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Effect of introduction of HACCP on the microbiological quality of some restaurant meals

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Abstract

The microbial quality of Spanish potato omelette and pork loin before and after implementation of the HACCP system in University restaurants was used as indicator of food safety in this work. The prevalence of aerobic plate counts and incidences of *Staphylococcus aureus*, *Escherichia coli*, *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp. and *Clostridium perfringens* were analysed. Results of implementation of the HACCP system show a lower incidence of studied microorganisms. On the other hand, a documented training in personal hygiene, good manufacturing practices (GMPs), cleaning and sanitation procedures and personal safety in addition to the rearrangement in the infrastructure of these establishments could improve yet more the microbial quality of the meals served.

Keywords: HACCP; Restaurants; Spanish potato omelette; Pork loin

1. Introduction

The use of restaurants has increased in the last years due to a growing tendency to eat in other places than at home (Martín Cerdeño, 1999). In some cases, meals served in these establishments are implicated in foodborne disease outbreaks (Bryan, 1988; Hedberg et al., 1991; Wieneke, Roberts, & Gilbert, 1993). The presence of *Staphylococcus aureus*, *Escherichia coli*, *E. coli* O157:H7, *Listeria* spp., *Salmonella* spp. and *Clostridium perfringens* has been studied by several authors (Gillespie, Little, & Mitchell, 2000; Hatakka, 1998; Martínez-Tomé, Vera, & Antonia Murcia, 2000; Nichols, Little, Mithani, & de Louvois, 1999; Soriano, Rico, Moltó, & Mañes, 2000, 2001b).

An adequate protection of the consumer from foodborne illness can be achieved by inspection and personnel training based on good manufacture practices and hygienic food preparation, moreover, the application of a systematic approach to the identification and evaluation of food safety hazards as is the HACCP system must be carried out to achieve food safety (Tebbutt & Southwell, 1997; Worsfold & Griffith, 1995). The HACCP system has been used in foodservice establishments (Beumer, Vrouwenvelder, & Brinkman, 1994; Bryan, 1990; Lambiri, Mavridou, & Papadakis, 1995; Mañes & Soriano, 1999; Martínez-Tomé et al., 2000) and European Commission are actively promoting harmonisation of HACCP principles according to the 1995 Food Safety regulations implemented in the Directive on Food Hygiene (93/43/EEC) (European Communities Council, 1993).

The aim of this study was to determine the microbiological quality as indicator of food safety of two of the most consumed meals in University restaurants, i.e. Spanish potato omelette (popular meal in Spain made of potatoes, eggs, oil, onion and salt) and pork loin, before and after implementation of HACCP system.

2. Material and methods

2.1. Sampling and pre-treatment of samples

'Ready-to-eat' samples were collected from 19 University restaurants in Valencia. All samples studied were placed in sterilised plastic bags, transported in ice chests to the laboratory and cultured on the same day.

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'Ready-to-eat' pork loin and Spanish potato omelette were taken aseptically at the end stages of preparation from the serving dish. Samples were collected from September 1996 to March 1997 and September 1999 to March 2000 before and after, respectively, the implementation of HACCP system.

The samples studied (25 g) were weighed aseptically into sterile stomacher bags, diluted with 225 ml buffered peptone water (BPW) (Oxoid, Unipath, Hampshire, UK) and homogenised in a Stomacher (Classic IUL, Barcelona, Spain) for 2 min (10^{-1} dilution) and serially diluted in BPW.

2.2. Microbiological methods

Aerobic plate counts (APC, 30 °C for 72 h) were determined by surface spreading of homogenate dilutions (1.0 ml) on Plate Count agar standard (PCA) (Oxoid). To detect *Clostridium perfringens*, the 10^{-1} dilution (1 ml) of each food sample was added on sulfite polymixin sulfadiazine (SPS) agar (Merck, Darmstadt, Germany) with anaerobic incubation at 44.5 °C for 24 h. Anaerobic conditions were generated using anaerobic jars Anaerocult® A (Merck) (Harmon, 1976).

