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Microbiological quality of meat-based meals and operation of control systems within a food service environment

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Abstract

The catering industry is subjected to general European Union regulations concerning food hygiene, which has made the application of HACCP principles mandatory for almost all the activities in the food sector. The present report is aimed at evaluating the microbiological quality of meat-based meals and operation of control systems within a university restaurant. Unfortunately, the effort made over eleven years in terms of monitoring the temperature of the sliced boiled beef and implementing some preventive and corrective actions was not enough to bring under control the key Critical Control Points (CCP) of the cold storage in a university canteen. This was most likely due to the persistence of some structural deficiencies concerning human resources associated with possible cross-contamination phenomena. As a consequence of the inability to control these key CCP efficaciously, the (suggested) limit for the counts of the common contaminants (total mesophilic aerobes) was overcome in all the years except three, those for *Staphylococcus aureus* and coliforms were exceeded in two out of the eleven years of monitoring, and that for *Bacillus cereus* only in one year. No samples were found positive for the presence of *Listeria monocytogenes, Salmonella* spp., or *Escherichia coli.*

Introduction

Access to safe foods has always been mankind's first concern. Food safety is indeed the basic condition for food quality, which implies the absence or occurrence,within acceptable limits, of chemical and microbiological contaminants and any other entity that may create risks for human health. Meat and meat products from different sources, more than any other types of foods, may become vehicles of harmful food-borne pathogens. It is well known that living animals are natural reservoirs of pathogenic bacteria which originate from carcasses contaminated at slaughtering. It is also known that pathogens may contaminate meats from the environment as well via cross-contamination. Therefore, contamination control and the effective inactivation of pathogenic bacteria are decisive to the safety of meat and meat products (Borch and Arinder, 2002) and as such, continuous vigilance on food hygiene is necessary as established by European Regulations (Nørrung and Buncic, 2008). The outbreaks and scandals in the meat sector in the late nineties have had a crucial role in inspiring, or at least in accelerating, the issuing of key European Regulations on food hygiene, such as Regulation 178/2002 and the so-called "Hygiene Package" including Regulation (EC) 852/2004, which abrogated the previous EC Directive 93/43 but re-affirmed the mandatory nature of the Hazard Analysis and Critical Control Point (HACCP) principles.

The assessment of microbiological quality of foods is a fundamental activity in HACCP programs since it is the only valid tool to verify whether the HACCP system is working properly and to find out possible troubles in the procedures that have been implemented (Cenci-Goga *et al*., 2005; Osimani *et al*., 2011; Osimani *et al*., 2013a; Osimani *et al*., 2013b; Osimani *et al*., 2013c; Osimani *et al*., 2015). In spite of the increasing attention of the public authorities and consequently of the food operators towards food hygiene and food safety, the consumption of foods contaminated with pathogenic microorganisms or their toxins remains one of the major causes of disease, hospitalization, and economic loss (CDC, 2013). In this context, the catering industry occupies a delicate position with important public health implications since this sector involves complex procedures which aim at preparing a large number of different meals, sometimes also destined for consumers with an increased degree of vulnerability (Petruzzelli *et al*., 2014a). Among the numerous types of meals served at catering, meat products deserve particular attention because, if not properly cooked and/or managed,

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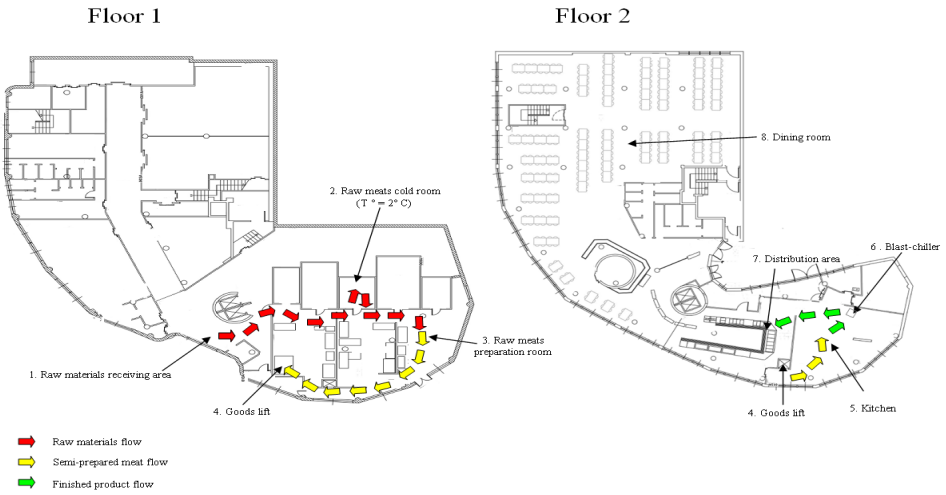


