



**EVALUATION OF MICROBIOLOGICAL SAFETY OF AN ICE CREAM
PRODUCTION LINE AFTER HACCP IMPLEMENTATION**

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ABSTRACT

The microbiological quality of an ice cream production line and the effect of correct application of Hazard Analysis and Critical Control Point (HACCP) principles with its prerequisites programs on the line were screened. The data of microbiological testing from the ice cream line were collected and analyzed before implementation of the system. A total of 340 samples (90 from personnel's hands swabs, 80 from sanitized surfaces swabs, 80 from water and 90 from the final product "ice cream") were collected and microbiologically examined after implementation of the HACCP system. The microbiological data of the samples before and after implementation of the system were compared. The spoilage markers (total bacterial count – TBC, total coliforms – TC and yeast & mould count – YMC) in ice cream samples were significantly reduced after HACCP implementation. Mean log CFU/g, for ice cream: APC reduced from 7.87 to 3.37, TC reduced from 1.68 to <1, and YMC from 1.64 to 0.05. The *Staphylococcus aureus* and *Escherichia coli* were not isolated from all ice cream samples after HACCP implantation. The hygienic condition of the production line due to effective implantation of HACCP was significantly improved and reflected on the microbiological quality of the final product.

Keywords: Ice Cream, Dairy Industry, Microbiological Safety, HACCP and Prerequisite Programs

INTRODUCTION

The safety of the food supply remains a high priority for consumers, producers and regulatory agencies. Therefore the safety of dairy products has received particular attention by several official bodies. The ice cream is a good medium for growth of

microorganisms due its high nutrient constituents and nearly neutral pH (6–7) [1]. The ice cream has been classified as a high risk potential hazard which has been implicated in numerous outbreaks of food poisoning [2]. Several pathogens including *Listeria monocytogenes*, *Salmonella* species, *Staphylococcus aureus*, *Yersinia enterocolitica* and *Bacillus cereus* have been reported as human pathogens isolated from ice cream [3-5]. Microbial contamination can be introduced at various stages of production line from different human and environmental sources [6]. Recently, several management systems were developed to improve the food safety in dairy industry, however, Hazard Analysis and Critical Control Point (HACCP) remains the best internationally recognized system for controlling foodborne pathogens and assuring food safety [7, 8]. The strength of HACCP in controlling food hazards comes from its capability to offer a more comprehensive and science-based alternative measures in comparison with traditional sanitation programs which based mainly upon good manufacturing practices [9, 10]. It provides effective methods for improving food quality/safety by preventing the introduction of hazards to the processing line and assuring a safer product for the consumer [11]. The effectiveness of HACCP depends on the correct

implementation of its principles, combined with implemented prerequisite programs [12]. However, it is important to maintain a successful implementation through a periodical evaluation of the system. The microbiological criteria are an important mirror to permit verification of the effectiveness HACCP implementation and the quality of foodstuffs at different stages of processing [13, 14].

Upon finding the microbial parameters exceeding the standard limits indicates failure of the HACCP-based controls. Therefore, the goal of the current study was to assess the actual changes in microbiological parameters of an ice cream production line after implementation of the HACCP principals.

MATERIAL AND METHODS

Description of the Production Line

This study was applied in an ice cream production line. The product of company which produces two tons per shift and there are 40 persons in the line.

The prerequisite program was applied and maintained in the production line before HACCP introduced by implementing specific employee hygiene practices, required cleaning and sanitation programs, specific facility design practices, equipment maintenance standards and supplier selection criteria. The microbiological safety of the final product and the general

hygienic practices of the line production were evaluated periodically by the quality control department.

Collection and Analysis of Microbiological Data Before HACCP Implementation

The data of microbiological testing, of previously examined hand workers swabs, sanitized contact surfaces swabs, water samples and final products of the ice cream production line were collected and analyzed before implementation of the HACCP system.

Implementation of HACCP

The HACCP system was documented and implemented through the following steps: the HACCP team was assembled and assigned their responsibility, the final product was described and flow charts constructed **Figure 1**, the seven HACCP principles were fully implemented: hazard analysis; determination of critical control points (CCPs) and critical limits; monitoring system establishment; corrective actions and verification procedures for each CCP; routine documentation and record keeping (CAC, 1997) **Table 1**.

The HACCP system was certified by an external to the factory company microbiological evaluation of the ice cream production line after the system application.

Collection of Samples

A total 340 samples were collected during the production of different batches of the ice

cream production. The collected samples included:

- Ninety from hand workers swabs.
- Eighty from contact surfaces swabs (preparation tank, packaging tables, pipes of inside and outside freezer). The samples were collected after the surfaces were cleaned and sanitized by hydrogen peroxide 2%.
- Eighty from water (water mains supply, water used in production and water for personnel's washing).
- Ninety from final product (ice cream).