To isolate *E. coli*, the homogenate in BPW was subcultured in Brilliant Green Bile (2%) broth (BGB) (Oxoid) at 35 °C for 24 h, the positive tubes were inoculated onto Rapid *E. coli* 2 agar (REC) (Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France) and incubated at 37 °C for 24 h. Violet colonies were tested for oxidase test strips (Microkit Ibérica, Madrid, Spain), Gram reaction and identified using the API 20E test strips (BioMérieux, Marcy l'Etoile, France). These isolated violet colonies on REC agar were also inoculated onto sorbitol-Mac Conkey agar (SMAC) (Oxoid) at 37 °C for 24 h. Colourless colonies on the SMAC agar were tested for agglutination using an *E. coli* O157:H7 latex agglutination test (Oxoid) (Blood & Curtis, 1995).

Isolation and identification of *Salmonella* and *S. aureus* were performed incubating the homogenate in BPW at 37 °C for 24 h. *Salmonella* were analysed using selenite cystine broth (Oxoid) and Rappaport–Vassiliadis enrichment broth (Oxoid) for 24 h at 37 and 42 °C, respectively, and the positive cultures were finally streaked onto Hektoen enteric agar (HEA) (Oxoid) and Brillliant Green agar (BGA) (Oxoid) and incubated 24–48 h at 37 °C. The purified suspect colonies were identified using the API 20E system (Busse, 1995). To detect *S. aureus*, the homogenate in BPW was subcultured in duplicate in Giolotti–Cantoni (GC) (Oxoid) supplemented with potassium tellurite solution 3.5% (Oxoid) and incubated at 37 °C for 24–48 h. The positive tubes were spread onto Baird–Parker (BP) agar (Oxoid) supplemented with egg-yolk emulsion (Oxoid) at 37 °C for 24 h. After incubation, the plates were examined for the typical black, convex colonies, with or without a light halo, and these were subcultured in tryptone soya agar (TSA) (Oxoid) at 37 °C for 24 h. Colonies obtained were examined microscopically, Gram stained, tested for catalase reaction and confirmed with an agglutination Staphytect Plus test (Oxoid) (Baird & Lee, 1995).

To isolate *L. monocytogenes*, 25 g sample was weighed into sterile Stomacher bags, diluted with 225 ml of *Listeria* enrichment broth (LEB) (Merck) and homogenised with a Stomacher. After enrichment at 30 °C for 48 h, a 0.1 ml portion of the homogenate was streaked on *Listeria* Palcam agar, consisting of *Listeria* selective agar base (Merck) and *Listeria* selective supplement (Merck). Plates were incubated at 30 °C for 48 h. Characteristic colonies were examined including Gram stain, morphology, motility and oxidase and catalase test followed by identification with the API *Listeria* system (BioMérieux) (Curtis & Lee, 1995).

2.3. Application of HACCP system

Personnel had been trained in personal hygiene, good manufacture practices (GMPs), cleaning and sanitation procedures and personal safety, implementation of HACCP was initiated. First, guidelines were drawn up detailing the various preparation stages involved from the preparation to final products. The following step was to establish, with all the personnel concerned, which procedures could involve risks and with the help of a decision tree, it was determined whether the risk could be controlled. Hazard analyses consisted of observing food preparation to identify sources and modes of contamination, measuring temperatures in internal regions of foods after cooking and collecting samples of foods after stages of preparation from the serving dish and testing them microbiologically.

According to the NACMCF (1992), HACCP system were applied in University restaurants based in the following seven principles:

1. Conduct a hazard analyses.
2. Identify the critical control points (CCPs).
3. Establish critical limits for preventive measures associated with each identified CCP.
4. Establish CCP monitoring requirements.
5. Establish corrective actions to be taken when monitoring indicates then a deviation from an established critical limit.
6. Establish verification procedures.
7. Establish record-keeping and documentation procedures.

