beverages

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Review

Molecular Methods for Detecting Microorganisms in Beverages

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Abstract: Beverages are an integral component of a person's food package. Various types of microorganisms widely contaminate beverages. This review presents current research data aimed at identifying dominant microorganisms in beverages and molecular methods for their detection. Wine, beer, dairy drinks, and fruit juices were selected as the main objects of the study. The most contaminated beverage turned out to be fruit juice. As a result of a large number of independent studies, about 23 species of microorganisms were identified in it. At the same time, they are represented not only by bacterial and fungal organisms, but also by protozoa. Milk turned out to be the least contaminated in terms of detected bacteria. The most common pollutants of these beverages were *Staphylococcus aureus*, *Bacillus cereus*, and *Vibrio parahaemolyticus*. It has been established that among pathogenic genera, *Salmonella* sp., *Campylobacter* sp. and *Shigella* sp. are often present in beverages. One of the main tools for the quality control of beverages at all stages of their production is different types of polymerase chain reaction. The sequencing method is used to screen for microorganisms in beverages. The range of variations of this technology makes it possible to identify microorganisms in alcoholic and non-alcoholic beverages. The high specificity of methods such as PCR-RFLP, Rep-PCR, qPCR, End-point PCR, qLAMP, the molecular beacon method, and RAPD enables fast and reliable quality control in beverage production. Sequencing allows researchers to evaluate the microbiological diversity of all the studied beverages, while PCR varieties have demonstrated different fields of application. For example, PCR-RFLP, RAPD-PCR, and PCR allowed the identification of microorganisms in fruit juices, qPCR, LAMP, and the molecular beacon method in wine, LAMP and multiplex PCR in milk, and End-point PCR and Rep-PCR in beer. However, it is worth noting that many methods developed for the detection of microbial contaminants in beverages were developed 10–20 years ago; modern modifications of PCR and isothermal amplification are still poorly implemented in this area.

Keywords: beverages; contamination; bacteria; pathogens; PCR; sequencing

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1. Introduction

The main aspect of food production is the safety of food for human health. Drinks are an integral component of an individual's diet. It has been proven that pathogenic microorganisms widely contaminate beverages [1]. The primary source of pollution is the production inventory and the room as a whole. Secondary contaminants include violations of the packaging, storage, and transportation conditions of food [2,3]. The following species are considered to be the main food pathogens: *Staphylococcus aureus*, *Bacillus cereus*, and *Vibrio parahaemolyticus*. Pathogenic genera include *Salmonella*, *Campylobacter*, and *Shigella* [4,5].

To assess the spoilage of manufactured products, the main visual parameters of quality deterioration are color change, turbidity, a specific non-characteristic odor, swollen packaging, and an altered aftertaste [6,7]. A lack of a timely comprehensive approach to quality control at all stages of food production can lead to contaminated products entering

the market. Some methods of monitoring and recognizing the sources of pathogens in food and beverages do not always correspond to modern production technologies and are therefore considered outdated. In this regard, any organization involved in the manufacture of food products should have or cooperate with laboratories, where there is a complex interaction of traditional routine microbiological techniques and molecular genetic analyses [8–10].

Approaches for detecting microorganisms are usually based on traditional cultivation methods and their modifications. Modern analytical methods are based on PCR, immunoassay, and high-throughput sequencing [11]. The method of surface-enhanced Raman spectroscopy (SERS) in combination with the use of solid-phase substrates based on Au nanostars makes it possible to quickly and effectively detect pathogens in beverages [12]. The detection of pathogens in milk using QCM immunosensors is possible, but only if high concentrations are reached after several hours of incubation [13].

The high-throughput sequencing of fresh milk samples obtained by mechanical and manual milking showed a high level of contamination of products with various species of fungi of the genera *Candida*, *Kluyveromyces*, *Pichia*, and *Kodamaea* [14]. Full-metagenomic sequencing is a culturally independent technique for determining potential bacterial pathogens in foods and beverages. This highly sensitive approach allows detecting microorganisms whose extraction from the sample is difficult. Based on it, *Brucella* spp., *Salmonella enterica*, and *E. coli* were found in raw milk without using the enrichment procedure [15]. Metagenomic sequencing using long reads for the identification of eae-positive STEC strains is a relatively new developing technique aimed at solving the problem of the identification of pathogenic microorganisms in food. With its help, the presence of shigatoxins produced by *E. coli* was revealed in samples of raw milk from cattle [16].

The polymerase chain reaction (PCR) method has become one of the main tools for the quality control of beverages and food at all stages of production [17,18]. Figure 1 shows the main molecular methods for identifying microorganisms in beverages found in the analysis publications in the open databases PubMed, Web of Science, and Scopus.

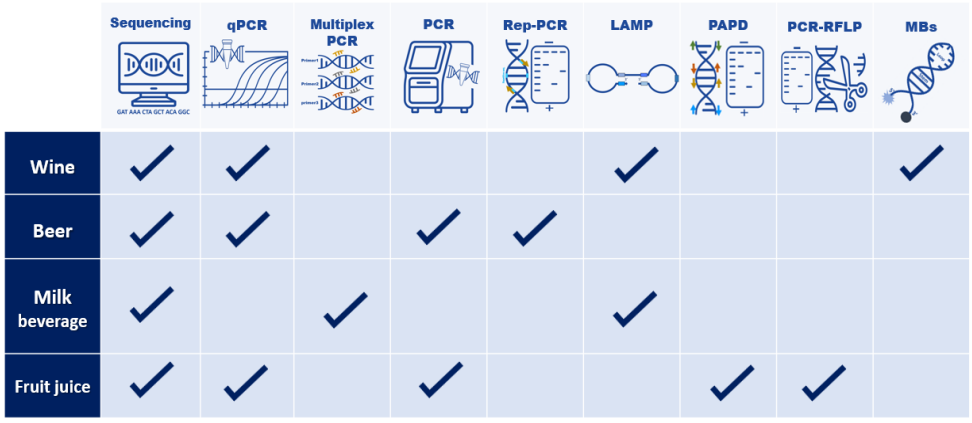


Figure 1. Molecular methods for the identification of microorganisms in beverages.

The main advantages of amplification are high specificity, low detection limit, fast results, and the automation of the process, starting from the sample preparation stage [19–22]. For example, the developed analysis based on multiplex PCR (mPCR) makes it possible to detect several pathogenic microorganisms at once in one reaction, which speeds up the time to obtain reliable results [23–25]. Unlike the culture method, which made it possible to determine the presence of staphylococci in 23% of samples, the qPCR method identified the *S. aureus* pathogen in 60% of the studied milk samples, which characterizes PCR as a highly sensitive technique [26].

It should be noted that raw foods, such as fruits and vegetables, are a source of a diverse microbiome, and their poor quality can lead to an increase in the likelihood of contamination of the final product of food production [27]. Due to the sugars, vitamins,

antioxidants, and polyphenols included in the composition, fruits have become an optimal and balanced environment for the growth of bacteria and fungi [28]. Microorganisms contaminate food and beverages due to their specific metabolic activities, accompanied by physicochemical reactions. Pathogens have high rates of adaptation to the environment, surviving in extreme conditions [28]. Members of the genera *Pichia*, *Candida*, *Saccharomyces*, and *Rhodotorula* have increased resistance to an environment with high acidity and a large amount of sugars. Pasteurized soft drinks packaged in plastic are particularly susceptible to contamination by such yeasts [29,30]. It is known that representatives of lactic acid bacteria *Lactobacillus* and *Leuconostoc* found in fruit juices demonstrated their resistance to such preservatives as benzoic and sorbic acid [31,32]. Fermented foods, such as wine, are a favorable environment for the growth of fungal microorganisms, among which there are also yeasts. They change the organoleptic properties of the drink due to excessive gas formation and the production of lipases, cellulases, and proteases [33]. A similar problem may affect not only wine and cider, but also traditional ethnogeographic drinks, including hardaliye [34], tepache [35], and khadi [36].

In addition to changing the taste, smell, and color of food products, contaminating fungi are sources of the low-toxic compounds patulin, aflatoxins, and ochratoxin A [37]. These toxic metabolic products have a negative impact on all organ systems of humans and animals. Therefore, mycotoxins are included in the list of teratogenic and carcinogenic compounds [38,39]. The ochratoxin A contamination of juice and wine during grape harvest has been proven [40]. In this regard, this study is devoted to the assessment of data from the existing literature related to the identification of microbiological contaminants of beer, wine, juice, and dairy drinks using molecular methods.

2. Microorganisms in Beer

Beer is a foamy drink made mainly from barley malt and hops [41]. This product has received its unique taste due to the peculiarities of its preparation and fermentation, in which microorganisms are directly involved [42]. It is known that beer is a microbiologically stable drink [43]. But, despite this, breweries face problems associated with the increased activity of lactic acid bacteria, including *Lactobacillus brevis*, *Lactobacillus lindneri*, and *Pediococcus damnosus*, as well as some Gram-negative bacteria such as *Pectinatus cerevisiiphilus*, *Pectinatus frisingensis*, and *Megasphaera cerevisiae* [44,45]. The main pollutants most frequently detected in beer drinks are shown in Figure 2A.

Competition of bacteria with yeast for the substrate consequently reduces the yield of ethanol, affects the taste characteristics of the finished product (high concentrations of lactic acid and diacetyl) and its quality (discoloration of the drink and turbidity), and causes premature spoilage [46]. It has been proven that the presence of bacteria of the genus *Pectinatus* and *Lactobacillus* in beer leads to the turbidity of the liquid and the formation of an unpleasant taste [47,48].

The analysis of the quality of beer drinks can be carried out by methods and technologies based on the use of molecular genetic developments, mainly PCR, in combination with cultural methods. The PCR-DGGE method made it possible to identify *Lactobacillus brevis* in finished products and in components used to make beer. The research conducted by Cristiana Garofalo et al. tracked *Lactobacillus brevis* within a brewery and during the craft beer production process by analyzing samples from indoor air, surfaces, yeast, and beer. Using the PCR-DGGE technique along with culture-dependent methods, *Lactobacillus brevis* was identified as responsible for beer spoilage. The study of samples obtained from the working surfaces in the brewery revealed the presence of lactic acid bacteria and bacteria of the genera *Staphylococcus* and *Acetobacter*, as well as bacteria of the Enterobaceriaceae family, which are potential pollutants of beer [49]. PCR analysis and the sequencing of the conservative 16S rRNA region of "light" beer samples showed a low content of lactic acid bacteria in beer. They turned out to be *Lactobacillus brevis*, *Lactobacillus backii*, and *Lactobacillus harbinensis* [46,50]. However, it should be noted that *L. brevis* is a common cause of beer spoilage [51]. Researchers from Argentina used ribosomal gene sequencing

(16S ribosomal subunit) to identify the beer microbiome. PCR was performed with primers 27F/1495R for bacterial isolates and ITS1/ITS4 for fungal isolates in beer [52,53]. Members of the following genera of lactic acid bacteria were found: *Levilactobacillus*, *Lactobacillus*, and *Pediococcus*; and acetic acid bacteria: *Flavobacterium* and *Proteus*. The fungal isolates were ascomycetes of the genera *Wickerhamomyces*, *Clavispora*, *Wickerhamiella*, and brazidiomycetes of the genera *Trichosporon* and *Naganishia* [53].

