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Microbiological analysis and evaluation of Good Manufacturing Practices during the processing of raw white cabbage (*Brassica oleracea* var. *capitata* f. alba) served in a self-service restaurant

Análise microbiológica e avaliação das Boas Práticas de Fabricação durante o fluxograma de processamento do repolho branco (*Brassica oleracea* var. *capitata* f. *alba*) cru servido em um restaurante self-service

ABSTRACT

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Introduction: The consumption of meals outside the home has become an increasingly common practice in the life of the Brazilian population and of everyone. **Objective:** The aim of this work was to evaluate the microbiological quality of raw white cabbage (Brassica oleracea var. capitata f. alba), served in an institutional self-service restaurant, in the different stages of processing (reception, sanitation, slicing, cooling and distribution). In addition, Good Manufacturing Practices (GMP) were evaluated through a checklist. Method: Total coliform, Escherichia coli and total aerobic bacteria were counted and Salmonella spp. was searched. Results: A sample collected at the reception stage showed Salmonella spp. Sanitization eliminated Salmonella spp. and reduced total coliforms and E. coli to undetectable numbers. The environment, the equipment and the manipulation strongly influenced the microbiological quality of food. Samples collected on day 4, after slicing, showed 3.2 log CFU of E. coli per g and at distribution 4.1 log CFU/g, which indicates unsatisfactory hygienic conditions. The restaurant had 55.75% compliance with GMP items, being classified as regular (Group 2), in accordance with RDC n° 275/2002. Conclusions: The non-conformities (37.00%) observed in the exposure to prepared food consumption may be influencing the microbiological quality of raw white cabbage salad served. In this way we highlight the importance of the application of GMP in the production process to obtain a safe food and the compliance with the four POP required by RDC n° 216/2004.

KEYWORDS: Vegetables; Microbiological Analysis; Good Manufacturing Practices; Food Services; Food Production

RESUMO

Introdução: O consumo de refeições fora do lar vem se tornando uma prática cada dia mais comum na vida da população brasileira e de todo mundo. Objetivo: O objetivo deste trabalho foi avaliar a qualidade microbiológica do repolho branco (Brassica oleracea var. capitata f. alba) cru, servido em um restaurante self-service, nas diferentes etapas do processamento (recepção, higienização, fatiamento, resfriamento e distribuição). Além de avaliar os procedimentos de Boas Práticas de Fabricação (BPF), através de uma lista de verificação. Método: Foram realizadas as contagens de coliformes totais, Escherichia coli e bactérias totais aeróbias, e pesquisa de Salmonella spp. Resultados: Uma amostra coletada na etapa de recepção apresentou Salmonella spp. A higienização eliminou Salmonella spp. e reduziu coliformes totais e E. coli a números indetectáveis. O ambiente, os equipamentos e a manipulação influenciaram fortemente na qualidade microbiológica do alimento. As amostras coletadas no dia 4, após o fatiamento, apresentaram contagem de 3,2 log UFC de E. coli por g, chegando a distribuição com 4,1 log UFC/g, valor que indica, condições higiênico-sanitárias insatisfatórias. O restaurante apresentou 55,75% de conformidade aos requisitos das BPF, sendo classificado no Grupo 2, de acordo com a RDC n° 275, de 21 de outubro de 2002. As não conformidades (37,00%) observadas no item exposição ao consumo do alimento preparado podem estar influenciando diretamente a qualidade microbiológica do repolho branco cru servido. Conclusões: Desta forma, destacamos a importância da aplicação das BPF no processo produtivo para a obtenção de um alimento seguro e o atendimento aos quatro POP exigidos pela RDC n° 216, de 15 de setembro de 2004.

PALAVRAS-CHAVE: Hortaliças; Análise Microbiológica; Boas Práticas de Fabricação; Serviços de Alimentação; Produção de Alimentos

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INTRODUCTION

The consumption of meals outside people's homes has become an increasingly common practice in the life of the Brazilian population as well as worldwide. To meet this demand, the collective meal market is growing and consolidating¹. In line with this expansion of the market, there is also a growing concern about food safety in Brazil among consumers who are more aware and informed about healthy eating and also among food services, which are responsible for the safety of the meals they produce^{2,3.}

Health promotion through safe and nutritionally adequate food pervades the Food Guide for the Brazilian Population, which recommends that the basis of a balanced diet should be fresh or minimally processed food in a wide variety and predominantly of vegetable origin⁴. It is also evidenced that the daily consumption of vegetables plays a fundamental role in human food, because they are rich sources of fiber, vitamins, minerals and antioxidants, which are even better when consumed raw⁵. However, the consumption of raw vegetables is often related to the transmission of infectious and parasitic diseases due to inadequate cultivation6 and handling during the production process, usually associated with the absence of adequate hygienic practices^{7,8,9}.

