Book Chapter

Microbial Dynamics in Sour–Sweet Wine Vinegar: Impacts on Chemical and Sensory Quality

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Published **October 05, 2023**

This Book Chapter is a republication of an article published by Alice Vilela at Applied Sciences in June 2023. (Vilela, A. Microbial Dynamics in Sour–Sweet Wine Vinegar: Impacts on Chemical and Sensory Composition. Appl. Sci. 2023, 13, 7366. https://doi.org/10.3390/app13137366)

How to cite this book chapter: Alice Vilela. Microbial Dynamics in Sour–Sweet Wine Vinegar: Impacts on Chemical and Sensory Quality. In: Prime Archives in Applied Sciences: 2nd Edition. Hyderabad, India: Vide Leaf. 2023.

Funding: This study was funded by the project e-Flavor [grant number POCI-01-0247-FEDER-049337], financed by the European Regional Development Fund (ERDF), through the

Competitiveness and Internationalization Operational Program (COMPETE2020), and by the National Funds FCT Portuguese Foundation for Science and Technology, Portugal, and COMPETE, under the projects CQ-VR [grant number UIDB/00616/2020 and UIDP/00616/2020].

Acknowledgments: The author would like to thank the CQ-VR—Chemistry Research Centre—Vila Real and the e-Flavor project for their financial support.

Conflicts of Interest: The author declares no conflict of interest.

Abstract

The most known and traditional vinegar is the one that is made from wine. For its production, the grape must undergo alcohol fermentation and the posterior oxidation of ethanol to acetic acid. Yeasts and acetic acid bacteria (AAB) sequence the biochemical processes. The process of wine acetification can be achieved by slow traditional techniques (the Orléans or French methods) or by a quick submerged industrial process. High-quality vinegar is usually produced by conventional methods using oak casks once the wood allows the continuous aeration of the acetic bacteria culture. Sour–sweet vinegar balances both bitter/sour and sweet flavors. The sourness typically comes from acetic acid, while the sweetness can come from the type of fruit or the amount of sugar present at the end of vinegar production. In general, sour-sweet vinegar has a more complex and nuanced flavor profile than regular vinegar, which is often simply sour. One kind of vinegar produced by wine acetification, where yeasts and bacteria co-exist and make savory vinegar, is traditional balsamic vinegar (TBV) from Italy. In Spain, fortified sherry wine has also been used for vinegar production. In Portugal, some wine companies have produced port wine vinegar since 2018. These three distinctive vinegar products have in common the sweetness that can be found, well balanced with the acetic acid sourness. This review analyzes the sour-sweat wine vinegar process, considering the possible interactions between yeasts and AAB during vinegar production and the symbiotic or competitive features of a diverse microbiota.

Keywords

Traditional Balsamic Vinegar; Orléans Method; Sherry Vinegar; Port Wine Vinegar; Yeasts; Acetic Acid Bacteria; Symbioses

Introduction

Vinegar production dates back to at least 200 BC and is an example of microbial biotransformation. Vinegar can be made from any fermentable sugary substrate and is a globally famous acetic acid condiment produced with dual fermentation (alcoholic and acetic). It possesses a pleasantly sour flavor and has been used as a diet condiment, food preservative, and remedy for people and animals [1].

The most known and traditional vinegar is the one that is made from wine. In the old days, wine vinegar production was considered a chemical process. In 1732, the Dutchman Boerhaave specified that the "mother of vinegar" was a living organism [1]. However, it was only in 1864 that Pasteur claimed that the conversion of wine into vinegar was due to the development of the veil of *Mycoderma aceti* on the wine's surface [1].

The methods for producing wine vinegar involve grape must, which goes through alcohol fermentation followed by ethanol oxidation to acetic acid [2]. These biochemical processes are carried out in sequence by yeasts and acetic acid bacteria (AAB), which produce acetic acid and several metabolic compounds responsible for the taste and flavor of vinegar, such as nonvolatile compounds (sugars, organic acids, amino acids) and volatile compounds (esters, aldehydes, among others) [3].

Wine vinegar can be differentiated by its production systems and technologies; two techniques are used to produce vinegar: the slower traditional processes and the quick submerged (industrial purpose) methods. In traditional vinegar, produced by the so-called Orléans or French method, the oxidation of ethanol into acetic acid occurs due to a static culture of AAB at the interface between the liquid and air. Usually, oak casks produce this kind of vinegar once the wood allows for continuous aeration of the

acetic bacteria culture. This is the case with vinegar such as Jerez/Xérès/sherry, where the oak used is *Quercus alba* (American oak), and it takes a minimum of six months in wood for the vinegar to be considered a sherry vinegar [4]. The wood barrels are filled to 2/3 capacity, have holes on the sides for air passage, and a funnel with an extension to the base of the barrel, allowing the wine to be added at the bottom and preventing the alteration of the biofilm formed by the AAB on the surface [5].

In the 18th–19th century, Schüzenbach (1793–1869) developed a dynamic acetification system called the "German rapid acetification system." This acetification process was industrialized and was named "Rundpump" (round pump) because the wine passed through wood shavings continuously sprinkled with the mash by a circulation pump and a rotating sprinkler. The wood shavings served as a support for the bacteria. Alternative materials such as bines, maize cobs, or rushes are cheaper than beechwood but less efficient [6,7].

Technological advances allowed the development of the submerged fermentation process, first used to produce antibiotics and vinegar, thanks to computerization. The Frings Acetator is an example of this advance. Annual production is half a million liters of vinegar at 10% acidity [6,7].

The submerged culture technique involves growing AAB in a liquid medium containing ethanol or other suitable carbon sources. This method is often used in laboratory-scale studies and industrial settings for large-scale acetic acid production. In submerged culture, the liquid medium is typically agitated to enhance oxygen transfer, which provides fast and efficient aeration [8]. According to Schlepütz and co-workers [9], in this system, a non-rotating stator supports a hollow-body turbine. Radial holes open in the opposite direction of rotation, allowing air from outside to be released inside, resulting in very fine air bubbles. A homogenous air–liquid emulsion is formed, pushed upwards, and diverted by deflectors. All of the mass is maintained in a continuous state of agitation, allowing an easy oxygen transfer from the medium to the bacteria. This prevents *Acetobacter* cell death and promotes efficient microbial growth [10].

Hence, the necessities for submerged processes are the availability of ethanol, continuous aeration, and acetic acid bacteria strains tolerant to high concentrations of acetic acid and ethanol that require small amounts of nutrients and are not sensitive to phage infections [10]. This last requirement is vital once phage infections impact AAB growth and, consequently, acetic acid production. Phages, also known as bacteriophages, are viruses that infect and replicate within bacterial cells. They specifically target and infect bacteria, including AAB, resulting in the lysis (bursting) of cells, leading to a decline in population, a disruption in their metabolic activity, and a decrease in acetic acid production [11,12]. Phages that infect AAB have been isolated from various sources, including vinegar production facilities, fruit juice processing plants, and natural environments. These phages can significantly impact industrial vinegar production, leading to decreased productivity, spoiled batches, and economic losses [11].

