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## Chapter

# Application of Hazard Analysis and Critical Control Point (HACCP) and Recent Technologies for Microbial Inactivation in Mozzarella Production

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## Abstract

The production of mozzarella cheese involves several steps that can lead to microbial contamination, which can pose a serious health risk to consumers. The use of Hazard Analysis and Critical Control Point (HACCP) has become a standard practice in the food industry to ensure the safety of mozzarella cheese. This involves identifying potential hazards and establishing critical control points to prevent or eliminate them. Recent technologies such as high-pressure treatment, pulsed electric fields, microfiltration, cold plasma and ultraviolet light treatment have also been developed to improve the safety and quality of mozzarella cheese by inactivating microorganisms. High-pressure treatment uses water at high pressure, while cold plasma treatment uses ionized gas to inactivate microorganisms. Ultraviolet light treatment uses UV-C light to kill bacteria and viruses, while pulsed electric field treatment uses short pulses of high-voltage current to destroy bacteria. The combination of HACCP and these technologies has proven effective in ensuring the safety and quality of mozzarella cheese. This approach has been widely adopted by the food industry to minimize the risk of microbial contamination and improve food safety. However, further research is needed to optimize the use of these technologies and provide better inactivation of microorganisms while maintaining the quality of mozzarella cheese.

**Keywords:** CCP cheese, HACCP, hazard, microbial inactivation, mozzarella

## 1. Introduction

The application of food safety guidelines plays an important role in producing wholesome and good quality cheese products because food safety in the dairy industry is a technical discipline that describes how milk is acquired, processed, handled, stored, and marketed in a way that prevents foodborne illness [1]. Food safety and prevention of foodborne diseases are promoted through the implementation of the Hazard Analysis and Critical Control Points (HACCP) system from dairy product

production to consumption [2]. It has been internationally recognized and accepted as an effective food safety management system [3], which has had a positive impact on minimizing economic losses and food poisoning outbreaks from all steps in the dairy production process via a systematic approach [2].

The Codex Alimentarius Commission has standardized the HACCP system [4] as a preventive approach that identifies, maintains, evaluates, controls, and monitors each production point that is crucial for food safety [5]. HACCP is governed by seven principles, including conducting a hazard analysis, identifying the process critical points, establishing critical limits, CCP monitoring requirements, corrective action verification, record-keeping procedures, and system documentation. The term “hazard” in the HACCP framework refers to substances or food-related conditions that affect consumer health. According to Suherman et al. [6] all physical, chemical, and biological hazards in the product might originate from raw milk, other raw materials, environmental contaminants, manufacturing equipment, and personnel. Therefore, implementing food safety standards like HACCP has been an effective and rational means of ensuring food safety and eliminating public health risks. It is appreciated as a worldwide systematic and defensive tactic to address biological, chemical, and physical hazards through deterrence and anticipation instead of end-product testing and inspection [1].

The critical control point is important in preventing, eliminating, or reducing a food safety hazard to an acceptable level. CCPs must be determined based on potential hazards that are reasonably likely to cause illness or injury if not controlled. The FDA stated that identifying CCPs in a complete, accurate, and timely manner is essential for controlling food safety hazards [7]. According to reports in the literature, the researcher’s use decision tree was used to identify CCP in cheese production [1, 8]. The most significant CCPs identified in mozzarella cheese manufacturing are Milk reception, pasteurization temperature, salt and rennet addition, coagulation, and cheese cutting [2, 8, 9]. While most countries develop regulations to ensure food safety through a preventive approach, such as HACCP principles, Codex, and ISO standards, the requirements’ fulfillment depends on many factors. Hazard identification and CCPs are the most crucial steps that need careful analysis and knowledge in HACCP implementation. HACCP plans to use Hazard Analysis to ensure product safety during and after processing to improve shelf life and make products safe for consumption [1]. The CCPs decision tree plays an important role in identifying the CCPs in each step of mozzarella cheese processing. Once the CCPs have been identified, they are monitored for critical limits and their frequency determined, and corrective actions can be taken if any critical limits fail. Temperature control checks, pH monitoring, microbial testing of the end product, and equipment calibration are required when a CCP fails because of improperly functioning equipment.

Moreover, effective microbial inactivation is essential in milk processing to eliminate harmful microorganisms and maintain product safety. Traditional thermal methods, such as pasteurization, have been widely used for microbial control in the dairy industry. However, there has been a growing interest in nonthermal processing techniques due to their potential to achieve microbial inactivation while minimizing the impact on milk quality attributes. Pulsed electric fields (PEF), high-pressure processing (HPP), and nonthermal plasma (NTP) technologies are among the notable nonthermal processing methods being explored for microbial control in milk. These techniques utilize electrical discharges, high pressures, or plasma to inactivate microorganisms while preserving the nutritional and sensory qualities of milk. However, challenges remain in optimizing these technologies for large-scale industrial

applications. Therefore, the implementation of HACCP and utilization of nonthermal processing methods have a significant impact on the production of excellent-quality products that are wholesome and suitable for human consumption by preventing contamination and using microbial inactivation. This chapter aims to review recent findings on CCPs in mozzarella cheese processing plants and microbial activation using nonthermal processing.

## 2. Literature review

### 2.1 HACCP

HACCPs are management systems that analyze, control and prevent biological, chemical, and physical hazards in everything from raw material production, procurement, and handling to final product manufacturing, distribution, and consumption [7]. According to the HACCP framework, the term “hazard” refers to any food agent or condition that has the potential to cause adverse health effects. Hazard analysis is the appraisal of the degree of severity of a hazard and its likelihood of occurrence. It is necessary to assess the survival and proliferation of potentially harmful bacteria, as well as the factors that lead to the existence or persistence of food. Therefore, this technique can be applied to a production line to determine the critical control points that can be measured and corrected [10]. In spite of this, HACCP has been shown to have greater effectiveness when used in conjunction with a quality management system and standard operating procedures, such as Good Manufacturing Practices (GMPs) and good hygiene practices (GHPs). In order to direct and control an organization’s food safety, the ISO introduced the Food Safety Management System (FSMS). This system has interrelated elements and was introduced to help organizations establish policies and objectives [11]. A wide variety of HACCP-based systems, including ISO 22000, BS PAS 220:2008, and other auditable commercial standards, have been used in the dairy industry for the implementation of cheese of many types [12–15].

Moreover, HACCP has been widely comprehended and adopted on a global scale as a trade promotion tool for effectively implementing the food safety management system, where countries face high levels of competition as well. The implementation of the HACCP plan in the global food market strengthens companies’ positions and improves their competitiveness [16]. This method identifies potential contaminants, evaluates the steps that must be taken to maintain food quality, and then develops a systematic approach to preventing food contamination. However, the effectiveness of the approach to preventing contamination depends on how it is implemented and applied [17]. The literature on food safety claims that a complex blend of managerial, organizational, and technical skills is required for the development, installation, monitoring, and verification of the HACCP system [18]. These significant flaws must also be present in medium-sized and small dairy businesses, while there are several factors that hinder its effectiveness, such as technical staff training, poor physical conditions, and the cost of implementing HACCP [19]. The HACCP system was implemented on the 12 steps given by the Codex Alimentarius Commission mentioned below.

