

Commercial nonindustrial production of pastries and ice cream in Naples, Italy: results from the inspection of 34 food businesses during a 2-year surveillance study

Lucia Tanucci Nannini¹, Antonio Pucino¹, Daniela Tufi², Maria Rosaria Martorelli¹, Maria Paola Vairano¹, Antonietta Della Rossa¹, Paola D'Agnese¹, Emilio Lemetre¹, Giuseppina Amispergh¹

¹Food Hygiene and Nutrition Service, Department of Prevention, Local Health Unit Naples1-center, ASL NA1Centro, Italy; ²Section of Clinical Medicine and Public Health, Department of Experimental Medicine, "Sapienza" University of Rome, Italy

Correspondence to: Antonio Pucino, Servizio Igiene degli Alimenti e della Nutrizione, Dipartimento di Prevenzione ASL NA1 Centro. Via S. Domenico, 17, 80131 Napoli (Italy). E-mail: antonio.pucino@inwind.it

Abstract

Background: Ice cream and cream-based pastries are an excellent media for the growth of pathogenic microorganisms. Foods that are prepared without due respect to the rules of food hygiene can carry microorganisms and/or toxins and could be responsible of toxinfections. The main objective of this study was to obtain an overall picture of the hygiene/sanitation conditions found in businesses that produce hand-made pastries and/or ice cream in Naples, Italy.

Methods: We inspected 34 businesses to assess the following aspects: hygiene/sanitation conditions of the work area and equipment, implementation of good food hygiene principles, evaluation of HACCP plans, licensing/authorization, quality control and sampling protocols, as well as systems for ensuring food traceability. In 28 of the businesses, samples (environment, foods, workers) were collected for microbiological analysis.

Results: Sanctions were issued for nonconformities in 8 businesses (23.5%), and 25 businesses (73.5%) received warnings with orders to correct minor nonconformities (inadequate documentation of compliance with current regulations, incorrect implementation of the quality-control protocol) within specified time periods. Microbiological analyses revealed irregularities in 24/28 businesses (85.7%), and 138 of the 280 samples collected displayed contamination levels exceeding the limits adopted for this study (49.3%). In particular, 80% of the surfaces sampled and 23.8% of the hand swabs collected were shown to be contaminated. All food samples collected met the process-hygiene and food-safety standards prescribed by the European Community. Results obtained were statistically significant (p < 0.05).

Conclusions: Our experience of food safety surveillance system indicates that Neapolitan food business operators involved in the production of hand-made ice cream or pastries do not fully understand the importance of the general preventive measures such as Good Hygienic Practices, Good Manufacturing Practice and Hazard Analysis Critical Control Point Codex requirements for providing consumers with safe, high-quality food products.

Key words: Ice cream, pastries, food safety

Introduction

Recent decades have witnessed remarkable advances in food production and preservation techniques, but food-borne diseases (FBDs) continue to cause substantial morbidity in industrialized nations [1]. Each year in the United States, FBDs cause approximately 76 million episodes of illness, 325,000 hospitalizations, and 5,200 deaths [2]. In 2007, a total of 2,161 epidemic

cases of food-related infection were reported in Italy [3], while in the European Union, there were 5,609 epidemics of FBD that involved almost 40,000 individuals and caused 19 deaths [4].

Most FBDs are caused by nonindustrial or homemade food products [5]. A study conducted by WHO in Europe showed that 25% of the FBD epidemics could be traced to recontamination of foodstuffs resulting from inadequate hygiene

conditions (1.6%), cross-contamination (3.6%), food storage in inappropriate settings (4.2%), use of contaminated utensils (5.7%), or contamination by food-handlers (9.2%) [6].

Ice cream and cream-based pastries are rich in fats and proteins, which represent an optimum medium for the growth of microorganisms, including potential pathogens. Products of this type produced in nonindustrial settings are particularly susceptible to contamination during production and distribution, which can lead to infections among consumers [7,8].