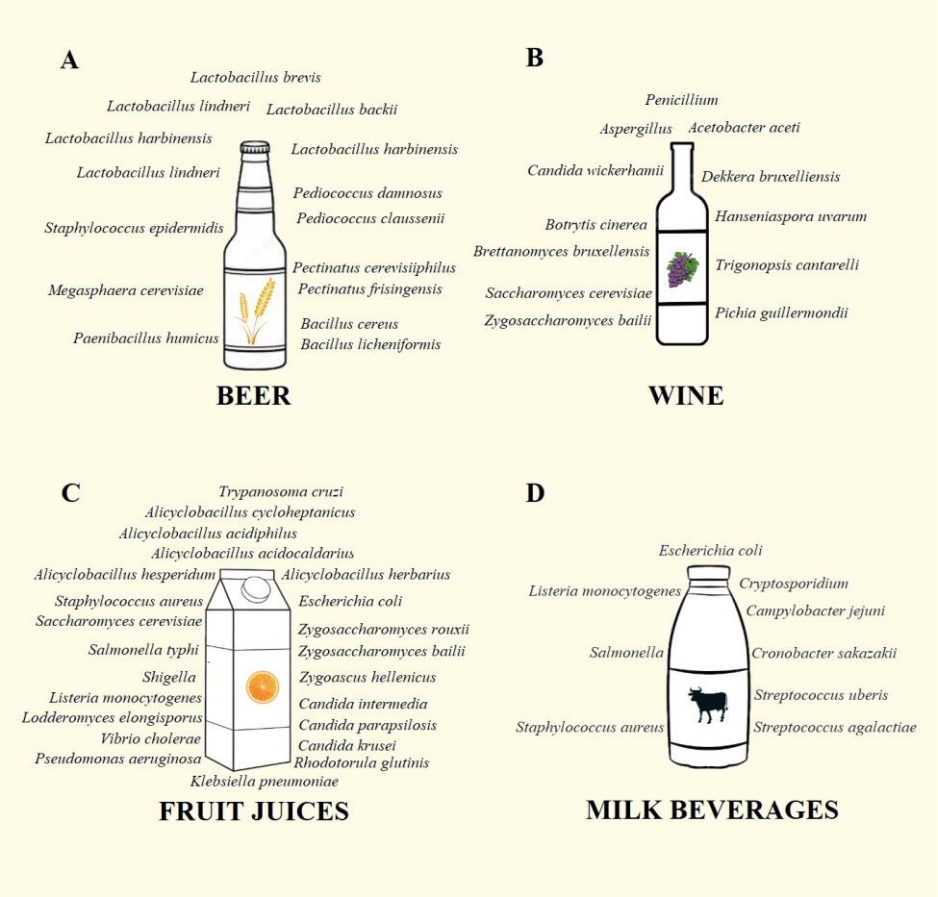


Figure 2. The main microbiological pollutants of beverages. (**A**) beer, (**B**) wine, (**C**) fruit juices, (**D**) milk beverages.

The results of Geissler et al.'s study indicate the important role of bacterial plasmid DNA in the contamination of brewing equipment, raw materials, and finished products. Thanks to sequencing technology, complete representations of such beer-contaminant lactic acid bacteria as *Lactobacillus paracollinoides*, *Lactobacillus lindneri*, and *Pediococcus claussenii* have been obtained [54]. The sequencing and genome assembly of two bacterial strains of *Loigolactobacillus backii* KKP 3565 and KKP 3566, found in production and in beer itself, revealed the presence of hop resistance genes *hitA*, *horA*, and *horC*. The genome of bacterial strains contained inserts from other contagious species. These genetic constructs contribute to the adaptation of representatives of lactic acid bacteria to the harsh environment of the brewery, including carrying out vital activities at high concentrations of hops [55]. A similar study, in which lactic acid bacteria were identified in beer, was carried out using the PCR method with primers for the hop resistance genes *horA* and *horC*. Twelve species of bacteria associated with beer spoilage belonging to the genera *Lactobacillus*, *Pectinatus*, *Pediococcus*, and *Megasphaera* were detected [56]. In Haakensen's work, the species *Bacillus cereus*, *Bacillus licheniformis*, *Staphylococcus epidermidis*, and *Paenibacillus humicus*, which were not previously associated with beer spoilage, were identified. Their identification was carried out using PCR with primers aimed at the multiple resistance gene MDR *horA*. It was

revealed that this gene in the genera *Lactobacillus* and *Pediococcus* is responsible for active growth in beer drinks [57]. Because of the developed PCR analysis of the variable region of the 16S rRNA gene, with a subsequent restriction reaction with KpnI, XmnI, BssHI, and ScaI nucleases, it was possible to identify representatives of anaerobic contaminants of the genera *Pectinatus*, *Megasphaera*, *Selenomonas*, and *Zymophilus* in beer [47,58,59].

3. Microorganisms in Wine

Wine is one of the most popular alcoholic beverages obtained as a result of the full or partial alcoholic fermentation of grapes [60]. Grape must is an intermediate stage in wine production, but despite this, in some regions, it is used as an independent product [61,62]. It does not have the noble organoleptic combinations characteristic of wine, is low in quality, and may contain microbial contaminants in its composition (*Aspergillus* spp. and *Penicillium* spp.) due to the fact that it is a by-product of production [63]. For example, the nested PCR method, based on the identification of representatives of the genus *Penicillium* due to specific primers to the gene encoding the β-tubulin protein, makes it possible to successfully identify this contagious microorganism not only in wine but also in all intermediate products of winemaking [60,64].

Depending on grape variety, fermentation conditions, and the amount of sugar syrup, the wine can be divided into white, red, dry, sweet, sparkling, and still [65]. A mandatory indicator of high-quality sparkling wine is its ability to foam. This issue is subject to active study in connection with the emergence of the problem of the reduced carbonation and volatility of wines [66–68]. It has been proven that metabolic products such as proteases produced by the pathogenic fungus *Botrytis cinerea* affect the quality and quantity of foaming sparkling wines. Proteases have a destructive effect on the vast majority of macromolecules of grape juice during fermentation reactions [69]. The SDS-PAGE method demonstrated the enzymatic activity of members of the genus *Botrytis* [70]. The contamination of wines of various varieties with non-saccharomycete yeasts can be controlled using the RFLP-PCR method. Such a study was conducted on Brazilian wines, where HinfI and HaeIII restrictases acted as restriction enzymes. The unique restriction pattern corresponded to representatives of *Brettanomyces/Dekkera bruxelliensis*, *Pichia guillermondii*, *Candida wickerhamii*, and *Trigonopsis cantarelli*. The bioinformatic analysis of the sequenced ITS1-5 site.8S-ITS2 and the D1/D2 domain confirmed the results obtained [71].

Members of the genus *Brettanomyces*, for example, *Brettanomyces bruxellensis*, are difficult-to-cultivate fungi and cause the spoilage of red wines [72]. They produce ethylphenols and ethyl guaiacols, which lead to the formation of biogenic amines in the product [73]. To control the fungal contamination of wines, a PCR method with primers to the D1/D2 domains of the *26S rRNA* gene was developed, which demonstrated high efficiency in detecting *S. cerevisiae*, *Hanseniaspora uvarum*, and *Dekkera bruxellensis* species [74,75]. An optimized amplification reaction technique with DBRUX F/R primers (26S rRNA gene), Brett F/R and Rad F/R (RAD4 gene), and Act F/R (actin gene) for the identification of members of *Brettanomyces* and *Dekkera* spp. has also been demonstrated in wine [76–79].

In wine production, high concentrations of weak acids are often used as preservatives; however, even in such environmental conditions, the species *Zygosaccharomyces bailii* is actively growing and multiplying [80]. PCR analysis with ZB F1/R1 primers effectively identifies this pathogen in wine samples, even in the presence of non-target DNA [81]. Fungi, which often burden food products, including wine, include representatives of the genera *Aspergillus* and *Penicillium*. They are producers of the carcinogen ochratoxin A (OTA), so it is necessary to control their presence in food. The identification of ATA in wine is effectively carried out using the molecular beacon method [63].

Another indicator of the quality of predominantly red wine is taste astringency [82]. The unpleasant sour taste and smell of acetic acid indicate the growth of acetic acid bacteria of the genus *Acetobacter* in the drink [83]. Even trace concentrations of such microorganisms cause wine spoilage. A real-time loop isothermal amplification (qLAMP) platform for the *16S rRNA* gene has been invented, capable of effectively detecting *Acetobacter aceti* in red

wine in a short time [84]. In addition, the identification of acetic acid bacteria in red wine by real-time PCR was demonstrated, thanks to the successful selection of primers for the *16S rRNA* gene [85,86]. The main pollutants most frequently detected in wine drinks are shown in Figure 2B.

4. Microorganisms in Fruit Juices

The main source of vitamins, minerals, and antioxidants are fruits and their processed products, for example, juices [87]. Fruit juices, with their high biologically active potential and valuable dietary fibers, are included in proper human nutrition [88,89]. The short shelf life increases the risk of contamination of such a product by pathogenic microorganisms. Special attention is paid to freshly squeezed juices, since they do not undergo a pasteurization process and are infected with microbial pathogens more often than others [90,91]. The main pathogenic bacterial species polluting fruit juices include *Escherichia coli*, *Listeria monocytogenes*, *Vibrio cholerae*, *Salmonella typhi*, and *Staphylococcus aureus*, as well as members of the genera *Shigella*, *Pseudomonas*, and *Alicyclobacillus* (Figure 2C) [92,93]. The sources of juice contamination can be the fruits themselves, as well as the conditions of their production, transportation, and storage [94]. The pH value of pasteurized products is acidic, which is a barrier to the growth and reproduction of microorganisms [95]. However, in the case of thermoacidophiles, whose prominent representatives are the genus *Alicyclobacillus*, a low pH does not interfere with their vital activity. This is possible due to the ability of bacteria to sporulate. The violation of storage conditions, for example, damage to packaging, induces the growth of thermoacidophiles and leads to the spoilage of fruit juices [96,97].

