



A trial for Application of Food Safety Tool (HACCP) on Small Cheese Processing Unit for Reduction of Microbiological and Chemical Contamination.



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THE study was to apply the (HACCP) system guidelines for the first time in a small processing unit of soft cheese production in a small-scale cheese plant in the Agriculture secondary school, Kafr El-sheikh governorate, Egypt, to obtain safe natural produced soft cheese. The (PRPs) were primarily executed. The detection of CCPs and OPRPs was made by applying a decision tree. The HACCP plan was investigated for microbial and chemical hazards. The results reflected that, raw milk was the most hazardous and important control point as a raw material that contains high levels of total bacterial, total coliform, total fungi, and *Staph. aureus* count ($3 \times 10^6 10^3 \times 2.3 \pm \text{CFU/ml}$, $0.72 \times 10^3 \pm 0.2 \times 10 \text{ CFU /ml}$, $1.08 \times 10^4 10 \times 0.9 \pm$, and $2 \times 10^3 10 \times 1.5 \pm \text{CFU/ml}$), as a microbial hazard, respectively. The greatest serious chemical hazard AflatoxinM1 was in all of the examined milk samples by (100%), 10% out of them exceeded PL. (50ppt), subsequently, cheese samples contained AflatoxinM1 by (100%), 30% of them exceeded the PL. Swab test results reflected heavy microbial contamination. *Staph. aureus* was detected from tables, food handlers, and hand washing basins with mean results of $1 \times 10^2 0.3 \pm \times 10$, $2.8 \times 10^4 1.1 \pm \times 10^2$, and $3 \times 10^6 2.3 \pm \times 10^3 \text{ CFU/cm}^2$, respectively. After HACCP application, the total bacterial and fungi count in the cheese product reduced to $0.6 \times 10 \pm 0.2 \times 10$ and $1 \times 10 \text{ CFU/gm}$ respectively, while coliform and *Staph. aureus* were not detected. Heavy metals (Cadmium, Lead, and Arsenic) were not detected. With PRPs application, HACCP system could be applied on small processing units.

Keywords: HACCP, Small dairy plant, Microbial hazard, Chemical hazard, ISO 22000

Introduction

Dairy products consider one of the human diet essential components, but various contaminants can act as causative agents for many food-borne diseases, as microorganisms, chemical pollutants, and toxins [1]. Milk and its products are tangled in 2-6% of foodborne diseases outbreaks in industrialized countries [2].

Food poisoning outbreaks and Economic losses through all the dairy production process

steps have been minimized by the development of food safety management systems (FSMSs) and standards revised by ISO [3,4].

HACCP was standardized by C A C [2]. As a systematic preventive approach for food safety that controls significant hazards and detects each critical point that is important for food safety throughout the food production chain [5]. The HACCP system aims to the prevention of the hazard before controlling and is intended to prevent problems before they occur [6]. Hazard

may be biological or chemical, deriving from raw materials, or finished products [7]. From hazard identification results the critical control points are determined through several parameters that can be assessed then corrective actions can be achieved [8].

HACCP-based systems, including ISO 22000 and various commercial standards have been widely applied in the dairy industry all over the world for cheese of various types [9, 10].

Problems related to *Salmonella enteritidis*, *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes*, have been documented during cheese manufacturing [11].

Additionally, chemical hazards may be found such as heavy metal contamination and Aflatoxin M1 which is a metabolite of aflatoxin B1 when ingested by dairy cattle through contaminated food and known to be carcinogenic in humans. The action level of aflatoxin M1 in milk that is strictly enforced by the (FDA) is 0.5 parts per billion; in addition, EU imposed an action limit of 0.05 parts per billion in milk [12].

HACCP was promoted as a microbiological safety system by Pillsbury Company in the 1960s, to ensure food safety for astronauts with NASA, while food safety systems at that time were based only on testing the end product, which was an unskillful way due to product waste. So, a preventative method gives a high food safety confidence needed to be developed [13].

HACCP system is now accepted as a safety system for food management worldwide as food safety awareness and interest have increased in both developed and developing countries so efforts are intensified by governments and various food processing industries for food safety improvement [14].

It is recognized as essential for FSMS and necessary pin down PRPs (Pre-requisite Programs) and GMPs (Good Manufacturing Practices) measures for additional hazards assessment and application of control measures before applying the FSMS [15].

All organizations can use HACCP in the food chain from farming to food services to cover all processes that impact the safety of the end product [16]. Milk and its products are fundamental components in the food supply chain and readily consumed by virtually all populations [17].

Possible contamination by microbiological hazard would affect product quality by spoilage micro-organisms like (molds) and hygiene indicator microorganisms such as (coliforms) [9]. Numerous studies have been applied for the HACCP implementation on a variety of cheeses [18]. The principles of Codex HACCP were included in all Food Safety Management Systems [2].

Codex HACCP was strengthened by adding three groups linked together: PRPs (prerequisite programs), OPRPs (operational prerequisite programs), and CCPs (Critical Control Points) [19]. ISO: 22000 is designed to be used by any organization (small or big-scale) within the food chain and demonstrates how to combine the hazard control plan (OPRPs and/or CCPs) with PRPs into a single integrated food safety hazard control system [20]. HACCP system applied for identification, and controlling hazards [21]. So the aim of this study is to apply a preventive system (HACCP) as a tool for ensuring food safety by conducting hazard analysis through integration of some microbiological and chemical hazards in (HACCP) system as safety parameters, identifying CCPs, monitoring, and corrective actions based on detected CCPs and result from documentation before and after HACCP application in small-scale cheese processing plant in agriculture secondary school, Kafr El-sheikh governorate, Egypt, to obtain efficient produced and safe natural soft cheese, with delivering training tool of FSMS for the first time on small-scale unit .