- 1. Establish a HACCP team:** The initial phase in implementing HACCP in the milk processing facility was to put together a team that has the knowledge and experience necessary to build a HACCP plan. The interdisciplinary team that was formed included employees from the production/sanitation, quality assurance,

microbiology, engineering, and inspection departments; all of this expertise was internal to the plant. HACCP teams that are highly effective have clearly defined roles and ensure proper representation of the team in order to achieve success.

2. **Define the product:** The HACCP team developed a comprehensive overview of the product based on the ingredients, processing methods, packaging materials, etc., utilized in the manufacturing process that would help in determining the presence of all potential hazards relating to the product.
3. **Identifying intended use:** Intended use refers to the use a product would be intended for by consumers or end users, such as infants, the elderly, or sick people. A few examples of this are: processed milk was used by infants to make baby foods, health drinks, and beverages such as tea and coffee, and all of these had to be kept ice-cold or cooked to be consumed; cheese was used in pizelles (or boiled and cooled dishes), etc.
4. **Establish a flow diagram:** Developing a flow diagram that represented the entire process made it easy to figure out any possible contamination streams and recommend control strategies. The purpose of a flow diagram is to provide a clear, concise overview of the paths involved in the operation. Every one of the stages in the production line that will be opened under the company's control was included in the flow diagram's coverage area. A schematic representation of the products has been established for the particular items based on the potential sequence components and their processing phases.
5. **On-site flow diagram verification:** Once a flow diagram had been developed, the HACCP team leader had to verify it on-site for accuracy and completeness. The flow diagram should be carefully examined by the HACCP team leader and other team members to ensure that it accurately depicts the actual processes that took place on-site. The various adjustments in procedures or actions that required on-site validation involved transporting raw materials and ingredients via machinery, manufacturing channels, and facility redeployment should also be clearly described. New ingredients used or products manufactured, product transfer to another line or piece of equipment, storage conditions for packaging, etc.
6. **List all the potential hazards and conduct a hazard analysis:** This is the most significant step of the HACCP plan, which ensures the safety of the product before and after processing, improves the product's shelf life, and makes it safe to consume. The HACCP team performs a hazard analysis based on the HACCP checklist (as recommended by the FAO), and all plausible risks associated with raw materials, components, processing steps, and postprocessing steps should be identified and labeled as biological (B), chemical (C), and physical (P) risks. Identification of potential biological, chemical, and physical dangers that could appear throughout each stage of processing is advantageous.
7. **Identifying CCPs:** Identifying CCPs in the HACCP plan is the most important part of the protocol, and the Codex decision tree in the appendices is used to identify CCPs. The use of decision trees varied depending on the type of procedure, such as production, processing, storage, transportation, or others.

**8. Establish a critical limit for each CCP:** In this step, a critical limit was defined and given for each CCP discovered. The parameters that were used to determine if a process was generating safe goods were referred to as critical limits. A number of parameters, including temperature, time, product measurements, water activity (ah), humidity level, etc., had critical limitations defined. The product's safety will be confirmed if these criteria are kept within acceptable limits. To decrease microbiological contamination and prevent foodborne illness, numerous nations have created various quality and safety standards. The tolerable microbial load in mozzarella cheese is summered in **Table 1**.

S/N	Quality	Requirement Cfu/g	Test method
1.	Total plate count /g	max $2 \times 10^4$ cfu/g	ISO 4833
2.	<i>Listeria monocytogenes</i>	Nil per gram	KS ISO 4833
3.	<i>Salmonella</i> spp	Nil per gram	KS ISO 4833
4.	<i>Shigella</i>	Nil per gram	KS ISO 4833 KS ISO 21,567
5.	<i>Clostridium botulinum</i>	Nil per gram	KS ISO 4833
6.	<i>Staphylococcus aureus</i>	Nil per gram	KS ISO 4833
7.	<i>E. coli</i>	Nil per gram	KS ISO 4833
8.	Fecal coliforms: max	Nil per gram	KS ISO 4832
9.	Nonfecal coliforms, max	100 cfu/g	KS ISO 4832
10.	Mold, max	100 cfu/g	KS ISO 6611
11.	Yeast, max	10 cfu/g	KS ISO 6611
12.	Mycotoxin residues (aflatoxin M1)	0.5 µg/kg	ISO 14501:2007/ AOAC 980.21
13.	Antibiotics (total antibiotic)	10.0 ppb	AOAC 962.16

Source: [9].

**Table 1.**  
 Microbiological requirements for mozzarella cheese.

**9. Establish monitoring procedures:** The producer relies on monitoring to demonstrate that the HACCP plan is being implemented. It gives the producer precise reports that the manufacturer can use to demonstrate that the production conditions adhere to the HACCP plan. The HACCP team primarily evaluated time-temperature treatments (thermograph), pH, moisture level, equipment, and suitable processing processes. These activities were monitored weekly and monthly. It was possible to take action in the case of a loss of control or to make a process modification if there was a trend toward a loss of control thanks to monitoring procedures that were carried out during the operation and recorded in documents for future reference.

**10. Establish corrective action:** Corrective actions will be taken to bring the process back under control, whenever the monitoring procedures detect a deviation from the CCP, based on the specific critical limit established for each CCP found during the process.

11. **Establish verification procedure:** The term “verification” refers to the systematic and diligent approach to methods, procedures, checks, and other appraisals, as well as the scrutiny of the HACCP plan to ensure compliance with it. The verification of contingency procedures is done on a daily, weekly, and monthly basis by a quality analyst and supervisor of the food safety team to ensure there are adequate procedures in place when critical limits are exceeded.
12. **Establish documentation and record-keeping.** Documentation is necessary for evaluating the HACCP plan’s suitability and the HACCP system’s devotion to the HACCP plan. A complete set of records was maintained for every step of the HACCP plan, including processing charts, written records, computerized records, and records generated by the HACCP system, including microbial and analytical testing records, verification records, and validation records. These records should be kept well and up-to-date in the industry’s record books.

## 2.2 Implementation of HACCP in mozzarella cheese processing plant

The successful implementation of a HACCP plan is facilitated by the commitment of top management. Establishing a strategy that outlines the person in control of designing, implementing, and maintaining the HACCP system is the next action. The HACCP coordinator and team are initially chosen and trained as needed. The team is then in charge of creating the initial plan and coordinating its execution. HACCP plans for specific products can be developed by product teams. After the HACCP plan is completed, operator procedures, forms, and procedures for monitoring and corrective action are developed. Implementing the HACCP system entails continuing to use the monitoring, record-keeping, corrective action procedures, and other activities outlined in the HACCP plan [20]. Peristeropoulou et al. [2] claim that a HACCP system must take into account raw materials, ingredients, food manufacturing practices, the role of manufacturing processes in controlling hazards, the likely end use of the product, the categories of consumers of concern, and epidemiological evidence regarding food safety during the identification, evaluation, and subsequent operation stages.

In the manufacturing of mozzarella cheese proper understanding of potential hazards and its preventive strategies, processing steps, and identifying primary ingredients has a significant impact on designing and implementing HACCP. According to Suherman et al., [6], milk, rennet, starter culture, and salt are the primary ingredients for cheese processing. The processing steps include milk reception, pasteurization and cooling, rennet addition and coagulation, cutting and whey draining, cooking and stretching, dry salting, molding, brining, packaging, and storage. Then the identification of the CCPs will be the next step for implementing the HACCP plan.

