

Application of quality assurance programs in small dairy plants

Abstract

Hazard Analysis and Critical Control Points (HACCP) is one of the most important systems in quality assurance programs, HACCP system is a plant-specific and product-specific quality system. So, application of HACCP system plan in small-scale cheese plant of fresh Domiati cheese was investigated to identify microbiological and chemical hazards and determine the Critical Control Points (CCPs). The results indicated that the raw milk was the most hazardous and very important CCP as a raw material, because it contained high total bacterial count (2.5×10^8 cfu/ml) and high total fungi (8.5×10^5 cfu/ml) as well as pathogenic bacteria such as *Campylobacter* and faecal coliform. Also, manufacture processing steps; raw milk receiving, pasteurization and cheese storage steps were the CCPs used to eliminate, prevent or minimize different hazards. The total bacterial count in the final cheese product decreased from 2.1×10^8 to 3.1×10^6 cfu/g after application of HACCP system. Also, total fungi, total and faecal coliform and *Campylobacter* were not detected in the final cheese product, which become acceptable according to the Egyptian Standards after HACCP application. On the other hand, pathogenic microorganisms; *Pseudomonas aeruginosa*, *Bacillus cereus*, *Yersinia enterocolitica*, *Salmonella* spp and *Listeria monocytogenes* were not detected at all before and after application of HACCP system. All guidelines for the implementation of HACCP system including a flow diagram of processing steps, identifying hazards, controlling at different CCPs through monitoring and corrective actions and verifying the HACCP plan as well as record keeping were also established.

Keywords: HACCP, domiati cheese, dairy product, pathogenic bacteria, quality assurance

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Abbreviations: HACCP, hazard analysis critical control points; FDA, food and drug administration; CCPs, critical control points; NASA, national aeronautics and space administration; MPN, most probable number; TBA, thiobarbituric acid

Introduction

Traditional quality assurance programs and facility inspections have proven to be inadequate in controlling many food-borne illnesses.¹ Also, results of the WHO surveillance program² indicate that the number of causative agents of food-borne diseases continues increase. The best way to achieve disease reduction and food product quality assurance is through implementation of the preventative system of Hazard Analysis Critical Control Points (HACCP) from production to consumption of dairy products.³ HACCP is a system that was developed for assuring pathogen free foods for the space program by the Pillsbury Company, the U.S. Army and the National Aeronautics and Space Administration (NASA) in the 1960s. Food and Drug Administration (FDA) has considered developing regulations of HACCP system for the dairy industry.^{1,4}

The HACCP system is a systematic approach to the identification and assessment of risk and use preventative actions to control of the microbiological, chemical and physical hazards associated with each segment of the food chain from the production to consumption following the seven basic principles, which are used to develop and implement a HACCP program as follows: Analyze hazards, determine Critical Control Points (CCPs), establish critical limits for CCPs,

monitor CCPs, take corrective action, record keeping and verify that the system is working.⁵ The goal of these CCPs is to ensure that food safety hazard can be prevented, controlled, reduced or eliminated. Most of the hazards are brought in by raw materials.⁶ Cheese is the most popular dairy products in the world, produced in a great range of types and forms throughout the world countries.⁷ Most large cheese manufactory companies have implemented HACCP system to produce safe and good quality product. HACCP is a plant-specific and product-specific quality system.⁶

This work aims to study application and implementation of HACCP system plan as a food safety tool during fresh Domiati cheese production in Dairy Technology Unit, Faculty of Agriculture, Cairo University as a small-scale cheese plant, to identify microbiological and chemical hazards and determine the Critical Control Points (CCPs) in fresh Domiati cheese production line.

Materials and methods

Samples

Processing steps of cheese production line were followed by collection samples at each step; also samples were taken from the raw materials such as; raw milk, salt and rennet enzyme as well as the water supply. The samples were collected in sterilized containers and transferred directly to the laboratory and analyzed without delay for microbiology, but preserved in freezer for chemical tests. Different swabs were taken from the equipments, utensils, work surfaces and food handlers during production processes.