One major disadvantage of the submerged method is the heat generated and the lack of temperature control. Acetic acid fermentation is an exothermic process that releases heat as a byproduct. Heat accumulation can be an issue in submerged systems, leading to increased temperature, which may negatively affect bacterial growth and acetic acid yield. Proper temperature control becomes crucial to maintaining optimal conditions [10].

Regarding organoleptic drawbacks, the liquid creates foam in submerged fermentations due to stirred conditions, leading to a reducing environment that compromises the acetification process [10]. Also, as acetic acid production progresses, the fermentation medium can become more viscous due to acetic acid and bacterial biomass accumulation. The increased viscosity can hinder efficient mixing and oxygen transfer, further impacting fermentation [13].

Contrasting submerged methods, the vinegar produced by traditional methods (Orléans or French method) is generally considered to be of high quality, both chemically and sensorially, due to the balance between organic acids and amino acids achieved through long-term surface culture fermentation [2].

However, in the traditional method, there is no microbial control, which delays the alcoholic fermentation (AF) and the acetic acid fermentation (AAF), causing contamination and low acidity. Therefore, it is essential to understand the interactions between acetic acid bacteria and yeasts to promote strategies that can allow a faster and higher acidity yield fermentation while maintaining quality, regardless of the method used.

One type of vinegar produced by wine acetification, where yeasts and bacteria co-exist and make savory vinegar, is traditional balsamic vinegar (TBV), which originated in Modena and Reggio Emilia, Italy. In Spain, fortified sherry has also been used for vinegar production with its distinctive nutty and oxidized flavor [14]. In Portugal, sour-sweet wine vinegar has recently emerged. Port wine vinegar has been produced by some wine companies since 2018. It has been described as having "a robust and naturally fruity flavor accented by a slight hint of acidity and a deep shade of ruby red."

Thus, in this review, we analyze the balsamic acetification system and the possible interactions between acetic acid bacteria and yeasts that occur during vinegar production, considering the positive features of a diverse microbiota, such as glucose and ethanol tolerance, alcohol and aldehyde dehydrogenase formation, thermotolerance, acetate and ethanol oxidation, the capability to grow in different acetic acid concentrations, and aroma compound production [15].

The acetification process of port wine and sherry wine vinegar will also be discussed since these types of wine, due to their chemical characteristics, pose some challenges to acetic acid bacteria regarding ethanol tolerance.

Traditional Balsamic Vinegar (TBV) Processing

The fabrication of TBV involves a three-stage process: cooking of must, microbiological transformations (two fermentations, alcoholic and acetic), and aging (Figure 1).

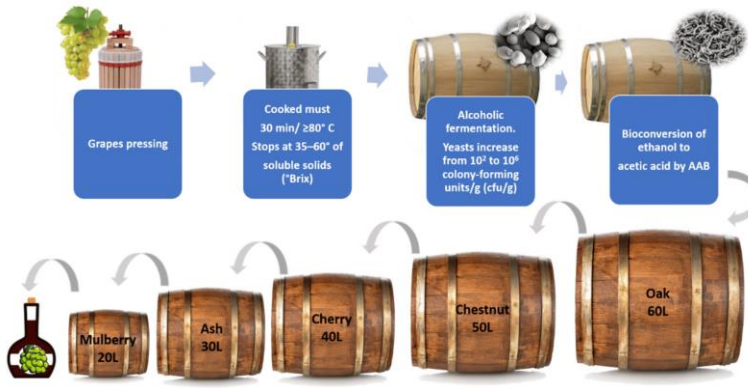

Figure 1: Schematic representation of TBV processing. The cooking of must and microbiological transformations are the first steps of the process. The aging involves using a set of 5/7 wood barrels made from different wood species, arranged in a series of decreasing volumes. A specific volume of the final product is removed from the smallest barrel. AAB—acetic acid bacteria.

Cooking of the Must

Grape must is obtained from grapes such as Ancellotta, Berzemino, Lambrusco, Occhio di Gatta, Sauvignon, Sgavetta, and Trebbiano, all Modena and Emilia Romano local grape-cultivars [16]. After crashing, it is boiled in uncovered vessels, which allows the removal of impurities and coagulated proteins by floating. Afterward, the temperature is kept below 85–90 °C. The cooking process stops when the must reach 35–60° Brix. Ultimately, the cooked must will present pigments formed by non-enzymatic browning and 5-hydroxymethyl-2-furaldehyde (5-HMF) due to hexose degradation by dehydration and cyclization [16,17]. The cooking of the must produces a highly concentrated and dark liquid that is very sweet to the palate.

Microbiological Transformations

The microbiological cooking process must occur in two steps: first, a natural alcoholic fermentation of sugars, followed by ethanol oxidation to acetic acid (the second step). In the first step, yeasts metabolize the sugars from the grapes into ethanol, carbon dioxide, and many secondary by-products. The cooked and sterile must is transferred into open wooden vessels already yeast-

contaminated. Over a few weeks, alcoholic fermentation occurs due to the rapid increase in yeast population (10^2 to 10^6 CFU/g) [18]. To perform this fermentation, no dried yeast is used (Figure 1). The bioconversion of ethanol to acetic acid by AAB is the following microbiological process (second step). Acetic acid bacteria occur naturally, making a thin coating or biofilm on the surface of fermented cooked must. Due to the presence of ethanol (9–10%, *v*/*v*), biofilm formation may be slow. To accelerate the process, bacterial biofilm can be transferred manually from one vessel to another in active oxidation [19] (Figure 2).

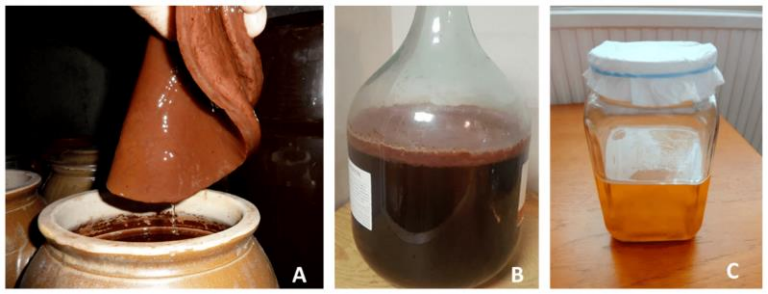

Figure 2: (**A**) The mother of vinegar is removed from a wine vinegar pot. (**B**) Bacterial layer growing in red wine vinegar. (**C**) Mother of vinegar formation in homemade vinegar.

Ageing Process

At least five casks (wooden barrels) are needed for the aging process. They are casks of different sizes and woods, as seen in Figure 1, arranged in decreasing scalar volumes. A small volume of aged vinegar (the smallest barrel) is spilled every year. This barrel is then refilled with the contents of the previous barrel. This procedure is repeated to the biggest cask, which receives newly oxidized and fermented cooked must [20]. The operation is called “rincalzo” (refilling), and it must be repeated for at least 12 years for the vinegar to be defined as a traditional DOP.