Materials and Methods

Working-out of PRPs as described by Baraka [22].

Some PRPs should be set up first; the corrective measures for improving the adaptation to PRPs were implemented by the HACCP team for improving (GMPs and GHPs). Production chain from farm to the final consumer was considered, habits for the proper and correct handling of foods, and better working environment were established.

Working - out the HACCP Plan: according to Kamboj et al.[23].

The twelve steps for developing the HACCP plan (Table 1)

Food safety team (HACCP team)

The research team was created to implement the requirements of the system. The members of the team were trained on the HACCP system and ISO 22000:2018 standard.

TABLE 1. HACCP principles and application steps (plan of HACCP).

Step 1	Assemble HACCP ¹ team (Food safety team)
Step 2	Describe product
Step 3	Identify intended use of the product
Step 4	Construct flow diagram of production process
Step 5	On-site confirmation of flow diagram
Step 6. Principle 1	conduct a hazard analysis, List all potential hazards, and consider control measures
Step 7. Principle 2	Determine CCPs ²
Step 8. Principle 3	Establish critical limits for each CCP
Step 9. Principle 4	Establish a monitoring system (Monitoring systems at CCPs and for OPRPs)
Step 10. Principle 5	Establish corrective actions
Step 11. Principle 6	Establish verification procedures
Step 12. Principle 7	Establish documentation and record-keeping

HACCP¹, CCPs.² according to Mureşan et al. [4].

Description and the intended use of the product

The product was described as follow:

Natural soft cheese (soft cheese category, ready-to-eat product). Moisture contents (65-75%).

Plastic bags are used as packaging material.

The shelf life (7days) depends on cheese quality, safety, storage, and transportation practices.

Product Name: White cheese

Product size: 1000 gm.

Distribution and storage: At a temperature of 4°C.

Consumer Requirements: Fresh in the cooling temperature and security of contaminants.

Intended Consumers: Consumers of all ages.

The ingredients: fresh milk, rennet, sodium chloride.

Shelf life: 7 days

Product user: Grocery store at the same layout of the plant.

Staphylococcus aureus / 1gm: <100 CFU/gm

Coliform / 1gm: <1000 CFU/gm

Yeast / 1gm: <1000 CFU/gm

Mold / 1gm: <10 CFU/gm Source [6]. (Milk

and soft cheese)

Flow chart (Diagram) establishing and verification

All stages of the process for making soft cheese were included in (Fig. 1).

This chart is a prerequisite for a detailed presentation of the conditions that could affect the safety of the product [24].

The flow diagram was checked on-site by the members of the HACCP team.

Identification of the hazards

According to the hazard nature biological or chemical, the probability of occurrence and severity level is done (Table 2), and then the rating of hazard is calculated [4].

The detection of CCPs

This was achieved by the decision tree pattern in Fig. 2, to identify control measures Based on the risk assessment results, and after using the decision tree, the standard efficiency points (CCP) or (OPRP) will be established [21].

