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Food Safety in Conventional and Innovative Catering Systems

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CHAPTER 1

Foreword

1. Foreword

The theme of this research originates from the growing importance that catering is playing in the contemporary social context and from the need to adapt the study of food safety to new trends.
Catering industry defines those businesses, institutions, and companies responsible for any meal prepared outside the home.
Caterers are likely to play an important role in the quality of diet of people, because of the increasing importance of eating out in contemporary lifestyle.
Within the last years, major changes have taken place in the catering sector. The consumers' demand varies over time. Nowadays people have wide food experiences, mostly related to increased opportunities for travelling abroad, globalization of culinary art and demand for ethnic foods influenced by the immigrant population.
In the food supply chain, retailers and caterers are in charge of delivering food to people and the quality of the available food is essential for the possibility of eating a healthy diet.
Considering the complexity of the organizations involved, the vulnerability of the people who receive the foodservices, and the consequences of possible adverse events, it is clear that catering services are of great strategic importance in the program to develop the right nutrition and to ensure food safety and nutritional quality.

1.1 The catering systems

The catering industry comprises a diverse and proliferative number of operations which may be sub-divided into two main groups, i.e. commercial or profit-orientated and non-commercial or cost-orientated.
In the profit-orientated group are the hotels, restaurants, fast food outlets, cafes, bars, public houses/taverns, leisure centres, country clubs, health spas and travel caterers (in-flight catering, catering on trains and ships, etc.). Many public service caterers can be categorized in the cost-oriented group, e.g. hospitals (patients), schools, social services, forces (army, navy, airforce), old peoples homes, fire stations, police, prisons. Each of the sub-divisions within the two main groups may be referred to as a sector.
A major sector not mentioned above and which is hard to categorize under the headings of profit- or cost-orientated is that of industrial catering providing meals for people at work, for example the provision of meals for hospital workers, hotel workers, school workers. Thus, this sector falls both into the commercial and non-commercial groups in that some businesses require that

their catering section is profit-making whilst others subsidize their catering unit heavily and regard it as a part of the commitment of the company to its staff.

The major methods of catering used today can be classified as follows:
- conventional or classical: i) cook-serve, ii) cook-warm-hold;
- innovative: i) cook-chill, ii) cook-freeze, and iii) *sous vide* cooking (cooking under vacuum).

The cook-serve method is characterized by the on-premises preparation of food from a raw state on a daily basis for each meal. Meals are assembled and served with a minimum holding period.

The cook-warm-hold is a method of catering in which, after cooking, food is maintained at precise temperatures for a few hours before consumption. All potentially hazardous foods, except those prepared for immediate consumption, shall be maintained in such a fashion as to prevent the growth or development of bacteria. Hot-holding equipment must be able to keep foods at temperatures between 60 and 65 °C, and cold-holding equipment must be capable of keeping foods at a temperature of 10°C of colder.

A cook-chill system is defined as a catering process whereby meals or meal components are fully cooked, then cooled by controlled chilling, e.g. blast chilling, and subsequently stored at a temperature above freezing point, prior to regeneration and/or service. Cook-chill system can be used in combination with modified atmosphere packaging in order to extend the shelf-life of food up to 21-28 days at chill storage (<4°C).

Cook-freeze is a specialized food production and distribution system for prolonging the life of prepared and cooked food by rapid freezing, storage at very low temperatures and regenerating (reheating) at the time of service. The process shares many of its advantages with cook-chill.

The *sous vide* cooking is a method of cooking food sealed in airtight plastic bags in a water bath for a long time at precise temperatures, which are much lower than other cooking methods, typically around 60 °C.

1.2 Foodborne diseases and the catering industry

Foodborne diseases remain responsible for high levels of morbidity and mortality in the general population, but particularly for at-risk groups, such as infants and young children, elderly and immunocompromised.

Cases of foodborne diseases (FBDs) occur daily throughout the world, from the most to the least developed countries. The consumption of foods contaminated by foodborne pathogenic microorganisms and toxins produced by the

microorganisms causes deaths, illnesses, hospitalization, and economic losses. In industrialized countries the percentage of the population suffering from FBDs each year has been reported to be up to 30% (World Health Organization (WHO), 2007).

The Community Summary Report on foodborne outbreaks in the European Union in 2008 indicated that up to 62% of FBDs were associated with catering or foodservice functions (European Food Safety Agency (EFSA), 2010), which highlights the importance of food safety in foodservice areas.

In foodservice environments, various factors may be related to FBDs. The major risk factors causing FBDs include foods from unsafe sources, inadequate cooking, improper holding temperatures, contaminated equipment, and poor personal hygiene. Also drinking water is a crucial factor related to foodborne diseases.

European Union legislation on food hygiene focuses on controls needed for public health protection and clarifies the responsibility of food business operators to produce food safely. Catering businesses have to apply a food safety management system based on the principles of Hazard Analysis and Critical Control Point (HACCP) (Regulation EC 852/2004).

1.2.1 References

European Food Safety Authority (EFSA) (2010). *The Community Summary Report – Trends and Sources of Zoonoses and Zoonitic Agents and Food-borne Outbreaks in the European Union in 2008.* EFSA Journal, 2010 8(1):1496. Available from: http://www.efsa.europa.eu/en/scdocs/doc/1496.pdf

Regulation (EC) No. 852/2004 of the European Parliament and the Council of 29 April 2004 on the hygiene of foodstuffs. Official Journal of the European Union L 139.

World Health Organization (WHO) (2007). *Food Safety and Foodborne Illness - Fact sheet No. 237.* Available from: http://www.who.int/mediacentre/factsheets/fs237/en/

CHAPTER 2

Objectives

2. Objectives

My research activity focused on the study of food safety in the Italian catering sector, including drinking water. This thesis consists of a collection of articles, which reports four research works published or under review onto peer-reviewed journals. Below, the specific objectives of each study are described.

2.1 Cook-serve method in mass catering establishments: is it still appropriate to ensure a high level of microbiological quality and safety?

The first work focused on three conventional "cook-serve" catering establishments in Lombardy, Italy (with an output ranging from 800 to 1700 meals a day).
The evaluation of food safety was performed by monitoring the microbiological quality and safety of foods ready for consumption, tap and microfiltered drinking water, food contact surfaces and food handlers.
The final goal was to determine whether the "cook-serve" production system is still able to ensure a high level of protection of human health and consumer interest in relation to food.

2.2 Food safety in commercial catering

In the second work, food safety was evaluated in 20 commercial catering centres (restaurants) by means of microbiological analyses of different categories of meals.
The objective of this study was to ascertain the levels of hygiene of different types of foods, measuring the results according to reference microbiological criteria and to compare the data collected over two years of monitoring. The food samples collected and analyzed came from public businesses/firms who voluntarily underwent analytical monitoring carried out by the trade association FIPE (Federazione Italiana Pubblici Esercizi), as well as the services of a hygienist consultant. These steps were taken as a part of the process of obtaining the "Bollino Blu della Ristorazione" (Blue Sticker Catering) awarded by FIPE and the Minister of Health.

2.3 Monitoring the bacteriological quality of Italian bottled spring water from dispensers

The third work focused on a particular category of drinking water: the large container bottled spring water for dispensers.

The aims of this research were to monitor the hygiene of bottled spring water and to evaluate the effectiveness of the dispenser sanitation procedure, reproducing field conditions.

2.4 Microbiological quality and safety of refrigerated precooked lasagna packaged under modified atmosphere for Italian school catering

The fourth work contains microbiological experiments with lasagna, one of the most popular recipes in Italian school catering.

The objective of this study was to evaluate and compare the microbiological quality and safety of an innovative production process of refrigerated precooked lasagna prepared by a conventional centralized school kitchen. This study was carried out using two production processes and two types of packaging materials, under a modified atmosphere, and stored at a refrigerator temperature for up to 28 days.

The ultimate goal was to determine the most suitable production method and type of packaging to ensure as high a level of microbial safety as possible.

Cook-serve method in mass catering establishments: is it still appropriate to ensure a high level of microbiological quality and safety?

With Editor in:
Food Control

3. Cook-serve method in mass catering establishments: is it still appropriate to ensure a high level of microbiological quality and safety?

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3.1 Abstract

The purpose of this study was to evaluate the hygienic quality of three traditional "cook-serve" catering establishments in Lombardy, Italy (with an output ranging from 800 to 1700 meals a day), by monitoring the microbiological quality and safety of foods ready for consumption ($n = 727$), tap water ($n = 32$), microfiltered drinking water ($n = 28$), food contact surfaces (n = 280), and food handlers ($n = 76$). The food contact surfaces showed unacceptable contamination in 17.1% of samples. The hygienic level of the washed hands of food workers was very high, since only 1.3% of samples showed unacceptable contamination. Food sample analyses highlighted a percentage of samples that did not conform to microbial reference standards: the presence of *Listeria monocytogenes* was found in 11.5% of "soft cheese" samples. *Staphylococcus aureus* non-conforming percentages ranged from 2.2% for "first and second courses" to 34.6% for "soft cheeses"; *Escherichia coli*, from 2.7% for "raw vegetables" to 7.7% for "soft cheeses"; total coliforms from 6.7% for "first and second courses" to 76.4% for "raw vegetables". The results of the water analysis indicated a high frequency of contamination with *Pseudomonas aeruginosa* (21.4% for microfiltered drinking water and 21.9% for tap water samples). In conclusion, the results suggest that more effort should be made in the application of HACCP principles. In addition in order to ensure a higher level of microbiological safety of meals, various changes in the timing of food preparation and holding temperatures are needed.

3.2 Introduction

The consumption of foods contaminated by foodborne pathogenic microorganisms and toxins produced by the microorganisms causes deaths, illnesses, hospitalization, and economic losses (Yoon et al., 2008). Due to their

widespread nature, foodborne diseases (FBDs), in particular gastro-intestinal infections, represent a very large group of pathologies with a strong negative impact on public health. However they are often given little consideration as the symptoms are often moderate and self-limiting. Many patients do not consult a doctor, faecal specimens are often not obtained, and most cases are sporadic rather than part of an outbreak (Evans et al., 2006). This has led to a general underestimation of their importance, and consequently to incorrect practices during the preparation and preservation of food. This results in the frequent occurrence of outbreaks involving varying numbers of consumers (Legnani et al., 2004).

In 2010 the European Food Safety Authority (EFSA) commissioned a survey in all 27 European Union Member States in order to assess how consumer views on food-related risks have evolved since an earlier survey carried out in 2005. In the 2010 survey, one of the key findings showed that the majority of respondents associated food and eating with pleasure, such as selecting fresh and tasty foods (58%) and with the enjoyment of meals with friends and family (54%). Food safety (37%) was less commonly associated with food and eating as such, with a considerable variation in opinion depending on the country, ranging from a low of 14% in Austria to a high of 75% in Cyprus; in Italy 40% of respondents were concerned about food safety (EFSA, 2010).

Nevertheless, in industrialized countries the percentage of the population suffering from FBDs each year has been reported to be up to 30% (WHO, 2007). Estimates of the incidence of FBDs are generally based on three main data sources: statutory notifications from doctors, isolates reported by laboratories, and outbreaks reported by public health departments. The Community Summary Report on foodborne outbreaks in the European Union in 2008 indicated that up to 62% of FBDs were associated with catering or foodservice functions (EFSA, 2010), which highlights the importance of food safety in foodservice areas.

In foodservice environments, various factors may be related to FBDs. USDHHS-FDA-CFSAN (2000) suggested that risk factors causing FBDs include foods from unsafe sources, inadequate cooking, improper holding temperatures, contaminated equipment, and poor personal hygiene. WHO (2007) suggested that drinking water is an important factor related to FBDs. Hygienic food preparation and the education of those involved in the preparation, processing and service of meals are crucial lines of defence in the prevention of most types of FBDs (Gibson et al. 2002, Dharod et al., 2009). Sixty percent of foodservice personnel in one study were reported not to wash their hands adequately (DeWit & Kampelmacher, 1984). According to the statistical results of the Centers for Disease Control and Prevention for the

period 1988-1992, the rate of food poisoning caused by poor personal hygiene was 22% (Aycicek et al., 2004).

European Union legislation on food hygiene focuses on controls needed for public health protection and clarifies the responsibility of food business operators to produce food safely (Bolton & Maunsell, 2004). Catering businesses have to apply a food safety management system based on the principles of HACCP (Reg. EC 852/2004). A flexible HACCP system is more suitable for foodservice operations, which due to the complexity of their recipes, menus, food varieties, and amounts of food involved, varies for different types of foodservice operations (Veiros, 2009). For the efficient management of food safety in mass catering foodservices, the microbial risks of all the foodservice components need to be evaluated.

In Italy the conventional ("traditional" or "cook-serve") foodservice system (CFS) is the most common in the catering sector. The cook-serve method is characterized by the on-premises preparation of food from a raw state on a daily basis for each meal. Meals are assembled and served with a minimum holding period (Light & Walker, 1990). Two of the advantages of the CFS are: i) high degree of perceived quality, because this system makes people think of fresh and homemade food, which people often equate with quality; ii) food is served soon after preparation, which does not impact the quality of the food product.

There are also several disadvantages of CFS: i) it is labor intensive, because preparation is timed in relation to when the food will be served and eaten, thus, this system is more affected by the highs and lows of demand for food than any of the other systems (i.e. cook & chill, freeze & chill); ii) this method is not possible in most catering operations, simply because the space needed for the storage, preparation, cooking and assembly of freshly cooked food and the number of staff needed to carry out the task for large numbers of people is prohibitive in terms of cost and organisation; iii) food safety, because there are more decisions that must be made at critical control points in a short period of time.

The objective of this study was to evaluate the hygienic quality of three traditional "cook-serve" catering facilities, in Lombardy, Italy. This involved monitoring the following: the microbiological quality and safety of foods ready for consumption, the tap and microfiltered drinking water, food contact surfaces, and food handlers.

The microbiological analysis focused on pathogenic and potential-pathogenic microorganism markers (*Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*). It also included spoilage-microorganisms and hygienic markers: aerobic plate counts, total coliforms, and intestinal Enterococci. The final goal was to determine whether the "cook-serve" production system is still able to ensure a high level of protection of human

health and consumer interest in relation to food, as defined by European law (Reg. EC No. 178/2002).

3.3 Materials and methods

3.3.1 Catering establishments and description of food preparation
The investigation was conducted from March 2008 to October 2010, involving three catering establishments with a production potential ranging from 800 to 1700 meals a day. These foodservice establishments prepare meals using the Conventional Foodservice System, also known as the "cook-serve" method, in which ingredients are assembled and where necessary cooked daily on site, held either heated or chilled, and served to customers.

No items are purchased fully prepared (i.e. only requiring portioning and serving), and meals are timed according to two rosters: lunch and dinner. Raw vegetables for salads are washed, disinfected with a 0.5% chlorine-based solution (Suma Chlor D4.4, Diversey Inc., USA), and rinsed with tap water in the vegetables area. Drinking water is provided to customers by filtration and dispensing systems (activated carbon filtration devices), which offer chilled, carbonated and ambient microfiltered water.

3.3.2 Sample collection
3.3.2.1 Food ready for consumption
The hygienic control of our investigation focused on food ready for consumption. Ready-to-eat food samples (n = 727) were collected monthly from the three establishments from March 2008 to October 2010. Food samples were collected in the morning between 11:00 a.m. and 12:30 p.m.. On each sampling day, approximately 250 g of each food type was placed in a sterilized plastic bag. Food temperature was recorded with a portable thermometer (Checktemp 1, Hanna Instruments, Italy) at the time of sampling.

All samples were transported to the laboratory in containers with ice, and were analyzed the same day.

For this study, RTE foods were divided into four groups according to their preparation methods. Group A (n = 462) consisted of first and second courses (cooked foods ready for consumption: pasta, cooked meats, dressing for meat, cooked ham, vegetables). Group B (n = 57) consisted of multi-ingredients (cooked and uncooked foods ready for consumption: sea-foods sauces, roast beef, salads, etc.). Group C (n = 26) consisted of soft cheeses (e.g. brie, gorgonzola, taleggio). Group D (n = 182) consisted of raw vegetables ready for consumption (e.g. julien carrots, sliced fennel, chopped lettuce and radicchio).

3.3.2.2 Food-contact surfaces

From March 2008 to October 2010, samples of the surfaces in contact with food (*n* = 280) - tables, cutting boards, vegetal cutter blades, knives, stainless steel recipients for the distribution of foods, plastic containers for foods to be distributed cold (such as vegetable salads), ceramic plates, ladles, steel pallets – were selected for evaluation.

The hygienic control of surfaces was performed after normal cleaning procedures had been completed, according to HACCP company plans. Surfaces were examined by flexible hygiene test slides "Contact-Slide" (International PBI S.p.A., Italy). Each slide was provided with two different types of agar.

The surface sampling technique consisted of unscrewing the cap with the paddle containing the medium, flexing the cap until it formed a corner of 90°, pushing the medium onto the surface by applying light pressure and rescrewing the slide into its tube. The contact-slides were transported in an ice container and analyzed immediately on arrival at the laboratory.

3.3.2.3 Food handlers

Samples (*n* = 76) were collected from the washed hands of workers by the flexible hygiene test Contact-Slide (International PBI S.p.A., Italy). A standard hand wash was the method used by food personnel (Tessi et al., 2002).

3.3.2.4 Water

Water samples (in total, *n* = 60) were collected, from tap (*n* =30) and microfiltered water (*n* =30), quarterly from March 2008 and October 2010. Samples were collected from taps previously disinfected externally by spraying a 70% ethanol solution, and internally with imbibed swabs. Before sampling, the water was left to run for about 2 min.

Approximately 1000 ml of water from the sinks of the vegetable washing areas, the food processing areas and from dispensing machines in the lunchroom were sampled and put into sterilized plastic bottles (International PBI SpA, Italy).

The bottles contained 10 mg of sodium thiosulfate in order to neutralize chlorine in the water. The samples were transported in an ice container and analyzed immediately on arrival at the laboratory.

3.3.3 Microbiological analyses

3.3.3.1 Microbiological analysis of food, environment and food handlers

For the food samples, an analytical unit (10 g) was aseptically taken from each unit, added to 90 ml of sterile diluent solution (0.85% NaCl and 0.1% peptone), and homogenized in a stomacher 400 (Colworth, UK) for 1 min at room

temperature and then serial 10-fold dilutions were made in a sterile saline solution.

Mesophilic aerobic plate counts (APC) were enumerated using a Petrifilm™ Aerobic Count (3M, St. Paul, Minnesota, USA), following the AFAQ/AFNOR 3M 01/1-09/89 method. Petrifilm™ plates were also used to determine total coliforms (TC), *Escherichia coli* (EC), *Staphylococcus aureus* (SA), in accordance with the following methods: AFNOR 3M 01/2-09/89A, AFAQ/AFNOR 3M 01/08-06/01 and AFNOR 3M 01/9-04/03, respectively .

Salmonella spp. detection was carried out using the method recommended by the International Organization for Standardization (ISO 6579:2002 Cor.1:2004). Detection of *Listeria monocytogenes* was performed according to AFNOR BRD 07/4-09/98 and the presence was confirmed according to AOAC N.060402 2006 methods. All analyses were performed in duplicate.

For the microbiological analysis of surfaces and food handlers, the microbial parameters taken as reference included the total bacterial count, which is correlated, although not specifically, with hygiene procedures, and the traditional total coliforms indicators, coagulase positive *Staphylococci* and *Enterococcus* spp.. The Contact-Slides (International PBI SpA, Italy) Nos. 3 and 5 were used in compliance with ISO 18593 (2004). Contact-Slide No. 3 is a system used for a total bacterial count (agar type: PCA+TTC+Neutralizing) and coagulase positive and mannitol-fermenting *Staphylococci* (CPS) (agar type: Vogel Johnson Agar) research. Contact-Slide No. 5 is a slide for coliform bacteria (agar type: V.R.G.B.+Neutralizing) and intestinal Enterococci (agar type: Bile Esculin Agar+Neutralizing) research. The Neutralizing compound consists of Tween 80, which eliminates the bactericidal activity of ammonium quaternary compounds contained in sanitizers. In the laboratory, both the slides were incubated at 36+/-1°C for 24-48 hours and the results were interpreted in accordance with the reference standards.

3.3.3.2 Microbiological analyses of water

Microbiological analyses were performed using the membrane filtration technique according to ISO protocols, for the detection of total coliforms (TC), (ISO 9308-1:2002, 2002); *E. coli* (EC), (ISO 9308-1:2002, 2002); *P. aeruginosa* (PA), (ISO 16266:2008, 2008); *Enterococcus* spp. (EN), (ISO 7899-2:2003, 2003); and heterotrophic plate count (HPC) at 22 and 37 °C, (ISO 6222:2001, 2001). Water samples of 250 ml each were filtered through a hydrophilic mixed cellulose ester membrane (International PBI Spa, 2054045) of pore size 0.45 μm and 47 mm in diameter for all organisms.