The collected samples were transferred as quickly as possible in an insulated ice-box to the laboratory.

Microbiological Examination

The Surface and worker hand swabs were examined according to [16] by streaking a tip of wetted swabs on an area of 20 cm². The tips were aseptically cut off and transferred into tubes containing diluents. The tubes were mixed, serially diluted and examined for aerobic plate count, Staphylococci counts according to [17] and Coliforms count according to [18]. Water samples were also evaluated for total colony count and Coliforms count according to [19]. The final product (Ice cream samples) had been examined for spoilage markers (total bacterial count, Coliforms content Staphylococci counts and total yeast & mould counts). The decimal dilutions were

done according to [20]. Total bacterial counts, Coliforms content (MPN/ml), Total staphylococci counts and Total Yeast & mould counts were performed according to [17, 20]. While the pathogens (*E. coli* and *S. aureus*) were isolated according [22] and Typical colonies were selected and their identity confirmed by standard biochemical reactions [23]. The results obtained before and after the implementation of HACCP system were analyzed and compared to evaluate the effectiveness and performance of HACCP-based programs in the ice-cream production line.

Statistical Analysis

The collected data were analyzed using SPSS statistics 17.0. Results were recorded as mean \pm SE. Analysis of variance was performed by ANOVA procedure to compare the results by at least significant (LSD) at $P < 0.05$.

RESULTS & DISCUSSION

This case study was conducted in an ice cream production line, which included the collection of data just before the application of HACCP system and the steps taken to put the system in place. Moreover, the microbiological safety was evaluated after the application of the system to assess its effectiveness the general hygiene as well as on the final product.

The results obtained in the **Table 2** revealed that the mean log values of total colony

count and coliforms (cfu/cm²) of examined personnel's hands were significantly reduced at ($P < 0.05$) after HACCP implementation. Our results are in agreement with the findings of [24] who observed that the implementation of personnel's training programs and Good Hygiene Practices (GHP) along with the job training had a beneficial effect on the personnel's general hygiene. After HACCP implementation, total colony count, coliform and *Staphylococcus aureus* counts (cfu/cm²) in personnel's hands were reduced by 98.009%, 100% and 100% respectively. The counts of coliforms and *Staphylococcus aureus* (cfu/cm²), of samples, after the implementation of HACCP system became at a satisfactory level to meet the standards of "Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs." [25] The food handler's swabs were used in this study to evaluate the effectiveness of the training programs, hand washing and the overall personal hygiene practices of food handlers for the entire assessment period. Food production workers were trained on the correct hand and fingertip washing procedures. Correct use of a fingernail brush to wash hands and fingertips was the best way to assure removal of transient microorganisms [26]. The critical control in hand washing is to reduce high levels of pathogenic

microorganisms, to a safe level; this requires correct scientific knowledge, management leadership, and employee training [8]. The hand sanitizers should only be used after properly washed hands, it can never replace hand washing; therefore, food workers must always wash their hands before the hand sanitizer is applied [27].

For effective implementation of HACCP system and production of safe food, it is important to implement and maintain prerequisite programs including good cleaning and disinfection of surfaces and equipments [28].

Determination of the total microbial number on the surfaces of utensils, work surfaces and other equipment in contact with food is very important to estimate the level of contamination during production and evaluate the effectiveness of cleaning and disinfecting protocols [16].

The total colony counts and coliforms of all examined surfaces swabs were more than 10^3 cfu/cm² and 10^2 cfu/cm², respectively before implementation of the system. After HACCP implementation the total bacteria counts and coliforms were reduced significantly ($P < 0.05$) **Table 3**. The Public Health Laboratory Service (PHLS) in the UK established limits for cleaned surfaces ready for use and stated that lower than 80 cfu/cm² is satisfactory, 80- 10^3 cfu/cm² is border line and more than 10^3 cfu/cm² is

unsatisfactory [29]. According to PHLS, 17.5 % only of our examined samples were satisfactory, 43.75 % were borderline and 38.75 % were classified as unsatisfactory samples after implementation of HACCP system, High levels were detected by [30] that attributed the unsatisfactory level of total colony count to poor hygiene and ineffective cleaning procedures, Therefore, a corrective measures should be identified and implemented to lower the counts to the recommended acceptable level.

Application of effective cleaning procedures combined with carefully planned cycle parameters and efficient pre-processing sanitization of preparation tank, packaging tables, pipes, inside and outside freezer and other surfaces significantly improved the bacteriological condition of the processing equipment and accordingly the final product.