Representatives of the genus *Alicyclobacillus* form phenolic impurities, including guaiacol, which cause a specific "medicinal" taste and odor in the juice [98–100]. Their vital functions and the ability to reproduce in fruit juices pose a problem for producers in the food industry. This contributed to the development of various approaches to the identification of *Alicyclobacillus* based on molecular methods [101,102]. For example, *A. acidoterrestris* from acidic juice was identified using RAPD PCR for 6 h [103]. Unique species patterns of the *16S rRNA* gene were found in all bacterial isolates of *A. acidoterrestris*, different from the bands on the electrophoregram for *A. acidocaldarius* and *A. hesperidum* [104]. The PCR method with primers and TaqMan probes aimed at the gene encoding 16S rRNA has shown its effectiveness in identifying one of the main contaminants of juices, *Alicyclobacillus* spp. [105]. The RFLP analysis of 16S rRNA was used to characterize *Alicyclobacillus* strains from concentrated apple and orange juices [104,106,107]. Based on the selection of the target sequence of 16S ribosomal DNA, PCR technology was developed to identify the species *A. acidoterrestris*, *A. acidiphilus*, *A. cycloheptanicus*, and *A. herbarius* with a sensitivity of 2.6×10^2 CFU/mL, 74×10^2 CFU/mL, 2.8×10^2 CFU/mL, and 3.1×10^2 CFU/mL, respectively [100]. The RFLP analysis of the *16S rRNA* and *rpoB* genes, as well as the vdc region, can be successfully used to identify and study the intraspecific heterogeneity of *Alicyclobacillus* species. The Hin6I enzyme for 16S rRNA provides the formation of special restriction patterns that allow for the species-specific differentiation of *Alicyclobacillus*. The analysis of the *rpoB* and *vdc* genes also revealed two main types among *A. acidoterrestris* isolates, one of which is similar to the reference strain *A. acidoterrestris* DSM 2498, and the other is similar to the reference strain *A. acidoterrestris* ATCC 49025 [107]. The *vdcC* gene is present in all strains of *Alicyclobacillus* that produce guaiacol, but is absent in strains that do not produce guaiacol, with the exception of *A. fastidiosus* DSM 17978. Based on the sequence of the *vdcC* gene, a pair of primers specific to *A. acidoterrestris* was constructed and real-time PCR was performed using SYBR Green I to directly quantify *A. acidoterrestris* in apple juice. A developed real-time PCR system was used to detect *A. acidoterrestris* in 36 artificially infected apple juice samples [108]. Effective real-time PCR analysis has been demonstrated for the identification of *Alicyclobacillus* bacteria in kiwi juice. In total, 86 samples were examined; 69 of them were taken on production lines, and 17 were bought in a supermarket in China. The control of the specificity of amplification reactions using

SYBR Green I was carried out by analyzing the melting curve. A melting point of 80.5 °C was observed for all *Alicyclobacillus* species, with average Ct values of 26.0 ± 1.0 [109].

Fruit juices were tested for the most frequently polluting bacterial pathogens to control and ensure the safety of production using multiplex PCR. Conditions were optimized and PMAxx technologies were developed to eliminate dead bacterial cells. This analysis allowed the identification of the following contaminants: *Escherichia coli*, *Staphylococcus aureus*, *Shigella*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. In a study conducted by Tiantian Huang et al., fruit juice samples were tested for the most frequent bacterial pathogen-pollutants, namely, *Escherichia coli*, *Staphylococcus aureus*, *Shigella*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The light-induced PMAxx technologies were developed with optimized treatment conditions to eliminate dead bacterial cells, followed by multiplex PCR. According to the results, the applied technique is an effective method for the simultaneous detection of living pathogenic bacteria in fruit juice samples [110]. Fruit juices and nectars are the optimal environment not only for the growth of bacteria but also yeast. This explains the reason for the frequent contamination of juices with fungi. PCR-RFLP methods with ITS primers for the 5.8S rRNA subunit and high-throughput sequencing have revealed taxa such as *Candida*, *Lodderomyces*, *Wickerhamomyces*, *Yarrowia*, *Zygosaccharomyces*, *Zygoascus*, *Cryptococcus*, *Filobasidium*, *Rhodotorula*/*Cystobasidium*, and *Trichosporon*, which polluted production inventory and equipment, as well as the fungi *Zygosaccharomyces bailii*, *Z. bisporus*, *Zygoascus hellenicus*, and *Saccharomyces cerevisiae*, which directly contaminated fruit juices. Widespread industrial distribution is generally characteristic of *Candida intermedia*, *C. parapsilosis*, and *Lodderomyces elongisporus* [111]. Real-time PCR with specific primers for the citrate synthase gene made it possible to establish the species of the fungus *Candida krusei*, which causes the spoilage of juices. Also, *Zygosaccharomyces bailii*, *Z. rouxii*, *Rhodotorula glutinis*, and *Saccharomyces cerevisiae* were identified in apple juice based on the values of melting curves by PCR with primers to 5.8S of the rRNA and ITS2 [112].

In Brazil, sugarcane juice is a popular dietary supplement and is actively consumed by the population as an independent drink [113]. In addition, sugarcane juice is considered the most popular freshly squeezed juice in Egypt. Indians and Pakistanis share the same habit as Egyptians regarding chewing raw sugarcane and consuming its juice [114,115]. Its inclusion in the diet often provokes the development of Chagas disease due to *Trypanosoma cruzi*. The effectiveness of the real-time PCR method with the developed specific pair of primers Cruzi32/Cruzi148 for the identification of the parasite in acai pulp and sugarcane juice was shown [116].

5. Microorganisms in Dairy Beverages

Dairy beverages are represented by a wide range of products: from milk and kefir to drinking yoghurts [117]. They contain a large number of elements necessary for the proper growth, development, and functioning of organisms, such as fatty acids, vitamins, micro-, and macro-elements [118]. A review of studies on milk microflora clearly shows that the most common genera of lactic acid bacteria in milk are *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus*, and *Enterococcus*. There are also psychrotrophic populations that became established during refrigerated storage, such as *Pseudomonas* and *Acinetobacter* spp. Other genera distinct from lactic acid bacteria, as well as various yeasts and molds, are also found in milk (Figure 2D) [119]. However, milk can often contain various microbiological contaminations (*Campylobacter* spp., *Salmonella* spp., *Brucella melitensis*, and *Mycobacterium bovis*) [120]. Such contamination can seriously threaten health, which is why molecular methods for detecting pathogens in dairy drinks are needed [121]. For example, methods based on nucleic acid amplification (NAA), including polymerase chain reaction (PCR), loop isothermal amplification (LAMP), recombinase polymerase amplification (RPA), rotating circle amplification (RCA), enzyme-free amplification, and others, are widely used to detect foodborne pathogens in milk [122].

LAMP was the first to detect the important environmental pathogen *Streptococcus uberis* in raw milk. To do this, bacteria were grown on sheep blood heart agar plates for

18 h at 37 °C under aerobic conditions. For DNA extraction, bacteria were collected by scraping the surface of the plate [123]. Three genes were selected as targets for the PCR detection of *Streptococcus uberis*: *sodA*, *pore*, and *cpn60* [124,125].

About 300 suspected isolates of *Staphylococcus aureus* have been confirmed using MALDI-TOF MS and real-time PCR. In addition, the pathogen was detected in several swabs from a bucket of milk, as well as in swabs from the nose and hands of milkers [126]. A multiplex PCR method has also been developed for the detection of *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli* using species-specific primers [127] aimed at species-specific DNA sites encoding 16S and 23S rRNA [128–130], as well as encoding the sip (surface immunogenic protein) sequence-specific (SSS) gene for GBS (Lancefield Group B *Streptococcus*). Checking the effectiveness of the primers showed that the SAU1 and SAU2, SAGA1 and SAGA2 kits, and Ecol1 and Ecol2 primers detected at least 8000 bacteria.mL^{-1} (*Staphylococcus aureus*), 3000 bacteria.mL^{-1} (*Streptococcus agalactiae*), and 3000 bacteria.mL^{-1} (*E. coli*) [130]. Standardized multiplex PCR showed good accuracy of detection of *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli* in goat's milk [127].

Magnetic beads, which are coated with monoclonal antibodies serve as another molecular technique to detect foodborne pathogens (such as *Escherichia coli*) in dairy products [131]. Also of scientific interest is the method of detecting *Salmonella* using magnetic capture probes by modifying oligonucleotides complementary to sequences on the surface of magnetic nanoparticles with an amino-modified silica coating. The sensitivity of detection was 104 CFU/mL, which could be increased to 10 CFU/mL after a 12 h enrichment step. Magnetic capture probes were used to separate *invA* mRNA, with a novel step of placing complexes of magnetic capture probes and *invA* mRNA in an RT-qPCR mixture without any denaturation, and purification steps to detect *Salmonella* in milk [132].

The molecular method of detecting *Cronobacter sakazakii* in raw milk is also interesting. The method is a combination of quantitative LAMP and propidium bromide (PMA-QLAMP). The *gyrB* gene is targeted for the development of primers. The DNA of six out of twenty-four strains of *C. sakazakii* was amplified using PMA-QLAMP. The ability of PMA-QLAMP to quantitatively detect live *C. sakazakii* in a 10% solution of dry infant formula (PIF) was limited to 4.3×10^2 CFU/mL concentrations and above. Pasteurizing raw milk containing 10^6 CFU/mL of viable C. *sakazakii* resulted in the maximum VBNC ratio of C. *sakazakii*. The PMA-QLAMP detection revealed that even though around 13% of the 60 samples were positive for *C. sakazakii* viability, the titers of *C. sakazakii* in these positive samples were low, and none of them entered the VBNC state during pasteurization. PMA-QLAMP has demonstrated potential as a specific and reliable method for detecting VBNC-*C. sakazakii* in pasteurized raw milk, thereby providing an early warning system indicating the potential contamination of PIF [133]. *GyrB* is a conserved gene that is a molecular marker of bacterial phylogenetic analysis [134].

Table 1 summarizes information from the available literature and publications showing primer sequences used to identify pathogenic microorganisms in beer, wine, juice, and milk beverages.

Table 1. Detection methods and primers for the identification of microorganisms in beverages.