Figure 1. University restaurant layout and the production flow of raw meat and finished-products

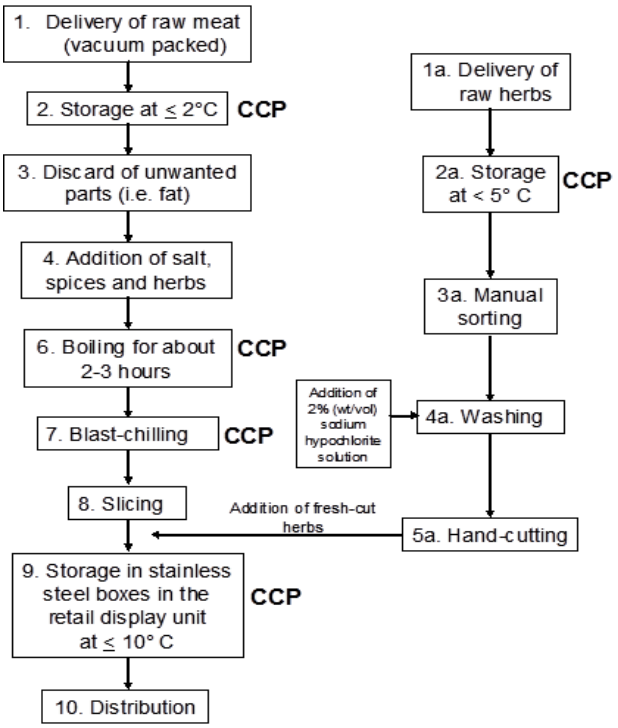
they can be the source of pathogenic and toxinogenic microorganisms (Borch and Arinder, 2002; Badrie *et al*., 2004; Jordan *et al.*, 2006; Petruzzelli *et al*., 2010; Petruzzelli *et al.*, 2014b). The effectiveness of the cooking process depends on several factors, such as: the microbiological quality of raw meats, the amount of meat to be cooked, the size of the piece(s), the cooking method (frying, roasting, boiling, etc.), the type of equipment used for cooking (oven, crock pot, stew pot, grill, etc.), the presence of additional ingredients (potatoes, stuffing, raw parsley, sauces, spices, etc.). In addition, efficient protection from different kinds of post-cooking contamination, rapid cooling down (by blast-chillers) and controlled cold storage are all of primary importance for guaranteeing safety of cooked meat preparations (Daelman *et al.*, 2013; Gibbons *et al.*, 2006; Juneja *et al*., 2001; Mataragas *et al.*, 2008).

On the basis of the above premises, an 11-year report (2002-2012) about the operation of a control system regarding the microbiological quality of boiled beef prepared and consumed at a university restaurant is presented. To this end, an extended monitoring of temperature of the ready-to-eat meat in cold storage in a retail display unit, together with a congruous number of microbiological analyses, were carried out between 2003 and 2012.

Materials and Methods

Description of the university canteen

This study concerns a University canteen, located in the city of Ancona (central Italy) that serves up to 1,200 students a day. The canteen, which is housed on two floors (Figure 1) of a building, was built in the 70 s and adapted to its current use in 1990. The HACCP system was originally implemented in accordance with the directions given by the European Directive



CCP Critical Control Point

Figure 2. Boiled beef preparation flow chart

43/93 and revised between the years 2003 and 2004. Figure 2 shows the boiled beef flow chart as reported in the revised HACCP manual. Furthermore, the appropriate anatomical part of raw beef is delivered at the receiving area of the canteen as a 5 kg vacuum-packaged piece, and from here its "journey" begins (Figure 1). According to the flow chart, the raw meat packages, purchased from reputable and approved stockists, are inspected on arrival for possible signs of spoilage or damage, and their temperature is checked in order to verify whether the legal limit of ≤ +2°C has been respected. If the on-arrival inspection is passed, the packages are immediately stored at ≤ +2°C (CCP – Critical Control Point) in a dedicated