Sampling

Through different steps of the cheese

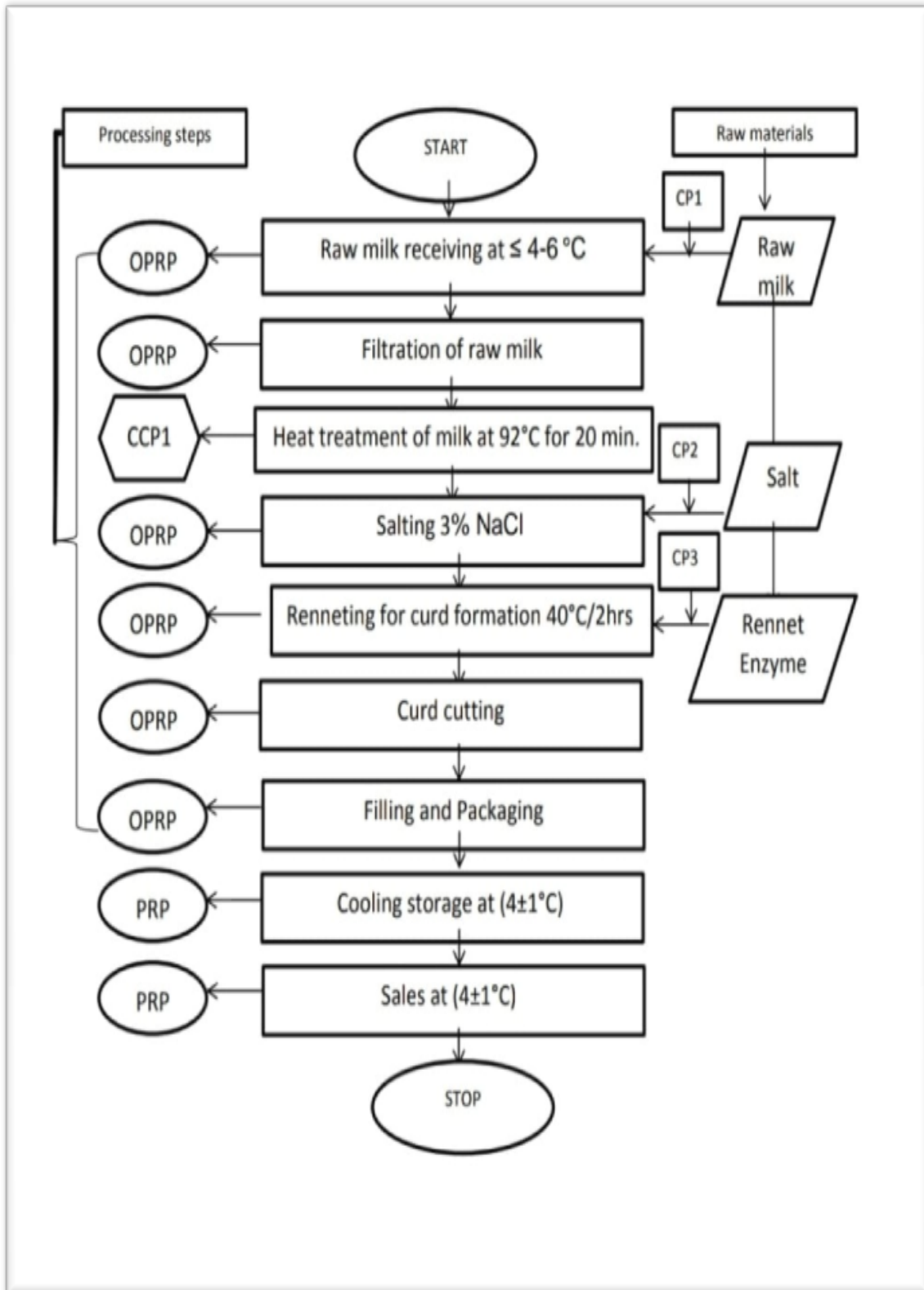


Fig. 1. Flow diagram of fresh soft cheese manufacturing steps carried out in Dairy Technology Unit, Agriculture school with CCPs and OPRPs inside the process regarding CPs, and PRPs out of the process related to the study.

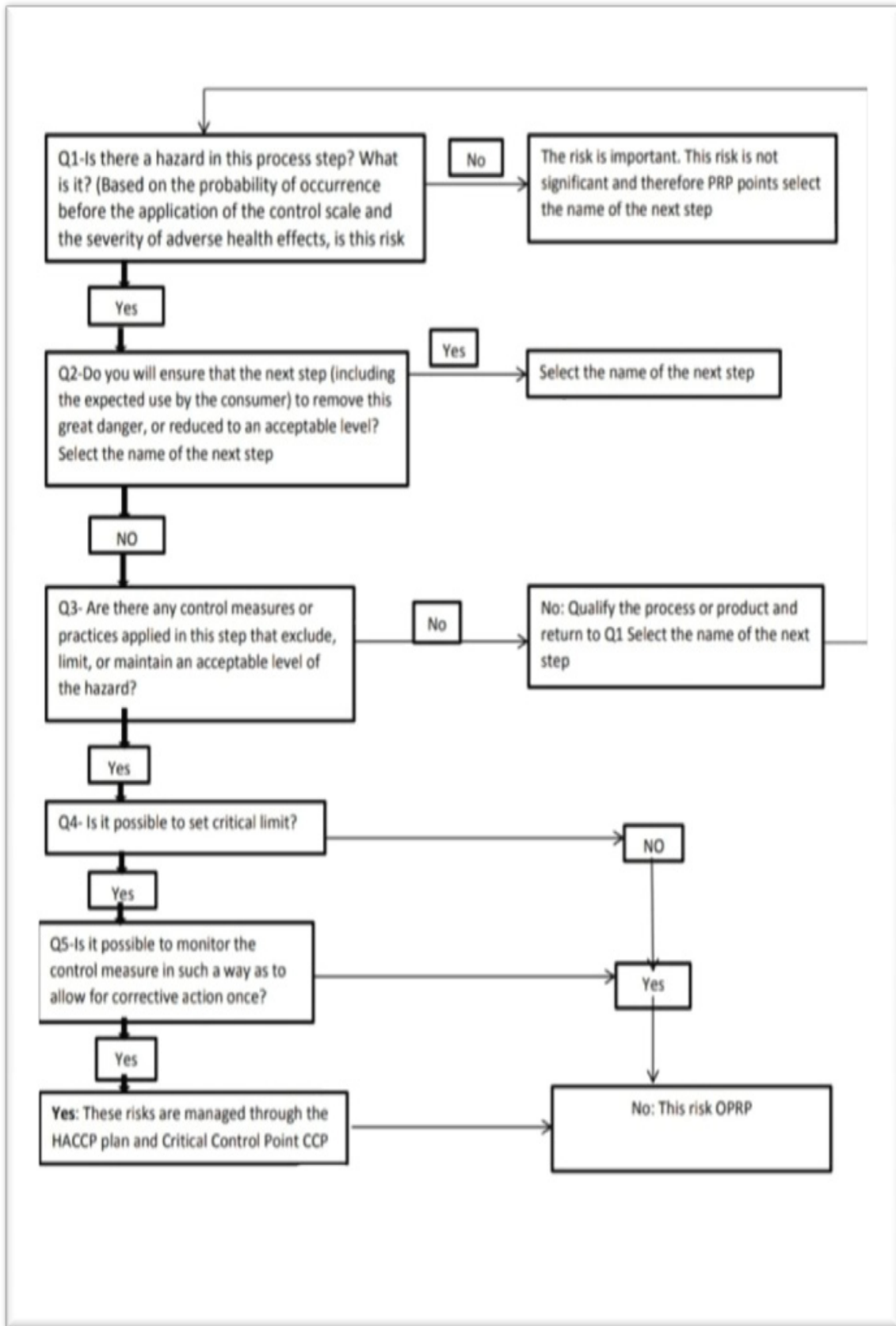


Fig. 2. Diagram of decision tree to identify CCPs.

