

Editorial

Microbial Diversity and Safety in Fermented Beverages

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For thousands of years, humans have exploited the natural process of fermentation of various foods to preserve them, and to enjoy the changes in the sensory characteristics that could be produced. Recently, the world of fermented beverages has gone through a rapid transformation linked to deep changes in consumer preferences, consumption habits, climate, new regulations and entry of emerging countries, accompanied by safety concerns and an important reduction in economic resources available to people. As with all food handling and preparation, we need to be sure the fermented food produced is safe. Fermentation is a complex biological process where microbial diversity takes place and the environment created inside of the fermented food provides the conditions to reduce the risk of pathogenic bacteria growth, thus providing safe food. In addition, food manufacturers fermenting food carefully control their processing and must comply with the National Food Standards Codes. Although these products have a generally good food safety record, sometimes inadequate manufacturing practices or the presence of acidophilic pathogens could compromise food safety. In fact, fermented beverages may adversely become contaminated with pathogens or microbial toxins and thereby transform into vehicles that can transmit diseases to the consumers. Moreover, many microorganisms can deteriorate the physical-chemical and sensory properties as well as the flavor of the final products. In this editorial, we present an overview of a review and six original research papers published in the Special Issue “Fermentation Process and Microbial Safety of Beverages” of the *Beverages* journal.

Nwaiwu et al. [1] proposed an overview of the prevalent microorganisms associated with nine traditional and artisanal fermented beverages in Nigeria such as soymilk, nono (fermented cow milk), tiger nut milk, yoghurt, kunu, zobo, palm wine and the local beers pito and brukutu. They compiled studies in which bacteria of the genera *Bacillus*, *Escherichia*, *Lactobacillus*, *Staphylococcus*, and *Streptococcus* were identified in all nine beverages. Regarding eukaryotic microorganisms isolated from these beverages, the genera *Saccharomyces*, *Aspergillus*, *Candida*, and *Penicillium* were the most representative in all beverages. The occurrence of fungal isolates could be responsible for producing mycotoxins and shows the need for post-production tests. In general, there is a low prevalence of pathogenic bacteria, especially those of the genus *Escherichia* in palm wine, pito and burukutu due to their low acidity and high ethanol content. However, the prevalence of hygiene indicator bacteria was higher in nonalcoholic drinks, probably because of incorrect practices during processing. This review confirms that the magnitude of the production and sales of unregulated local beverages in Nigeria has reached the stage where significant regulation and food safety standards are required to safeguard public health. In its study, the authors concluded that it is necessary to monitor and characterize the microbiota of artisanal beverages using molecular methods at all stages of production and storage.

Commercial yeast inoculation is a widespread practice in winemaking since they can control the must fermentation and they are recognized as safe. However, it is well known that the use of indigenous wine yeasts can enrich wine quality and differentiate wine styles. Yeast cream preparation (CRY), recently accepted by the International Organization of Vine



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and Wine, could allow easier usage of autochthonous yeasts. Thus, Fracassetti et al. [2] investigated the actual Italian wine industry's attitude towards the available formulations of commercial wine yeasts with attention to CRY. They also evaluated the perception of wineries toward indigenous yeasts in both winemaking and marketing viewpoints. Results have shown different levels of knowledge and use of the available yeast formulations. In general, there is not a predominantly positive or negative opinion regarding the use of indigenous yeasts. Wineries using CRY (4% of the sampled wineries) mainly adopted them as a part of the production in order to compare the wines with the ones traditionally obtained with commercial yeasts. CRY is perceived by some interviewees as a potential tool to increase communication and product differentiation. This survey could anticipate future trends in the use of yeast formulations, determined by the market demands for diversified, unique, and environmentally sustainable products, that can allow an accessible application of a safe and precision enology.

However, can an indigenous yeast colonize a winery where a commercial yeast was used? Abdo et al. [3] performed a study to check the fungal colonization of a new winery over time, specifically for *Saccharomyces cerevisiae*. They analyzed the microbiota present before the arrival of the first harvest on the floor, the walls and the equipment of the new winery by Illumina MiSeq. The genus *Saccharomyces* was poorly detected on floor and equipment but the presence of *S. cerevisiae* species was not reported. Then, wild *S. cerevisiae* strains were isolated from a 'Pied de Cuve' used during the first vintage to ensure alcoholic fermentation (AF). Among the 25 isolates belonging to this species, 17 different strains were identified highlighting a great intraspecific diversity. *S. cerevisiae* strains were also isolated from different vats throughout the spontaneous fermentations during the first vintage. The following year, some of these strains were isolated again during AF. Four strains were found in the winery equipment before the arrival of the third harvest suggesting potential colonization by these strains. They concluded that *S. cerevisiae* has the ability to form a biofilm on solid surfaces confirming their possible capacity to colonize the winery.