When consumed raw, vegetables may be related to foodborne diseases and become the vehicles of pathogenic microorganisms of public health relevance, like Escherichia coli O157: H7, Salmonella spp., Listeria monocytogenes and Shigella sp.¹⁰. Vegetables that are purchased fresh are not free from infectious agents: studies report that they are closely linked to pesticide and pathogen contamination¹¹. Microbiological quality control of food, which was based on the analysis of the final product, gave way to the control of the processing stages of the product, more efficiently guaranteeing the harmlessness of the final products^{2,12}, which may include dishes with raw vegetables.

The inclusion of vegetables in the menu of a Food and Nutrition Unit (FNU) is based on the planning of its functional physical structure, through the sizing of the areas for reception, storage, pre-preparation and distribution, in co-management with the human and material resources available for the validation of the process^{13,14}.

Risk mitigation in the provision of safe food is closely linked to Food Safety Management Systems (FSMS), which are, in turn, based on Good Manufacturing Practices (GMP). GMP cover requirements related to facilities, equipment, utensils, raw materials, sanitation, water supply, food handling and vector and urban pest control^{15,16}.

With that in mind, the present study had the objective of evaluating the microbiological quality and the GMP procedures of raw white cabbage (Brassica oleracea var. capitata f. alba), served in an institutional self-service restaurant, at various processing stages.

METHOD

Study Design

This cross-sectional study was conducted in a self-service restaurant in the city of Niterói, Rio de Janeiro, Brazil. The restaurant serves between 6,500 and 7,000 meals between lunch and dinner every day.

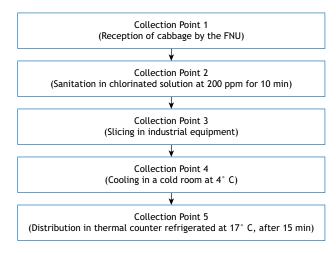
Sample collections and microbiological analyses were done from June to August 2016. Samples of raw white cabbage were collected in four consecutive weeks (one day per week), when present on the menu in the form of raw salad. For each day of the experiment (four days), five samples were collected, one at each determined collection point, totaling 20 samples of raw white cabbage. Five collection points were defined for each day, referring to the different stages of the production process: Reception, Sanitation, Slicing, Cooling and Distribution (Figure 1).

Approximately 100 g of each sample were collected using firstuse vinyl gloves. The samples were individually packaged in sterile plastic bags with the identification defined for each sample at each collection point. The samples were stored in a thermal container with reusable ice, transported to the laboratory and analyzed in a maximum period of 3 hours.

Microbiological analyses

25 g of the sample were added to 225 ml of buffered peptone water (BPW), which was homogenized in a MK1204 Stomacher Type Homogenizer for 120 s (10^{-1} dilution). Then, we proceeded to the other decimal dilutions (10^{-2} and 10^{-3}) in 0.85% (p/v) saline^{17.}

To count total coliforms and E. coli, we used a fraction of 1.0 ml of each dilution, which was deposited at the center of the



UAN: Unidade de Alimentação e Nutrição

Figure 1. Collection points of the samples within the raw white cabbage production flowchart.



EC 3M Petrifilm plate (3M Company, St. Paul, MN, USA). The inoculum was spread with a plastic diffuser and formed a gel; the plates were incubated in an oven at 35° C for 48 hours. Later on, we counted the colony forming units (CFU) of total coliforms (blue and red colonies, with gas) and E. coli (blue colonies, with gas).