TABLE 2. Probability (likelihood) of Hazard occurrence and level of severity. High-risk Controlled by HACCP/CCPs or OPRPs. Adapted according to. Mureşan et al. [4].

Level of risk	Probability of the hazard	Severity of the hazard
High risk (3)	Highly probable: known history in the sector.	Life-threatening or long-term chronic illness: (e.g., infection, intoxication, or anaphylaxis), Chronic effects or death.
Medium risk (2)	Could occur; minimal history within the sector but has happened.	Injury or intolerance: Not usually life-threatening.
Low risk (1)	Unlikely to occur; no known examples.	Minor or no effect: short duration.

manufacturing process, samples were taken and collected for analysis from 10 lots, before and after HACCP applying, from raw materials such as; raw milk, salt, and rennet enzyme, additionally water supply, samples were taken throughout the ten lots of production (10 samples for each). Swabs also from equipment, utensils, work surfaces, walls, hand washing basins, packaging materials, refrigerators, and food handlers during the production process were taken (80 swabs, 10 swabs for each). Swabs were taken by the swab method according to Stinson and Tiwari [25]. In clean and sterilized plastic bags samples were collected and directly transferred to the laboratory for analysis and examination.

Technique of fresh soft cheese manufacturing

The technique of manufacturing in the flow chart (Fig.1) was verified and indicated that: receiving raw milk was at 4°C followed by the filtration step. Pasteurization was at 85-95 °C for 20-30 min. Then cooling to 40°C, and salting with 3% salt (Na Cl) followed by addition of rennet enzyme at 40°C for 2 h., for curd formation. Cheese curd was cut, packaged, and stored at 4±1°C for one week on the market which is located at the same layout of the small dairy plant under study. This environmental condition could affect the product shelf life. These conditions must be taken into consideration because of their importance for consumer health [24].

Microbiological examination

Preparation of samples: Serial dilutions were prepared from liquid samples (milk, water, and rennet enzyme), and solid samples (salt and cheese), 25ml/gm. were mixed with 225 ml saline in a blinder. Then ten-fold serial dilutions were done for counting some microbial groups.

Microbial count

Each count was occurred according to the spe-
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cific medium and the determination technique of each microbial group.

Total bacterial count [26]

By using standard plate count agar (SPC) one milliliter from each previously prepared serial dilution was aseptically transferred into duplicate sterile Petri dishes. About 10-12 ml of sterile melted and cooled at (45 + 1 °C). SPC medium was poured into each plate and mixed carefully. After solidification, the inoculated plates including the control one (inoculated with sterile distal water) incubated at 32 ± 1 °C for 48 ± 3 h.

Total coliform count [26]

Using the most probable Number technique, One ml from each prepared serial dilution was inoculated into a series of three fermentation tubes of Lauryl Sulphate Tryptose broth (LST) supplemented with inverted Durham's tubes. Inoculated and control tubes were incubated at 35°C for 48 h.

Total fungi count [26]

One ml from each previously prepared serial dilution was transferred into each of the duplicate sterile Petri dishes containing about 10-12 ml of sterile melted and cooled Sabaroud dextrose agar medium at (45 ± 1°C). After solidification, the inoculated plates including the control one (inoculated with sterile distal water) incubated at 21-25 °C for 5-7 days. Total mold and yeast count were obtained by direct counting cultured agar plates multiplying the number with dilution factor.

Count of Staph. aureus [27]

Ten grams from the sample were homogenized with 90 ml 1/4 ringer's solution to make serial tenfold dilution up to 10⁶ from the original dilution (1:10). Only 0.1 ml from each dilution was spread over double plates of Baird Parker using a sterile bent glass spreader then incubated 48hr at 37

°C (opaque black shining convex colonies with narrow white margins and surrounded by clear zone) were counted and the average number per gram was calculated.

Isolation and identification of Staph. aureus [28]

For isolation of *Staph. aureus*, we take (0.1ml) from the mixture of prepared samples and suspended instruments swabs were spread on the surface of Baird Parker agar (BPA) and from air settled plate of Baird Parker agar, medium supplemented with egg yolk tellurite emulsion incubated in buffered peptone water (BPW) at 37 °C for 18-24 h. Black colonies surrounded by whitish halo zone formation on BPA were considered presumptive *Staph. aureus*, confirmed with Gram's staining, coagulase, catalase, and other biochemical tests. Selective isolate enriched culture streaked onto mannitol salt agar, colony chosen for more examination, and presumptive staphylococci were identified according to colony morphology, Gram stain, and coagulase test with rabbit plasma, and biochemical characterization of isolates by Vitek-2 compact (Vitek-2 is bioMerieux, Inc., Durham, NC). Confirmation of *Staph. aureus* was done using API STAPH identification test strips (BioMerieux, Marcy-I, and Etoile, France) according to the manufacturer's instructions.

Isolation and identification of Bacillus cereus [29]

Use selective media mannitol-egg yolk-poly-myxin (MYP) gives colonies with a violet-red background and surrounded by a zone of egg-yolk precipitate [30].

E. Coli O157:H7 isolation and identification [31, 21]

Ten gm. Or ml. of the prepared sample were homogenized with MacConkey broth and then incubated at 37°C overnight. One ml of the incubated samples in enrichment broth were streaked out onto specific media: consisting of Cefixime (0.05mg/l), Potassium tellurite (2.5mg/l) Sorbitol MacConkey agar (C-T SMAC), then overnight incubation at 37°C, gives delayed fermentation of D-sorbitol within more than 99%, and resists several antibiotics and antimicrobial agents, cultured on Sorbitol - MacConkey agar with Cefixime and Potassium tellurite (C-T S MAC). Positive colonies appear either pale or colorless colonies.