In addition, ice cream is often consumed by children and infants, as well as by pregnant women, elderly persons, and individuals with compromised immunity, all of whom are particularly susceptible to food-borne disease. *Listeria monocytogenes* and *Salmonella* spp. display enhanced pathogenicity in immunocompromised, and the presence of AIDS increases the risk of *Listeria monocytogenes* infection by approximately 300-fold [9].

These data highlight the importance of close, active surveillance of all phases of nonindustrial food production. As specified in Regulation (EC) 852/2004 [10], the primary responsibility for this surveillance lies with the owner of the food business, referred to as the "food business operator" (FBO). This regulation [10] states that FBOs shall be responsible for implementing a set of procedures for monitoring and improving production processes known as the Hazard Analysis Critical Control Point (HACCP) system. According to Regulation (EC) 178/2002 [11], the FBO is also responsible for setting up and implementing a system that ensures the traceability of each food product and for establishing a system for sampling ready-to-eat to ensure that they meet the microbiological standards prescribed in EC Regulations 2073/2005 and 1441/2007 [12, 13].

We report the results of a 2-year public health surveillance study conducted by the staff of the Food Hygiene and Nutrition Service (FHNS) of Local Health Unit Naples 1-Center in Naples, which is in the Campania Region in southern Italy. The focus of this study was the commercial production of hand-made ice cream and fresh pastries in the city of Naples and its conformity with the provisions of Regulation (EC) 882/2004 [14]. The aims of the study were: 1) to obtain an overall view of the structural/hygiene/public health characteristics of workplaces in the city where ice cream and pastries are made; 2) to verify the presence and application in these workplaces of a quality-control surveillance

protocol; 3) to identify possible sources of infection and means of contamination in the food-production chain; 4) to determine whether sanification procedures are being carried out correctly by the workers; 5) to determine whether the finished, ready-to-eat food products sold by these business meet current microbiological standards.

Methods

During the 2 years of surveillance (2007-2008), FHNS staff carried out 34 inspections in 34 businesses involved in the production of handmade ice cream and fresh pastry products sold in the city of Naples.

Each inspection included assessments of the hygienic/sanitary condition of the work areas; the check of the application of appropriate principles of hygiene during the preparation, sale, and storage of foodstuffs; personal hygiene of the workers; the presence at the work-site of complete documentation regarding: licensure and registration, evaluation of HACCP plans, records of Critical Control Point (CCP) monitoring activity, implementation of the protocol for collecting environmental and food samples for testing, implementation of EC Regulation 178/2002 [11] on the traceability of foodstuffs, and the presence of certification of staff training in compliance with the standards prescribed by Executive Decree No. 46/2005 of the Campania Region [15].

In addition, in the course of the years 2007-2008, 280 samples were collected from 28 of the 34 businesses inspected and analysed. They included 28 specimens of ready-to-eat foods (ice cream and fresh pastry products), 28 samples of indoor air from production areas, 140 swabs of surfaces that had just been sanitized (equipment, work counters, utensils, walls), and 84 swabs taken from staff members (clothes or uniforms [n=28], hands during work activities [n=28], hands that had just been washed [n=28]).

Sample collection

Indoor air

Indoor air samples were collected from production areas during normal work hours. A Surface Air System (SAS Super 180 - International PBI S.p.A., Milan, Italy) sampler equipped with a RODAC (Replicate Organism Detecting and Counting) plate (diameter 55 mm) containing specific agar-based growth medium for determining the total mesophilic bacteria counts and total fungal counts was used. Each sample consisted of 500 liters of air. Results were expressed in colony-forming units (cfu) per cubic



meter. Results were interpreted according to the criteria shown in Table 1 [16].

Table 1. Microbial loads in air and corresponding air quality ratings.