## 2.3 The hazard analysis and critical control point framework for mozzarella cheese manufacture

### 2.3.1 Hazard identification

Identification of potential hazards and their sources have a significant role in describing the full process of risk assessment, which includes three important steps: (1) identify hazards and risk factors that have the potential to cause harm (hazard identification), (2) analyze and evaluate the risk associated with that hazard (risk analysis, and risk evaluation) and (3), determine appropriate ways to eliminate the

hazard, or control the risk when the hazard cannot be eliminated (risk control) [21]. Hazard identification is helpful in identifying potential microbiological, chemical, and physical hazards that may occur during each step of processing mozzarella cheese [22]. Microbiological hazards are pathogens or harmful bacteria introduced during production, *E.coli*, and *Staphylococcus aureus*, are harmful bacterial in the raw milk, and *Salmonella* could contaminate the end product. These microbiological hazards may be originated from a different source [21]. Microbial potential hazards include pathogenic bacteria such as *Listeria monocytogenes*, *verotoxigenic E. coli*, *S. aureus*, and *Salmonella* from raw materials and *Salmonella*, *Cl. perfringens*, *Cl. botulinum*, *B. cereus*, and *L. monocytogenes* from ingredients. Besides, mycotoxins and bacterial toxins such as *staphylococcal enterotoxin* are found in certain raw milk and ingredients. Additional hazards associated with flavoring foods such as spices, herbs, ham, fruit, mushrooms, etc., pathogens that may be transferred from staff, re-contaminating molds, and pathogenic bacteria from the processing and packaging environment are also considered biological hazards [6, 23].

Chemical contaminants include plant toxins and chemicals added during processing and other chemicals originating from the raw materials [21]. Chemical hazards in the cheese manufacturing industry include pesticide residues, environmental contaminants in raw materials and ingredients (e.g., products high in fat, dioxins, and dioxin-like PCBs, heavy metals), contaminants from processing activities (coolants, lubricants, sanitizers), carryover additives (with numerical ADIs specified) from raw materials and ingredients, and contaminants from food contact materials, including coatings, waxes, and soft plastics like ripening films [1, 23]. Physical contamination is foreign material that could come from incorrect personal handling or bad environmental conditions. The hazards originated from the raw material and the processing steps [21]. Physical hazards include glass, bone or insect debris, metal fragments, hard plastic, etc.; the glass may come from unsecured windows, lamps, bulbs, neon tubes, thermometers, laboratory glass, spy holes on the tanks, insect-killing tubes, or other glass equipment; the insect debris may come from raw materials, ingredients, and packaging material; the metal may come from improperly built or maintained machines or equipment; the stones may come from damaged floors or walls; the wood from pallets or wooden equipment; and the plastic from buckets, boxes, and brushes are among the potential physical hazards in cheese manufacturing industries [1, 23]. The potential hazard, source, and preventive measures in the processing line are summarized in the **Table 2**.

### 2.3.2 Critical control points (CCPs) in mozzarella cheese

According to evidence in the literature, different researchers identify different CCPs in cheese manufacturing plants depending on the type of product produced, the sophistication of the manufacturing plant, the ingredients added during production, and the legislation of the country and international standards. For instance, Peristeropoulou et al. [2] established four CCPs in the storage of raw milk during its stay in self-cooler tanks in the factory, pasteurization of raw milk, heating of whey with stirring and Transport and distribution to consumers for the mizithra cheese production, whereas milk reception, dry salting, and brine salting also considered as CCP areas by other authors. Critical points are controlled through a monitoring system of planned measurements and observations for early detection of any deviations from the critical limits of CCPs and to ensure each CCP is always under control [8].



Processing steps	Hazards	Preventive measure
Milk reception	<p><b>Microbial:</b> Insects, pests, antibiotic residues, microbe aflatoxin</p> <p><b>Chemical:</b> urea, detergents, boric acid, ammonium sulfate, sugar, hydrogen peroxide, melamine, salicylic acid, benzoic acid, NaCl, antibiotic and insect side residues</p> <p><b>Physical:</b> Stones, husk, hair, glass, metal, plastic fragments, wood chips</p>	proper equipment setting, sanitize all the transfer equipment
Pasteurization	<p><b>Microbial:</b> <i>Bacillus</i> spp., <i>Clostridium</i> spp., <i>Coxiella burnetii</i>, spores, and <i>thermoduric</i> bacteria</p> <p><b>Chemical:</b> Cleaning disinfectant, sodium hypochlorite, hydrogen peroxide, iodine, isothiazolinones, ozone, peracetic acid, phenolics, and surfactants.</p> <p><b>Physical:</b> Stones, husk, hair, glass, metal, plastic fragments, wood chips</p>	72°C, 15 s, proper pasteurizer setting, sanitize all the equipment
Rennet	<p><b>Microbial:</b> <i>Bacillus</i> spp., <i>Clostridium</i> spp., <i>Coxiella burnetii</i>, spores, and <i>thermoduric</i> bacteria</p> <p><b>Chemical:</b> Cleaning disinfectant, sodium hypochlorite, hydrogen peroxide, iodine, isothiazolinones, ozone, peracetic acid, phenolics, and surfactants.</p> <p><b>Physical:</b> Stones, husk, nut, bolt</p>	sanitize the container used for diluting rennet, proper personal hygiene and handling
Coagulation	<p><b>Microbial:</b> <i>Bacillus</i> spp., <i>Clostridium</i> spp., <i>Coxiella burnetii</i>, spores, and <i>thermoduric</i> bacteria,</p> <p><b>Chemical:</b> Cleaning disinfectant, sodium hypochlorite, hydrogen peroxide.</p> <p><b>Physical:</b> Stones, husk, nut, bolt</p>	40°C, 60 min, proper personal hygiene and handling
Cutting	<p><b>Microbial:</b> <i>Bacillus</i> spp., <i>Clostridium</i> spp., <i>Coxiella burnetii</i>, spores, and <i>thermoduric</i> bacteria</p> <p><b>Chemical:</b> Cleaning disinfectant, sodium hypochlorite, and hydrogen peroxide</p> <p><b>Physical:</b> Stones, husk, nut, bolt</p>	correct knife size for optimum curd size, sanitize the cutting tools and the cutter's hands and arms, proper personal hygiene, and handling
Salting	<p><b>Microbial:</b> <i>Bacillus</i> spp., <i>Clostridium</i> spp., <i>Coxiella burnetii</i>, spores, and <i>thermoduric</i> bacteria</p> <p><b>Chemical:</b> Cleaning disinfectant,</p> <p><b>Physical:</b> Corrosive equipment</p>	2.5–4.5% salt, moisture content is optimum at 60–65%, sanitize the salt container and the stirring tools, supply quality water, proper personal hygiene and handling
Storage and distribution	<p><b>Microbial:</b> <i>Pseudomonas</i>,</p> <p><b>Chemical:</b> Cleaning disinfectant, sodium hypochlorite, hydrogen peroxide, iodine, isothiazolinones, ozone, peracetic acid, phenolic, and surfactants.</p> <p><b>Physical:</b> Falling shocks, vibration, unsuitable temperature, humidity, glass, bone or insect debris, metal fragments, and plastic</p>	The temperature of storage is ≤45°F. Distributed using refrigerated (≤45°F), proper building setting, proper storage condition setting, pest control

Source: [1, 6, 21–23].