№	Object	Target	Method	Primer	Subsequence (5′-3′)	Literature
1	Beer	*Lactobacillus*	Rep-PCR	REP1R-I	IIIICGICGICATCIGGC	[46,50]
				REP2-I	IIICGNCGNCATCNGGC	
				BOXA1R	CTACGGCAAGGCGACGCTGACG	
				PRIMER	(GTG)5 (5GTGGTGGTGGTGGTG3)	
2		*Pectinatus*	qPCR	F	GCTTTTAGCTGTCGCTTGGA	[47]
				R	TGCATCTCTGCATACGTCAA	
3		Bacteria	qPCR	27F	GAGAGTTTGATCCTGGCTCAG	[53]
				1495r	CTACGGCTACCTTGTTACGA	
4		Yeasts	qPCR	ITS1	TCCGTAGGTGAACCTGCGG	[52,53]
				ITS4	TCCTCCGCTTATTGATATGC	
5		Resistance gene HorA/HorC	qPCR	LbHC-1	ATCCGGCGGTGGCAAATCA	[56]
				LbHC-2	AATCGCCAATCGTTGGCG	
		Lactobacillus brevis	qPCR	LBP2	CTGATTTCAACAATGAAGC	
				UNP1	CCGTCAATTCCTTTGAGTTT	
		Pectinatus cerevisiiphilus	qPCR	16C-F	CGTATGCAGAGATGCATATT	
				IC-R	CACTCTTACAAGTATCTAC	
		Pediococcus damnosus/*Pediococcus inopinatus*	qPCR	PIDF1	TGTGAGAGTAACTGCTCATG	
				PIDR8	ACGCCTAATCTCTTTGGTTA	
		Megasphaera cerevisiae	qPCR	Mc-f4	ACCGAATACGATCTAAAG	
				Mc-r4	TTAAGACCGACTTACCGA	
6		*Clostridia*	End-point PCR	An-0279f	ACGATCAGTAGCCGGT	[59]
				An-0603r	AGCCCCGCACTTTTAAG	
7		*Megasphaera cerevisiae*	qPCR	F	CACTGAATAGTCTATCGC	[58]
				R	AAGACCGACTTACCGAAC	
1		*Penicillium expansum*	qPCR	PE F	ATCGGCTGCGGATTGAAAG	[64]
				PE R	AGTCACGGGTTTGGAGGGA	
2	Wine	*Acetobacter aceti*	qLAMP	F3	AGGTGGGGATGACGTCAAG	[84]
				B3	CGGGAACGTATTCACCGC	
				FIP	CTAGCTTCCCACTGTCACCG	
					TCCTCATGGCCCTTATGTC	
				BIP	AACCGTCTCAGTTCGGATTGCATCCGCGATTACTAGCGATTC	
				LF	AGCACGTGTGTAGCCCA	
				LB	CTCTGCAACTCGAGTGCATG	
				F	CGGAATGACTGGGCGTAAG	
				R	CAGTAATGAGCCAGGTTGCC	
				PROBE	6FAMCGGGCTTAACCTGGGAGCTGCATTBHQ1	

Table 1. *Cont.*

№	Object	Target	Method	Primer	Subsequence (5′-3′)	Literature
3		Acetic acid bacteria	qPCR	AAB F AAB R	TGAGAGGATGATCAGCCACACT TCACACACGCGGCATTG	[85]
4		Yeasts	qPCR	YEAST F YEAST R	GAGTCGAGTTGTTTGGGAATGC TCTCTTTCCAAAGTTCTTTTCATCTTT	[74]
5		*Brettanomyces*/*Dekkera* spp.	qPCR	DBRUX F DBRUXR	GGATGGGTGCACCTGGTTTACAC GAAGGGCCACATTCACGAACCCCG	[76,78,79]
6		*Brettanomyces bruxellensis*	qPCR	BRETT 1 BRETT 2 RAD 1 RAD 2 ACT 1 ACT 2	CGAAGAAGTTGAACGGCCGCATTTG TCTTCGATATGCCGTCCAAAAGCTC GTTCACACAATCCCCTCGATCAAC TGCCAACTGCCGAATGTTCTC TGTCAGAGACATCAAGGAGAAGCT CGTCTGCATTTCCTGGTCAA	[77] [78] [75]
7		*Zygosaccharomyces bailii*	qPCR	ZB F1 ZB R1	CATGGTGTTTTGCGCC CGTCCGCCACGAAGTGGTAG A	[81]
8		Ochratoxin A	Molecular Beacon Method	*APTABEACON*	6FAMCGCGCTGGATCGGGTGTGGGTGGCGTAAAGGGAGCATC GGACACAGCGCGBHQ1	[63]
1		*Alicyclobacillus acidoterrestris* *Alicyclobacillus acidiphilus* *Alicyclobacillus cycloheptanicus* *Alicyclobacillus herbarius*	PCR	*A1-92-3 F* *A1-92-3 R* *A2-16S-1 F* *A2-16S-1 R* *A3-16S-3 F* *A3-16S-3 R* *A4-5472 F* *A4-5472 R*	TCGCAACCTGCTTCTCCA TGGTGGACGGGATTGTTT ATGCAAGTCGAGCGAAC GCAACTTTCCTCAACGG TGCAAATGCACCGCAGAT GGCTTTCCACTCCCCTTG TGAGTCGCTTCTTCGTTCTT CTACGGGATGACGGAAGC	[100]
2	Fruit juices	*Alicyclobacillus acidoterrestris*	RAPD	Ba-lO F -61 F-64	AACGCGCAAC CCTGTGATGGGC GCCGCGCCAGTA	[103]
3		*Alicyclobacillus acidoterrestris*	PCR-RFLP Restriction endonuclease: HhaI	P1 P4	GCGGCGTGCCTAATACATGC ATCTACGCATTTCACCGCTAC	[104]
4		*Alicyclobacillus*	qPCR	CC16S-F CC16S-R CC16S-Probe	CGTAGTTCGGATTGCAGGC GTGTTGCCGACTCTCGTG QUASAR670CGGAATTGCTAGTAATCGCBHQ-2	[105]
5		*Alicyclobacillus*	PCR–RFLP Restriction endonucleases: BsuR I, Hinf I, Msp I, Rsa I	P1 P2	CGGGATCCAGAGTTTGATCCTGCGTCAGAACGAACGCT CGGGATCCTAGGGCTACCTTGTTACGACTTCACCCC	[106]

Table 1. *Cont.*

№	Object	Target	Method	Primer	Subsequence (5′-3′)	Literature
6		*Alicyclobacillus acidoterrestris*	PCR-RFLP Restriction endonuclease: HhaI	P1	GCGGCGTGCCTAATACATGC	[104]
				P4	ATCTACGCATTTCACCGCTAC	
				vdc fr	CTGTTGGCTCAATGGCGGCTGAGCGAT	
				vdc rev	TTATCAGCGGTTTATCCGCGGTGGAACAGTC	
7		*Alicyclobacillus*	PCR-RFLP Restriction endonucleases: BsuRI, Hin6I, HphI	vdc1 fr	AACGACGCAGGTGTGGAAAC	[107]
				vdc1 rev	AGCGTGGGCAAGTTGTCATGTG	
				vdc K and vdc S	TTGGCAACGGAGAAGTGGGAG	
					AATCACGCGCTGATGATGGG	
				Bur 5	GCCGACGTGATGCTCAARGAGCGCA	
				Bur 6	GTSGCRTCGAGAATCATCTTGTG	
				Gru3	CGYGACGTDCACTAYTCBCACTA	
				Gru4	GCCCANACYTCCATCTCRCCRAA	
				Gru5	CGCGACGTACACTATTCGCACTA	
				Gru6	GCCCAAACCTCCATCTCACCAAA	
8		*Alicyclobacillus acidoterrestris*	qPCR	vdcCF1	TAYGAAATGGCMGGTGC	[108]
				vdcCR1	GGAAGGTTGAAYGGATC	
9		*Alicyclobacillus*	qPCR	F	ATGCGTAGATATGTGGAGGA	[109]
				R	CAGGCGGAGTGCTTATTG	
10		Yeast	PCR-RFLP Restriction endonucleases: CfoI, HaeIII, HinfI	ITS1	TCCGTAGGTGAACCTGCGG	[111]
				ITS4	TCCTCCGCTTATTGATATGC	
11		*Zygosaccharomyces bailii*, *Zygosaccharomyces rouxii*, *Candida krusei*, *Rhodotorula glutinis*, *Saccharomyces cerevisiae*	qPCR	ITS3	GCATCGATGAAGAACGCAGC	[112]
				ITS4	TCCTCCGCTTATTGATATGC	
				CS Fwd	GCATATGGTGGTTATGAGAGG	
				CS Rev	AGCAGAAACATTACCACCTTC	
12		*Trypanosoma cruzi*	qPCR	32F	TTTGGGAGGGGCGTTCA	[116]
				148R	ATATTACACCAACCCCAATCGAA	
				probe71	FAMCATCTCACCCGTACATT3NFQ	
1		*Streptococcus uberis*	LAMP	Su sodA F3	TGGCGTTATTATCTGATGTGT	[124]
				Su sodA B3	AGAYCCAAAACGTCCCGT	
				Su sodA FIP	ATGGTTAAGATGTCCGCCTCCCATCAATTCCAGAAGATATTCGT	
				Su sodA BIP	TTCACCTGAGAAAACAGAAATCACTTCTTTAAATGCATCAAAA GAACC	
				Su sodA Bloop	CGGAAGTAGCTTCTGCTATTGAT	

Table 1. *Cont.*

№	Object	Target	Method	Primer	Subsequence (5′-3′)	Literature
				Su sodA FIP	DIG-ATGGTTAAGATGTCCGCCTCCCATCAATTCCAGAAGA TATTCGT	
				Su sodA BIP	Biotin-TTCACCTGAGAAAACAGAAATCACTTCTTTAAATG CATCAAAAGAACC	
2	Milk beverages	*Streptococcus aureus*	multiplex PCR	Sau 327/SAU1	GGACGACATTAGACGAATCA	[130]
				Sau 1645/SAU2	CGGGCACCTATTTTCTATCT	[131]
				STAUR4	ACGGAGTTACAAAGGACGAC	[129]
				STAUR6	AGCTCAGCCTTAACGAGTAC	
3		*Streptococcus agalactiae*		Sag 432/SAGA1	CGTTGGTAGGAGTGGAAAAT	[130]
				Sag 1018/SAGA2	CTGCTCCGAAGAGAAAGCCT	
				SIP3/SIP3	TGAAAATGCAGGGCTCCAACCTCA	[131]
				SIP4/SIP4	GATCTGGCATTGCATTCCAAGTAT	
4		*Escherichia coli*		Eco 2083/Ecoli1	GCTTGACACTGAACATTGAG	[130]
				Eco 2745/Ecoli2	GCACTTATCTCTTCCGCATT	
				FOP	CCGTTAAAGTGCC	[131]
				BOP	GCTTTACGTGCCGCC	
5		*Cronobacter sakazakii*	QLAMP	FIP	TGCTGCTCAACCGCCGATTTCTCCCCCCCCCCCCCACCACCAA AGACA	[133]
				BIP	GATGAACGAGCTGCTGGCCGTCGATAATTTTGCCGA	
				FLP	CACCTCGGAGGAGACC	
				BLP	CTGCTGGAGAACCC	

6. Conclusions

The unique composition of drinks, rich in micro- and macronutrients, is a good environment for the growth and development of various microorganisms that cause the spoilage of products and even the poisoning of consumers. The most common pollutants are *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella* sp., *Campylobacter* sp., *Shigella* sp., and *Vibrio parahaemolyticus*. Nonetheless, several studies using molecular and PCR-based technique have identified other species of microorganisms in beverages. In this regard, Rosalinda Urso et al. investigated yeast biodiversity during sweet wine production by performing PCR-DGGE and detected species of *Candida zemplinina* and *Hanseniaspora uvarum* [135]. Moreover, Fatemeh Zendeboodi et al. identified the dominant spoilage fungal species in non-alcoholic beer manufacturing by performing PCR. These species include *Saccharomyces*, *Pichia*, *Rhodotorula*, *Alternaria*, *Hansenia*, *Wickerhamomyces*, and *Cladosporium* [136]. The study conducted by Phattaraporn Sarikkha et al. detected bacterial and yeast species in sugary kefir, which is an acid-alcoholic fermented beverage, using PCR-DGGE. This research identified the species *Gluconobacter japonicus*, *Bacillus cereus*, *Lactobacillus rhamnosus*, *Saccharomyces cerevisiae*, and *Candida ethanolica* [137]. Sources of pollution can be both production equipment and premises, as well as violations of the packaging, storage conditions, and transportation of products.

