Scientific Research Publishing

Health, 2020, 12, 1620-1631
https://www.scirp.org/journal/health
ISSN Online: 1949-5005
ISSN Print: 1949-4998

Identification and Characterization of Risk Factors Linked to the Bacteriological Contamination of Street Foods: Case of Porridge Made from Rice Sold in the City of Bangui, Central African Republic

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How to cite this paper: Lango-Yaya, E., Agboko, F.M., Bondom, R.L., Saravolia, M., N'yetobouko, S.J., Sezongo, O. and Rafai, D.C. (2020) Identification and Characterization of Risk Factors Linked to the Bacteriological Contamination of Street Foods: Case of Porridge Made from Rice Sold in the City of Bangui, Central African Republic. *Health*, **12**, 1620-1631.
https://doi.org/10.4236/health.2020.1212118

Received: September 10, 2020
Accepted: December 28, 2020
Published: December 31, 2020

Abstract

Traditional ready-to-eat food products are topical in Central African Republic They are sold in most cases on the roadside and present a variety of productions including the porridge made from cereal derivatives (Peanut and Rice), commonly called "potato" These foods are sold under precarious hygienic conditions, which constitute a major public health risk for consumers, especially children under five and vulnerable people. It is in this context that a study was carried out in eight districts of the city of Bangui on porridge made from rice and peanuts in order to assess the levels and risks of contamination of these foods. The results obtained showed that this activity is performed most often by women between the ages of 20 and 29. Of these, 17.85% have never been to school; 44.64% have a level of primary school. The results of the microbiological analysis carried out revealed a strong proliferation of germs indicators of faecal contamination, hygiene indicators in different points of sale, the average content of the contamination of aerobic mesophilic flora, fecal coliforms, total coliforms and Staphylococcus aureus are respectively 4.33×10^3, 1.845×10^3 and 1.508×10^3 CFU. No presence of *Salmonella* and *Shigella* was observed. The various stages of porridge production until sale requires the application of hygiene rules in order to avoid intoxication and toxi-infection of consumers.

Keywords

Porridge, Bacteria, Rice, Peanut, CAR

1. Introduction

Fast-food is a characteristic of developing countries. It is a source of food for the population, working during the day or away from their homes. Fermented foods represent an important part of the diet of the populations of Central Africa countries where agriculture is mainly based on food products including cereals. These food products are often transformed into ready-to-eat foods, including fermented porridge, which occupies a prominent place among this population [1]. The cereals used for the production of these porridges through various traditional processes are frequently millet, sorghum, maize and rice [2]. These cereals therefore represent an important source of energy (70% of energy intake on the continent) and of micronutrients in the African countries [3] [4]. In developed countries, food fermentations are often integrated as marketing strategies to build nutritional claims in order to offer consumers a "healthy" lifestyle or to meet specific organoleptic expectations [5]. This is not the case in Central African Republic like the countries of the sub-region where fermentations are spontaneous, but with an advantage involving heterogeneity of microorganisms. These microorganisms involved in the fermentations of cereals are mainly lactic acid bacteria, yeasts, molds and certain non-pathogenic strains of Escherichia coli [6]. In addition to their role in the natural preservation of food through the production of compounds such as organic acids and bacteriocins which greatly reduce the growth of pathogens, these microorganisms have probiotic potential, that is to say provide a beneficial effect to the host when sufficiently ingested [7] [8]. Among the benefits provided, we can mention the prevention and treatment of diarrheal diseases, the absorption of fibers during digestion, and the reduction in severity of symptoms of irritable bowel syndrome, stimulation of the immune system, reductions in signs and symptoms of lactose intolerance and many others [9]. Thus, consumer products such as fermented porridge containing the microorganisms with desirable effects could provide answers to unsolved questions about the hypotheses attributed to the beneficial effects of probiotics. In the case of traditional processes, the fermentation process takes place spontaneously thanks to the development of the epiphytic microflora, which can lead to products of undesirable organoleptic, microbiological or toxicological quality [3] [10]. It is in this context that this study was conducted, the objective of which is to assess the degrees and risks of contamination of traditional porridge made from rice and peanuts.

2. Material and Methods

2.1. Biological Material

Rice or ruzi in Greek and peanut or aràpiko fistiki in Greek are the biological material used in this study [11] [12] [13].