Manufacturing technique of fresh domiati cheese

Manufacture is illustrated in flow diagram (Figure 1) and could be summarized as follows: Raw milk was received and kept at 4°C and standardized. Then milk was pasteurized at 72°C for 15sec and cooled to 40°C, then salted with 3% salt (NaCl). Rennet enzyme was added to the milk which kept at 40°C for 2hr for protein coagulation and curd forming. After coagulation, the curd was cut and pressed for 24hr to remove the whey. Cheese was cut and packaged and stored at 5°C for 1-2weeks until consumption.

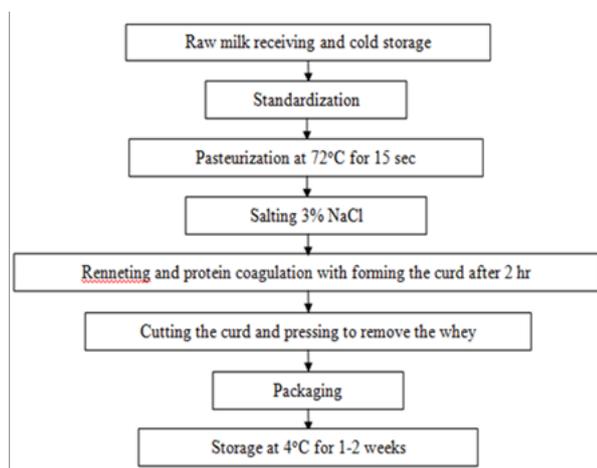


Figure 1 Flow diagram of fresh Domiati cheese manufacturing steps carried out in Dairy Technology Unit, Faculty of Agriculture, Cairo University.

Microbiological determinations

Serial dilutions were directly prepared from the liquid samples such as milk, whey, water and rennet enzyme, but with the solid samples such as salt, cheese curd and cheese product, ten grams of the sample were mixed in a blender with 90ml of saline to obtain the first dilution. Samples were examined for counting of some microbial groups as well as the detection of some pathogenic bacteria.

Counting of microbial groups: Each group was mentioned in relation to its specific medium and the technique of determination.

- Total viable bacterial count, Trypticase soy agar medium+0.6% yeast extract,⁸ pour-plate method (cfu/ml or g) at 30°C for 48hr.
- Total spore-forming bacteria, Trypticase soy agar medium+0.6% yeast extract,⁸ pour-plate method (cfu/ml or g) at 30°C for 48hr with pasteurization the dilutions at 80°C for 15min.
- Total fungi, Rose Bengal chloramphenicol agar medium,⁸ pour-plate method (cfu/ml or g) at 25°C for 5-7days.
- Total coliform, MacConkey broth purple medium,⁹ most probable Number (MPN) technique at 37°C for 24hr.
- Faecal coliform, MacConkey broth purple medium,⁹ most probable Number (MPN) technique at 44.5°C for 24hr.

Counting of some pathogenic bacteria: Some of pathogenic bacteria can be counted directly using specific selective culture media.

i. *Pseudomonas aeruginosa*

Asparagine enrichment broth medium¹⁰ could be used for counting of *Pseudomonas aeruginosa* by using Most Probable Number (MPN) technique at 35°C for 48hr. The positive tubes, where presence of a green blue fluorescent pigment.

ii. *Bacillus cereus*

It could be counted using *Bacillus cereus* selective agar medium¹¹ and pour plate technique (cfu/ml or g) at 37°C for 48hr. *B. cereus* colonies are characterized as 5mm in diameter, turquoise blue in color, surrounded by distinct opaque zone of egg yolk precipitation with the same color.

iii. *Staphylococcus aureus*

Staphylococcus aureus cells were counted by pour plate technique using Vogel - Johnson agar medium.¹² Plates were incubated at 37°C for 48hr with the examination after 24hr. typical colonies appeared as black colonies surrounded by a yellow zone.

iv. *Yersinia* spp

Cefsulodin Irgasan Novobiocin agar¹³ was used for counting of *Yersinia* spp. using pour plate technique with spreading inoculation (cfu/ml or g) at 22-32°C for 24-48hr. Colonies of *Yersinia* appeared dark purple (bull's-eye) with translucent edge sometimes surrounded by precipitated bile zone.