TOTAL MICROBIAL COUNT (CFU/ m³)	POLLUTION LEVEL		
< 50	VERY LOW		
50 – 100	LOW		
101 – 500	MODERATE		
501 – 2000	HIGH		
> 2000	VERY HIGH		

Source: Maroni et al., 1993 [16]

Surfaces

For each commercial food-production site, samples were taken from 5 surfaces (work counters, equipment, utensils, and walls) that had just been sanitized. RODAC contact plates (diameter 55 mm) were placed in direct contact with the sampling area, which measured 24 cm². For each sample collected, we determined the total mesophilic bacteria count and the total fungal count expressed in CFU/24 cm². The criteria used to evaluate the results are shown in Table 2 [17].

Table 2. Level of contamination on sanitized surfaces.

TOTAL MICROBIAL COUNT (CFU/24 cm²)	SURFACE CLASSIFICATION GOOD ACCEPTABLE	
≤ 25		
26 – 50		
> 50	NOT ACCEPTABLE	

Source: Nota applicativa International Pbi S.p.A., 2007 [17]

Each site sampled was also analyzed for the presence/absence of the following microbes: *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus*, Enterobacteriacee, and enterococci. As far the presence/absence of these bacteria is concerned, a sample was considered as "not acceptable" only if one of these microorganism was present, even if the total microbial count was <50 CFU/24 cm², because the swabs were performed on surfaces that had just been sanitized. For this analysis, the surface was

sampled with a sterile swab dipped in sterile normal saline (0.9% NaCl) and passed over an area measuring 100 cm².

Workers

The clothes worn by the workers during food production activities were sampled with sterile swabs as described above for surface sampling. Samples were analyzed for the presence/absence of *Salmonella* spp., *S. aureus*, *B. cereus*, enterococci, and Enterobacteriaceae.

The hands of workers were sampled during production activities and immediately after handwashing. The samples were collected by pressing all five of the fingers onto the surface of a Petri dish (diameter: 120 mm) containing agar-based growth medium specific for the growth of microbes like Enterobacteriaceae, enterococci, *S. aureus* and *B. cereus*.

Testing results for the workers' uniforms and hands were expressed in qualitative terms (i.e., presence vs. absence of each contaminant category).

Foodstuffs

Foodstuffs were collected and placed in a sterile plastic bag. Each sample was analyzed for the presence of Listeria monocytogenes, Salmonella spp., and Enterobacteriaceae, as specified in the European Commission Regulation (EC) No. 1441/2007 on microbiological criteria for foodstuffs, which modifies Regulation (EC) No. 2073/2005 on the same subject [12, 13]. In addition to these parameters, we also determined the numbers per gram of: total mesophilic bacteria, coliforms, S. aureus, Escherichia coli, fungi, and B. cereus. Since acceptable ranges for these parameters have not been established by the EC, the results were interpreted according to the standards adopted by the Local Health Unit Naples1-center (ASL NA1Centro) [18, 19] (Table 3). All samples were refrigerated and transported to the Regional Agency for Environmental Prevention of the Campania Region (ARPAC) Laboratories in Naples, where they were analyzed in accordance with nationally and internationally approved reference methodology [20-29].

Statistical analysis

Statistical analyses of the results were performed using a descriptive statistics and a univariate analysis (chi square test) for testing for differences between groups. A p-value < 0.05 was considered statistically significant.

Results

The inspections conducted in the 34 food businesses resulted in the following disciplinary



Table 3. Microbiological standards adopted by the Campania Region for parameters not included in EC Regulations.

MICROBIOLOGICAL PARAMETERS	LIMITS (CFU/g)		
Total mesophilic bacteria	1 x 10 ⁶ *		
Coliforms	1.1 x 10 ³ *		
Escherichia coli	< 10 *		
Staphylococcus aureus	1 x 10 ² *		
Bacillus cereus	5 x 10 ² ^		
Fungi	1 x 10 ³ ^		

Note: Standards for ice cream/fresh pastries established in *Resolution no. 1120/2005 of the Umbria Region [18] or in ^the *Accordo Direzione Regionale Sanità Pubblica* (Administrative Agreement Regional Public Health – A.R.P.A. Piedmont Region [19]

actions:

- a) Eight (23.5%) of the 34 businesses received immediate sanctions for:
- unacceptable hygiene conditions constituting a threat to food safety (Legislative Decree No. 193/07 [30]). These businesses were also ordered to undertake immediate routine and special cleaning measures and to suspend production activities in the affected area(s)/sector(s) until acceptable hygiene conditions had been restored;
- licensing irregularity (failure to update the Public Health Authorization with the name of the business' current legal representative) (Legislative Decree No. 193/07 [30]);
- lack of certification of food safety training for workers.
- b) Twenty-five (73.5%) of the 34 businesses received warnings (Legislative Decree No. 193/07 [30]) with orders to eliminate one or more minor nonconformities within the specified deadlines.

The nonconformities documented in these businesses were as follows:

- twenty-five businesses had sampling protocols that failed to satisfy the norms set forth in EC Regulations 2073/2005 and 1441/2007 [12,13];
- in 24 businesses, the documentation regarding CCP monitoring was incomplete or not sufficiently specific;
- twenty-two businesses were cited for inadequate food-safety training of workers;
- twenty-one businesses were cited for minor hygiene / structural nonconformities that did not represent an immediate threat to the health of the consumer;
- in 16 businesses, the documentation provided was not sufficiently organized to ensure product traceability.

Appropriate deadlines were set for the correction of each nonconformity, and follow-up inspections after these dates confirmed that all irregularities had been fully resolved.

Microbiological analyses revealed nonconformity in 138 (49.3%) of the 280 samples collected during the inspections.

The statistical analysis, used to compare the differences between groups (surfaces, indoor air, foods, workers), showed significant differences (p < 0.05) among the samples analysed in the two years (Table 4).

These nonconformities involved 24 (85.7%) of the 28 businesses inspected which had failed to meet the quality control standards used in this study

The quality of air circulating in the work areas was fair with microbial loads ranging from 100 to 300 CFU/m². In contrast, the surfaces in the work areas were grossly contaminated with microbial loads that were inacceptable in 80% of the cases (p< 0.0001) (Table 4). Hygiene problems involving surface contamination with mesophilic bacteria, fungi, enterococci, and Enterobacteriaceae emerged in 24 (85.7%) of the 28 businesses

Table 4. Conformity with microbiological standards of the samples analyzed.

Samples		non-conformity		conformity		p-value	
type	n	n	%	n	%		
Surfaces	140	112	80.0	28	20.0	< 0.000	
Indoor air	28	0	0.0	28	100.0	< 0.000	
Foods	28	6	21.4	22	78.6	0.0019	
Workers	84	20	23.8	64	76.2	< 0.000	
Total	280	138	49.3	142	50.7		



inspected. In 3/28 businesses (10.7%), these organisms were found together with pathogens like *B. cereus* and *S. aureus*. There was no evidence of *Salmonella* spp. in any of the specimens (Figure 1).

The FBOs were informed of the results of these microbiological analyses and ordered to provide retraining for all of the staff members with special emphasis on sanification, forms of cross-contamination, and personal hygiene. All businesses were reinspected to ensure that these orders had been carried out, and in all cases the problems of contamination had been fully resolved.

Microbiological analysis of samples collected from the workers' uniforms and hands revealed nonconformity in 15 (53.6%) of the 28 businesses inspected. Twenty (23.8%) of the 84 samples examined failed to meet the required standards (p< 0.0001) (Table 4). Seven (25.0%) of the 28 uniforms sampled were contaminated with microorganisms at levels exceeding the limits used in the study. Of the seven uniforms, five were contaminated with Enterobacteriaceae and enterococci, and 3 were found to be contaminated with *S. aureus* and *B. cereus*. *Salmonella* spp. were not found on any of the uniforms.

As for the workers' hands, the samples collected after washing were contaminated more frequently than those collected during food production activities (positivity rates: 10 out of 28 samples [35.7%] vs. 3 out 28 samples [10.7%]); they were also found to be contaminated with *S. aureus*. The latter was isolated from 3 of the 10 positive

samples (30.0%), collected after hand-washing vs. 1 of the 3 positive samples (33.3%) collected during food production activities (Figure 2). The contamination of the workers' hands after washing stemmed from errors made during washing and drying of the hands.