The membranes were placed in each petri dish filled with a specific medium: Tergitol TTC (Oxoid Corporation, 502948) for total coliforms and *E. coli*, Pseudomonas Agar Base/CN-Agar (Oxoid Corporation, 502946) for *P.*

aeruginosa, and Slanetz and Bartley (Oxoid Corporation, PO5018A) for *Enterococcus* spp.

 The pour-plate method was used for the enumeration of HPC, and a sterile petri dish was filled with 1 ml of a water sample. Amounts of 15 ml to 20 ml of a Water Plate Count Agar Medium (Oxoid Corporation, CM1012B) were added. Petri dishes were incubated at 37 °C for 24 h, at 42 °C for 24 h, at 37 °C for 48 h, at 37 °C for 24 h and at 22-37 °C for 24-72 h, for each medium and temperature, respectively. *P. aeruginosa* was confirmed using an oxidase test, a fluorescence test and cetrimide agar, followed by 24 h of incubation at 42 °C.

3.3.4 Evaluation of microbiological quality and safety
3.3.4.1 Food ready for consumption
The foods examined in this study can, on the whole, be considered as "gastronomic products" and as such most are not covered by Italian and European specific legislation regarding microbiological quality standards. For the purposes of this study, standards for specific types of foods were therefore taken from national or international regulations. Several reference values were established for Group A "First and second courses" and Group B "Multi-ingredient preparations" taking into account the standards proposed by various regional regulations and authors. The microbiological criteria taken as references are reported in Table 1.

3.3.4.2 Food-contact surfaces and food handlers
As suggested by Henroid et al. (2004), and Sneed et al. (2004), we referred to the following microbial counts as standards for cleaned and sanitized food-contact surfaces and hands: APC <1.3 $\log_{10}$ cfu/cm^2, TC, CPS and EN < 1.0 $\log_{10}$ cfu/cm^2.

3.3.4.3 Water
For the purposes of this study, the results were interpreted according to current European regulations for drinking water (Council of the European Economic Community Directive 98/83/EEC), which state that *E. coli*, *Enterococcus* spp., *P. aeruginosa* should not be detectable in 250 ml samples of water and total coliforms in 100 ml samples of water, while HPC, at 22 °C, and 37 °C, should not exceed 100/ml, and 20/ml CFU, respectively.

Table 1: Microbiological reference standards for the various foodstuffs submitted for microbiological investigation.

Groups	Bacteriological tests	Standards (CFU/g)*	References
A – First and second courses (cooked food ready for consumption)	Aerobic plate counts	10^5	Commission Regulation (EC) No. 1441/2007, (2007) Lombardia Region (2001) O.M. 7.12.1993, (1993)
	Total coliforms	10^2	
	E. coli	<10	
	S. aureus	10	
	Salmonella spp.	Absence in 25 g	
	L. monocytogenes	Absence in 25 g	
B – Multi-ingredients preparations (cooked and uncooked foods ready for consumption)	Aerobic plate counts	10^6	Commission Regulation (EC) No. 1441/2007, (2007) Lombardia Region (2001) Piemonte Region (2008) O.M. 7.12.1993, (1993)
	Total coliforms	10^3	
	E. coli	<10	
	S. aureus	10^2	
	Salmonella spp.	Absence in 25 g	
	L. monocytogenes	Absence in 25 g	
C – Soft cheeses	Total coliforms	10^4	Commission Regulation (EC) No. 1441/2007, (2007) Lombardia Region (2001)
	E. coli	10^2	
	S. aureus	10^2	
	Salmonella spp.	Absence in 25 g	
	L. monocytogenes	Absence in 25 g	
D – Raw vegetables ready for consumption	Aerobic plate counts	10^6	Commission Regulation (EC) No. 1441/2007, (2007) Lombardia Region (2001) Piemonte Region (2008)
	Total coliforms	2×10^3	
	E. coli	10^2	
	S. aureus	10^2	
	Salmonella spp.	Absence in 25 g	
	L. monocytogenes	Absence in 25 g	

*Standards: acceptable limits, CFU: colony forming units.

3.4 Results and Discussion

3.4.1 Food samples

The incidence and levels of microorganisms in the four groups of foods are shown in Table 2. Overall the data revealed a high level of microbiological safety in the foods examined. All the food samples ($n = 727$) appeared to carry no *Salmonella* spp. in 25 g. Of particular interest was the isolation of *Listeria*

monocytogenes in 11.5% of the soft cheese samples (3 out of 26), in particular in one sample of sliced Taleggio and in two samples of sliced Gorgonzola.

In Group A (cooked first and second courses), the data showed a low contamination frequency with aerobic plate counts (29.2%), total coliforms (8.2%), *E. coli* (1.9%) and *S. aureus* (2.2%). This could be due to the effectiveness of cooking plus the hot and cold holding temperatures before consumption.

The percentage of positive samples increased considerably in Groups B (multi-ingredient preparations, cooked and uncooked), C (soft cheeses) and D (raw vegetables for salads). High percentages were observed for aerobic plate counts, ranging from 78.9% for Group B to 100% for Group D. With regard to total coliforms, the incidence ranged from 42.3% for Group C to 94% for Group D. The percentage of *E. coli* positive samples ranged from 5.3% for Group C to 8.8% for Group D. Of particular interest was the incidence of *S. aureus* in the soft cheese group, which was found in 34.6% of the samples examined. The source of this contamination were probably due to a primary contamination in the cheese factories. This would seem to be confirmed in Table 4, which highlights that the level of contamination with coagulase positive *Staphylococci* on washed hands and surfaces in contact with food was very low.

In this study, geometric mean bacterial counts (Table 2) were calculated considering only the positive samples; negatives were defined as samples with counts <10 CFU/g, because <10 CFU/g was the detection limit of the assays. APC mean values ranged from 3.42 $\log_{10}$ CFU/g for Group A to 5.64 $\log_{10}$ CFU/g for Group D; TC counts ranged from 3.18 $\log_{10}$ CFU/g for Group A to 4.44 $\log_{10}$ CFU/g for Group D; EC counts ranged from 1.47 $\log_{10}$ CFU/g for Group B to 3.14 $\log_{10}$ CFU/g for Group C, and SA counts ranged from 2.07 $\log_{10}$ CFU/g for Group D to 2.71 for Group C.

Table 3 shows the percentages of conformity to microbiological standards (Table 1) for the four groups of foods. The microbiological analysis of Group A (cooked foods) showed that more than 93.0% of the samples could be classified as conforming to all microbial parameters. Group B (cooked and uncooked multi-ingredient preparations) showed that 77.2% and 59.6% of the samples could be classified as conforming to APC and TC counts; 94.7% and 93.0% of the samples were considered as conforming to EC and SA counts.

These findings suggest that some changes in manufacturing practices should be made to enhance the microbiological quality of these foods, in particular with regard to the APC and TC levels. On the other hand, the bacteriological analysis of Group C (soft cheeses) showed that 34.6% of the samples could be classified as non-conforming with respect to TC and SA counts, and 7.7% of the samples did not conform to *E. coli* standards.

These results may be explained by a primary source of contamination. The microbiological monitoring of Group D (raw vegetables) showed that 76.4% of

the samples did not conform to TC standards, 36.8% did not conform to APC standards, and 2.7% and 1.1% did not conform to *E. coli* and *S. aureus* standards. A possible reason for the heavy contamination of raw vegetables could be due to the ineffectiveness of the washing and sanitizing procedures carried out prior to the service.

Table 2: Incidence and levels of microorganisms on the four groups of foods sampled in the catering establishments.

Groups	Bacteriological tests	Number of positive samples[a]	Incidence (%)[b]	GM (log$_{10}$ CFU/g)	SD (log$_{10}$ CFU/g)	Minimum-Maximum (log$_{10}$ CFU/g)
A – First and second courses (cooked food ready for consumption) *n*: 462	Aerobic plate counts	135	29.2	3.42	1.37	1.60-8.81
	Total coliforms	38	8.2	3.18	1.15	1.30-5.71
	E. coli	9	1.9	2.02	1.10	1.00-4.15
	S. aureus	10	2.2	2.46	0.75	1.48-3.88
	Salmonella spp.[c]	0	0.0			
	L. monocytogenes[c]	0	0.0			
B – Multi-ingredients preparations (cooked and uncooked foods ready for consumption) *n*: 57	Aerobic plate counts	45	78.9	4.70	1.63	2.00-7.69
	Total coliforms	29	50.9	4.02	1.46	1.78-6-64
	E. coli	3	5.3	1.47	0.50	1.00-2.00
	S. aureus	5	8.8	2.37	0.74	1.30-3.08
	Salmonella spp.[c]	0	0.0			
	L. monocytogenes[c]	0	0.0			
C – Soft cheeses *n*: 26	Total coliforms	11	42.3	3.46	1.32	1.78-5.72
	E. coli	2	7.7	3.14	0.93	2.48-3.80
	S. aureus	9	34.6	2.71	0.35	2.08-3.30
	Salmonella spp.[c]	0	0.0			
	L. monocytogenes[c]	3	11.5			
D – Raw vegetables ready for consumption *n*: 182	Aerobic plate counts	182	100.0	5.64	1.15	3.00-9.11
	Total coliforms	171	94.0	4.44	1.24	2.00-8.48
	E. coli	16	8.8	1.74	0.68	1.00-3.23
	S. aureus	4	2.2	2.07	0.46	1.68-2.59
	Salmonella spp.[c]	0	0.0			
	L. monocytogenes[c]	0	0.0			

GM: geometric mean, SD: standard deviation, CFU: colony forming units.
[a] For the quantitative tests, negative were considered for samples that had counts < 1.00 log10 CFU/g. 1.00 log10 CFU/g was the detection limit. For the presence/absence test, negative were considered for samples that had no Salmonella spp. and/or L. monocytogenes.
[b] The incidence (%) was calculated *per* group.
[c] Presence/absence test.

Table 3: Percentage of conformity of the various foodstuffs to microbiological reference standards.

Groups	Assessment	Percentage of conformity					
		APC	TC	EC	SA	S spp.	LM
A – First and second courses (cooked food ready for consumption) *n*: 462	Conforming	96.5	93.3	98.1	97.8	100.0	100.0
	Non-conforming	3.5	6.7	1.9	2.2	0.0	0.0
B – Multi-ingredients preparations (cooked and uncooked foods ready for consumption) *n*: 57	Conforming	77.2	59.6	94.7	93.0	100.0	100.0
	Non-conforming	22.8	40.4	5.3	7.0	0.0	0.0
C – Soft cheeses *n*: 26	Conforming	-[a]	65.4	92.3	65.4	100.0	88.5
	Non-conforming	-[a]	34.6	7.7	34.6	0.0	11.5
D – Raw vegetables ready for consumption *n*: 182	Conforming	63.2	23.6	97.3	98.9	100.0	100.0
	Non-conforming	36.8	76.4	2.7	1.1	0.0	0.0

APC: aerobic plate counts, TC: total coliforms, EC: *Escherichia coli*, SA: *Staphylococcus aureus*, S spp.: *Salmonella* spp., LM: *Listeria monocytogenes*.
[a]-, not detected.

3.4.2 Food-contact surfaces and food handlers

Table 4 shows the percentage of conformity to microbiological reference standards of surfaces in contact with food and the washed hands of food workers. The hygiene level of surfaces ($n = 280$) was adequately high. Only 17.1% of surfaces did not conform with the advisory standards for the total bacterial count; 7.9%, 2.5% and 1.1% were found to be contaminated with total coliforms, coagulase positive *Staphylococci* and *Enterococcus* spp. at $\geq 1.0 \log_{10}$ CFU/cm^2 respectively. These results show that the current cleaning and sanitizing procedures applied by food operators were effective. An analysis of the samples taken from the washed hands of workers immediately after the washing and disinfecting procedure showed that there was no contamination with total coliforms and *Enterococcus* spp.. The total bacterial count and coagulase

positive *Staphylococci* exceeded the satisfactory limit only in one out of 76 samples examined. These results suggest that hands were washed adequately.

Table 4: Conformity of surfaces in contact with food and washed hands to advisory standards[a,b]. Both surfaces and hands were cleaned and disinfected before sampling.

Samples	Total bacterial count		Total coliforms		Coagulase positive *Staphylococci*		*Enterococcus* spp.	
	Sat. < 1.3 Log_{10} CFU/ cm^2 (%)	Unsat. $\geq$ 1.3 Log_{10} CFU/ cm^2 (%)	Sat. < 1.0 Log_{10} CFU/ cm^2 (%)	Unsat. $\geq$ 1.0 Log_{10} CFU/ cm^2 (%)	Sat. < 1.0 Log_{10} CFU/ cm^2 (%)	Unsat. $\geq$ 1.0 Log_{10} CFU/ cm^2 (%)	Sat. < 1.0 Log_{10} CFU/ cm^2 (%)	Unsat. $\geq$ 1.0 Log_{10} CFU/ cm^2 (%)
Surfaces in contact with food *n*: 280	82.9	17.1	92.1	7.9	97.5	2.5	98.9	1.1
Food handlers *n*: 76	98.7	1.3	100.0	0.0	98.7	1.3	100.0	0.0

Sat.: satisfactory, Unsat.: unsatisfactory.
CFU: colony forming units.
[a] Henroid et al., 2004.
[b] Sneed et al., 2004

3.4.3 Water

The microbial characteristics of water sampled in the three catering establishments are presented in Tables 5 and 6. As shown in Table 5, 34.4% of the tap water and 64.3% of the microfiltered drinking water were found to be inappropriate for human consumption, as defined by European law. These percentages were calculated considering all the samples that were contaminated by at least one parameter exceeding the bacteriological limits set by law. With regard to the heterotrophic plate counts at 37°C, 21.9% of the tap water samples, and 53.6% of the microfiltered drinking water samples did not conform to limits.

The most significant result was the high contamination frequency with *P. aeruginosa* that was found in 21.4% of the microfiltered drinking water samples and in 21.9% of the tap water samples. Liguori et al. (2010) investigated the microbiological quality of water in commercial stores in Italy and reported 3.3% of tap water samples positive for *P. aeruginosa*. In Southern Greece, Papapetropoulou et al. reported 9% of tap water samples contaminated by this microorganism. Instead in Brazil, Zamberlan da Silva et al. (2008) reported

29.1% of tap water samples were positive for *P. aeruginosa*. This bacterium is known to be an important agent of nosocomial infections, with multidrug-resistant strains in hospitals being of particular concern (Morais et al., 1997, Huq et al., 2008, Felfoeldi et al., 2010, Papapetropoulou et al., 2010). Since elderly and immunocompromised people are at greatest risk of infection, drinking water should contain as few *P. aeruginosa* as possible.

Total coliforms and *Escherichia coli* were not detected in any of the samples analyzed. Table 6 shows the incidence and levels of heterotrophic plate counts. HPC values in positive tap water samples ranged from 0.30 to 3.04 $\log_{10}$ CFU/mL, and the mean counts, at 37 °C and at 22°C, were found to be 1.32 and 1.58 $\log_{10}$ CFU/ml, respectively. HPC values in microfiltered drinking water samples ranged from 0.00 to 3.23 log10 CFU/mL, and the mean counts were 1.72 (at 37 °C) and 1.81 $\log_{10}$ CFU/mL (at 22 °C).

The results obtained from the water analysis are of great importance, because the waters examined in this study were treated and disinfected, and for this reason should not have been contaminated at these levels.

Table 5: Conformity of water samples to microbiological standards[a].

Water samples	Assessment	Percentage of conformity						
		HPC 37°C	HPC 22°C	TC	EC	EN	PA	**Total**[b]
Tap water (vegetables washing areas, food processing areas, etc.) *n*: 32	Conforming	78.1	93.8	96.9	96.9	96.9	78.1	65.6
	Non-conforming	21.9	6.3	3.1	3.1	3.1	21.9	**34.4**
Filtered drinking water (chilled, carbonated chilled and ambient) *n*: 28	Conforming	46.4	78.6	100.0	100.0	96.4	78.6	35.7
	Non-conforming	53.6	21.4	0.0	0.0	3.6	21.4	**64.3**

HPC: heterotrophic plate counts, TC: total coliforms, EC: *Escherichia coli*, EN: *Enterococcus* spp., PA: *Pseudomonas aeruginosa*.
[a] HPC at 37°C should no exceed 20 CFU/mL, HPC at 22°C should no exceed 100 CFU/mL, TC should not be detectable in 100 ml samples of water, EC, EN and PA should not be detectable in 250 mL samples of water.
[b] The total percentage of conformity was calculated considering the samples that were contaminated by at least 1 microbial parameter exceeding limits set by law.

Table 6: Incidence and levels of heterotrophic plate counts on the water samples.

Water samples	Bacteriological tests	Number of positive samples[a] (%)[b]	Geometric Mean ($\log_{10}$ CFU/mL)	Standard Deviation ($\log_{10}$ CFU/mL)	Minimum ($\log_{10}$ CFU/mL)	Maximum ($\log_{10}$ CFU/mL)
Tap water (vegetables washing areas, food processing areas, etc.) n: 32	HPC 37°C (CFU/mL)	19 (59.4)	1.32	0.76	0.30	3.04
	HPC 22°C (CFU/mL)	20 (62.5)	1.58	0.56	0.30	2.70
Filtered drinking water (chilled, carbonated chilled and ambient) n: 28	HPC 37°C (CFU/mL)	22 (78.6)	1.72	0.82	0.00	3.11
	HPC 22°C (CFU/mL)	23 (82.1)	1.81	0.56	0.60	3.23

CFU: colony forming units, HPC: heterotrophic plate counts.
[a] Negative were considered for samples that had HPC 0 CFU/mL.
[b] Values in parentheses are positive percentages.

3.5 Conclusions

This study investigated the food safety of three Italian catering establishments, serving from 800 to 1700 meals a day, using the traditional cook-serve method for all the preparations. On the basis of our findings, it was concluded that the microbiological quality and safety of Group A were satisfactory. On the other hand, the detection of the presence of *Listeria monocytogenes* in soft cheeses, and the heavy contamination by APC and TC in Groups B, C, and D showed that a better application of HACCP principles is needed.

The most important drawback of the traditional cook and serve system is the very short period of time between the meal preparation and the service to customers. The limited number of hours required to prepare thousands of meals a day induces food operators to do some preparations early in the morning, especially raw vegetables, which could increase bacterial growth.

In order to ensure a higher level of microbiological safety, some changes in the timing of food preparation and holding temperatures need to be made. Regarding the hygienic education of staff, the results from the microbial

assessment showed a high level of hygienic safety on the cleaned food contact surfaces and on washed hands, which is an important prerequisite to prevent cross-contamination. The microbial characteristics of the water samples analyzed showed that in order to protect human health, food operators should increase the frequency of the sanitisation procedures both of the microfiltered water dispensing systems and the washing areas in the catering establishments.

3.6 References

AOAC International The Scientific Association Dedicated to Analytical Excellence® (2010). Validation Certificate N.: 060402. *Microgen Listeria-ID Kit for confirming the presence of Listeria monocytogenes.*

Association Française De Normalisation (AFNOR) (2010). Validation Certificate N.: 3M-01/2-09/89A. 3M Petrifilm™ Coliform Count Plate. Application for the Enumeration of Coliform Colonies With and Without Gas (as compared to ISO 4832 VRBL method).

Association Française De Normalisation (AFNOR) (2010). Validation Certificate N.: BRD 07/4-09/98. Application for detection of Listeria Monocytogenes (as compared to NF EN ISO 11290-1 including the amendment A1 (2004) method).

Association Française De Normalisation (AFNOR) (2010). Validation Certificate N.: 3M-01/9-04/03. 3M Petrifilm™ Staph Express Count System. Application for the Enumeration of Coagulase Positive Staphylococci (as compared to EN ISO 6888-1 method).

Association Française pour l'Assurance de la Qualité (AFAQ) et **Association Française De Normalisation (AFNOR)** (2010). Validation Certificate N.: 3M-01/1-09/89. 3M Petrifilm™ Aerobic Count Plates. Application for the Enumeration of Total Viable Count (as compared to ISO 4833 method).

Association Française pour l'Assurance de la Qualité (AFAQ) et **Association Française De Normalisation (AFNOR)** (2010). Validation Certificate N.: 3M-01/08-06/01. 3M Petrifilm™ Select *E. coli* Count Plate. Application for the Enumeration of *Escherichia coli* (as compared to ISO 16649-2 *E. coli* method).

Ayçiçek H., Aydoğan H., Küçükkaraaslan A., Baysallar M., Başustaoğlu A.C. (2004). Assessment of the bacterial contamination of hands of hospital food handlers. *Food Control*, 15, 253-259.

Bolton D.J. & Maunsell B. (2004). *Guidelines for food safety control in European restaurants*. Dublin: Teagasc – The National Food Centre.
Council of The European Economic Community Directive 98/83/EEC (1998). On the quality of water intended for human consumption. The Council of the European Communities. (OJ No. L330, 5.12.1998).