**Table 2.**  
Hazard analysis in mozzarella cheese processing steps.

HACCP implementation requires the completion of a special form in order to create monitoring files in all CCPs, including:

Processing stage

- Type of the CCPs
- The controlling parameter and its limits
- The method, frequency, records, and the monitoring agent
- The corrective action in case the control parameter is out of control, verification/evaluation of the effectiveness of the applied corrective action, and the person responsible for the corrective action
- The evaluation officer of the corrective action

Generally, documentation plays a significant role in verifying that HACCP controls are being adhered to and maintained in accordance with their objectives. The CCPs in mozzarella cheese manufacturing plants are summarized in **Table 3** [1, 2, 6, 9, 22–24].

## **2.4 Nonthermal milk microbial inactivation and preservation techniques**

The conventional method of microbial elimination and inactivation process cause major change in some nutritional components and physical and chemical properties of the food products, which leads to the development of detrimental flavor, loss of vitamins, and volatile flavor compounds [25]. Consumers also want foods that have undergone minimal processing with clear labels and goods made without the use of heat, which led to the invention and introduction of nonthermal processing methods to the food industry. The nonthermal processing is a revolutionary processing approach that is used not only in the milk processing industry but also in other food products. These processes eliminate microbes or other biological organisms without raising the temperature significantly and preventing a sequence of undesirable reactions in foods. Due to their capacity to produce safe, wholesome foods with a longer shelf life that are fresh and nutritious, nonthermal processing technologies can be utilized as a replacement for thermal processing. High-pressure processing (HPP), nonthermal plasma (cold plasma), ultrasonic, pulsed electric field (PEF), ultraviolet irradiation, and membrane microfiltration techniques are some common nonthermal procedures that are used in milk processing [26].

### *2.4.1 High-pressure processing (HPP)*

HPP is a nonthermal technique of inactivating microbes and enzymes in food products using high pressure. HPP is a nonthermal technique for inactivating microbes and enzymes in food products using high pressure. It is an ideal substitute for conventional heat processing because it has no effect on the nutritional or sensory qualities of food. Sousa et al. [27] conducted an experiment to evaluate the impact of HPP on human milk and found that only ionic and hydrophobic interaction of macromolecules (proteins) was disrupted without denaturation of biologically active proteins. The microbial inactivation has been revealed with very little to no effect on the

CCPs	Hazards	Preventive measure	Critical limits	Monitoring procedure	Monitoring frequency	Corrective action
Milk reception	Microbial chemical and physical contamination e.g.	Reception of natural raw milk at $\leq 4$ $-6^{\circ}\text{C}$ , milk clarification, preheat treatment, separation	No unqualified material be used	Apply supply quality assurance	Each supply	Refusing raw materials with defects Cleaning the reception area and equipment Operator training & cooling $< 4$
	Total plate count/g	Proper transfer equipment Sanitize equipment Proper personal hygiene and handling	Max $2 \times 10^4$ cfu/g	Inspection of personal and equipment hygiene	Every time during collection and reception	Cleaning of milk collection and storage equipment, pasteurization  Operator training & cooling $< 4$
	Clostridium botulinum	Avoid cross-contamination and contact with the soil	Nil per gram	Inspection of personal, environmental, and equipment hygiene	Every time during collection, reception, and at the end of production	Inspection of personal, environmental, and equipment hygiene
	Mycotoxin residues (aflatoxin M1) checking	Checking the feed staff and another source of contamination	$0.5 \mu\text{g}/\text{kg}$	Regular detection	Each supply and collection center	
	Antibiotics (total antibiotics)	Checking the supply and proper withdrawal	$10.0 \text{ ppb}$	Regular detection	Each supply and collection center	Discard
Pasteurization	Survival of pathogens such	Pasteurizer checks: Check the heat plate,	Temperature set at $72^{\circ}\text{C}$ , 15 s,	Check thermometer and time,	Each batch	Adjust the temperature and time by setting
	<i>As E. coli</i> , <i>Staphylococcus aureus</i> , <i>Bacillus cereus</i> , etc.	Check the temperature Controller, and Check the flow diversion	and pathogenic bacteria must be Nil per gram, phosphatase test negative	Check equipment is properly running Supervisor managing and record Keeping	Routinely Each batch	The equipment well Call the engineer to repair

CCPs	Hazards	Preventive measure	Critical limits	Monitoring procedure	Monitoring frequency	Corrective action
Starter culture and Rennet addition	Microbiological contamination	Proper additional rate	Rennet: 100 ml/100 kg concentrate agitator set	Check the additional rate of the rennet and pH	Each batch	Applying more testing on ph adjust agitate rate and store temperature < -40°C
	Physical contamination	Agitate properly	At medium	Check the rate of the agitator record-keeping	Each batch	Operator training
Coagulation	Microbiological, physical, and chemical contamination	Proper time setting and recording	Temperature set at 40–45°C Time is set at 30–60 min	Check the temperature/time and the stirring tools	Each batch	Reject product
		Take the stirring tools out of the tank	Tools prevent coagulation	Record keeping	Each batch	Operator training
Cutting	Microbiological Contamination	Proper time and temperature setting		Check the temperature/time record-keeping	Each batch	Adjust the heater to change the temperature Operator training
Dry salting	Microbiological contamination e.g. Salmonella	The correct level of salt Correct mixing during salting The salt-free from any contamination	Salt% = 5.0% The product must be free from Salmonella and shigella	Records and testing	Each batch	Incorrectly salted curd must not be allowed To progress
Scalding	Microbiological, physical, and chemical	Proper time and temperature setting	Temperature is set at 38° C, scalding for 30 min, Stirring for 20 min	Check the temperature and the time, record keeping, check fine cutting of coagulum into cubes	Carried out after every 15 minutes	Adjust the heater to change temperature
Molding	Microbiological contamination	Proper temperature Setting	Temperature set at 32°C	Check thermometer and record-keeping	Each batch	Adjust the heater to change the temperature

CCPs	Hazards	Preventive measure	Critical limits	Monitoring procedure	Monitoring frequency	Corrective action
Packaging	Microbiological, physical, and chemical	screens, filters Use specific sizes for the products Low-level shocks, less impact with other packages		Visual inspection of packages and packing material	Every 3 Hour	Build proper structural frame Cabinet for placement of pallets in warehouse
Storing & Distribution		Maintain refrigeration temperature, control humidity, cooling the facility in the carrier vehicle	Temperature 4 °C	Record keeping, documentation	Check after every 60 min	Avoid fluctuations during cold storage

*Source: [1, 2, 6, 9, 22–24].*

**Table 3.**  
*HACCP for mozzarella cheese processing plant.*

small molecules of milk components (vitamins, taste, and amino acids), color, and other nutritional components. However, microbial inactivation is irreversible due to changes in membrane protein and other factors under greater pressure in microbial cellular membranes.

Moreover, HPP brought about some important modifications, such as denaturation, coagulation, and protein aggregation, which can influence the final product yield. Several researchers have successfully used HPP to increase the shelf life of milk [28] and milk products, including cheddar cheese [29], gorgonzola cheese [30], and queso fresco cheese [31]. In recent years, HPP has been demonstrated to be an effective tool for changing milk's functional properties and pressure-induced molecular changes and successfully applied in the sector [32].