Detection of some pathogenic bacteria: Some of pathogenic bacteria can be detected and isolated using selective procedures.

a. *Salmonella* spp

Bottle of selenite cystine broth medium¹⁴ was inoculated with 10-25ml or g of the sample and incubated at 37°C for 24hr. Full loop from this bottle was streaked onto plates of XLD agar medium,¹⁵ then plates were incubated at 37°C for 24-48hr. Typical colonies of *Salmonella* spp. were red colonies with black centers.

b. *Listeria monocytogenes*

Detection and isolation procedures of *Listeria* spp. were followed according to the method of IDF.¹⁶ It is a provisional International Dairy Federation recommended method for milk and milk products and the following steps were applied: Twenty -five ml or g of the product were added to 225ml of *Listeria* enrichment broth medium,¹⁷ then mixed and carefully homogenized and incubated at 30°C for 48 hours. A loopfull of enriched culture was streaked onto plates of Agar *Listeria* ottavian & agosti (ALOA) medium¹⁸ and incubated at 37°C for 48hr. Colonies of *Listeria monocytogenes* appeared light blue surrounded by an opaque halo, while, other *Listeria* spp. appeared light blue without opaque halo.

c. *Campylobacter* spp

Detection method of *Campylobacter* spp. was followed according to Oosterom et al.¹⁹ Two ml or g of the sample were put in 20ml of supplemented Thioglycollate broth medium and incubated at 37°C for 24hr under microaerophilic conditions (6% O₂ and 10% CO₂). One loopfull of the broth was streaked on supplemented Skirrow's agar. The plates were incubated at 43°C for 48hr under microaerophilic conditions. *Campylobacter* spp. colonies were small grey flat colonies with irregular edge.

Chemical analysis

i. Aflatoxins

Aflatoxin M1 was determined by Thin-Layer Chromatographic method according to AOAC Official Methods of Analysis.²⁰

ii. Thiobarbituric acid (TBA)

TBA test was used to determine the amount of TBA reactive

substances as indicator for oxidized fat in the milk and cheese samples.²¹

iii. Heavy metals

Heavy metals (Cd, Se, Pb and Co) were determined using Atomic Absorption method according to AOAC Official Methods of Analysis.²²

Statistical analysis

All samples have been taken in triplicate. The average (X) and standard deviation (SD) were calculated.

Developing the HACCP Plan

Hazard analysis and determination of critical control points of the raw materials (Figure 2) and processing steps (Figure 3) were carried out according to Mortimore & Wallace.²³

