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RISKS OF MICROBIAL SPOILAGE OF WINE: A REVIEW

Kiro Mojsov[1*], Meri Petreska[2], Jugoslav Ziberoski[3]

[1]**Technological -Technical Faculty, University Goce Delcev, Krste Misirkov,** P.O. Box. 201, **Stip, Republic of Macedonia**
[2]**Ministry of Agriculture, Forestry and Water Economy, Jurij Gagarin 15, 1000 Skopje, Republic of Macedonia**
[3]**Faculty of Agricultural Sciences and Food, Ss Cyril and Methodius University, Edvard Kardelj bb, 1000, Skopje, Republic of Macedonia**

[*]e-mail: kiro.mojsov@ugd.edu.mk

Abstract

Wines are alcoholic drinks obtained from the fermentation of grapes. The main role of microorganisms in winemaking is to convert grape sugars to alcohol, reduce wine acidity and contribute to aroma and flavour. They can also cause numerous unwelcome wine spoilage problems, which reduce wine quality and value.

Winemaking processes include multiple stages at which microbial spoilage is likely to occur and ends up with altering the quality and hygienic status of the wine. This may render the wine unacceptable, since the spoilage can include bitterness and off-flavours, and cosmetic problems such as turbidity, viscosity, sediment and film formation. The main microorganisms associated with wine spoilage are yeasts, acetic acid bacteria and lactic acid bacteria.

A microbial spoilage is the consequence of inadequate working practices. These inadequate practices are derived from two principle attitudes: (1) non-application of known practices for well identified risks and (2) insufficient evaluation of risk levels.

One of the aims of winemaking is to minimize potential for microbial spoilage and in this review are presented risks of microbial spoilage of wine and their prevention.

Key words: *Winemaking, microbial spoilage, quality of the wine, hygienic status of the wine, prevention.*

1. Introduction

Wines are alcoholic drinks obtained from the fermentation of grapes. Their composition is determined by the composition of the grape, which depends on genetic characters, vine growing conditions, and by winemaking practices, which involve a series of operations. In white wine making, the first step after crushing is pressing. This separates the solid parts from the juice, and the juice is then fermented separately. In red wine making, fermentation is achieved on the whole must obtained after crushing, and pressing is performed after the maceration phase. Winemaking is a biotechnology which is centuries old. The basic activities of modern wineries are fundamentally the same as those undertaken traditionally. The main role of microorganisms in winemaking is to convert grape sugars to alcohol, reduce wine acidity and the development of the flavour and aroma of the wine. Although grape must have a relatively complete nutrient composition, it can support only a limited number of microorganisms. One of the aims of winemaking is to minimize potential for microbial spoilage and this review focuses on preventing spoilage of wine by microorganisms. The main microorganisms associated with wine spoilage are yeasts, acetic acid bacteria and lactic acid bacteria. Numerous types of microorganism are involved in winemaking, and can be described as endogenous (from grapes or winery surfaces) or exogenous (from selected starter cultures). Yeasts and bacteria can make either beneficial or detrimental contributions to wine quality. The principal microbial genera associated with grapes are:

- yeasts: mainly *Kloeckera* and *Hanseniaspora*, with lesser representations of *Candida, Metchnikowia, Cryptococcus, Pichia* and *Kluyveromyces* and very low populations of *Saccharomyces cerevisae,*
- lactic acid bacteria: *Lactobacillus, Leuconostoc, Oenococcus and Pediococcus,*

- acetic acid bacteria*: Gluconobacter, Acetobacter,*
- fungi: *Botrytis, Penicillium, Aspergillus, Mucor, Rhizopus, Alternaria, Ucinula. Cladosporium.*

These microorganisms involved are at the core of the winemaking process, whether for good or ill and they affect the quality of wine (Du Toit and Pretorius [1]). However, from the onset of alcoholic fermentation the influence of the wild yeasts diminishes quickly as the increase of ethanol concentrations drastically influences their growth (Loureiro and Malfeito-Ferreira [2]). Yeast and bacteria found in grape must and wine originate from the vineyard, grapes, and winery processing equipment (Fleet [3]).