So, what happens if these indigenous *S. cerevisiae* yeasts interact with other yeast species in a winery? *Lachancea thermotolerans* is a non-*Saccharomyces* yeast appreciated for its potential of acidification due to the production of lactic acid; however, this species also synthesizes other metabolites that positively modulate sensory wine properties. Blanco et al. [4] evaluated the enological potential of the strain *L. thermotolerans* Lt93 as monoculture to ferment 'Treixadura' and 'Mencia' musts and its impact on resident yeast population dynamics and wine characteristics. They not only used monocultures of *L. thermotolerans* Lt93 and *S. cerevisiae* strains, but also applied sequential inoculation and spontaneous fermentations. After a microbiological analysis of the native yeast population and chemically studying the wine composition, their results showed that *L. thermotolerans* Lt93 was unable to overgrow wild yeast population in 'Treixadura' white must. However, in the wines produced with 'Mencia' red must, *L. thermotolerans* Lt93 was the predominant yeast at the beginning of fermentation and remained at high frequency until the end. Moreover, this non-*Saccharomyces* yeast strain was able to modulate the sensory characteristics of red wines made with the 'Mencia' grape variety.

Other grape varieties were also analyzed taking into account their aromatic characteristics. Tsiakkas et al. [5] evaluated the anthocyanins and volatile compounds of two monovarietal wines from indigenous varieties, 'Yiannoudi' and 'Maratheftiko', grown in the island of Cyprus from the vintages 2014 to 2016. The experimental analysis comprised the determination of anthocyanin's profile and the fermentation derived volatiles and a sensory evaluation. Both the analytical results and the blind wine tasting showed that wines, at their early stage, were easily differentiated by variety, especially in terms of anthocyanins composition, while, in aged wines, the differences among samples were influenced in time by the winemaking procedures and it was not possible to differentiate varieties in such conditions.

Taking into account the alcoholic beverages with low alcohol content (2–4%) and a high pH safety, what would happen if these beverages were contaminated with *L. monocytogenes*?

Paramithiotis et al. [6] assessed the transcriptomic response of *L. monocytogenes* during co-culture with three *S. cerevisiae* strains. Their results highlighted that the transcriptomic response of *L. monocytogenes* key virulence genes was in the majority of the cases dependent on the yeast strain. In addition, this work demonstrates the complex trophic relationships that take place during co-existence between *L. monocytogenes* and *S. cerevisiae* according to the temperature applied to the bioprocess.

Though fermented beverages production practices may vary, it is fairly common for the finished product to be pasteurized in its final package to preserve product quality and product safety. The vast majority of fermented beverages is in practice similar to winemaking, but beer, cider and other similar beverages typically contain much lower ethanol levels (around 5–7%) compared to wine (11–15%). It is therefore more prone to microbial spoilage such as the mentioned *L. monocytogenes*. Thus, Valliere and Harkins [7] used in-package water bath heat pasteurization for hard cider production which is commonly employed to improve product safety and stability, and they affirmed that there is a considerable lack of research-based guidelines to inform industry practices. Because of this, they performed an experiment where fermented cider was bottled and inoculated with high populations of *Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii* yeast strains. Then, bottles were subjected to water bath pasteurization 60 °C at varying lengths of time. For both yeast species, populations were reduced to undetectable levels after just 1 min of processing time. They recommended that cider producers may be able to sufficiently reduce the risks of spoilage organisms with minimal water bath pasteurization, especially when combined with other methods to reduce the presence of spoilage organisms.

As the above studies demonstrated, food safety draws from a wide range of academic fields, including chemistry, microbiology and engineering. These diverse areas of thought converge to ensure that beverages processing safety is carried out wherever beverages products are sourced, manufactured, prepared, stored, or sold. In this sense, food and fermented beverage safety is a systemic approach to hygiene and accountability that concerns every aspect of the global food and beverages industry.

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