

Microbiological Quality of Ice Cream after HACCP Implementation: a Factory Case Study

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Abstract

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The microbiological quality of the final product and the safety of the production procedures were screened in an ice cream factory, after implementation of a Hazard Analysis Critical Control Points (HACCP) system. We analysed 30 vanilla (IC1), 30 strawberry (IC2), and 30 chocolate flavoured (IC3) samples of ice cream; 30 of water; 90 of personnel's hands flora; 150 of plastic ice cream containers flora; 50 of sanitised equipment-surfaces flora. After HACCP introduction, *Staphylococcus aureus* was not further detectable in ice cream and *Escherichia coli* was mostly less than 10 CFU/g, while the spoilage markers (total coliforms – TC, aerobic plate counts – APC) in ice cream and the environment were reduced by 20–35%. Mean log CFU/g, for IC1: TC from 2.20 reduced to 1.57, APC from 4.58 reduced to 3.62. For IC2: TC from 2.29 reduced to 1.65, APC from 4.61 reduced to 3.49. For IC3: TC from 2.67 reduced to 1.76, APC from 5.08 reduced to 3.81.

Keywords: ice cream; microbiological quality; Hazard Analysis Critical Control Points

The microbiological quality of ice cream can be low, as it is a good growth-medium for microbes due to its nutrients (lactose, proteins, etc) and to its almost neutral pH of 6–7 (KANBAKAN *et al.* 2004). There are numerous reports on human pathogens incidence in ice cream: *Listeria monocytogenes* (WHO 1998), *Salmonella species* (VOUGHT & TATINI 1998), *Staphylococcus aureus* (MASSA *et al.* 1989), *Yersinia enterocolitica* (EL-SHERBINI *et al.* 1999), *Bacillus cereus* (TORKAR & MOZINA 2000), etc.

Hazard Analysis Critical Control Points (HACCP) is a systematic approach used in the food industry

for the identification, assessment, and control of biological, chemical, and physical hazards (CAC 1997), providing an effective way to advance food quality/safety, focusing on preventing hazards and improving processes (SWANSON & ANDERSON 2000). The effectiveness of HACCP depends on the correct application of its principles, combined with other programs (prerequisites), such as Good Manufacturing Practices (GMPs), Good Hygiene Practices (GHPs), and Sanitation Standard Operating Procedures (SSOPs) (WHO 1999; ROBERTO *et al.* 2006). For a successful food quality/safety control, prerequisite programs should be developed,

linked to HACCP and managed within a quality-management system (ISO 9001:2000; WALLACE & WILLIAMS 2001).

HACCP implementation generally improves the microbiological quality of food products (SORIANO *et al.* 2002). The evaluation of the microbiological testing results can give important information not only on the quality of the foodstuff at a specific stage of its processing, but on the overall efficiency of HACCP design and implementation as well (KOKKINAKIS & FRAGKIADAKIS 2007). The aim of this study was to evaluate the actual changes of the microbiological quality of ice cream and of the general production hygiene-conditions, following full implementation of HACCP system in an ice cream producing factory that had only partially applied HACCP prerequisites in the past.

MATERIAL AND METHODS

Sampling. This study was carried out during 2006, in a factory that could produce 5 tons of ice cream per day, at Siteia, Crete, Greece. The mixing of the ice cream fresh material was done at 60°C, after which it was pasteurised at 70°C for 35 minutes. Three flavoured ice cream products were tested: vanilla – IC1, strawberry – IC2, chocolate – IC3. The sampling was carried out when semisolid ice cream was packed into 5 kg plastic containers for hardening and storage. The semi-solid product was chosen because hardening is carried out at –30°C to –40°C and the final product is kept at –25°C, i.e. the temperatures where microbial growth does not exist. The sampling was carried out initially in June, and then in September after HACCP implementation. In each sampling, three different ice cream lots were tested (a lot is made and packed under the same conditions), with 5 samples per lot, that is 15 per IC flavour. In total, 90 ice cream samples (45 samples before HACCP and 45 samples after HACCP implementation) were microbiologically analysed. Water quality was also evaluated; 30 samples analysed: W1 – water mains supply, W2 – water used as ice cream ingredient, W3 – water for personnel's washing. In addition, 90 samples of personnel's hands; 150 from plastic ice cream containers, and 50 from the surfaces of sanitised equipment were evaluated.