The sugar concentration increases along the barrel set due to the evaporation through the opening on the top of the barrel and the wood. In this procedure, every barrel will contain a blend of different vinegar ages; naturally, the age increases from the bigger

barrel to the smaller one. However, it is not easy to define the age of balsamic vinegar. In 2007, Giudici and Rinaldi [21] developed a theoretical model that allows predicting the age of traditional balsamic vinegar, considering the volume of vinegar transferred from barrel to barrel and the amount of newly cooked-must to be added, providing a way to define the age of traditional balsamic vinegar and verify the requirements of the DOP legislation. The referred authors proved that the age of traditional vinegar correlates to the amount of vinegar withdrawn and/or the amount of cooked must added. With their mathematical model, any producer could validate the age of their vinegar, letting the consumer buy it at the stated age [21].

At the end of the process, it is possible to obtain a dark-sugary/acid liquid with more or less 40% (*w*/*v*) of sugars (glucose and fructose in a 1:1 ratio) and with a pH of 2.3–3.2 and 2–5% (*w*/*v*) in acetic acid [22].

Traditional Balsamic Vinegar Yeasts and Alcoholic Fermentation

Zygosaccharomyces Strains

The genus *Zygosaccharomyces* was first described in 1901 by Barker. Long after, in 2011, in the fifth edition of *The Yeasts, a Taxonomic Study* [23], James and Stratford described six *Zygosaccharomyces* species within the genus, namely, *Z. bailii*, *Z. bisporus*, *Z. kombuchaensis*, *Z. lentus*, *Z. mellis*, and *Z. rouxii*. Since then, novel species have been proposed, and *Z. osmophilus* and *Z. seidelii* are most recently studied by Matos and co-workers [24] and Brysch-Herzberg et al. [25], respectively.

Up until the study of Solieri et al. [18], only the spoilage species *Zygosaccharomces bailii*, *Z. rouxii*, and *Saccharomycodes ludwigii* were associated with balsamic vinegar samples (Table 1). Turtura [26] were the ones that first identified, based on morpho-physiological reports, the presence of *Z. rouxii* and *Z. bailii*, such as their ability to grow at 1% acetic acid concentration. Afterward, Giudici [27] discovered the occurrence of *Saccharomycodes ludwigii* strains, together with *Z. bailii* and *Z. rouxii*. This author also proposed the model of a two-stage fermentation: first, an

alcoholic fermentation, where yeasts convert sugars to ethanol, followed by ethanol oxidation to acetic acid by AAB, the biochemical process usually called acetic fermentation.

In 2006, Solieri and collaborators [18] found that many different indigenous yeast species could be found and recovered from the traditional balsamic vinegar initial processing stage, that is, in cooked must, leading to the conclusion that the population of the yeasts is more complex than what was reported in the literature. In fact, besides *Z. bailii*, found in 41% of the samples, *Z. rouxii*, *Z. mellis*, *Z. bisporus*, and *Z. pseudorouxii* were also found. Strains of *S. cerevisiae*, *Candida* (*stellate* and *lactis-condensi*), and *Hanseniaspora* (*valbyensis* and *osmophila*) were also detected [16,17]. However, it is worth noting the prevalence of *Zygosaccharomyces* strains.

Z. bailii is a yeast species broadly present in several food fermentation processes, such as wine, vinegar, and tea [28,29]. In many cases, *Z. bailii* may be considered a contaminant yeast due to its high resistance to preservers, such as SO_2 and sorbate, and increased tolerance to various stresses (alcohol concentration, low pH, low water activity, and presence of toxic weak acids, such as lactic, ascorbic, and acetic acid) [30,31]. Notwithstanding their association with spoilage, some food systems have also studied their potential benefits [32,33]. In wine fermentation, *Z. bailii* in a mixed culture starter with *S. cerevisiae* enhanced the production of ethyl esters [28]. In Kombucha and Haipao tea fermentations, *Z. bailii*, in symbiosis with other microorganisms, helps obtain beverages rich in fiber, lysine, and crude protein [34].

As mentioned before, besides Z. bailii strains, Z. rouxii strains have also been described in traditional balsamic vinegar processes. In contrast to *Z. bailii*, *Z. rouxii* can grow at salt concentrations up to 16% (*w*/*v)* and glucose concentrations up to 75% (*w*/*v*) [29,35]. Moreover, Hong et al. [36] demonstrated that *Z. rouxii* cells, under glucose stress, decrease GCY1 (glycerol 2-dehydrogenase ($NADP^+$) GCY1) gene expression to retain glycerol. This high sugar tolerance may explain how this yeast can be found in the cooked must of traditional balsamic vinegar fermentation. 2007, Solieri and co-workers [37] isolated two

strains, coded ABT301 and ABT601, from traditional balsamic vinegar. After a multi-gene sequence approach, cloning, and sequence analysis of 5.8S-ITS rDNA, they found that their genomic background differed from the other *Zygosaccharomyces* species and could be related to *Z. rouxii*. Later, in 2013, after performing physiological and morphological tests, ABT301 was renamed *Zygosaccharomyces sapae* sp. nov. This strain was described as maltose-fermentation-negative, non-psychrotolerant, osmotolerant, and halotolerant [38].

Non-*Zygosaccharomyces* Strains

Interestingly, most yeasts coexisting in balsamic vinegar are fructophilic, a behavior contrary to that of *Saccharomyces cerevisiae* [39]. This fructophilic behavior depends on the pattern of fructose and glucose transporters, which prioritize fructose [40]. Other less osmotolerant species, such as *Saccharomycodes ludwigii*, formerly referred to by Solieri et al. [18] and Solieri and Giudici [16], seem to be similar to *S. cerevisiae* and discreetly choose glucose over fructose.

S. ludwigii is known for spoiling fruit juices, wines, and ciders [41]. This yeast species also presents a high tolerance to SO_2 [42] and resistance to pressurized CO_2, showing the aptitude to deteriorate carbonated beverages [43]. It has also been isolated from sweet wines [44] and balsamic vinegar [27], thus showing its tolerance to high sugar levels. *S. ludwigii* (strain APRE2) showed the ability to grow at temperatures of up to 43 °C [39,45]. Moreover, this yeast is described as a high producer of secondary metabolites such as isobutyl alcohol, acetoin, ethyl acetate, and acetaldehyde [42,46]. The high production of isobutanol [46] and acetaldehyde [42] can be considered discriminant characteristics of *S. ludwigii*, although this metabolic feature seems to depend on the strain. Some strains also produce considerable amounts of glycerol (up to 11.7 g/L) and succinic acid (up to 1.4 g/L) [47], giving the vinegar interesting sensory properties (Table 1).