The studied restaurant meals are summarised with reference to CCPs and their monitoring on the HACCP worksheet for Spanish potato omelette (Table 1) and pork loin (Table 2) and they are illustrated in flow diagram (Figs. 1 and 2). Hazards associated to the studied

Table 1
HACCP worksheet for critical control points in University restaurants: Spanish potato omelette

Item	Hazard	Control	Limit	Monitoring frequency/ documentation	Action (for exceeding limit)	Personnel responsible
1. Receiving	Chemical	Certified supplier with HACCP program	Specified tolerances	Certificates of conformance for each lot	Reject as supplier	Receiving operator
2. Storage at room temperature	Physical	Compliance with raw material specifications	Free of foreign material	Operator check	Eliminate product	Storaging operator
3. Refrigerated storage	Biological	Temperature records	Product temperature of $\leqslant 3$ °C	Record product temperature each shift	Place product on hold (i.e. retain, release or destroy)	Storaging operator
		Product disposition records		Continuous monitoring of storage temperature	Adjust temperature according to specification and evaluate risk	
4. Frying	Chemical	Temperature Heating time	$\leqslant 180$ °C Avoid intermittent heating	Check time Check heating time	Investigate temperature/ time abuse and evaluate risk	Cooking operator
5. Cooking	Biological	Temperature/time control specifications	$T^a > 80$ °C during a $t > 10$ min	Check temperature Check time	Adjust T^a/t of cooking	Cooking operator
6. Slicing	Biological	Good handling practices	Use clean utensils Good hygiene	Observe procedures	Modify procedures	Cooking operator
7. Hot holding display	Biological	Temperature and time control specifications	Internal $T^a > 70$ °C Time of display < 4 h	Measure T^a of holding display	Increase T^a	Cooking operator

Table 2
HACCP worksheet for critical control points in University restaurants: pork loin

Item	Hazard	Control	Limit	Monitoring frequency/ documentation	Action (for exceeding limit)	Personnel responsible
1. Receiving	Chemical	Certified supplier with HACCP program	Specified tolerances	Certificates of conformance for each lot	Reject as supplier	Receiving operator
2. Storage at room temperature	Physical	Compliance with raw material specifications	Free of foreign material	Operator check	Eliminate product	Storaging operator
3. Refrigerated storage	Biological	Temperature records	Product temperature of $\leqslant 3$ °C	Record product temperature each shift	Place product on hold (i.e., retain, release or destroy)	Storaging operator
		Product disposition records		Continuous monitoring of storage temperature	Adjust temperature according to specification and evaluate risk	
4. Frozen storage	Biological	Temperature records	Product temperature of $\leqslant -18$ °C	Record product temperature each shift	Place product on hold (i.e., retain, release or destroy)	Storaging operator
		Product disposition records		Continuous monitoring of storage temperature	Adjust temperature according to specification and evaluate risk	
5. Thawing	Biological	T^a/time control specifications	Final T^a 0–7 °C	Check temperature Check thawing time	Investigate time/T^a and evaluate risk	Cooking operator
6. Cooking	Biological	T^a/time control specifications	$T^a > 80$ °C during a $t > 10$ min	Check temperature Check time	Adjust T^a/t of cooking	Cooking operator
7. Hot holding display	Biological	T^a/time control specifications	Internal T^a of > 70 °C Time of display < 4 h	Measure temperature of holding display	Increase T^a	Cooking operator