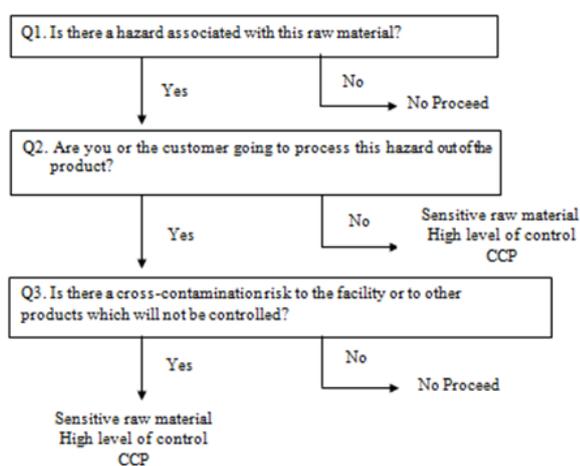


Figure 2 Diagram of decision tree to identify CCPs of the raw materials.²³

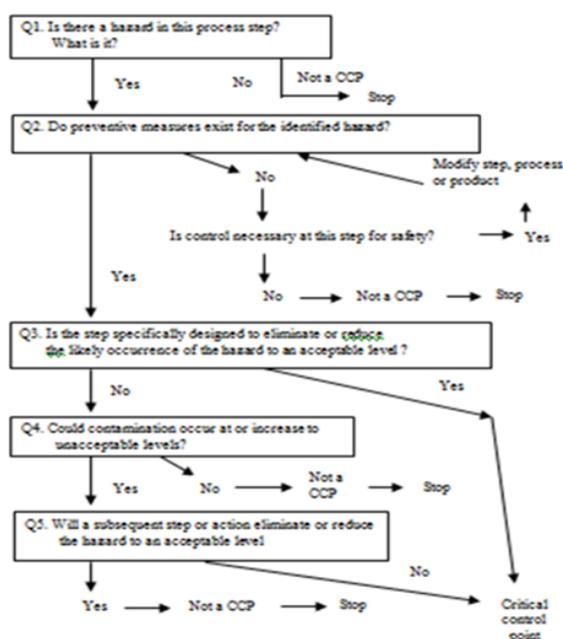


Figure 3 Diagram of decision tree to identify CCPs of processing steps.²³

Results and discussion

Application of Hazard Analysis and Critical Control Points (HACCP) program in fresh Domiati cheese production line:

Conduct hazard analysis

The hazard analysis for soft cheese manufacture is to identify different hazards in the various raw materials and steps of processing and consideration of control measures for the hazards.²⁴

Biological hazards: Raw materials are considered the main source of biological hazards and the raw milk is the most important source of pathogenic bacteria. Table 1 shows the average of microbiological analysis of raw materials, where the raw milk contained high load of total bacterial count 2.5×10^8 cfu/ml and contained 1.2×10^3 and 8.5×10^5 cfu/ml spore-forming bacteria and total fungi. Also, total and faecal coliforms were found 4.5×10^3 and 2.5×10^3 /ml, this result indicate that the production of milk lack hygienic practices. In the same direction, Aboul-Khier et al.,²⁵ reported that total coliform occurred in all of 40 raw milk samples ranged from 6.0×10^5 to 1.2×10^6 /ml, but faecal coliform detected only in 85% of samples ranged from 2.4×10^4 to 3.4×10^4 /ml in Sohage city, Egypt. On the other hand, Campylobacter was detected in raw milk, but Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus, Yersinia enterocolitica, Salmonella spp and Listeria monocytogenes were not detected in raw milk samples which were acceptable according to Egyptian Standards.²⁶ On contrary, Listeria monocytogenes was recovered from 2% and 4% of cow and goat milk samples, respectively, from Mansoura city, Egypt.²⁷ On the other hand, although rennet enzyme contained 8.3×10^6 , 1.0×10^2 and 1.2×10^6 cfu/ml total bacterial counts, spore-forming bacteria and total fungi, respectively, and 2.5×10^2 total coliform count/ml, but it was not in the same importance of raw milk as hazards source, because pathogenic bacteria were not detected in rennet enzyme, which also added in small amount for coagulation. On contrary, salt and water contained low counts of microorganisms.

Equipments, utensils, work surfaces and food handlers are very important sources for microbial contamination of cheese product during processing steps, so swabs from containers, utensils, food handlers, tables, walls, packaging material and refrigerator were microbiologically investigated. The results (Table 2) indicated that, hazardous pathogenic bacteria were not detected in all swabs except Campylobacter that was detected from utensils, tables, walls and food handlers which contained total bacterial counts around 10^4 cfu/cm², while the packaging material has the lowest total bacterial count 2.2×10^6 cfu/cm². Total fungi were found in all swabs in counts from 1.1×10^1 to 4.8×10^3 cfu/cm². Spore-forming bacteria not detected in packaging material and food handlers swabs, but found in other swabs between 2.1×10^1 to 5.7×10^3 cfu/cm². Also, total coliform was not found in walls and refrigerator swabs and found in other swabs in few numbers, but faecal coliform not detected in all swabs.