DeWit J.C., Kampelmacher (1984). Some aspects of bacterial contamination of hands of workers in foodservice establishments. *Journal of Bacteriological Hygiene*, 186, 9-12.

Dharod J.M., Paciello S., Bermudez-Millan A., Venkitanarayanan K., Damio G., Perez-Escamilla R. (2009). Bacterial contamination of hands increases risk of cross-contamination among low-income Puerto Rican meal preparares. *Journal of Nutrition Education and Behavior*, 41(6), 389-397.

Evans M.R., Sarvotham T., Thomas D.R., Howard A.J. (2006). Domestic and travel-related foodborne gastrointestinal illness in a population health survey. *Epidemiology and Infection*, 134(4), 686-693.

European Food Safety Authority (2010). *Special Eurobarometers 354 – Food-related risks – Conducted by TNS Opinion & Social at the request of the European Food Safety Authority*. Available from: http://ec.europa.eu/public_opinion/archives/ebs/ebs_354_en.pdf

European Food Safety Authority (2010). *The Community Summary Report – Trends and Sources of Zoonoses and Zoonitic Agents and Food-borne Outbreaks in the European Union in 2008*. EFSA Journal, 2010 8(1):1496. Available from: http://www.efsa.europa.eu/en/scdocs/doc/1496.pdf

Felfoeldi T., Heeger Z., Vargha M., Marialigeti K. (2010). Detection of potentially pathogenic bacteria in the drinking water distribution system of a hospital in Hungary. *Clinical Microbiology & Infection*, 16(1), 89-92.

Gibson L.L., Rose J.B., Haas C.N., Gerba C.P., Rusin P.A. (2002). Quantitative assessment of risk reduction from hand washing with antibacterial soaps. *Journal of Applied Microbiology*, 92(s1), 136S-143S.

Henroid D.H., Mendonca A.F., Sneed J. (2004). Microbiological evaluation of food contact surfaces in Iowa Schools. *Food Protection Trends*, 24, (9), 682-685.

Huq A., Whitehouse C.A., Grim C.J., Anam M., Colwell R.R. (2008). Biofilms in water, its role and impact in human disease transmission. *Current Opinion in Biotechnology*, 19, 244-247.

International Organization for Standardization (ISO) 6579:2002 Cor.1:2004) (2004). *Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Salmonella spp.*. Geneva: International Organization for Standardization.

International Organization for Standardization (ISO) 9308-1:2002 (2002). *Water quality – detection and enumeration of Escherichia coli and coliform bacteria – Part 1: Membrane filtration method,* Geneva: International Organization for Standardization.

International Organization for Standardization (ISO) 7899-2:2003 (2003). *Water quality – Detection and enumeration of intestinal enterococci – Part 2: Membrane filtration method,* Geneva: International Organization for Standardization.

International Organization for Standardization (ISO) 6222:2001 (2001). *Water quality – Enumeration of culturable micro-organisms – Colony count by inoculation in a nutrient agar culture medium*, Geneva: International Organization for Standardization.

International Organization for Standardization (ISO) 16266:2008 (2008). *Water quality – Detection and enumeration of Pseudomonas aeruginosa – Method by membrane filtration*, Geneva: International Organization for Standardization.

International Organization for Standardization (ISO) 18593:2004 (2004). *Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plate and swabs. ISO/TC 34/SC 9 N 374, March, 1999.* Geneva: International Organization for Standardization.

Legnani P., Leoni E., Berveglieri M., Mirolo G., Alvaro N. (2004). Hygienic control of mass catering establishments, microbiological monitoring of food and equipment. *Food Control*, 15, 205-211.

Light N. & Walker A. (1990). *Cook-chill catering:technology and management*. Elsevier Science Publishers LTD, Crown House, Linton Road, Barking, Essex IG11 8JU, England. ISBN 1-85166-437-8.

Liguori G., Cavallotti I., Arnese A., Amiranda C., Anastasi D., Angelillo I.F. (2010). Microbiological quality of drinking water from dispensers in Italy. *BMC Microbiology*, 10:19. Available from: http://www.biomedcentral.com/1471-2180/10/19

Lombardia Region General Direction of Health, DDUO 11 luglio 2001 No. 16901 (2001). Linee guida della Regione Lombardia per la ristorazione ospedaliera. Direzione Generale Sanità. Bollettino Ufficiale della Regione Lombardia 190, 02.08.2001.

Morais P.V., Mesquita C., Andrade J.L., Milton S.C. (1997). Investigation of persistent colonization by Pseudomonas aeruginosa like strains in a spring water bottling plant. *Applied and Environmental Microbiology*, 63(3), 851-856.

Ordinanza del Ministero della Sanità 07.12.1993 (OM 7.12.1993) (1993). Limiti di *Listeria monocytogenes* in alcuni prodotti alimentari. *Gazzetta Ufficiale*, 291, 13.12.1993.

Papapetropoulou M., Iliopoulou J., Rodopoulou G., Detorakis J., Paniara O. (1994). Occurrence and antibiotic-resistance of Pseudomonas species isolated from drinking water in Southern Greece. *Journal of Chemotherapy*, 6(2), 111-116.

Piemonte Region - Determinazione Dirigenziale No. 520 dell'11.08.2008 (2008). Allegato 2 rev. 03/2008. Criteri microbiologici per prodotti alimentari non soggetti a specifica normativa di legge. Available from: http://www.regione.piemonte.it/sanita/sanpub/igiene/dwd/dd520_all.pdf

Regulation (EC) No. 178/2002 of the European Parliament and the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Communities L 31.

Regulation (EC) No. 852/2004 of the European Parliament and the Council of 29 April 2004 on the hygiene of foodstuffs. Official Journal of the European Union L 139.

Regulation (EC) No. 1441/2007 of the European Parliament and the Council of 5 December 2007 on microbiological criteria for foodstuffs. Official Journal of The European Union L 322.

Sneed J., Strohbehn C., Gilmore S.A., Menconca A. (2004). Microbiological evaluation of foodservice contact surfaces in Iowa assisted-living facilities. *Journal of the American Dietetic Association*, 104(11), 1722-1724.

Tessi M.A., Arìngoli E.E., Pirovani M.E., Vincenzini A.Z., Sabbag N.G., Costa S.C., Garcìa C.C., Zannier M.S., Silva E.R., Moguilevsky M.A. (2002). Microbiological quality and safety of ready-to-eat cooked foods from a centralized school kitchen in Argentina. *Journal of Food Protection*, 65(4), 636-642.

[USDHHS-FDA-CFSAN] U.S. Department of Health and Human Services, Food and Drug Administration, Centers for Food Safety and Applied Nutrition (2000). Report of the FDA retail food program database of foodborne illness risk factors. Available from: http://vm.cfsan.fda.gov/~acrobat/retrsk.pdf

Veiros M.B., Proença R.P.C., Santos M.C.T., Kent-Smith L., Rocha A. (2009). Food safety practices in a Portuguese canteen. *Food Control*, 20, 936-941.

World Health Organization (2007). *Food Safety and Foodborne Illness - Fact sheet No. 237*. Available from: http://www.who.int/mediacentre/factsheets/fs237/en/

Yoon Y., Kim S.R., Kang D.H., Shim W.B., Seo E., Chung D.H. (2008). Microbial assessment in the school foodservices and recommendations for food safety improvement. *Journal of Food Science*, 73, (6), M304-M313.

Zamberlan da Silva M.E., Santana R.G., Guilhermetti M., Filho I.C., Endo E.H., Ueda-Nakamura T., Nakamura C.V., Filho B.P.D. (2008). Comparison of the bacteriological quality of tap water and bottled mineral water. *International Journal of Hygiene and Environmental Health*, 211, 504-509.

CHAPTER 4

Food safety in commercial catering

4. Food safety in commercial catering

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4.1 Abstract

The catering industry plays a very important role in public health management, because about 30% of total daily meals are consumed in catering industry establishments (restaurants, cafeterias *bars*). In this context, food safety was evaluated in 20 catering centres by means of microbiological analyses of different categories of meals. Results demonstrate that there was a significant decrease in microbial contamination between 2006 and 2007; no pathogens were found in 217 samples examined; this result was obtained by improving voluntary controls.

4.2 Introduction

There has been a significant change in the dietary habits of Italians who have less time available to devote to buying and preparing food at home. This has led to a substantial modification in both the places of consumption and the supply of products. Within the EU, eating meals outside the home has reached 33.8% of total daily food consumption.

The term "eating out" means, in its strictest sense, consuming meals provided by the catering industry, both institutional (e.g. hospitals, schools) and commercial (e.g. restaurants, cafés). In a broader sense, the term refers also to alternative channels for selling prepared foods, including vending machines, food trucks or mobile canteens, food retailers such as bakeries and delicatessens, and finally, other large scale distribution outlets that have special departments for delicatessen items or takeaway foods.

13% of food consumption away from home is provided by institutional catering services, which include hospitals, company and school canteens., whereas the other 87% takes place through commercial catering establishments such as restaurants, pizzerias, and sandwich bars. Over 17 million Italians eat at least one meal outside the home every day [1].

Food is still prepared in many businesses, such as restaurants or family-style eating houses, according to the *cook-serve* system in which meals are cooked within a few hours before they are consumed [2].

In these establishments, the consumer usually has a rather limited choice of dishes, especially if they are elaborate or special; however this is often associated with high quality because it evokes memories of typical home cooking [3].

If a catering or food service provider increases the number of dishes offered or adds dishes that require more elaborate preparation, then *cook & chill* systems are adopted, which require quick chilling of food immediately after it is cooked. These systems utilize a combined technology such as blast-chillers and refrigerators that maintain a constant temperature between 0-2° C, which allows for interruption of the production process between the cooking phase and the moment of serving the food.

The shelf-life of meals produced with this system can be extended up to 10-15 days by adopting either (vacuum/gas) packaging post-cooking or vacuum cooking. Along with commercial catering, which produces dishes within its own facilities , new food companies are created producing and supplying ready-to-eat dishes packaged under vacuum with a shelf-life of about 30-40 days targeted to meet specific commercial catering demands.

Given the high cost of the technology and the uniqueness of the raw materials, these products are aimed at a sophisticated and elite catering market. However, at the moment of serving, the final consumer will find it impossible to detect the applied food technologies, and there are few components of the product that make it possible to distinguish the difference among prepared dishes that have undergone one or more temperature treatments, since the recipe has been deconstructed to the appropriate extent prior to suit the subsequent preservation[4].

The World Health Organization ranks the commercial catering industry as the second most frequent cause of food contamination with a frequency of 21.5%, institutional catering as the fourth with 7.2%, school and company canteens as the 5th most frequent with 1.7%. The reasons for this ranking are to be found in the lack of staff training, the low level of sensitivity of caterers and mistrust towards health and hygiene consultants.

The purpose of this study, which was carried out between January 2006 and December 2007, was to ascertain the levels of hygiene of different types of foods, measuring the results against microbiological criteria (Table 1) and to compare the data collected over two years of monitoring.

The food samples collected and analyzed came from public businesses/firms who voluntarily underwent analytical monitoring carried out by the trade association FIPE (Federazione Italiana Pubblici Esercizi), as well as the services of a hygienist consultant. These steps were taken as a part of the process of

obtaining the "Bollino Blu della Ristorazione" (Blue Sticker Catering) awarded by FIPE and the Minister of Health.

The objective of the B.B. was to achieve certification of catering companies compliance with food safety guidelines drawn up with mutual agreement by all parties who signed the protocol: The guidelines focus on critical areas relating to food safety within small businesses [5].

4.3 Materials and methods

A total of 217 food samples ready for consumption were analyzed, including cooked meat, cooked and raw seafood, cooked and raw vegetables, milk and egg-based crèmes, deli products, and egg-based products. 71 food samples were analyzed in 2006; 142 samples were analyzed in 2007. It was not possible to identify the production systems that were used: fresh- hot or cook & chill.

The parameters studied were: aerobic plate counts (APC) (method AFNOR 3M 01/1-09/89), *E. coli* (EC) (method AFNOR 3M 01/8-06/01), total coliforms (TC) (method AFNOR 3M 01/2-09/89A), *Staphylococcus aureus* (method AFNOR 3M 01/9-04/03), *Listeria monocytogenes* (presence/absence test: AFNOR BRD 07/4-09/98, count: AFNOR BRD 07/05-09/01) and *Salmonella* spp. (6579-2002/Cor an ISO 2004). All analyses were performed in duplicate.

4.4 Results

All samples analyzed showed an absence of *Listeria monocytogenes* and *Salmonella* spp in 25 g of product, and *E.coli* counts were below the detection limit of the assay (<10 CFU/g) in all the samples, thus demonstrating that food safety criteria are well managed and achieved by food handlers and personnel.

Table 1 shows the microbiological reference standards adopted for the assessment of compliance for each type of food ready for consumption.

Figure 1 shows the general trend of compliance assessments of health and hygiene in 2006 and 2007.

Figures 2 and 3 show the percentage breakdown of non-compliance by type of product in 2006 and 2007.

Figures 4, 5 and 6 show the levels of contamination for aerobic plate counts in meat-based cooked recipes, seafood-based cooked recipes, and cooked vegetable recipes.

Table 1: Microbiological reference standards for the evaluation of microbiological quality and safety of food samples analyzed.

|	Foods ready for consumption, cooked Log$_{10}$ CFU/g	Foods ready for consumption, uncooked Log$_{10}$ CFU/g	Raw and washed vegetables ready for consumption Log$_{10}$ CFU/g
Aerobic plate counts	4.00	5.00	6.00
Total coliforms	2.00	3.00	3.00
Escherichia coli	2.00	2.00	2.00
Staphylococcus aureus	2.00	2.00	2.00
Listeria monocytogenes	absence in 25g	absence in 25g	absence in 25g
Salmonella spp.	absence in 25g	absence in 25g	absence in 25g

CFU: colony forming units.
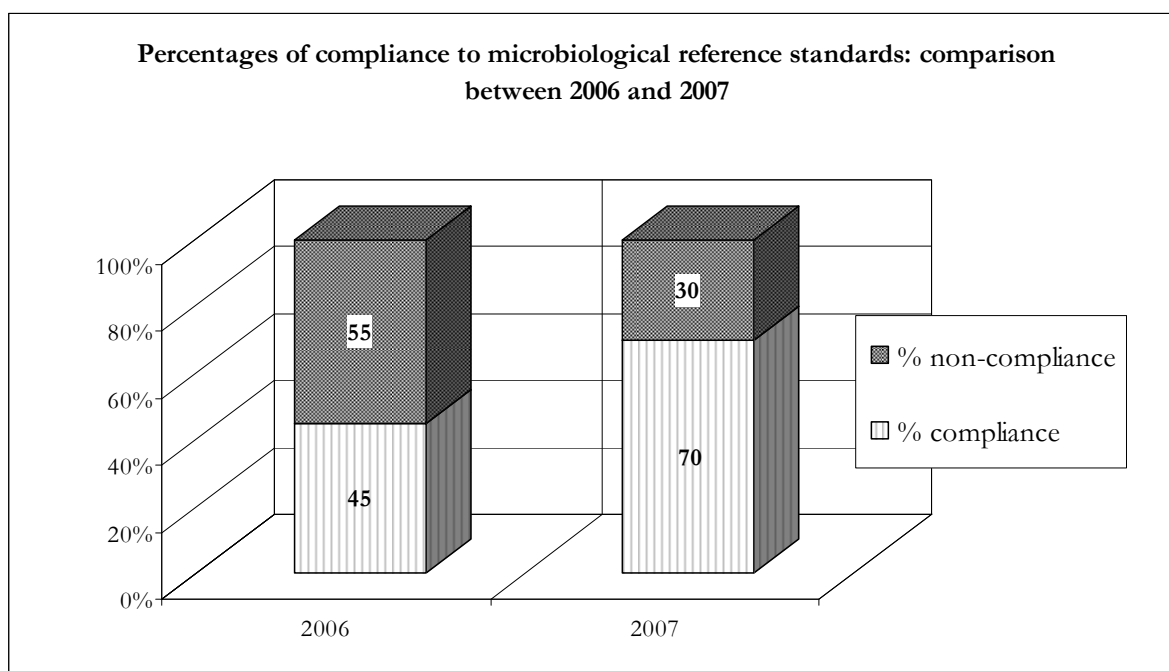
Figure 1: Overall percentages of compliance to microbiological limits in 2006 and in 2007. The calculation of the percentages included all the samples that resulted non-compliant to reference standards with at least one microbial parameter.

Non-compliance percentages in different types of food samples in 2006

uncooked vegetables 11%

cooked vegetables 5%

egg-based recipes 0%

gastronomy 14%

sauces 3%

cooked meat-based recipes 34%

uncooked seafood-based recipes 19%
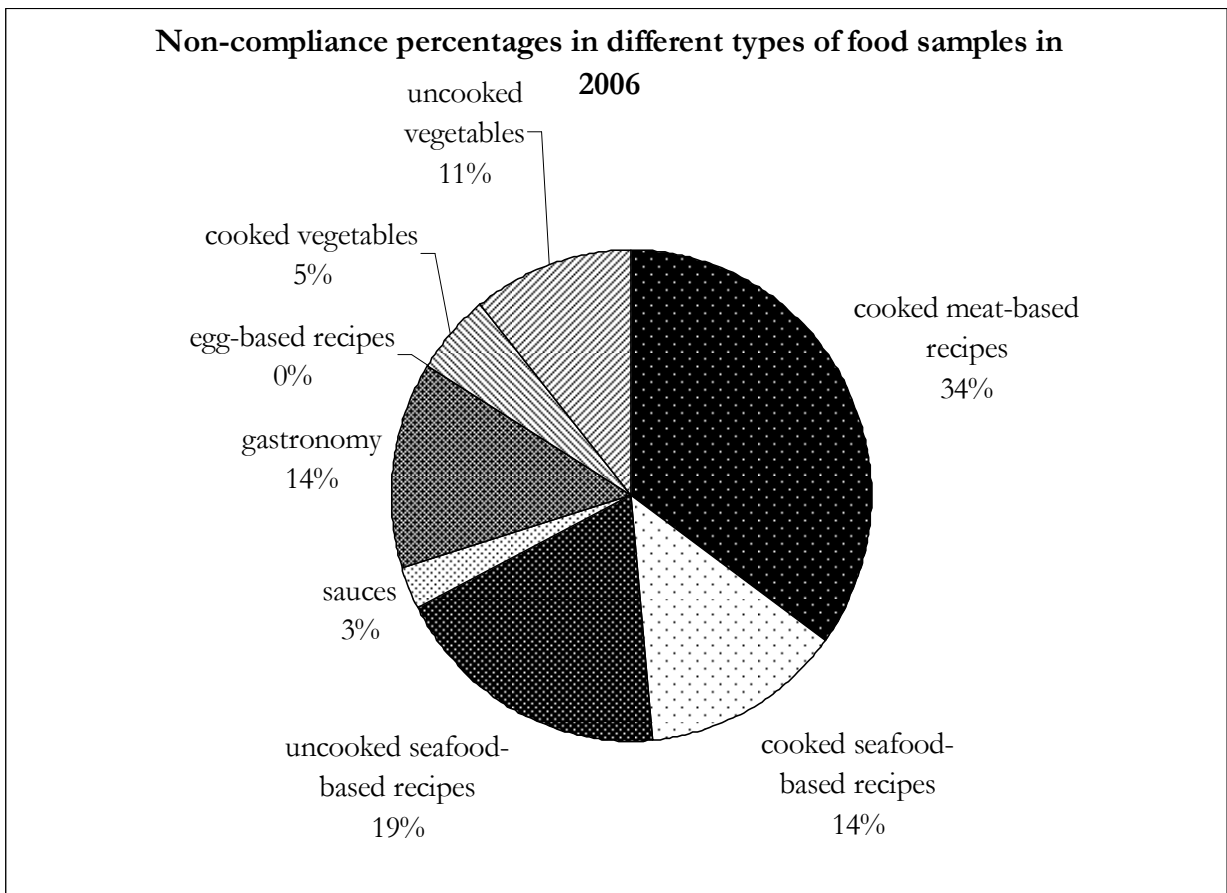
cooked seafood-based recipes 14%