2.2. Methods

2.2.1. Processes of Preparation of the Porridge

Once the mixture of rice flour and ground peanut are prepared, the porridge can

be made by putting the rice mixture previously diluted in water on fire until it boils, then add the rice flour and ground peanut, keeping it on the fire with constant stirring, for 5 to 10 minutes after the appearance of bubbles on the surface. But the action of the enzymes contained in the flour of the rice is more effective if one proceeds in the following way:

- Dilute the compound flour in a little less than half of the water that will be used for the preparation of the porridge;
- Bring the rest of the water that will be used for preparation to a boil;
- Pay the diluted flour into the container of boiling water after removing from heat;
- Wait 5 minutes before returning the container to the heat;
- Keep boiling for 5 to 10 minutes while stirring.

The cooking time is 45 minutes to 1 hour, with stirring, after the mixture of flour and ground peanut have reached a temperature of 85˚C, from which one can observe a simmering of the mixture and the appearance of bubbles on the surface. Once the cooking is complete, the sugar and the lemon juice are added, and then the porridge is left to cool to 45˚C, the temperature at which the measurements of their viscosity are carried out.

2.2.2. Sampling

A total of four hundred samples were taken from the vendors in a random fashion. These samples were taken, packaged in carefully closed sterile bags and labeled with the sample code, date, time, place of collection and placed in an insulated box. These samples were sent to the National Laboratory of Clinical Biology and Public Health in Bangui under aseptic conditions for microbiological analysis. A questionnaire was administered to the saleswomen in order to collect their socio-demographic characteristics.

2.2.3. Analysis Protocol for All Studies, We Used Standardized Methods [11]

1) Preparation of the stock suspension and decimal dilutions

The preparation of the initial suspension and the decimal dilutions is carried out according to the directives of standard NF V 08-010 relating to the preparation of dilutions for microbiological examination.

2) Weighing

Each type of sample is introduced into sterile bags (fitted with a filter) previously placed on a device (Dilumat). This apparatus simultaneously includes weighing and dilution. To 30 g of product are added 120 ml of buffered peptone water in order to produce a stock solution diluted to the fifth.

3) Homogenization and grinding

Homogenization will allow the homogeneous distribution of microorganisms in the sample [13]. It is an important step in the analysis. The sample, placed in a bag fitted with a filter, is ground for 1 to 2 min using a peristaltic-type homogenizer-grinder (STOMACHER). The stock solution consists of the grinding product diluted to the fifth.

4) Revivification

Food microorganisms are often in a precarious physiological state (damaged cells, sublethal lesion) during various technological treatments (dehydration, heat treatment, cold). Therefore, to isolate or count them on a selective medium, they must first be applied a restorative treatment called revivification. For the finished products and raw materials, a revivification of the grinding product was carried out.

5) Decimal dilutions

At the time of inoculation, decimal dilutions (10^{-1} to 10^{-5}) from the stock solution were made depending on the microbiological species sought. The dilutions were carried out in tubes containing 9 ml of Tryptone Salt beforehand. Each tube is vigorously shaken with a vortex to help distribute the suspended germs.

2.2.4. Microbiological Analysis Techniques

For enumerations and microbiological research, the techniques used are mostly standard methods. The germs sought are: germs indicative of hygienic quality; *Salmonella; Shigella*; suspected pathogenic staphylococci; sulfite-reducing anaerobes; germs indicative of commercial quality; fecal or thermo-tolerant coliforms; total aerobic mesophilic microflora [14].

