fermentation

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Review

Comprehensive Study on Hygiene and Quality Assessment Practices in the Production of Drinkable Dairy-Based and Plant-Based Fermented Products

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Abstract: Hygiene practices are crucial for the production of fermented products, as they affect both product quality and safety. Fermented products, including dairy-based such as kefir, kombucha, and traditional ethnic drinks, rely on beneficial microbes. However, poor cleanliness might introduce dangerous microorganisms, jeopardizing customer health and product stability. This study aims to discuss the key hygiene measures required for safe and high-quality drinkable dairy-based and plant-based fermented product production and to avoid cross-contamination, fermentation vessels, utensils, and storage containers should be cleaned and sterilized regularly. Personal hygiene for workers is also critical, including adequate handwashing, the use of protective equipment, and hygiene protocol training. Another key part of industrial facility management is environmental control and furthermore, adopting Hazard Analysis and Critical Control Points (HACCP) systems allows for the systematic identification and mitigation of production-related risks. Regular microbiological examination of items and surfaces helps to ensure that hygiene methods are effective and that the products fulfill safety requirements. Therefore, strict hygiene measures must be followed when creating fermented drinks to provide safe, high-quality products. Such procedures not only protect consumer health, but also improve product shelf life and sensory properties, increasing consumer trust and satisfaction.

Keywords: hygiene; quality assessment; dairy based; plant based; fermented products

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1. Introduction

Fermentation is a technique that utilizes microorganisms to break down complex organic substances into smaller ones. The process results in desired biochemical alterations that develop the product with chemical and physical attributes. It enhances the nutritional profile of food products by improving the food characteristics comprising vitamins, proteins, and essential amino acids, in addition to aroma, appearance, texture, and taste [1]. Fermentation is considered an important process for producing next-generation food products. The approach has been used for a long time to increase the product's shelf life and enhance its nutritional content [2]. The first use of fermented products by humans dates back to the Neolithic period [3]. The cultural, religious, social, and economic aspects define the use of a variety of ingredients for fermented foods and products [2]. The common fermented foods include food products such as cheese, sauerkraut, miso, kimchi, tempeh, sourdough bread, and natto, as well as fermented meat and fish [4,5]. The use of fermented products has been in practice for millennia. Wine, beer, distilled spirits, kombucha, kefir, boza, kvass, cider, sake, and mead are some important fermented products popular in different communities all across the globe [3,6]. The use of fermentation in dairy products has been prominent in Indian, European, and Middle Eastern countries while fermented animal-based food products have been dominant in China, Japan, and Korea [2]. The consumption of fermented drinks, like yogurt, is reported to reduce the risk of developing

type 2 diabetes, cancers (breast and colorectal), nervous disorders, manage weight, and enhance bone strength, gastrointestinal as well as cardiovascular health [7]. Furthermore, fermentation is also used to produce forages, which are efficient feed for ruminants, like cows. Studies have identified maize as the prevalent crop frequently ensiled due to its high soluble carbohydrate content and the efficient fermentation enhances the nutritional profile of the animal feed after the process of ensiling. Bacterial inoculants are the earliest additives used to manage silage fermentation and chemical additives are now commonly used to preserve silage. However, as these are associated with toxic effects and expensive prices, it has become critical to develop new and safer additives [8].

In the process of fermentation, the microbial strains of bacteria (potential probiotic microbes), filamentous fungi, and yeast strains, like *Saccharomyces cerevisiae*, *Kluyveromyces* spp., and *Komagataella phaffii*, are engineered for application in the food industry [2]. The production of fermented foods is largely dependent on the lactic acid fermentation pathway contributed by lactic acid bacteria (LAB) that are further categorized into hetero-fermenters, homo-fermenters, and facultative fermenters. Hetero-fermenters utilize the phosphoketolase enzyme to generate ethanol, carbon dioxide, and lactate. These include bacteria like *Fructilactobacillus sanfranciscensis*, *Limosilactobacillus fermentum*, *Limosilactobacillus reuteri*, *Levilactobacillus brevis*, and *Leuconostoc* spp., while homo-fermenters generate two moles of lactate from one mole of glucose using the enzyme aldolase enzyme. The class of homofermenters includes LAB like *Lactobacillus acidophilus*, *Lactobacillus delbrueckii*, *Lactobacillus helveticus*, *Enterococcus* spp., and *Pediococcus* spp. The facultative fermenters, like *Latilactobacillus curvatus*, *Lactiplantibacillus plantarum*, and *Lacticaseibacillus casei*, follow the homo- or hetero-fermentation pathway based on the availability of substrate or environmental conditions [9].

The improved technologies with the advancement of science have evolved the fermentation process enhancing the product yield and quality. This includes the development of state-of-the-art equipment and instruments that focus on saving energy, reducing operation time, increasing yield, enhancing quality, and producing safer products [10]. However, improper sanitation conditions in the production facility, use of sub-standard ingredients, improper storage, and a lack of adequate safety measures can result in an increased risk of producing contaminated products containing pathogenic microbes or hazardous toxins. The contaminated products compromise product stability and quality as well as consumer health. Many low- and medium-income countries in Asia and Africa are frequently exposed to safety concerns on fermented products due to poor-quality processing. The presence of biological contaminants like *Salmonella* spp., *Escherichia coli*, *Bacillus cereus*, *Shigella* spp., *Listeria monocytogenes*, and enterotoxigenic *Staphylococcus aureus* are detected in the poor-quality fermented foods [11].

This study aims to understand the production of fermented products, the importance of maintaining hygienic conditions, and the approaches to achieve it. The study aims to address the concerns of biosafety and biosecurity about fermented products.

2. Fermented Products

Fermented products exhibit benefits like enhanced nutritional profile, probiotic properties, improved digestibility, increased shelf life, production of antioxidants, and improvement in sensory parameters [12]. The drinkable fermented products are classified into dairy and plant-based fermented drinks.

Studies have reported that fermented milk and yogurts constitute a market of EUR 46 billion in Asia, North America, and Europe [13]. A wide range of fermented milk is produced in different parts of the world. Most of the dairy-based products have a yogurt-like consistency and are produced from cow, goat, sheep, camel, yak, and coconut milk. Buttermilk, sour milk, ayran, acidophilus milk, kefir, and kumys are some of the common dairy-based fermented products (Figure 1) [14,15]. Daaaaaaaairy-based fermented drinks are also considered rich sources of probiotic bacteria. The bacterial strains involved in fermentation are associated with good gut health stimulating the growth of beneficial

bacteria in the human gut region, thus, the consumption of fermented products improves the composition of gut microbiome improving digestion [16]. A large proportion of these drinks utilizes the natural microflora of the film with LAB like *Lactobacilli*, *Lactococcus*, *Streptococcus*, and *Leuconostoc* [17]. The fermentation of milk by LAB facilitates the elimination of lactose and galactose consequently preventing the conditions of lactose intolerance and galactose accumulation [18]. The shelf life of this class of drinks can be increased to 45 days maintaining its texture and flavor by using techniques like high-pressure processing (HPP). The processing facilitates the maintenance of bacterial count to the optimal levels that ensure the safety of the consumer ingesting the product [19].

