Microbiological Quality Assessment of the Main Food Consumed in Collective Catering in The City of N'Djamena-Chad

Abdelsalam Adoum Doutoum[1,2], Abdelsalam Tidjani[2]*, Nazal Alhadj Markhous[2], Djimadoum Kimassoum[2], Bessimbaye Nadlaou[2]

[1]Institut National Supérieur des Sciences et Technique d'Abéché, Tchad

[2]Laboratoire de Recherche en Sciences des Aliments et Nutrition (LARSAN), Université de N'Djamena-Tchad, Tchad

*Corresponding author: Abdelsalam Tidjani, Laboratoire de Recherche en Sciences des Aliments et Nutrition (LARSAN), Université de N'Djamena-Tchad, Tchad. Tel: +2350023566352119; Email: abdelti@yahoo.fr

Abstract

Background: Collective catering is important in the diet and nutrition of the population. However, the handling and production of these foods under unhygienic conditions could lead to food poisoning for consumers. In order to improve the microbiological quality of meals served in restaurants, surveys have been conducted with restaurant owners.

Methods: We collected and analyzed 90 samples of meat, fruit and fruit juice, drinking water and rinsing water. These samples were sampled aseptically and analyzed according to standard methods.

Results: The study shows that 82.75% of the manipulators did not wash their hands with soap and 79.31% did not carry out medical examinations. Compared to microbiological analyzes, the compliance rate varies between 73.33% and 100% for all samples. Total Mesophilic Aerobic Flora, (FAMT) Total Coliforms (TC), Fecal Coliforms (FC) were found in the samples of minced meat sauce "sarkima", grilled meats and rinsing. Fruit and vegetable samples do not contain coliforms, but there is fungal flora with 30% and 20% non-compliance, respectively. As for the samples of drinking water, they were compliant for all the germs sought. Salmonellae are absent in all samples.

Conclusion: The results of the analyzes are generally satisfactory, but there are still some failures in terms of hygiene to ensure a healthy production of meals. Good hygiene practices and good manufacturing practices are still needed to promote collective restoration advantage to protect the health of consumers.

Introduction

Urbanization and the many constraints faced by urban dwellers have facilitated the emergence of new consumption patterns in the collective catering system [1]. Although it contributes to the availability of food that is ready to eat and at a good price, poor hygiene practices in these restaurants can be the source of the presence of pathogenic organisms responsible for food poisoning. Foodborne illnesses are conditions that, in general, are infectious or toxic in nature and caused by microbes that enter in the body through ingested foods [2]. These food-borne diseases are a common and growing public health problem in both developed and developing countries. A large proportion of cases of these diseases come from the consumption of water or contaminated food [3]. In Africa, food safety remains precarious. This is due to the unhygienic preparation premises, the sale of food in poor hygienic conditions, but especially the non-application of food hygiene rules [4-6].

The agents causing diarrhea were the main cause of food-borne illnesses in the majority of sub regions. Non-typhoid *Salmonella enteritica* represented a significant burden in all sub regions, particularly in the African region [7]. In addition, microbiological analyzes carried out on foods sold in catering in several countries have shown deficiencies in microbiological

quality, which could increase the risk of food poisoning [8,9]. Many hazards can be related to food, including those responsible for the ills such as poisoning, mainly due to the microbial flora from the raw materials or contamination during handling and some representatives' pathogens [10]. Among the various risks to human health, the risk of microbiological nature is the most important [11]. The microorganisms most frequently associated with the hazards are *Salmonella*, *Staphylococcus*, *Clostridium*, *Listeria* and *Bacillus*, to which should be added the pathogenic strains of *Escherichia coli*. Collective Toxi-Infections (TIAC) of *Salmonella* are mainly caused by contaminated food of animal origin, such as meat, poultry, milk and eggs [12]. The use of contaminated materials, the significant delay as well as the non-respect of the cold chain during the conservation of the food and the insalubrity are factors which were mentioned in the occurrence of the collective food poisoning infections [13]. Also, in food service establishments, food safety is all the more important because a large number of meals are prepared and served to customers, most of whom attend mass catering. Therefore, our study proposed to assess the hygienic practices and to analyze the microbiological qualities of some samples of the main foods consumed in catering in the city of N'Djamena in Chad in order to raise awareness about the good practices and prevent food poisoning.