1) Enumeration of spoilage germs

Principle: each living microorganism introduced into the mass of an agar medium generally gives rise to a colony visible to the naked eye. Consequently, if a product or its dilution is inoculated in an agar medium, the number of colonies (CFU: Colony Forming Unit) which have developed corresponds to the number of microorganisms present in the volume considered [12]. Mesophilic aerobic flora (MAF) is a set of microorganisms capable of multiplying at average temperatures, more precisely those whose optimum growth temperature is between 25˚C and 45˚C [15]. The enumeration of the aerobic mesophilic flora was carried out according to the method described in the French standard NF V 08-051. Seeding is carried out in depth (in the mass). Using a sterile pipette, 1 ml of the stock solution or successive dilutions (MS, 10^{-1}, 10^{-2} for the finished products and 10^{-3}, 10^{-4}, 10^{-5} for the wind products and raw materials) is transferred to a Petri dish. The medium is maintained in super cooled in a water bath at 37˚C., is poured (approximately 15 ml) into each Petri dish. The combination of these two operations should not exceed 15 min. To ensure the uniform distribution of the germs throughout the dish, the inoculum-medium mixture is carefully homogenized in circles and back and forth movements. The boxes solidified at laboratory temperature are placed in the oven (boxes turned upside down). After 72 hours of incubation at 30˚C, all the colonies which have developed are counted using a colony counter. The "sandwich" inoculation technique was used. The dishes are inoculated deeply with 1 ml of stock solution, 10^{-1} and 10^{-2}. About 15 ml of molten VRBL agar brought to 47˚C. are poured into the Petri dishes. When the medium is solidified, a second coat of the same medium is applied; this is the "sandwich" or "double layer" seeding technique. The purpose of this technique is to accentuate the selective characteristics of the environment by

establishing anaerobic conditions which inhibits many strict aerobic bacteria. After 24 hours of incubation at 37˚C, the count of the characteristic colonies: purplish red (lactose fermentation) of diameter 0.5 mm or more, sometimes surrounded by a reddish zone due to the precipitation of bile salts gives the number of coliforms totals in CFU/g.

The detection of coliforms is based on the use of the lactose contained in the medium. Thermo-tolerant coliforms or fecal coliforms (CF), these are coliforms which present the same characters as total coliforms but which develop at 44˚C - 45˚C. They are sometimes called "presumptive *Escherichia coli*". The taxonomic group is poorly defined. We can mention: *Escherichia coli*, *Klebsiella pnemoniae*, *Enterobacter cloacae*, *Citrobacter freundii*, etc. The NF V 08-060 standard served as a reference for the enumeration of thermo-tolerant coliforms. The procedure and the characteristic colonies are the same as for total coliforms. The distribution is linked to the volume of the inoculum, *i.e.* 5 ml of stock solution is distributed in 3 Petri dishes for samples of sales products and samples of finished products and 1 ml of stock solution and 10^{-1} for the raw materials, and the incubation takes place at 44˚C for 24 hours.

2) Enumeration of pathogenic and toxigenic germs

The search for pathogenic and toxigenic germs must be carried out directly or after enrichment. It is based on the use of solid selective media and often accompanied by identification or confirmation of the strain. *Staphylococcus aureus* belong to the *Micrococcaceae* family. *Staphylococcus aureus* is a heat-sensitive germ; it is also sensitive to the acidity of the medium but tolerates high concentrations of Nacl. The search and enumeration method is essentially based on the detection of lecithinase and coagulase (standard routine method NF V 08-057). Colony confirmation technique for the enumeration of coagulase positive *Staphylococcus* by colony count at 37˚C was adopted. Inoculation, it is carried out on the surface, *i.e.* 0.1 ml of stock solution, 10^{-1} for finished products and sales products and 1 ml of stock solution, 10^{-1} divided into 3 tubes for raw materials are deposited on the surface of a Baird Parker medium previously poured and to which Potassium tellurite (1 ml/box) and egg yolk emulsion (1 ml/box) are added before use. Spreading each inoculum is spread as quickly as possible with a sterile glass support (Pasteur pipette) over the entire surface of the medium. Incubation and reading after drying at laboratory temperature, the dishes are incubated at 37˚C. for 24 to 48 hours. The characteristic colonies of *Staphylococcus aureus* appear on the medium, black, shiny, convex, 1 mm to 1.5 mm in diameter after 24 hours of incubation and 1.5 to 2.5 mm in diameter after 48 hours. Each colony is surrounded by a halo of light (about 2 to 5 mm in diameter) due to the hydrolysis of the egg's lecithin (lecithinase). This step was undertaken for the search for free coagulase from selected characteristic colonies. Using a sterile Pasteur pipette, part of each selected colony is removed. This inoculum is inoculated into a brain-heart broth (BCC) tube for enrichment. Incubation is done at 37˚C for 20 to 24 hours. After incubation of the BCC, 0.1 ml of each culture is added sterile to 0.1 ml of lyophilized rab-

bit plasma in sterile hemolysis tubes. These tubes are incubated at 37˚C. After 4 to 6 hours of incubation, the coagulation of the medium is examined. The reaction to coagulase is considered positive when the coagulum occupies more than three quarters of the volume initially occupied by the liquid, the tube can be inverted without the contents being spilled.