Figure 2: Non-compliance percentages in different types of food samples in 2006. The calculation of the percentages included all the samples that resulted non-compliant to reference standards with at least one microbial parameter.
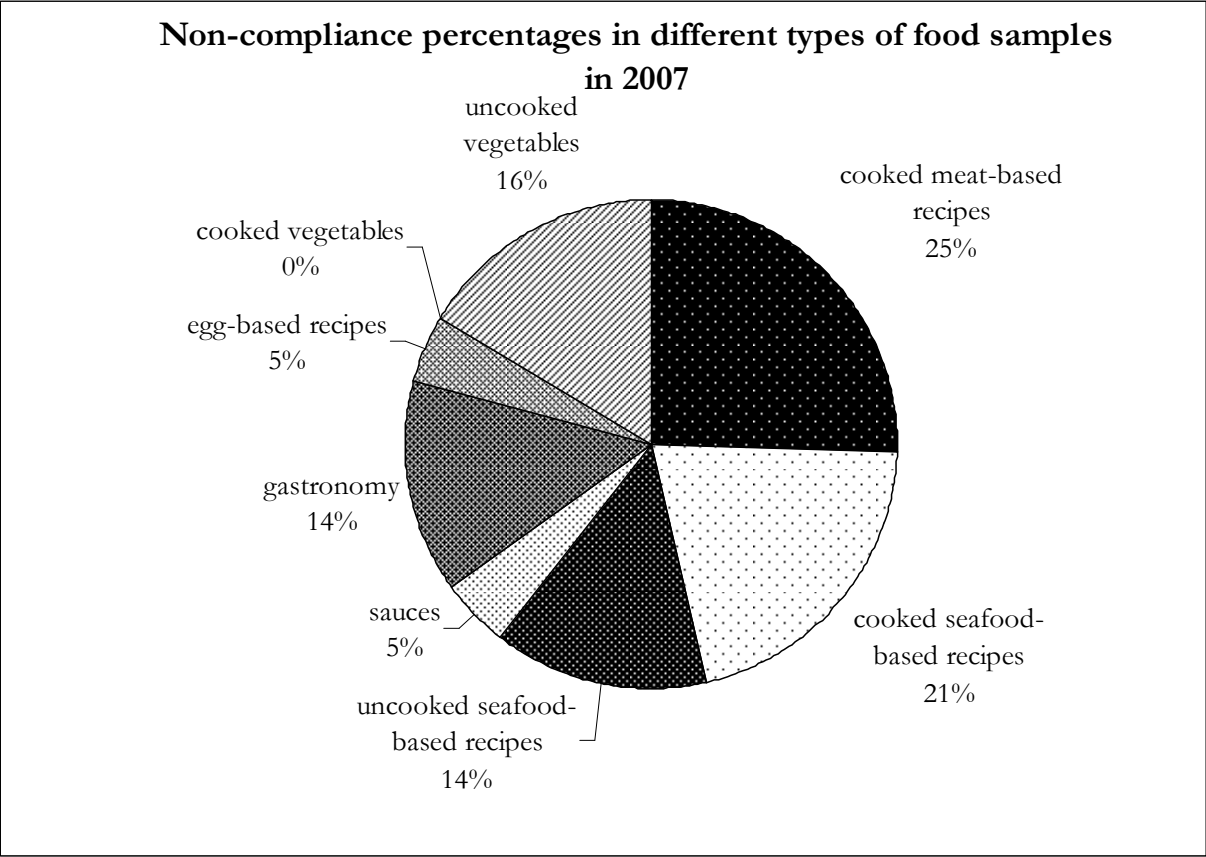
Non-compliance percentages in different types of food samples in 2007

uncooked vegetables 16%

cooked vegetables 0%

egg-based recipes 5%

gastronomy 14%

sauces 5%

uncooked seafood-based recipes 14%

cooked meat-based recipes 25%

cooked seafood-based recipes 21%

Figure 3: Non-compliance percentages in different types of food samples in 2007. The calculation of the percentages included all the samples that resulted non-compliant to reference standards with at least one microbial parameter.
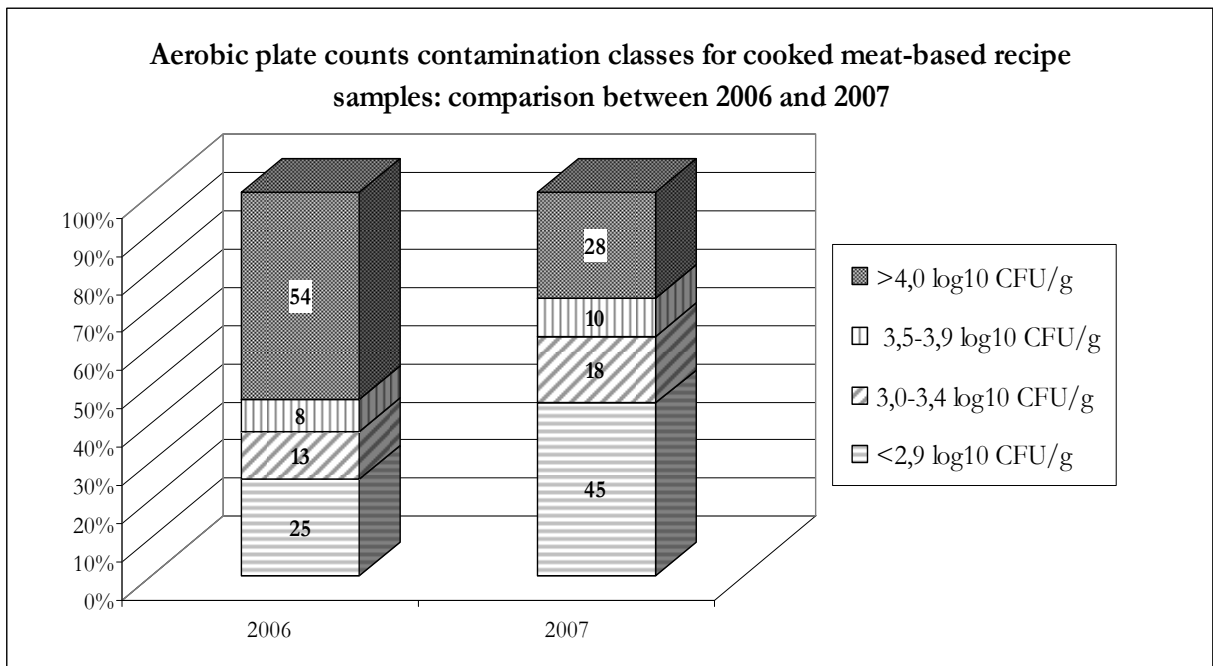
Figure 4: The levels of contamination for the microbial indicator "aerobic plate counts" are represented. The values in the areas indicate the percentages of the samples belonging to the four classes of contamination.
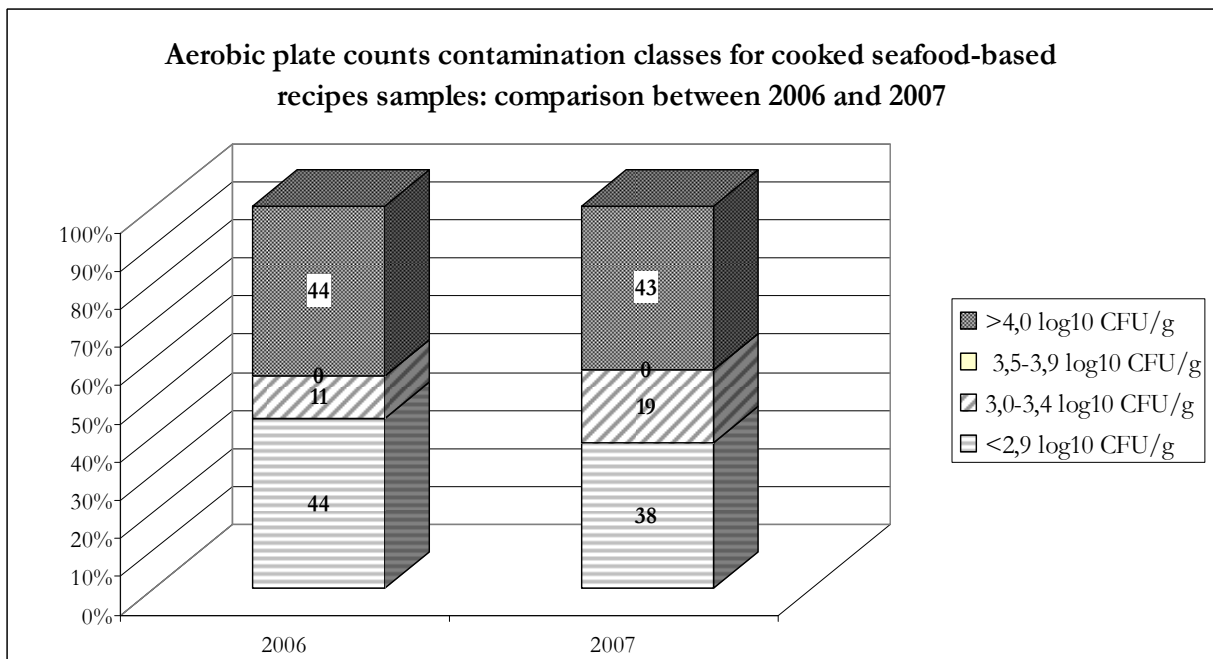

Figure 5: The levels of contamination for the microbial indicator "aerobic plate counts" are represented. The values in the areas indicate the percentages of the samples belonging to the four classes of contamination.

Figure 6: The levels of contamination for the microbial indicator "aerobic plate counts" are represented. The values in the areas indicate the percentages of the samples belonging to the four classes of contamination.
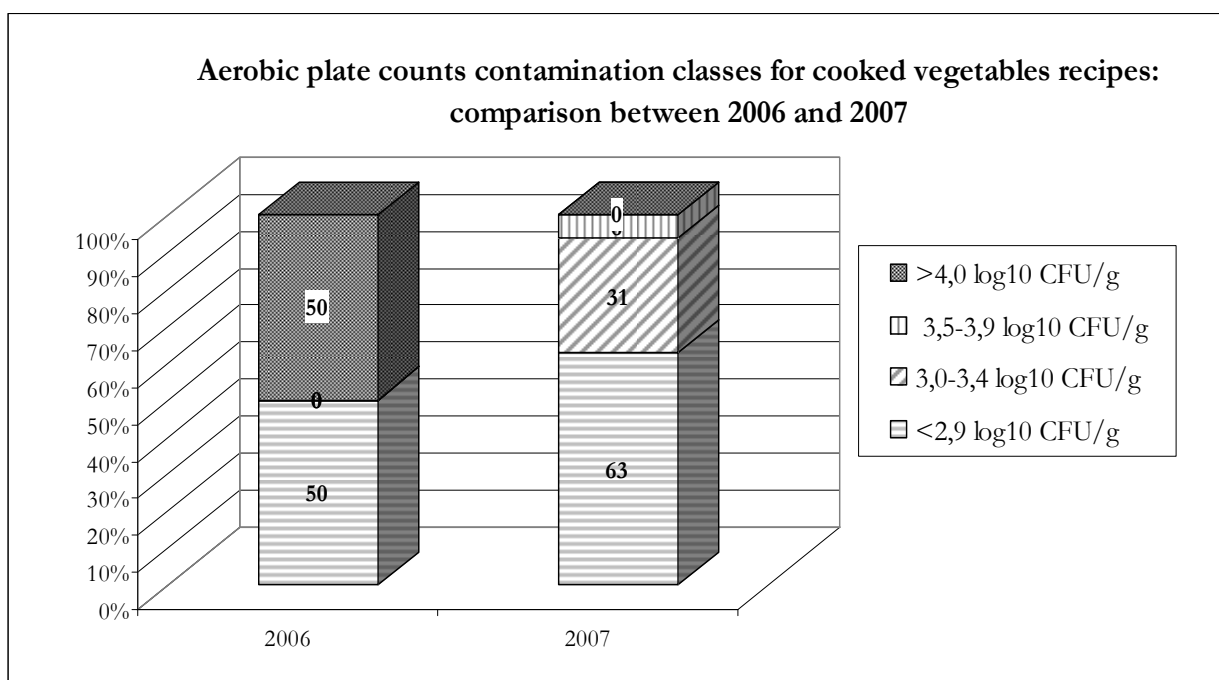
4.5 Discussion and conclusions

The results are reassuring regarding the absence of pathogenic microorganisms in both the years 2006 and 2007.

In 2006, non-compliant samples amounted to 55% , while in 2007 this value fell to 30% (Figures 1). Figures 2 and 3 show the percentage breakdown of non-compliance by type of preparation in 2006 and 2007. It is to be noticed that the decrease of non-compliances was generally found in cooked preparations, whereas the percentages remained unchanged in raw preparations. In Figures 4, 5 and 6, an improvement is observed, especially regarded the preparation of cooked meat and vegetables as well as cooked seafood.

The reasons for this result could be that operators in the food sector have become more sensitized to the risks linked to these food preparations, and that consultants' suggestions and corrective measures have been more easily adopted within the establishment of production by adopting workflows with suitable. timing and temperature conditions.

Changes in the commercial catering sector and the adoption of production systems that differ from traditional ones (where the time lag between cooking and serving is relatively short) expose consumers to greater risk. The results of this survey are clear and encouraging, demonstrating that operators of small

restaurants have felt the need to be supported by external professionals to monitor their production chain and levels of hygiene, thereby improving the food safety of their catering activity [6].

4.6 References

www.fipe.it (2007) *Indagine congiunturale sulla ristorazione commerciale*

www.uniprom.it (2005) Nuovi sistemi di cottura.

Montanari A. (2004) *Mangiare Fuori. Logiche e Tecniche della ristorazione italiana dall'osteria al fast food.* I libri di ristorando. Edifis.

Dragoni I. (2005) *Nutrition School System: un progetto di ricerca per la sicurezza alimentare nella scuola.* 6th Neotron International Symposium. 24 Febbraio 2005, Villa Cesi.

Ministero della Salute (2004) *Bollino blu della ristorazione, protocollo di verifica.* FIPE Servizi S.r.l. Roma.

Istituto Superiore di Sanità (2007) *Le tossinfezioni alimentari: sorveglianza e controllo.* VI Workshop internazionale Enter-net Italia, sistema di sorveglianza delle infezioni enteriche. Roma, 17-18 maggio 2007.

CHAPTER 5

Monitoring the bacteriological quality of Italian bottled spring water from dispensers

5. Monitoring the bacteriological quality of Italian bottled spring water from dispensers

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5.1 Abstract

The bacteriological quality of unprocessed bottled spring water was evaluated, through enumeration of heterotrophic plate count at 22 and 37 °C, total coliforms, *Escherichia coli*, intestinal enterococci and *Pseudomonas aeruginosa*. The aims of the research were to monitor the hygiene of bottled spring water and to evaluate the effectiveness of the dispenser sanitation procedure, reproducing field conditions. In total, 120 water samples were analyzed. The results indicated a high contamination frequency with *P. aeruginosa* (57.5%) and therefore the need to refine hygiene in the bottling plant and in the dispenser sanitation procedures.

5.2 Introduction

In Italy the large container bottled water consumption is a relatively recent phenomenon. The Italian market is the third largest in Western Europe after the United Kingdom and France (Beverfood, 2007). Water dispensers (coolers) are increasingly popular in office buildings, shopping centres, schools and hospitals. The format of the 18.9 litre bottle is the most widespread.

The expression "water for human use" includes all types of water which, under current legislation, are identified as follows: water intended for human consumption (drinking or tap water), natural mineral waters, spring waters. Drinking water is defined by Italian law (Legislative Decree 31/2001) in accordance with the Council of the European Economic Community Directive (1998) 98/83/EEC legislation.

Drinking water is often disinfected before it enters the distribution systems. Natural mineral water is bacteriologically pure water. The term "spring water" is reserved for water intended for human consumption in its natural state, and bottled at source. Like mineral waters, spring waters must have microbiological purity, and cannot undergo any treatment for disinfection.

The safety and microbiological quality of 18.9 litre spring water bottles depend on both the sources of contamination that might arise from the spring, after bottling, storage and transportation, but also from problems due to the efficacy of the dispensers sanitation before bottling takes place (Morais et al., 1997, Baumgartner et al., 2006, Kokkinakis et al., 2008).

The cleaning treatments of the dispensers are especially sensitive not only due to their structural characteristics but also because bacterial growth is often associated with the formation of a biofilm consisting of microbial cells, extra cellular and inorganic products, which can cause the corrosion and clogging of water pipes. The biofilm enables bacteria to survive and to transfer genetic material encoding virulence factors and antibiotic resistance (Grobe et al., 2001, Huq et al., 2008, Mena et al., 2009).

The aims of this research were to check the microbial flora of 18.9 litre spring water bottles through a determination of the heterotrophic plate count (HPC) at 22 and 37 °C, total coliforms, *Escherichia coli*, *Enterococcus* spp., *Pseudomonas aeruginosa,* and to evaluate over time the efficacy of the dispenser sanitation.

5.3 Materials and methods

5.3.1 Sampling

From October to April 2008-2009, water samples were collected from 40 sampling points as follows: 120 samples of non-carbonated spring water from refillable 18.9 litre polycarbonate plastic (PC) bottles, all from a single Northern Italy supplier, placed on five dispensers identified by a number from 1 to 5. All the bottles have a device at the cap that should prevent the reflux of water from the dispenser reservoir. Each dispenser was equipped with two taps, identified by a letter from "a" to "j" which dispensed, depending on the model, room-temperature (range 20.4-23.4 °C), chilled (range 4.2-6.4 °C) and hot water (85 °C) (Figure 1). All the samples were analyzed in triplicate.

The sampling process was divided into four experiments. During the first and the second experiments, samples were collected reproducing field use, without a previous dispenser sanitation procedure; during the third and fourth experiments, a standard disinfection procedure was performed before the sampling in order to test its efficacy.

The dispensers disinfection was performed spraying a 3% chlorine solution (contact time: 15 minutes) followed by rinsing off with potable water. Each day, at irregular intervals of time, 1 litre of water in total was collected with a graduated cylinder, in order to simulate daily use. Water samples for analysis were collected from dispensers taps previously disinfected externally spraying a

70% ethanol solution, and internally with imbibed swabs. Before sampling, was let the water run for about 1 minute. Each sample consisted of 900 mL.

Experiment 1- water samples were collected the same day (T0) and 7 days (T7) after installing the coolers in the laboratory.

Experiment 2- a microbial water analysis was performed on samples collected with a sterile syringe directly from three bottles, one *per* lot.

Experiment 3- the aim was to assess the effectiveness of the cooler sanitation procedure. Five spring water bottles belonging to a single new lot were installed, and sampling was performed immediately after the sanitation procedure.

Experiment 4- the coolers were sanitized again before sampling, and two new PC bottles were filled with sterile water (SW) and installed on dispensers.

SW was analysed before filling the bottles, and then samples from dispenser taps were analyzed at 0, 7 and 14 days. In this final experiment, the microbiological parameters were restricted to HPC and *P. aeruginosa.*

5.3.2 Microbiological analyses

Microbiological analyses were performed using the membrane filtration technique according to International Standards Organization (ISO) protocols, for the detection of total coliforms (TC), (ISO 9308-1:2002, 2002); *E. coli* (EC), (ISO 9308-1:2002, 2002); *P. aeruginosa* (PA), (ISO 16266:2008, 2008); *Enterococcus* spp. (EN), (ISO 7899-2:2003, 2003); heterotrophic plate count (HPC) at 22 and 37 °C, (ISO 6222:2001, 2001).

Water samples of 250 ml each were filtered through a hydrophilic mixed cellulose ester membrane (International PBI Spa, 2054045) of 0.45 µm pore size and 47 mm in diameter for all organisms, and membranes were placed in each petri dish filled with a specific medium: Tergitol TTC (Oxoid Corporation, 502948) for total coliforms and *E. coli*, Pseudomonas Agar Base/CN-Agar (Oxoid Corporation, 502946) for *P. aeruginosa*, Slanetz and Bartley (Oxoid Corporation, PO5018A) for *Enterococcus* spp.

For the enumeration of HPC the pour-plate method was used, and a sterile petri dish was filled with 1 ml of water sample, and 15 ml to 20ml of Water Plate Count Agar Medium (Oxoid Corporation, CM1012B) were added. Petri dishes were incubated at 37 °C for 24 h, at 42 °C for 24 h, at 37 °C for 48 h, at 37 °C for 24 h and at 22-37 °C for 24-72 h, for each medium and temperature, respectively. For *P. aeruginosa*, the organism was confirmed using an oxidase test, a fluorescence test and cetrimide agar, followed by 24 h of incubation at 42 °C.