Material and Methods

In our study, we surveyed 58 restaurants in the city of N'Djamena. As a result, 90 samples of the main foods were aseptically collected from 3 randomly selected pilot restaurants. There are fifteen (15) samples of grilled meats, fifteen (15) "sarkima" minced meat sauce, fifteen (15) peeled fruit (mangoes) for juice production, fifteen (15) fruit juice (mangoes), fifteen (15) rinsing water for washing kitchen equipment and utensils and fifteen (15) drinking water (mineral water). The survey and maintenance sheet is based on the BTSFA (Better Training for Safety Food in Chad) inspection form template, based on the food quality control model as recommended by FAO. The samples were taken under aseptic conditions and each placed in a sterilized polyethylene bag on which are written codes for their identification. Then, the samples taken were analyzed at the Food Sciences and Nutrition Research Laboratory (LARSAN) of the FSSH of the University of N'Djamena. For the microbiological analyzes, 10 g of each ground material are suspended in 90 ml of tryptone-salt solution containing 0.03 g / 1 of tween 80; then the revivification is done for 45 minutes at laboratory temperature (25°C). Then the decimal dilutions were obtained by adding one ml of each of the preceding, by means of a new graduated pipette, to the 9 ml contained in the tubes. The preparation of the decimal dilutions was made according to standard NF V 08-010. Microorganisms were searched for using standard, routine reference methods [14,15]. The search for *Salmonella* was made by the SS culture medium by incubation at

37°C. for 48 hours according to the standardized method NF EN ISO 6579: 2002.

Enumeration and Research of Microorganism

Total Mesophilic Aerobic Flora

We used the Plate Count Agar medium to count Total Mesophilic Aerobic Flora at 30°C for 72 hours. 1 ml aliquots of the stock suspension or its dilutions are put in sterile Petri dishes. Each dilution is seeded in duplicate. Then, 10 to 15 ml standard count agar (PCA) is poured into each dish. The medium and inoculum of each box are mixed well by gently shaking the plates in the direction of clockwise tracking movements in the opposite direction. The plates are left at laboratory temperature (25°C) to solidify the medium. They are then incubated at 30°C for 48 to 72 hours. The results have been noted and the calculations are done.

Total and Faecal Coliforms

To enumerate coliforms, Mac Conkey crystal violet was used for enumeration of total and faecal coliforms. The double layer seeding technique was used. Dilutions of 10^{-1} to 10^{-4} and 10^{-1} to 10^{-3} were used respectively for total and faecal coliforms. The dishes were incubated for 24 hours at 37°C for total coliform and 44°C for faecal coliforms.

Fongic Flora

The molds were searched by the Sabouraud medium with chloramphenicol. For each dilution, two Petri dishes were seeded and incubated at 30°C for 72 hours after which, colonies of fongic flora were counted.

Salmonella

This search includes several steps:

- Pre-enrichment which consists in incubating at 37° C, 25 g of the sample in 225 ml of buffered peptone water for 24 hours;

- **Enrichment:** in this case, 1 ml of pre-enriched culture is removed using a sterile pipette and seeded in duplicate into tubes containing 9 ml of Rappaport broth. One tube is incubated at 37° C and the other at 44° C for 24 hours;

- **Isolation:** The isolation media used are *Salmonella*/ *Shigella* (SS). Using an assay, colonies were isolated from tubes incubated at 37°C. The plates are incubated at 37°C for 24 hours. The characteristic colonies are subjected to the identification test. The lactose (-) colonies (clear colonies) H2S (+) (black center) and the lactose (-) colonies H2S (-) (without black center) are isolated on medium SS.

The formula below was used to express the results relating to the calculation of the CFU/g number.

$$[N] = \frac{\sum c}{(n_1 + 0.1\, n_2)\, dV}$$

N: Number of germs / g

ΣC: Sum of colonies counted on all retained boxes of two successive dilutions

V: Volume of inoculum applied to each box (in ml)

n1: Number of boxes retained at the first dilution

n2: Number of boxes retained at the second dilution

d: Dilution rate of the first box held

V: Inoculum volume applied to each box

Data Processing

The data collected was captured and analyzed using the Excel software. The results were processed according to analytical variability to compare the level of contamination with the different products analyzed. The chi-square test (χ^2) was used to compare the qualitative variables with a significance level of 5%.