Winemaking processes include multiple stages at which microbial spoilage is likely to occur. The first stage involves the fruit material to be processed and equipment to be used. One must attempt to reduce the numbers of microbes in the juice and on the equipment. This is achieved through processing the pulp by applying food hygiene practices and following the hazard analysis critical control point (HACCP) system. The second stage of microbial spoilage may occur during fermentation because at this stage, the fruit juice contains both the natural flora of the fruit and flora that may be harboured by the wine cellar and its equipment. The microbial spoilage ends up altering the quality and hygienic status of wine. This may render the wine unacceptable, since the spoilage can include bitterness and off-flavours and cosmetic problems such as turbidity, viscosity, sediment and film formation. The major spoilage organisms of the yeast genera include *Brettanomyces, Candida, Hanseniaspora, Pichia* and *Zygosaccharomyces.* The genera of lactic acid bacteria include *Lactobacillus, Leuconostoc* and *Pediococcus*, while the acetic acid bacteria genera are *Acetobacter* and *Gluconobacter* (Du Toit and Pretorius [4]).

2. Spoilage yeasts in the wine industry

Yeasts play a central role in the spoilage of beverages, mainly those high acidity and reduced water activity. The spoilage caused in wine by yeasts is important because they cause refermentation, ester formation, hydrogen sulphide and volatile sulphure compounds, volatile acidity, the formation of volatile phenols, mousiness, film formation, deacidification and the formation of ethyl carbamate. *Schizosaccharomyces pombe* has been associated with wine spoilage when growing in bottled wine and forming a sediment at the bottom of the bottle (Boulton *et al.* [5]). The yeast *Zygosaccharomyces bailii* is one of the major wine spoilage yeasts, re-fermenting juice or wine during storage (Sponholz [6]). Yeasts *Hansenula anomala, Kloekera apiculata and Hanseniaspora uvarum* are associated with ester taint of faulty wines, which correlates with large amounts of acetic acid. These three species are associated with grape juice and result in spoilage at the early stages of alcoholic fermentation (Fleet [7]). The ester taint can be linked to the presence of ethyl acetate and methyl butyl acetate. Hydrogen sulphide is produced by yeasts during fermentation through the sulphate reduction pathway and has a flavor threshold of 50-80 mg/L and when exceeding this value will produce the rotten-egg off-flavor (Wenzel *et al.* [8]). One of the yeasts that can withstand the toxicity of ethanol levels and which has become the latest concern for most winemakers as a result of phenolic off-flavors, is *Brettanomyces/Dekkera* (Licker *et al.* [9]). *Dekkera* is the sporogenous form (ascospore-forming) or sexual anamorph of *Brettanomyces*. Wines typically associated with a "Bretty character" is commonly recognized by aromatic defects ranging from medicinal smells to farmyard-like odors and even spicy clove-like aromas (Licker *et al.* [9]).

2.1 Bacterial spoilage in wine

Bacteria are part of the natural microbial ecosystem of wine and play an important role in winemaking by reducing wine acidity and contributing to aroma and flavour. They can cause numerous unwelcome wine spoilage problems, which reduce wine quality and value. Lactic acid and acetic acid bacteria are the only families of bacteria found in grape must and wine.

The spoilage caused by lactic acid bacteria is associated particularly with acetification of the wine through the production of acetic acid, mousy taints, bitterness, ropiness, buttery flavour and increased viscosity of the wine. Many secondary metabolites produced by bacteria are volatile and potentially affect wine sensory qualities. The main spoilage caused by acetic acid bacteria is associated with oxidation of the ethanol to acetaldehyde and eventually acetic acid. Gram-negative acetic acid bacteria require oxygen for growth. They carry out incomplete oxidation of alcohols, leading to the accumulation of organic acids as end products (Bartowsky and Henschke [10]). Even though the optimum pH for the growth of acetic acid bacteria is 5.5 to 6.3 (Holt *et al.* [11]). they are able to survive at wine pH (3.0-4.0). pH of 3.3 or lower is inhibitory to most lactic acid bacteria, but not to acetic acid bacteria (Du Toit and Lambrechts [12]). The presence of sulphur dioxide (SO_2) in wine should prevent the growth of acetic acid bacteria, but the molecular form being the most effective (Bartowsky and Henschke [10]). The brief aeration of red wine during racking and transfer operations is sufficient to encourage the growth of acetic acid bacteria and cause wine spoilage, even when SO_2 has been added (Millet and Lonvaud-Funel [13]). The control of oxygen is an essential tool, in preventing wine spoilage by acetic acid bacteria. All acetic acid bacteria species are considered spoilage bacteria. The occurrences of most spoilage scenarios are uncommon and can be avoided with correct hygiene management during the vinification and maturation process. Careful winemaking practices include use of the correct dosage of SO_2 during wine maturation and filtering the wine prior to bottling, which can reduce the risk of wine spoilage by acetic acid bacteria in bottled wine.