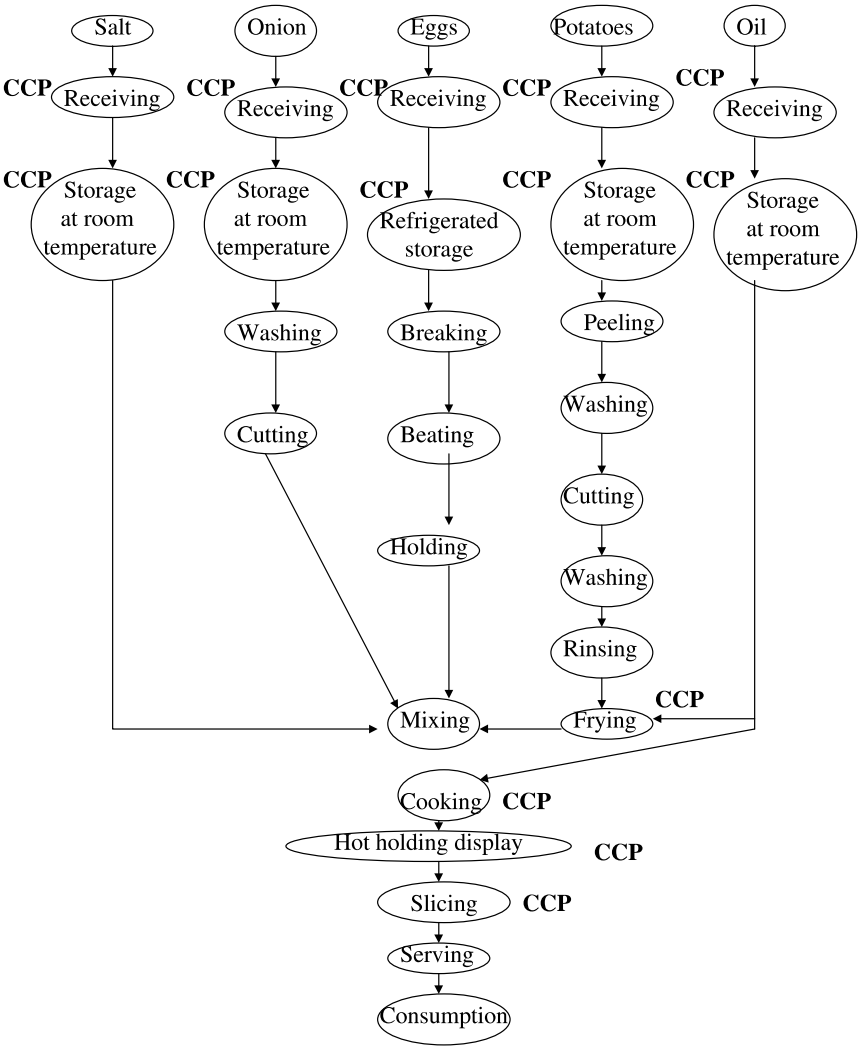

Fig. 1. Flow diagram of Spanish potato omelette in restaurants (CCP: critical control point).

meals may be physical (stone, glass, metal), chemical (polar compounds of frying oil, pesticides) and biological (presence of microorganisms, bacterial toxins and bacterial spores). Furthermore, temperatures of studied meals during hot holding display in each establishments are shown in Fig. 3. These temperatures were measured with a Crison 638 pT digital thermometer (Crison, Instruments, Barcelona, Spain).

3. Results and discussion

Tables 3 and 4 show the effects of processing on microbial populations before and after implementation of the HACCP system in University restaurants. APC of Spanish potato omelette and pork loin before implementation of the HACCP system ranged from 2.12 to 5.77 and from 1.84 to 5.30 $\log_{10}$ CFU g^{-1}, respectively (Tables 3 and 4). Solberg et al. (1990) suggested a maximum concentration in ready-to-eat foods of APC of 5.0 $\log_{10}$ CFU g^{-1}. This limit was exceeded in the restaurant identified as number 16 (Tables 3 and 4); however, after establishing the HACCP system, none of the restaurants gave values higher than 5.0 $\log_{10}$ CFU g^{-1} (Tables 3 and 4). Arranz Santamaría, Pérez-Melero Gómez, and Escoín Peña (1995) found values of 4.8 and 7.5 $\log_{10}$ CFU g^{-1} in omelettes and pork loin, respectively, from bars due to incorrect processing and handling practices. In a previous work (Soriano et al., 2000), microbial analysis of Spanish potato omelette and pork loin samples resulted in APCs from < 1.00 to 2.90 and from < 1.00 to 3.77 $\log_{10}$ CFU g^{-1}, respectively, when GMP were carried out in these establishments.