Nasal and throat swabs were also collected from workers whose hands were contaminated with *S. aureus* to determine whether they were healthy carriers. Positive results emerged for only one of these individuals. A precautionary leave of absence with appropriate antibiotic therapy was ordered for this worker, and he was only allowed to resume work after three throat swabs produced negative results.

In spite of the documented hygiene deficiencies regarding work surfaces and the workers themselves, microbiological testing of the food samples revealed that all 28 products tested met the process hygiene and food safety criteria established by EC regulations [12, 13]. *L. monocytogenes, S. aureus,* and *B. cereus* were not isolated from any of these foods. Six of the 28 products analyzed (21.4%; p = 0.0019) (Table 4) had total mesophilic bacterial counts and coliform counts that exceeded the limits adopted for these parameters by the Local Health Unit Naples1-Center [18, 19] (Table 3).

Discussion

Over 10 years have passed since the Italian Parliament issued Legislative Decree No. 155/97 [31]. This decree was abrogated in 2007 by Legislative Decree No. 193 of 6 November 2007 [30], which provided for the implementation of

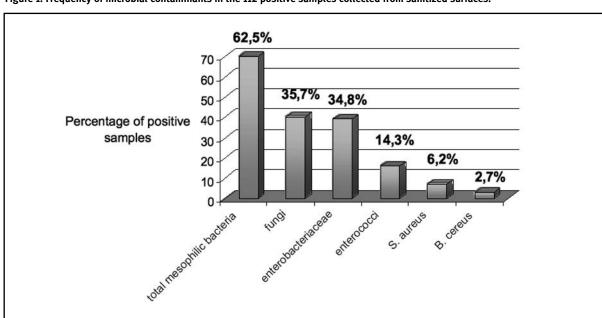


Figure 1. Frequency of microbial contaminants in the 112 positive samples collected from sanitized surfaces.



EC Regulation 852/2004 in Italy [10]. Despite these efforts, most FBOs in Naples involved in the artisanal production of ice creams or pastries still do not seem to have grasped the importance of general preventive measures such as Good Hygienic Practices (GHP), Good Manufacturing Practices (GMP), and the HACCP system in ensuring the delivery of safe, high-quality food products to consumers. This justifies the high percentages of disciplinary actions documented in the food businesses inspected (73.5% and 23.5%) and the positive results of environmental samples (surfaces, workers).

In many cases, the HACCP plans were considered a mere formality, and some FBOs were not even aware of the content of these plans. The frequent failures to implement the food-sampling protocols (pursuant to EC Regulations 2073/2005 and 1441/2007 [12, 13]) reflects the difficulties on the part of the FBOs in adapting to the new regulations and highlights the need for information and training.

The detection of mesophilic bacteria, fungi, enterococci, and Enterobacteriaceae together with important pathogens like *B. cereus* and *S. aureus* on surfaces sanitized indicate that sanification procedures are not being carried out correctly. In the businesses we inspected, these deficiencies could often be attributed to sanification protocols that were incomplete or inadequate (in terms of the procedures to be used, intervention sites, frequency or monitoring practices) or to inadequate training of staff members involved in sanification activities.

The presence of *S. aureus* was also found on the workers' hands after washing. The contamination of the workers' hands after washing stemmed from errors made during washing and drying of their hands. Transfer of pathogens by food handlers, in particular from their hands, is of particular importance in food service establishments [32]. Our results provide further support to the importance of possible recontamination of ready-to-eat foods by workers' hands and food contact surfaces.

Poor hygienic conditions, in terms of work surfaces and the workers themselves, did not compromise the microbiological quality of the products tested which always met the food safety criteria established by EC regulations [12, 13]. Pathogens were not found as reported by other Authors [33, 34].

Unfortunately, our results cannot be compared with other Italian regions as there are no published national data on surveillance system concerning commercial non-industrial production of pastries and ice creams.