Results

The parameters for assessing the hygiene of staff, working methods and raw materials in catering establishments are summarized in (Table 1). It was apparent from Table 1 that there is generally no wearing of appropriate clothing during handling, the separation between the healthful areas was 6.90% compared to that of the unhealthy which was 93.10 % (significant difference: $x^2 = 17.760$, ddl = 1, p = 0.001). The principle of walking forward was not respected. The same was true for the cleaning and disinfection of the equipment, which was 3.45% for disinfected equipment against 96.55% for non-disinfected equipment (significant difference: $x^2 = 19.183$, ddl = 1, p = 0.001). It was the same for the compliance or not of the general hygiene of the staff with the proportions of yes or no of 8.62% and 91.38% respectively (significant difference:

$x^2 = 18.944$, ddl = 1, p = 0,001). The hand washing was 17.24% against the non-washing of hands which was 82.72% (Significant difference $x^2 = 18.720$, ddl = 1, p = 0,001).

Study parameters (N = 58)	Yes	%	No	%
Wearing the appropriate dress code	0	0	58	100
Hand washing with soap	10	17.24	48	82.75
Compliance of general staff hygiene	5	8.62	53	91.38
Knowledge of GPH* or HACCP** plan	0	0	58	100
Qualification and ability to monitor a production	0	0	58	100
Medical control	12	20.69	46	79.31
Cleaning and disinfection of handling equipment	2	3.45	56	96.55
Temperature control system	2	3.45	56	96.55
Separation of raw materials and finished products	4	6.90	54	93.10
GPH* = Good Practice Hygiene HACCP* = Hazard Analysis Critical Control Point				

Table 1: Assessment of hygiene practices.

(Table 2) shows the average values obtained in the samples of grilled meat, minced meat "sarkima" and fruit. Grilled meats, sarkima and fruits (mangoes) are found to be contaminated by the FAMT which are found with low values in fruits. Total coliforms are also present in meats but absent in fruits. The same is true of faecal coliforms, which are not present in fruits. The fungal flora was found with an average of 0.52×10^2 in the fruits. *Salmonella* are absent.

Average number of germs (CFU/g)					
Samples	FAMT 30°C	Total coliform (37°C)	Fecal coliform (44°C)	*Salmonella* (37°C)	Fongic Flora (25°C)
Grilled meat	$2.12 \pm 0.81 \times 10^5$	$0.025 \pm 0 \times 10^3$	2 ± 0.70	0	-
Sarkima	$3.01 \pm 0.23 \times 10^5$	$0.72 \pm 0,55 \times 10^3$	0.4 ± 0.38	0	-
Mangoes fruits	$1.21 \pm 0.91 \times 10^5$	Absent	Absent	0	$0.52 \times 10^2 \pm 0.50 \times 10^2$

Table 2: Average sought germs in grilled meat, minced meat "sarkima" and fruit.

In (Table 3), it is noted that the FAMT is present in fruit juices and rinsing waters. Except for rinsing water samples that are contaminated with total and fecal coliforms, all other samples do not contain these germs. The fungal flora was counted for an average value of 0.52×10^2. All samples do not contain *Salmonella*.

Average number of germs (CFU/100 ml)					
Samples	FAMT 30°C	Total Coliform (37°C)	Fecal Coliform (44°C)	*Salmonella* (37°C)	Fongic Flora (25°C)
Fruit Juice	$1.18 \pm 0.90 \times 10^5$	0	0	Absent/25ml	$0.45 \pm 0.10 \times 10^2$
Drinking water	0	0	0	Absent/25ml	-
Rinse water	$4.11 \pm 0.18 \times 10^5$	$0.64 \pm 0.23 \times 10^3$	60 ± 0	Absent/25ml	$0.52 \pm 0.01 \times 10^2$

Table 3: Average number of sought germs in fruit juices, drinking water and rinsing water.

The results in (Table 4) show that the compliance rate was 100% or 33.33% satisfactory and 66.66% acceptable for the FAMT. As for total coliforms, the satisfactory sample rate was 80% as against 26.66% unsatisfactory for fecal coliforms.

Germs sought -norm [m-M] / CFU/g	Germs	Analytical variabiity	Number of samples	(%)	Appreciations
$3.10^5 - 3.10^6$	FAMT	$N \leq m$	5	33.33	Satisfactory
		$3m \leq N \leq 10m$	10	66.66	Acceptable
$10^3 - 10^4$	TC	$N > 10m$	3	20	Unsatisfactory
		$3m \leq N \leq 10m$	12	80	Acceptables
$10 - 10^2$	FC	$N \leq m$	11	73.33	Satisfactory
		$N > 10m$	4	26.66	Unsatisfactory
No Germs in 25g	SS	No Germs	15	100	Satisfactory

Table 4: Compliance rates of grilled meat samples (n = 15).