In some vinegar fermentations, the oxidation of ethanol to acetic acid is an exothermic reaction that triggers a temperature increase as high as 50 °C [45,48]. Thus, although acetification does not

increase the temperature much for the balsamic vinegar process, natural, thermo-resistant yeast strains allow for better fermentative performance, thermo-tolerant strains can increase the length of the cytoplasmic membrane lipid layer of acyl chains, saturation, branching, and/or cyclization of the fatty acids, stabilizing the native configuration of the enzymes [48]. Moreover, they improved the use of Na^+ ions instead of protons as coupling ions in energy transduction. Due to the high H^+ permeability, predominantly at high temperatures, a Na^+ motive force is a more energetic advantage than a proton motive force [49].

S. ludwigii also exhibits a significant multi-stress tolerance toward ethanol, acetic acid, furfural, and 5-hydroxymethyl furfural (5-HMF) when using lignocellulosic biomass as feedstock for ethanol production at high temperatures [45]. These features are also interesting for vinegar production: tolerance toward 5-HMF. In the first stage of balsamic vinegar production, the cooked must is obtained after heating the grape juice in an open container over a direct flame. This typical process leads to glucose and fructose oxidation via non-enzymatic ways to different chemical mixtures [50]. In acidic media, furfural derivatives, 5-HMF, are produced [51], and this compound is proven toxic to biological species, including humans.

Indeed, Cocchi and co-workers [52] proposed a grape must-heating strategy after ten h of heating (after the moisture level falls to 70%), consisting of using controlled conditions to prevent the reactions that lead to furfural formation, which are enhanced in acidic media, high sugar concentrations, and high temperatures [51,53].

Another example of *S. ludwigii's* resilience and resistance to acetic acid and sugar is the study by Saeki [54]. *S. ludwigii* cells entrapped in calcium alginate beads, together with immobilized *Acetobacter aceti* cells, were able to perform the continuous production of vinegar using a saccharified rice medium as feedstock, containing 81 g/L of glucose and 11 g/L of acetic acid. The vinegar produced 4.7% to 5.3% acetic acid [54].

Candida spp. is a beverage- and food-associated yeast. *C. stellata* has been isolated in must from botrytized grapes, wines from overripe grapes, and cooked musts associated with TBV [18,55].

C. stellata presents a strong fructophilic character [56,57], presenting a considerably lower fermentation rate for glucose than fructose. Gonçalves et al. [58] detected the presence of the Ffz1 transporter as a requirement for fructophily in *S. bombicola* (the anamorph of *C. bombicola* and the synonym of *Torulaspora bombicola* and *C. bombicola* [59]. Ffz1 is a definite fructose carrier codified by the FFZ1 gene [60].

Besides fermenting fructose, *C. stellata* yeast species are characterized by low fermentation rates and high production of secondary metabolites, such as acetaldehyde, acetoin, glycerol, and succinic acid [61] (Table 1). Regarding glycerol formation, the low alcohol dehydrogenase activity and high glycerol-3-phosphate dehydrogenase activity cause a substantial deviation of the metabolic pathways toward glycerol production [61].

C. stellata yeast species are also attractive due to their ability to produce a variety of enzymes in wine, namely, polygalacturonase and pectin methyl esterases [62], proteases [63], cellulases and hemicellulases [63,64], and β-glucosidases [65], giving the wine flowery and fruity aromas as documented by Cordero-Bueso et al. [66] (Table 1). All the above features make *C. stellate* an interesting yeast that promotes pleasant aromas in traditional balsamic vinegar.

Another Candida species, also found in balsamic vinegar, is *Candida lactis-condensi*. This yeast has been isolated from high-sugar wines (Tokaj Essence wines with a sugar concentration ranging from 365 to 752 g/L) [67]. *C. lactis-condensi* and *C. zemplinina* were recently transferred to the *Starmerella* genus, which anchorages yeast species frequently occurring on flowers and flower-visiting insects [67,68].

Hanseniaspora valbyensis is an uncommon yeast frequently found in TBV [16]. This yeast can also survive and increase in a demanding environment with high acid and sugar content and is

rich in pectins [16]. *H. valbyensis* presents substantial pectinolytic activity [69], justifying its prevalence in TBV. This yeast produces low levels of ethanol yet forms significant amounts of acetate esters, such as ethyl and phenyl-ethyl acetate [70], giving the vinegar fascinating glue- and rose-like aromas (Table 1).

In wine fermentation, *H. osmophila* presents a glucophilic nature and may harm both the development and fermentation rate of *Saccharomyces* populations [71]. Generally, *H. osmophila* produces high levels of ethanol and ethyl acetate [71].

Selective fermentation of glucose is a critical characteristic of a yeast strain for TBV production. This is an attractive feature, considering that the most osmotolerant species found in the TBV process show, in general, a fructophilic metabolism, resulting in glucose crystallization in the vinegar [16]. Saccharomyces strains are known to be glucophilic, so this yeast strain is also crucial for TBV production [72].

Table 1: Main *Zygosaccharomyces* and non-*Zygosaccharomyces* strains found in traditional balsamic vinegar fermentations, metabolic features, and sensory properties.

Microorganisms	General Metabolic Features	Sensory Properties	Ref.
Zygosaccharomyces species			
Z. bailli, *Z. rouxii* *Z. mellis* *Z. bisporus* *Z. pseudorouxii* *Z. sapae* *Z. lentus* *Z. osmophilus* *Z. seidelii*	High resistance to SO_2 and sorbate; High tolerance to alcohol, low pH, low water activity, and presence of toxic weak acids, such as lactic, ascorbic, and acetic acids; Fructophilic character (possesses FFZ genes that encode specific	Redberry aroma, due to the production of linear esters; Blackberry aroma due to the production of branched esters; Pungent aroma due to higher alcohol content; Rose-like aroma due to the biosynthesis of 2-phenyl ethanol via the Shikimate or Ehrlich	[**18**,**26**,**27**,**28**,**73**,**74**,**75**]

	fructose facilitators); Halotolerant, osmotolerant, and non-psychrotolerant.	pathways (mainly *Z. rouxii*)	
Non-*Zygosaccharomyces* species			
Saccharomycodes ludwigii	Tolerance to sulfur dioxide (SO_2) and pressurized carbon dioxide (CO_2); Can produce up to 12% (*v*/*v*) of ethanol and <1.0 g/L acetic acid; Resistance to acetic acid and osmotolerant.	Sweet, musty odor (isobutyl alcohol), buttery odor (acetoin), fruity, glue-like aroma (ethyl acetate), and an oxidative aroma (acetaldehyde); Acid/salty taste due to succinic acid and some softness induced by glycerol.	[**40**,**41**,**42**,**43**,**44**,**45**,**46**,**47**,**70**]
Candida stellate *C. bombicola* *C. lactis-condensi*	Fructophilic character presenting the Ffz1 transporter, codified by the FFZ1 gene; Low fermentation rates and high production of secondary metabolites; Its growth is variable at 37 °C but is sensitive to heat, while it can grow at lower temperatures and higher pH values; Low sensitivity to ethanol.	Buttery odor (acetoin), an oxidative aroma (acetaldehyde), and a softness in the mouth (glycerol); Acid/salty taste due to succinic acid; Flowery and fruity aromas due to β-glucosidases activity.	[**15**,**56**,**57**,**58**,**61**,**65**,**66**]
Hanseniaspora valbyensis *H. osmophila*	Significant pectinolytic activity;	Gluey and rose-like aromas (acetate esters	[**69**,**70**,**71**]