Microbiological quality analysis. Ice cream microbiological analysis was based mainly on the Council Directive 92/46/EEC (EEC 1992) requirements for certain food pathogens. *Listeria mono-*

cytogenes detection followed horizontal methods (ISO 11290-1:1997) with two-stage enrichment (HARRIGAN 1998). Each ice cream sample (25 ml) was added into 225 ml “half Fraser” broth (Biokar Diagnostics, Pantin Cedex, France), homogenised, and incubated at 30°C for 24 hours. A loopful of each primary enrichment broth was inoculated on a plate of Oxford agar (Biokar Diagnostics) and incubated at 35°C for 48 hours. In parallel, 0.1 ml of each primary enrichment broth was inoculated in 10 ml Fraser broth for secondary enrichment, incubated at 35°C for 48 h and used to inoculate Oxford agar plates, which were also incubated at 35°C for 48 hours. Suspect colonies, of both primary and secondary enrichments from each sample, were sub-cultured onto tryptone soya yeast extract agar (TSYEA, Difco, Sparks, USA) and incubated at 35°C for 48 hours. Further confirmation tests were carried out using the API *Listeria* kit (bioMerieux, Marcy-l'Etoile, France).

Salmonella spp. detection followed a horizontal method (HARRIGAN 1998; ISO 6579:2002). Each ice cream sample (25 ml) was homogenised with 225 ml buffered peptone water (BPW, Biokar Diagnostics) and incubated at 37°C for 24 hours. After this pre-enrichment, 10 ml were added to both 100 ml of selenite-cystine broth and 100 ml of Rappaport-Vassiliadis (RV, Biokar Diagnostics) broth, and incubated at 37°C and 42°C, respectively, for 24 hours. Each broth was used to inoculate both brilliant green phenol red (BGA, Oxoid, Basingstoke, UK), and xylose lactose deoxycholate (XLD, Biokar Diagnostics) agar plates, incubated then at 37°C for 24 h and 48 h, respectively. Further confirmation tests were carried out with the API 20E kit (bioMerieux).

Baird-Parker agar (BPA, Biokar Diagnostics) with egg yolk telluride emulsion (Oxoid) was used for enumeration of *Staphylococcus aureus* (ISO 6888-1:1999). Each ice cream sample (25 ml) was added into 225 ml peptone water (PW, Biokar Diagnostics), homogenised and used to inoculate BPA plates. After incubation at 37°C for 48 h, BPA plates were examined for typical black colonies of *S. aureus*; while further confirmation tests were carried out with the API Staph kit (bioMerieux). The 3M Petrifilm *Escherichia coli*/Coliform Count (EC), and the Aerobic Count (AC) methods were used for enumeration of *E. coli*, Total Coliforms (TC), and APC (GRACIAS & MCKILLIP 2004). Microbiological analysis of water was carried out by the membrane-filtration technique (ISO

9308-1:2000) for *E. coli*, and TC, and ISO 7899-2: 2000 for *Enterococcus faecalis*. For the evaluation of personnel's hands, ice cream containers, and sanitised surfaces hygiene, the Hy-Giene Monitor (Hy-Laboratories Ltd., Rehovot, Israel) kit was used (RAUGEL 1999).