There is a significant difference (x^2 = 56.893, ddl = 1, p = 0.001) between the proportion of satisfaction of CT (40%) and that of non-satisfaction of CF (66.66%) for grilled meat.

M = 10m = maximum limit set; m = fixed standard; N = number of germs determined per gram of sample analyzed.

In (Table 5), the acceptability of FAMTs of "sarkima" samples was 53.33%, fecal coliforms were 86.66% satisfactory, and *Salmonella* was absent in all samples analyzed.

Germs sought -norm [m-M] / CFU/g	Germs	Analytical variabiity	Number of samples	(%)	Appreciations
3.10^4-3.10^5	FAMT	$3m \leq N \leq 10m$	8	53.33	Acceptable
		N > 10m	7	46.66	Unsatisfactory
10^3-10^4	TC	$N \leq m$	8	53.33	Satisfactory
		N > 10m	7	46.66	Unsatisfactory
10-10^2	FC	$N \leq m$	13	86.66	Satisfactory

Table 5: Compliance rates of "sarkima".

(Table 6) shows that Total Mesophilic Flora (FAMT) is present with a non-satisfaction rate of 26.66%. The Fungal Flora (FF) was counted giving a satisfactory assessment at 66.66% and the 20% unsatisfactory considering the maximum limit. Total and fecal coliforms and *Salmonella* were not found in the mango fruit samples.

Germs sought -norm [m-M] / CFU/g	Germs	Analytical variabiity	Number of samples	(%)	Appreciations
10^7-10^8	FAMT	$N \leq m$	13	86.66	Satisfactory
10^4-10^5	FF	N > 10/100ml	2	13.33	Unsatisfactory
		$m \leq N \leq 3m$	12	80	Acceptable
Absent in 25g	SS	N > 10m	3	20	Unsatisfactory
		No Germs	15	100	Satisfactory
Absent in 25g	SS	No Germs	15	100	Satisfactory

Table 6: compliance rates of mango fruits samples (n = 15).

It is apparent from (Table 7) that the rate of non-compliance with FAMT is 13.33% in fruit juice samples. Similar to the mango fruit samples, total and fecal coliforms as well as *Salmonella* were not found, but the fungal flora is present with a 20% non-compliance rate.

Germs sought -norm [m-M] / CFU/g	Germs	Analytical variabiity	Number of samples	(%)	Appreciations
10^7-10^8	FAMT	N > m	4	26.66	Unstaisfactory
		$N \leq m$	11	73.33	Satisfactory
10^4-10^5	FF	$m \leq N \leq 3m$	10	66.66	Acceptable
		N > 10m	3	20	Unsatisfactory
Absent in 25g	SS	No Germs	15	100	Satisfactory

Table 7: Compliance rates of mango juice samples (n=15).

The total mesophilic aerobic flora value must be less than or equal to 100 per ml. When coliforms, the limit values are of 10 per 100 ml. The analyzed samples of rinse water are unsatisfactory with respect to the FAMT and CTs as recorded in (Table 8), with a compliance percentage of 60% and 40%, respectively. FCs are 20% satisfactory and 80% are unsatisfactory while *Salmonella* are absent in all samples of the rinsing water analyzed.

Germs sought -norm [m-M] / ml	Germs	Analytical variabiity	Number of samples	(%)	Appreciations
≤100/ml	FAMT	N > m/ 100 ml	6	40	Unsatisfactory
		N ≤ m	9	60	Satisfactory
≤10/100ml	CT	N >10/100ml	5	33.33	Unsatisfactory
		N ≤ m	10	66.67	Satisfactory
Undetectable	CF	N ≤ m	6	40	Satisfactory
		N > 10/100ml	9	60	Unsatisfactory
Undetectable	*Salmonella*	No Germs	10	100	Satisfactory

Table 8: Compliances of rinse water samples (n=15).