A majority of research studies on milk processed using HPP were focused on determining whether harmful and spoilage microorganisms had been eliminated. Due to the resistance of spores to high pressure, the process at 400–600 MPa was comparable to heat pasteurization (72.8°C, 15 s) [33, 34]. By contrast, sterilized milk could not be compared to 400–600 MPa processing. While no research was conducted on the effect of HPP on mozzarella cheese, the experiment conducted on Turkish white cheese by treating with high pressure from 50 to 600 MPa for 5 or 10 min at 25°C found reductions in *L. monocytogenes*, total aerobic *mesophilic* bacteria, molds and yeasts, *Lactococcus* spp., and *Lactobacillus* spp. [35]. The process had no effect on pH and water activity of the cheese. Another study conducted on goat's milk cheese manufactured from raw milk processed at 450 MPa for 10 minutes or at 500 MPa for 5 minutes showed that the counts of *L. monocytogenes* were reduced by more than 5.6 log cfu g<sup>-1</sup> without significantly altering the organoleptic properties of the final product.

The HPP process at 400 to 600 MPa for 7 minutes has been shown to eliminate *mesophilic* and aerobic bacteria, *Enterobacteriaceae*, lactic acid bacteria, and *Listeria* spp. and have no effect on the cheese's texture [36]. However, trained panelists and customers failed to notice the differences between the control and pressure-treated samples. According to Lopez-Pedemonte et al. [37], ultrahigh-pressure homogenization (UHPH) and high-pressure homogenization (HHP) are effective methods for inactivating *S. aureus* CECT 976 in milk used for cheese production. A primary and secondary homogenization stage using the UHPH was conducted at 300 and 30 MPa, respectively, and was then followed by an HHP treatment at 400 MPa/10 min/20°C. They discovered that cheese contained *S. aureus* at a starting load of 8.5 log<sub>10</sub> CFU/g in control. Following UHPH and HHP treatment of the milk, the cheese showed full inactivation of *S. aureus* and its enterotoxin after 15 days of ripening.

A study on Gorgonzola cheese rind by pressure treatment between 400 to 700 MPa for 1 to 15 min at 30°C inactivated seven hemolytic strains belonging to serotype 1/2a of *L. monocytogenes*.

In addition, De Lamo-Castellvi et al. [38] revealed that all viable cells of *E. coli* O59:H21 and O157:H7 were eliminated in washed-curd cheese after being treated at 500 MPa. Besides, pressures of 300 and 400 MPa applied to cheese at a pH of around 4.8 completely inactivate both *S. Enteritidis* CECT 4300 and *S. Typhimurium* CECT 443 [39].

In general, milk and its products retain significant flavor, color, and nutrients after being pressure-treated. However, a strategic combination of HPP with other thermal and nonthermal treatments may be considered in order to prevent the recovery of injured cells during storage (**Table 4**).

Product	Target microorganisms	Treatment conditions	References
Washed-curd cheese	<i>Yersinia enterocolitica</i> CECT 4055 (serotype O3) CECT 559 CECT 4054	Strain CECT more baro tolerant at 300 MPa	De Lamo-Castello et al. [38]
Washed-curd cheese	<i>Listeria monocytogenes</i> NCTC 11994 Scott A	Strain NCTC 11994 was more sensitive to HPP at 400–500 MPa for 10 min	Lopez-Pedemonte et al. [37]
Swiss cheese slurry	Coliforms, yeasts, molds, presumptive coagulasepositive <i>Staphylococcus</i> , starter lactic acid bacteria	The counts of all microbial groups reduced greatly at 345–550 MPa for 10–30 min	Ding et al. [40]
Gouda cheese D-values	<i>Aeromonas hydrophila</i>	D-value of 32.05, 12.97, and 2.43 min at 100, 200, and 300 MPa at 50 °C, respectively	Fonberg-Broczek et al. [41]
Washed-curd cheese	<i>Staphylococcus aureus</i> CECT 4013 ATCC 13565 <i>Staphylococcal enterotoxin A</i> (artificially inoculated)	Rate of inactivation increased with increasing pressure from 300 to 500 MPa	Lopez-Pedemonte et al. [37]
Mato cheese	<i>Staphylococcus carnosus</i> 4491	No remarkable decrease in counts of bacteria at 500 MPa for 30 min at 10 or 25 °C. 7 log reduction at the same pressure at 55 °C for 5 min	Capellas et al. [42]

**Table 4.**  
Effect of high hydrostatic pressure on the inactivation of some pathogenic bacteria in cheese.

#### 2.4.2 Microfiltration (MF)

Microfiltration is a type of membrane filtration that uses an open membrane structure and is operated at low pressure on the filter material [26]; it uses porous membranes with typical pore sizes of between 0.1 and 10  $\mu\text{m}$ . MF membranes can retain particles, cells, and large macromolecules (e.g., polymers). The dairy industry uses MF membranes for a variety of purposes, including the removal of bacteria [43, 44], whey defatting [45], and the production of concentrates enriched in casein micelles [44]. It extends the milk storage lifespan by decreasing the microbial load and eliminating spores, while preserving organoleptic quality. The low cost and fouling of cellulose acetate membranes make them very popular. Membrane separation was first used to separate milk components in the late 1960s and is now routinely used in whey and cheese processing.

MF can be used to reduce the microbial load in liquid milk and increase its shelf life without any changes in its composition and sensory qualities [46]. Using modified membrane structures, the microbial load can be reduced significantly without affecting milk composition [47]. García and Rodríguez [48] developed a process for extending the shelf life (ESL) of milk by 33 days using a combination of microfiltration and thermal treatment. There was a minimal change in the main composition of ESL milk compared to that of raw untreated milk. Low-fat milk is preferred for microfiltration processing, although slight changes in protein, calcium, and lactose levels were observed after the treatment [47].

Several studies have been conducted to verify the efficiency of microfiltration in microbial and spore removal [49–54]. However, García and Rodríguez [48] claimed that the exclusive utilization of MF is not granite for the removal of spores and some

pathogenic bacteria because of the survival nature of some bacteria, which multiply during the ripening and storage periods. Bacteria are maintained and concentrated in the retentate if the milk quality is poor and membrane pore sizes of 0.1  $\mu\text{m}$  are not used in the separation of milk proteins. In contrast, Skrzypek and Burger [55] revealed that ceramic-membrane-based microfiltration is risk-free and nonintrusive.

According to France et al. [56] the dairy sector frequently filters at temperatures where microorganisms can flourish, which, combined with high TMP, a lack of mechanical cleaning, and constant feed flow, makes membranes vulnerable to biofilm formation. The temperature at which filtration is performed is an important consideration for processors because it influences microbial growth and diversity. High temperatures (45–55°C) provide optimal conditions for the growth of *thermophilic* bacteria that can produce heat-stable proteases and lipases. These bacteria can affect the quality of dairy products, produce acid on growth, and reduce pH [57, 58]. The growth of microorganisms and formation of biofilms can be more rapid at high temperatures than at low temperatures. As reported by Chamberland et al. [59], skimmed milk processing at 15°C led to a much slower occurrence of biofilms on UF membranes than at 50°C. *Psychrophiles* and *psychrotrophic* bacterial genera can grow at low temperatures, but their optimal growth temperature is –20–30°C. The authors revealed that the number of 16S rRNA gene copies on the membrane increased from 3.21 to 8.83 log<sub>10</sub> gene copies per cm<sup>2</sup> after 15 hours of processing, indicating that bacterial growth was much higher at 50°C. A study conducted by Rodríguez-González et al. [60], using a cross-flow MF of 1.4  $\mu\text{m}$  pore size, reported a reduction of 2.1-log in mesophilic microorganisms in skim milk. Another study by Maubois [61] also revealed that the vegetative cells of skim milk showed a reduction of >3.5-log after MF processing at 55°C in 1.4  $\mu\text{m}$  pore size. The MF-treated milk was free from somatic cells, and the spore reduction was >4.5-log.