Microbiological quality evaluation. Ice cream microbiological analysis was based mainly on Council Directive 92/46/EEC (EEC 1992), as repealed by Directive 2004/41/EC (EC 2004) and Regulation 2073/2005 (EC 2005). Other guidelines (EC 1999; ADASC 2000; GILBERT *et al.* 2000; NZICMA 2002) were also considered. The *E. coli* presence in ice cream is not mentioned in directive 92/46/EEC (EEC 1992), possibly because it represents a part of total coliforms, where *E. cloacae* and *E. aerogenes* can predominate (MASSA *et al.* 1989). The presence of *E. coli* and coliforms after pasteurisation mainly indicates recontamination (REIJ *et al.* 2004), although if the raw materials are heavily contaminated, low numbers can be found in the products even after pasteurisation. In addition, (ICA 2004): (I) strains of *E. coli* are pathogenic. (II) *E. coli* inhabits human and animal guts and its presence can indicate that other organisms of the same origin, causing food poisoning, can be possibly present. *E. coli* enumeration in ice cream is mentioned in ADASC guidelines (2000), with a value less than 10 CFU/g required. Directive 98/83/EEC (EEC 1998) was used for the evaluation of water samples and Hy-Giene Monitor visual interpretation guide (RAUGEL 1999) for the evaluation of microbial levels on personnel's hands, containers, and surfaces.

Implementation of HACCP. The HACCP system was certified by an external to the factory company. Three stages were involved in HACCP implementation. In the first one, the factory established procedures in order to: deal with environmental hazards; reconsider plant ergonomics; reorganise raw materials acceptance and products transportation; monitor storage conditions; reorganise equipment sanitation; reevaluate personnel training, surfaces sanitation etc. In the second stage, the HACCP team was assembled, protocols were described and flow charts constructed. In the third stage, the 7 HACCP principles were fully implemented: Hazard analysis; determination of Critical Control Points (CCPs) and critical limits for each; monitoring system establishment; corrective actions and verification procedures for each CCP; routine documentation and record keeping (CAC 1997).

RESULTS AND DISCUSSION

This case study concerns an ice cream factory, which at the time of the first sampling had just started to design a HACCP system. The first sampling shows the initial microbial quality of products (ice cream samples), ingredients (water), personnel's hygiene (hands), packaging materials (ice cream containers), and sanitation procedures efficiency (equipment surfaces). The HACCP effects were apparent at the second sampling. Prerequisites introduced by HACCP, Good Manufacturing Practices (GMPs), and Good Hygiene Practices (GHPs), significantly improved the ice cream quality, and the hygiene-level inside the plant as indicated by analysis of the *Staphylococcus aureus* levels (all samples negative at the second sampling), as well as the *Escherichia coli*, total coliforms, and aerobic plate counts levels.

All ice cream samples (IC1, IC2, and IC3) contained no *Listeria monocytogenes* and/or *Salmonella* spp., both before and after HACCP implementation (Table 1). On the other hand, the bacterium *S. aureus* is a most important agent of dairy products quality-deterioration worldwide (Asao *et al.* 2003). According to directive 92/46/EEC (EEC 1992), for 5 ice cream samples representing 1 lot, all should contain *S. aureus* less than 100 CFU/g (Colony Forming Units per gram); however 3 should be at the satisfactory level of < 10 CFU/g, only 2 at the acceptable 10–10² CFU/g, and none > 10² CFU/g. Within the 3 ice cream lots we tested (3 lots vs. 5 samples each) before HACCP: IC1 had 5 samples containing *S. aureus* (2 < 10² CFU/g, 3 > 10² CFU/g); IC2 had 7 positive samples (3 < 10² CFU/g, 4 > 10² CFU/g); IC3 had 9 positive samples (6 < 10² CFU/g, 3 > 10² CFU/g) (Table 1). Overall, none of the three lots tested before HACCP was within 92/46/EEC requirements. After HACCP introduction, all samples, from 3 more lots tested, were found negative for *S. aureus* (Table 1). The sensitivity and specificity of the individual test procedures in a given microbiological test system are not necessarily the same (BARRY *et al.* 1979), but the final identification should be the same. In this study, for obtaining more precise results, the identification and enumeration concerning *L. monocytogenes*, *Salmonella* spp., and *St. aureus*, were carried out using both the recommended broth/agar ISO methods as well as API kits.

