22
10 ¹ -10 ²	15	0	5	17	0	0	11	4	2	3	0	0	1	0	1
10 ² -10 ³	31	3	7	37	4	2	45	3	2	2	0	0	0	0	0
10 ³ -10 ⁴	50	7	4	35	5	2	22	2	0	2	1	0	1	0	0
10 ⁴ -10 ⁵	41	8	1	13	1	0	3	2	0	0	0	0	0	0	0
10 ⁵ -10 ⁶	19	1	1	2	0	0	0	0	1	0	0	0	0	0	0
10 ⁶ -10 ⁷	10	1	0	0	1	1	1	0	0	0	0	0	0	0	0
>10 ⁷	5	2	1	3	0	0	0	0	0	0	0	0	0	0	0

The distribution of non-compliance by seed and by category demonstrated that a high number of salads of all categories were classified as unsatisfactory due to the presence of *E. coli* (Figure 2). Comparison between the categories of salads revealed that salads were contaminated by the germs sought: total aerobic mesophilic flora, total coliform, *E. coli* whereas only raw salads were contaminated by Sulphite-Reducing Anaerobic bacteria (SRA) and *Salmonella* spp. By against, *Staphylococcus aureus* was detected both in raw and cooked salads.

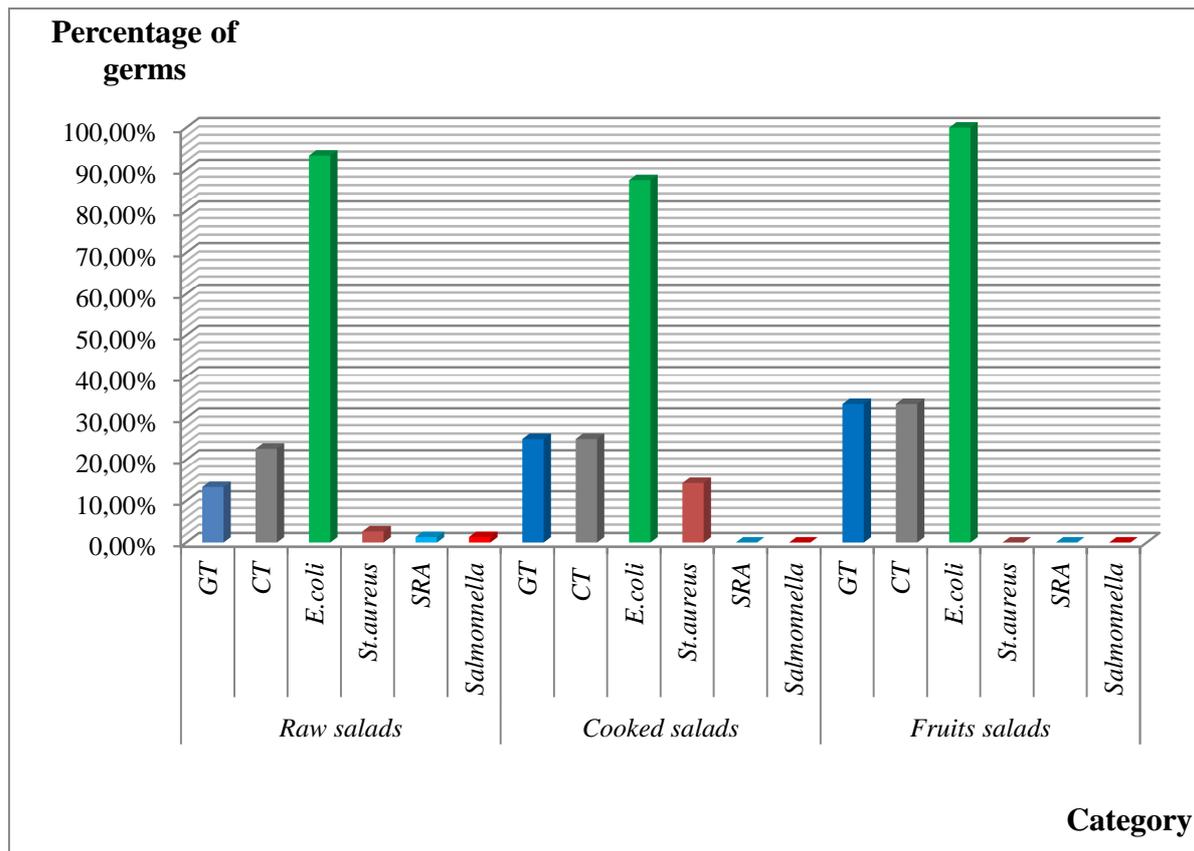


Figure 2 : Non-compliance by seed and by category

4 DISCUSSION

This is the first report on the microbiological quality of salads conducted in the north central region of Morocco. The study is representative since the samples were carried out in different restaurants. It showed that 38.4% of analyzed salads presented unsatisfactory microbiological quality according to Moroccan guidelines [12]; [13] in Porto region (86%).