Salmonella isolation and identification [26]

From the prepared sample, 25 gm. was aseptically added to 225mL. sterile buffered peptone water, thoroughly mixed, at 24 ± 2 h. at 35 ± 1°C, then 1 mL inoculated into sterile tubes

containing 10 mL Selenite F broth and incubated at 35 ± 1 °C for 24 ± 2 h.

A loopful of selective enrichment on 2 plates of Xylose lysine deoxycholate at 35± 1°C for 24 ± 2 h. Suspected Colonies were purified for further identification according [32]. Suspected colonies appeared as red colonies with or without black center identified biochemically and serologically. And confirmed using API strips (BioMerieux, Mary- I' Etoile, France). In general, serological identification according to Kauffman - White scheme [33]. Was done, using Salmonella antiserum (DENKA SEIKEN Co., Japan)

Listeria monocytogenes isolation and identification [34]

Sample (25 ml. or gm.) were added to 225ml. of BLEB, three selective agents aseptically added to attain final concentrations of 10 mg/L acriflavine, the antifungal agent 40 mg/L cycloheximide, and 50 mg/L sodium nalidixic acid in the BLEB with pyruvate pre-enrichments, the enrichment mixed with additives and incubation at 30°C for 24 - 48 h.

A loopful was streaked onto selective Oxford agar for 24 h., 2 incubations at 35°C typical *Listeria* colonies were 1 mm diameter, gray to black surrounded by a black halo. Typical *Listeria* species colonies are nearly 2-3 mm diameter, black with a black halo and sunken center.

Chemical examination

Aflatoxin M1 determination in milk and cheese samples [35]

ELISA assays are widely used for the detection of aflatoxin M1 in milk with high sensitivity and selectivity. So according to the official method of Analysis using, the Max Signal Aflatoxin M1 ELISA Kit we validate the accuracy and precision of the kit (Cat# FOOD-1060-05) at the EU MRL of 0.05 ppb [12]. Using, (high quantity repeat of ELISA platform technician hands-on time) for the screening of contaminated milk samples.

Heavy metals

Lead (Pb), cadmium (Cd), and arsenic (As) were determined using the Atomic Absorption method according to Chen et al. [21]. By (FAAS) all measurements, blanks, triplicate measurements of elements in extracts and analysis of certified reference materials for each metal (Merck) were routinely included for quality control. Samples have been taken in triplicate. The average and standard deviation were calculated.

Results and Discussion

Application of PRPs.

According to Kauffman [33]. PRPs are the primary theoretical programs for security bases establishment that provide foundations for HACCP. So, all PRPs programs were evaluated.

(OPRPs) [19]

OPRP is an operational prerequisite program to control a non-measurable significant hazard. It is a control measure, but not considered a CCP, and could be identified through risk assessments. Through determining what can be measured and then determining how. They should be controlled just like a CCP (identifying, monitoring, verification, etc.). And they should reduce the probability of exposure to a hazard or other contamination sources, such as sanitation, hand washing and Glass/metal control but without defining a critical limit but has action criteria.

Implementation of HACCP guidelines on the fresh white soft cheese production line (Steps 7-12) Table 1 [22].

Conduct a hazard analysis

The steps of processing from raw material reception to product sale were analyzed, and consideration any measures to manage the identified hazards were taken.

Biological hazards

The microbiological analysis of raw materials and cheese curd illustrated in Table 3 reflect the high load of total bacterial count in raw milk as a mean of $\times 10^6 \pm .3 \times 10^3$ CFU/ml and contained $0.72 \times 10^3 \times 10 \pm 0.2 \pm$ and $1.08 \times 10^4 \times 10 \pm 0.9 \pm$ CFU/ml for total coliform and total fungi respectively. Staph. aureus count was found as $2 \times 10^3 \times 10 \pm 1.5 \pm$ CFU/ml, which is less than the result dedicated by [36]. As 1×10^2 CFU/ml; this result indicates a lack of hygienic practices while milking production. On the other hand, pathogenic bacteria were not detected. These microbiological results agreed with [28]. Which showed means results of ($2.5 \times 10^8 \pm 2.1 \times 10^8$ CFU/ml, $4.5 \times 10^3 \pm 3.2 \times 10^3$ CFU/ml, and $8.5 \times 10^5 \pm 1.5 \times 10^5$ CFU/ml) for total bacterial count, coliform count, and total fungi count respectively. However, Staph. aureus, B. cereus, Salmonella spp, L. monocytogenes, were not detected. Also, these pathogenic bacteria failed to be detected by Aboul-Khier et al [38]. And this is agreed with the EOS [6]. Abdel-Khalek et al [39]. Could detect L. monocytogenes in raw milk. Salt, rennet enzyme, and water contained microbial count lower than Egyptian standard, additionally free from pathogenic micro-organisms. Thus the main

source of microbial hazard in the raw materials is raw milk which is considered the important source of pathogenic bacteria such as Staph. aureus which exceeds the Egyptian standard.

Table 4 shows the results of different swabs were taken from the equipment, utensils, food handlers, tables, hand washing basins, walls, packaging materials, and refrigerators, which may be the sources for microbial contamination during processing. The pathogenic bacteria were not detected in all the swabs except S. aureus which was detected in tables, food handlers and hand washing basins by mean results of $1 \times 10^2 0.3 \pm \times 10$, $2.8 \times 10^4 1.1 \pm \times 10^2$ and $3 \times 10^6 2.3 \pm \times 10^3$ CFU/cm², respectively. These high results point to the loss of good hygienic practices, shortage in the cleaning and sanitization process.