The bacterium *E. coli* may be transmitted through water/equipment contamination or poor hygiene

Table 1. Microbial pathogen levels of ice cream, before and after HACCP implementation

Microbe	Sam- ples	IC1 – ice cream vanilla flavoured			IC2 – ice cream strawberry flavoured			IC3 – ice cream chocolate flavoured		
		before HACCP	after HACCP		before HACCP	after HACCP		before HACCP	after HACCP	
		number of positives samples	range (CFU/g)	number of positives samples	number of positives samples	range (CFU/g)	number of positives samples	number of positives samples	range (CFU/g)	number of positives samples
LMO	15	0	–	0	0	–	0	–	–	–
SLM	15	0	–	0	0	–	0	–	–	–
SAU	15	5	2 < 102	no positives	7	3 < 102	9	6 < 102	no positives	no positives
			3 > 102			4 > 102		3 > 102		

LMO: *Listeria monocytogenes*; SLM: *Salmonella* spp.; SAU: *Staphylococcus aureus*
Negative were considered the samples that had no LMO, or SLM, or SAU

of food handlers (SNYDER 1998), and its presence may indicate recontamination (REIJ *et al.* 2004) of ice cream after the pasteurisation stage, i.e. during homogenisation where various ingredients are added. The *E. coli* values, before HACCP, in IC1 samples were ($13 = 10-10^2$ CFU/g, $2 > 10^2$ CFU/g); in IC2 ($1 < 10$ CFU/g, $8 = 10-10^2$ CFU/g, $6 > 10^2$ CFU/g); IC3 ($10 = 10-10^2$ CFU/g, $5 > 10^2$ CFU/g) (Table 1). After HACCP, the *E. coli* values were lower: for IC1 ($10 < 10$ CFU/g, $5 = 10-10^2$ CFU/g); IC2 ($12 < 10$ CFU/g, $2 = 10-10^2$ CFU/g); IC3 ($14 < 10$ CFU/g, $1 = 10-10^2$ CFU/g) (Table 2).

Total coliforms (TC) and aerobic plate counts (APC) values (NZICMA 2002) were also determined. The TC values represent heat sensitive microorganisms that do not survive through pasteurisation but can, however, re-contaminate foods during further handling. The APC values quantify the total number of aerobic bacteria present in the foodstuff. According to most hygiene guidelines (i.e. EEC 1992; EC 1999; NZICMA 2002), TC should be in the range of $0-10^2$ CFU/g ($0-2 \log$ CFU/g), while APC in the range of $0-10^5$ to ($5 \log$ CFU/g or less). Both TC and APC values were found decreased after HACCP implementation: mean \log CFU/g for TC and APC respectively, for IC1 (TC from 2.20 decreased to 1.57, and APC from 4.58 to 3.62); for IC2 (TC from 2.29 decreased to 1.65, and APC from 4.61 to 3.49); and IC3 (TC from 2.67 decreased to 1.76, and APC from 5.08 to 3.81) (Table 3). The microbiological quality of the chocolate ice cream screened was lower, when compared to the vanilla and strawberry flavoured ice creams (Table 3).

As the food industry evolves, the need emerges for regulatory agencies to assess its current technological status concerning the ability to control pathogenic micro-organisms, and to identify areas where additional technologies are necessary to be applied (BUCHANAN 1995). In the ice cream industry, the concentration of micro-organisms is not always easy to ascertain since a part of the viable population loses the ability to form colonies because of sublethal injuries after freezing (FOSCHINO 2002). The potential of the molecular detection/typing of microorganisms in ice cream (TORKAR & MOZINA 2000), a methodology not affected by freeze injuries, is not fully utilised as yet, even not considered by the guidelines of the international or national agencies. This is important for toxinogenic pathogens, where rapid differentiation of strains by classical tests is not

Table 2. *Escherichia coli* levels (CFU/g) of ice cream, before and after HACCP implementation