The results were interpreted according to current regulations for bottled water, which state that *E. coli*, *Enterococcus* spp., *P. aeruginosa* should not be detectable in 250 ml samples of water and total coliforms in 100 ml samples of water, while HPC, at 22 °C, and 37 °C, should not exceed 100/ml, and 20/ml CFU, respectively.
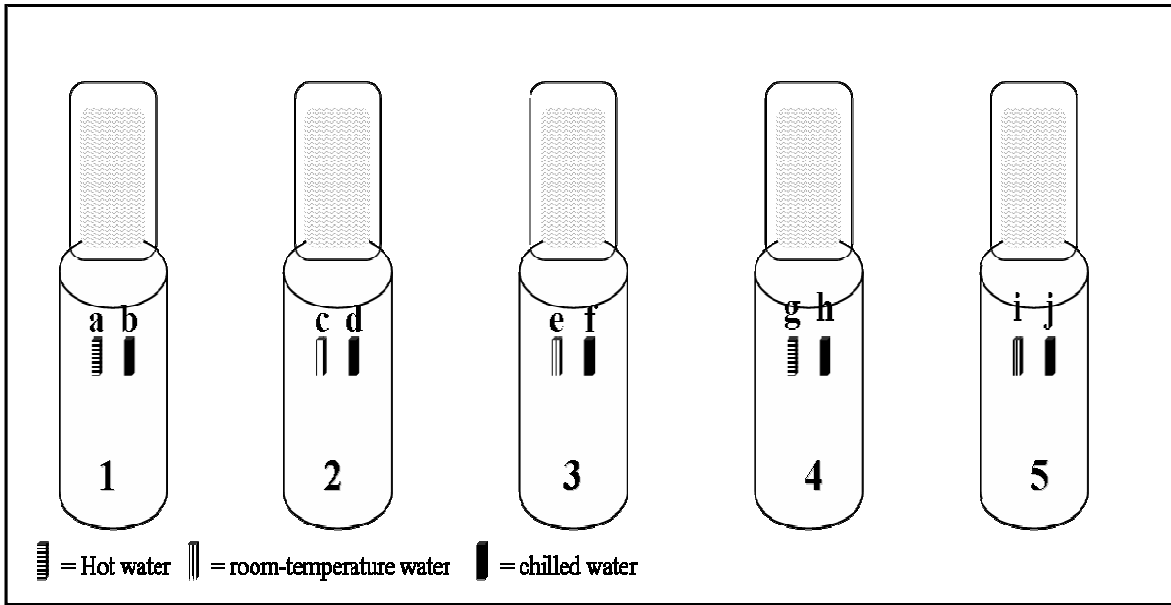
Figure 1: schematic representation of water sampling points

5.4 Results

The microbial characteristics of water are presented in Tables 1-4. TC, EC and EN were not detected in all the samples analyzed.

An examination of the results of Experiment 1 (Table 1) shows non-compliance of the water analyzed, both at T0 to T7. Out of a total of 60 analyses, corresponding to a number of 20 sampling points examined, 90% were found to exceed the limits set by law for HPC at 22 °C and 37 °C. *P. aeruginosa* was the major contaminant bacterium, and was found in 8 out of the 20 sampling points examined. Dispensers 1 and 4 showed values exceeding the HPC limits, while dispensers 2, 3 and 5 also revealed contamination by *P. aeruginosa*. Results of the analyses performed on hot water at T7 showed a complete destruction of the microbial flora previously recognized at T0.

The water collected directly from bottles (Table 2) showed evidence of high contamination by viable micro-organisms, including *P. aeruginosa*, that was found in 2 out of the 3 sampling points. The microbiological efficiency of the dispensers sanitation is shown in Table 3. The results showed values of HPC and *P. aeruginosa,* which represent the residual contaminant that survived the sanitation, both HPC and *P. aeruginosa* exceeded the limits imposed by law in 9 sample points out of a total of 10.

The results of the fourth experiment (Table 4) showed that at T0 there was no bacterial growth. An increase in bacterial growth was detected after 7 days of sanitizing treatment, and at T14 a further increase of bacterial growth was

revealed. Contamination is attributable exclusively to micro-organisms surviving in the dispensers after the sanitation procedures.

Table 1: microbial characteristics of water samples from dispensers. All data represent the mean of three separate determinations.

Water sample*	HPC 22°C Mean ($\log_{10}$ CFU/ml)	Range ($\log_{10}$ CFU/ml)	HPC 37°C Mean ($\log_{10}$ CFU/ml)	Range ($\log_{10}$ CFU/ml)	PA Mean ($\log_{10}$ CFU/250 ml)	Range ($\log_{10}$ CFU/250 ml)
1a, T0(R)	3.49	3.39-3.57	3.36	3.29-3.42	0	
1a, T7(B)	0		0		0	
1b, T0(R)	3.41	3.26-3.52	3.36	3.30-3.41	0	
1b, T7(C)	4.49	4.35-4.60	4.74	4.63-4.83	0	
2c, T0(R)	2.88	2.79-2.95	2.83	2.74-2.91	>2.48	
2c, T7(R)	3.15	3.08-3.21	2.97	2.88-3.04	>2.48	
2d, T0(C)	3.08	3.01-3.14	2.90	2.81-2.98	>2.48	
2d, T7(C)	3.59	3.46-3.69	3.20	3.13-3.26	>2.48	
3e, T0(R)	4.08	4.04-4.12	3.15	3.08-3.21	1.85	1.79-1.90
3e, T7(R)	5.00	4.73-5.16	5.11	5.03-5.18	0	
3f, T0(C)	4.07	4.02-4.11	3.15	3.08-3.21	2.30	2.27-2.32
3f, T7(C)	4.99	4.91-5.06	4.98	4.89-5.05	0	
4g, T0(R)	4.54	4.51-4.57	4.04	3.96-4.11	0	
4g, T7(B)	0		0		0	
4h, T0(C)	4.62	4.56-4.67	4.30	4.23-4.36	0	
4h,T7(C)	4.46	4.32-4.57	4.45	4.31-4.56	0	
5i, T0(R)	2.60	2.48-2.70	2.76	2.66-2.84	0	
5i, T7(R)	3.53	3.40-3.63	3.15	3.08-3.21	0.48	0.01-0.70
5j, T0(C)	2.59	2.46-2.69	2.53	2.40-2.63	0	
5j, T7(C)	4.08	4.01-4.14	3.67	3.55-3.76	1.11	0.95-1.23

HPC: heterotrophic plate count, CFU: colony forming unit, PA: *Pseudomonas aeruginosa*, T0: time zero, T7: after seven days. 2.48 $\log_{10}$ CFU/250ml: detectable limit of the assay
*The number indicates the dispenser, the letter indicates the dispenser tap. In parentheses: B: hot, R: room-temperature, C: chilled

Table 2: microbial characteristics of water samples from bottles, one *per* lot. All data represent the mean of three separate determinations.

Bottle lot – water sample*	HPC 22°C Mean ($\log_{10}$ CFU/ml)	Range ($\log_{10}$ CFU/ml)	HPC 37°C Mean ($\log_{10}$ CFU/ml)	Range ($\log_{10}$ CFU/ml)	PA Mean ($\log_{10}$ CFU/250 ml)	Range ($\log_{10}$ CFU/250 ml)
Lot 1(R)	3.68	3.58-3.76	4.88	4.78-4.96	0	
Lot 2(R)	3.41	3.27-3.52	3.04	2.96-3.11	>2.48	
Lot 3(R)	3.00	2.93-3.06	5.15	5.08-5.21	1.41	1.25-1.53

HPC: heterotrophic plate count, CFU: colony forming unit, PA: *Pseudomonas aeruginosa.* 2.48 $\log_{10}$ CFU/250ml: detectable limit of the assay

*Lot 1 was associated with dispensers 1, 3 and 4. Lot 2 was associated with dispenser 2, and lot 3 was associated with dispenser 5. In parentheses: R: room-temperature

Table 3: first microbial evaluation of dispensers sanitation. Five new spring water bottles of a single lot were installed. Microbial characteristics of water samples from coolers after sanitation are illustrated. All data represent the mean of three separate determinations.

Water sample*	HPC 22°C Mean ($\log_{10}$ CFU/ml)	Range ($\log_{10}$ CFU/ml)	HPC 37°C Mean ($\log_{10}$ CFU/ml)	Range ($\log_{10}$ CFU/ml)	PA Mean ($\log_{10}$ CFU/250 ml)	Range ($\log_{10}$ CFU/250 ml)
1a(B)	0		0		0	
1b(R)	3.75	3.72-3.77	3.66	3.63-3.69	>2.48	
2c(R)	3.32	3.28-3.36	3.04	2.81-3.19	>2.48	
2d(R)	3.36	3.32-3.40	3.04	2.81-3.19	>2.48	
3e(R)	2.74	2.34-2.94	1.54	1.43-1.63	>2.48	
3f (R)	2.85	2.83-2.87	1.69	1.59-1.77	>2.48	
4g(R)	2.48	2.35-2.58	2.36	2.32-2.40	1.45	1.37-1.52
4h(R)	2.36	2.21-2.47	2.28	2.23-2.32	0.48	0.31-0.60
5i(R)	3.85	3.83-3.87	3.80	3.78-3.82	0.90	0.77-1.00
5j(R)	3.76	3.73-3.78	3.78	3.75-3.80	0.95	0.84-1.04

HPC: heterotrophic plate count, CFU: colony forming unit, PA: *Pseudomonas aeruginosa,* 2.48 $\log_{10}$ CFU/250ml: detectable limit of the assay

*The number indicates the dispenser, the letter indicates the dispenser tap. In parentheses: B: hot, R: room-temperature

Table 4: second evaluation of the efficacy of dispensers sanitation. Two new bottles filled with sterile water were installed. Microbial characteristics of water samples from coolers after sanitation are illustrated. Sterile water was separately analyzed at time zero. All data represent the mean of three separate determinations.

Water sample*	HPC 22°C Mean (log_{10} CFU/ml)	Range (log_{10} CFU/ml)	HPC 37°C Mean (log_{10} CFU/ml)	Range (log_{10} CFU/ml)	PA Mean (log_{10} CFU/250 ml)	Range (log_{10} CFU/250 ml)
SW, T0(R)	0		0		0	
2d, T0(C)	0		0		0	
2d, T7(C)	1.65	1.55-1.73	1.95	1.88-2.01	2.26	2.22-2.29
2d, T14(C)	2.61	2.50-2.70	2.82	2.69-2.95	>2.48	
3f, T0(C)	0		0		0	
3f, T7(C)	2.79	2.70-2.86	2.94	2.84-3.04	>2.48	
3f, T14(C)	2.94	2.87-3.00	3.12	3.01-3.23	>2.48	

HPC: heterotrophic plate count, CFU: colony forming unit, PA: *Pseudomonas aeruginosa,* SW: sterile water, T0: time zero, T7: after seven days, T14: after fourteen days. 2.48 log_{10} CFU/250ml: detectable limit of the assay
*The number indicates the dispenser, the letter indicates the dispenser tap. In parentheses: R: room-temperature, C: chilled

5.5 Discussion

The spring water analyzed in this study was found to be inappropriate for human consumption as defined by law, since 85% of the water samples were found to exceed the limits for HPC and 57.5% were positive for *P. aeruginosa.*
Like Lévesque and coworkers (1994), and Baumgartner and coworkers (2006), we were be able to show that the aerobic plate counts increase in dispensers. It is well known that, as has been described in previous studies (Baumgartner et al., 2006, Kokkinakis et al., 2008), the unprocessed bottled water is naturally colonized with bacteria at high levels (up to 5.11 CFU/ml in our study), and therefore HPC is not a suitable criterion for estimating the hygienic quality of bottled water. Nevertheless, plate counts may be useful to analyze the effect of dispenser cleaning.
The most considerable result is the high contamination frequency with *P. aeruginosa.* Baumgartner and co-workers (2006) reported 25% of the spring water samples from bottles and 24.1% of the water samples from coolers positive for

P. aeruginosa, instead in Kokkinakis and co-workers (2008) study no *P. aeruginosa* was found.

This bacterium is known to be an important agent of nosocomial infections, with multidrug-resistant strains in hospitals being of particular concern (Morais et al., 1997, Huq et al., 2008, Felfoeldi et al., 2010). Because elderly and immunocompromised people are at greatest risk of infection, drinking water in hospitals and institutions should contain as few *P. aeruginosa* as possible.

The *P. aeruginosa* isolates recovered in samples coming from bottles indicates that they likely originated from the water-production plant. This bacterial contamination could be due to the survival of microorganisms even after washing and refilling of PC bottles back to the bottling plants.

Alternatively, the bacteria may have survived in the water in quantities that were not found in the first analysis, but were then able to reproduce since they were protected by biofilm.

The current procedure for dispensers cleaning was not effective, as can be confirmed by comparing the results obtained in the fourth experiment. The results of our study show that in order to protect human health, the following concrete actions are recommended: microfiltration or pasteurization in order to eliminate unwanted bacteria, such as *P. aeruginosa*; accurate environmental and water microbiological controls at critical points in the bottling plants; the study of new dispenser sanitizing procedures; an increase in the frequency of sanitations.

The places of use of water from dispensers are often private places, and are not subject to official surveillance and official microbiological type controls. Therefore it is essential continue to study the microbiological characteristics of bottled waters in order not to underestimate the microbiological risks associated with their consumption.

5.6 References

Baumgartner A., Grand M. (2006). Bacteriological quality of drinking water from dispensers (coolers) and possible control measures. *Journal of Food Protection*, vol. 69, No 12, 3043-3046.

Beverfood (2007). Bottled water consumption in Europe. http://www.beverfood.com .

Council of The European Economic Community Directive 98/83/EEC (1998). On the quality of water intended for human consumption. The Council of the European Communities. (OJ No. L330, 5.12.1998).

Felfoeldi T., Heeger Z., Vargha M., Marialigeti K. (2010). Detection of potentially pathogenic bacteria in the drinking water distribution system of a hospital in Hungary. *Clinical Microbiology & Infection*, 16(1), 89-92.

Grobe S., Wigender J., Hans-Curt F. (2001). Capability of mucoid *Pseudomonas aeruginosa* to survive in chlorinated water. *International Journal of Hygiene and Environmental Health*, 204, 139-142.

Huq A., Whitehouse C.A., Grim C.J., Anam M., Colwell R.R. (2008). Biofilms in water, its role and impact in human disease transmission. *Current Opinion in Biotechnology*, 19, 244-247.

International Organization for Standardization (ISO) 9308-1:2002 (2002). *Water quality – detection and enumeration of Escherichia coli and coliform bacteria – Part 1: Membrane filtration method,* Geneva: International Organization for Standardization.

International Organization for Standardization (ISO) 7899-2:2003 (2003). *Water quality – Detection and enumeration of intestinal enterococci – Part 2: Membrane filtration method,* Geneva: International Organization for Standardization.

International Organization for Standardization (ISO) 6222:2001 (2001). *Water quality – Enumeration of culturable micro-organisms – Colony count by inoculation in a nutrient agar culture medium*, Geneva: International Organization for Standardization.

International Organization for Standardization (ISO) 16266:2008 (2008). *Water quality – Detection and enumeration of Pseudomonas aeruginosa – Method by membrane filtration*, Geneva: International Organization for Standardization.

Khan A., Adesiyun A. A. (2005). Bacteriological quality of drinking water from refrigerated coolers in a university community. *Journal of Food, Agriculture & Environment*, 3(2), 32-34.

Kokkinakis E. N., Fragkiadakis G. A., Kokkinaki A. N. (2008). Monitoring microbiological quality of bottled water as suggested by HACCP methodology. *Food Control,* 19(10), 957-961.

Levesque B., Simard P., Gauvin D., Gingras S., Dewailly E., Letarte R. (1994). Comparison of the microbiological quality of water coolers and that of municipal water systems. *Applied and Environmental Microbiology*, 60 (4), 1174-1178.

Mena K.D., Gerba C.P. (2009). Risk assessment of *Pseudomonas aeruginosa* in water. *Reviews of Environmental Contamination and Toxicology*, 201, 71-115.

Morais P.V., Mesquita C., Andrade J.L., Milton S.C. (1997). Investigation of persistent colonization by Pseudomonas aeruginosa like strains in a spring water bottling plant. *Applied and Environmental Microbiology*, 63(3), 851-856.

Sellerio S. (2006). Il mercato delle acque in boccioni. *Annuari del bere Beverfood*, 361-366.

CHAPTER 6

Microbiological quality and safety of refrigerated precooked lasagna packaged under modified atmosphere for Italian school catering

6. Microbiological quality and safety of refrigerated precooked lasagna packaged under modified atmosphere for Italian school catering

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6.1 Abstract

The aim of this study was to evaluate the microbiological quality and safety of refrigerated precooked lasagna prepared in a traditional "cook-serve" centralized kitchen for up to 28 days of storage. Two preparation methods (hot and cold sauce layering) and two materials (polyethylene and cellulose) for packaging under a modified atmosphere were tested. Microbiological analyses focused on pathogenic and potential-pathogenic microorganism markers (*Salmonella* spp., *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, and spores of sulphite-reducing clostridia), and on the evolution of spoilage-hygiene markers: mesophilic and psychrotrophic aerobic bacteria, total coliforms and lactic acid bacteria. In total, 81 samples were analysed at intervals of 7 days. No pathogenic microorganisms were detected in all the samples examined for up to 28 days of storage. 37% of samples were positive for *S. aureus*. After cooking all the samples were in a situation of conformity according to the limits established. In conclusion, the hot sauce layering method associated with polyethylene packaging resulted the most effective productive solution for prolonging the shelf-life of packaged precooked lasagna for up to 21 days.

6.2 Introduction

In Italy it is estimated that state school catering services serve more than 2,700,000 students daily. This represents a huge "social restaurant" in which the State, through local authorities, basically chooses the organization, raw materials, and the menus (Emilia-Romagna, Italy, 2009; Conferenza Permanente Stato-Regioni, 2010). Considering the complexity of the organizations involved, the vulnerability of the people who receive the foodservices, and the consequences

of possible adverse events, it is clear that catering services are of great strategic importance in the program to develop the right nutrition and to ensure food safety and nutritional quality. Cases of foodborne diseases (Veiros et al., 2009) occur daily throughout the world, from the most to the least developed countries. As most of these cases are not reported, the true dimension of the problem is unknown (WHO, 2007).

Data from Europe and the United States show that the greatest benefits in reducing foodborne illness levels have come from implementing controls in farm-to-retail processing. (Veiros et al., 2009). European food regulations contain all seven principles of the HACCP (Reg. EC 852/2004). Catering businesses need to have a food safety management system based on the principles of the HACCP. A flexible HACCP system is more suitable for foodservice operations since these may vary due to the complexity of the recipes, menus, food varieties, and amounts involved.

Data show that the places where incriminated food was consumed in Europe, from 1999 to 2000, increased 30% in restaurants, hotels and bars 30%; 7% in schools; 8% in canteens; 53% in catering areas and 36% in private homes 36%, comparatively to the period from 1993 to 1998 (Veiros et al., 2009).

Worldwide, numerous foodborne disease outbreaks in schools have been reported (Santana et al., 2009), but the extent of these outbreaks has not been systematically described, because under-notification of outbreaks is still common.

According to Michino and Otsuki (2000), in Japan between May and December 1996, there were 11,826 cases, including two deaths, from Escherichia coli O157:H7 infection. The major sources of infection were school-lunches served at elementary and nursery schools.

In Italy, Genghi and others (2004), reported that in April 2004, there was an outbreak caused by *Salmonella thompson* which involved 77 children attending kindergartens, nursery schools and primary schools on the island of Elba. The schools involved received the meals prepared by a central kitchen.

In foodservice environments, various factors may be related to foodborne diseases. Paulson (2002), and Green and others (2006) suggested that the hygiene of food workers may be involved in spreading foodborne illnesses. This is also supported by Guzewich and Ross's (1999) study, which reviewed 81 foodborne outbreaks from 1975 to 1998, and found that 89% were associated with the transmission of pathogens to foods by the workers' hands.

Ha and others (2003) suggested that drinking water, employees' hands, refrigerators, and aprons obtained from school foodservices were also possible factors related to foodborne illnesses. USDHHS-FDA-CFSAN (2000) suggested that risk factors causing foodborne illnesses are foods from unsafe sources, inadequate cooking, improper holding temperatures, contaminated equipment,

and poor personal hygiene. Adopting the most appropriate foodservice system therefore plays a major role in determining food safety.

In Italy the traditional "cook-serve" school foodservice is the most common, in which ingredients are assembled and food is produced onsite, kept either heated or chilled for up to two hours, and served to consumers. This system is the most accepted by Italian parents because of its similarities to the timing and methods of home-made meals. However from the point of view of the controls of critical points, it is the most critical foodservice system because there is insufficient time to check the microbiological quality of the raw materials and meals before consumption.

Another foodservice system that has been increasing, especially in larger Italian cities, is the centralized system. In this system meals are prepared in one single kitchen, and are transported to receiving kitchens where they are served to customers usually within two hours. Food can be transported either hot or cold. In the centralized foodservice system, meals may be produced immediately before the service or may be cooked or precooked and then packaged, stored chilled or frozen even for long periods of time, and finally heated or reheated and served to customers.

The extended shelf-life type of precooked meals is not very popular in Italy with its deep cultural roots in the culinary arts and on the important role of the mother in the preparation of home meals. For these reasons mass food production and long-term storage are often perceived as negative factors, given the existing trend for consumption of fresh rather than frozen products. Currently in Italian school foodservices, there are a few dishes that, due to their complex preparation needed are purchased already precooked and often frozen, such as lasagna. Lasagna is a classic Italian pasta casserole dish, which consists of alternate layers of pasta, cheese and often other ingredients such as minced beef with tomato sauce (Bolognese sauce). Lasagna is one of the most popular recipes in Italian school foodservices (Dragoni et al., 2005).