Table 1. Sanitary assessment of food environment (SAFE) at critical control points (CCP)

Phases	Risks	Preventive actions	Limits	Monitoring	Corrective actions	Registrations (on dedicated forms)
Raw meat storage in cold room	Microbial contamination and/or growth	Check of raw meat expiration date. Visual check of the cold room temperature display	T°= 2 °C	Use of a temperature data logger (including over-temperature alarm)	In case of temperature abuse, raw meat pieces must be destroyed. Processing of the abused meat (within the same day) is permitted if the temperature abuse period ranges from 0 to 3 hours (with a recorded temperature = 10°C)	Daily recording of raw meat storage temperature
Raw herbs storage in cold room	Microbial contamination and/or growth	Visual check of the cold room temperature display.	T° = 5 °C	Use of a temperature data logger (including over-temperature alarm)	In case of temperature abuse, raw herbs must be discarded or processed within the same day	Daily recording of vegetable storage temperature
Meat cooking	Microbial survival	time/temperature values need to be respected??	Core temperature = 75° C for at least 10 minutes	Manual temperature measurement (with portable thermometer); visual check of the cooking progress	In case of incomplete cooking, the meat pieces are subjected to further cooking	Recording of cooking temperature
Meat blast-chilling	Microbial contamination and/or growth	time/temperature values need to be respected??	Core temperature = 10° C after 2 hours of chilling	Automatic temperature measurement.	In case of inappropriate cooling, the meat pieces are discarded; hence a technical action on the blast-chiller is required.	Recording of blast-chilling temperature
Sliced boiled meat storage in the retail display unit	Microbial contamination and/or growth	maintenance temperature to be respected??; check?? cleanliness of the stainless steel pans	T° = 10 °C	Manual temperature measurement (with portable thermometer)	In case of temperature abuse, the pans containing sliced meats must be put in the blast-chiller again (if temperature values = 30 °C are recorded, meat must be discarded)	Recording of ready-to-eat meat temperature

cold room (equipped with an internal data logger) and managed in accordance with the "first-in-first-out" stock rotation.

The meat packages are again subjected to a visual inspection of the vacuum seal. Then, once unpacked, the condition of the meat is checked by a visual and olfactory examination. Boiled beef is prepared by portioning pieces of about 1.5-2.0 kg from the whole muscle that are trimmed before being put into boiling water containing rosemary, celery, carrots, garlic and, marjoram. All the pre-cooking steps are carried out in a dedicated room and with dedicated chopping-boards. Cooking (CCP) is monitored by both visual examination and temperature measuring at the meat core (with a portable thermometer), as specified in the standard operating procedures (SOPs). Finally, cooked meat is subjected to rapid cooling in order to reach a temperature < 10°C within 2 hours in a blast-chiller (CCP). Afterwards, the meat piece is sliced with a slicing machine, and raw parsley and olive oil are added to it; then, the slices are stored (for a maximum of three hours) under refrigerated conditions (CCP) in the retail display unit dedicated to cold-served meals. All the equipment used in the procedure is sanitized at the end of work using detergents and a 2% benzalkonium chloride-based solution, as specified in the standard operating procedures (SOPs). The CCPs are monitored as reported in Table 1.

Microbiological analyses of ready-to-eat boiled beef

Sixty-four boiled beef samples were collected from 2002 to 2012 with a casual frequency, using sterile instruments and sterile bags (Sto-Circul-Bag, Pbi International, Milan, Italy), quickly stored under refrigerated conditions, and analysed within 2 hours. The following microbiological analyses were carried out, as previously described by Osimani and colleagues (2011): presence/absence of *Listeria monocytogenes* and *Salmonella* spp. (in 25 g); viable counts of coliforms, sulphite-reducing clostridia, total mesophilic aerobes (TMA) and of presumptive *Escherichia coli, Bacillus cereus,* and *Staphylococcus aureus.*

As previously described by Osimani *et al.* (2011), unacceptable samples were defined on the basis of the microbiological limits set by EC Regulation 2073/2005, whereas those considered unsatisfactory were established on the basis of the Italian guidelines for the microbiological quality of cooked and cold-served meals in accordance with the microbiological limits suggested by the Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, which is a reference Public Institution on food hygiene and veterinary public health. More specifically, the limits were the following. In 25 g of meat, there had to be absence of *Salmonella* spp. and *Listeria monocytogenes*; ≤ 3 Log cfu/g of coliforms; ≤ 1 Log cfu/g of *E. coli*; ≤ 2 Log cfu/g of *B. cereus*; ≤ 2 Log cfu/g of *S.aureus*; ≤ 1 Log cfu/g of sulphite reducing clostridia; ≤ 4.0 Log cfu/g TMA.