Samples	IC1 – ice cream vanilla flavoured						IC2 – ice cream strawberry flavoured						IC3 – ice cream chocolate flavoured					
	before HACCP			after HACCP			before HACCP			after HACCP			before HACCP			after HACCP		
	< 10	10–10 ²	> 10 ²	< 10	10–10 ²	> 10 ²	< 10	10–10 ²	> 10 ²	< 10	10–10 ²	> 10 ²	< 10	10–10 ²	> 10 ²	< 10	10–10 ²	> 10 ²
15	0	13	2	10	5	–	1	8	6	12	3	–	0	10	5	11	1	–

Table 3. Total coliforms and aerobic plate count levels (log CFU/g) of final products, before and after HACCP implementation

Microbe	Samples	IC1 – ice cream vanilla flavoured				IC2 – ice cream strawberry flavoured				IC3 – ice cream chocolate flavoured			
		before HACCP		after HACCP		before HACCP		after HACCP		before HACCP		after HACCP	
		mean	range	mean	range	mean	range	mean	range	mean	range	mean	range
TC	15	2.20	1.75–2.65	1.57	1.18–1.95	2.29	1.69–2.88	1.65	1.40–1.90	2.67	2.17–3.16	1.76	1.54–1.98
APC	15	4.58	4.30–4.86	3.62	3.40–3.83	4.61	4.23–4.98	3.49	3.20–3.79	5.08	4.94–5.22	3.81	3.65–3.96

TC: – Total coliforms; APC – Aerobic plate counts

Table 4. Incidence and levels of microorganisms in water samples before and after HACCP implementation

Microbe	W1 – water mains supply			W2 – water used as ingredient			W3 – water used for personnel's hygiene		
	before HACCP			after HACCP			before HACCP		
	number of analysed samples	number of positive samples*	incidence (%)	number of analysed samples	number of positive samples*	incidence (%)	number of analysed samples	number of positive samples*	incidence (%)
Before HACCP									
<i>E. coli</i>	5	0	0	5	0	0	5	0	0
<i>E. faecalis</i>	5	1	20	5	0	0	5	0	0
Total coliforms	5	2	40	5	1	20	5	1	20
After HACCP									
<i>E. coli</i>	5	0	0	5	0	0	5	0	0
<i>E. faecalis</i>	5	1	20	5	0	0	5	0	0
Total coliforms	5	1	20	5	0	0	5	0	0

*Negatives were considered the water samples that had *Escherichia coli*, *Enterococcus faecalis* and total coliforms of 0 CFU/100 ml of water

Table 5. Microbial contamination of personnel's hands, containers, and surface samples before and after HACCP implementation

Microbe	Personnel's hands		Containers		Surface after CIP	
	before HACCP	after HACCP	before HACCP	after HACCP	before HACCP	after HACCP
<i>E. coli</i>	–	–	75 (75 LC)	75 (75 LC)	25 (20 LC, 5 MC)	25 (25 LC)
Total coliforms	45 (15 LC, 30 HC)	45 (40 LC, 5 MC)	75 (75 LC)	75 (75 LC)	25 (10 LC, 15 HC)	25 (25 LC)
APC	45 (5 LC, 40 HC)	45 (40 LC, 5 MC)	75 (50 LC, 25 MC)	75 (70 LC, 5 MC)	25 (5 LC, 20 HC)	25 (25 LC)

APC – Aerobic Plate Counts; LC – Low Contamination; MC – Moderate Contamination; HC – Heavy Contamination

feasible (TORKAR & MOZINA 2000). The application of new enrichment media, in combination with serological confirmation of the pathogens (ISO 6579:2002), as well as molecular and immunochemical methodologies (BAYLIS *et al.* 2000), can improve the recovery and detection of pathogenic bacteria in ice cream.