Total aerobic bacteria count was performed using the deep seeding technique. A 1 ml fraction of each dilution was deposited in a sterile disposable plate, in duplicate, and then this fraction was homogenized in tryptone soybean agar (TSA; Himedia). After solidification of the culture medium, the plates were incubated at 30° C for 72 hours for further counting of CFU^{18.}

To search for Salmonella spp., the BPW samples were incubated at 35° C for 24 hours for recovery of the injured cells. After enrichment, a 1 ml fraction of the BPW culture was transferred to 10 ml of Tetrathionate Broth (TT, Oxoid) plus iodine iodide (0.2 ml). Another 0.1 ml fraction was transferred to 10 ml of modified Rappaport Vassiliadis broth (RV, Sigma-Aldrich), and the tubes were incubated at 35° C and 42° C for 24 hours, respectively. A fraction of each selective enrichment broth was streaked in the selective media of Hectoen Enteric agar (HE, Becton Dickson), Bismuth Sulfite agar (BS, Acumedia) and Xylose Lysine Deoxycycolate agar (XLD, Kasvi), in duplicate. The plates containing the selective media for Salmonella spp. were incubated at 35° C for 24 hours. The typical suspicious colonies of Salmonella in each culture medium (HE: transparent colony, blue-green with or without black center, BS: brown or black colony with or without metallic luster; XLD: transparent colony, dark pink, with or without black center), were used for biochemical identification using Lysine Iron agar (LIA, Himedia) and Triple Sugar Iron agar (TSI, Himedia). After incubation at 35° C for 24 hours, the colonies identified as Salmonella spp. [TSI: alkaline ramp (red) and acidic background (yellow), with or without production of H2S; LIA: alkaline background and ramp (purple, without color change of the medium) with or without production of H2S] were subjected to confirmation by serum agglutination using polyvalent antiserum O for Salmonella (Probac do Brasil, São Paulo, Brazil)^{17.}

Evaluation of Good Manufacturing Practices procedures and production flow

A checklist of 11 categories based on RDC n. 216, of September 15, 2004¹⁹, was used for the evaluation of the GMP in the FNU we studied. The categories were divided into: 1- Building, facilities, equipment, furniture and utensils; 2- Hygiene of facilities, equipment, furniture and utensils; 3- Integrated control of vectors and urban pests; 4- Water supply; 5- Waste management; 6- Handlers; 7- Raw materials, ingredients and packaging; 8- Food preparation; 9- Storage and transportation of prepared food; 10- Exposure to the consumption of prepared food; 11- Documentation and records. The items in each category were rated as compliant, non-compliant or not applicable.

In order to evaluate the results, we used the parameters of RDC n. 275, of October 21, 200220, which classifies the establishments

according to their percentage of compliance with GMP. Those belonging to Group 1 meet 76 to 100% of the GMP requirements; those in Group 2, 51 to 75%; and in Group 3, they meet from 0 to 50% of the requirements^{20.}

The production flow was monitored and recorded during all days of collection, from the arrival of the raw material until the moment of its distribution. We measured the temperature in the stages of the production process whenever it was necessary (cooling and distribution).

Statistical analyses

The results of total coliforms, *E. coli* and total aerobic bacteria counts were converted into log CFU/g. The statistical significance was analyzed by the variance test (ANOVA) and Tukey's test. The Prisma 5.0 statistical program was used to determine the correlation between the different processing stages of raw white cabbage. Values of p <0.05 were considered significant.

RESULTS

Raw white cabbage samples collected at the receiving stage had mean total coliforms and *E. coli* of 4.1 log CFU/g. The sanitizing process significantly eliminated total coliforms and *E. coli* of raw white cabbage (p < 0.001 and p = 0.0481, respectively). However, in the stages after sanitation, the number of these microorganisms increased, presenting 3.1 log CFU/g of total coliforms and 2.0 log CFU/g of total coliforms and *E. coli* (Table 1). There was a significant difference in the increase of total coliforms in the cooling and distribution stages, in relation to the sanitation stage (Table 1).

The sanitation step reduced the population of *E. coli* in raw white cabbage to undetectable numbers (0.1 log CFU) on all collection days. *E. coli* remained undetectable (0.1 log CFU/g) in the slicing and cooling steps on days 1 and 2 and in the slicing step on day 3. Samples collected in the cooling stage on day 3 presented 1.8 log CFU of *E. coli* per g of raw white cabbage. In the samples of raw white cabbage collected on day 4, the count of *E. coli* was 3.2

Table 1. Mean of total coliform counts, *E. coli* and total aerobic bacteria in the samples of raw white cabbage collected at the different stages of the process flow diagram of an institutional self-service restaurant.