Temperature monitoring of ready-to-eat boiled beef

Since 2003, the temperature of ready-to-eat boiled

Table 2. Microbiological quality of the boiled beef rolls

Year	Number of samples exceeding reference limits						% unsatisfactory
	TMA	C	Ec	Sa	Bc	SRC	samples*
2002	6 (6.2; 5.3; 5.4; 6.0; 5.2; 5.3)	1 (2.3)	0	3 (2.0; 2.3; 2.7)	0	0	64.3 [9 out of 14]
2003	9 (5.8; 6.0; 4.7; 4.4; 6.5; 6.4; 4.8; 4.6; 5.3)	0	0	6 (2.0; 2.8; 2.9; 2.0; 2.3; 2.9)	0	0	62.5 [15 out of 24]
2004	4 (5.0; 6.0; 7.4; 7.1)	0	0	0	0	0	66.6 [4 out of 6]
2005	2 (5.2; 5.3)	0	0	0	0	0	50.0 [2 out of 4]
2006	1 (4.6)	0	0	0	0	0	50.0 [1 out of 2]
2007	0	0	0	0	0	0	0 [0 out of 2]
2008	0	0	0	0	0	0	0 [0 out of 2]
2009	1 (6.0)	1 (3.8)	0	0	0	0	50.0 [1 out of 2]
2010	1 (4.6)	0	0	0	1 (2.3; 2.4)	0	100 [2 out of 2]
2011	1 (7.5)	0	0	0	0	0	50.0 [1 out of 2]
2012	0	0	0	0	0	0	0 [0 out of 4]
Total unsatisfactory samples	25	2	0	9	1	0	54.7 [35 out of 64]

*Unsatisfactory samples exceeding reference limits for one or more parameters. TMA = Total Mesophilic Aerobes; C = Coliforms; Ec = *Escherichia coli*; Sa = *Staphylococcus aureus*; Bc = *Bacillus cereus;* SRC = Sulphite-Reducing Clostridia. In round brackets, the values of viable counts expressed as Log cfu/g; In square brackets, the number of unsatisfactory samples out of the total number of samples analyzed

beef to be subjected to microbiological analyses was recorded during cold-storage in a dedicated retail display unit using a high precision thermometer (Checktemp 98509-1 Hanna Instruments, Milan, Italy). The values recorded were compared with the limit (T < 10°C) set by D.P.R. no. 327 of 26/03/1980 (Art. 31), published in the Official Gazette of the Italian Republic no. 193 of 16/07/1980.

Results and Discussion

Microbiological analyses of ready-to-eat boiled beef

The results of the microbiological analyses based on the (legal or suggested) reference limits are shown in Table 2. Samples exceeding the limit of TMA (with counts ranging from 4.4 to 7.5 Log cfu/g) were found in all the years except for 2007, 2008 and 2102. Moreover, in 40% of the samples not conforming to TMA, these microorganisms reached populations ≥ 6.0 Log cfu/g. Even if a heavy load of TMA is not necessarily a risk factor for the health of the consumer, it is used as a broad indicator of the food's poor microbiological quality. As previously reported by Hamasaki *et al.* (2013), for cooked meat products stored below +10°C, a high load of TMA in meat-based preparations might even be due to the occurrence of spoilage bacteria which is able to grow on a non selective PCA medium (Egan, 1983).