Conversely, psychrophiles and psychrotrophic bacterial species, such as *Pseudomonas*, *Psychrobacter*, and *Corynebacterium*, can be developed during milk filtration at low temperatures (15°C). Schiffer and Kulozik [62] examined microbial growth under feed-and-bleed filtration of skim milk at temperatures of 10, 14, 16, 20°C, and 55°C and a sudden drop in pH, associated with microbial activity, forced them to stop filtration at 55°C after 10 h. In cold MF, multiplication is minimal because most psychrotrophic bacteria prefer temperatures in the range of 20 to 30°C. Higher temperatures (15–20°C) facilitate the growth of mesophilic bacteria and are close to the optimal metabolic activity of psychrotrophic bacteria; therefore, microbial filtration during dairy processing is generally avoided. However, the bacterial communities responsible for increasing microbial counts at 16°C and 20°C were not determined, making it unclear whether mesophilic bacteria significantly contributed to microbial counts at the upper end of the cold MF range.

Moreover, the effectiveness of MF in eliminating microbes, spores, and somatic cells from skim milk at cold temperatures was examined by several scholars and found a positive result [62–65]. According to an experiment conducted by Fritsch and Moraru [64] the entire vegetative cells and spores were eliminated after MF processing, while the skimmed milk initially had a count of 5.25 and 2.15-log CFU/mL of vegetative bacteria and spores, respectively, following the application of MF treatment (pore size of 1.4  $\mu\text{m}$  at 6°C) and somatic cell count was reduced to 3.0-log. Gosch et al. [65] conducted an experiment to examine the impact of pore size on the elimination of microorganisms and discovered that a membrane with a pore size of 0.8  $\mu\text{m}$  was more effective than a membrane with a pore size of 1.4  $\mu\text{m}$  in terms of >3.5 log decrease in the count and > 5.4-log reduction in the total viable count.



Conversely, two MF treatments reduced the total viable count of skim milk to  $>2.3$  log CFU/mL. When compared to a ceramic membrane with 1.4 mm pores, MFs with 0.14 mm and 0.2 mm pore diameters showed a similar drop in bacterial cell density in their permeate.

Sterilox membranes (Pall-Exekia Company) are much more effective because of their narrower pore distribution sizes and can reduce the microbial load by 5–6-log and 3–4-log CFU/mL, respectively, with 0.8 and 1.4 mm microfiltration filters. Elwell and Barbano [63] used ceramic membranes with 1.4 m pore sizes to investigate the quality and storage stability of skim milk after MF. They discovered a 3.79-log reduction in the bacterial count and reported that the spore count decreased from an initial count of 2-log CFU/mL in raw milk to an undetectable level.

Another investigation found that filtering skim milk at 50°C via a membrane with a pore size of 1.4  $\mu\text{m}$  resulted in  $>3.5$ -log reductions in the bacterial count and retention of all somatic cells. Comparing the results with 0.5  $\mu\text{m}$  membrane processing, the bacterial reduction was increased to 2–3-log when a smaller pore-size membrane was used [66]. After filtering skim milk with a 1.4- $\mu\text{m}$  membrane. Trouvé et al. [67] observed a  $> 4.5$ -log reduction in spore-forming bacteria. Furthermore, Brans et al. [68] investigated the use of 0.5  $\mu\text{m}$  micro-sieves, an advanced membrane filtering device. The narrow pore size distribution of this membrane is able to work at low trans-membrane pressure and has achieved a 6.6-log reduction in the amount of *Bacillus subtilis* inoculated in SMUF.

#### 2.4.3 Pulsed electric fields (PEF)

Pulsed electric fields is nonthermal processing technique that includes passing brief bursts of a strong electric field through fluid or semi-fluid meals. This breaks the microbial cell membrane, leading to cell rupture and, ultimately microbial cell death [69]. Milk is a suitable product that can receive PEF treatment because it is a fluid food. According to Bendicho et al. [70], milk that had PEF treatment underwent fewer unfavorable alterations and had a lower bacterial burden. According to research by Sharma et al. [71], the effects of PEF treatment were found to rise as the treatment temperature rose. According to Sharma et al. [71], PEF-treated milk has microbiological stability comparable to thermally treated pasteurized milk but without any thermally caused damage.

The main purpose of PEF processing milk samples is to examine the impact of PEF on various bacteria that are probably present in milk. One of the earlier investigations used PEF with  $36.7 \text{ kV cm}^{-1}$  and 40 pulses for 25 minutes to inactivate *Salmonella* Dublin in homogenized milk. The desired microbe was totally destroyed inactive under these circumstances in samples kept at 7 to 9 °C for eight days; nevertheless, a 3-log reduction was achieved in *E. coli* cells under the same circumstances [72]. Untreated milk had an increase in the population of native milk microflora to  $107 \text{ cfu ml}^{-1}$ , whereas PEF treatment reduced the bacterial load to about  $4102 \text{ cfu ml}^{-1}$  [73]. Skim milk (SM) and whole milk (WM) were subjected to PEF processing (30.76 to  $53.84 \text{ kV cm}^{-1}$  electric field intensity and 12, 24, and 30 pulse numbers), along with light heating (20, 30, and 40°C), which resulted in minor alterations in the physicochemical parameters of both milks after processing. After PEF processing, mesophilic bacteria grew more slowly in both SM and WM than they did in psychrophilic bacteria, reaching up to 6 and 7 log  $\text{cfu ml}^{-1}$  growths after 25 days of storage at 4°C, respectively [74].

Despite the fact that the majority of studies have concentrated on PEF processing of milk, a small number of studies have also looked into PEF's potential applications in the dairy industry. For instance, *Lactobacillus brevis*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Saccharomyces cerevisiae* counts were reduced by approximately 2 log cfu g<sup>-1</sup> when yogurt was processed using PEF [73]. There were no appreciable variations in L, a, and b values, oBrix, pH, or a few selected sensory qualities between the control and treated samples after PEF and heat processing (at 60°C for 30 s). In comparison to the control samples held at 4 or 22°C, the microbial counts of the samples treated with heat (at 60°C) and PEF were reduced [75].

PEF-treated dairy products show strong customer acceptability and sensory characteristics that are comparable to those of heat-treated products [76, 77]. The flavor profile of cheddar cheese created from PEF-treated milk was superior to that of cheese samples made from milk pasteurized at 63°C for 30 minutes. The values of the cheese's hardness and springiness increased while those of the other textural characteristics, such as adhesiveness and cohesiveness, remained unchanged [78].