Figure 1. Dairy-based fermented products (created by Vuppu et al. using Biorender.com).

The coliform bacteria, *Candida* spp. and *Saccharomyces* spp. are frequently present in fermented milk products, during unhygienic preparation conditions, as observed in some African fermented milk products [17]. The dairy industry in Africa detects the persistence of pathogenic *Brucella abortus*, *Mycobacterium bovis*, and *Coxiella burnettii.* These are considered as serious threats to public health. The high nutrient profile of milk makes it susceptible to contamination by food-borne microbes [20]. Contaminants or hazards to food safety are classified into three classes: biological, physical, and chemical [20]. The contamination can enter the dairy-based fermented product in three important phases: (1) entry into milk through animal feces, air, water, feed, soil, people, and farm equipment, etc.; (2) chances of contaminant entry into the product during the production process due to lack of hygienic conditions in the manufacturing unit; and (3) the entry of contaminants at the storage tank, transportation vessels, and distribution stages due to improper handling of storage containers [11,20]. Therefore, to maintain the safety profile of the product, an efficient sanitation methodology and hygiene approach were necessary to be established at the production facility, which can eliminate the risk factors that can be introduced in each step.

The plant-based fermented products (as presented in Figure 2) are derived from plants, like maize, barley, millets, quinoa, oats, rice, wheat, soy, sorghum, coconut, sesame, rye,

hemp, hazelnut, and almond, utilizing the natural microbial community present in the grains [17,21]. Some examples of traditional plant-based fermented products include kvass, boza, kile, taar, kaera, hulumur, kali, and borsh [14]. Microbes like *Lactobacillus confuses*, *Leuconostoc mesenteroides*, and *Saccharomyces cerevisiae* are often used for the fermentation of several drinks [17].

Figure 2. Plant-based fermented products (created by Vuppu et al. using Biorender.com).

Plant-based fermented drinks are categorized into alcoholic and non-alcoholic products. Chhang, jau chhang, chicha, tarubá, and apple cider are some traditional alcoholic products [22].

Kombucha and water kefir are common non-alcoholic fermented products, however they have a trace amount of alcohol in their composition due to fermentation [22].

Each type of fermented drink requires specific raw materials and a defined set of bacterial or yeast strains to form the product. Table 1 presents an overview of the details of dairy-based fermented drinks and Table 2 presents details of plant-based fermented drinks.

Table 1. Details of dairy-based fermented products.

Product	Geographic Distribution	Raw Materials (Substrate)	Microorganisms Used in Fermentation (Starter Culture)	Fermentation Time	Reference
Buttermilk	Europe	Cow milk	Mesophilic LAB	16 h	[12,23]
Sour milk	Iceland, Denmark, Sweden, and Southern Norway	Milk	Mesophilic LAB	6–8 h	[14,24]
Ayran	Asia (Central Asia and the Middle East) and Europe	Cow milk	*Lactobacillus bulgaricus* and *Streptococcus thermophilus*	4–6 h	[12,17,25]
Acidophilus milk	America and Europe	Cow milk	*Lactobacillus acidophilus*	18–24 h	[12,15,26]
Kefir grains with milk	Eastern Europe	Milk	*Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus fermentum*, *Lactobacillus acidophilus*, *Lactococcus* spp., *Leuconostoc* spp., *Acetobacter* spp., *Kluyveromyces marxianus*, *Saccharomyces cerevisiae*, *Saccharomyces unisporus*, and *Saccharomyces exiguus*	16 h	[15,17,18]

Table 1. *Cont.*

Product	Geographic Distribution	Raw Materials (Substrate)	Microorganisms Used in Fermentation (Starter Culture)	Fermentation Time	Reference
Koumiss	Asia and Russia	Horse milk	*Lactobacillus helveticus* NS8	Primary fermentation: 2 h Secondary fermentation (after packaging): 2–3 days	[17,26]

Table 2. Details of plant-based fermented products.

Product	Geographic Distribution	Raw Materials (Substrate)	Microorganisms Used in Fermentation (Starter Culture)	Fermentation Time	Reference
Kvass	Russia	Rye and barley malt or flour and rye bread	*Leuconostoc mesenteroides*, *Lactobacillus casei*, and *Saccharomyces cerevisiae*	4–10 h	[17,27]
Boza	Turkey and Bulgaria	Barley, wheat, rye, rice, millet, oats, or maize	*Leuconostoc mesenteroides*, *Leuconostoc paramesenteroides*, *Leuconostoc sanfranciscensis*, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, *Saccharomyces uvarum*, *Candida* spp., and *Pichia fermentans*	24–48 h	[17,28,29]
Kaera	Estonia	Oat	LAB	6–12 h	[14]
Hulumur	Turkey	Rice, millet, and sorghum	LAB	24 h	[14,30]
Borsh	Hungary and Romania	Beetroot (for red borsh) and cereals (for white borsh)	LAB	7 days	[14,31,32]
Chhang	Himalayan belt of India	Rice	*Lactobacillus pentosus*, *Pediococcus pentosaceus*, *Bacillus aerophilus*, *Bacillus subtilis*, *Saccharomyces* spp., *Saccharomycopsis malanga*, *Saccharomycopsis fibuligera*, and *Kluyveromyces marxianus*	12–24 h	[33]
Jau chhang	Himalayan belt of India	Barley	*Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Serratia* spp., *Saccharomyces cerevisiae*, and *Candida tropicalis*	3–5 days	[22,34]
Chicha	South America	Rice, corn, peanuts, cassava, and fruits	*Lactobacillus plantarum*, *Streptococcus* spp., *Leuconostoc* spp., *Weissella* spp., *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*, *Candida* spp., and *Pichia* spp.	1–3 days	[22,35]
Tarubá	Amazonas, Brazil	Cassava	*Bacillus subtilis*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Torulaspora delbrueckii*, *Pichia exigua*, and *Candida* spp.	12 days	[22,36]

Table 2. *Cont.*

Product	Geographic Distribution	Raw Materials (Substrate)	Microorganisms Used in Fermentation (Starter Culture)	Fermentation Time	Reference
Apple cider	Global	Apple	*Lentilactobacillus diolivorans*, *Lentilactobacillus buchneri*, *Secundilactobacillus collinoides*, *Secundilactobacillus paracollinoides*, *Lactobacillus plantarum*, *Limosilactobacillus fermentum*, and *Paucilactobacillus suebicus*	2–3 weeks	[22]
Kombucha	Global, prominent in China	Tea	*Lactobacillus* spp., *Acetobacter* spp., *Gluconacetobacter xylinus*, *Candida* spp., *Saccharomyces* spp., *Pichia* spp., *Zygosaccharomyces* spp., *Dekkera* spp., *Torulaspora* spp., and *Hanseniaspora* spp.	14 days	[17,22,37]
Water kefir	Global, prominent in Mexico	Fruits, vegetables, and molasses	*Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus brevis*, *Lactobacillus hilgardii*, *Lactobacillus pentosus*, *Lactococcus lactis*, *Leuconostoc mesenteroides*, *Zymomonas* spp., *Saccharomyces cerevisiae*, *Zygosaccharomyces Florentina*, *Zygosaccharomyces lentus*, *Dekkera bruxellensis*, *Sekkera anomola*, *Hanseniaspora vinea*, *Hanseniaspora valbyensis*, and *Lachancea fermentati*	2–4 days	[17,22,38]

3. Production of Fermented Products

Fermentation converts carbohydrates into acids or alcohols mediated by microorganisms like yeast and LAB. LAB comprise *Lactobacillus* spp., *Lactococcus* spp., and *Enterococcus* spp., and play an important role in bio-control strategies by demonstrating antibacterial properties that result in the inhibition of the growth of pathogenic microbes. These bacterial strains have potential to remove mycotoxins [13]. The chemical reaction of lactic acid fermentation is presented in Figure 3.