Before implementation of the HACCP system, *E. coli* and coagulase-positive staphylococci were detected in 21.0% and 17.9%, respectively, of Spanish potato omelette (Table 3) and in 20.0% and 23.2%, respectively, of pork loin samples (Table 4). Implementation of the HACCP system resulted in lower incidence of *E. coli* (1% in Spanish potato omelette) and coagulase-positive

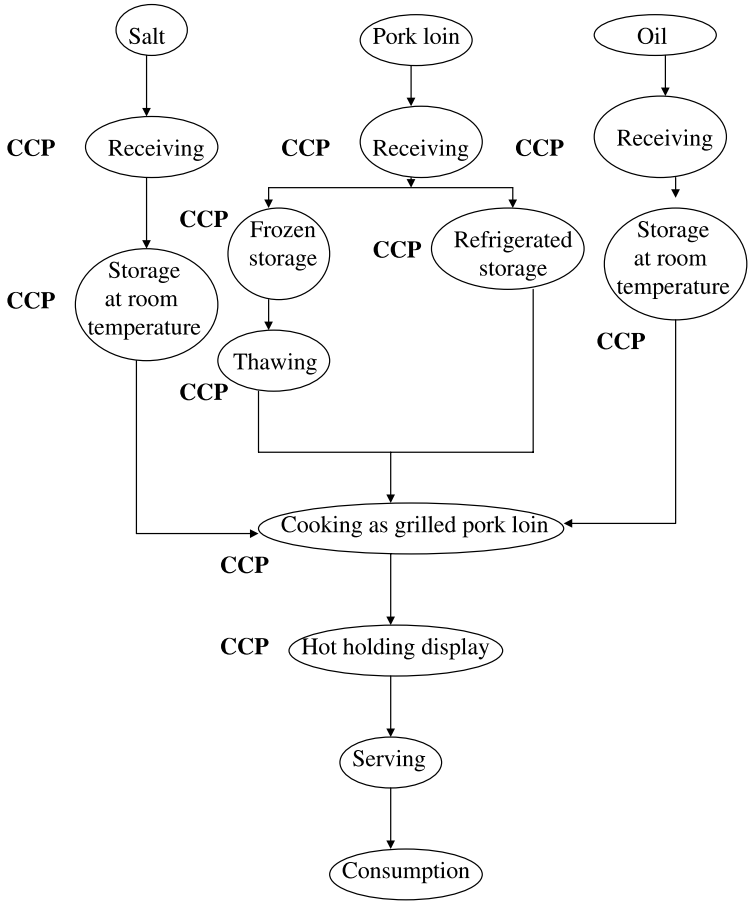

Fig. 2. Flow diagram of pork loin in restaurants (CCP: critical control point).