The most contaminated drink turned out to be fruit juice. A large number of independent studies have made it possible to identify about 23 types of juice microbiological contaminants. Milk turned out to be the least contaminated in terms of the detected types of bacteria. An analysis of the data from the literature on the molecular detection of wine and beer microbiological contaminants revealed a relatively equal number of pathogenic species for both drinks. One of the main tools for quality control of beverages at all stages of their production is the polymerase chain reaction method. A wide range of variations of this technology makes it possible to identify microbiological pollutants in alcoholic and non-alcoholic beverages. The high specificity of such methods as PCR-RFLP, RAPD-PCR, qPCR, End-point PCR, LAMP, and the molecular beacon method allows for fast and reliable quality control in production. Sequencing allows evaluating the microbial diversity of all the beverages we study. PCR-RFLP, RAPD-PCR, and PCR allowed the identification of bacterial contaminants in fruit juices, qPCR, LAMP, and the molecular beacon method in wine, LAMP and multiplex PCR in milk, and End-point PCR and Rep-PCR in beer.

The results that we obtained were formulated in relation to the analysis of publicly available scientific publications and are incomplete due to the small number of modern studies in this field. Due to the persistent concern about contamination and food safety, molecular genetic methods can become effective and promising methods for solving these problems. However, it is crucial to consider all the challenges encountered when implementing these molecular genetic techniques in beverage production practices. First and foremost is the concern regarding the cost-effectiveness of PCR methods, since PCR-based techniques require advanced equipment, specific machinery, and high-quality reagents. Additionally, procedures such as the regular calibration of PCR instruments as well as the maintenance of equipment, in order to keep the instruments reliable for a high degree of data accuracy over time, along with the training of expert technicians, increase overall expenses. Another technical limitation of the PCR-based methodology is detection sensitivity. Due to the presence of some specific chemical components acting as PCR inhibitors in beverages, and also the low concentration of microorganisms in these samples, attaining optimal sensitivity, which is critical for clinical applications, is more challenging. Moreover, the adaptability of these PCR-based methods with different types of beverages should be carefully considered, as they have diverse chemical compositions, highlighting the necessity of implementing customized PCR protocols. It is noteworthy to mention that developing sensitive and high-throughput molecular approaches for screening large-scale beverage production is a significant challenge.

Many of the methods developed to detect microbiological contaminants in beverages were developed 10–20 years ago. It is necessary to develop new modern modifications of PCR and isothermal amplification for the detection of microorganisms in beverages.

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References

1. Zhang, M.; Wu, J.; Shi, Z.; Cao, A.; Fang, W.; Yan, D.; Wang, Q.; Li, Y. Molecular methods for identification and quantification of foodborne pathogens. *Molecules* **2022**, *27*, 8262. [CrossRef] [PubMed]
2. Skowron, K.; Budzyńska, A.; Grudlewska-Buda, K.; Wiktorczyk-Kapischke, N.; Andrzejewska, M.; Wałecka-Zacharska, E.; Gospodarek-Komkowska, E. Two faces of fermented foods—The benefits and threats of its consumption. *Front. Microbiol.* **2022**, *13*, 845166. [CrossRef] [PubMed]
3. Avîrvarei, A.C.; Salanță, L.C.; Pop, C.R.; Mudura, E.; Pasqualone, A.; Anjos, O.; Barboza, N.; Usaga, J.; Dărab, C.P.; Burja-Udrea, C.; et al. Fruit-based fermented beverages: Contamination sources and emerging technologies applied to assure their safety. *Foods* **2023**, *12*, 838. [CrossRef] [PubMed]
4. Velusamy, V.; Arshak, K.; Korostynska, O.; Oliwa, K.; Adley, C. An overview of foodborne pathogen detection: In the perspective of biosensors. *Biotechnol. Adv.* **2010**, *28*, 232–254. [CrossRef] [PubMed]
5. Franz, C.M.A.P.; den Besten, H.M.W.; Böhnlein, C.; Gareis, M.; Zwietering, M.H.; Fusco, V. Reprint of: Microbial food safety in the 21st century: Emerging challenges and foodborne pathogenic bacteria. *Trends Food Sci. Technol.* **2019**, *84*, 34–37. [CrossRef]
6. Juvonen, R.; Virkajärvi, V.; Priha, O.; Laitila, A. Microbiological spoilage and safety risks in non-beer beverages. *VTT Tied. Res. Notes* **2011**, *2599*, 107. [CrossRef]
7. Azeredo, D.R.; Alvarenga, V.; Sant'Ana, A.S.; Srur, A.U.S. An over- view of microorganisms and factors contributing for the micro-bial stability of carbonated soft drinks. *Food Res. Int.* **2016**, *82*, 136–144. [CrossRef]
8. Zhao, X.; Lin, C.W.; Wang, J.; Oh, D.H. Advances in rapid detection methods for foodborne pathogens. *J. Microbiol. Biotechnol.* **2014**, *24*, 297–312. [CrossRef] [PubMed]
9. Li, W.L.; Wu, A.; Li, Z.C.; Zhang, G.; Yu, W.Y. A new calibration method between an optical sensor and a rotating platform in turbine blade inspection. *Meas. Sci. Technol.* **2017**, *28*, 035009. [CrossRef]
10. Zhong, J.; Zhao, X. Detection of viable but non-culturable *Escherichia coli* O157:H7 by PCR in combination with propidium monoazide. *3 Biotechnology* **2018**, *8*, 28. [CrossRef]
11. Fusco, V.; Quero, G.M. Culture-dependent and culture-independent nucleic-acid-based methods used in the microbial safety assessment of milk and dairy products. *Compr. Rev. Food Sci. Food Saf.* **2014**, *13*, 493–537. [CrossRef] [PubMed]
12. Zeng, P.; Guan, Q.; Zhang, Q. SERS detection of foodborne pathogens in beverage with Au nanostars. *Microchim. Acta* **2024**, *191*, 28. [CrossRef] [PubMed]
13. Länge, K. Bulk and surface acoustic wave biosensors for milk analysis. *Biosensors* **2022**, *12*, 602. [CrossRef] [PubMed]
14. da Silva Campos, J.; Júnior, A.C.V.; Boniek, D.; da Silva, D.L.; de Paula Lana, U.G.; Fernandes, J.V.; de Resende Stoianoff, M.A.; Andrade, V.S. Identification and evaluation of thermotolerance of yeasts from milk in natura exposed to high temperature and slow and fast pasteurization. *Braz. J. Microbiol.* **2023**, *54*, 1075–1082. [CrossRef]
15. Grützke, J.; Gwida, M.; Deneke, C.; Brendebach, H.; Projahn, M.; Schattschneider, A.; Hofreuter, D.; El-Ashker, M.; Malorny, B.; Al Dahouk, S. Direct identification and molecular characterization of zoonotic hazards in raw milk by metagenomics using *Brucella* as a model pathogen. *Microb. Genet.* **2021**, *7*, 000552. [CrossRef]
16. Jaudou, S.; Deneke, C.; Tran, M.L.; Schuh, E.; Goehler, A.; Vorimore, F.; Malorny, B.; Fach, P.; Grützke, J.; Delannoy, S. A step forward for Shiga toxin-producing *Escherichia coli* identification and characterization in raw milk using long-read metagenomics. *Microb. Genet.* **2022**, *8*, mgen000911. [CrossRef] [PubMed]
17. Palomino-Camargo, C.; Gonzalez-Munoz, Y. Molecular techniques for detection and identification of pathogens in food: Advantages and limitations. *Rev. Peru. Med. Exp. Y Salud Publica* **2014**, *31*, 535–546.
18. Mangal, M.; Bansal, S.; Sharma, S.K.; Gupta, R.K. Molecular detection of foodborne pathogens: A rapid and accurate answer to food safety. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 1568–1584. [CrossRef]
19. Jiyeon, H.; Ingyun, H.; Hyosun, K.; Chankyu, P.; Insoo, C.; Kunho, S. Evaluation of PCR inhibitory effect of enrichment broths and comparison of DNA extraction methods for detection of *Salmonella enteritidis* using real-time PCR assay. *J. Vet. Sci.* **2010**, *11*, 143–149. [CrossRef]