2.2.5. Data Analysis

Data processing was done with Excel and Epi info version 6 software. Descriptive statistical tests were used to calculate central trends.

With a p value < 0.05 bilaterally and the confidence interval considered was 95%. Some results were expressed as frequency, percentage and SPSS software is used to calculate trends.

3. Results

3.1. Socio-Demographic Characteristics

The socio-demographic results obtained showed that the sale of traditional porridge made from rice and peanuts is most often carried out by women whose age varies from 20 to 29 years. Figure 1 shows 17.85% of saleswomen who have never been to school, 44.64% have left school at the primary level, 35.71 at the secondary level and 1.78 have a university level.

3.2. Hygienic Characteristics of Saleswomen

98% of saleswomen do not have a medical certificate.

Figure 2 provides information on sales attire, skin lesions, condition of hands and nails.

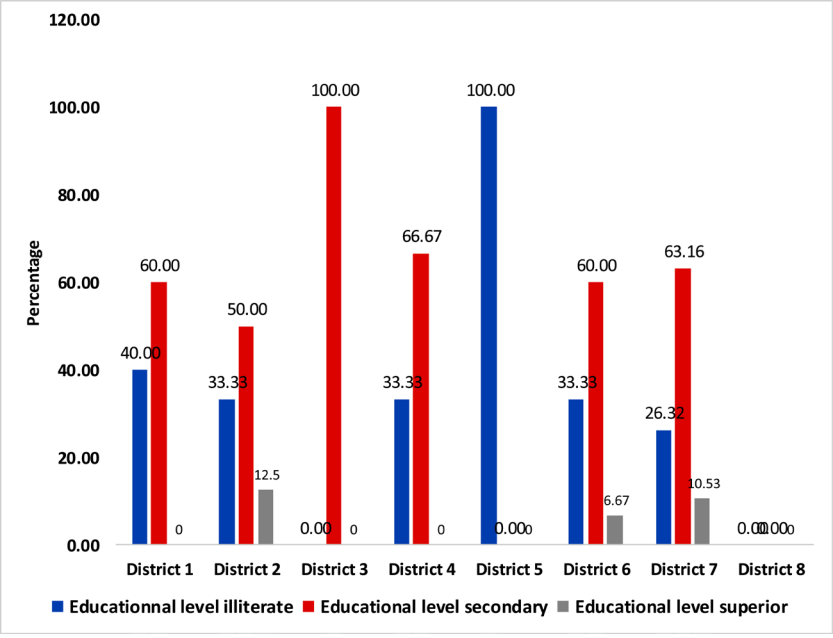

Figure 1. Education level of saleswomen in the 8 districts of Bangui.

3.3. Enumeration of Germs

3.3.1. Characteristic of Proximity of Places of Sale Porridge

23.21% of sales are located respectively next to landfills, 41.07% on public roads and 12.5% next to latrines (Table 1).

3.3.2. Count of Germs in Porridge Made from Rice

There is a strong proliferation of water intended for rinsing serving plates with germs indicating faecal contamination, indicators of hygiene in the various points of sale. No presence of salmonella and shigella was observed (Table 2).

3.3.3. Bacterial Load of Ground Peanut plus Sugar Mixture

It shows 10% of the samples that are microbiologically acceptable; we will notice that in some samples we observe the absence of *Staphylococcus aureus* while 90% are unsatisfactory. None of the samples at the sites are satisfactory (Table 3).

Table 2 shows values which are worrying in almost all the Arrondissements of Bangui, only in the 1st, 2nd and 7th Arrondissements that the absence of Staphylococcus aureus is recorded.

4. Discussion

The presence of microorganisms in raw materials is explained by the fact that they are often contaminated, in fields, during storage and during production [3]. The use of soiled materials, unhygienic working conditions during handling (dirty hands, unsanitary premises, etc.) as well as poorly adapted storage conditions (damp premises, excessive congestion, poor stock rotation, etc.) could explain the