In our case study, the coliforms were not isolated from water samples (mains-supply water, water used in production and water used for personnel's washing) before and after implementation of the system, while the mean log of total colony counts were 2.82 ± 1.2 , 2.75 ± 1.3 respectively, however, the total colony counts remained at unsatisfactory level after HACCP implementation according to [19] guidelines which recommended total bacterial count lower than 100 cfu/ml for potable water to

be accepted. These high bacteria counts of water may be reflected on the microbial counts of the surface and equipments.

HACCP is an effective system that can provide an appropriate level of protection via implementation and monitoring of preventative control measures and applying corrective actions for points out of control to prevent reoccurrence of inconformity [31, 32]. However, evaluation and monitoring the microbiological quality of final product is an important step to verify the effectiveness of these measures [33, 34]. The hygienic quality of the final product is dependent on the effective heat treatment of the ice cream mix base and the microbiological quality of added ingredients including water and packaging materials, the cleanliness of surfaces and the efficiency of the line sterilization.

The mean counts (log cfu/g) for the final product (ice cream) were significantly ($P < 0.05$) reduced after implementation of the HACCP system. The total bacterial count reduced from 7.87 before implementation to 3.37 after implementation, coliform counts reduced from 1.68 before implementation to less than 1 after implementation and yeast and mold counts reduced from 1.64 before implementation to 0.05 after implementation. Our results coincided with the study of [35], who obtained a significant

reduction in the population of aerobic plate count and coliforms count in 108 items of examined dairy company following implementation of GMP.

According the Egyptian Standard ES (1185-2005) for ice cream, 16.67% and 10% of examined samples were unsatisfactory for *Staphylococcus aureus* and *Escherichia coli*, respectively before HACCP implementation, while after implementation of the system all final product samples were classified as satisfactory due to absence of *Staphylococcus aureus* and *Escherichia coli* from all examined samples. This may indicate a correct pasteurization. Our results are in agreement with the findings of [36, 37] who examined 351 and 339 of ice cream samples respectively and did not isolate coagulase positive staphylococci, however, these results were in disagreement with the results of [38].

Although, the microbiological criteria confirmed the effectiveness of prerequisites and HACCP system, 3.33% of the ice cream samples exceeded the established limits for coliforms (Table 5). Higher coliform counts were detected by [14] who found 12 % of examined ice cream samples were not in the line with the microbial criteria. They attributed the unsatisfactory limits of coliforms by post-contamination resulted from small failures in some aspects of the system of process management which need

continue working to improve the hygiene and cleaning to ensure consumer safety.

In conclusion, implementation of HACCP system is an effective method for ensuring the safety and consumer satisfaction of the food products. This will strengthen the company's positions and improves their competitiveness. The Continual evaluations of the microbiological quality give important information of efficiency of HACCP design and the correct implementation of its principles.

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Table 1: HACCP Plan for the Ice Cream Production Line

CCP	Hazards	Control Measures	Critical limits	Monitoring Procedure	Corrective Action	Verification
Pasteurization CCP 1	Survival of pathogens (Bacillus cereus, Listeria monocytogenes, and Salmonella, etc.	Properly pasteurization for killing vegetative pathogens to make the product safe and high in quality	Temperature set at 85°C. / 20 seconds.	Check thermometer/ time (flow meter) - Check equipment properly running - Supervisor managing and record keeping	If ice cream mixture doesn't fulfill the critical limits, it must be re-pasteurized once again.	Review records
Aging CCP 2	Recontamination	-Rapid chilling of the ice cream mixture at 4°C and keeping it during the aging step is important, so micro-organisms are unable to grow.	<4°C	-Record Thermometer	-Discarding or Re-pasteurization	-Review records
Labelling CCP 3	Allergenic ingredients	- labeling instruction	- complete instruction on label	-Visually monitored by personnel as the correct labeling.	-If ice cream has been packed with any missing instruction, it has to be discarded.	-Records on labeling instruction and review records -review customers complaints

Table 2: Total Bacterial Counts, Coliform Counts and *Staphylococci* counts of Hand Workers Swabs Before and After Implementation of HACCP System

Samples of handworker swabs (n''= 90).	Before implementation of HACCP system	After implementation of HACCP system	Percentage of reduction Calculated to mean	Significance value
1. Total bacterial count	4.81± 4.1*	3.11± 2.2	98.009	0.00**
2. Coliforms	1.53± 0.5	<1	100	0.00
3. <i>Staphylococci</i> count	1.22±0.3	<1	100	0.00