20. Löfström, C.; Hansen, F.; Hoorfar, J. Validation of a 20-h real-time PCR method for screening of *Salmonella* in poultry faecal samples. *Vet. Microbiol.* **2010**, *144*, 511–514. [CrossRef]
21. Cantekin, C.; Ergun, Y.; Solmaz, H.; Özmen, G.Ö.; Demir, M.; Saidi, R. PCR assay with host specific internal control for *Staphylococcus aureus* from bovine milk samples. *Maced. Vet. Rev.* **2015**, *38*, 97–100. [CrossRef]
22. Forghani, F.; Wei, S.; Oh, D.H. A rapid multiplex real-time PCR high-resolution melt curve assay for the simultaneous detection of Bacillus *cereus, Listeria monocytogenes*, and *Staphylococcus aureus* in Food. *J. Food Prot.* **2016**, *79*, 821–824. [CrossRef] [PubMed]
23. Elizaquível, P.; Aznar, R. A multiplex RTi-PCR reaction for simultaneous detection of *Escherichia coli* O157:H7, *Salmonella* spp. and *Staphylococcus aureus* on fresh, minimally processed vegetables. *Food Microbiol.* **2008**, *25*, 705–713. [CrossRef] [PubMed]
24. Kawasaki, S.; Fratamico, P.M.; Horikoshi, N.; Okada, Y.; Takeshita, K.; Sameshima, T.; Kawamoto, S. Multiplex real-time polymerase chain reaction assay for simultaneous detection and quantification of *Salmonella* species, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in ground pork samples. *Foodborne Pathog. Dis.* **2010**, *7*, 549–554. [CrossRef]
25. Singh, J.; Batish, V.K.; Grover, S. Simultaneous detection of *Listeria monocytogenes* and *Salmonella* spp. in dairy products using real time PCR-melt curve analysis. *J. Food Sci. Technol.* **2012**, *49*, 234–239. [CrossRef] [PubMed]
26. Bastam, M.M.; Jalili, M.; Pakzad, I.; Maleki, A.; Ghafourian, S. Pathogenic bacteria in cheese, raw and pasteurised milk. *J. Vet. Med. Sci.* **2021**, *7*, 2445–2449. [CrossRef] [PubMed]
27. Harris, L.J.; Farber, J.N.; Beuchat, L.R.; Parish, M.E.; Suslow, T.V.; Garrett, E.H.; Busta, F.F. Outbreaks associated with fresh produce: Incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Compr. Rev. Food Sci. Food Saf.* **2003**, *2*, 78–141. [CrossRef]
28. Grumezescu, A.; Holban, A.M. *Preservatives and Preservation Approaches in Beverages*; Academic Press: Cambridge, MA, USA, 2019; Volume 15, pp. 540–562.
29. Aneja, K.R.; Dhiman, R.; Aggarwal, N.K.; Kumar, V.; Kaur, M. Microbes associated with freshly prepared juices of citrus and carrots. *Int. J. Food Sci.* **2014**, *2014*, 408085. [CrossRef] [PubMed]
30. Kregiel, D.; James, S.A.; Rygala, A.; Berlowska, J.; Antolak, H.; Pawlikowska, E. Consortia formed by yeasts and acetic acid bacteria *Asaia* spp. in soft drinks. *Antonie Leeuwenhoek* **2018**, *111*, 373–383. [CrossRef]
31. Kregiel, D. Health safety of soft drinks: Contents, containers, and microorganisms. *BioMed Res. Int.* **2015**, *2015*, 128697. [CrossRef]
32. Bintsis, T. Lactic acid bacteria: Their applications in foods. *J. Bacteriol. Mycol.* **2018**, *6*, 89–94. [CrossRef]
33. Hernández, A.; Pérez-Nevado, F.; Ruiz-Moyano, S.; Serradilla, M.J.; Villalobos, M.C.; Martín, A.; Córdoba, M.G. Spoilage yeasts: What are the sources of contamination of foods and beverages? *Int. J. Food Microbiol.* **2018**, *286*, 98–110. [CrossRef] [PubMed]
34. Coskun, F.A. Traditional turkish fermented non-alcoholic grape-based beverage, "Hardaliye". *Beverages* **2017**, *3*, 2. [CrossRef]
35. Romero-Luna, H.E.; Peredo-Lovillo, A.; Davila-Ortiz, G. Tepache: A pre-hispanic fermented beverage as a potential source of probiotic yeasts. In *Chemistry of Fermented Foods*; American Chemical Society: Washington, DC, USA, 2022; pp. 135–147. [CrossRef]
36. Motlhanka, K.; Lebani, K.; Boekhout, T.; Zhou, N. Fermentative microbes of khadi, a traditional alcoholic beverage of Botswana. *Fermentation* **2020**, *6*, 51. [CrossRef]
37. Azam, M.S.; Ahmed, S.; Islam, M.N.; Maitra, P.; Islam, M.M.; Yu, D. Critical assessment of mycotoxins in beverages and their control measures. *Toxins* **2021**, *13*, 323. [CrossRef]
38. Larcher, R.; Nicolini, G. Survey of ochratoxin A in musts, concentrated musts and wines produced or marketed in Trentino (Italy). *J. Commod. Sci.* **2001**, *40*, 69–78.
39. Yazdanpanah, H. Mycotoxin contamination of foodstuffs and feedstuffs in Iran. *Iran. J. Pharm. Res.* **2006**, *5*, 9–16. [CrossRef]
40. Mule, G.; Susca, A.; Logrieco, A.; Stea, G.; Visconti, A. Development of a quantitative real-time PCR assay for the detection of *Aspergillus carbonarius* in grapes. *Int. J. Food Microbiol.* **2006**, *111*, 28–34. [CrossRef]
41. Rani, H.; Bhardwaj, R.D. Quality attributes for barley malt: "The backbone of beer". *J. Food Sci.* **2021**, *86*, 3322–3340. [CrossRef]
42. Zugravu, C.A.; Medar, C.; Manolescu, L.S.C.; Constantin, C. Beer and Microbiota: Pathways for a Positive and Healthy Interaction. *Nutrients* **2023**, *15*, 844. [CrossRef]
43. Kordialik-Bogacka, E. Biopreservation of beer: Potential and constraints. *Biotechnol. Adv.* **2022**, *58*, 107910. [CrossRef] [PubMed]
44. Sakamoto, K.; Konings, W.N. Beer spoilage bacteria and hop resistance. *Int. J. Food Microbiol.* **2003**, *89*, 105–124. [CrossRef] [PubMed]
45. Xu, Z.; Luo, Y.; Mao, Y.; Peng, R.; Chen, J.; Soteyome, T.; Bai, C.; Chen, L.; Liang, Y.; Su, J.; et al. Spoilage lactic acid bacteria in the brewing industry. *World J. Microbiol. Biotechnol.* **2020**, *30*, 955–961. [CrossRef] [PubMed]
46. Tsekouras, G.; Tryfinopoulou, P.; Panagou, E. Detection and identification of lactic acid bacteria in semi-finished beer products using molecular techniques. In Proceedings of the 2nd International Electronic Conference on Foods—"Future Foods and Food Technologies for a Sustainable World", Virtual, 15–30 October 2021. [CrossRef]
47. Satokari, R.; Juvonen, R.; Wright, A.; Haikara, A. Detection of *Pectinatus* beer spoilage bacteria by using the polymerase chain reaction. *J. Food Prot.* **1997**, *60*, 1571–1573. [CrossRef] [PubMed]
48. Suzuki, K.; Hill, A.E. 7—Gram-positive spoilage bacteria in brewing. In *Technology and Nutrition*; Woodhead Publishing: Sawston, UK, 2015; pp. 141–173. [CrossRef]
49. Garofalo, C.; Osimani, A.; Milanović, V.; Taccari, M.; Aquilanti, L.; Clementi, F. The occurrence of beer spoilage lactic acid bacteria in craft beer production. *J. Food Sci.* **2015**, *80*, M2845-52. [CrossRef] [PubMed]

50. Gevers, D.; Huys, G.; Swings, J. Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species. *FEMS Microbiol. Lett.* **2001**, *205*, 31–36. [CrossRef] [PubMed]
51. Ma, Y.; Deng, Y.; Xu, Z.; Liu, J.; Dong, J.; Yin, H.; Yu, J.; Chang, Z.; Wang, D. Development of a propidium monoazide-polymerase chain reaction assay for detection of viable *Lactobacillus brevis* in beer. *Braz. J. Microbiol.* **2017**, *48*, 740–746. [CrossRef] [PubMed]
52. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. *Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics*; Academic Press: Cambridge, MA, USA, 1990; pp. 315–322. [CrossRef]
53. Latorre, M.; Bruzone, M.C.; de Garcia, V.; Libkind, D. Microbial contaminants in bottled craft beer of Andean Patagonia, Argentina. *Rev. Argent. Microbiol.* **2023**, *55*, 88–99. [CrossRef] [PubMed]
54. Geissler, A.J.; Behr, J.; Vogel, R.F. Multiple genome sequences of important beer-spoiling lactic acid bacteria. *Genome Announc.* **2016**, *4*, e01077-16. [CrossRef]
55. Kiousi, D.E.; Bucka-Kolendo, J.; Wojtczak, A.; Sokołowska, B.; Doulgeraki, A.I.; Galanis, A. Genomic analysis and in vitro investigation of the hop resistance phenotype of two novel *Loigolactobacillus backii* strains, isolated from spoiled beer. *Microorganisms* **2023**, *11*, 280. [CrossRef]
56. Asano, S.; Shimokawa, M.; Suzuki, K. PCR analysis methods for detection and identification of beer-spoilage lactic acid bacteria. *Methods Mol. Biol.* **2019**, *1887*, 95–107. [CrossRef] [PubMed]
57. Haakensen, M.; Ziola, B. Identification of novel horA-harbouring bacteria capable of spoiling beer. *Can. J. Microbiol.* **2008**, *54*, 321–325. [CrossRef] [PubMed]
58. Satokari, R.; Juvonen, R.; Mallison, K.; von Wright, A.; Haikara, A. Detection of beer spoilage bacteria Megasphaera and Pectinatus by polymerase chain reaction and colorimetric microplate hybridization. *Int. J. Food Microbiol.* **1998**, *45*, 119–127. [PubMed]
59. Juvonen, R.; Koivula, T.; Haikara, A. Group-specific PCR-RFLP and real-time PCR methods for detection and tentative discrimination of strictly anaerobic beer-spoilage bacteria of the class *Clostridia*. *Int. J. Food Microbiol.* **2008**, *125*, 162–169. [CrossRef] [PubMed]
60. Sanzani, S.M.; Miazzi, M.M.; di Rienzo, V.; Fanelli, V.; Gambacorta, G.; Taurino, M.R.; Montemurro, C. A rapid assay to detect toxigenic *Penicillium* spp. contamination in wine and musts. *Toxins* **2016**, *8*, 235. [CrossRef] [PubMed]
61. Jorgensen, K. Occurrence of ochratoxin A in commodities and processed food—A review of EU occurrence data. *Food Addit. Contam.* **2005**, *1*, 26–30. [CrossRef] [PubMed]
62. Majerus, P.; Hain, J.; Kölb, C. Patulin in grape must and new, still fermenting wine (Federweißer). *Mycotoxin Res.* **2008**, *24*, 135–139. [CrossRef] [PubMed]
63. Sanzani, S.M.; Reverberi, M.; Fanelli, C.; Ippolito, A. Detection of ochratoxin A using molecular beacons and real-time PCR thermal cycler. *Toxins* **2015**, *7*, 812–820. [CrossRef] [PubMed]
64. Sanzani, S.M.; Montemurro, C.; Di Rienzo, V.; Solfrizzo, M.; Ippolito, A. Genetic structure and natural variation associated with host of origin in *Penicillium expansum* strains causing blue mould. *Int. J. Food Microbiol.* **2013**, *165*, 111–120. [CrossRef]
65. Silva, P. First science & wine world congress. *J. Agric. Food Chem.* **2020**, *68*, 3299–3301. [CrossRef]
66. López-Barajas, M.; López-Tamames, E.; Buxaderas, S.; Tomás, X.; de La Torre, M.C. Prediction of wine foaming. *J. Agric. Food Chem.* **1999**, *47*, 3743–3748. [CrossRef] [PubMed]
67. Liu, P.H.; Vrigneau, C.; Salmon, T.; Hoang, D.A.; Boulet, J.C.; Jégou, S.; Marchal, R. Influence of grape berry maturity on juice and base wine composition and foaming properties of sparkling wines from the champagne region. *Molecules* **2018**, *23*, 1372. [CrossRef]
68. Amaro, F.; Almeida, J.; Oliveira, A.S.; Furtado, I.; Bastos, M.L.; Guedes de Pinho, P.; Pinto, J. Impact of cork closures on the volatile profile of sparkling wines during bottle aging. *Foods* **2022**, *11*, 293. [CrossRef]
69. Marchal, R.; Warchol, M.; Cilindre, C.; Jeandet, P. Evidence for protein degradation by Botrytis cinerea and relationships with alteration of synthetic wine foaming properties. *J. Agric. Food Chem.* **2006**, *54*, 5157–5165. [CrossRef]
70. Marchal, R.; Salmon, T.; Gonzalez, R.; Kemp, B.; Vrigneau, C.; Williams, P.; Doco, T. Impact of *Botrytis cinerea* contamination on the characteristics and foamability of yeast macromolecules released during the alcoholic fermentation of a model grape juice. *Molecules* **2020**, *25*, 472. [CrossRef] [PubMed]
71. Echeverrigaray, S.; Randon, M.; da Silva, K.; Zacaria, J.; Longaray, A.P. Delamare Identification and characterization of non-saccharomyces spoilage yeasts isolated from Brazilian wines. *World J. Microbiol. Biotechnol.* **2013**, *29*, 1019–1027. [CrossRef]
72. Millet, V.; Lonvaud-Funel, A. The viable but non-culturable state of wine micro-organisms during storage. *Lett. Appl. Microbiol.* **2000**, *30*, 136–141. [CrossRef]
73. Caruso, M.; Fiore, C.; Contursi, M.; Salzano, G.; Paparella, A.; Romano, P. Formation of biogenic amines as criteria for the selection of wine yeasts. *World J. Microbiol. Biotechnol.* **2002**, *18*, 159–163. [CrossRef]
74. Hierro, N.; Esteve-Zarzoso, B.; González, A.; Mas, A.; Guillamón, J.M. Real-time quantitative PCR (QPCR) and reverse transcription-QPCR for detection and enumeration of total yeasts in wine. *Appl. Environ. Microbiol.* **2006**, *72*, 7148–7155. [CrossRef]
75. Willenburg, E.; Divol, B. Quantitative PCR: An appropriate tool to detect viable but not culturable *Brettanomyces bruxellensis* in wine. *Int. J. Food Microbiol.* **2012**, *160*, 131–136. [CrossRef]
76. Phister, T.G.; Mills, D.A. Real-time PCR assay for detection and enumeration of *Dekkera bruxellensis* in wine. *Appl. Environ. Microbiol.* **2003**, *69*, 7430–7434. [CrossRef] [PubMed]