Even though only a small number of food businesses were inspected and a relatively small number of samples for each typology were collected, a full sanitation assessment (visual inspection, documentation, laboratory tests) for each business was carried out. This allowed us to obtain an overall view of the structural/hygiene/public health characteristics of workplaces where ice cream and pastries are made and to verify the correct application of a quality-control surveillance protocol.

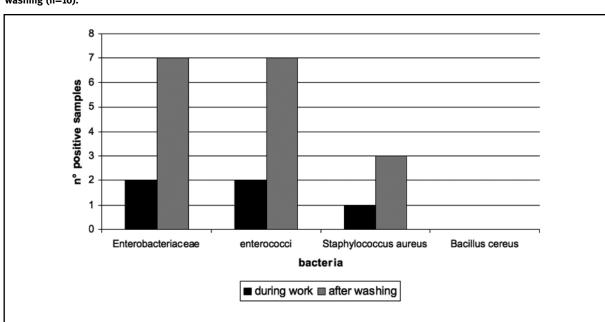


Figure 2. Microbial contaminants present in positive samples collected from workers' hands during work activities (n=3) and after washing (n=10).



knowledge is essential in order to correct errors in food production and to improve the implementation of preventive measures.

It was encouraging to note that in almost all cases, the FBOs targeted by the inspections reacted positively to the advice and information offered by inspectors and promptly carried out the prescribed corrective measures. This favorable outcome probably reflects the manner in which the inspections themselves were carried out. These encounters were not limited to the identification of short-comings and the prescription of corrective measures: they also included activities aimed at increasing the FBO's

knowledge and understanding of current foodsafety norms, the potential hazards associated with poor hygiene, and of his/her indispensable role in ensuring the quality/safety of the food consumed in Naples.

Additionally, environmental biomonitoring is very useful during inspections regarding hygiene/public health: identification of dubious hygienic conditions allows closer inspection of critical points in the food production chain and the prescription of corrective measures that can improve food safety and reduce the risks to consumers.

References

- 1) Käferstein F, Abdussalam M. Food safety in the 21st century. Bull World Health Organ 1999; 77(4): 347-51.
- 2) Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, et al. Food-related illness and death in the United States. Emerg Infect Dis 1999; 5(5): 607-25.
- 3) Department of Health. Epidemiological Bulletin. Available from:

http://www.ministerosalute.it/malattieInfettive/paginaInterna MenuMalattieInfettive.jsp. [Accessed on december 2009].

- 4) The Community Summary Report on Food-borne Outbreaks in the European Union in 2007. The EFSA Journal 2009; 271:21.
- 5) Scott E.A review of foodborne disease and other hygiene issues in the home. J Appl Bacteriol 1996;80:5-9.
- 6) World Health Organisation (WHO). Surveillance Programme. Sixth Report of WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe, 2005. Berlin: FAO/WHO Collaborating Centre for Research and Training in Food Hygiene and Zoonoses.
- 7) De Schrijver K, Buvens G, Possè B, Van den Branden D, Oosterlynck O, De Zutter L, et al. Outbreak of verocytotoxin-producing E. coli 0145 and 026 infections associated with the consumption of ice cream produced at farm, Belgium. Eurosurveillance 2007; 13 (1-3): 1-4.
- 8) Morgan D, Mawer SL, Harman PL. The role of home-made ice cream as a vehicle of Salmonella enteritidis phage type 4 infection from fresh shell eggs. Epidemiol Infect 1994; 11381:21-9.
- 9) Bortolussi R. Listeriosis: a primer. CMAJ 2008; 179(8):795-7. 10) Regulation (EC) No 852/2004 of the European Parliament and of the Council
- of 29 April 2004 on the hygiene of foodstuffs. Official Journal of the European Union L 139/1, 30/04/2004.
- 11) Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 janury 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 Union L 31/1,01/02/2002.
- 12) Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union L338/1, 22/12/2005.
- 13) Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on