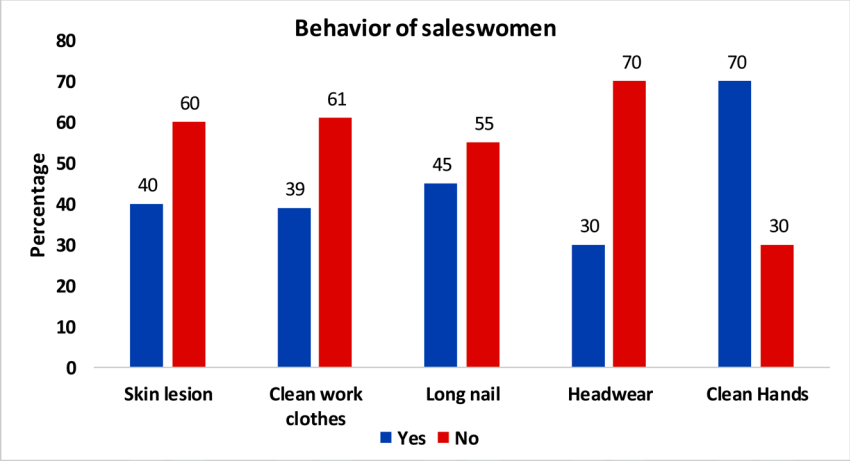

Figure 2. Hygienic situation of the sales environment in the 8 districts of Bangui.

Table 1. Characteristic of Proximity to places of sale porridge.

	Latrine	Hospital	Dump	Water polluted	Public highway			
					0 at 2 m	2 at 4 m	4 at 8 m	8 at +
Effective	7	4	13	9	6	14	3	0
Percentage (%)	12.5	7.14	23.21	16.07	41.07			

Table 2. Count of germs in porridge made from rice.

Sa/Bo	Germs Wanted			
	MAF	CF	CT	SF
	Standards			
	$<2 \times 10^5$	<10	$<10^3$	<10
1er	5.255×10^3	3.253×10^3	2.845×10^3	1.142×10^3
2e	6.715×10^3	3.134×10^3	3.14×10^2	1.648×10^3
3e	3.096×10^3	1.489×10^3	1.535×10^3	7.9×10^2
4e	1.942×10^3	3.77×10^3	1.72×10^2	8.66×10^2
5e	3.771×10^3	1.366×10^3	1.170×10^3	1.083×10^3
6e	3.199×10^3	1.407×10^3	1.407×10^3	7.96×10^2
7e	1.991×10^3	4.37×10^2	2.10^2	5.62×10^2
8e	3.200×10^3	1.171×10^3	9.76×10^2	1.02×10^3

Sa: Sample Bo: borough, MAF: Mesophilic Aerobic Flora, FC: Fecal Coliforms, TC: Total Coliforms FS: *fecal Staphylococcus*.

Table 3. Bacterial load results of the ground peanut-sugar mixture.

Sa/Bo	Germs Wanted			
	MAF	FC	TC	FS
	Standards			
	$<2 \times 10^5$	<10	$<10^3$	<10
1er	1.9×10^6	23	2.5×10^3	Abs
2e	2.8×10^5	34	4.14×10^3	Abs
3e	3.5×10^5	1.4×10^2	1.35×10^3	1.9×10^2
4e	1.7×10^6	3.1×10^2	3.72×10^3	1.86×10^2
5e	3.71×10^5	66	1.278×10^3	1.83×10^2
6e	3.5×10^5	1.7×10^2	1.47×10^3	Abs
7e	1.1×10^6	1.3×10^2	2.5×10^3	25
8e	3.1×10^5	1.11×10^2	2.67×10^3	36

Sa: Sample Bo: borough, MAF: Mesophilic Aerobic Flora, FC: Fecal Coliforms, TC: Total Coliforms FS: *fecal Staphylococcus*.

large number of samples highly contaminated both by spoilage germs (FAM) and hygiene indicator germs (CT and CF) as well as pathogenic germs (*Staphylococcus aureus*) [3]. There are three types of flora in food. The initial flora: this is the flora naturally present in foodstuffs from the raw material, this flora may or may not have an interest in the transformation of the food. The exogenous flora, also called the contamination flora, is responsible for the degradation of the food (loss of organoleptic characteristics), it comes from the workforce, the equipment used or the environment. Useful or technological flora is the flora involved in the processing of food, providing the organoleptic characteristics de-