Total bacterial count and total fungi count, both exceeded the Egyptian standard in all swabs in count from $2 \times 10^4 \pm 1.2 \times 10^2$ to $3 \times 10^6 1.8 \pm \times 10^3$ CFU/cm², except walls have no fungi or coliform. Also, other swabs have no coliform except food handlers and hand washing basins have a total coliform count of $0.3 \times 10 \pm 0.06 \times 10$ and $7.5 \times 10 \pm 1.7 \times 10$ CFU/cm², respectively. This means the probability of the presence of contamination from food handlers to hand washing basins by coliform bacteria, near result dedicated by Nasr et al. [37]. For the total coliform count, but he found total fungi in 100% of swabs by count from 1.1×10 to 4.8×10^3 CFU/cm².

As a result of microbiological contamination of raw materials (Table 3) and swabs before HACCP application (Table 4), the cheese product was subsequently contaminated because processing steps of manufacturing affect the cheese microbial load; the microbiological analysis during processing steps before HACCP application illustrated in Table 3. After pasteurization, the microbial load should be decreased because pasteurization considers the most potent step for reduction of microbial load and killing pathogenic bacteria, therefore we noticed the absence of pathogenic bacteria in cheese, where total bacterial, total coliform, and total fungi count increased by means results of $3 \times 10^7 10 \pm 0.1 \times 10 \times 0.3$, $10^3 \times 1.2 \pm$, and $8.8 \times 10^3 10 \times 1.4 \pm$ CFU/gm. respectively. The total bacterial count is lower than 2×10^8 CFU/gm. that recorded by Eltakra et al. [6]. And the count was higher than (3.1×10^2 CFU/gm.) for total fungi count. This explains cheese contamination during processing steps and handling such as in salting, renneting, and packaging. Or from salt and rennet

enzyme, Or from equipment, work surfaces, and food handlers, or maybe also as a result of inefficient heat treatment step which assessed here as a (CCP). So after establishing critical limit, corrective action application, checking (OPRs), and controlling GMP with GHP, The cheese product improved microbiologically after HACCP application as shown in table 4 where total bacterial and total fungi count decreased to less than Egyptian standard $0.6 \times 10 \pm 0.2 \times 10$ and 1×10 CFU/gm. Respectively, coliform is not detected.

Pathogenic bacteria were not detected, this result is in disagreement with those of Nasr et al. [37]. Who detect *S. aureus* after HACCP application and conversely with Said and Fahmy [40]. Who isolated *B. cereus* and *Staph. aureus* from cheese samples after HACCP application.

The objectives of heat treatment in the processing are to eliminate vegetative food poisoning bacteria, reduce food spoilage bacteria to an acceptable level that will not hinder the starter micro-organism growth.

The filling step also has the greatest effect on the products, while contamination may occur from condensation formed on the material of packaging such as properly prepared plastic materials [41].

Effective cleaning procedures and efficient sanitization of the work environment will improve the hygienic condition and consequently the final product, as well as good quality raw materials will play an important role in producing high-quality products [42].

Hoolasi [43]. Investigated milk and milk product samples before and after HACCP application and recorded that the strong control was implemented during the system positively affected the microbial quality of the end product.

Chemical hazards

Pollution of food products by chemical hazards could be occurred in processing or from poorly examined raw materials or by chemical spoilage. Table 5 illustrated that raw milk and cheese, both were contained aflatoxin M1. (100%) milk samples contain aflatoxin M1, one sample out of them exceeded MPL. This affected the product subsequently, while cheese contained more aflatoxin M1 concentration as follows, (100%)cheese samples contain aflatoxin M1, (30%) out of them exceeded MPL according to EC [15]. This is explained as one kg of cheese needs 3litres of milk, so the concentration of

aflatoxin will be more in cheese. Aflatoxin B1 is the highest genotoxic mycotoxin in animal food, and, converted to aflatoxin M1 after ingestion by ruminants, and causes liver cancer, and has a cumulative effect on humans, and is carcinogenic. So it is very important to check the supply of dairy-based products for aflatoxin contamination. Raw materials and cheese were free from (Pb, Cd and As) heavy metals, this agreed with the result recorded by Nasr et al. [37].

Detecting (CCPs) and (OPRPs)

CCP steps in the process of manufacturing, that control specifically identified hazard, and lack of its control results in presence of unacceptable hazard [32]. Control points (CPs) in raw materials must be checked previously with the supplier and before beginning the processing operation. CCPs in the processing steps (Fig.1) of soft cheese were detected according to the decision tree (Fig.2). The raw milk was the most important CP before receiving and (OPRP) at the process line because it was the main source of chemical hazard (Aflatoxin m1) in the final product so it must be secured before the start of the processing operation. on the other hand, pasteurization at 92 °C for 20 min. was the main CCP in the processing due to microbial contamination which was detected in the end product and must be controlled by the Time and temperature mentioned in the flow diagram of the product. Except for this step, other steps were considered OPRPs which are not less important than the CCP step, except that it is not measurable, so its control was done visually through good inspection and monitoring as follow:

- Reception of milk at $\leq 4-6^{\circ}\text{C}$ --- OPRP1 --- Receive milk at $< 4-6^{\circ}\text{C}$ and check/ change supplier.
- Clarification of raw milk --- OPRP2 --- Visual inspection at every clarification.
- Heat treatment of milk at (92°C for 20 min.) --- CCP1--- Time and temperature measurements.
- Salting --- OPRP3--- Visual inspection clean salt, avoid contamination.
- Renneting at (42°C for 2-3h.) ---OPRP4--- Visual inspection and chick temperature/ time for curd formation.
- Curd cutting ---OPRP5---use clean instruments, and avoid contamination
- Filling and packaging (Manual packaging) ---OPRP6---Visual inspection at every packaging and check packaging materials.