NOTE: n'': number of the Samples; * Mean log (10)±SE; **The Mean Difference is Significant at the 0.05 Level

Table 3: Total Bacterial Counts and Coliform Counts of Surfaces Swabs Before and After Implementation of HACCP System

Samples of Surfaces swabs (n''=80).	Before implementation of HACCP system	After implementation of HACCP system	Percentage of reduction Calculated to mean	Significance value
1. Total colony count	4.78± 4.1*	3.18±2.3	97.509	0.00**
2. Coliform	2.03± 1.1	<1	99.299	0.00

Table 4: Total Bacterial Counts, Coliform Counts and Yeast & Mold Counts of Ice Cream Samples Before and After Implementation of HACCP System

Samples of Ice cream samples(n''=90)	Before implementation of HACCP system	After implementation of HACCP system	Percentage of reduction Calculated to mean	Significance value
1.Total colony count	7.86± 7.5*	3.37± 2.5	99.996	0.00**
2. Coliform	1.68± 0.5	<1	98.375	0.00
3. Yeast & mold counts	1.64±0.5	<1	97.417	0.00

NOTE: n'': Number of the Samples; * Mean Log (10)±SE; **The Mean Difference is Significant at the 0.05 Level

Table 5: Acceptability of Ice Cream Samples (Final Product) According Egyptian Microbiological Standards No. 1185/2005

NOTE: n'': Number of the Samples; * Mean log (10)±SE; **The Mean Difference is Significant at the

The Examined Organisms	Egyptian Microbiological Standards	Before implementation				After implementation			
		Acceptable		Unacceptable		Acceptable		Unacceptable	
		No.	%	No.	%	No.	%	No.	%
<i>Total bacterial counts</i>	<1.5×10 ⁴ cfu /g	40	44.44	50	55.56	90	100	0	0
<i>Coliforms count</i>	<10 cfu /g	25	27.78	65	72.22	87	96.67	3	3.33
<i>Staphylococcus aureus</i>	absent in 1 g	75	83.33	15	16.67	90	100	0	0
<i>Escherichia coli</i>	absent in 1 g	81	90	9	10	90	100	0	0

0.05 Level

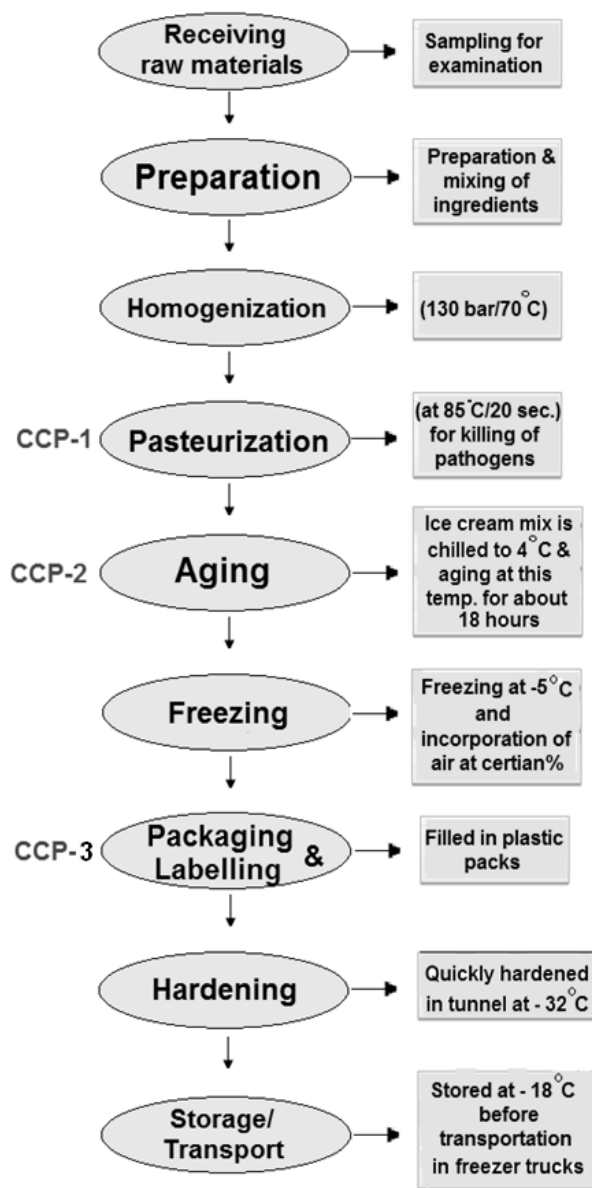


Figure 1: Flow Diagram of Ice Cream Line Production