The (Figure 1) below shows the compliance rate of all samples analyzed. It is found that all samples are 100% compliant with *Salmonella*. But the other microorganisms sought are present with diversified values in the analyzed samples. As for bottled mineral waters (n = 15), no germs were detected and 100% of the samples are compliant and fit for consumption.
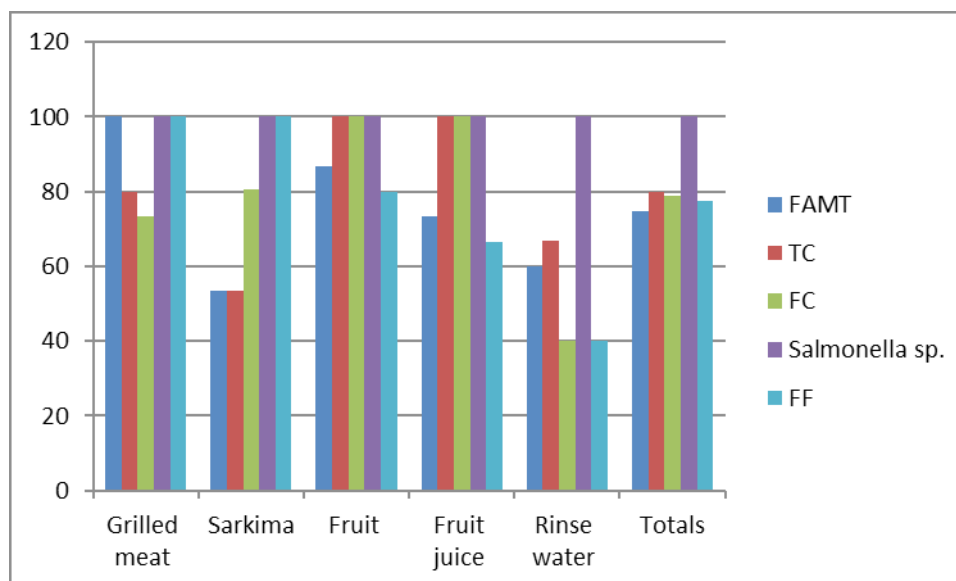

Figure 1: Compliance rates of analyses samples.

Discussion

The study showed that 93% of restaurateurs surveyed stored their food at room temperature in disorder. This is in contradiction with the principles of conservation. These poor food storage practices which unrefrigerated be explained by lack of knowledge of food hygiene by the restaurant. This observation could explain to a large extent the microbial overload obtained in our results, because according to Barro et al. [16], refrigeration limits the multiplication of microorganisms. The consequences of poor food preservation have been demonstrated in the study by Tidjani et al. [17] who had worked on the microbiological quality of meat consumed in Chad. Lack of knowledge of good hygiene practices and good manufacturing practices for catering obtained in (Table 1) would also be one of the causes of non-compliance of some results. These deficiencies in hygiene have been highlighted by several authors [18,16]. Low levels of education and training in

hygiene and manufacturing rules are other factors that influence, but are mostly neglected [19,20]. Sanitation deficiencies related to street food were also reported by Akindele and Ibrahim [5] who worked on street foods in Nigeria and by Somda et al. [21] who worked on the quality of chickens sold on the street in Burkina Faso. Materials and surfaces intended to be in contact with raw materials and finished products are generally unsanitary and often kept in unhygienic and poorly cleaned areas. The rate of non-cleaning and non-disinfection of materials and surfaces (96.55%). Other studies have indicated the risk of contamination of food by materials such as knives [22,23]. However, Pérez-Rodríguez et al. [24] have confirmed the lack of hygiene in small and medium-sized enterprises that produce food. An unhygienic environment, unsanitary materials and flies are true carriers of micro-organisms [3]; this could explain the presence of fecal coliforms found in some samples analyzed. The finished products were sometimes stored near or even together with the raw materials or handled with the same materials. This finding noted that the use of the same materials on raw materials and finished products could be a source of cross-contamination as indicated by the work of Tidjani et al. [25] and Barro et al. [26] in their studies. Compared to microbiological analyzes, FAMT exceeded the limits in 46.66% of "sarkima" samples, 26.66% in mango fruit, 13.33% in fruit juice and 60% of rinse water. These results are not similar to those of Djibrine et al. [4] who found high FAMT values in street foods such as sandwiches with a 34% non-compliance rate. Pradeep et al. [4] also obtained values that exceed the limits of the FAMT. When total coliforms were used, 20% of the grill samples, 46.66% of the "sarkima" minced meat and 33.33% of the rinse water samples were unsatisfactory.