Preservation methods that extend the shelf-life of meals are of the utmost importance. Modified atmosphere packaging (MAP) in combination with chill storage (<4°C) is a well-known food preservation method, used for a great variety of foods including fresh beef, poultry, fish, sausages, cheese, bakery products, pasta, vegetables (Speranza et al., 2009). The right combination of gases (CO_2, N_2 and O_2) in the headspace of food packs suppresses the microbial flora of perishable foods developed under aerobic conditions (Patsias et al., 2003). A minimum CO_2 concentration of 20 – 30% is necessary to produce inhibitory effects (Stiles, 1991).

Chilling slows the deterioration of stored foods but if the atmosphere surrounding the product is also modified to reduce oxygen concentration, the shelf-life is increased considerably because of a further reduction in the rate of

chemical oxidation by oxygen and in the growth of aerobic microorganisms (Phillips, 1996). Storage temperatures play a major role in slowing bacterial growth, as the increase in shelf-life of MAP products provides sufficient time for human pathogens to multiply to levels which render the food unsafe yet still edible (Jay, 1992).

Some of the major microbiological hazards associated with modified atmosphere packaging are: 1) *Clostridium botulinum* type E, which is capable of growth and toxin production at 3°C; 2) *Listeria monocytogenes*, enterotoxigenic *Escherichia coli* and spore-formers such as *Bacillus cereus* can survive inadequate heat and then grow during the chilled storage of the product; 3) any leaks in the seal and packaging material can lead to post-thermal processing contamination by pathogens (Nyati, 2000).

However, the use of MAP with precooked meals that are subsequently cooked is considered less hazardous because cooking (if correctly carried out) kills vegetative pathogens such as *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Clostridia* (Hotchkiss, 1988).

The aim of this study was to evaluate and compare the microbiological quality and safety of refrigerated precooked lasagna prepared by a centralized school kitchen. The study was carried out using two production processes and two types of packaging materials, under a modified atmosphere, and stored at a refrigerator temperature for up to 28 days.

The ultimate goal was to determine the most suitable production method and type of packaging to ensure as high a level of microbial safety as possible.

The microbiological analysis focused on pathogenic and potential-pathogenic microorganism markers (*Salmonella* spp., *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, and spores of sulphite-reducing clostridia); as well as spoilage-microorganisms and hygienic markers: mesophilic aerobic and psychrotrophic bacteria, total coliforms and lactic acid bacteria.

6.3 Materials and methods

This investigation was conducted from June 2010 to July 2010, involving the centralized kitchen of an Italian state school catering service located in Lombardy, which provides 80,000 meals a day for 3 to 14 year-old children.

6.3.1 Preparation of lasagna
The lasagna was made on 23 June 2010. The ingredients consisted of: a) Bolognese sauce (40%) (Table 1), b) Béchamel sauce (40%) (Table 2) and c) lasagna sheets (20%) (durum wheat, wheat flour type 00, pasteurized whole egg (20%) and water). Bolognese and Béchamel sauces were cooked in the same

kitchen, lasagna sheets were provided precooked and frozen. The lasagna consisted of five layers of sheets of pasta interleaved with three layers of Bolognese sauce and three layers of Béchamel.

The first production method, named hot sauce layering (HSL) was divided into the following steps: 1) preparation and cooking of Bolognese and Béchamel sauces; 2) layering sauces (temperature of sauces at the moment of assembly: Bolognese 79 °C and Béchamel 85 °C) with frozen precooked lasagna sheets; 3) rapid chilling in a blast chiller (BCF 180A, Zanussi Professional, Italy), (cooling time: 2 hours, temperature at the end of chilling: 3 °C); 4) modified atmosphere packaging in half size gastronorm containers.

The second production method, named cold sauce layering (CSL) was divided into the following steps: 1) preparation and cooking of Bolognese and Béchamel sauces; 2) rapid chilling of samples in the blast chiller (cooling time: 2 hours, temperature of Bolognese and Béchamel sauces after thermal reduction: 2 °C); 3) layering of sauces with frozen precooked lasagna sheets; 4) modified atmosphere packaging in half size gastronorm containers.

Two types of packaging were tested and compared: 1) crystalline polyethylene terephthalate (CPET), with a top layer of amorphous polyethylene terephthalate (APET) (Arcoplastica S.r.l., Italy) and 2) cellulose with PET (92%+8% respectively) (S.I.S. S.p.A., Italy), hereafter CELL-PET. The top film was a polyethylene film type, 30 μm in thickness, with an O_2 permeability of 56 ml/m^2/d/atm at 65% RH/23 °C and a water vapour transmission rate of 13 g/m^2/d at 90% RH/38 °C (Tecnofood Pack S.p.A., Italy).

The following gas mixture was used: 50%/50% (CO_2/N_2), according to Phillips (1996). The gas mixture was prepared using an automatic packaging machine (Mondini, CV/PN-50VG, Italy).

Table 1: Bolognese sauce recipe.

Bolognese sauce	
Ingredients	%
Minced Italian beef	44.0
double concentrated tomato	27.5
frozen diced onion	3.5
frozen diced carrot	3.5
frozen diced celery	3.5
extra virgin olive oil	2.3
red wine	2.5
Salt	1.0
Water	2.9
tomato pulp	2.9
tapioca starch	1.0
Butter	2.7
wheat flour	2.7

Table 2: Béchamel sauce recipe.

Béchamel sauce	
Ingredients	**%**
water	75.0
wheat flour	7.0
butter	9.5
screamed milk powder	6.2
salt	0.9
sugar	0.8
tapioca starch	0.5
nutmeg	0.1

6.3.2 Storage conditions and sampling

The lasagna ($n = 81$) was stored in a cold room set at 2 °C for 28 days. The temperature of the cold room was monitored every 30 minutes by a temperature recorder (iLOG, Escort Data Loggers Inc., USA)). For this study, precooked lasagna portions were divided into four groups according to their preparation and packaging methods (Figure 1). Group A ($n = 15$): HSL method and CPET/APET packaging; Group B ($n = 15$): CSL method and CPET/APET packaging; Group C ($n = 15$): HSL method and CELL-PET packaging; Group D ($n = 15$): CSL method and CELL-PET packaging.

During the storage, the analysis of the headspace gas concentration and sampling for the microbiological tests were performed on day 0 and after 7, 14, 21 and 28 days of storage. At time 0, the frozen precooked lasagna sheets, and the Béchamel and Bolognese ($n = 9$) sauces after cooking were analysed separately. At time 28, three samples *per* group ($n = 12$) were analysed after cooking at 180 °C for 30 minutes. The cooking was carried out using a convection oven (Zanussi S.p.A., Italy) set at "dry cooking".

All samples were transported to the laboratory in containers with ice and were analysed the same day.
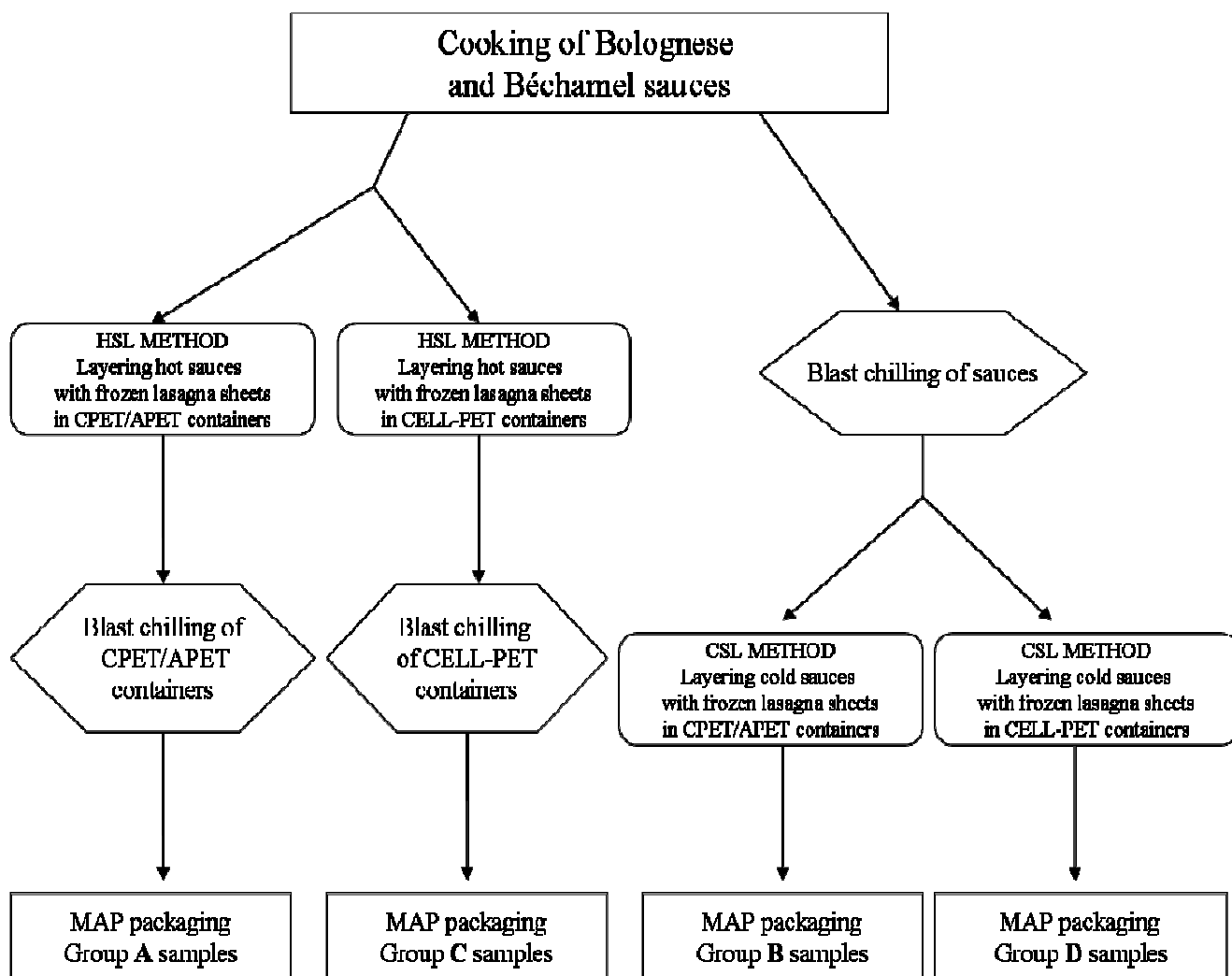
Figure 1: Flow-diagram of lasagna groups preparation.

6.3.3 Headspace gas analysis

Gas concentrations in the headspace of packages (CO_2 and O_2) were determined using an OXYBABY®-M headspace analyzer (WITT GasetechniK GmbH & CO., England).

6.3.4 Microbiological analysis

An analytical unit (10 g) was aseptically taken from each sample unit, added to 90 ml of sterile diluent solution (0.85% NaCl and 0.1% peptone), and homogenized in a stomacher 400 (Colworth, UK) for 1 min at room temperature and then serial 10-fold dilutions were prepared in a sterile saline.

Psychrotrophic aerobic plate counts (PAPC) were determined using a pour plate technique on Plate Count Agar (Oxoid, Basingstoke, UK); plates were incubated at 10 °C for 10 days. This medium was chosen since it is a well known and frequently applied tool for the enumeration of bacteria in food quality control

programs in the food industry (Espe et. al., 2004, Bernardi et al., 2009), and it is the medium indicated by official methods (ISO 17410: 2001).

Mesophilic aerobic plate counts (MAPC) were enumerated using a Petrifilm™ Aerobic Count (3M™, St. Paul, Minnesota, USA), following the AFAQ/AFNOR 3M 01/1-09/89 method.

Petrifilm™ plates were also used to determine total coliforms (TC), *Escherichia coli* (EC), *Staphylococcus aureus* (SA), using the following methods AFNOR 3M 01/2-09/89A, AFAQ/AFNOR 3M 01/08-06/01 and AFNOR 3M 01/9-04/03 respectively.

Lactic acid bacteria (LAB) were enumerated on de Man-Rogosa–Sharpe agar (Oxoid, Basingstoke, UK), at pH 5.5. Plates were incubated at 30 °C for 48 h under anaerobic conditions in an anaerobic jar (Oxoid, Basingstoke, UK) with an AnaeroGen™ sachet oxygen absorber (Oxoid, Basingstoke, UK); *Salmonella* spp. detection was in accordance with ISO 6579:2002 Cor.1:2004.

The detection of *Listeria monocytogenes* were performed in accordance with AFNOR BRD 07/4-09/98 and AOAC No. 060402 2006. For *Bacillus cereus* enumeration, 0.1 mL of each dilution sample was put onto polymixin-piruvate-egg yolk-mannitol-bromothymol blue agar (PEMBA, Oxoid, Basingstoke, UK) with 50,000 IU of polymixin per litre and egg yolk emulsion (Oxoid, Basingstoke, UK). The plates were air dried and incubated at 30 °C for 24 to 48 h (Tessi et al., 2002).

For the isolation and enumeration of spores of sulphite-reducing clostridia (SSC), the first step was the heat treatment of the tubes containing the first decimal dilution in a thermostatically controlled water bath at 80±0.1 °C for exactly 10 min after the temperature reached 80 °C in a control tube, in order to eliminate vegetative cells. In the second step, spores were enumerated by the pour plate method (Swanson et al., 1992) onto tryptose-sulfite-cycloserine (TSC) agar without egg yolk (CM587, Oxoid, Basingstoke, UK). The agar was supplemented with a 1% filter-sterilized D-cycloserine (Oxoid, Basingstoke, UK) solution (4% wt/vol). An amount of 0.1 ml of heated dilution was put onto empty sterile Petri dishes, poured plates were overlaid with a thin layer (5 ml) of freshly prepared TSC agar (supplemented with D-cycloserine as previously described). Upon solidification of the TSC overlay, plates were placed in anaerobic jars (Oxoid, Basingstoke, UK) with an AnaeroGen™ sachet oxygen absorber (Oxoid, Basingstoke, UK) and incubated at 37.0 °C for 48 h. Characteristic black colonies, if present, were enumerated. All analyses were performed in three replications.

6.3.5 Evaluation of microbial safety

Codex Alimentarius Commission guidelines (1997) were followed for evaluating the microbial safety of samples: *L. monocytogenes* (not detected in 25 g), *Salmonella*

spp. (not detected in 25 g), *E. coli* (satisfactory <1.00 $\log_{10}$ CFU/g, unsatisfactory ≥ 1.00 $\log_{10}$ CFU/g), *B. cereus* (satisfactory <2.00 $\log_{10}$ CFU/g, unsatisfactory ≥ 2.00 $\log_{10}$ CFU/g), *S. aureus* (satisfactory <2.00 $\log_{10}$ CFU/g, unsatisfactory ≥ 2.00 $\log_{10}$ CFU/g), spores of sulphite-reducing clostridia (satisfactory <1.00 $\log_{10}$ CFU/g, unsatisfactory ≥ 1.00 $\log_{10}$ CFU/g).

Mesophiles and psychrotrophic aerobic plate counts, total coliforms and lactic acid bacteria provide a general estimation of the total number of microorganisms on produce, and are very helpful in estimating/comparing the microbial quality of each group of samples analysed (Kokkinakis et al., 2007).

In our study we established the following acceptance limits: MAPC/PAPC = 5.00 $\log_{10}$ CFU/g; TC = 3.00 $\log_{10}$ CFU/g and LAB = 9.00 $\log_{10}$ CFU/g.

6.4 Results and discussion

Salmonella spp. and *Listeria monocytogenes* were not detected in any of the samples analysed. *Escherichia coli* was <1.00 $\log_{10}$ CFU/g, *Bacillus cereus* was <2.00 $\log_{10}$ CFU/g, spores of sulphite-reducing clostridia were <1.00 $\log_{10}$ CFU/g in all the samples examined.

Lactic acid bacteria counts were lower than the acceptance limit up to 28 days of storage (mean counts ranged from 2.30 at day zero to 6.98 $\log_{10}$ CFU/g on day 28).

Staphylococcus aureus was found with a frequency of contamination of 37% (mean counts varied from 1.30 to 2.88 $\log_{10}$ CFU/g). In half of the positive samples SA was unsatisfactory (counts were ≥ 2.00 $\log_{10}$ CFU/g), and the unsatisfactory counts were reported in Groups B and D (cold sauce layering) from day 14 (Group B) and from day 28 (Group D), respectively. No difference in contamination was found between CELL-PET and CPET/APET packages.

It is well known that *S. aureus* lives on humans, food equipment, environmental surfaces, and animals, and exists in the nasal passages, throats, hair, and skin of 50% or more of healthy individuals. This bacterium is highly vulnerable to heat treatment and nearly all sanitizing agents (Yoon et al., 2008). For these reasons we can assume that the presence of *S. aureus* may be an indication of the poor efficacy of blast chilling, because the contamination was detected in the groups in which the lasagna had been prepared with the cold sauce layering (CSL) method. Time and temperature parameters set for rapid chilling may have allowed the survival of *S. aureus* in such low quantities that they were not detected at days 0 and 7.

The evolution of microbial populations (MAPC, PAPC, TC, LAB) in the four Groups are shown in Figures 2-5. The trend in the percentage of carbon dioxide measured in the headspace of packages is shown in Figure 6. During the period

of 28 days, the mean temperature in the cold room recorded by the data logger was 2.1 °C. As regards the enumeration of mesophilic (MAPC) and psychrotrophic (PAPC) microorganisms, we found that the counts were the same order of logarithmic magnitude (Fig. 2 and Fig. 3). We can thus hypothesize that almost all the mesophilic bacteria detected had the capacity to grow at refrigeration temperatures.

At day 0, precooking was the most important factor to affect initial bacterial populations, as mean mesophilic and psychrotrophic counts ranged from 2.00 to 3.98 $\log_{10}$ CFU/g and total coliforms counts (Fig. 4) ranged from <1.00 to 2.00 $\log_{10}$ CFU/g. The lowest levels of contamination were found in Groups A and B (CPET/APET packaging with HSL and CSL methods). The results of the tests performed on the three elements of lasagna (on day 0) showed that mean MAPC and PAPC in frozen precooked lasagna sheets ranged from 4.00 to 4.04 $\log_{10}$ CFU/g, in Béchamel sauce they varied from 2.00 to 2.60 $\log_{10}$ CFU/g, and in the Bolognese sauce they were <1.00 $\log_{10}$ CFU/g, and all the other parameters (TC, SA, LAB) were lower than the detection limits.

At day 14, the inhibitory effect of CO_2 was particularly important as the populations reached were low (mean MAPC and PAPC ranged from 2.60 to 4.18 $\log_{10}$ CFU/g, TC counts from <1.00 to 2.38 $\log_{10}$ CFU/g); only in Group D (CELL-PET packaging with CSL method) were mesophilic, psychrotrophic and total coliforms counts higher than the acceptance limits after 14 days of storage.

Our results demonstrate that bacterial growth also occurred in the presence of CO_2 but the beginning of the growth was retarded with higher CO_2 concentrations. These results agree with those reported by Gonzales-Fandos et al. (2000), who studied the evolution of bacteria on cheese packaged under a modified atmosphere. They also agree with Murcia et al. (2003) who studied the extension of the shelf-life of ready-to-eat foods in vacuum or modified atmosphere packaging, and with those reported by Speranza et al. (2009), who studied the microbiological quality of ready-to-cook seafood products under a modified atmosphere.

At day 21, the growth of MAPC, PAPC and TC increased considerably, in particular mesophile and psychrotroph counts exceeded acceptance limits in Groups A, B and D (mean counts varied from 5.53 to 6.96 $\log_{10}$ CFU/g), and total coliforms counts exceeded the limit in Group D (TC count was 3.28 $\log_{10}$ CFU/g). Only in Group C (CELL-PET packaging with HSL method) were counts lower than the acceptance limits after 21 days of storage.

At day 28, the populations detected were higher than the acceptance limits (mean MAPC and PAPC ranged from 6.18 to 8.08 $\log_{10}$ CFU/g, TC counts varied from 3.11 to 4.95 $\log_{10}$ CFU/g). The maximum levels were reached by Groups B and D (both prepared with the CSL method).

These results demonstrate that the CO_2 concentration inhibited the growth of microorganisms as long as a sufficient concentration of dissolved CO_2 was maintained on the surface of the lasagna. As shown in Figure 6, the carbon dioxide percentage decreased rapidly until day 7, then decreased more slowly up to day 28. C and D were the groups in which the CO_2 decreased to the lowest levels, which were those groups in which we used cellulose as a packaging material.

These data indicate that probably the heat sealing of CELL-PET containers with a PET top film was less effective over time compared to the polyethylene containers. The inefficiency of heat-sealing was also demonstrated by the percentage of oxygen (Fig. 7) measured in the headspace. In fact, during the 28 days of storage, in Groups C and D oxygen increased from 0.0 to 18.8%, while in Groups A and B it increased from 0.0 to 2.2%.

The level of oxygen may have inhibited the surface growth of pathogenic anaerobic bacteria, which were not detected in this investigation. No difference in aerobic bacterial counts was found between higher and lower percentage oxygen packages, most likely because the bacterial populations were also able to grow also in an environment with an oxygen deficiency (<1%).