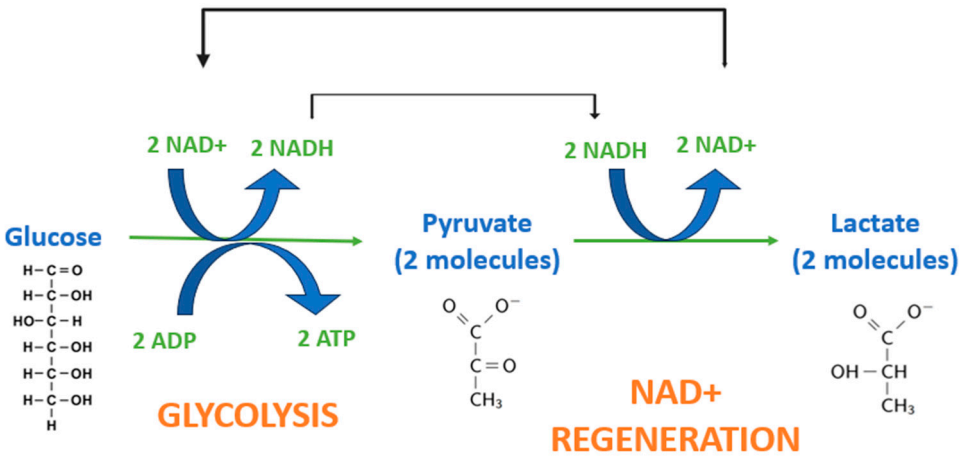

Figure 3. Lactic acid fermentation.

The majority of the fermented products follow submerged fermentation. It is the most prevalent biotechnological application in the industry and constitutes the most advanced

design and operation system. The solid composition of the mass represents 5–10% of the fermentation materials [39]. The schematic representation of the production of fermented products is presented in Figure 4.

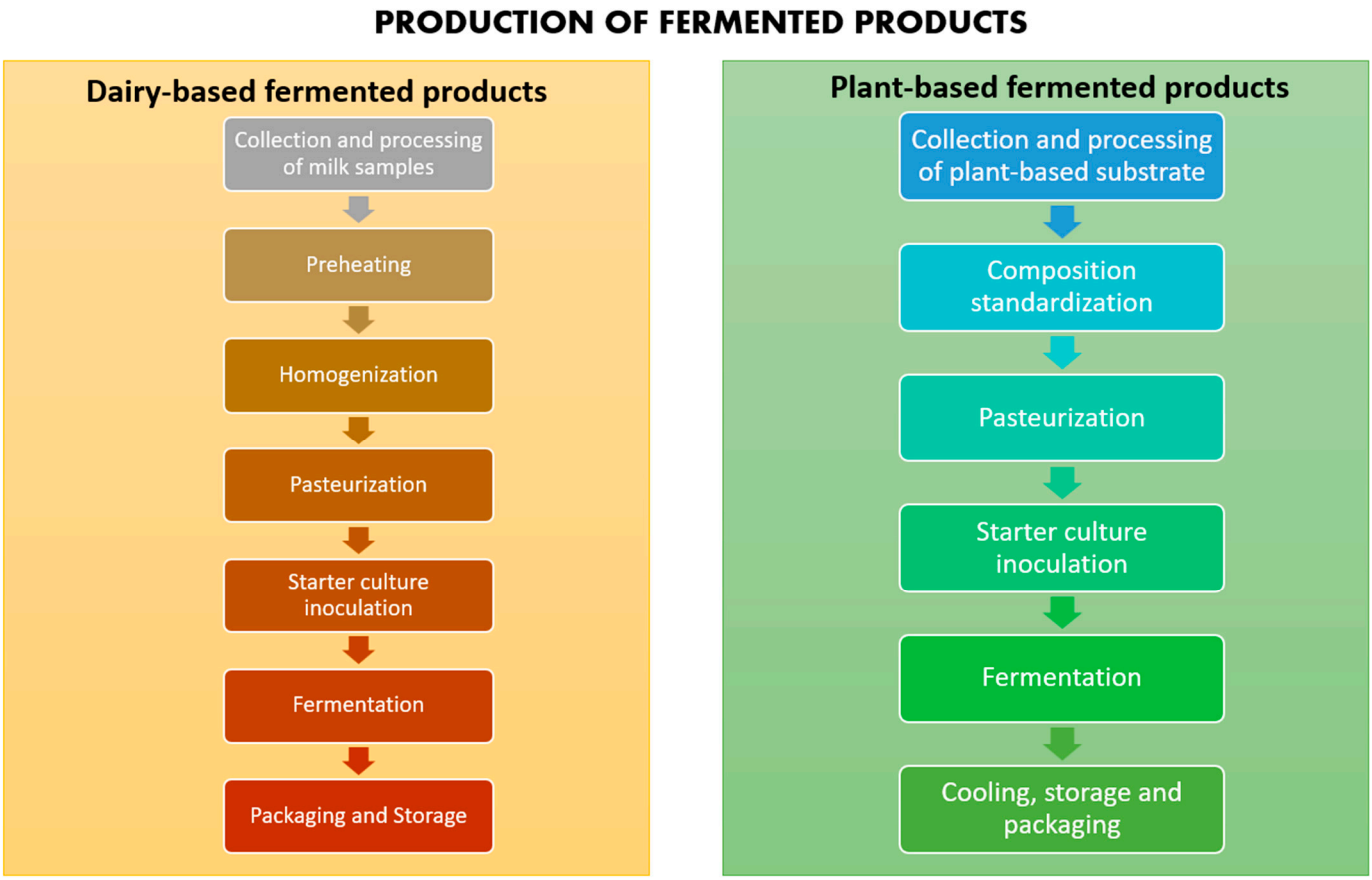

Figure 4. Schematic representation of the production of fermented products.

3.1. Preparation of Dairy-Based Fermented Products

The important components of fermentation include the substrate, starter culture, microbial inoculum for fermentation, and suitable fermenters designed for a specific product. The general steps involved in the production of dairy-based products include a collection of milk, pasteurization of milk, the addition of skimmed milk, cooling, inoculation of starter culture, fermentation at an optimal temperature and pH followed by cooling overnight [15,40]. During the fermentation of milk, LAB ferment lactose causing a reduction in the pH that prevents the proliferation of pathogenic microbes and increases the shelf-life of the fermented products [41]. The fermentation of drinkable dairy products involves metabolic processes like proteolysis, carbohydrate metabolism, and lipolysis [42].