The amount of proteolysis in cheese curds made from milk that had undergone PEF treatment (2 s pulse width, 2 Hz pulse frequency, and up to 120 pulses) was higher than that of cheese curds created from pasteurized milk but lower than that of cheese curds made from raw milk. The rennet coagulation time of milk treated with PEF was recently reported to have increased by 10% [79]. The potential of PEF treatment on the inactivation rate of selected pathogens in milk and milk products is summarized in **Table 5**.

Product	Target microorganism	Processing conditions	References
Homogenized milk	<i>Salmonella</i> Dublin (full reduction) <i>Escherichia coli</i> (3 log reduction)	36.7 kV cm <sup>-1</sup> , 40 pulses, 25 min	Dunn [72]
Raw skim milk	<i>Listeria innocua</i> (4.3 log reduction)	30 and 40 kV cm <sup>-1</sup> , 1–30 pulses, 20–72°C, <10 s. Best balance of inactivation was achieved at 55°C with 40 kV cm <sup>-1</sup>	Guerrero- Beltrán et al. [80]
Raw skim milk	<i>Listeria innocua</i> (2.5 log reduction)	30, 40 or 50 kV cm <sup>-1</sup>	Calderon- Miranda et al. [81]
Raw skim milk	<i>Listeria innocua</i> (2.0, 2.7 and 3.4 log reductions)	30, 40 or 50 kV cm <sup>-1</sup> plus 10 IU nisin application	Calderon- Miranda et al. [81]
UHT milk	<i>Geobacillus stearothermophilus</i> (3 log reduction)	60 kV cm <sup>-1</sup> , 26–210 μs	Shin et al. [82]
UHT milk	<i>Pseudomonas fluorescens</i> , <i>Bacillus cereus</i> , <i>Lactococcus lactis</i> (0.3–3.0 log reductions)	35 kV cm <sup>-1</sup> with 64 pulses of bipolar square wave for 188 μs	Michalac et al. [83]
UHT milk (whole)	<i>Pseudomonas</i> isolates (complete inactivation)	31 kV cm <sup>-1</sup> , 20 μs, 55°C	Craven et al. [84]
Cheese whey <i>Zygosaccharomyces bailii</i>	<i>Listeria innocua</i> , (3.0–5.0 log reductions)	(7.9–8.8 log reductions) 40 kV cm <sup>-1</sup> , 4937 μs PEF plus UV (with 7.7 s, 229 mJ ml <sup>-1</sup> dosage)	Dave et al. [85]

**Table 5.**  
 Effect of PEF treatment on the inactivation rate of selected pathogens in milk and milk products.

#### 2.4.4 Ultraviolet light (UV)

The wavelength of the ultraviolet (UV) radiation employed for food processing ranges from 100 to 400 nm. Raw milk absorbs UV light at a rate of  $290 \text{ cm}^{-1}$  at a wavelength of 253.7 nm. Milk is photosensitive; hence, UV light radiation usually has a detrimental effect on it [86]. However, Krishnamurthy et al. [87] revealed that pulsed UV radiation has the capacity to completely inactivate *Staphylococcus aureus*, a milk pathogen. Moreover, ultraviolet (UV) light has shown promise as a nonthermal method for microbial inactivation in various food products, including dairy items. Recent studies have explored the antimicrobial effects of UV-A light on processed cheese, demonstrating its potential as a surface decontamination method. According to Altic et al. [88], milk flowing through a UV chamber caused *Mycobacterium avium* subsp. *paratuberculosis* cell clumps to break apart. The synthesis of vitamin D may be aided by short-wavelength UV radiation. Matak et al. identified some alterations in sensory perception and dietary habits.

When UV light treatment is utilized for pasteurization, milk turbidity is a significant difficulty. Turbidity in milk reduces microbial inactivation because it allows less UV radiation to penetrate. Milk becomes turbid when suspended and colloidal materials are present in high concentrations, which results in milk's opaqueness. Two methods have been employed in contemporary UV reactors to boost the UV light penetration into milk based on fluid flow, opening the door for the employment of this technology in the dairy and food industries for pasteurization. The first method uses the laminar flow of milk or fluid to create an extremely thin film over a UV-irradiated surface, allowing light to pass through the milk. The second method uses turbulent milk flow to reduce the necessary route length and increase UV light penetration in milk [89]. It does this by placing all liquid components in close proximity to UV-exposed surfaces. The studies on the impact of UV processing on the quality of whole milk found no significant changes in the milk's viscosity, color, pH, soluble solid contents, or viscosity. When pasteurized whole milk was exposed to UV light with a dose of  $10 \text{ mJ/cm}^2$  for 12 to 235 minutes, the pH range of the milk treated with UV light was 6.66 to 6.70, the viscosity was typically 2.00–0.01 (m Pa s), the color change  $E^*$  was in the range of 0–0.5, and the contents of soluble solids were 12.78–0.10 (% g/g) [90].

The primary goal of UV light processing of milk and other dairy products is the elimination of harmful and spoilage bacteria. According to Krishnamurthy et al. [87], the effectiveness of pulsed UV light at 5, 8, or 11 cm from a UV light strobe with a 20, 30, or  $40 \text{ ml min}^{-1}$  flow rate up to three times via recirculation for continuous flow milk treatment resulted in a 0.55 to 7.26 log cfu  $\text{ml}^{-1}$  reduction in *S. aureus* counts. Raw cow's milk was processed in a continuous flow coiled tube ultraviolet reactor for 17 seconds at a cumulative dose of  $16.822 \text{ mJ cm}^{-2}$ , which reduced the total microbial count by  $2.3 \text{ log cfu ml}^{-1}$  but had no discernible effect on the odor compounds in the treated, untreated, or control samples. Milk samples treated with UV caused a detectable change in odor, but no significant difference in malondialdehyde or other reactivity was detected after treatment or during storage between untreated and UV-treated milk samples. Moreover, Bandla et al. [91] revealed that UV-treated milk had higher lipid oxidation products than fresh or untreated raw milk samples.

Recently Hales and Bastarrachea conducted a research to determine the antimicrobial mechanism and microbial inactivation kinetics of UV-A light on processed cheese inoculated with *Escherichia coli* K12 and *Listeria innocua*. The results revealed that an exposure of approximately 70 minutes of UV-A light was required to achieve

an  $\sim 6$  log reduction in *E. coli* K12, while *L. innocua* L2 required around 130 minutes of exposure. The UV-A light exposure also led to increased oxidative stress and membrane damage in both bacteria. Furthermore, infrared spectroscopy indicated no significant changes in the surface chemistry of processed cheese after UV-A exposure, except for a decrease in moisture content and an increase in lipid concentration. The study concluded that UV-A light could be a suitable alternative for surface decontamination of dairy products, including processed cheese [92].

In general ultraviolet (UV) light has emerged as a nonthermal method for microbial inactivation in various food products, and recent studies have investigated its effectiveness on processed cheese. UV light shows promise as a nonthermal method for microbial inactivation in dairy products. However, its effectiveness can vary depending on the target microorganism and specific product characteristics. Further research and optimization of UV treatment parameters are necessary to fully harness its potential for preserving dairy products' quality and safety.

#### 2.4.5 Cold plasma

Plasma (quasi-neutral gas) technology (PT) is one of the most recent technologies with numerous uses in the food sector. It is defined as the fourth stage of matter, which is electrically charged or ionized but does not have a fixed shape or volume [93, 94]. It is an entirely or partially ionized state made up of neutrally charged molecules and atoms, free radicals, negatively and positively charged ions, intermediate highly reactive species, and negatively and positively charged ions [95].