	Production of high levels of ethanol and ethyl acetate; Glucophilic nature.	such as ethyl and phenyl-ethyl acetate).	
S. cerevisiae	Tolerance to sulfur dioxide (SO_2); Glucophilic nature (avoids glucose crystallization in the final vinegar).	Esters, alcohols, and aldehydes (depending on the strain).	[72]

Traditional Balsamic Vinegar Acetic Acid Bacteria (AAB) and Acetic Fermentation

Acetic acid bacteria (AAB) are a cluster of Gram-negative, obligately aerobic bacteria belonging to the family *Acetobacteraceae* [76] essential in producing fermented foods such as vinegar. They are rod-shaped, facultative anaerobes with a size of approximately 0.5–1.5 µm in width and 1–5 µm in length. They are also catalase- and oxidase-positive, which means they produce the enzymes that break down hydrogen peroxide and can use oxygen as a terminal electron acceptor in respiration. They can fix nitrogen and use nitrogen gas as a source of nitrogen for growth [77].

AAB grows optimally at a pH range of 4.0–6.0 and 25–30 °C temperature range. They require a source of ethanol or other alcohol for growth and energy production. The metabolism of AAB is highly aerobic, and they need oxygen for the oxidation of ethanol to acetic acid. This oxidation reaction involves acetaldehyde production as an intermediate [78].

Many AAB strains can partly oxidize carbohydrates, alcohols, and related compounds to yield industrial products such as acetic acid. The main metabolic pathway of AAB is the oxidative fermentation of ethanol into acetic acid. This pathway involves the following steps [78]: (i) Ethanol is oxidized to acetaldehyde

by the enzyme alcohol dehydrogenase. (ii) Acetaldehyde is then oxidized to acetic acid by the enzyme acetaldehyde dehydrogenase. This reaction produces NADH, which the electron transport chain uses to produce ATP. (iii) The electron transport chain in AAB involves several electron carriers, including quinones and cytochromes. The final electron acceptor in the chain is oxygen, which is reduced to water. These partial oxidation processes of AAB are named AAB oxidative fermentation (AOF) [79].

The PPP (Pentose Phosphate Pathway) and ED (Entner–Doudoroff) pathway provide the necessary energy and reducing power for the AOF process. The PPP generates NADPH, which is used for the biosynthesis of cellular components and reducing acetaldehyde to ethanol. The ED pathway generates ATP and NADPH, which are used for cellular metabolism and reducing acetaldehyde to ethanol [79,80]. The AOF process depends on various factors, such as the type and concentration of AAB present, pH, temperature, and oxygen availability. AABs are highly adaptable and thrive in multiple environmental conditions [20].

The production of acetic acid results in a decrease in the extracellular pH, which can inhibit the growth of AAB. AAB produces and excretes large amounts of gluconic acid to maintain pH homeostasis, which can act as a buffer [78]. In addition to ethanol's oxidative fermentation, many AAB species also produce other metabolic products, such as dihydroxyacetone, acetoin, and 2,3-butanediol [78]. After ethanol exhaustion, acetate accumulates in the cell (cytosol) and can be utilized via acetyl-CoA synthase and phosphoenolpyruvate carboxylase [81]. AAB, namely *Gluconobacter oxydans,* can also metabolize glucose by an oxidative pathway, generating many metabolites such as glucono-δ-lactone by glucose dehydrogenase (GDH). Glucono-δ-lactone is then hydrolyzed into D-gluconate and, depending on the pH of the medium, D-gluconate is oxidized to 2-ketogluconate and 2,5-diketogluconate. Gluconic acid is the main oxidation product of *Acetobacter* and *Gluconobacter* strains and indicates TBV authenticity [81,82].

The acetic acid bacteria involved in traditional balsamic vinegar (TBV) production are mainly of the genus *Acetobacter*, including *Acetobacter aceti*, *Acetobacter pasteurianus*, and *Gluconacetobacter europaeus* [20]. These bacteria are naturally present in the environment and on the surface of grapes, and they play a crucial role in the production of TBV. During vinegar production, the acetic acid bacteria consume the ethanol produced during the initial fermentation process and convert it into acetic acid, which gives TBV its characteristic sour taste and aroma. This acetic fermentation process occurs in wooden barrels, providing a unique environment for the acetic acid bacteria to thrive [20].

Studies have shown that the composition of the acetic acid bacteria community in vinegar can vary depending on factors such as the type of grape used, the production methods, and the location of the production site [83]. However, certain species of Acetobacter have been identified as a critical factor in determining the quality of TBV [19]. Solieri et al. [38] analyzed the microbial communities present during the production of TBV using a combination of culture-independent and culture-dependent techniques. They found that *Acetobacter* was the dominant genus of bacteria present and that particular species, such as *A. aceti* and *A. pasteurianus*, were consistently associated with high-quality TBV.

Apart from acetic acid, AAB produces a range of volatile organic compounds (VOCs) that contribute to the aroma of vinegar: (i) Acetaldehyde, a key intermediate in converting ethanol to acetic acid by AAB. It is a potent aroma compound contributing to vinegar's characteristic pungent and fruity aroma. Acetaldehyde is produced in high concentrations during the initial stages of vinegar fermentation and then gradually decreases as acetic acid production increases [84]. (ii) Acetoin, a neutral aroma compound that contributes to vinegar's buttery and creamy notes. AAB produces it as a byproduct of acetic acid production and is present in relatively low concentrations in vinegar [85]. Diacetyl is another neutral aroma compound that contributes to vinegar's buttery and creamy notes. AAB produces it as a by-product of acetic acid production and is present in relatively low

concentrations in vinegar [85]. Ethyl acetate is also produced, presenting a fruity/glue aroma compound. AAB produces it as a byproduct of acetic acid production and is present in relatively high concentrations in vinegar [84].

Fortified Wines and Fortified Vinegar

Fortified wines usually present a high alcohol content (15–22%, *v*/*v*), resulting from the addition of distilled spirits and an oxidative character once they are produced under oxidative conditions. These wines can be produced using fermented and unfermented grape must. According to Council Regulation (EC) No. 491/2009 of 25 May, port and sherry are the most common styles of fortified wines. All of them can be used to produce sour-sweet vinegar that maintains some of the original wine's characteristics.