Manufacture processing steps affect the microbial load of the cheese product; (Table 3) shows the microbiological analysis during processing steps of cheese before HACCP system application. After receiving of raw milk, the microbial load was not affected by standardization of milk composition step, but pasteurization was the most effective step to reduce the microbial counts and destroy pathogenic bacteria, where total bacterial count and spore-forming bacteria decreased from 1.2×10^8 and 1.1×10^3 to 1.5×10^4 and 1.3×10^2 cfu/ml, also fungi, coliform and Campylobacter were

completely destroyed, but *Staphylococcus aureus* appeared after pasteurization with 7.2×10^2 cfu/ml. During salting, renneting and coagulation, cutting the curd and removing the whey and packaging steps, the milk and cheese curd were contaminated by fungi, coliform,

Staphylococcus aureus and *Campylobacter* from raw materials (salt and rennet enzyme), equipments, utensils, work surfaces and food handlers. The growth of these microorganisms during these steps caused an increase in microbial counts.

Table 1 Microbiological examination of raw materials used in cheese manufacture ($X \pm S_b$).

ND, not detected; +ve, positive; -ve, negative

Microbiological tests	Raw materials			
	Raw milk	Salt	Rennet enzyme	Water
Total Bacterial Count (cfu/ml or g)	$2.5 \times 10^8 \pm 2.1 \times 10^8$	$8.7 \times 10^3 \pm 1.5 \times 10^3$	$8.3 \times 10^6 \pm 1.7 \times 10^6$	$5.2 \times 10 \pm 4.1 \times 10$
Spore-Forming Bacteria (cfu/ml or g))gg)	$1.2 \times 10^3 \pm 1.1 \times 10^3$	$4.0 \times 10^3 \pm 3.5 \times 10^3$	$1.0 \times 10^2 \pm 0.5 \times 10^2$	$1.0 \times 10 \pm 0.7 \times 10$
Total Fungi (cfu/ml or g)	$8.5 \times 10^5 \pm 1.5 \times 10^5$	ND	$1.2 \times 10^6 \pm 0.9 \times 10^6$	$1.0 \times 10 \pm 0.5 \times 10$
Total Coliforms /ml or g	$4.5 \times 10^3 \pm 3.2 \times 10^3$	ND	$2.5 \times 10^2 \pm 1.7 \times 10^2$	ND
Faecal Coliforms /ml or g	$2.5 \times 10^3 \pm 1.1 \times 10^3$	ND	ND	ND
<i>Pseudomonas aeruginosa</i> /ml or g	ND	ND	ND	ND
<i>Bacillus cereus</i> (cfu/ml or g)	ND	ND	ND	ND
<i>Staphylococcus aureus</i> (cfu/ml or g)	ND	ND	ND	ND
<i>Yersinia enterocolitica</i> (cfu/ml or g)	ND	ND	ND	ND
<i>Salmonella spp</i>	-ve	-ve	-ve	-ve
<i>Listeria monocytogenes</i>	-ve	-ve	-ve	-ve
<i>Campylobacter spp</i>	+ve	-ve	-ve	-ve

Table 2 Microbiological examination of the swab samples taken from different locations in production line

ND, not detected; +ve, positive; -ve, negative

Microbiological tests	Swabs						
	Containers	Utensils	Food handlers	Tables	Walls	Packaging material	Refrigerator
Total Bacterial Count (cfu/cm ²)	2.2×10^2	3.4×10^4	1.1×10^4	2.6×10^4	7.2×10^4	2.2×10	3.1×10^2
Spore-Forming Bacteria (cfu/cm ²)	1.3×10	2.4×10^2	ND	5.7×10^3	2.5×10^3	ND	2.1×10
Total Fungi (cfu/cm ²)	5.1×10	2.1×10	1.1×10^2	4.8×10^3	2.3×10^2	1.1×10	2.1×10^2
Total Coliforms /cm ²	4.0×1	1.1×10	2.5×10	3.0×10	ND	0.6×1	ND
Faecal coliform/cm ²	ND	ND	ND	ND	ND	ND	ND
<i>Pseudomonas aeruginosa</i> /cm ²	ND	ND	ND	ND	ND	ND	ND
<i>Bacillus cereus</i> (cfu/cm ²)	ND	ND	ND	ND	ND	ND	ND
<i>Staphylococcus aureus</i> (cfu/cm ²)	ND	ND	ND	ND	ND	ND	ND
<i>Yersinia enterocolitica</i> /cm ²	ND	ND	ND	ND	ND	ND	ND
<i>Salmonella spp</i>	-ve	-ve	-ve	-ve	-ve	-ve	-ve
<i>Listeria monocytogenes</i>	-ve	-ve	-ve	-ve	-ve	-ve	-ve
<i>Campylobacter spp</i>	-ve	+ve	+ve	+ve	+ve	-ve	-ve