Throughout history, dairy-based fermented items have been made from the milk of all domesticated animals. Traditionally, the spontaneous fermentation technique was based on the utilization of natural microflora of the substrate (milk in the case of dairy-based products) for fermentation. With advancements in science, scientists have isolated beneficial bacteria and analyzed their characteristics. Consequently, the identification of LAB and their health benefits as well as their role in lactic acid fermentation were reported [43].

The standard for fermented milk is provided by CODEX (CXS 243-2003) [44]. The products should be developed as per the guidelines provided by the Code of Hygienic Practice for Milk and Milk Products (CXC 57-2004) [44] and the General Principles of Food Hygiene (CXC 1-1969) [44]. These guidelines prevent the removal of whey after fermentation during the production of fermented drinks [45].

3.1.1. Collection and Processing of Milk Samples

High-quality milk samples that fulfill the regulatory guidelines are selected for the preparation of the product. The samples are homogenized for breaking fat globules creating a consistent texture and preventing separation of cream.

3.1.2. Pasteurization of Dairy-Based Fermented Drinks

Pasteurization is broadly categorized into two categories: long-time pasteurization at low temperatures (also known as the holder method) and short-time pasteurization at higher temperatures (also known as the flash method). The holder method involves heating samples to around 62 °C for approximately 30 min, while in the case of the flash method, the samples are heated to over 72 °C for around 15 min. The type of pasteurization employed is decided based on the desired end product. The majority of the drinkable fermented products undergo the flash method of pasteurization [46].

The milk sample is heated using hot water, an electric current, or dry heat, and maintained at 62.8 °C for 30 min. Following this, the milk is cooled at 4 °C [47]. This approach eliminates pathogenic microbes with no detrimental impact on the nutritional and sensory characteristics. However, an inadequate temperature for pasteurization may prove to be inefficient in the elimination of pathogenic microbes in contaminated milk samples. Instead, one could promote the rapid multiplication of the microbes. Poor modulation of the temperature and time can also increase the risk of contamination [20]. Different dairy-based fermented products require different pasteurization conditions. For instance, in the case of the production of milk kefir, the milk is pasteurized for 15 min at 90 °C followed by cooling to 25 °C. The cooled milk is mixed with kefir grains and processed to form milk kefir [48]. In the case of Koumiss, the mare or cow milk is pasteurized by heating at 90–92 °C for a period of 5 to 10 min. This is followed by cooling at 26–28 °C [49]. The fermented drink kombucha requires pasteurization at 82 °C for 30 s [50].

The pasteurization conditions can vary for different geographic locations. Regions with warmer temperatures require pasteurization regularly to avoid microbial contamination, while the process can be less frequent in places with cooler temperatures. The pathogens eliminated by pasteurization vary in different regions based on the dominant pathogen prevalent in that region. The conditions of pasteurization are also calibrated based on the quality and efficiency of the pasteurization facility [46].

3.1.3. Inoculation with Starter Cultures

The inoculum of starter cultures specific to the type of fermented drink is added to the obtained pasteurized milk. *Bifidobacterium* spp., *Lactobacillus* spp., and *Streptococcus* spp. are the common bacterial strains involved in fermentation. The volume of inoculum used is generally around 1–3% of the total milk volume.

3.1.4. Fermentation of Dairy-Based Products

The fermentation of dairy-based products is performed at the optimal temperature range of 24–32 °C for mesophiles or 35–55 °C for thermophiles [40]. The incubation time varies according to the product being produced. The pH is constantly monitored during fermentation till it reaches the required acidity. Until the minimal durability date, the starter culture will continue to be active, viable, and present in large numbers inside the composition. A starter culture should have a minimum of 107 CFU/g of microorganisms. In the case of probiotic drinks, the count of the microorganisms added as a supplement to enhance the nutrition profile should be 106 CFU/g [12]. The colony-forming units per gram (CFU/g) are calculated using the formula

$$\text{CFU/g} = (\text{Number of colonies} \times \text{dilution factor})/\text{volume of culture (mL)}.$$

The production of lactic acid during fermentation by breaking down lactose results in a reduction in pH and a thickened texture. During the process, metabolic by-products, like

ethanol, carbon dioxide, and diacetyl, are released into the fermenter. Once the product obtains the required texture and pH, the process of fermentation is stopped, and the temperature is brought below 10 °C. The obtained product is then stored and packaged for distribution to outlets and consumers. Cooling post-fermentation is an essential approach to prevent the proliferation of contaminants in the product, consequently enhancing the shelf life. According to the guidelines of the European Union, fermented dairy products are cooled to temperatures around 4 °C or lower, while the FDA mandates cooling to 7 °C or lower after fermentation [51–53].

3.2. Preparation of Plant-Based Fermented Products

The plant-based fermented drinks derived from fruits, vegetables, legumes, tea, grains, and protein-based whey are becoming a popular aspect of research, as they are advantageous to people who are lactose intolerant or have high cholesterol [54]. In addition to alcoholic products, the category of plant-based fermented products also includes plant-based milk substitutes, like soy milk and peanut soymilk [16].

3.2.1. Collection and Processing of Substrates

The plant-based ingredients are the substrates for the preparation of plant-based fermented products. The ingredients are collected and washed thoroughly to remove the physical impurities. Following this, the samples are soaked and ground to obtain liquid, which is further filtered to remove the residual mass.

3.2.2. Composition Standardization

The composition of the liquid is standardized by adding nutrients required for fermentation.

3.2.3. Pasteurization of Plant-Based Fermented Drinks

Pasteurization is an important aseptic technique to produce plant-based fermented drinks. Usually, plant extracts obtained by different methods undergo pasteurization at 80 °C for 5 minutes (flash fermentation). This is useful for the elimination of non-sporulating pathogenic microbes [55]. A plant-based traditional Turkish fermented drink called Shalgam, prepared using bulgur flour, black carrot, turnip, sourdough, water, and salt, requires pasteurization at 85 °C for 26 s [56]. A fermented drink developed from discarded bread flour requires heating at 70 °C for 5 min, followed by cooling for 10 min at 37 °C [57]. In the case of cider, pasteurization is performed by heating at 76.7 °C for 10 min [58].

3.2.4. Starter Culture Inoculation

The starter culture is inoculated based on the desired product, and mixed evenly for a uniform distribution. The starter culture can comprise bacterial strains, yeast strains, or a combination of both. Before inoculation, the liquid derived from the plant part is pre-heated at 37 °C in a water bath.

3.2.5. Fermentation of Plant-Based Products

The fermentation time can vary from days to weeks for different fermented drinks. The parameters, like temperature, pH, and oxygen level (in the case of aerobic fermentation), are set at optimal conditions. The majority of the fermented products are prepared by fermentation at the temperature range of 20 to 40 °C for 24 h to a few weeks based on the desired product [59]. The process of fermentation enhances the activity, like antioxidant activity, and bioavailability of phenolic acids by facilitating their biotransformation from polyphenols using LAB [54].