Port wine is produced in the Douro Demarcated Region (DDR), Portugal, namely in Douro sub-regions "Baixo Corgo," "Cima Corgo," and "Douro Superior." Among the many authorized grape cultivars of DDR, Mourisco Tinto, Tinta Amarela, Tinta Barroca, Tinta Francisca, Tinta Roriz, Tinto Cão, Touriga Nacional, and Touriga Franca are most commonly used to produce port wine [86]. Considering the wine-making process, two significant groups of wines can be differentiated: Bottle-aged and wood-aged ports. During the fermentation of the grapes, once the concentration of remaining sugars reaches the desired degree of sweetness, the wines are fortified by adding grape spirit (77.0 ± 0.5%, *v*/*v* ethanol at 20 °C) to the fermenting must. This is an essential step in port production. The average ratio of grape spirit added is 115 L:435 L (grape spirit and must, respectively) [86]. Due to ethanol toxicity, fortification stops yeast metabolism. Grape spirit also favors polyphenol solubilization and triggers the precipitation of insoluble matter [87].

According to the aging method, oxidative or reductive wines can be designated as "ruby" or "tawny" wines. Ruby ages in large wooden vats for 3–5 years, followed by bottling. They are wines with a red color, a full-bodied structure, and fruity notes. Tawny wines age in smaller wooden vessels (about 500–600 L) and

usually show spicy, nutty, and woody notes and explicit brown and yellow colors [88].

Sherry wine is a fortified wine made from white grapes primarily grown in the Sherry Triangle in southwestern Spain. Grape harvesting typically occurs in late summer or early fall when the grapes are fully ripe. Pedro Ximenez (PX), Palomino, and Muscat of Alexandria are the most commonly used grape varieties for sherry production. The fermentation process of sherry wine involves using yeast species such as *S. cerevisiae beticus, cheresiensis, montuliensis*, and *Zygossacharomyces rouxii* [89], also known as "flor" yeast. These yeasts grow on the surface of the wine and produce a layer of foam and yeasts known as the "flor," which gives sherry its distinctive nutty and oxidized flavor [14]. The veil of "flor" comprises multicellular masses composed of fungi, bacteria, and yeasts, the later essential microorganisms [90]. This veil is primarily responsible for the sensory features of sherry wine and its oxidative metabolism; however, the functional utilization of oxygen and the separation effect exercised by the yeast veil help to prevent excessive wine oxidation. Acetaldehyde inhibits veil formation at concentrations above the yeast's tolerance threshold [90].

During fermentation, the wine is kept at a constant temperature of around 20–22 °C to promote the growth of the veil. After fermentation, sherry wine is fortified with distilled grape spirits, typically around 15–18% alcohol by volume. This fortification process helps stabilize the wine and prevent spoilage. The wine is then aged in oak barrels, undergoing a complex aging process lasting several years or even decades [14]. During the biological aging of sherry wines, microorganisms, such as LAB (*Leuconostoc*, *Pediococcus*, and *Lactobacillus*) and fungi (*Botrytis cinerea*), can cooperate and coexist with the "flor" yeast strains mentioned above [91].

To make fortified sherry vinegar, high-quality wine is selected and transferred to oak barrels. The barrels are partially filled to allow for the formation of the acetic acid bacteria film, and the quality of vinegar is heavily determined by the specific wooden casks used for its aging [92]. After acetic acid bacteria inoculation, the

wine is then allowed to acetify, and this process can take several months to a few years, depending on the desired flavor profile of the vinegar. Once the wine has been acetified, the resulting vinegar is aged in oak barrels for a minimum of six months to allow it to develop its unique flavor and aroma characteristics. The longer the vinegar is aged, its flavor becomes more complex and intense [92].

Port wine vinegar is often described as having a rich, sweet flavor with a hint of acidity. It is commonly used in salad dressings, marinades, and sauces and can also be used as a substitute for other types of vinegar in recipes. One difficulty in the acetification process is the control of oxygen levels. Oxygen can accelerate the acetification process, leading to an excessively acidic wine, or slow down the process, resulting in insufficient acidity. Using oxygen-permeable membranes and carefully controlling the wine's exposure to oxygen can help maintain optimal oxygen levels during the acetification process [93].

For port wine acetification, the major constraint may be the wine alcohol content (usually higher than 19%, *v*/*v*). According to Giudici et al. [22], AAB having a unique set of alcohol dehydrogenase enzymes not inhibited by ethanol may be the reason for acetic bacteria's resistance to alcohol content. However, a high ethanol concentration can inhibit acetic acid bacterial cell growth and limit acetification fermentation productivity [20,94]. According to the work of Tonello [95], the bacteria's acclimatization to the high-alcohol environment will allow for better acetification. Hence, the first stage in the acetification process must be to use wine with a lower alcohol degree and let the bacteria adapt to the fermentation conditions, namely alcohol content, temperature, aeration, and stirring. Another possibility is to dilute the wine with distilled water, lowering the alcohol content to around 6–7% or the appropriate ethanol content. This last hypothesis will also reduce all the pleasant flavor components of the wines and, thus, the final vinegar.

While port wine vinegar is a relatively recent product, sherry vinegar, or Jerez vinegar, has existed since the corresponding

wine. Spontaneous high volatile acidities may occur due to the traditional methods of production. If the "flor" layer is disrupted or the wine is not adequately maintained, it can lead to spontaneously high volatile acidities. The "spoiled" wines are separated in standalone cellars and aged following the traditional process employed for wines [96]. Consequently, the unplanned wine's acetification may result in high-quality vinegar marketed as Protected Denomination of Origin Jerez Vinegar. This enological product is sold in glass bottles of different volumes, but the official label of the DO Council is compulsory to guarantee its origin.

According to the exceptional regulations for sherry vinegar, wines can be acetified by the Orléans method or by the submerged process in stainless steel vessels. Traditionally, sherry vinegar is elaborated in oak casks, with the acetic acid bacteria inoculated on the surface of the wine once it needs oxygen to metabolize [96]. Long periods are required to obtain a satisfactory degree of acetic acidity. Barrels (500 L volume) are filled to two-thirds of their total volume, and acetification occurs during the aging of wine/vinegar. Once the vinegar is obtained, it remains in the oak barrels to gain the expected chemical and sensory characteristics (Figure 3).

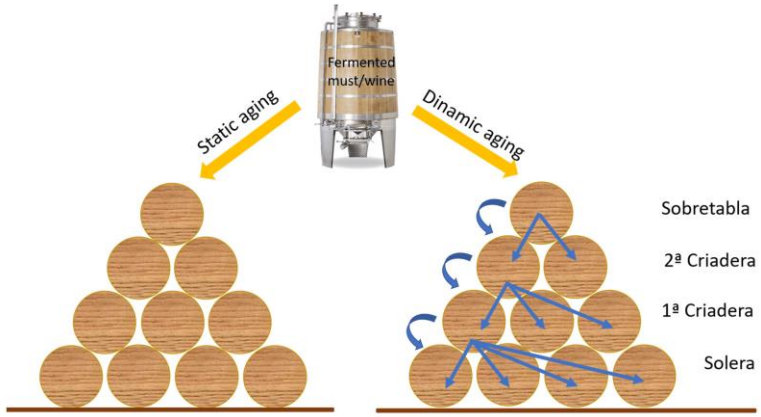

Figure 3: Dynamic and static aging of Jerez vinegar. Adapted from Tesfaye et al. [96].