Our results suggest that the carbon dioxide concentration was the most effective in inhibiting aerobic bacterial growth rather than the lack of oxygen.

At day 28, the results on the samples analysed after cooking showed almost a complete destruction of microbial populations, in all the groups, thus demonstrating the effectiveness of cooking time and temperature.

Figure 2: Evolution of Psychrotrophs during storage of Groups: Group A, hot sauce layering method and CPET/APET packaging; Group B, cold sauce layering method and CPET/APET packaging; Group B, hot sauce layering method and CELL-PET packaging; Group D, cold sauce layering method and CELL-PET packaging. The data are the mean of three experiments.

Figure 3: Evolution of Mesophiles during storage of samples: Group A, hot sauce layering method and CPET/APET packaging; Group B, cold sauce layering method and CPET/APET packaging; Group B, hot sauce layering method and CELL-PET packaging; Group D, cold sauce layering method and CELL-PET packaging. The data are the mean of three experiments.
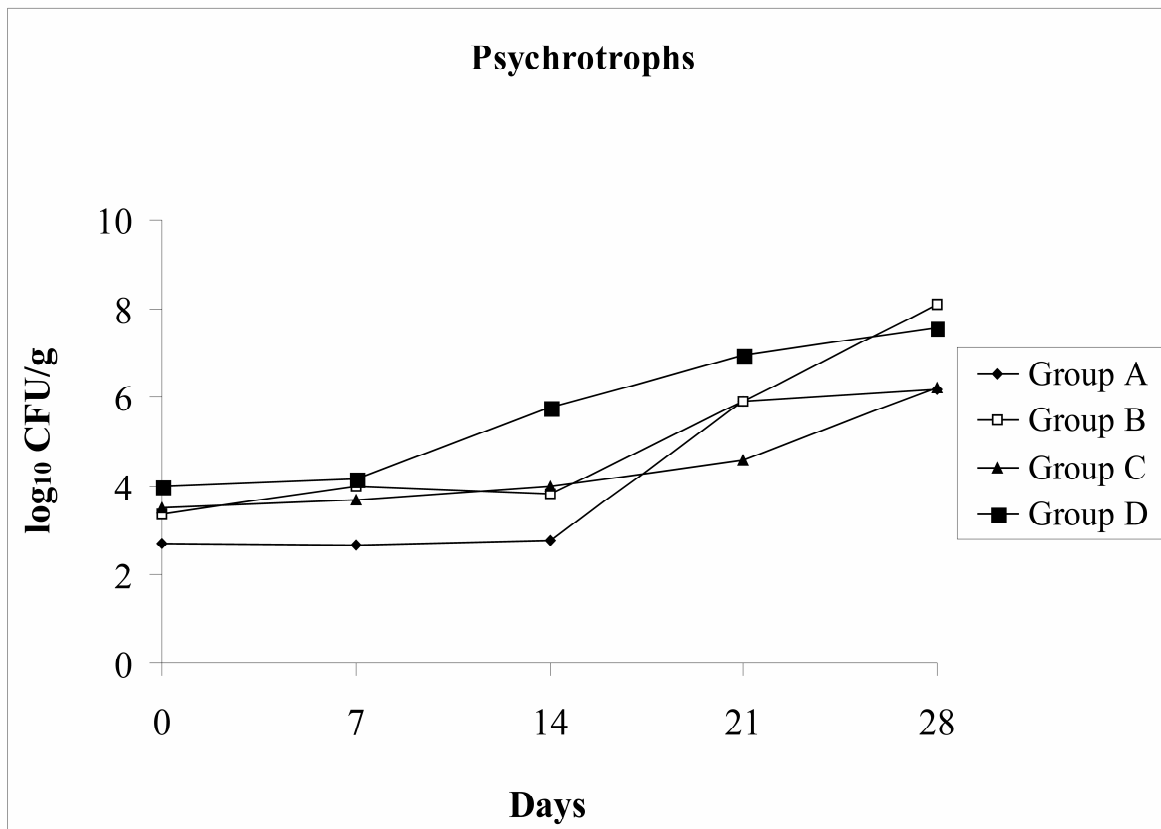
Figure 4: Evolution of Total coliforms during storage of samples: Group A, hot sauce layering method and CPET/APET packaging; Group B, cold sauce layering method and CPET/APET packaging; Group B, hot sauce layering method and CELL-PET packaging; Group D, cold sauce layering method and CELL-PET packaging. The data are the mean of three experiments.
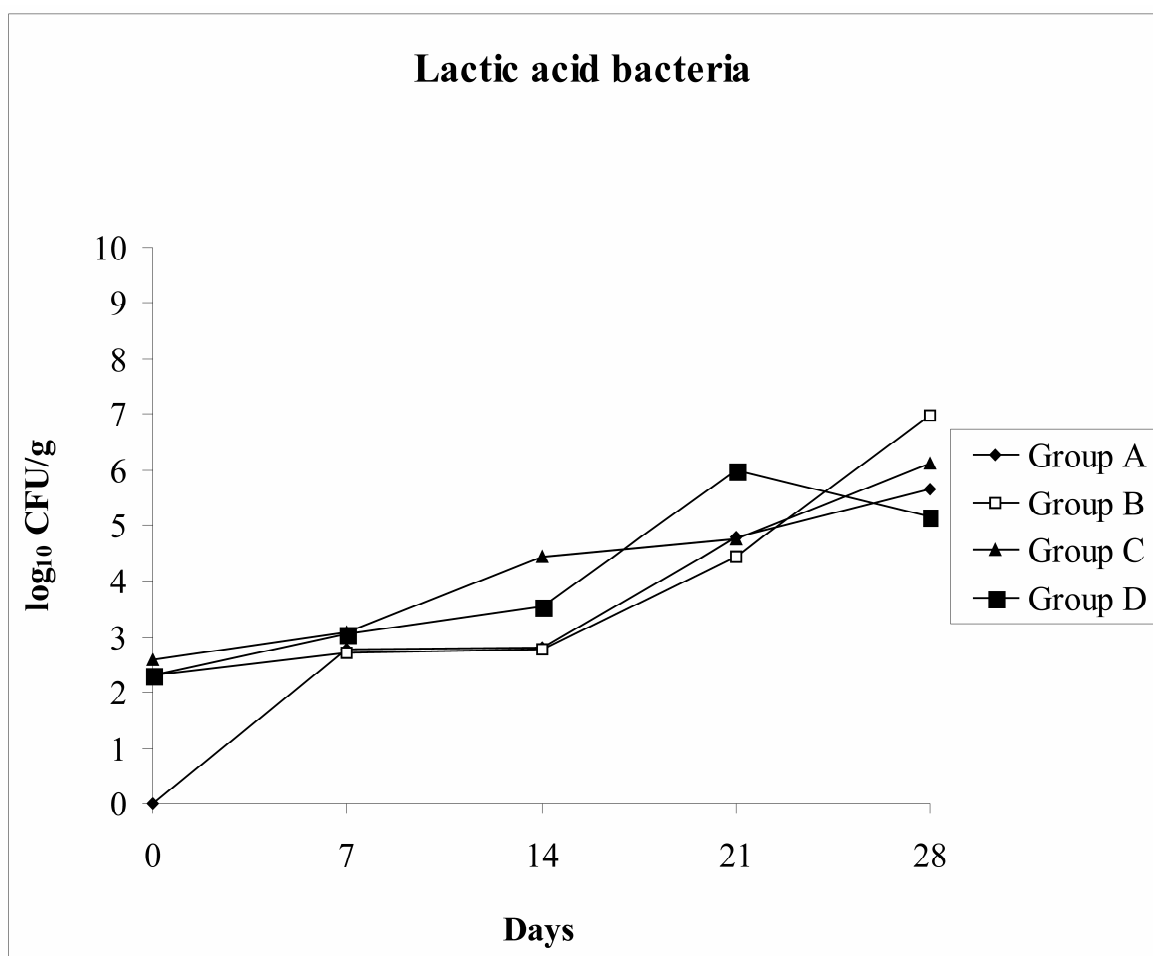
Figure 5: Evolution of Lactic acid bacteria during storage of samples: Group A, hot sauce layering method and CPET/APET packaging; Group B, cold sauce layering method and CPET/APET packaging; Group B, hot sauce layering method and CELL-PET packaging; Group D, cold sauce layering method and CELL-PET packaging. The data are the mean of three experiments.
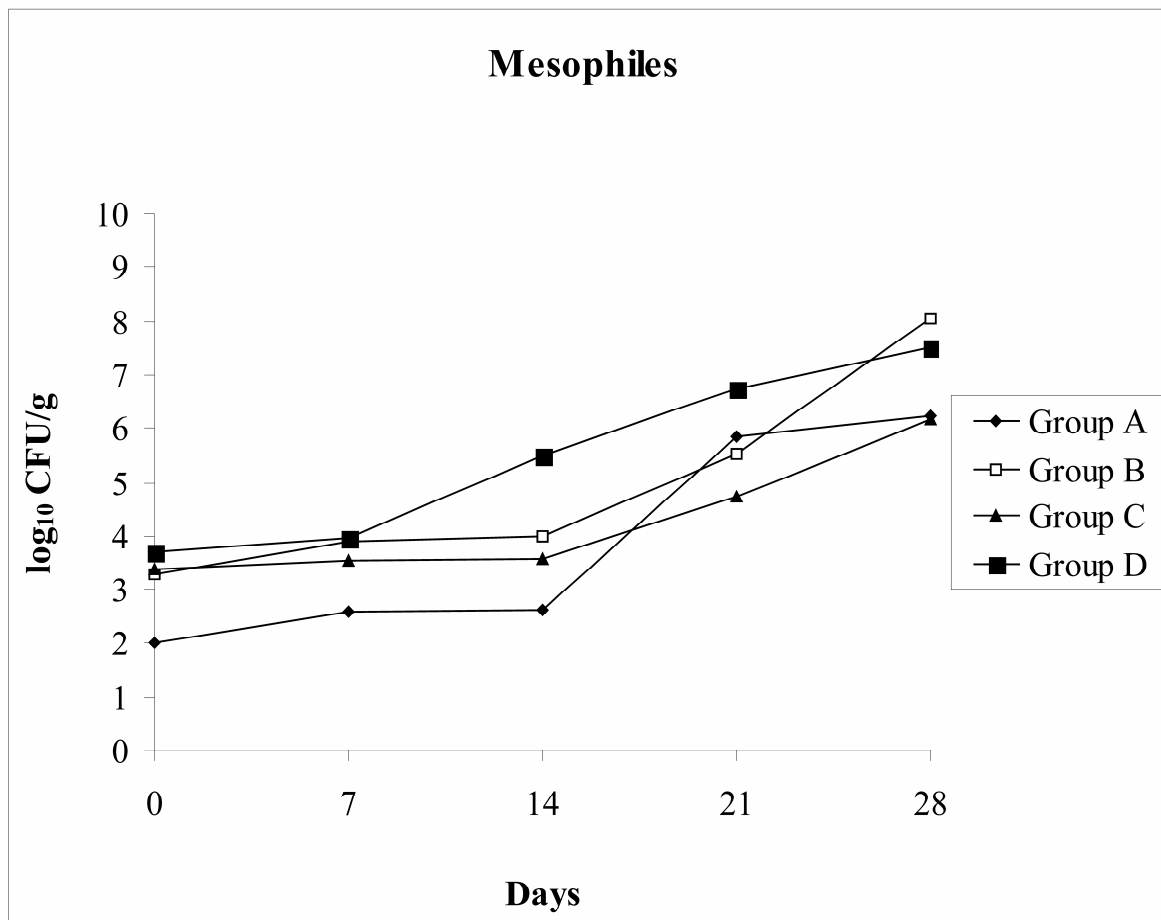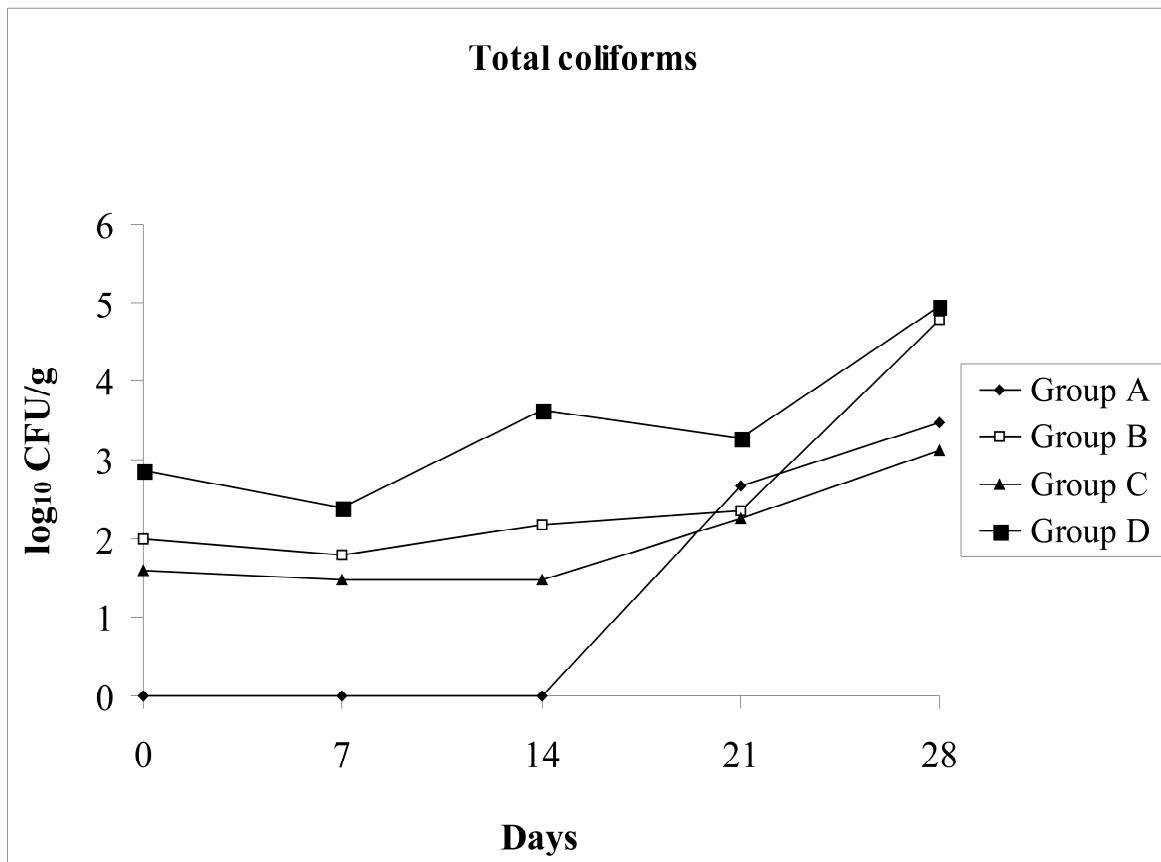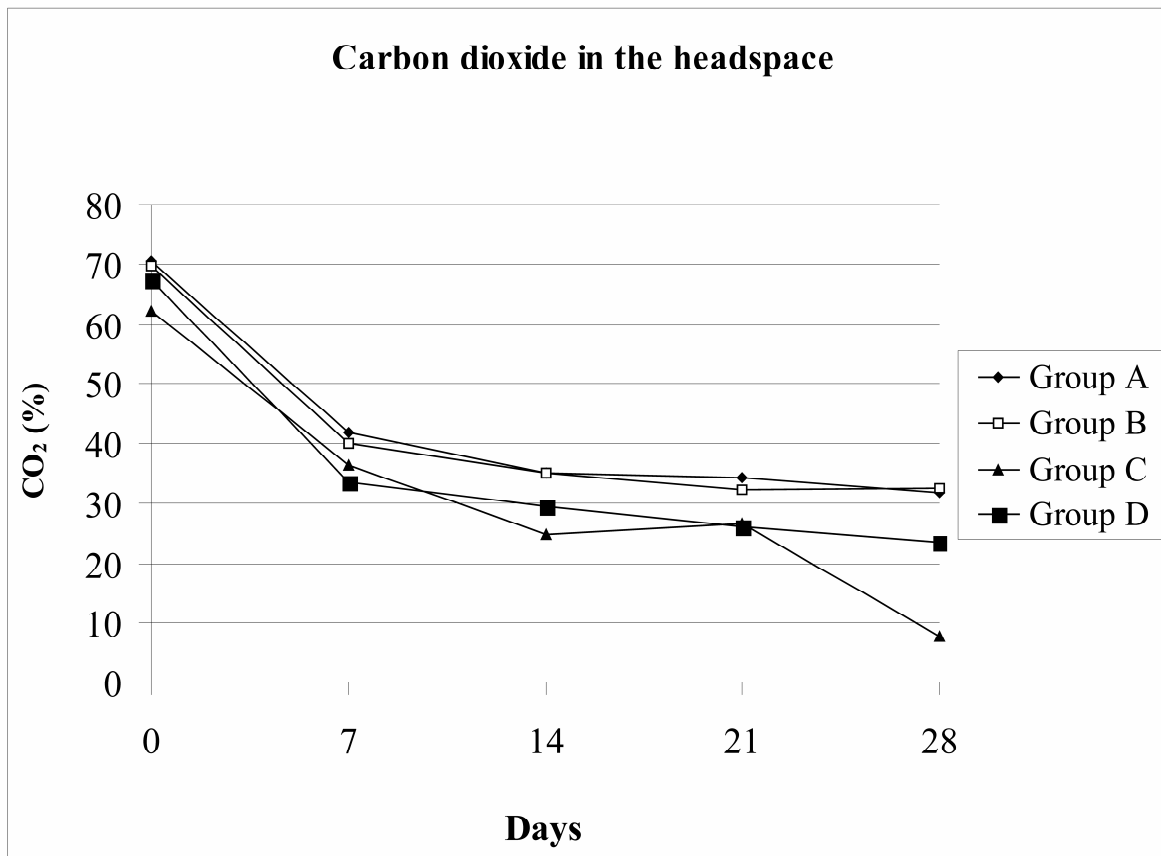
Figure 6: Concentration of CO_2 during storage of lasagna sample Groups: Group A, hot sauce layering method and CPET/APET packaging; Group B, cold sauce layering method and CPET/APET packaging; Group B, hot sauce layering method and CELL-PET packaging; Group D, cold sauce layering method and CELL-PET packaging.
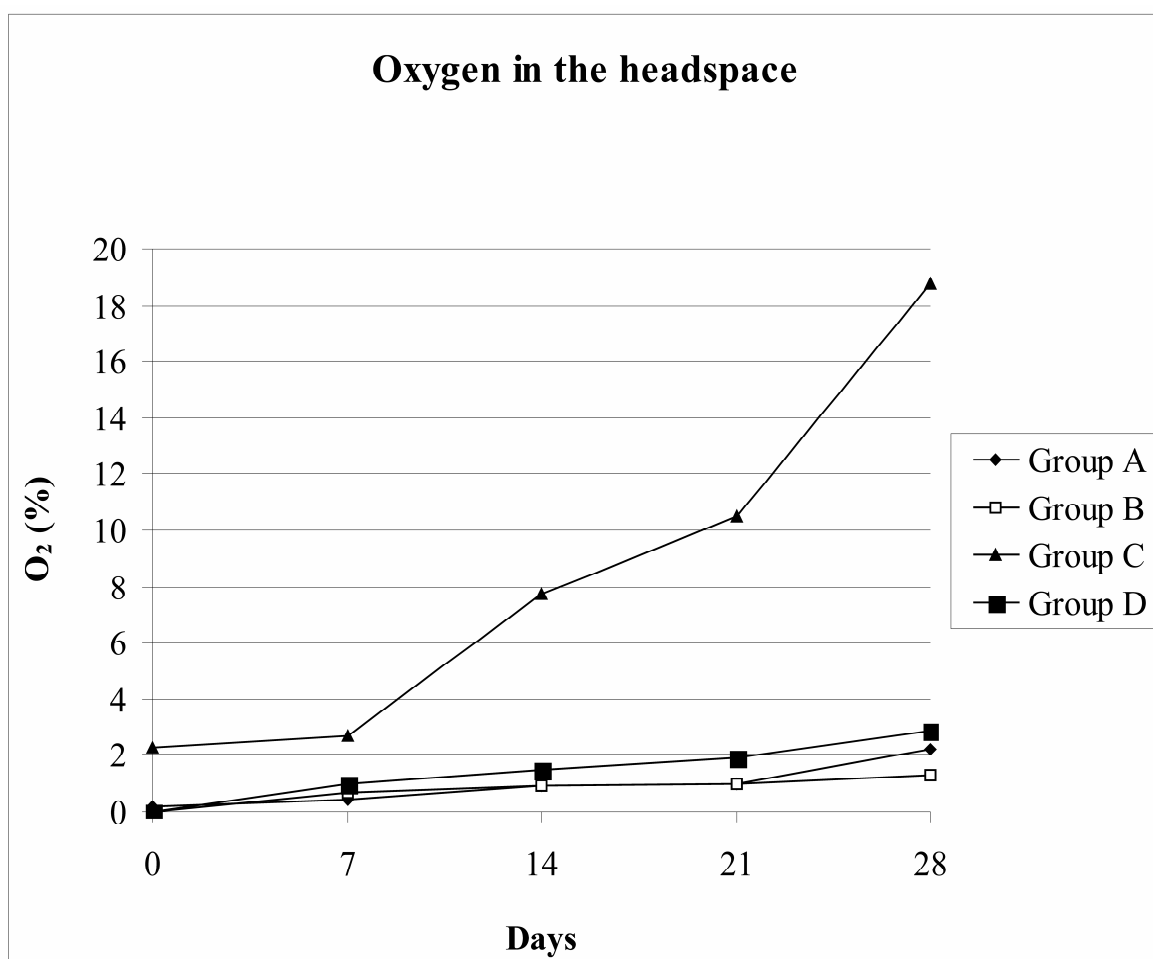
Figure 7: Concentration of O_2 during storage of lasagna sample Groups: Group A, hot sauce layering method and CPET/APET packaging; Group B, cold sauce layering method and CPET/APET packaging; Group B, hot sauce layering method and CELL-PET packaging; Group D, cold sauce layering method and CELL-PET packaging.