3.2.6. Cooling, Storage, and Packaging

The produced fermented products are cooled to arrest bacterial activity and prevent further growth. The product is stored at 4 °C to extend its shelf life and the liquid is transferred to clean, sterile bottles or containers to prevent the risk of contamination, sealed, and sent to retail outlets for the consumers.

4. Sanitation and Hygiene during the Production of Fermented Drinks

Fermentation is a process that was developed to extend the shelf life of products. It promotes the growth of probiotic microorganisms, like LABs, which are advantageous to human health. These beneficial bacteria also facilitate the elimination or inhibition of the growth of pathogenic microbes. However, there exists a risk of contamination of the fermented product by pathogenic microbes due to the lack of hygiene compliance during the production stages [60]. Hygiene and sanitation are essential during the production of fermented drinks and are required at each step to prevent the entry of hazardous contaminants or the release of toxins in the composition of the fermented drinks. It is important to ensure food safety during the production process. The important strategies to ensure the hygienic production of contamination-free products include the control of the ingredients to prevent the entry of contaminants, and regular sterilization of fermentation vessels, equipment, tools, and storage containers. The staff and workers working in the facility should also practice proper hygiene, like frequent hand washing or sanitation. The facility should be developed with environmental controls to regulate parameters like pH, temperature, oxygen level, pressure, agitation, and foam. It also includes keeping surfaces clean and sterilized, monitoring air quality to reduce airborne pollutants, and ensuring proper waste disposal.

Quality control checks using microbiological techniques at every step are also necessary to ensure the good quality of the product and avoid the entry of undesired pathogens. Hazard Analysis and Critical Control Points (HACCP) systems can be employed for quality control facilitating the systematic identification and mitigation of production-related risks. It is useful for the management of food quality and safety. The guidelines and regulations for HACCP are provided by the World Health Organization (WHO) and the United Nations Food and Agriculture Organization (UN FAO). The HACCP help to detect the presence of biological, chemical, and physical contaminants during the production and post-production processes. It ensures the food safety compliance of fermented products. Quality assessments of the product utilize scientific techniques, like microbiological studies (e.g., the plate culture technique), to detect pathogenic contaminants, and assay studies for the detection of biological and chemical contaminants [61,62]. The guarantee of food safety relies on compliance to Good Manufacturing Practices (GMPs), Food Safety Management Systems (FSMSs), Good Hygiene Practices (GHPs), and Sanitation Standard Operating Procedures (SSOPs). Serious monitoring of the production process and taking corrective measures will facilitate the reduction in the risk of contamination. Thus, the HACCP monitor and validate the efficiency of the safe production process of fermented products [63]. The laboratory tests employed for hygiene assessment include an enumeration of the microbial count, determination of pH [64], visualization of fluorescent indicator using UV light, the contact-agar technique, cultivation of surface microbes by swabbing and swiping with non-woven cloth [65], and the aerobic plate count test, as provided by the Bacteriological Analytical Manual from the Food and Drug Administration (FDA) [60]. Cultivation of surface microbes by swabbing and an enumeration of the bacterial count: The swabs of the samples for enumeration to ensure good hygiene at the facility are collected from the floors, walls, equipment like fermenters, containers, and personal protective equipment of the working staff following the reference method ISO18593 (2018) [66]. The swabs are placed aseptically in a sterile container and transported to the testing lab, where they are plated using the swab culture technique. The plates are incubated at 37 °C for 24 h. Following this, the bacterial colonies are enumerated using a colony counter [67].

Ultraviolet fluorescence markers for the detection of contamination: The utility of this technique to detect contamination gained prominence after the COVID-19 outbreak. It is performed using program image processing software that calculates the intensity and area of the fluorescent dyes. The image is captured orthogonally using a camera. The processing requires a homology matrix to correct and stitch images, estimate pose, and construct vision. The result is analyzed using visualization software on a computer [68].

Aerobic plate count test: This test is employed to identify the population of aerobic bacteria in the fermented product to detect the presence of pathogens. The method involves the procurement of a sample of the product, dilution of the sample, plating the sample on agar plates, incubation at 37 °C for 24 h, enumerating the colonies using a colony counter, and calculating the number of probable colonies per milliliter (mL) of a sample. The approach is useful for screening psychrotrophic, thermodurics, coliforms, and proteolytic or lipolytic microorganisms [69].

The presence of toxins, such as bacterial toxins, mycotoxins, and biogenic amines, were detected using a series of chromatographic, immunoassay-based, nano-sensor, and culturing techniques. The identified toxins can be efficiently removed from the product by using a particular starter culture for aflatoxin binding, the degradation of biogenic amines, and bio-preservation [70]. The detection of mycotoxins in the sample is a complicated task due to its complex structure and chemical profile. Usually, mixture of methanol with water and acetonitrile with water are used as solvents to extract the toxins from fermented products. However, in the case of fermented products that have a high-fat profile, 1-octanol, ethyl-acetate and formic acid, toluene, chloroform, acetone, and dichloromethane are used to efficiently extract mycotoxins. This is followed by a clean-up step for enhanced selectivity and the removal of interfering compounds. The clean-up step is performed using techniques like liquid-liquid extraction, solid phase extraction, solid-liquid extraction, microwave-assisted extraction, supercritical fluid extraction, aptamer affinity columns, and immunoaffinity columns. The mycotoxins are detected and identified using analytic methods, like high-performance liquid chromatography, gas chromatography, TLC, and immunoassays, which have been modified with detectors, including diode array, MS, UV, and FL. While the biogenic amines are detected and quantified using colorimetric, and fluorometric techniques, advanced chromatographic techniques, like HPLC-UV and HPLC-FL, are dominantly used [71]. The most suitable approach to remove the toxins from the product includes a "clean-substrate strategy" that focuses on the prevention of the contamination and accumulation of mycotoxins at the initial stage of production, that is, the harvest of crops. The process of fermentation is also reported to control the toxins in the product by eliminating or reducing their quantity during the fermentation stage. Several beneficial microorganisms used in fermentation have demonstrated the ability to decrease the toxin load or even eliminate it efficiently [71].

The sanitation and hygiene strategies for dairy-based fermented drinks and plant-based fermented products are discussed below.

4.1. Sanitation Strategies for Sample Collection and Processing

Milk is the key ingredient in the case of dairy-based fermented drinks. Good hygiene practices can be assured by maintaining sanitized conditions during milking. The facility should be clean and sanitized regularly to protect the cows from developing infections. The udder of the cow is wiped or sprayed with disinfecting sprays [72]. Since this part of the cow is exposed to milk, it is preferable to use a natural sanitizer to avoid any side effects due to the presence of chemicals in the synthetic formulations. The natural formulations developed are to be used during the process of milking cows to create a sterile environment for the prevention of initial adulteration of milk by contaminants.

The antimicrobial activity of the natural sanitizers is contributed by the phytocompounds present in the composition, which are derived from plant sources. Aloe vera can also be used in the formulation as it provides moisturization to the tissues of the udder of the cow and also exhibits antimicrobial activity. In the case of plant-based fermented drinks,

the plant-based raw materials or substrates are cleaned thoroughly by washing with clean sterile distilled water. The containers, vessels, and catheters handling the raw materials are washed effectively with soap and water after every use. The containers carrying milk have to be sealed immediately after the addition of the sample. Similarly, containers used for placing soaked and ground plant parts are also cleaned properly and kept closed to avoid the entry of any physical or biological contaminants.