Determining critical limit (CL) for the detected CCP

Each CCP has a critical limit at which the ef-

TABLE 3. Microbial examination result of Raw materials and cheese curd (X±SD) before and after HACCP application to verify the advantages of FSMS.

Microbiological Tests	Raw Materials														
	Raw Milk			Salt			Rennet Enzyme			Water			Cheese curd		
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	
Total Bacterial Count (CFU/ml or g)	3×106±2.3×103	3×105	1×104±0.3×102	2×102	8.6×102	5×102	4×103±0.9×10	4×102	3×107±1.2×103	0.6×10±0.2×10					
Total Coliforms ml or g	0.72×103±0.2×10	0.4×10	0.36×10±0.1×10	-ve	0.3×10±0.05×10	-ve	-ve	-ve	0.3×103 ±0.1×10	-ve					
Total Fungi (CFU/ml or g)	1.08×104±0.9×10	-ve	3.6×103±1.6×10	-ve	1×103±0.6×10	-ve	2×102±0.7×10	-ve	8.8×103±1.4×10	1×10					
Bacillus cereus (CFU/ml or g)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve					
Staphylococcus aureus (CFU/ml or g)	2×103±1.5×10	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve					
Salmonella spp	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Listeria monocytogenes	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
E.coli O157:H7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					

ND: Not detected; -ve: Negative; CFU: Colony forming units.

Cheese [44]. TBC: 10⁴ cells/gm., mold: 10 cells/gm., yeast: 400 cells/gm., free from *Staph. aureus*

Coliform; 10² CFU/g, *Staph. aureus*; 10² (CFU/g)

TBC: (CFU /ml max) 1x10⁶ in raw milk

TABLE 4. Microbiological examination of swab samples along processing line before HACCP application.

Microbiological Tests	Swabs							
	Containers	Utensils	Food Handlers	Tables	Hand washing basins	Walls	Packaging Material	Refrigerator
Total Bacterial Count (CFU/cm ²)	3x10 ⁶ ±1.7 x10 ³	3x10 ⁶ ±1.9 x10 ³	3x10 ⁶ ±1.8 x10 ³	3x10 ⁶ ±1.6 x10 ³	3 x10 ⁶ ±1.5 x10 ³	2x10 ⁴ ±1.2 x10 ²	3x10 ⁸ ±1.7 x10 ³	3x10 ⁶ ±1.8 x10 ³
Total Coliforms (CFU/cm ²)	-ve	-ve	0.3 x10 ±0.06 x10	-ve	7.5x10 ±1.7 x10	-ve	-ve	-ve
Total Fungi (CFU/cm ²)	3x10 ⁶ ±2.1 x10 ³	2.9x10 ⁴ ±0.9 x10 ²	3x10 ⁶ ±1.4 x10 ²	3x10 ⁶ ±1.8 x10 ³	3x10 ⁶ ±1.1 x10 ³	-ve	3x10 ⁵ ±2.1 x10 ²	2.1x10 ⁶ ±3.1 x10 ²
Bacillus cereus (CFU/cm ²)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Staphylococcus aureus (CFU/cm ²)	-ve	-ve	2.8x10 ⁴ ±1.1 x10 ²	1x10 ² ±0.3 x10	3x10 ⁶ ±2.3 x10 ³	-ve	-ve	-ve
Salmonella spp	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Listeria monocytogenes	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
E.coli O157:H7	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D

Dairy equipment surfaces were: ≤ 10 CFU/cm² Coliform count
 Indicated standard for swabs: ≤ 103 CFU/cm². TBC [44].

TABLE 5. Chemical hazards in raw milk and cheese product before and after HACCP application .

Type of samples	No. of examined samples	³ No. (%) of positive samples for Aflatoxin M1 (ppt.) [EOS7136/2010]13	Heavy metal residues					
			Pb		Cd		AS	
			Before	After	Before	After	Before	After
Raw milk	10	10(100%): .out of them exceed MPL (10%)1	None of (70%)7 .them exceed MPL	ND	ND	ND	ND	ND
Cheese	10	10(100%): .out of them exceed MPL (30%)3	7(70%): None of them exceed MPL.	ND	ND	ND	ND	ND
Salt	10	-	-	ND	ND	ND	ND	ND
Rennet	10	-	-	ND	ND	ND	ND	ND

ND: Not determined, Detection of aflatoxin M1 in milk and cheese by ELISA. EOS [44]. Guided with EU which imposed a limit of 0.05 parts per billion in milk = (50ppt).
³No. (%) of positive Aflatoxin M1 samples, by ppt. [44].

fectiveness of the procedure will be ensured. By minimizing or eliminating significant hazards. The CL for detected CCP was shown in Fig. 1, for the heat treatment step, the temperature/time must be not less than 92 °C for 20 sec.

OPRPs do not have critical limits, but have acceptable limits such as for raw milk receiving step total bacterial load has accepted limit, raw milk standards and testing for microbiological and chemical hazards in addition to receiving of milk which must not less than 6 °C affect this step to be the critical point as shown in Fig. 1, and for storage step of cheese, the temperature of storage must be secured as shown in Fig. 1 as a good preservation practice (PRP).