Vinegar aging occurs similarly to the aging process used for sherry wine; it may be a dynamic process called the "criaderas and solera system" or a static method (Figure 3). The dynamic solera system consists of a sequence of casks arranged, varying from three to eight. The wine (substrate for the acetification) is introduced at the system's top, and the final product is withdrawn from the bottom. The vinegar is homogenized throughout the whole system. In the static method, the vinegar is aged in a single barrel [96]. The dynamic aging system allows an upgrade in oxygen and oxygen concentration transfer, similar to what occurs with the classical Orléans process [97].

During biological aging, a large amount of acetaldehyde is produced, along with acetic acid and glycerol consumption, with a concomitant ethanol metabolism that, due to the absence of glucose, is used by the yeasts as a carbon source. It also occurs in consuming all the amino acids, particularly proline [98]. The aging in oak barrels, exposure to air, and the growth of a complex community of microorganisms, including yeast and lactic acid bacteria, contribute to the vinegar's sensory properties. For example, it is well known that the characteristic aroma of sherry vinegar includes several volatile compounds besides acetaldehyde and acetic acid, such as diacetyl (butter aroma), isoamyl acetate (banana aroma), isovaleric acid (cheesy-like aroma), ethyl acetate (glue or fruit-like aroma), and sotolon (aroma of curry, cumin, honey, nuts (almonds, walnuts), caramel, maple syrup, molasses, and burnt sugar, depending on the concentration) [99]. Also, this kind of vinegar may have a significantly higher content of short- and medium-chain acids, especially isovaleric acid (cheesy-type odor) [100,101].

Wine Yeasts and Acetic Acid Bacteria Interactions

Biotic Interactions

In vinegar production, the interaction between wine yeasts, acetic acid bacteria, and even lactic acid bacteria—the so-called biotic factor—plays a crucial role in converting ethanol to acetic acid. Wine yeasts, such as S. cerevisiae and other non-Saccharomyces species carry out the initial fermentation stage. These yeasts

convert the sugars in the wine into ethanol and other by-products, including glycerol, acetaldehyde, and organic acids. The presence of these by-products can affect the activity and growth of AAB during the subsequent acetification stage [6]. As a result, the metabolism of yeasts impacts the flux of accessible substrates for AAB [102]. Moreover, yeast releases free amino acids during extracellular proteolytic activity and/or autolysis, which also benefits AAB [103]. The interaction between wine yeasts and acetic acid bacteria in vinegar production can be either symbiotic or competitive, depending on various factors such as temperature, pH, and nutrient availability. The symbiotic relationship between wine yeasts and acetic acid bacteria benefits vinegar production by promoting both microorganisms' growth and enhancing the fermentation process's efficiency. Wine yeasts produce ethanol as a substrate for acetic acid bacteria, while acetic acid bacteria consume ethanol and produce acetic acid. This symbiotic relationship has been shown to increase acetic acid yield during vinegar production [5,20,104]. Furthermore, it was found in Kombucha, a sugared tea fermented by SCOBY (Symbiotic Culture of Bacteria and Yeast), that AAB re-routed the metabolism of *S. cerevisiae* towards elevated invertase and fermentative activities [102].

Hutchinson et al. [105] evaluated the performance of a consortium of non-saccharomyces and AAB capable of achieving a complete ethanol-acetic acid fermentation for producing a Balsamic-styled vinegar in South Africa. Two inoculation strategies were followed: the co-inoculation of yeasts and AAB at fermentation (ethanol 0%, *v*/*v*) and the sequential inoculation (yeast only at the start and AAB at ethanol 6%, *v*/*v*). They found that co-inoculation was faster, and there were minimal differences between yeast and bacteria growth kinetics, meaning they could coexist.

In the particular case of sherry wine vinegar, the microbiology of sherry wine must be considered. Molecular and physiological characterization of the yeasts implicated in the veil of sherry wines has described the presence of different *S. cerevisiae* strains, mainly var. *cheresiensis*, *beticus*, *montuliensis*, and *Zygosaccharomyces rouxii*, which can grow aerobically in wine [106]. During this first fermentation process, the yeasts produce a

variety of aroma compounds, such as esters and higher alcohols, that contribute to the sensory properties of the final vinegar product.

Lactic acid bacteria (LAB) are also present in vinegar, albeit in smaller numbers compared to yeasts and AAB. They are implicated in the production of lactic acid and other organic acids, such as acetic and succinic acids, which contribute to the flavor and acidity of the vinegar. Some LAB strains may also produce antimicrobial compounds (bacteriocins) that may inhibit the growth of other microorganisms [107], including the AAB. Moreover, according to the recent work of Menglei and co-workers [108], cereal vinegar LAB and AAB production showed a negative interrelatedness during the fermentation process. *L. helveticus* CGMCC 12062 and *A. pasteurianus* CGMCC 3089 showed no nutritional competition when co-cultured in vitro. However, the growth and metabolism of *L. helveticus* were reduced during fermentation due to the presence of the AAB, indicating an amensal phenomenon involving these two species [109].

The interactions between yeasts, AAB, and LAB in vinegar fermentation are complex. They can vary depending on the specific conditions of the fermentation process, such as the substrate, temperature, and pH. Yeasts may provide essential nutrients, such as vitamins and amino acids, for the growth of AAB and LAB, while LAB may compete with AAB for nutrients and oxygen. Additionally, the development of one microbial group may also influence the growth of others through the production of metabolic by-products or the modification of environmental conditions [109].

Abiotic Interactions

In vinegar production, abiotic interactions refer to non-living factors or processes that influence the production process or the quality of vinegar. The primary abiotic interaction in vinegar production is the fermentation process, where AAB converts ethanol into acetic acid in the presence of oxygen. Oxygen is typically provided through aeration systems or by allowing air

exposure to the fermenting liquid [9,13]. While a sufficient oxygen supply is necessary to support the AAB's growth and metabolism, insufficient oxygen can result in slower fermentation or the development of undesirable microorganisms. For yeasts, excessive exposure to oxygen can be detrimental. High oxygen levels can cause oxidative stress, forming ROS (reactive oxygen species) that can injure cellular components, including DNA, proteins, and lipids. This oxidative damage can inhibit yeast growth and viability [110,111]. However, in some cases, oxygen increases yeast viability by increasing sterol and fatty acid biosynthesis. The Crabtree effect has been observed in TBV species, such as *Z. bailii* and *S. cerevisiae*, but not in *Candida* spp, which, in aerobic conditions, respire sugars. Hence, the oxygen level may negatively affect ethanol production if the dominant species in cooked must are Crabtree-negative yeasts [16].

Osmotic stress caused by sugar concentration is also a biotic stress that must be considered. Some yeast species are less affected by increased sugar content in cooked must, namely, *Zygosaccharomyces* spp. And *Candida* spp. Strains that can produce the highest ethanol concentration at the highest sugar value. On the other hand, *S. cerevisiae* strains isolated from TBV were shown to be more sensitive to the high sugar concentration [16].