sired by processors and appreciated by consumers [16]. In this study, the analysis shows disturbing results that show the values of strains of Fecal Staphylococcus, Fecal Coliforms and Total Coliforms that exceed international standards. The strains of *Staphylococcus aureus* are pathogenic germs but also an indicator of hygiene, in particular in the event of manual intervention (sorting, handling of products processed by traditional means [17]. This is reflected in the high frequency of isolation of *Staphylococcus aureus* from groundnut ground much more from sugar. In the finished products the analysis shows hygiene control germs (CT, CF,) and pathogenic germs (*Staphylococcus aureus*) among the samples of "pôpôtö". Microorganisms capable of presenting a health risk for fermented foods such as porridge, mainly Bacillus cereus strains and certain *Enterobacteriaceae* [18]. Strains of Lactobacillus plantarum isolated from ben-saalga produce bacteriocins that inhibit the growth of Bacillus cereus. For other pathogens, studies have shown that the lack of control of the processes made it possible to have a very low level of contamination and below the detection threshold. Fermentation and especially cooking are important points to control for the reduction of pathogens [3] [19]. The natural stabilizers produced by microorganisms (organic acids, carbon dioxide, hydrogen peroxide, diacetyl, ethanol and bacteriocins) participate in the preservation and harmlessness of these fermented porridge thus guaranteeing their safety. sanitary [7]. In the fermented porridge known in certain areas of Burkina Faso with different names, there are other species of lactic acid bacteria such as *Lactobacillus caseiO*3*, Lactobacillus pentosusO*4, *Lactobacillus fermentumO*5*, Lactobacillus sp.O*6*, Pediococcus spp, Lactobacillus breillus fermentobactum Mv*2 [20]. In general, lactic bacteria carry out lactic fermentation by degrading certain substrates (glucose, malic acid) into lactic acid which acidifies the medium inhibiting the growth of strains sensitive to an acidic pH, this fermentation can be homolactic or hetero-lactic. In the case of homolactic fermentation, glucose by the reaction of glycolysis is transformed into pyruvate, which is completely degraded into lactate by an enzyme which is lactate dehydrogenase [21]. Hetero-lactic fermentation leads to degradation by glycolysis of glucose into pyruvate then into lactate using the pentose phosphate pathway with the formation of other compounds, for example, in addition to lactate, there is the formation of ethanol, carbon dioxide and acetate [22]. Indeed, the cooking of the "pôpôtö" requires a relatively long time with high temperatures. Similar microbiological analysis results were found in samples of rice and beans cooked at 60˚C to 90˚C. Cooking may select for sporulated sprouts and subsequent slow cooling is a favorable factor for their multiplication [23]. In other words, although the high and sufficient temperature used to cook these foods to kill vegetative forms and spores, but these microorganisms could still survive. Heat-stable toxins such as *Staphylococcus aureus* enterotoxin may also persist after cooking. The relatively low level of contamination of spoilage flora (MAF) can be explained by the fact that they are generally killed by heat [23]. Out of a total of 56 samples analyzed, some were found to be unsatisfactory. In

the absence of the means implemented by the authorities, "pôpôtö" is sold almost everywhere: near hospitals, schools, institutes, etc. Nevertheless, the irregular presence of hygiene witness germs (TC, FC, SA) is almost automatically attributed to poor personal and clothing hygiene of the saleswomen as well as to the unsanitary conditions of the production plan and equipment. Exposure to the open air are two major factors in the contamination and proliferation of MAF in the "pôpôtö". This germ indicates the state of freshness and the general hygiene of the food. On the other hand, the absence of *Salmonella* and *Shigella* was recorded. These results are similar to the results found by E. B.Z. N'goran-Aw [24]. It may also turn out that the analysis period (seasonal factor) plays a non-negligible role on the analysis samples.

5. Conclusion

Consumers have a right to expect that the food they eat is safe and suitable for consumption. The current situation of the sale of food on the public highway in C.A.R allows us to highlight the importance of studying the bacteriological quality of "pôpôtö". The results of the microbiological analyzes have shown that the raw materials are contaminated. Although the sprouts are reduced and are even found to be absent after the cooking step, they reappeared at the time of sale. The vectors of food contamination are mainly vendors, the environment, the production process and inappropriate sales conditions. However, the producers and sellers of "pôpôtö" provide consumers with nutritious and tasty food at an affordable price for all social strata. This is why the hygienic quality of "pôtôtö" should be monitored and improved. It is necessary to undertake Information, Education and Communication campaigns. Further studies on the quality control of other types of food sold on the street could be considered to complement this study.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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