Process Step	Quality Indicator*				
	Total coliforms	E. coli	Total aerobic bacteria		
Reception	4.1 ^{ab} ± 0.50	4.1 ^a ± 0.17	5.0 ^a ± 0.60		
Sanitation	$0.1^{acd} \pm 0.00$	$0.1^{a} \pm 0.00$	2.3 ^{ab} ± 1.38		
Slicing	1.6 ^b ± 1.34	1.1 ± 1.79	4.4 ± 0.72		
Cooling	2.8° ± 0.45	1.1 ± 1.13	5.3 ^b ± 0.63		
Distribution	3.1 ^d ± 1.07	2.0 ± 1.79	4.4 ± 0.63		

*Mean log CFU/g and standard deviation of the various repetitions. Equal lowercase letters indicate a significant difference (p < 0.05) between the different stages of the process, according to Tukey's test, for the different quality indicators we evaluated.



log CFU/g in the slicing and cooling stages. In the distribution step, the count of *E. coli* was 1.0 log CFU/g on days 1 and 2, 1.8 log CFU/g on day 3 and 4.1 log CFU/g on day 4. Samples of raw white cabbage collected on day 4, at the slicing, cooling and distribution stages, presented counts of *E. coli* above the maximum limit established by Brazilian legislation, which is 2 log CFU/g²¹ (Figure 2).

A high number of aerobic total bacteria was observed in samples of raw white cabbage collected at the reception, with an average of 5 log CFU/g. After the sanitation, there was a significant decrease (p = 0.0125) of the microbial load with a mean of 2.3 log CFU of total aerobic bacteria/g. In the post-sanitation stages, the mean aerobic total bacteria was 4.4 log CFU/g in the slicing, 5.3 log CFU/g in the cooling, and 4.4 log CFU/g in the distribution, with increase of the total microbial load between the sanitation and cooling stages (Table 1).

Of the 20 samples of raw white cabbage we analyzed, Salmonella spp. was found in one (5%), at the Reception stage, on day 1 of collection. The study was able to identify the sanitation stage as an important biological hazard control point in the raw white cabbage production flowchart.

A total of 117 items were analyzed for the GMP assessment through a checklist based on RDC n. 216/200419. Four items did not apply to the studied FNU. Of the 113 items that applied, 63 (55.75%) were compliant and 50 (44.25%) were non-compliant. The FNU was classified as Regular (Group 2), according to RDC 275/200220. Of the 11 categories evaluated, five presented compliance between 25% and 50%, three presented compliance between 56% and 57% and three showed compliance between 80% and 100%. The percentage of compliance with GMP items by category is shown in Figure 3, where the category of water supply with the highest percentage of conformities (100%) is highlighted and the category of storage and transportation of the prepared food has the lowest percentage of compliance (25%).

The four Standard Operating Procedures (SOP) recommended as required by RDC n. 216/200419, "Sanitation of Facilities,

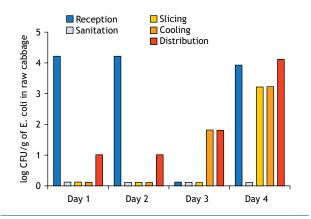


Figure 2. Count of *E. coli* in the different stages of the production process of raw white cabbage, over the four days of collection. Legislation standard 2 log CFU/g^{21} .

Equipment, Furniture and Appliances", "Integrated Control of Vectors and Urban Pests", "Sanitation of the Reservoir" and "Hygiene and Health of Handlers", achieved a percentage of compliance of 50%, 33.33%, 100% and 57.14%, respectively (Figure 3).

During the production process, over the four days of collection, we recorded the temperatures and the collection points at the cooling and the distribution stages, as shown in Table 2.

The production flow was carried out in the forward direction. The vegetable pre-preparation area was insufficient for the unit's production volume (approximately 7,000 meals per day). The white cabbage supplier was not monitored for good delivery conditions, and the microbiological and physico-chemical control of the raw material was not performed on a regular basis. The pre-preparation site was not isolated from the preparation area by physical barriers, the handlers performed several activities during the cleaning and slicing of the white cabbage, such as handling food trolleys, utensils and equipment and the fresh raw material, wearing the same disposable gloves throughout the process. Few customers had the habit of sanitizing their hands, and the part of the utensil that came into contact with the clients' hands (handle) was constantly seen inside the container where the raw cabbage was being served.

DISCUSSION

Sanitizing raw vegetables is fundamental for the preparation of food that is safe for the health of the consumer. This process must ensure the elimination, reduction and/or removal of microbial contamination present in the raw material, especially pathogens²². Sanitizing with sodium hypochlorite at 200 ppm for 10 min was efficient, as it significantly reduced and/or eliminated all microbiological quality indicators (E. coli, total coliforms, total aerobic bacteria and Salmonella spp.) we evaluated (Table 1). However, there was increase and/or contamination of the product, with the exception of Salmonella in the post-sanitation stages.