As regards coliforms, whose counts are generally adopted as an efficient parameter for estimating the overall hygiene of foods, samples exceeding the reference limits (with counts ranging between 2.3 and 3.8 Log cfu/g) in this report were detected only in the years 2002 and 2009. Given the thermolabile nature of these microorganisms, they cannot survive the boiling procedure. Therefore, their presence in the boiled beef can most likely be ascribed to cross contamination from the added raw parsley (not appropriately washed), or the slicing machines (not appropriately sanitised). Notwithstanding the finding of nonconforming samples for coliforms, E. coli was never detected as requested by the European Regulations and in compliance with the recommendations of the European Food Safety Authority (EFSA) for the reduction of public health risks due to verotoxigenic *E. coli* (VTEC) contamination of meat–based preparations.

As far as *Bacillus cereus* is concerned, only one sample with counts exceeding the limits was found in the year 2010. However, the counts detected ranging between 2.3 and 2.4 Log cfu/g were only slightly higher than the (suggested) limit and significantly lower than the amount of cells (5 Log cfu/g) that probably have to be ingested along with the foods before they can cause disease (Kramer and Gilbert, 1989). The detection of this toxin-forming microorganisms at lower levels might be ascribed to cross-contamination due to improper handling of meat (EFSA, 2005), or to the addition of parsley carrying spores of Bacillus cereus, as previously highlighted by Te Giffel *et al.* (1996).

The suggested limits for *S. aureus* exceeded in 3 and 6 samples in the years 2002 and 2003, respectively, representing 21.4 % and 25.0% of the total samples for each year. Also in these samples, the counts were only slightly higher than the limits, with viable counts comprising between 2.0 and 2.9 Log cfu/g. Though *S. aureus* is an ubiquitous organism, the largest reservoir of enterotoxin-producing staphylococci is human beings (Sollid *et al.*, 2013). Therefore, the presence of staphylococci in cooked or processed foods could indicate poor hygiene of

Table 3. Temperature of the boiled meat rolls during cold storage

Year	Number of samples exceeding reference limits	% of unsatisfactory samples
2003	18 (22.9; 22.8; 15.0; 30.0; 13.0; 24.7; 24.8; 24.7; 19.3; 22.0; 21.4; 14.0; 16.3; 12.3; 24.6; 28.3; 20.1; 22.1)	75.0 [18 out of 24]
2004	6 (15.7; 15.9; 13.0; 14.2; 16.7; 17.2)	100 [6 out of 6]
2005	2 (20.0; 19.4)	50.0 [2 out of 4]
2006	2 (11.5; 20.0)	100 [2 out of 2]
2007	2 (27.1; 24.3)	100 [2 out of 2]
2008	2 (23.0; 36.2)	100 [2 out of 2]
2009	2 (28.0; 17.8)	100 [2 out of 2]
2010	1 (29.3)	100 [1 out of 1]
2011	2 (17.2; 21.3)	100 [2 out of 2]
2012	4 (21.2; 29.5; 22.3;18.1)	100 [4 out of 4]
Total unsatisfactory samples	41	83.7 [41 out of 49]

In round brackets, temperature values expressed as °C; in square brackets, the number of unsatisfactory samples out of the total number of samples analyzed

food handlers and handling procedures.

Sulphite reducing clostridia were never detected in all the years of monitoring (counts < to the limit of 1 Log cfu/g). Primarily, none of the samples analysed were found positive for the presence of the pathogens Salmonella spp. and Listeria monocytogenes which may be associated to meat meals when appropriate cooking and separation of cooked from raw meat are not accomplished. Since it is well known that no increase of microbial load is possible in food when temperature is maintained below the minimum growth value, it was plausible to expect that the ready-to-eat beef samples analysed underwent severe temperature abuse.

Temperature monitoring of ready-to-eat boiled beef

The results of the microbiological analyses carried out in the year 2002, which showed a percentage as high as 64.3% of samples exceeding the legal or suggested limits for TMA, coliforms and *S. aureus*, strongly suggest temperature abuse during cold storage of ready-to-eat boiled beef. Consequently, since 2003, the temperature of all the samples to be subjected to microbiological analyses was regularly recorded. The results of the temperature monitoring carried out from 2003 to 2012 during cold storage of ready-to-eat boiled beef are shown in Table 3. Even though cold storage was considered as a CCP in the flow chart of boiled beef, it is clearly evident that its control was not achieved during the present study. In agreement with the results of the microbiological analyses, almost 100% of the samples actually exceeded the legal temperature limit (with the exception of a few samples, in 2003 and 2005). Still worse, 24 of the 49 samples exceeding the temperature limit had temperature values ranging between 20.0 and 36.2 °C.