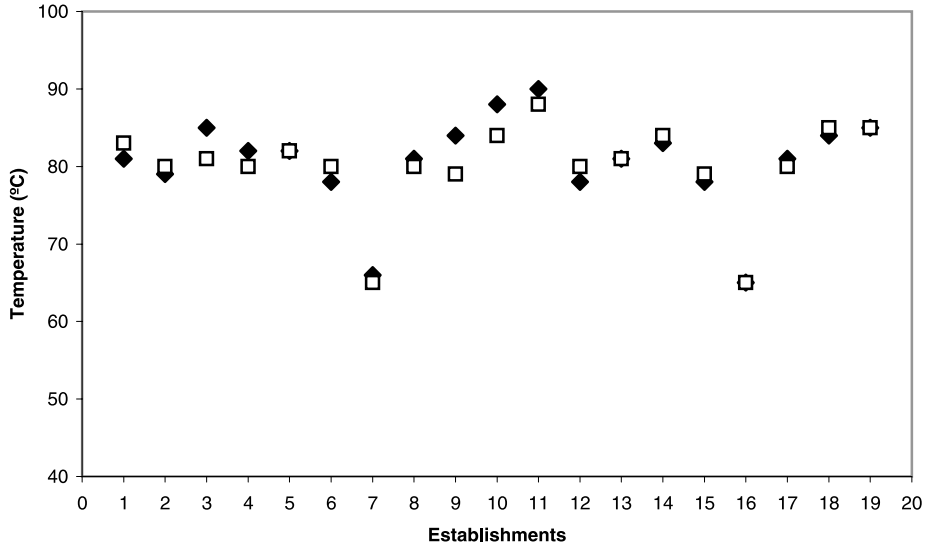

Fig. 3. Temperature of studied meals during hot holding display in each establishments (◆: Spanish potato omelette, □: pork loin).

staphylococci (2% in pork loin), as shown in Tables 3 and 4. These percentages are lower than in a previous work when our team started to work with the HACCP system (Soriano et al., 2001b). In the literature, percentages of isolated *E. coli* from omelettes are reported as 8.0% (Arranz Santamaría et al., 1995), 3.8%

Table 3
Microbial profiles, before and after implementation of the HACCP system, of Spanish Potato omelette samples collected from 19 University restaurants

Establishment	Aerobic plate count (log CFU/g) range		Number of positive samples (%) out of five analysed			
			Escherichia coli		*Staphylococcus aureus*	
	Before	After	Before	After	Before	After
1	3.18–3.39	1.92–2.97	3 (60)	0	2 (40)	0
2	2.97–3.27	1.77–2.00	1 (20)	0	0	0
3	2.52–3.39	1.87–2.02	0	0	0	0
4	2.73–3.93	1.90–1.97	0	0	1 (20)	0
5	2.88–3.74	1.88–2.02	0	0	0	0
6	2.38–2.94	<1.00	0	0	0	0
7	2.12–2.52	<1.00	0	0	0	0
8	2.77–3.86	1.39–1.73	1 (20)	0	1 (20)	0
9	2.79–3.98	1.92–2.15	1 (20)	0	1 (20)	0
10	3.04–3.72	1.60–2.02	1 (20)	0	2 (40)	0
11	2.40–2.74	1.90–2.11	0	0	1 (20)	0
12	2.34–2.94	1.99–2.39	2 (40)	0	0	0
13	2.62–3.69	1.50–2.73	1 (20)	0	1 (20)	0
14	2.64–3.99	1.92–2.50	1 (20)	0	1 (20)	0
15	3.04–3.56	1.97–2.56	2 (40)	0	0	0
16	4.83–5.77	<1.00	3 (60)	1 (20)	4 (80)	0
17	3.40–4.25	2.07–2.38	0	0	1 (20)	0
18	3.17–3.76	2.72–297	1 (20)	0	2 (40)	0
19	3.25–3.61	2.16–2.91	3 (60)	0	3 (60)	0
Grand total			20/95 (21)	1/95 (1)	17/95 (17.9)	0/95 (0)

Table 4
Microbial profiles, before and after implementation of the HACCP system, of pork loin samples collected from 19 University restaurants