2.2 Preventing spoilage of wine by microorganisms

Preventing microbial spoilage of wine include pasteurization and chemical preservation. Studies on pasteurization showed that most potential spoilage microbes possess little resistance to heating of table wine: e.g. at 109°F, 99.9% of organisms were killed within 1 min (Splittstoesser [14]). The heat resistance of three strains of *Dekkera/Brettanomyces* was evaluated at different temperatures between 32.5 and 55°C. When heating was performed in wine, significant inactivation begins at 35°C (Couto *et al*. [15]). Heating wine prior to bottling has been explored, including flash pasteurization, however, concerns on the impact of this on wine sensory characteristics have meant that this technology is not widely used (Ribéreau-Gayon *et al*. [16]). Wine containing 10, 11 and 12% alcohol and inoculated with large populations of yeasts required a very high conc. of sorbic acid (0.05%) to prevent spoilage (Splittstoesser [14]). Sorbic acid (2,4-hexadienoic acid) can be used as a chemical preservative in sweetened wines at bottling to prevent yeast fermentation after packaging. However, several lactic acid bacteria species, including *O. Oeni* strains, are able to metabolize sorbic acid resulting in the formation of 2-ethoxyhexa-3,5-diene, which has an odour reminiscent of crushed geranium leaves (*Pelargonium spp*.) (Riesen [17]). Thus, care is needed when bottling wine preserved with sorbic acid to ensure that the bacterial population has been eliminated. How best to avoid wine spoilage is not always clear-cut. As an initial barrier, the high ethanol concentrations (up to 16% v/v), high wine acidity (pH as low as 2.9) can inhibit development of bacterial populations. Storage of wine at temperatures below 15°C might assist with minimizing the ability of bacteria to proliferate in wine, but will also delay wine maturation.

Traditionally, sulphur dioxide has been used to control unwanted micro-organisms during winemaking, where it is usually added to bins of machine-harvested grapes and after malolactic fermentation. Sulphur dioxide acts as both an antimicrobial agent and an antioxidant in wine (Romano and Suzzi [18]). Physical removal of microorganisms through filtration of juice or wine can also be used. However, filtration typically is mainly conducted prior to bottling and hence is not used to remove microorganisms during winemaking.

There are several chemical inhibitors and natural products that can be used for the control of microorganisms in wine. Dimethyl dicarbonate is a chemical inhibitor of microorganisms inactivating cellular enzymes (Daudt and Ough [19]). More recent studies in red wine suggest that the permitted rate of dimethyl dicarbonate addition (200 mg L^{-1}) does not effectively inhibit lactic acid bacteria or acetic acid bacteria implying that dimethyl dicarbonate might not be a good preservative against undesired bacterial contamination of wine (Costa *et al*. [20]).

Natural products such as lysozyme and bacteriocins to inhibit bacterial growth have been successfully utilized in various pharmaceutical and food industries for almost 50 years, and lysozyme has recently been approved for use in winemaking. Lysozyme is ineffective against eukaryotic cells and it cannot be used to control spoilage yeast, such as *Dekkera/ Brettanomyces* (McKenzie and White [21]). The addition of lysozyme must be considered carefully, because it is able to bind with tannins and polyphenols in red wines and typically results in a slight decrease in wine colour or might result in the formation of a wine haze (Gerbaux [22]).