6.5 Conclusions

This study investigated the extension of the shelf-life of refrigerated precooked lasagna cooked with two different processes and packaged using two materials, under MAP. The microbiological results suggest that the potential risk of the growth of pathogenic microorganisms is minimal.

The most effective production method was the HSL (hot sauces layering) because microbial counts were lower than the CSL method counts, for up to 28 days of storage.

Both packaging methods (CPET-APET and CELL-PET) ensured a sufficiently long shelf-life for most catering purposes. The only difference worth noting was that cellulose had higher values of oxygen in the headspace, which could affect

its future use in catering due to the greater probability of aerobic bacterial growth.

In conclusion, the HSL method associated with CPET-APET packaging in MAP was the most effective solution for prolonging the shelf-life of lasagna for up to 21 days.

The results obtained could be useful to implement new technological processes within the traditional Italian foodservice industries, which currently, due to the large number of consumers to be served, acquire ready-to-cook lasagna from outside suppliers in order to optimize time and labour.

We think that there are several advantages in the application of the production technology analysed in this study: a) increase of the level of food safety, more efficient controls of microbiological hazards; b) greater flexibility in menus; c) the possibility of producing onsite recipes for ethnic and religious minorities (for example using poultry meat instead of beef); d) re-introduction of simple processes in order to enhance internal production, thus enabling users to test and taste the quality of raw materials.

6.6 References

AOAC International The Scientific Association Dedicated to Analytical Excellence® (2010). Validation Certificate No.: 060402. *Microgen Listeria-ID Kit for confirming the presence of Listeria monocytogenes.*

Association Française De Normalisation (AFNOR) (2010). Validation Certificate N.: 3M-01/2-09/89A. 3M™ Petrifilm™ Coliform Count Plate. Application for the Enumeration of Coliform Colonies With and Without Gas (as compared to ISO 4832 VRBL method).

Association Française De Normalisation (AFNOR) (2010). Validation Certificate N.: BRD 07/4-09/98. Application for detection of Listeria Monocytogenes (as compared to NF EN ISO 11290-1 including the amendment A1 (2004) method).

Association Française De Normalisation (AFNOR) (2010). Validation Certificate N.: 3M-01/9-04/03. 3M™ Petrifilm™ Staph Express Count System. Application for the Enumeration of Coagulase Positive Staphylococci (as compared to EN ISO 6888-1 method).

Association Française pour l'Assurance de la Qualité (AFAQ) et **Association Française De Normalisation (AFNOR)** (2010). Validation

Certificate N.: 3M-01/1-09/89. 3M™ Petrifilm™ Aerobic Count Plates. Application for the Enumeration of Total Viable Count (as compared to ISO 4833 method).

Association Française pour l'Assurance de la Qualité (AFAQ) et **Association Française De Normalisation (AFNOR)** (2010). Validation Certificate N.: 3M-01/08-06/01. 3M™ Petrifilm™ Select *E. coli* Count Plate. Application for the Enumeration of *Escherichia coli* (as compared to ISO 16649-2 *E. coli* method).

Bernardi C., Ripamonti B., Campagnoli A., Stella S., Cattaneo P. (2009). Shelf-life of vacuum packed Alaskan, Scottish and Norwegian cold-smoked salmon available on the Italian market. *International Journal of Food Science & Technology*, 44, 2538-2546.

Codex Alimentarius Commission (1997). Joint FAO/WHO Food Standards Programme, Codes Committee on Food Hygiene. Food Hygiene, Supplement to Volume JR-1997. *Principles for the Establishment and Application of Microbiological Criteria For Foods. CAC/GL 21-1997*. Secretariat of the Joint FAO/WHO Food Standards Programme. Rome: Food and Agriculture Organization of the United Nations.

Conferenza Permanente Stato-Regioni (2010). *Linee di indirizzo nazionale per la ristorazione scolastica.* Rome, Italy, 29 April 2010. Available from: http://www.statoregioni.it/Documenti/DOC_026408_2CU.pdf

Direzione Generale Sanità e Politiche sociali, Regione Emilia-Romagna (2009). *Linee strategiche per la ristorazione scolastica in Emilia-Romagna.* Bologna, Italy. Available from: http://www.saluter.it/wcm/saluter/pubblicazioni/tutte_le_pubblicazioni/contributi/contributi.htm

Dragoni I.G., Vallone L., Balzaretti C.M. (2005). Alunni in cattedra: indagine sulle metodologie di rilevazione della Customer Satisfaction nella ristorazione scolastica. *Ristorando*, 5(2005), 54-56.

Espe M., Kiessling A., Lunestad B.T., Torrissen O.J., Bencze Røra A.M. (2004). Quality of cold-smoked salmon collected in one French hypermarket during a period of 1 year. *Lebensmittel-Wissenschaft und – Technologie*, 37, 617-638.

Genghi L., Ferrini R., Vai L., Dionisi A.M., Filatici E., Scalfaro C., Luzzi I. (2004). *Salmonella thompson: episodio epidemico in una mensa scolastica.* In: Caprioli A., Luzzi I., Lana S., ed. 4th Workshop nazionale Enternet Italia Sistema di sorveglianza delle infezioni enteriche. Diagnostica ed epidemiologia delle zoonosi trasmesse da alimenti. Istituto Superiore di Sanità, 2004, Rome, Italy, p. 26.

Gonzales-Fandos E., Sanz S., Olarte C. (2000). Microbiological, physicochemical and sensory characteristics of Cameros cheese packaged under modified atmospheres. *Food Microbiology*, 17, 407-414.

Green L.R., Selman C.A., Radke D., Mack J.C., Reimann D.W., Stigger T., Motsinger M., Bushnell L. (2006). Food worker hand washing practices: an observation study. *Journal of Food Protection*, 69, 2417-2423.

Guzewich J., Ross M.P. (1999). Evaluation of risks related to microbiological contamination of ready-to-eat food by food preparation workers and the effectiveness of interventions to minimize those risks. Available from: http://www.cfsan.fda.gov/~ear/rterisk.html.

Ha K.S., Park S.J., Shim W.-B., Chung D.-H. (2003). Screening of MRSA (methicilline resistant *Staphylococcus aureus*) and *seb* gene in producing strains isolated from food service environment of elementary schools. *Journal of Food Hygiene and Safety*, 18, 79-86.

Hotchkiss J.H. (1988). Experimental approaches to determining the safety of food packaged in modified atmospheres. *Food Technology*, 42, 55-64.

International Organization for Standardization (ISO 17410:2001) (2001). *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of psychrotrophic microorganisms.* Geneva: International Organization for Standardization.

International Organization for Standardization (ISO 6579:2002 Cor.1:2004) (2004). *Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Salmonella spp..* Geneva: International Organization for Standardization.

Jay J.M. (1992). Microbiological Food Safety. *Critical Reviews in Food Science and Nutrition*, 31, 177-190.

Kokkinakis E., Boskou G., Fragkiadakis G.A., Kokkinaki A., Lapidakis N. (2007). Microbiological quality of tomatoes and peppers produced under the good agricultural practices protocol AGRO 2-1 & 2-2 in Crete, Greece. *Food Control*, 18(2007), 1538-1546.

Michino H., Otsuki K. (2000). Risk factors in causing outbreaks of foodborne illness originating in school lunch facilities in Japan. *Journal of Veterinary Medicine Science*, 62, 557-560.

Murcia M.A., Martinez-Tomè M., Nicolàs M.C., Vera A.M. (2003). Extending the shelf-life and proximate composition stability of ready to eat foods in vacuum or modified atmosphere packaging. *Food Microbiology*, 20, 671-679.

Nyati H. (2000). An evaluation of the effect of storage and processing temperatures on the microbiological status of sous vide extended shelf-life products. *Food Control*, 11(2000), 471-476.

Patsias A., Chouliara I., Badeka A., Savvaidis I.N., Kontominas M.G. (2003). Shelf-life of a chilled precooked chicken product stored in air and under modified atmospheres: microbiological, chemical, sensory attributes. *Food Microbiology*, 23(2006), 423-429.

Paulson D. (2000). Hand washing, gloving, and disease transmission by the food preparer. *Dairy Food and Environmental Sanitation*, 20, 838-845.

Phillips C.A. (1996). Review: Modified Atmosphere Packaging and its effects on the microbiological quality and safety of produce. *International Journal of Food Science and Technology*, 31, 463-479.

Regulation (EC) No. 852/2004 of the European Parliament and the Council of 29 April 2004 on the hygiene of foodstuffs. Official Journal of the European Union L 139, pp. 1-54.

Santana Nadja G., Almeida Rogeria C.C., Ferreira Jeane S., Almeida Paulo F. (2009). Microbiological quality and safety of meals served to children and adoption of good manufacturing practices in public school catering in Brazil. *Food Control*, 20(2009) 255-261.

Speranza B., Corbo M.R., Conte A., Sinigaglia M., De Nobile M.A. (2009). Microbiological and sensorial quality assessment of ready-to-cook seafood

products packaged under modified atmosphere. *Journal of Food Science*, 74(9), M473-M478.

Stiles M.E. (1991). Scientific principles of controlled/modified atmosphere packaging. In: Ooraikul B., Stiles M.E., (Eds), Modified Atmosphere Packaging of Food. Ellis Horwood Ltd., England, pp. 18-15 and 118-147.

Swanson K.M.J., Busta F.F., Peterson E.H., Johnson M.G. (1992). *Colony count methods*. In: Vanderzant C., Splittstoesser D.F. (Eds.), Compendium of methods for the microbial examination of foods. American Public Health Association, Washington D.C., 75-95.

Tessi M.A., Arìngoli E.E., Pirovani M.E., Vincenzini A.Z., Sabbag N.G., Costa S.C., Garcìa C.C., Zannier M.S., Silva E.R., Moguilevsky M.A. (2002). Microbiological quality and safety of ready-to-eat cooked foods from a centralized school kitchen in Argentina. *Journal of Food Protection*, 65(4), 636-642.

Veiros M.B., Proença R.P.C., Santos M.C.T., Kent-Smith L., Rocha A. (2009). Food safety practices in a Portuguese canteen. *Food Control*, 20(2009) 936-941.

[USDHHS-FDA-CFSAN] U.S. Dept. of Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition (2000). Report of the FDA retail food program database of foodborne illness risk factors. Available from: http://vm.cfsan.fda.gov/~acrobat/retrsk.pdf

World Health Organization (WHO) (2007). *General information related to foodborne disease*. Available from: http://www.who.int/foodsafety/foodborne_disease/general/en/index.html

CHAPTER 7

General Discussion

7. General discussion

All the studies have pursued the unique goal of identifying strengths and weaknesses of conventional and innovative catering systems, in order to draw the lines of future research activity and to provide a scientific basis to the caterers and to the institutions that oversee the food safety. Below the most relevant findings of the four works are described.

7.1 Cook-serve method in mass catering establishments: is it still appropriate to ensure a high level of microbiological quality and safety?

This study investigated the food safety of three Italian catering establishments, serving from 800 to 1700 meals a day, using the traditional cook-serve method for all the preparations. On the basis of our findings, it was concluded that the microbiological quality and safety of Group A (cooked food ready for consumption) were satisfactory.

On the other hand, the detection of the presence of *Listeria monocytogenes* in soft cheeses, and the heavy contamination by aerobic plate counts and total coliforms in Groups B (multi-ingredient preparations cooked and uncooked, C (soft cheeses), and D (raw vegetables ready for consumption) showed that a better application of HACCP principles is needed.

The most important drawback of the traditional cook and serve system is the very short period of time between the meal preparation and the service to customers. The limited number of hours required to prepare thousands of meals a day induces food operators to do some preparations early in the morning, especially raw vegetables, which could increase bacterial growth.

In order to ensure a higher level of microbiological safety, some changes in the timing of food preparation and holding temperatures need to be made. Regarding the hygienic education of staff, the results from the microbial assessment showed a high level of hygienic safety on the cleaned food contact surfaces and on washed hands, which is an important prerequisite to prevent cross-contamination.

The microbial characteristics of the water samples analyzed showed that in order to protect human health, food operators should increase the frequency of the sanitisation procedures both of the microfiltered water dispensing systems and the washing areas in the catering establishments.

7.2 Food safety in commercial catering

The results are reassuring regarding the absence of pathogenic microorganisms in both the years 2006 and 2007.
In 2006, non-compliant samples amounted to 55% , while in 2007 this value fell to 30%. It is to be noticed that the decrease of non-compliances was generally found in cooked preparations, whereas the percentages remained unchanged in raw preparations. An improvement is observed, especially regarded the preparation of cooked meat and vegetables as well as cooked seafood.
The reasons for this result could be that operators in the food sector have become more sensitized to the risks linked to these food preparations, and that consultants' suggestions and corrective measures have been more easily adopted within the establishment of production by adopting workflows with suitable. timing and temperature conditions.
Changes in the commercial catering sector and the adoption of production systems that differ from traditional ones (where the time lag between cooking and serving is relatively short) expose consumers to greater risk. The results of this survey are clear and encouraging, demonstrating that operators of small restaurants have felt the need to be supported by external professionals to monitor their production chain and levels of hygiene, thereby improving the food safety of their catering activity.

7.3 Monitoring the bacteriological quality of Italian bottled spring water from dispensers

The spring water analyzed in this study was found to be inappropriate for human consumption as defined by law, since 85% of the water samples were found to exceed the limits for HPC and 57.5% were positive for *P. aeruginosa.*
The most considerable result is the high contamination frequency with *P. aeruginosa.* This bacterium is known to be an important agent of nosocomial infections, with multidrug-resistant strains in hospitals being of particular concern. Because elderly and immunocompromised people are at greatest risk of infection, drinking water in hospitals and institutions should contain as few *P. aeruginosa* as possible.
The *P. aeruginosa* isolates recovered in samples coming from bottles indicates that they likely originated from the water-production plant. This bacterial contamination could be due to the survival of microorganisms even after washing and refilling of PC bottles back to the bottling plants. Alternatively, the bacteria may have survived in the water in quantities that were not found in the first analysis, but were then able to reproduce since they were protected by

biofilm. The current procedure for dispensers cleaning was not effective, as can be confirmed by comparing the results obtained in the fourth experiment. The results of our study show that in order to protect human health, the following concrete actions are recommended: microfiltration or pasteurization in order to eliminate unwanted bacteria, such as *P. aeruginosa*; accurate environmental and water microbiological controls at critical points in the bottling plants; the study of new dispenser sanitizing procedures; an increase in the frequency of sanitations.

7.4 Microbiological quality and safety of refrigerated precooked lasagna packaged under modified atmosphere for Italian school catering

This study investigated the extension of the shelf-life of refrigerated precooked lasagna cooked with two different processes and packaged using two materials, under modified atmosphere. The microbiological results suggest that the potential risk of the growth of pathogenic microorganisms is minimal.

The most effective production method was the "hot sauces layering" because microbial counts were lower than the "cold sauces layering" method counts, for up to 28 days of storage.

Both packaging methods (CPET-APET and CELL-PET) ensured a sufficiently long shelf-life for most catering purposes. The only difference worth noting was that cellulose had higher values of oxygen in the headspace, which could affect its future use in catering due to the greater probability of aerobic bacterial growth.

In conclusion, the HSL method associated with CPET-APET packaging in MAP was the most effective solution for prolonging the shelf-life of lasagna for up to 21 days.

The results obtained could be useful to implement new technological processes within the traditional Italian foodservice industries, which currently, due to the large number of consumers to be served, acquire ready-to-cook lasagna from outside suppliers in order to optimize time and labour.

We think that there are several advantages in the application of the production technology analysed in this study: a) increase of the level of food safety, more efficient controls of microbiological hazards; b) greater flexibility in menus; c) the possibility of producing onsite recipes for ethnic and religious minorities (for example using poultry meat instead of beef); d) re-introduction of simple processes in order to enhance internal production, thus enabling users to test and taste the quality of raw materials.

CHAPTER 8

Summary

8. Summary

Cook-serve method in mass catering establishments: is it still appropriate to ensure a high level of microbiological quality and safety?

AUTHORS: M. A. Marzano, C. M. Balzaretti.
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Abstract
The purpose of this study was to evaluate the hygienic quality of three traditional "cook-serve" catering establishments in Lombardy, Italy (with an output ranging from 800 to 1700 meals a day), by monitoring the microbiological quality and safety of foods ready for consumption ($n = 727$), tap water ($n = 32$), microfiltered drinking water ($n = 28$), food contact surfaces (n = 280), and food handlers ($n = 76$). The food contact surfaces showed unacceptable contamination in 17.1% of samples. The hygienic level of the washed hands of food workers was very high, since only 1.3% of samples showed unacceptable contamination. Food sample analyses highlighted a percentage of samples that did not conform to microbial reference standards: the presence of *Listeria monocytogenes* was found in 11.5% of "soft cheese" samples. *Staphylococcus aureus* non-conforming percentages ranged from 2.2% for "first and second courses" to 34.6% for "soft cheeses"; *Escherichia coli*, from 2.7% for "raw vegetables" to 7.7% for "soft cheeses"; total coliforms from 6.7% for "first and second courses" to 76.4% for "raw vegetables". The results of the water analysis indicated a high frequency of contamination with *Pseudomonas aeruginosa* (21.4% for microfiltered drinking water and 21.9% for tap water samples). In conclusion, the results suggest that more effort should be made in the application of HACCP principles. In addition in order to ensure a higher level of microbiological safety of meals, various changes in the timing of food preparation and holding temperatures are needed.

Food safety in commercial catering

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Abstract

The catering industry plays a very important role in public health management, because about 30% of total daily meals are consumed in catering industry establishments (restaurants, cafeterias *bars*). In this context, food safety was evaluated in 20 catering centres by means of microbiological analyses of different categories of meals. Results demonstrate that there was a significant decrease in microbial contamination between 2006 and 2007; no pathogens were found in 217 samples examined; this result was obtained by improving voluntary controls.

Monitoring the bacteriological quality of Italian bottled spring water from dispensers

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Abstract

The bacteriological quality of unprocessed bottled spring water was evaluated, through enumeration of heterotrophic plate count at 22 and 37 °C, total coliforms, *Escherichia coli*, intestinal enterococci and *Pseudomonas aeruginosa*. The aims of the research were to monitor the hygiene of bottled spring water and to evaluate the effectiveness of the dispenser sanitation procedure, reproducing field conditions. In total, 120 water samples were analyzed. The results indicated a high contamination frequency with *P. aeruginosa* (57.5%) and therefore the need to refine hygiene in the bottling plant and in the dispenser sanitation procedures.

Microbiological quality and safety of refrigerated precooked lasagna packaged under modified atmosphere for Italian school catering

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Abstract

The aim of this study was to evaluate the microbiological quality and safety of refrigerated precooked lasagna prepared in a traditional "cook-serve" centralized kitchen for up to 28 days of storage. Two preparation methods (hot and cold

sauce layering) and two materials (polyethylene and cellulose) for packaging under a modified atmosphere were tested. Microbiological analyses focused on pathogenic and potential-pathogenic microorganism markers (*Salmonella* spp., *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, and spores of sulphite-reducing clostridia), and on the evolution of spoilage-hygiene markers: mesophilic and psychrotrophic aerobic bacteria, total coliforms and lactic acid bacteria. In total, 81 samples were analysed at intervals of 7 days. No pathogenic microorganisms were detected in all the samples examined for up to 28 days of storage. 37% of samples were positive for *S. aureus*. After cooking all the samples were in a situation of conformity according to the limits established. In conclusion, the hot sauce layering method associated with polyethylene packaging resulted the most effective productive solution for prolonging the shelf-life of packaged precooked lasagna for up to 21 days.

CHAPTER 9

Acknowledgements

9. Acknowledgements