In our case study, mains-supply water (W1) was found contaminated with *E. faecalis* and TC (Table 4), possibly due to problems in the water supply pipelines systems. Following HACCP implementation (Table 4) that introduced water-sanitation techniques (water chlorination, with free residual chlorine 0.2–0.4 ppm), the water used for the ice cream production (W2) and for personnel's washing (W3) contained small numbers of TC (Table 4). All W2 and W3 samples were found microbiologically safe, that is of potable quality as required (EEC 1998). Active carbon filters (NZICMA 2002) were fitted into the water lines supplying ice cream ingredient tanks, to hold any residual chlorine from affecting ice cream sensory characteristics. Microbial contamination of personnel's hands and surface samples decreased after HACCP implementation (Table 5), indicating that the introduction of personnel's training programs and Good Hygiene Practices (GHP) along with on the job training had a beneficial effect on the personnel's general hygiene. Plastic ice cream containers were found lowly contaminated, both before and after HACCP implementation (Table 5).

The introduction of the HACCP methodology reinforced the existed functional and hygiene prerequisites, while introducing new ones (Table 6). Commercial ice cream production involves two levels of the supply chain. The first, or production supply-chain, includes all the procedures

of transportation, quality control, traceability, and processing of the raw materials, until the final product is prepared and initially packed. The management of the production supply-chain is a major target of HACCP, and concerns the handling, the flow, and the hygiene of different materials as milk, water, flavourings, wooden sticks, paper and plastic containers, package materials etc. The second level, or distribution supply-chain, includes logistics, management of the “frozen transport”, storage and distribution to the ice cream retailers; procedures that require a distribution network. The distribution supply-chain must be also included in HACCP standardisation, in cooperation with ice cream retailers. The quality control and the logistics departments must cooperate on the ice cream storage conditions, the preparation of orders to be delivered by the First In First Out (FIFO) principle, the transportation conditions, the traceability through the products “bar codes”, etc.

CONCLUSION

The results of this study show the extent of the positive effects that a Hazard Analysis Critical Control Points (HACCP) system, introduced in an ice cream factory, had on both the microbiological quality of the final product and on the total quality/hygiene management as well. Among others, two main hygiene problems concerning the presence of the pathogen *Staphylococcus aureus* in the final product and the contamination of the mains-supply water with *Enterococcus faecalis* were detected and corrected. Further linkage of the HACCP system introduced in the factory to quality management systems, such as ISO 9001:2000, can be possibly proved to provide higher quality/hygiene stand-

Table 6. Indicative HACCP prerequisites application in the ice cream factory studied

	Before HACCP	After HACCP
GMPs, GHPs		
Factory environmental hazards monitoring	+	+
Factory transportation vehicles hygiene	–	+
Equipment hygiene maintenance	–	+
Pest control	+	+
Sanitation procedures: Clean-In-Place (CIP)	–	+
Water hygiene monitoring	–	+
Personnel hygiene monitoring	–	+
Personnel training	?	+
Raw materials specifications	–	+
Final products specifications	–	+
Accreditation procedures	–	+
Traceability and final product recall	–	+
Management of non-conforming products	–	+
Corrective actions	–	+
Auditing methodologies	–	+
Raw materials delivery		
Supplier evaluation	+	+
Temperature control	+	+
Macroscopic characteristics evaluation	+	+
Shelf life monitoring	+	+
Immediate storage	+	+
Suppliers transportation vehicles hygiene	+	+
Storage of materials		
Adequate air ventilation	+	+
Storage space cleanliness	+	+
Separate storage of chemical substances	+	+
Use of materials		
Use of First In First Out (FIFO)	+	+
Identification of expired materials	+	+
Macroscopic check prior to use	+	+
Food processing		
Separate processing departments	+	+
Adequate air ventilation	+	+
Existence of working instructions	–	+
Measurement and recording processes	–	+
Product labeling	+	+
Final product storage and transport		
Separation of raw materials, final products	+	+
Coverage of stored materials	+	+
Temperature control	+	+
	60%	100%

ards, along with higher awareness of the factories customers (i.e. ice cream retailers).

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