77. Delaherche, A.; Claisse, O.; Lonvaud-Funel, A. Detection and quantification of *Brettanomyces bruxellensis* and 'ropy' *Pediococcus damnosus* strains in wine by real-time polymerase chain reaction. *J. Appl. Microbiol.* **2004**, *97*, 910–915. [CrossRef] [PubMed]
78. Tessonniere, H.; Vidal, S.; Barnavon, L.; Alexandre, H.; Remize, F. Design and performance testing of a real-time PCR assay for sensitive and reliable direct quantification of *Brettanomyces* in wine. *Int. J. Food Microbiol.* **2009**, *129*, 237–243. [CrossRef] [PubMed]
79. Tofalo, R.; Schirone, M.; Corsetti, A.; Suzzi, G. Detection of *Brettanomyces* spp. in red wines using real-time PCR. *J. Food Sci.* **2012**, *77*, M545-9. [CrossRef] [PubMed]
80. Palma, M.; Sa-Correia, I. Physiological genomics of the highly weak-acid-tolerant food spoilage yeasts of *Zygosaccharomyces bailii* sensu lato. *Prog. Mol. Subcell. Biol.* **2019**, *58*, 85–109. [CrossRef]
81. Rawsthorne, H.; Phister, T.G. A real-time PCR assay for the enumeration and detection of *Zygosaccharomyces bailii* from wine and fruit juices. *Int. J. Food Microbiol.* **2006**, *112*, 1–7. [CrossRef] [PubMed]
82. González-Muñoz, B.; Garrido-Vargas, F.; Pavez, C.; Osorio, F.; Chen, J.; Bordeu, E.; O'Brien, J.A.; Brossard, N. Wine astringency: More than just tannin-protein interactions. *J. Sci. Food Agric.* **2022**, *102*, 1771–1781. [CrossRef]
83. Bartowsky, E.J.; Henschke, P.A. Acetic acid bacteria spoilage of bottled red wine—A review. *Int. J. Food Microbiol.* **2008**, *125*, 60–70. [CrossRef] [PubMed]
84. Zhang, J.; Wang, L.; Shi, L.; Chen, X.; Liang, M.; Zhao, L. Development and application of a real-time loop-mediated isothermal amplification method for quantification of *Acetobacter aceti* in red wine. *FEMS Microbiol. Lett.* **2020**, *367*, fnaa152. [CrossRef]
85. Valera, M.J.; Torija, M.J.; Mas, A.; Mateo, E. Acetic acid bacteria from biofilm of strawberry vinegar visualized by microscopy and detected by complementing culture-dependent and culture-independent techniques. *Food Microbiol.* **2015**, *46*, 452–462. [CrossRef]
86. Longin, C.; Guilloux-Benatier, M.; Alexandre, H. Design and performance testing of a DNA extraction assay for sensitive and reliable quantification of acetic acid bacteria directly in red wine using real time PCR. *Front. Microbiol.* **2016**, *7*, 831. [CrossRef] [PubMed]
87. Mandappa, I.M.; Basavaraj, K.; Manonmani, H.K. *Analysis of Mycotoxins in Fruit Juices*; Academic Press: Cambridge, MA, USA, 2018; pp. 763–777. [CrossRef]
88. Karasawa, M.M.G.; Mohan, C. Fruits as prospective reserves of bioactive compounds: A review. *Nat. Prod. Bioprospect.* **2018**, *8*, 335–346. [CrossRef] [PubMed]
89. Dhalaria, R.; Verma, R.; Kumar, D.; Puri, S.; Tapwal, A.; Kumar, V. Bioactive compounds of edible fruits with their anti-aging properties: A comprehensive review to prolong human life. *Antioxidants* **2020**, *9*, 1123. [CrossRef]
90. Raybaudi-Massilia, R.M.; Mosqueda-Melgar, J.; Soliva-Fortuny, R.; Martin-Belloso, O. Control of pathogenic and spoilage microorganisms in fresh-cut fruits and fruit juices by traditional and alternative natural antimicrobials. *Compr. Rev. Food Sci. Food Saf.* **2009**, *8*, 157–180. [CrossRef] [PubMed]
91. Jimma, F.I.; Mohammed, A.; Adzaworlu, E.G.; Nzeh, J.; Quansah, L.; Dufailu, O.A. Microbial quality and antimicrobial residue of local and industrial processed fruit juice sold in Tamale, Ghana. *Food Technol.* **2022**, *2*, 26. [CrossRef]
92. Salomao, B.D.C.M. *Pathogens and Spoilage Microorganisms in Fruit Juice: An Overview*; Academic Press: Cambridge, MA, USA, 2018; pp. 291–308. [CrossRef]
93. Sharma, N.; Singh, K.; Toor, D.; Pai, S.S.; Chakraborty, R.; Khan, K.M. Antibiotic resistance in microbes from street fruit drinks and hygiene behavior of the vendors in Delhi, India. *Int. J. Environ. Res. Public Health* **2020**, *17*, 4829. [CrossRef] [PubMed]
94. Tasnim, F., Jr.; Anwar Hossain, M.; Kamal Hossain, M.; Lopa, D.; Formuzul Haque, K.M. Quality assessment of industrially processed fruit juices available in Dhaka city, Bangladesh. *Malays. J. Nutr.* **2010**, *16*, 431–438. [PubMed]
95. Keller, S.E.; Miller, A.J. Microbiological safety of fresh citrus and apple juices. In *Microbiology of Fruits and Vegetables*; CRC Press: Boca Raton, FL, USA, 2005; pp. 227–246.
96. Jensen, N. *Alicyclobacillus*: A new challenge for the food industry. *Food Aust.* **1999**, *51*, 33–36.
97. Cacho, P.; Danyluk, M.; Rouseff, R. GC–MS quantification and sensory thresholds of guaiacol in orange juice and its correlation with *Alicyclobacillus* spp. *Food Chem.* **2011**, *129*, 45–50. [CrossRef]
98. Chang, S.S.; Kang, D.H. *Alicyclobacillus* spp. in the fruit juice industry: History, characteristics, and current isolation/detection procedures. *Crit. Rev. Microbiol.* **2004**, *30*, 55–74. [CrossRef]
99. Molva, C.; Baysal, A.H. Evaluation of bioactivity of pomegranate fruit extract against *Alicyclobacillus acidoterrestris* DSM 3922 vegetative cells and spores in apple juice. *LWT-Food Sci. Technol.* **2015**, *62*, 989–995. [CrossRef]
100. Hui, L.; Hong, C.; Bin, L.; Rui, C.; Nan, J.; Tianli, Y.; Zhouli, W. Establishment of quantitative PCR assays for the rapid detection of *Alicyclobacillus* spp. that can produce guaiacol in apple juice. *Int. J. Food Microbiol.* **2021**, *360*, 109329. [CrossRef]
101. Osopale, B.A.; Adewumi, G.A.; Witthuhn, R.C.; Kuloyo, O.O.; Oguntoyinbo, F.A. A review of innovative techniques for rapid detection and enrichment of *Alicyclobacillus* during industrial processing of fruit juices and concentrates. *Food Control* **2019**, *99*, 146–157. [CrossRef]
102. Sourri, P.; Tassou, C.C.; Nychas, G.E.; Panagou, E.Z. Fruit juice spoilage by *Alicyclobacillus*: Detection and control methods-a comprehensive review. *Foods* **2022**, *11*, 747. [CrossRef] [PubMed]
103. Yamazaki, K.; Okubo, T.; Inoue, N.; Shinano, H. Randomly amplified polymorphic DNA (RAPD) for rapid identification of the spoilage bacterium *Alicyclobacillus acidoterrestris*. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 1016–1018. [CrossRef]
104. Sourri, P.; Doulgeraki, A.I.; Tassou, C.C.; Nychas, G.E. A single enzyme PCR-RFLP assay targeting V1-V3 region of 16S rRNA gene for direct identification of *Alicyclobacillus acidoterrestris* from other *Alicyclobacillus* species. *J. Appl. Genet.* **2019**, *60*, 225–229. [CrossRef]