Finally, after storage step, Domiati cheese (Table 3) contained 2.1×10^8 , 2.3×10^2 and 6.7×10^5 cfu/g total bacterial count, spore-forming bacteria and total fungi, respectively, and faecal coliform count 1.4×10^4 /g, this result is in agreement with those of Said and Fahmy²⁸ who isolated cells of *Escherichia coli* from 32 samples out of 50 samples of Domiati cheese collected from Assiut city, Egypt. The result indicated that, the final cheese product was unacceptable according to Egyptian Standards.²⁹ *Campylobacter* was isolated from the final cheese product, but *Pseudomonas aeruginosa*, *Yersinia*

enterocolitica, *Salmonella* spp, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus* were not detected. On contrary, Said and Fahmy²⁸ detected *Bacillus cereus* and *Staphylococcus aureus* in 48 and 72% of 50 Domiati cheese samples taken from Assiut city, Egypt. From the previous, it is clear that biological hazards were fungi, coliform, *Staphylococcus aureus* and *Campylobacter* which come from raw milk and rennet enzyme as raw materials and food handlers, utensils and work surfaces during manufacture.

Table 4 Chemical hazards of raw materials used in cheese manufacture, cheese product and whey
ND, not determined

Samples	Thiobarbituric acid (TBA) mg/kg	Aflatoxin M ₁ mg/kg	Heavy metals mg/kg			
			Cd	Se	Pb	Co
Raw Milk	11.07	0	Nil	Nil	Nil	Nil
Salt	ND	ND	Nil	Nil	Nil	Nil
Rennet Enzyme	4.029	0	ND	ND	ND	ND
Cheese Curd	0.027	0	Nil	Nil	Nil	Nil
Whey	0.096	0	Nil	Nil	Nil	Nil

Chemical hazards: Food products can be polluted by chemical hazards from raw materials, during manufacture or as a result of chemical spoilage. As shown in Table 4 raw materials; raw milk and salt and cheese curd were free of Cd, Se, Pb and Co heavy metals, also raw milk, rennet enzyme and cheese curd were free of Aflatoxin M₁, but raw milk and rennet enzyme contained 11.070 and 4.029mg/kg Thiobarbituric acid (TBA) as a result of fat oxidation.

Determine critical control points (CCPs)

CCPs are points or areas in a process that are required to control the identified hazards and lack of control is likely to result in unacceptable health hazards.³⁰ CCPs in raw materials and processing line steps (Figure 4) of fresh Domiati cheese were determined according to CCP decision tree (Figure 2) (Figure 3) of Mortimore & Wallace.²³ For the raw materials, it is clear that the raw milk was very important CCP; on the other hand, (Figure 4 & Table 5) show that, raw milk receiving at 4-6°C, pasteurization at 72°C for 15sec and storage of cheese product at 4-6°C steps were the main CCPs in the processing line of fresh Domiati cheese.

Establish critical limits

The critical limits provide the base line for measuring the effectiveness of food safety procedures. These limits are needed to minimize or eliminate significant food safety hazards. Table 5 Summarizes the critical limits for each processing steps, the critical limits for raw milk receiving step were raw milk standards and receiving milk at temperature less than 6°C; for pasteurization step, the heat temperature must not less than 72°C for 15sec and for storage of cheese product step, the storage temperature must not more than 6°C.

Establish monitoring procedures

The temperature should be monitored by a calibrated thermometer

at every step with monitoring the time with some steps such as pasteurization. A number of non continuous monitoring procedures could be used such as rapid platform tests with raw milk receiving step and microbiological and chemical tests for raw materials and packaging material (Table 5).