We detected a variation of unsatisfactory microbiological quality salad from one category to another. The difference between the categories was significant ($P= 0.024$). Lowest hygienic quality was noted in the raw category, which may be associated with their direct contact with soil and/or water. [4] noticed poor microbiological quality among salads containing carrot. [14] found that most salads had a poor microbiological quality due to contamination of the raw material by untreated sewage.

We noted that the rate of unsatisfactory microbiological quality salad was 30.9% in 2011, 42.9% in 2012 and 44.8% in 2013. The difference between the groups wasn't significant.

The count of total aerobic mesophilic flora varied from 1 to $>7 \log_{10} \text{CFU.g}^{-1}$. Most samples had a count ranging under $6 \log_{10} \text{CFU.g}^{-1}$, while 22.1% of unsatisfactory microbiological quality salad had a count over $6 \log_{10} \text{CFU.g}^{-1}$. The latter could be related to the non-mastery of ambient conditions. In fact, although total aerobic mesophilic flora count is not related to the safety of the product, it is indicative of good practices throughout the processing, from production to marketing, including the storage temperature [10]. Total aerobic mesophilic flora counts are also useful for indicating the shelf-life duration and microbial quality of foods [15].

Total coliforms counts varied from 1 to $7 \log_{10} \text{CFU.g}^{-1}$ for raw, cooked and fruits salads. Most samples had counts under $5 \log_{10} \text{CFU.g}^{-1}$. These microorganisms are widely distributed in nature and commonly found in raw vegetables. Hence, they are not associated with fecal contamination [16]; [17].

In this study, the salads unsatisfactory microbiological quality was related mostly to *E. coli*. It was found in 93.33% of raw unsatisfactory salads, 87.5% of cooked unsatisfactory salads and in all fruits salads. Similar results were found in other studies. [18] reported on samples of salads, a high level of non-compliance caused by fecal coliforms (98%) including *E. coli*.

[14] found in a study conducted in 2012 in Mexico a total of 130 salads collected from 6 restaurants, a percentage of 99% salads contaminated with fecal coliforms. In reality, *E. coli* is the best indicator of fecal contamination because it is found exclusively in the digestive tract of men and animals and is frequently used for monitoring the sanitary quality of foods [17]; [18]. Furthermore, some strains are pathogenic to men, such as *E. coli* O157:H7, which has been associated with many foodborne illness outbreaks [20]; [21].

The existence of *Staphylococcus aureus* was observed in 3.48% analyzed salads. It concerned mainly cooked salads. That could be due to human contamination of food by *Staphylococcus*, which is a commensal of the skin and mucous membranes of Man [22].

The sulphite-reducing anaerobic bacteria were found in 3.48% of unsatisfactory microbiological quality salads. This rate is lower than that reported by [23].

The presence of *Salmonella* spp in foods is unacceptable because of its serious health hazard. We isolated it from a sample of raw salads vegetables. This pathogen has been found in vegetables produced in some countries, namely Brazil, Canada, Mexico and Turkey [1].

5 CONCLUSION

The results of this study showed that 38.4% of salads analyzed were unsatisfactory. A significant variation of unsatisfactory microbiological quality was observed between the salads categories ($P=0.024$). Furthermore, all categories of salads were polluted by total aerobic mesophilic bacteria, Total Coliform and *E. coli*, while only the raw salads had been contaminated by Sulphite-Reducing anaerobic (SRA) and *Salmonella* spp. The *Staphylococcus aureus* was detected both in raw and cooked salads. The presence of the microorganisms isolated from the samples analyzed indicates that the conditions of preparation, handling and storage were not hygienic. Most outbreaks of food borne infections are generally caused by foods that have been mishandled or mistreated during preparation or storage.

In order to prevent contamination and bacterial growth in salads served to the consumer, good hygiene and correct handling practices, efficient cleaning, sanitization of containers, maintaining refrigerating conditions and implementation of pre-requisite programs and HACCP plan are needed.

ACKNOWLEDGMENTS

We gratefully thank all health environment technicians and Pr EL FAKIR S., Dr KEITA S., from Laboratory of Epidemiology, Faculty of Medicine, Fez, Morocco.

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