Distribution was the most important point of increase and/ or contamination by E. coli, since this type of bacteria was detected in all samples of raw white cabbage at this stage, as shown in Figure 2. RDC n. 12, of January 2, 200121, does not establish an E. coli count, however, it establishes the maximum limit of 10^2 (2 log CFU) thermotolerant coliforms per g for "vegetables that are fresh, prepared (peeled or selected or sliced), sanitized, chilled or frozen, for direct consumption, except for mushrooms"²¹. Thus, we compared the results obtained for E.

 Table 2. Temperature of raw white cabbage during the cooling and in the distribution counter over the four days of collection.

Process Step	Collection Day/Temperature				
Process Step	1	2	3	4	
Cooling	21°C	17°C	16°C	16°C	
Distribution	21°C	18°C	17°C	19°C	



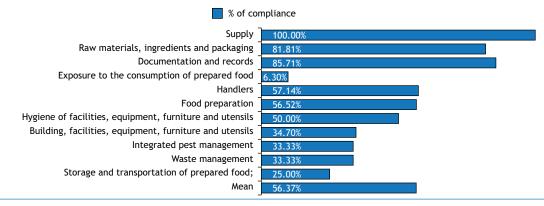


Figure 3. Percentage of items compliant with the requirements of Good Manufacturing Practices, according to RDC n. 216/200419

coli with the maximum limit established for thermotolerant coliforms, and the sample collected at day 4 was classified as unfit for human consumption (Figure 2). The distribution was also a point of increase and/or contamination of total coliforms and total aerobic bacteria in raw white cabbage. In the distribution stage, the product is exposed and handled by the customers, thus facilitating the increase and/or contamination of the product by different microorganisms. The GMP checklist item for ready-to-eat food exposure presented 63% of compliance, however, non-compliance with this item was also observed (37%). This non-compliance associated with the item of ready-to-eat food may be directly interfering with the contamination and/ or increase of the microbial population, especially the significant increase of total coliforms in the raw white cabbage at the distribution point. Bacterial contamination seems to be directly related to the conditions of distribution, disposal of containers at the counter, consumer access, collection of food or contamination of collection utensils.

The increase and/or contamination of raw white cabbage by total coliforms, E. coli and total aerobic bacteria in the slicing and cooling stages may be related to the fact that post-sliced raw white cabbage presents a high percentage of humidity and a bigger area of contact with the air, which renders the ready product more susceptible to microbial development than the fresh product²³. Furthermore, the high percentage of non-compliance (43.42%) found in the food preparation item, especially the control of cross contamination, may have influenced the microbiological quality of raw white cabbage in these stages. In the cooling stage, raw white cabbage was stored for an average time of 1 hour between 17° C and 20° C. The percentage of compliance of 25%, referring to the item of storage and transportation of the prepared food, warrants the inadequate conditions presented in this stage of the process, since the refrigeration is contemplated in the storage item. The incidence of microorganisms in vegetables reflects the hygienic-sanitary quality of the processing steps and the microbiological condition of the product at the time of processing^{23.}

Salmonella spp. has been isolated from raw vegetables consumed in various parts of the world^{24,25}. Sodium hypochlorite is the most common and efficient sanitizer of fruit and vegetables; it can eliminate several pathogens, including Salmonella spp.²⁶. RDC n. 12/2001 recommends the absence of Salmonella spp. per 25 g of vegetables that are fresh, whole, selected or not, excluding mushrooms"^{21.}

Studies indicated the presence of microorganisms such as Staphylococcus aureus, coliforms at 45° C, Salmonella, aerobic mesophilic bacteria, molds and yeasts on counters and cutting boards^{27,28}, in industrial equipment (blenders and processors) and pans at the FNU^{29,30}. In the present study, microbiological analyses of surfaces of counters, equipment and utensils were not performed, but the category of "Sanitation of facilities, equipment, furniture and utensils" of the checklist achieved an adequacy index of 50%, indicating that the lack of hygiene may be contributing to the inadequate microbiological quality of the final product.

Laboratory diagnosis facilitates the tracking of pathogens transmitted to the production, storage, transport and handling of vegetables³¹. The control of the stages of the productive process of meals provides a better view of the whole process, which is fundamental for the supply of safe food for human consumption. But for this, a set of actions is necessary.