The seasonings and spices that go into the preparation of "sarkima" are treated under unhygienic conditions that may present microbiological hazards, but according to the studies of Prince and Maalekuu, [27] spices are often kept in conditions not always good, frequently soiled land, in addition they represent a favorable substrate for the multiplication of many microorganisms. Fecal coliforms were 33.33% non-compliant for grilled meats, 13.33% for sarkima and 40% for rinse water. The study by Nutili et al. [28] showed that 55% of processed foods contained more fecal coliforms in South Africa. The presence of fecal coliforms is a proof of the unhygienic environment of the sales and the non-compliance of hygiene rules by the staff. The same observations are made by other authors [29,30]. Also, several authors [31-34] have found faecal coliforms and *E. coli* in foods. The presence of *E. coli* in the food indicates poor hygiene conditions during processing, preparation, sale and consumption. Results of non-compliance of FAMT and total and fecal coliforms in "sarkima" and rinse water samples are consistent with studies by Campos et al. [35] who obtained a percentage of 55.6% in food handled in public places in Brazil. Also the studies of Barro et al. [36] reported inadequacies

related to the microbiological potability of water used for washing utensils by street food producers. The low presence of FAMT and the absence of coliforms in samples of fruit and mango juice are explained by the fact that once purchased; the products are immediately disinfected with bleach 12° diluted chlorine, before being used. At least this technique has been mastered by those responsible for the preparation of fruit juices following a training that was carried out for the benefit of street food handlers by the Research and Food Safety Support Project. Street (PRASAR) [25]. But unfortunately many of the food handlers sold on public roads including catering have not benefited from this training. On the other hand, the acidic pH of the fruits did not allow the growth of indicator germs of non-compliance with hygiene rules and pathogenic germs. This observation was also made by Lynda & Naouel [37] who conducted a study on the Microbiological Quality of soft drinks and fruit juices in Algeria. However, our results differ somewhat from those of Barro et al. [38] who found germs indicative of poor hygiene in fruit and fruit juice sold on the street in Burkina Faso and Kasse et al. [39] in mango samples in Senegal. *Salmonella* were not identified in the grilled meat, "sarkima", fruit, fruit juice and water samples analyzed as Hamiroune et al. [40] in the food samples analyzed in Algeria. *Salmonella* results observed with a frequency of 6.67% [41] in meat in Benin are not similar with the 100% compliance frequency obtained in our study. Other authors [42] reported low prevalence of *Salmonella* in food. Howover, studies of Ire and Imuh [43] indicated that ready-to-eat foods were contaminated with different bacterial pathogens such as *S. aureus*, *E. coli*, *Salmonella* sp. and *Vibro* sp. The presence of these pathogens in the gastrointestinal tract has been shown to be of great importance to the health of the population. As for drinking water, all samples are 100%, are consistent with all the desired germs that are FAMT, total and fecal coliforms and *Salmonella*. This is explained by the fact that the analyzed water samples are in fact bottled mineral waters well preserved and served directly to customers. But it is possible that the desired germs can be found in bottled water if the packaging does not respect the rules of hygiene. As for the fungal flora, it was found in 30% of the fruit samples and 50% of the fruit juices. These results are not similar to the work of Ntuli et al. [28] found the fungal flora in all samples analyzed. The non-compliance results obtained in this study are consistent with Kimassoum et al. [44] who identified fungal flora in some foods and Firew et al. [45] in a study of foods sold in restaurants and on the streets. As far as fruit juices are concerned, some fruit juice handlers follow the good practices taught by PRASAR [45], which justified the low microbial overload in these products.

Conclusion

This study revealed inadequacies in hygiene for both staff and meals. Certainly pathogenic germs such as *Salmonella* sp. samples are absent, but there are microorganism's indicative of

non-compliance with hygiene rules such as total mesophilic flora, total coliforms and fecal coliforms, in particular in the samples of minced meat "sarkima" fruit, fruit juice and the rinsing water. But overall, taking into account the totals of compliance, the quality of the products analyzed is acceptable with regard to certain germs. This work shows that good hygiene practices and good manufacturing practices, prerequisites for the HACCP system to guarantee the safety of food offered to consumers, are rarely observed in most restaurants, but there are still some handlers who respect it after receiving training on good practices. Thus, in order to control the critical points in order to reduce microbial loads to acceptable levels, it is necessary to apply the rules of hygiene, cleaning and disinfection and other corrective measures for protect the health of the consumer. For this awareness and training must continue in the sector of catering.

Thanks

Competing Interests

Authors have declared that no competing.

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