105. Connor, C.J.; Luo, H.; Gardener, B.B.; Wang, H.H. Development of a real-time PCR-based system targeting the 16S rRNA gene sequence for rapid detection of *Alicyclobacillus* spp. in juice products. *Int. J. Food Microbiol.* **2005**, *99*, 229–235. [CrossRef] [PubMed]
106. Chen, S.; Tang, Q.; Zhang, X.; Zhao, G.; Hu, X.; Liao, X.; Xiang, H. Isolation and characterization of thermo-acidophilic endospore-forming bacteria from the concentrated apple juice-processing environment. *Food Microbiol.* **2006**, *23*, 439–445. [CrossRef] [PubMed]
107. Dekowska, A.; Niezgoda, J.; Sokołowska, B. Genetic heterogeneity of *Alicyclobacillus* strains revealed by RFLP analysis of vdc region and rpoB gene. *BioMed Res. Int.* **2018**, *2018*, 9608756. [CrossRef]
108. Wang, Z.; Yue, T.; Yuan, Y.; Zhang, Y.; Gao, Z.; Cai, R. Targeting the vanillic acid decarboxylase gene for *Alicyclobacillus acidoterrestris* quantification and guaiacol assessment in apple juices using real time PCR. *Int. J. Food Microbiol.* **2021**, *338*, 109006. [CrossRef]
109. Cai, R.; Wang, Z.; Yuan, Y.; Liu, B.; Wang, L.; Yue, T. Detection of *Alicyclobacillus* spp. in fruit juice by combination of immunomagnetic separation and a SYBR Green I Real-Time PCR assay. *PLoS ONE* **2015**, *10*, e0141049. [CrossRef] [PubMed]
110. Huang, T.; Shi, Y.; Zhang, J.; Han, Q.; Xia, X.S.; Zhang, A.M.; Song, Y. Rapid and simultaneous detection of five, viable, foodborne pathogenic bacteria by photoinduced PMAxx-coupled multiplex PCR in fresh juice. *Foodborne Pathog. Dis.* **2021**, *18*, 640–646. [CrossRef] [PubMed]
111. Corbett, K.M.; de Smidt, O. Culture-dependent diversity profiling of spoilage yeasts species by PCR-RFLP comparative analysis. *Food Sci. Technol. Int.* **2019**, *25*, 671–679. [CrossRef] [PubMed]
112. Casey, G.D.; Dobson, A.D.W. Potential of using real-time PCR-based detection of spoilage yeast in fruit juice—A preliminary study. *Int. J. Food Microbiol.* **2004**, *91*, 327–335. [CrossRef] [PubMed]
113. Oliveira, A.C.; Seixas, A.S.; Sousa, C.P.; Souza, C.W. Microbiological evaluation of sugarcane juice sold at street stands and juice handling conditions in Sao Carlos, Sao Paulo, Brazil. *Cad. Saude Publica* **2006**, *22*, 1111–1114. [CrossRef] [PubMed]
114. Ahmed, A.; Dawar, S.; Tariq, M. Mycoflora associated with sugar cane juice in Karachi city. *Pak. J. Bot.* **2010**, *42*, 2955–2962.
115. Abbas, S.R.; Sabir, S.M.; Ahmad, S.D.; Boligon, A.A.; Athayde, M.L. Phenolic profile, antioxidant potential and DNA damage protecting activity of sugarcane (*Saccharum officinarum*). *Food Chem.* **2014**, *147*, 10–16. [CrossRef] [PubMed]
116. Mattos, E.C.; Meira-Strejevitch, C.D.S.; Marciano, M.A.M.; Faccini, C.C.; Lourenço, A.M.; Pereira-Chioccola, V.L. Molecular detection of *Trypanosoma cruzi* in acai pulp and sugarcane juice. *Acta Trop.* **2017**, *176*, 311–315. [CrossRef]
117. Rettedal, E.A.; Altermann, E.; Roy, N.C.; Dalziel, J.E. The Effects of unfermented and fermented cow and sheep milk on the gut microbiota. *Front. Microbiol.* **2019**, *10*, 458. [CrossRef]
118. Paszczyk, B.; Czarnowska-Kujawska, M.; Klepacka, J.; Tońska, E. Health-promoting ingredients in goat's milk and fermented goat's milk drinks. *Animals* **2023**, *13*, 907. [CrossRef]
119. Quigley, L.; O'Sullivan, O.; Stanton, C.; Beresford, T.P.; Ross, R.P.; Fitzgerald, G.F.; Cotter, P.D. The complex microbiota of raw milk. *FEMS Microbiol.* **2013**, *37*, 664–698. [CrossRef]
120. Aliyo, A.; Teklemariam, Z. Assessment of Milk Contamination, Associated Risk Factors, and Drug Sensitivity Patterns among Isolated Bacteria from Raw Milk of Borena Zone, Ethiopia. *J. Trop. Med.* **2022**, *2022*, 3577715. [CrossRef]
121. Koskinen, M.T.; Holopainen, J.; Pyorala, S.; Bredbacka, P.; Pitkala, A.; Barkema, H.W.; Bexiga, R.; Roberson, J.; Solverod, L.; Piccinini, R.; et al. Analytical specificity and sensitivity of a real-time polymerase chain reaction assay for identification of bovine mastitis pathogens. *J. Dairy Sci.* **2009**, *92*, 952–959. [CrossRef]
122. Pang, L.; Pi, X.; Yang, X.; Song, D.; Qin, X.; Wang, L.; Man, C.; Zhang, Y.; Jiang, Y. Nucleic acid amplification-based strategy to detect foodborne pathogens in milk: A review. *Crit. Rev. Food Sci. Nutr.* **2022**, *8*, 1–16. [CrossRef]
123. Cornelissen, J.B.W.J.; De Greeff, A.; Heuvelink, A.E.; Swarts, M.; Smith, H.E.; Van der Wal, F.J. Rapid detection of *Streptococcus uberis* in raw milk by loop-mediated isothermal amplification. *J. Dairy Sci.* **2016**, *99*, 4270–4281. [CrossRef]
124. Alber, J.; El-Sayed, A.; Lammler, C.; Hassan, A.A.; Zschock, M. Polymerase chain reaction mediated identification of *Streptococcus uberis* and *Streptococcus parauberis* using species-specific sequences of the genes encoding superoxide dismutase A and chaperonin 60. *J. Vet. Med. B Infect. Dis. Vet. Public Health* **2004**, *51*, 180–184. [CrossRef]
125. Gillespie, B.E.; Oliver, S.P. Simultaneous detection of mastitis pathogens, *Staphylococcus aureus*, *Streptococcus uberis*, and *Streptococcus agalactiae* by multiplex real-time polymerase chain reaction. *J. Dairy Sci.* **2005**, *88*, 3510–3518. [CrossRef]
126. Bruno, S.J.; Phiri, B.M.; Hang'ombe, M.E.; Mubanga, M.; Maurischat, S.; Wichmann-Schauer, H.; Schaarschmidt, S.; Fetsch, A. Prevalence and diversity of *Staphylococcus aureus* in the Zambian dairy value chain: A public health concern. *Int. J. Food Microbiol.* **2022**, *375*, 109737. [CrossRef]
127. Machado, G.P.; Silva, R.C.; Guimarães, F.F.; Salina, A.; Langoni, H. Detection of *Staphylococcus aureus*, *Streptococcus agalactiae* and *Escherichia coli* in Brazilian mastitic milk goats by multiplex-PCR. *Pesqui. Vet. Bras.* **2018**, *38*, 1358–1364. [CrossRef]
128. Straub, J.A.; Hertel, C.; Hammes, W.P. A 23S rDNA-targeted polymerase chain reaction-based system for detection of *Staphylococcus aureus* in meat starter cultures and dairy products. *J. Prot.* **1999**, *62*, 1150–1156. [CrossRef]
129. Riffon, R.; Sayasith, K.; Khalil, H.; Dubreuil, P.; Drolet, M.; Lagacé, J. Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. *J. Clin. Microbiol.* **2001**, *39*, 2584–2589. [CrossRef]
130. Chotár, M.; Vidova, B.; Godany, A. Development of specific and rapid detection of bacterial pathogens in dairy products by PCR. *Folia Microbiol.* **2006**, *51*, 639–646. [CrossRef]
131. Luciani, M.; di Febo, T.; Zilli, K.; di Giannatale, E.; Armillotta, G.; Manna, L. Rapid detection and isolation of *Escherichia coli* O104:H4 from milk using monoclonal antibody-coated magnetic beads. *Front. Microbiol.* **2016**, *7*, 942. [CrossRef]

132. Bai, Y.; Cui, Y.; Suo, Y.; Shi, C.; Wang, D.; Shi, X. A Rapid method for detection of *Salmonella* in milk based on extraction of mRNA using magnetic capture probes and RT-qPCR. *Front. Microbiol.* **2019**, *10*, 770. [CrossRef]
133. Hu, L.; Zhang, S.; Xue, Y.; Zhang, Y.; Zhang, W.; Wang, S. Quantitative detection of viable but nonculturable *Cronobacter sakazakii* using photosensitive nucleic acid dye PMA combined with isothermal amplification LAMP in raw milk. *Foods* **2022**, *11*, 2653. [CrossRef]
134. Dauga, C. Evolution of the gyrB gene and the molecular phylogeny of Enterobacteriaceae: A model molecule for molecular systematic studies. *Int. J. Syst. Evol. Microbiol.* **2002**, *52*, 531–547. [CrossRef]
135. Urso, R.; Rantsiou, K.; Dolci, P.; Rolle, L.; Comi, G.; Cocolin, L. Yeast biodiversity and dynamics during sweet wine production as determined by molecular methods. *FEMS Yeast Res.* **2008**, *8*, 1053–1062. [CrossRef]
136. Zendeboodi, F.; Jannat, B.; Sohrabvandi, S.; Khanniri, E.; Mortazavian, A.M.; Khosravi, K.; Gholian, M.M.; Sarmadi, B.; Javadi, N.H.S. Detection of non-alcoholic beer spoilage microorganisms at critical points of production by polymerase chain reaction. *Biointerface Res. Appl. Chem.* **2021**, *11*, 9658–9966. [CrossRef]
137. Phattaraporn, S.; Rachnarin, N.; Issara, P.; Siwarutt, B. Identification of bacteria and yeast communities in a Thai sugary kefir by polymerase chain reaction-denaturing gradient gel electrophoresis (pcr-dgge) analyses. *J. Ind. Technol.* **2015**, *11*, 25–39.

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