- microbiological criteria for foodstuffs. Official Journal of the European Union L 322/12, 7/12/2007.
- 14) Regulation (ec) no 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. Official Journal of the European Union L 165/6, 30/4/2004.
- 15) Decreto Dirigenziale Regione Campania n° 46 del 23 febbraio 2005. Bollettino Ufficiale della Regione Campania n. 30,13/6/2005.
- 16) Maroni M, Alcini D, Cavallo D, Carter P. I limiti proposti in ambito internazionale per la qualità dell'aria indoor. [International proposed limits to indoor air quality]. Acts of 56° Congresso Nazionale della Società Italiana di Medicina del Lavoro e Igiene Industriale, Venezia, 20-23 ottobre 1993. Vol.1:355-64.
- 17) Note applicative pbi S.p.A. "Il controllo microbiologico ambientale". [Microbiological environmental control]. N.99/23/03/2007.
- 18) Regione Umbria. "Sicurezza alimentare: norme vincolanti Regione Umbria- aggiornamento DGR n.5310/1994 art. 4 D.L.vo 123 del 3.3.93: particolari tipologie di alimenti e modalità di analisi". Deliberazione della Giunta Regionale n. 1120 del 06/07/2005.
- 19) Accordo Direzione Sanità Pubblica Regione Piemonte e Agenzia Regionale per la Protezione Ambientale del Piemonte. Regolamentazione dell'attività inerente il controllo di alimenti, acque, cosmetici ed altro. [Regulation in matter of food, water, cosmetics and others controls]. 31/3/2006
- 20) UNI EN ISO 11290. Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of Listeria monocytogenes. Part 2: Enumeration method. 2005.
- 21) UNI EN ISO 6579. Microbiology of food and animal feeding stuffs. Horizontal method for the detection of Salmonella spp. 2008.
- 22) UNI EN ISO 6888. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species). Part 1:Technique using Baird-Parker agar medium.
- 23) UNI EN ISO 4833. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony-count technique at 30 degrees C. 2004.



- 24) UNI EN ISO 7932. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of presumptive Bacillus cereus Colony-count technique at 30 $^{\circ}$ C. 2005.
- 25) International Organization for Standardization. ISO 21527. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of yeasts and moulds Part 1: Colony count technique in products with water activity greater than 0,95. 2008.
- 26) International Organization for Standardization. ISO 21527. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of yeasts and moulds Part 2: Colony count technique in products with water activity less than or equal to 0,95. 2008.
- 27) International Organization for Standardization. ISO 16649. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli. Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. 2001.
- 28) International Organization for Standardization. ISO 4832. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coliforms. Colony-count technique. 2006.

- 29) International Organization for Standardization. ISO 21528. Microbiology of food and animal feeding stuffs. Horizontal methods for the detection and enumeration of Enterobacteriaceae. Part 2: Colony-count method. 2004.
- 30) Decreto Legislativo 6 Novembre 2007, n. 193. Attuazione della direttiva 2004/41/CE relativa ai controlli in materia di sicurezza alimentare e applicazione dei regolamenti comunitari nel medesimo settore. GU n. 261 del 9/11/2007. Suppl. Ordinario n.228.
- 31) Decreto Legislativo 26 maggio 1997, n. 155. Attuazione delle direttive 93/43/CEE e 96/3/CE concernenti l'igiene dei prodotti alimentari. Supplemento ordinario alla "Gazzetta Ufficiale" n. 136 del 13 giugno 1997 Serie generale.
- 32) Montville R, Chen YH, Schaffner DW. Glove barriers to bacterial cross-contamination between hands to food. J Food Prot 2001; 64:845-9.
- 33) Maifreni M, Civilini M, Domenis C, Manzano M, Di Prima R, Comi G. Microbiological quality of artisanal ice cream. Zentralbl Hyg Umweltmed 1993; 194(5-6):553-70.
- 34) Delia S, Mauro A. Microbiological testing of commercial ice cream in Messina as required by the Ministerial Ordinance of 11 October 1978. Ann Sclavo 1980; 22(4):624-32.