Establishment	Aerobic plate count (log CFU/g) range		Number of positive samples (%) out of five analysed			
			Escherichia coli		*Staphylococcus aureus*	
	Before	After	Before	After	Before	After
1	1.90–2.60	<1.00	0	0	2 (40)	0
2	2.48–3.01	<1.00	0	0	1 (20)	0
3	3.01–3.47	<1.00	0	0	0	0
4	2.36–2.54	1.83–2.04	0	0	0	0
5	2.29–2.34	1.69–1.97	0	0	1 (20)	0
6	2.80–3.08	1.49–1.81	1 (20)	0	2 (40)	0
7	2.50–3.80	1.36–1.63	2 (40)	0	3 (60)	1 (20)
8	2.65–3.60	1.15–1.60	1 (20)	0	0	0
9	1.84–3.01	1.46–2.26	0	0	0	0
10	2.52–3.77	2.00–2.06	0	0	1 (20)	0
11	3.67–4.08	1.94–2.02	1 (20)	0	2 (40)	0
12	2.08–3.44	1.18–2.22	0	0	1 (20)	0
13	2.69–2.87	2.03–2.05	0	0	0	0
14	2.55–2.90	2.08–2.46	0	0	0	0
15	3.47–4.49	2.03–2.30	3 (60)	0	1 (40)	0
16	5.04–5.30	1.95–2.84	3 (60)	0	4 (80)	1 (20)
17	3.94–4.03	3.00–3.17	3 (60)	0	1 (20)	0
18	3.86–4.00	2.92–3.01	2 (40)	0	2 (40)	0
19	3.77–4.55	2.72–2.97	3 (60)	0	1 (20)	0
Grand total			19/95 (20)	0/95 (0)	22/95 (23.2)	2/95 (2)

(Ferrer, de Simón, & Tarragó, 1992) and 1.7% (Soriano et al., 2000). For pork loin, coagulase-positive staphylococci were isolated in 2.0% of this meat product in restaurants (Ubach, Miguel, Jaume, & Puig, 1988) and in 4.8% of pork loin in University restaurants (Soriano et al., 2000).

Neither *E. coli* O157:H7, *Salmonella* spp., *Clostridium perfringens* nor *L. monocytogenes* were detected in any samples before and after implementation of HACCP system.

The effects of personnel training (Soriano, Moltó, & Mañes, 2001a) and of application of the HACCP system (Mañes & Soriano, 1999) in meals served in University restaurants are shown in Tables 3 and 4 as mentioned above. Beumer et al. (1994) suggested that the evidence shows that in foodservice establishments, inspection alone is not sufficient to guarantee food safety and that to solve this problem, it is necessary to train food handlers in food microbiology and hygiene and in the implementation of the HACCP system. Temperatures of studied meals during hot holding display in each establishment are shown in Fig. 3. In the samples studied after implementation of this food safety system, the presence of *E. coli* (20%) (Table 3) and/or coagulase-positive staphylococci (20%) (Table 4) in the establishments identified as number 7 and 16, would suggest that the system was incorrectly implemented (in Fig. 3, establishment nos. 7 and 16 holding temperatures are below 70 °C) and both establishments present a reduced workspace as well as a too reduced number of employees to cope simultaneously with continuous monitoring in this food safety system and the foodservice operations. In our view, the presence of *E. coli* in 'ready-to-eat' pork is due to the reduced place to work in the establishment no.16 studied. This reduced workspace probably is the origin of a cross-contamination between raw and cooked food. Mossel and Struijk (1995) suggested that *E. coli* can be used as an appropriate marker to assess the bacteriological safety of raw foods but not in 'ready-to-eat' food. On the other hand, the assumption of cross-contamination is based on the presence of *S. aureus* in samples from the 16th and the seventh establishments studied. A question that we put to ourselves was: 'what can be done to solve this problem?'. Our solution has been twofold: first, we continue offering a documented training in personal hygiene, GMP, cleaning and sanitation procedures and personal safety, and second, we recommended an amplification of the space allocated for the kitchens. In this way, it would be possible to increase the number of employees and consequently accomplish the development of the HACCP system in its entirety, and at the same time avoid the problem of cross-contamination. In the literature, the application of this system in foodservice establishments has considerably improved the microbial quality of the meals served (Beumer et al., 1994; Bryan, 1990; Lambiri et al., 1995; Mañes & Soriano, 1999; Martínez-Tomé et al., 2000). Tebbutt and Southwell (1997) suggested that the combination of microbiology and hazard analysis proved helpful in assessing compliance with food hygiene legislation.

The microbial results of this study demonstrate that personnel training and HACCP application contribute to improve the food safety in these establishments, and also, that the use of microbial quality is a good indicator of food safety. Moreover, a rearrangement in the infrastructure of some restaurants could improve yet more the microbial quality of the meals served.

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