The most appealing characteristics of PT are its low-temperature properties and increased efficacy in microbial inactivation [96]. The fundamental idea behind PT is a straightforward physical one: by feeding the gas with extra energy through an electrical discharge, the gas is transformed into an energy-rich plasma state, the fourth state of matter. The application of PT enhances the product's quality and maintains its safety from pathogenic and spoilage-causing bacteria without compromising its functional, sensory, or nutritional profile [97]. Furthermore, it is crucial to remember that PT only causes changes to the food's surface because plasma-reactive species lack penetrating power [98]. PT is frequently used in the food industry for enzyme inactivation, wastewater treatment, food packaging modification, hazardous elimination, and food decontamination [99].

Despite its widespread use in the medical, chemical, and polymer industries, cold plasma has yet to be tested in the dairy industry, and little research has been conducted. A study was conducted on whole, semi-skimmed, and skimmed milk stored at 4°C for 42 days by applying plasma at 20 kV to determine its potential for reducing *E. coli*, *S. Typhimurium*, and *S. aureus* inactivation rates and found a reduction of 3.63 log cfu, 2.00 log cfu ml<sup>-1</sup>, and 2.62 log cfu ml<sup>-1</sup> for *E. coli*, *S. Typhimurium*, and *S. aureus*, respectively. Neither the pH nor the color of the milk samples changed significantly. It was found that no viable cells were detected in whole milk samples after a one-week examination and that the samples remained stable after being stored for more than six weeks.

The rate of microbial reduction increased with increased input power and plasma exposure time, as evidenced by the inactivation of *L. monocytogenes* inoculated into sliced cheese by atmospheric pressure plasma (APP), which is capable of operating at atmospheric pressure in air with 75, 100, 125, and 150 W input powers and 60, 90, and 120 s plasma exposure times. After 120 s of APP treatments at 75, 100, and 125 W, the viable cells of *L. monocytogenes* in sliced cheese were reduced by 1.70, 2.78, and 5.82 log

Microorganisms	Dairy food	Reduction	References
<i>E. coli</i> O157:H7 ATCC43895	Milk	≥ 3.94-log	Ruan [101]
<i>Salmonella</i> (5 strain mixture)		2.95-log	
<i>Listeria monocytogenes</i> (5 strain mixture)		2.74-log	
<i>B. cereus</i> (3 strain mixture)	Skim milk	0.18-log	
<i>E. coli</i> O157:H7 (5 strain mixture)		4.36-log	
<i>Salmonella</i> (5 strain mixture)		5.55-log	
<i>L. monocytogenes</i> (5 strain mixture)		4.73-log	

**Table 6.**  
Effect of plasma technology on different microbes in milk.

cfu g<sup>-1</sup>, respectively. When employing APP (with 75, 100, 125, and 150 W), the exposure times needed to inactivate 90% of the cheese's microbial population were 71.43, 62.50, 19.65, and 17.27 s, respectively. When sliced cheese was treated with 125 and 150 W of APP, no viable cells were detected [100]. **Table 6** provides an overview of how plasma technology influences certain milk bacteria.

## 2.5 Cost effectiveness of non-thermal technologies

The cost-effectiveness of high-pressure processing, pulsed electric field, microfiltration, cold plasma, and ultraviolet light treatment in dairy industries depends on several factors. The initial cost of equipment, maintenance costs, and the cost of energy and materials are important considerations. However, these technologies are generally considered cost-effective because they improve the safety and quality of dairy products, reduce waste, and extend shelf life. According to a recent article, although the initial investment in non-thermal processing equipment is higher, it can result in long-term cost savings by reducing energy consumption and extending product shelf life, thereby reducing waste and production costs [102]. In addition, researchers reported that non-thermal treatments are energy efficient [103] and have a lower environmental impact compared to traditional thermal processing, which is in line with the sustainability goals of the dairy industry [103, 104].

Although the study is somewhat older, Campus [105] reports that capital and operating costs of HPP plants will continue to decline in line with demand for the plants. For example, the average processing cost (depending on processing conditions) of HPP is US\$0.05–0.5 per liter or kilogram of various foods, which is lower than the cost of thermal processing. HPP technology is suitable and can be used cost-effectively for high-value products [106]. Jermann et al. [107] found that HPP is the second most widely used non-thermal technology in the world after microwave treatment. Microfiltration is the most widely used non-thermal processing, and its cost is even lower than that of UHT. According to Skrzypek and Burger [55], the installation of a microfiltration with a capacity of 25,000 l/h with a cream heater costs about €600,000, while a UHT ESL installation for the same capacity costs about €1,000,000, which have a significant effect on wide application of this technique in the dairy industry.

On the other hand, the cost of PEF is higher than that of the conventional method, which must be compensated by a high-priced product [108]. PEF can effectively accelerate the drying process in the food industry compared to conventional drying

with elevated heat by precisely controlling the process temperature, which leads to a reduction in energy costs and gas consumption [109]. Therefore, upscaling of PEF systems for dairy applications remains a challenge and should be a topic of future studies. The same is true for HPP, cold plasma, and ultrasound [110, 111].

In general, non-thermal processing in the dairy industry is not only effective in maintaining product quality and safety by removing or inactivating microorganisms, but can also be cost-effective in the long term, making it a valuable option for dairy producers. Therefore, research and development of these non-thermal processes for food processing should focus on cost-effectiveness, food safety, especially spore inactivation, and customer appeal.

### **3. Conclusion**

In recent years, the demand for dairy products has increased dramatically, especially mozzarella cheese, due to the popularity of pizza. Despite this, these products are also known to have the potential to pose a threat to consumers due to several sources of contamination, including equipment, personnel, additives, packaging materials used during the production process, and nonconforming conditions. The integrated implementation of Hazard Analysis Critical Control Point (HACCP) principles and nonthermal processing for microbial inactivation in Mozzarella cheese manufacturing is critical for guaranteeing food safety and quality. In the HACCP system, hazards and risks are identified and assessed as well as the appropriate controls are implemented at specific points within the production line in order to eliminate or reduce these hazards from occurring during the manufacture, storage, and distribution of food. Once the identification of potential hazards, the critical limit monitoring procedures, and frequency are established, corrective actions can be taken if the CCP fails. This includes temperature control checks, pH maintenance, microbial testing of the end product, and equipment calibration when a failure occurs due to improper equipment operation. The application of HACCP in the mozzarella cheese-making industry proved beneficial and profitable because the industry was able to reduce raw material (milk) and final product (cheese) losses while also increasing consumer confidence by producing safe cheese of improved and consistent quality.

In addition to the HACCP system, the incorporation of nonthermal processing procedures is beneficial for eliminating microorganisms during the production of mozzarella cheese. Nonthermal processing methods, such as high-pressure processing, pulsed electric fields, and ultraviolet light treatment, can be an alternative to traditional thermal processes such as pasteurization. These methods can successfully inactivate pathogenic and spoilage microorganisms but have little influence on product quality. These processing methods can be used at specific, important control points specified by the HACCP system, providing an additional layer of microbiological control and maintaining the safety of mozzarella cheese. Therefore, implementing HACCP procedures and nonthermal processing techniques for the production of mozzarella cheese plays an important role in maintaining the quality of the cheese as well as extending its shelf life.

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