Establishing monitoring procedures for each CCP or OPRP during processing

The time-temperature factor should be monitored and the instrument must be calibrated, like a thermometer at the pasteurization step. Monitoring procedures such as milk receiving rapid tests in the plan, as well as microbial and chemical examination procedures, and results of raw materials and packaging materials were applied.

Establishing corrective actions in the process

When the monitoring system shows any deviation in the critical limits, corrective actions must be done such as:

- Rejection of contaminated received raw materials [45]. Rejection of received milk if contamination is evident, such as in our study raw milk was contaminated with (AflatoxinM1), and 10% exceeded permissible limit as shown in Table 5.
- Resetting of pasteurizer for temperature and time correction.
- Re-clarification and reheat treatment of milk.
- Reject for damaged packaging materials and testing of the final product.

Corrective actions affect positively the process and the final product while the final cheese product has improved microbiologically and chemically and become acceptable after HACCP application. As shown in Tables 4 and 5.

Verification of HACCP system procedures

HACCP system verification procedures occurred through routine calibration of CCPs, Checking Heat treatment records, checking the calibration of monitoring devices, Check storage temperature, records, random samples were taken

for testing them microbiologically and chemically.

Documentation and Recordkeeping

- Documented information and accurate records are essential for a successful HACCP plan and system while recording all actions that have been taken such as measurements that show standards should be monitored, HACCP plan which contains responsibilities of the HACCP team, product description, intends use, temperature information, flow chart, corrective actions, and verification procedures.
- All expected hazards to be occurring in the production process are shown in Tables 3, 4, and 5.
- Risk assessment after identification of hazard should be documented. Then a suitable control plan is selected from the OPRP or CCP step in relation to the type of control measure that will be done. It is considered an OPRP step if a prerequisite program (PRP) is used as the control measure. However, a CCP step is used when a measurable critical limit (CL) is used as the control measure. Consequently, the actual hazard must be controlled by securing OPRP and CCP by proving that each CCP is always under control
- Control of (CCP and OPRP) based on the results of the hazard analysis in Tables 3, 4, and 5, cheese production process includes one CCP and six OPRPs as mentioned before. In general, the documentation help to verify that HACCP controls are being appropriately maintained.

Conclusion

- The HACCP system in our study is developed in the same literature review based on the twelve steps of codex HACCP.
- The PRPs were applied to get rid of some hazards before the process, thus simplifying the HACCP application.
- Finally, the HACCP system application ensures food safety and improves the product, so we recommended that:
- Compliance with the microbiological and chemical standards of the final product.
- Raw materials should be of good quality; this is ensured by microbiological and chemical analysis of raw materials before processing.
- Continues to check for suppliers, auditing, and evaluating them, especially new suppliers.

- Developing training programs for technicians, periodically for improving habits regarding hygienic practices and manufacturing practices, and the presentation of work instructions.
- For maintaining the sustainability of HACCP system application in low scale plants and compliance with the system, efforts must be intensified by legal authorities and encourage researchers to do more trials for HACCP application on small dairy units which excessively dispersed all over the country.

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Conflict of interest

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محاولة تطبيق نظام سلامة الغذاء (HACCP) على وحدة تصنيع الجبن الأبيض الطري للحد من التلوث الميكروبي والكيميائي للمنتج

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 البحوث الزراعيه - مصر.

معظم الجبن الأبيض الطري يتم إنتاجه بالطرق التقليدية العاديه دون استخدام النظم الحديثه لاكتشاف النقاط الحرجه ومكامن الخطر المسؤوله عن إنتاج منتج غير مطابق للمواصفات الصحيه وخاصة في وحدات التصنيع الصغيره، لذا في هذه الدراسه تم تطبيق خطة الهاسب على وحده صغيره تحتوي علي خط إنتاج الجبن الابيض الطبيعي في مصنع لمنتجات الالبان باحدي المدارس الزراعيه بكفر الشيخ وذلك لتحديد المخاطر الميكروبيه والكيميائيه في المنتج ومحاولة لتحسين المنتج بعد الشكوي من قصر مدة صلاحيته، وتم اجراء تقييم شامل للبنيه التحتيه للمصنع ككل والعمل على تطويرها من خلال برامج PRPs وتقييم بيئه العمل، وتم عمل تحليل المخاطر بدايه من استلام المواد الخام الداخلة في عملية التصنيع، ثم على خط الإنتاج، ثم المنتج النهائي من خلال الفحص الميكروبي والكيميائي حيث تم تحديد ثلاث نقاط حرجه وهى:

- ١- استلام اللبن الخام واحتوائه على الخطر الكيميائي الافلاتوكسين الذي تم اكتشافه في المنتج النهائي.
- ٢- درجة حرارة البستره.
- ٣- مرحلة التغليف اليدوي للمنتج.

حيث تواجد الخطر الميكروبي في المنتج النهائي وهوارتفاع العد الميكروبي الكلي وتواجد ميكروب الاستافيلوكوكاس اوريس. وتم عمل الاجراءات التصحيحيه اللازمه لنقاط التحكم الحرجه بعد مقارنتها بالحدود الحرجه لكل نقطه. وتم الحصول على جبن خالي من المخاطر وامن ومطابق للمواصفات القياسيه المصريه. لذا ينصح بتطبيق نظام الهاسب مع برامج PRPs التي تسهل من تطبيق النظام على تلك الوحدات الصغيره المنتشره بعدد كبير والرفع من كفاءتها الانتاجيه وكفاءة المنتج وفتح اسواق جديده ومن ثم التصدير للخارج.