Revision of GMP and SOPs

Microbiological criteria may be applied by food business operators to formulate design requirements and to examine end-products as one of the measures to verify and/or validate the efficacy of the HACCP plan (Codex Alimentarius, CAC/GL 21- 1997), even though a well implemented HACCP system should require the analysis of a relatively low amount of end-products (Sun and Ockerman, 2005). In order to improve the microbiological quality of boiled meat rolls, a careful revision of the good manufacture practices (GMP) and standard operative procedures (SOPs) was carried out in the year 2003 on the basis of the series of microbiological evidence that emerged in 2002. In 2003, the use of a blast-chiller was also introduced to allow all the cooked-cold-served preparations to be rapidly cooked to ≤10 °C within 2 hours. Dedicated microbiological analyses carried out to verify the correct implementation and effectiveness of such a procedure (data not shown) demonstrated the appropriateness of such action.

Again in 2003, the preparation of a flow chart of the boiled beef was revised (Figure 2). As the addition of raw herbs is acknowledged to affect the microbiological quality of cooked meals, 2% (wt/vol) food-grade sodium hypochlorite solution was introduced in the washing step of parsley to be added to the sliced meat. Also in this case, specific microbiological analyses validated the effectiveness of such an operation (data not shown). In the year 2004, the HACCP manual underwent deep revision and hence new registration procedures were implemented in addition to the use of dedicated forms. Due to the problem of the equipment not being able to maintain refrigeration temperature, since 2003, the dedicated retail display unit was replaced with new refrigeration equipment. Despite this action, the proper maintenance of temperatures

≤10° C remained a critical issue. As evinced by the (often severe) temperature abuse commonly recorded since 2005, not different from two years before (Table 3), the introduction of this new refrigerator had practically no effect in terms of improving the temperature maintenance limit.

In addition to the issues related to maintaining the temperatures, a recent study carried out in the same environment (Osimani *et al*., 2014a; Osimani *et al*., 2014b) has shown that the high microbial loads observed along the years may have in part resulted from an improper sanitization of the equipment used for the portioning of cooked meat. In particular, the microbial contamination observed could have resulted from an incorrect sanitization of the slicing machine with consequent transfer of the microbial load to the cooked meat (Pérez-Rodríguez *et al*., 2010). The unacceptable microbial values could also probably be due to possible handling errors carried out by the staff in addition to their failure to comply with the hygiene standards laid down in the SOPs and GMP; it is reported (Aarnisalo *et al*., 2006) that the staff in food plants usually moves between the production and non-production areas, and it is probable that sometimes gloves were not used while working in the food production area. Hence, since food hygiene in restaurants is affected by knowledge and attitudes of the operators (Läikkö-Roto and Nevasa, 2014), a new and more incisive theoretical and practical approach for staff training is desirable.

Conclusions

The HACCP system is a dynamic tool that is supposed to have a positive impact on food safety. Nevertheless, even a fully implemented HACCP system may not guarantee food safety since some risks associated with lack of personal hygiene and an incomplete respect for the SOPs and GMP cannot always be promptly monitored and prevented. Regarding the results of this long-period survey, it seems in fact plausible that some inadequacies in staff organization and logistics structurally occur at the canteen.

It is useful to point out that since 2007, due to retirements, the staff has been reduced by approximately 23%, from 22 to 17 units while at the same time the number of canteen users have increased from 1,000 to about 1,200 units. For these reasons, it seems likely that the ineffectiveness of corrective measures implemented (even if they were correctly planned in the HACCP manual) is largely due to the inadequacy of human resources. This issue was referred to the staff who manage the canteen, but unfortunately, it has not been unsolved since the hiring of new staff is not an easy task in a public body.

Given such a situation, a possible way to improve the HACCP functioning at the catering services managed by public institutions is by strengthening staff training that Regulation (EC) 852/2004 considers mandatory without specifying further details. An increased number of training sessions, with punctual analysis of the results of microbiological examinations carried out at the canteen and the (possible) problems that might emerge, may help the staff to better understand their role to ensure consumer safety and to act consequently, provided that a minimum standard of personnel resources is always guaranteed for all the activities at the canteen.

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