All liquids are pasteurized before the fermentation step to eliminate all contaminated microbes that could potentially interfere with the fermentation step. Pasteurization also maintains the quality of the drinkable fermented products. All instruments and equipment required are sanitized, cleaned, and dried for the efficient prevention of entry of contaminants.

4.2. Significance of Sanitation during the Inoculation of Starter Culture

Studies have found that bacterial isolates may contain genes coding for antibiotic resistance. Such microbes, when introduced with the starter culture, impart the resistance properties to the desired microbes of the fermentation starter culture, thus impacting the safety profile of the fermentation microbes and making the fermentation product a reservoir of resistance genes. Microorganisms with characteristics like antibiotic resistance properties should not be utilized in the food industry [73].

The inoculation of the starter culture into the substrate should be performed aseptically using a Laminar Air Hood to avoid contamination with undesired microbes that would impact the quality, safety, and yield of the product.

4.3. Hygiene and Sanitation Approaches in Fermentation

Inadequate or poor sanitation practices can have serious consequences on the fermentation process. The presence of biological contaminants leads to a reduced count of beneficial bacteria (good bacteria) and increased proliferation of harmful pathogenic bacteria. Conventionally, the risk of contamination is lowered by the utilization of chemical agents, like sulfur dioxide (a microbial inhibitor and antioxidant). However, the chemical agent impacts the organoleptic properties of the fermented product, like altering the aroma of the drink. It is also associated with serious health complications, like hypersensitivity reactions, increased risk of asthma, respiratory distress, rashes on the skin, and abdominal pain. It is also bacteriostatic in contrast to the desirable bactericidal properties. The chemical preservative, dimethyl dicarbonate (DMDC), is also used to prevent the growth of yeast during wine fermentation. The efficacy of this compound against yeast is greater than that against molds and bacteria. It can also react with methyl ethyl carbonate, methyl carbamate, and dimethyl carbonate, generating toxic byproducts [74]. Ascorbic acid, sodium hypochlorite (NaOCl), colloidal silver complex (CAgS), ethanethiol, and chitosan are examples of other commonly used preservatives during fermentation. Killer toxins and bacteriosins, like nisin, pediocin PA-1, killer toxins CpKT1 and CpKT2, and lacticin 3147 are also used as alternatives to sulfur dioxide [75]. All these compounds and approaches have limitations that further emphasize the importance of developing natural substitutes for these agents that are safer, eco-friendly, and inexpensive. Plants rich in antioxidants offer an effective alternative to chemical preservatives. Several studies have identified plant parts, like oak, vine shoot, wood tannin, almond peel, eucalyptus peel, essential oil of thyme, stilbenes extract, glutathione, hydroxytyrosol, oleuropein, and black radish, as replacements for sulfur dioxide in wine fermentation [75,76]. Usually, these compounds are added to the fermentation setup at a mass range of 200 to 400 mg/L [75].

We propose a preservative composition derived from plant extracts of ginger, turmeric, rosemary, tea, mint, and cinnamon exhibiting antioxidant and antimicrobial properties [77–82] with the potential to prevent the contamination of fermentation media. Phenolic compounds are a class of secondary metabolites responsible for pharmacological activities like antioxidant, anti-microbial, anti-diabetic, and anti-cancer. These compounds also remove free radical molecules and metal chelators, as well as prevent lipid peroxidation [83].

The natural preservative can be prepared by washing the plant parts thoroughly with sterile, distilled water. Following this, the plant tissue is crushed or ground, and filtered to obtain the extract. The sugars are removed from the obtained extract using the ultrafiltration technique. Furthermore, acacia gum or Arabic gum is added to the filtered liquid, and the mixture is lyophilized. The lyophilized powder is then stored in a clean, dry container under vacuum. Around 200 mg of the developed natural preservative powder are added to the fermentation setup [76].

Regular sanitation and sterilization of fermentation vessels and all the equipment and containers required in the food processing industry are essential as per the regulatory standards [84]. The fermentation facility and surface are also sanitized frequently. A novel formulation of a sanitizing agent for disinfecting the surfaces and containers in a fermentation facility can also be developed with natural, chemical-free ingredients, like tulsi, lemongrass, orange, mint, and cinnamon, in sterile water. To prepare this solution, 5 g of tulsi leaf powder, 8 g of lemongrass powder, 8 g of orange peel powder, 5 g of mint powder, and 2 g of cinnamon powder are mixed with sterile water to obtain a volume of 100 mL. The values were determined based on experimental studies like antimicrobial susceptibility tests and MIC studies. The solution is filtered to remove the residual matter, and the filtrate is ready to be used as a sanitizing agent. This solution can even be utilized by the working staff for frequent sanitation. Tulsi (*Ocimum sanctum*) is a beneficial medicinal herb that exhibits a wide array of pharmacological properties, including antimicrobial, antiviral, anti-oxidant, antidiabetic, and antistress. These properties are attributed to the presence of phytocompounds, like eugenol, apigenin, rosamarinic acid, linalool, urosolic acid, carvacrol, and β-caryophyllene. The plant extracts are reported to be effective against various bacterial and fungal strains such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Shigella dysentriae*, *Salmonella typhi*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Bacillus pumilus*, *Candida albican*, *Penicillium* spp., and *Aspergillus* spp. [85]. Lemongrass (*Cymbopogon citratus*) is known for its pharmacological activities due to the properties of phytocompounds like citral, citronellal, iso geranial, geranial, isoneral, neral, geraniol, citronellol, germacrene-D, geranyl acetate, and elemol. The extract of lemongrass reportedly inhibits the proliferation of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus cereus*, *Salmonella* spp., and *Candida albicans* [86,87]. The use of citrus fruit-based products like orange peel powder (*Citrus sinensis*), is associated with antibacterial and antifungal activities in addition to their pleasing fragrance [88]. Orange is rich in phytochemicals including linalool, limonene, ascorbic acid, linoleic acid, stearic acid, pentacyclic acid, and palmitic acid [89]. Studies have identified that orange peel powder is effective against bacterial strains like *Staphylococcus aureus*, *Staphylococcus epidermis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexineri*, and *Bacillus subtilis* [90]. Mint (*Mentha* spp.) is utilized for its antimicrobial activity facilitated by the properties of phytochemicals like limonene, β-pinene, pulegone, thymol, citronellal, citral, m-cresol, piperitone, and β-phellandrene [91]. Reports suggest that mint is effective in inhibiting the growth of both Gram-positive and Gram-negative bacteria [92]. Cinnamon (*Cinnamomum zeylanicum*) exhibits effective antimicrobial properties against *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria innocua* due to the properties of cinnamaldehyde, eugenol, eucalyptol, linalool, and β-caryophyllene [93]. The use of natural ingredients provides protection from any side effects that are common when using chemical sanitizers.