We can emphasize the importance of functional physical planning of the FNU in order to provide suitable hygienic-sanitary conditions to prevent the transmission of pathogenic microorganisms^{32.} Farias et al.³³, in a study conducted at a hospital FNU in Pará, Brazil, and Calil et al.³⁴, in a study carried out in a self-service restaurant in São Bernardo do Campo, São Paulo, Brazil, noticed that all the samples collected from raw salad presented hygienic-sanitary conditions within the standards established by the legislation.

Although the studied FNU was classified in Group 2 or as regular in relation to compliance with GMP requirements, most of the evaluated categories presented less than 50% of compliance. This indicates the need for the FNU to conform to GMP requirements for safe food production.

Of the four SOP established by RDC n. 216/200419, only the SOP referring to the "Sanitation of the water tank" achieved 100% of compliance. Compliance with SOP is directly related to the quality of the final product, as their inadequacy may lead to risks of chemical, physical and/or biological contamination of the product. With this, we highlight the need to adapt the studied FNU to



the SOP required by RDC n. 216/200419. Ongoing education and onsite training in food safety may help maintain and ensure the production of safe food 35 .

The SOP of "Water supply" and the "Documentation and records" GMP requirement presented the highest percentage of compliance among the eleven categories we evaluated (Figure 3). These activities are the sole responsibility of the Technical Officer of the unit, who elaborates the Good Practices Manual (GPM), the SOP, contracts for outsourced services, among other documents required by the Brazilian Health Surveillance Agency (Anvisa)³⁶. Some authors have described that the lack of professional dietitians in food services is directly related to the non-compliance with the items of "Water supply" and "Documentation and records"37,38. This is justified because the dietitian is the qualified professional in charge of planning, coordinating, directing, supervising and evaluating the area of food and nutrition^{37,38}. The high percentage of compliance presented for these categories in the studied FNU probably can be attributed to the fact that the establishment has a professional dietitian in its staff.

A study carried out in the city of Santa Maria, Rio Grande do Sul, Brazil, evaluated 23 commercial food services regarding their hygienic conditions; 70% of the establishments were classified in Group 2 (regular), and the category of water supply was the one that presented the highest percentage of adequacy $(82\%)^{39}$, similar to the results of this study.

The category of "Buildings, facilities, equipment, furniture and utensils" presented a low compliance percentage (34.7%) and is one of the possible factors related to cross contamination. Structural changes arise as alternatives to adapt this category to GMP.

RDC n. 216/2004¹⁹, which provides for the Technical Regulation of Good Practices for Food Services, does not determine the time and temperature during the distribution of ready-to-eat cold foods in FNU. The Ordinance of the Sanitary Surveillance Center of the São Paulo State Health Secretariat (CVS) n. 6, of March 10, 199940, establishes that potentially dangerous cold foods that favor rapid microbial multiplication must be distributed at a temperature between 10° C and 21° C for up to 2 hours or at a maximum of 10° C for up to 4 hours and cold foods that exceed the established time and temperature criteria should be discarded. The raw white cabbage salad served at the restaurant we studied remained for up to 1 hour in the refrigeration chamber at 4° C before proceeding to the distribution counter where it was exposed for up to 35 min. The temperatures of the raw white cabbage salad were in accordance with the standard established by CVS n. $6/1999^{40}$. The increase in microbial load in the samples collected in the cooling and distribution stages may be related to hygiene failures during slicing and/or hygiene failure in the cooling and distribution environment.

In order for the FNU we studied to meet GMP standards, it must undergo some changes. Considering the importance of the FNU in question, the high number of meals served daily and the usual supply of raw vegetables in its menu, the presented data highlights the need to perform greater control in the stages of the production process of raw white cabbage served in the form of salad, to review the GMP requirement of "Exposure to consumption of prepared food" and to adjust to the SOP-related items required by RDC n. 216/2004^{19.}

CONCLUSIONS

The sanitation step is a Critical Control Point (CCP) in the raw white cabbage production flowchart, including for the elimination of *Salmonella* spp. However, non-compliance with various items referring to GMP requirements in the post-sanitation stages led to an increase and/or contamination of the product by total coliforms, *E. coli* and total aerobic bacteria. The distribution stage was the most important point of contamination, especially for *E. coli*. This is mainly due to the non-compliance observed in the GMP requirement for the exposure of ready-to-eat food. Failure to comply with SOP may lead the FNU to serve raw white cabbage that is not fit for human consumption and poses a risk to consumer health.

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Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.



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