Establish corrective actions

Corrective actions should be taken when monitoring system indicate that, any of the critical limits was out of control. Corrective actions with every step were summarized in Table 5 such as rejection of contaminated raw materials,³¹ correction and resetting of pasteurizer temperature and time, maintenance or repairing of separator and pasteurizer and discarding the product if contamination was evident.

Record keeping

Documentation is needed to record measurements that show standards are being monitored. Effective HACCP record-keeping plan contains; listing of the HACCP team and responsibilities, description of the food and its intend use, list all regulations that must be met, temperature monitoring logs, flow chart from receiving to consumption and corrective actions. Accurate record keeping is essential part of successful HACCP plan.

Verification of HACCP system working

Verification of HACCP system includes routine calibration of CCPs monitoring system, testing of finished product and random collection of raw materials and end product, and then testing them chemically and microbiologically. The overall HACCP process must be verified periodically to be sure that, the HACCP system is effective and work well. describe the microbiological analysis during processing steps of cheese after HACCP application, the result indicate that, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Yersinia enterocolitica*, *Salmonella* spp and *Listeria monocytogenes* were not detected at

all, while, fungi, total and faecal coliform and *Campylobacter* were not detected after pasteurization step and in the final cheese product which become acceptable according to Egyptian Standards³⁰ after HACCP application. On the other hand, *Staphylococcus aureus* appeared after pasteurization step with 1.1×10^2 cfu/ml, but its count decreased during the next steps to be not detected in the final cheese product. Total bacterial count and spore-forming bacteria in the final cheese product were 3.1×10^6 and 7.2×10^2 cfu/g, in comparison with 2.1×10^8 and 2.3×10^2 cfu/g in the final cheese product before HACCP application (Table 3 & Table 6).

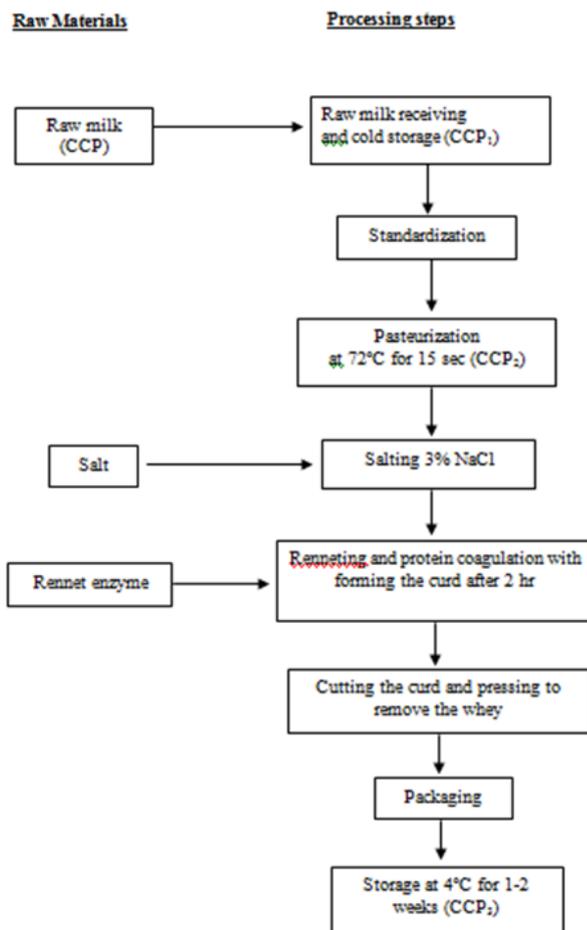


Figure 4 Flow diagrams of the fresh Domiati cheese manufacturing steps and CCPs for controlling hazards.

Conclusion

From the obtained results, it could be concluded that:

- The development and implementation of HACCP program is reliable to secure the safety of food.
- HACCP was developed as a “zero defects” program for food safety.
- HACCP is a plant-specific and product-specific quality system.
- HACCP system can be applied in small-scale cheese plant of fresh Domiati cheese.
- Quality Assurance programs can be applied in small-scale dairy plants.

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None.

Conflict of interest

The author declares no conflict of interest.

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