The advanced technology has reduced the difficulties of sterilizing fermentation facilities and instruments. Configuring valve systems in conjunction with steam traps is a crucial aspect of a fermentation unit. The systems can be modified based on the requirements to ensure adequate sterilization and prevent contamination [94].

4.4. Sanitation Methods for Storage and Packaging

The fermented products are transferred aseptically to clean, sterile containers, sealed, and packed. The packed products are stored at cooler temperatures of around 4 to 8 °C.

However, storage at a higher temperature (around 14 °C) can result in acidity by increasing the pH, altering the sensory characteristics, and exhibiting an increased count of aerobic bacteria [95].

The practices of proper sanitation and hygiene measures are essential to prevent the incidences of food spoilage and food poisoning inhibiting the growth of disease-causing pathogenic microbes. The overview of the sanitation strategies is presented in Figure 5. For proper hygiene control, human intervention in the production process should be minimized.

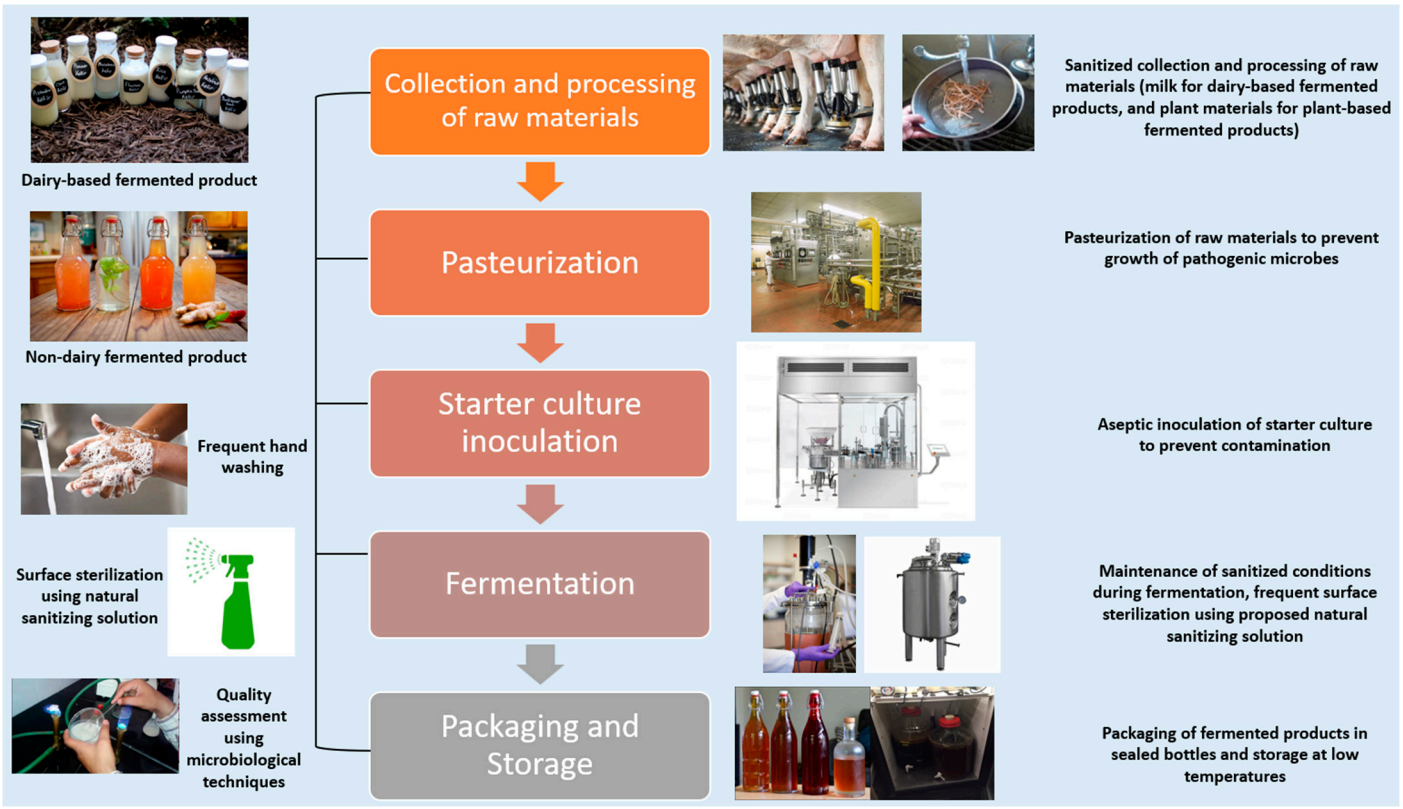

Figure 5. Overview of hygiene strategies at different steps of the production process.

5. Product Yield on Compliance and Non-Compliance to Hygiene Standards

The contamination of raw materials is an important factor causing failure during production and processing, resulting in the spoilage of fermented products. It also causes an increase in foam production and flavor loss. Each fermented drink requires specific conditions for production, which may be conducive to the undesirable microbes that enter the system by contaminated raw materials or introduced at any stage during processing. This proliferation of undesired microbes in the product results in reduced quality and the production of toxic by-products that are harmful to humans upon consumption. Enteric pathogens comprising bacteria, viruses, and protozoans are majorly responsible for the negative consequences on human health [96]. Studies have reported that spore-producing bacteria are among the common contaminants of fermented products that are introduced due to non-compliance with hygiene and sanitization guidelines. They include *Clostridium butyricum*, *Clostridium perfringens*, and *Clostridium tyrobutyricum* [97]. Also, bacteria, like *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*, reportedly release toxins, like biogenic amines, which are hazardous to the health of consumers. Other prevalent pathogens *include Shigella* spp., *Brucella* spp., *Salmonella enterica*, *Listeria monocytogenes*, and *Campylobacter* spp. [98]. The presence of these microbes results in the reduced effectiveness of fermentation. Such bacteria utilize the sugars from the product and generate chemical compounds undesirable for industrial application and deteriorate the quality of the fermented product [99].

On the other hand, ensuring the practice of adequate hygiene methods during the production process, the microbes dominating the product are the fermenting LAB and yeast, which belong to the class of microbes called GRAS (generally recognized as safe) [100].

Compliance with the guidelines and regulations during the production of fermented drinks ensures the quality and safety of the product. It prevents the entry of contaminants or the release of endotoxins into the composition. On the other hand, the lack of hygiene and sanitization strategies can result in contamination by the class of microbes called extremophiles, which can survive in in extreme environmental conditions, such as high temperature, high pH (alkalinity), low pH (acidity), high salt concentration, and low water availability. The detection of thermophiles corresponds to contamination with hazardous toxic consequences. These organisms can also cause the spoilage of acidic food products due to reduced water activity, like in the case of contamination by *Debaryomyces hansenii* [2]. Water activity is the percentage of equilibrium relative humidity, and is calculated as presented in Equation (1). The moisture content is usually obtained using the gravimetric method.

$$\text{Water activity (aw)} = \text{ERH}/100 \quad (1)$$

where ERH is the equilibrium relative humidity [101].