Temperature plays a significant role in vinegar production as it affects the growth and activity of AAB. These bacteria thrive in a temperature range of 25–30/35 degrees Celsius [78]. Lower temperatures can slow down fermentation, while higher temperatures can lead to the growth of unwanted microorganisms, affecting the quality of vinegar. However, in other symbiotic products such as Kombucha, where we can also find symbiotic microbial consortia of yeasts, acetic acid bacteria (AAB), and occasionally lactic acid bacteria, the effects of temperature in fermentation, product shelf life, and microbial prevalence have already been studied [112]. The literature on the subject is scarce for vinegar, namely, symbiotic vinegar such as TBV. However, Solieri and Giudice [16] mention that during the full-fermentation phase, exothermal metabolic reactions raise the temperature,

which sometimes rises above 40 °C, reducing yeast vitality independent of the species present in the fermentation vessel. Hence, while AAB thrives at those temperatures, yeast may stop fermenting, leading to stuck fermentations and a decrease in ethanol for AAB. Interestingly, the resistance of AAB to high temperatures was also found in other vinegar productions. Seven AAB (all *A. pasteurianus*) that tolerated temperatures above 40 °C and ethanol above 10% (*v*/*v*) were isolated from Chinese vinegar Pei (a moistened solid mixture of alcohol, wheat bran, rice hull, water, and vinegar seeds), showing that the isolated strains could be helpful for other fermentations besides TBV, such as submerged fermentation processes [113]. Matsumoto and co-workers [114] were able to adapt the *A. pasteurianus* K-1034 strain thermally, capable of performing acetic acid fermentation in moromi rice (the industry standard term for the fermenting mixture, yeast, koji, steamed rice, and water for brewing), by experimental evolution using a "pseudo" rice moromi culture. The adapted strain was able to grow up to 40 or 39 °C.

The ability of AAB to use less biomass to produce large amounts of acetic acid compared to other bacteria that also produce organic acids (such as LAB) may be considered the greatest strength of AAB [115]. Up until a specific concentration (7–8%; Qiu et al. [115]), the presence of acetic acid in vinegar alters the flavor of vinegar and increases the survival advantages of AAB, namely in *Acetobacter* strains [19]; however, if acetic acid accumulates beyond a specific concentration, it causes acid stress that inhibits AAB growth [116]. 7–8% acetic acid concentrations are lethal for yeasts, and high acetic acid levels are commonly associated with arrested fermentations [117,118]. Results from Ribeiro et al. [119] indicate that yeast cell wall resistance to lyticase activity increases during yeast population adaptation to sudden exposure to sub-lethal acetic acid concentrations. This response correlates with increased cell stiffness, increased content of cell wall β-glucans, and a slight increase in the transcription of the GAS1 gene, which encodes a β-1,3-glucanosyltransferase that leads to an extension of (1→3)-β-d-glucan chains. These alterations guarantee an adaptive response that limits the cycle associated with re-entering the toxic acid form after the active exclusion of acetate from the cell's interior. This phenomenon was also observed in *S.*

cerevisiae cells. Individual *Z. bailii* cells, a yeast species present in TBV, in a population exposed to different weak acids will exhibit variable tolerance to acetic acid, among other weak acids [120]. The most tolerant sub-population presents a lower pH, leading to reduced intracellular dissociation of weak acids and reduced counterion accumulation in the cell's interior (cytoplasm), thus conferring tolerance to any weak acid, including acetic acid [121].

While for yeasts, acetic acid is lethal at vinegar's expected acidity, for AAB, the concentration of alcohol (ethanol) in the initial liquid is an essential abiotic factor. AAB bacteria require a specific concentration of alcohol (typically around 5–7%) to convert into acetic acid. If the alcohol concentration is too low, fermentation may be slow or incomplete. Conversely, high alcohol concentrations can be toxic to the bacteria, depending on the strain used. El-Askri et al. [122] found among 400 strains of AAB isolated from different substrates, six strains (FAGD1, FAGD10, FAGD18, GCM2, GCM4, and GCM15) isolated from apple and grape that can produce acetic acid at 16% (*v*/*v*) of ethanol and can tolerate acetic acid concentrations in the fermentation medium up to 6% (*v*/*v*). Furthermore, the isolated strains are also metabolically active at temperatures up to 48 °C.

Regarding nutrient availability, it must be considered that if acetic acid fermentation is carried out after the AF, or in some cases, relatively at the same time, AAB will have a poor nutrient medium or compete for nutrients with the yeasts. Hence, depending on the raw material, adding nutrient sources (amino acids and proteins), minerals, and vitamins may be necessary to a significant or minor extent [10]. Santos Júnior et al. [123] studied the need for vitamins and minerals to increase cell mass production by AAB isolated from the vinegar industry (086/06) and a standard *Acetobacter aceti* strain (CCT 2565). Five minerals (Mn, Zn, Mo, B, and Fe) and eight vitamins (niacin, *p*-aminobenzoic acid, pantothenic acid, thiamine, pyridoxine, biotin, cyanocobalamin, and inositol) were tested in a fractional factorial design. The results showed that the 086/06 strain required a lower number of different micronutrients than the CCT 2565 strain, which may be because of the adaptation of the 086/06 strain to the conditions of industrial

production, reinforcing the fact that knowledge of microbial nutrition is necessary for the nutrients to be supplied in the appropriate form and amount.

Final Remarks

Sour–sweet wine vinegar is a flavorful product that can be used in the human diet in many culinary dishes. It is made from grape-must fermented with the simultaneous or sequential action of yeasts and acetic acid bacteria. These vinegar products are usually aged in oak wood for a long time.

The acetification process is slow and dependent not only on abiotic factors, such as alcohol content, oxygenation, and temperature, but also on abiotic factors, for example, the interaction with wine yeast and lactic acid bacteria.

The intervenient microorganisms (yeasts and AAB) may be co-inoculated sequentially. However, it must be considered for all the abiotic factors that determine acetification success, such as temperature, oxygen availability, and nutrients.

There are already studies referring to the existence of promising AAB strains that can be used in the vinegar industry to minimize the loads due to cooling systems, especially in countries with a summer temperature higher than 35 °C, such as Portugal (for producing port wine vinegar) and Spain (sherry wine vinegar). Moreover, those strains seem to be efficient at higher ethanol concentrations.

The nutritional requirements also depend on AAB strain adaptation to acetification conditions. Less demanding strains are those already adapted and used in industrial processes. These abiotic interactions collectively influence the fermentation rate, acetic acid production, and the quality of vinegar. Proper control and optimization of these factors ensure a successful vinegar production process.

The interaction between wine yeasts and acetic acid bacteria in vinegar production is a complex process that various factors can

influence. Understanding the symbiotic and competitive relationships between these microorganisms can help optimize the vinegar production process and increase the yield of acetic acid and the quality of the final product.

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