There are alternative technologies that have been used successfully for removing microorganisms from wine (Cheftel *et al*. [23] and Smelt *et al*. [24]. These include ultrahigh-pressure processing, ultrasound, ultraviolet irradiation and pulsed electric fields.

2.3 Preventing the risk of microbial spoilage at each stage

Microbiological spoilage results from the multiplication of microorganisms that consume elements of wine and which release their products there. A microbial spoilage cannot always be seen by the naked eye so some cells of certain microorganisms can produce several milligrams of extremely negative oenological compounds (ethyl-4-phenol, scatol, 3 methyl-indol). In order for a microbial spoilage to occur, three elements are required: contamination + conditions favourable to development + time. Microorganisms coming into the wine. These spoilage microorganisms need sources of energy, nitrogen, fatty acids, vitamins and minerals. Favourable conditions for their development include absent or insufficient microbiologically active SO_2, high pH levels, raised temperatures, etc. Microbial growth takes about 10 hours for a contaminating yeast population to multiply by two.

A microbial spoilage is the consequence of inadequate working practices. These inadequate practices are derived from two principle attitudes: (1) non-application of known practices for well identified risks and (2) insufficient evaluation of risk levels. An antiseptic addition or centrifugation is useful in reducing a population by a certain percentage without totally eliminating it. There is no 100 percent sure and effective miracle recipe for all cases. The risk of rapid redevelopment at a critical level of microorganisms is much higher, above all if other factors are favorable (nutrients, pH, temperature, oxygen).

Increased temperatures during the harvest and increased pH levels favor the multiplication of spoilage microbes on the juice and winery equipment. Halts in alcoholic fermentation and languid fermentation favor all spoilage microorganisms. Actions to carry out are place wines between 20-22 °C as soon as the alcoholic fermentation has finished, and until the end of malolactic fermentation and place wines at less than 16 °C after the end of their malolactic fermentation.

While emptying the tanks, the risk of acetic bacterial development is very high. Moreover, a well-filled tank is not a wine protected from spoilages. If the temperature is favorable, and there is not a sufficient level of active SO_2 and if there has been contamination, the quantity of dangerous microorganisms can multiply. Action to carry out is verified twice a week the fullness of the tanks.

Hygiene is obligatory and it is a strategic technique of all stages of winegrowing. Action to carry out is having a hygiene plan prepared before the harvest, with adapted procedures and material, and a sufficiently trained staff.

The good practices are really effective for wine if it is vinified while taking into account prevention of spoilage at each stage. A regular and complete alcoholic fermentation allows real management of malolactic fermentation and prevention of the risk of microbial spoilage during one of the most critical phases of winemaking. For competition with spoilage microorganisms, yeast inoculation with the correct dose of selected ferment is one of the principle key points.

Heavy lees are vegetative and agglomerates of colloidal material, yeast, bacteria and tartarate crystals, that settle at the bottom of the tank in the 24 hour period after the last stirring of the wine. They increase the combining of SO_2 and allow the protection of certain microorganisms included in the agglomerates. Action to carry out is quickly eliminating heavy lees.

In the case of insufficient hygiene in the winery, high-risk microorganisms are protected from the effects of SO_2. The risks are also grater when the pH is high and the temperature above 18 °C.

3. Conclusions

- Microbial wine spoilage continues to be of concern in grape vinification. They can cause numerous unwelcome wine spoilage problems, which reduce wine quality and value. A microbial spoilage is the consequence of inadequate working practices. These inadequate practices are derived from two principle attitudes: (1) non-application of known practices for well identified risks and (2) insufficient evaluation of risk levels. Microbial spoilage is a result of undesired growth of microorganisms in wine. Factors such as pH, temperature, residual sugar, nutrients, oxygen will affect the growth if they are not controlled within the range. The only way to prevent their growth is to minimize their contact with air and keep the wine cool.
- One of the aims of winemaking is to minimize potential for microbial spoilage and in this review are presented risks of microbial spoilage of wine and their prevention at each stage. Maintaining an adequate SO2 level, low pH, and sanitary conditions during processing can prevent the spoilage.

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