The antioxidant activity is an important feature of fermented products. The product exposed to contamination will have lower antioxidant activity compared to contamination-free fermented products. The activity was determined using DPPH (1,1-diphenyl-2-picrylhrdrazyl) by adding it to the test sample, followed by incubation in a dark room for 20 min. The sample was centrifuged at 4000 rpm for 10 min at 4 °C, and the absorbance of the supernatant was estimated at 517 nm using a spectrophotometer. A methanol solution with 1 mM of DPPH serves as the standard for the analysis. The antioxidant activity is calculated using Equation (2) [102]:

$$\text{Antioxidant activity (in percentage)} = [\text{Standard absorbance} - \text{Sample absorbance}]/\text{Standard absorbance} \times 100 \quad (2)$$

pH is another important characteristic determining the quality of the product. Contamination or spoilage due to a lack of hygiene practices during manufacturing can result in a deviation of the pH from the desirable range for the product. The pH is generally calculated using a digital pH meter [102].

The rheological properties, like shear rate, shear stress, and viscosity, of the fermented products depend on the fraction of their volume due to the high protein content [103,104]. The viscosity is measured using a viscometer. The consistency of the fermented products is determined using Equation (3), which represents the power law model:

$$\delta = K(\gamma)^n \quad (3)$$

where δ represents the shear stress (in Pa) and γ represents the shear rate (in s^{-1}) [104]. The Arrhenius equation (Equation (4)) is used to calculate the apparent viscosity, where η denotes the apparent viscosity, A represents the frequency factor, E_a is the activation energy, R is gas constant, and T is the temperature in K [104].

$$\eta = A\, e^{(-E_a/RT)} \quad (4)$$

Viscosity, in the case of fermented products, is due to the coagulation of protein. It is influenced by the concentration of total dissolved solids in the product. The greater the amount of total dissolved solids, the greater the viscosity of the fermented product [102]. Therefore, in the case of contaminated fermented drinks, an increase in the viscosity may be observed, attributed to the generation of polysaccharides by the proliferating pathogenic microbes in the product. Studies have also reported the relation between viscosity and the concentration of the key ingredient. The increase in the concentration of ingredients increased the apparent viscosity of the product. Usually, all products exhibit an increase in viscosity initially, however they may demonstrate variations during the period

of storage [105]. Studies have reported a reduction in the viscosity of a fermented drink after undergoing fermentation when ingredients with a high moisture content were added [102]. The increase in viscosity due to polysaccharides like starch is achieved during fermentation at a temperature called the gelatinization temperature. At this temperature, the starch granules undergo swelling and amylose is leached from the starch granules. Maximum viscosity occurs when all granules become completely swollen during the heating-and-holding cycle. This is indicative of the water-holding capacity of the polysaccharide [106].

The results of these tests are indicators that the properties of fermented products change when production is not conducted in an aseptic, sanitized, and hygienic facility. The quality of the product deteriorates under such conditions. The compliance to the guidelines for the hygienic production of fermented foods reportedly enhances the properties and shelf life of the product.

6. Conclusions

The use of strict hygiene standards is required for the production of drinkable fermented drinks to ensure their safety, quality, and consumer acceptance. The risk of contamination can be considerably reduced by rigorously controlling raw materials (substrates), equipment sterilization, and human hygiene, as well as imposing environmental controls within manufacturing facilities. Furthermore, systematic procedures, like Hazard Analysis and Critical Control Points (HACCP), and regular microbiological testing improve the consistency of these practices. Finally, these procedures not only protect consumer health but also help to extend the shelf life and improve the sensory attributes of fermented products, increasing consumer acceptance and contentment.

7. Future Directions

The future potential for researching hygienic techniques in the manufacture of fermented products is bright and varied. As the market for fermented products grows due to their health benefits, guaranteeing their quality and safety will remain a top priority. Future development and research priorities include the following:

- The development of new and more efficient sanitation methods, such as ozone therapy and ultraviolet (UV) sterilization, could improve hygiene standards and lower the risk of contamination. The use of ozone in the preservation of food products, including fermented foods, is gaining popularity as a result of its antimicrobial properties, oxidative activities, and absence of any residue in foods after decomposition. Ozone exhibits efficacy on pathogenic microbes, biofilms, and molds. Ozone technology has the potential to effectively control microbial growth, improve food safety, as well as provide shelf life extension [107]. Moreover, the utilization of UV treatment in food production is a promising decontamination approach. Shortwave UV radiation exhibits germicidal activity against numerous pathogenic microbes, including viruses, bacteria, yeasts, fungi, and molds. The technology has benefits like cost-effectiveness, low maintenance, and reduced energy requirements. These non-thermal approaches prevent nutrient damage that is commonly observed in thermal techniques [108].
- The development of fast and sensitive microbial detection methods will allow for the real-time monitoring of contamination, resulting in faster reactions to hygiene violations. Advanced techniques, like multiplex the Polymerase Chain Reaction (PCR), reverse transcriptase PCR, real-time PCR, quantitative PCR, nucleic acid sequence-based amplification, next-generation sequencing, DNA microarray, and nanotechnology-based approaches, are used for the effective and quicker detection of pathogens in food products, including fermented products [109]. Additionally, immunological assays and techniques like Enzyme Linked Immuno-Sorbent Assay (ELISA), antibodies, and latex agglutination methods are also used for the rapid detection of pathogens. However, the biosensors approach is identified to be the most effective technology, with benefits like portability, rapid identification, and sensitivity for microbial detection in food production. Biosensor technology in the food industry includes immune sensors,

electrochemical sensors, enzyme-based sensors, optical sensors, and magnetoelastic sensors [110].

- Integrating automated systems for cleaning and smart sensors into industrial facilities could improve hygiene practices by making them more reliable and efficient. The scientific advancements have resulted in the increased implementation of automated systems and technologies in food industries, including fermented food production units. Computer software robotics are used to control every process of food production. Automated systems have several advantages, including enhanced productivity, improvement in quality, and increased profitability [111]. Furthermore, sensors and artificial intelligence (AI) are also utilized for the efficient automation of food processing units. Machine learning and data mining approaches can be used to develop intelligent sensors that modulate and control the production process in addition to maintaining adequate hygiene conditions [112].
- Studies on sustainable hygiene procedures that reduce water consumption and chemical waste while maintaining safety and quality will be critical for reducing the environmental impact of fermented drink production. The sustainable hygiene approaches include strategies like cleaning out of place and cleaning in place [113]. Sustainable hygiene is greatly dependent on the design of the equipment and facility to avoid the introduction of contaminants at any stage. It promotes the use of mild green detergents, which are natural, safe, environment friendly, and efficient substitutes for chemical detergents and sanitizing agents [114].
- The use of the artificial intelligence (AI)-based approach for tracking and monitoring the hygiene and sanitation of the fermentation facility. With simulation studies, an efficient strategy for the sanitation of the complete facility can be devised. Internet of Things (IoT) technologies of AI have potential applications in the food industry for controlling the hygiene and sanitation of the fermentation unit. The study of this approach is in its initial stages and requires a large amount of research to establish its efficacy in ensuring the good hygiene condition of production facilities and preventing spoilage [115].

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