Some Issues in Malolactic Fermentation Acid Reduction and Flavor Modification*

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Lactic acid bacteria (LAB) play an important role in winemaking. They are involved in malolactic fermentation (MLF), which is also called secondary fermentation. Malolactic fermentation causes acid reduction, flavor modification and also contributes to microbiological stability. Acid reduction can be desirable in a high acid must provided it is consistent with wine style. Flavor improvement can be a good tool for enhancing overall wine quality, and achieving biological stability is essential for making sound wine. While MLF can be beneficial in wine, it can sometimes produce undesirable results. Among these include: excess acid reduction leading to high pH, increased risk of wine spoilage, formation of off flavors, poor color and production of amines.

It is important to remember that MLF can occur spontaneously during or after the alcoholic fermentation. It can be brought about by many kinds of lactic acid bacteria and some of these are not desirable. The changes in wine's flavor can be desirable or undesirable and although the wine is considered biologically stable following MLF, it can support the growth of spoilage causing microorganisms such as lactic acid and acetic acid bacteria and various undesirable yeasts. For this reason, it is necessary to add sulfites to wine soon after MLF and take special care of the wine during storage. A winemaker needs to have a good understanding and control of MLF in order to realize the benefits of the process for making better wines and at the same time avoid all the risks associated with it.

Question of Acid Reduction

It is a well-established fact that MLF causes acid reduction. The extent of acid reduction will depend on the amount of malic and citric acid in the must; but generally, the reduction in titratable acidity varies between 0.1 to 0.3%, with subsequent rise in pH of 0.1 to 0.3 units. In MLF dicarboxylic malic acid is converted to mono-carboxylic lactic acid. As a result, quantitatively, the acid reduction should be predictable. But in practice this is difficult due to several complicating factors. The LAB can also form lactic acid from glucose, and yeast can also degrade malic acid. The rise in pH following MLF can render some of the bitartrate insoluble and thus contribute to the loss of titratable acidity.

Grapes grown in a cooler climate tend to have higher acidity levels, generally due to the significant amount of malic acid in them. Wines from these high acid musts can benefit from MLF. On the other hand, grapes produced in a warm to hot climate have low acidity and MLF in these wines could reduce acidity to unacceptably low levels. For this reason, in these wines, MLF is discouraged.

Even in high acid must where MLF could be employed to lower acidity, the decision to choose MLF should be based on the style of wine and the acid profile of the must and the variety of the grapes. For example, when making a dry, crisp, light-to medium-bodied white wine with simple varietal character, MLF may not be an appropriate choice due to inappropriate flavor modification. Another case where MLF may not be desired is the production of white wine from strongly aromatic varieties such as Riesling, Gewurztraminer and Muscat, particularly where the winemaker wishes to preserve varietal character. Some winemakers, however, prefer to use MLF in wines from these aromatic varieties since it can improve the body or mouth feel of the wine, without significantly diminishing the fruity component.

If aromatic wines are to be made with residual sugar, then the danger of developing geranium-like off odors by spoilage-causing lactic acid bacteria also needs to be considered. In such cases it is best not to use MLF for the purpose of deacidification.

In producing full-bodied, rich and complex white wines, MLF is often employed. In this style of wine the goal is to achieve flavor complexity in addition to acid reduction. Examples of this style include Chardonnay and Burgundy wines with fuller body, and complex flavors.

Red wines contain tannins, which contribute to the astringent taste. Acidity in wine tends to enhance astringency; therefore, red wines (rich in tannins) are often made with lower acidity. Generally, the higher the tannin level in the wine, the lower the acidity needs to be to achieve proper balance. Red wines also have more complex flavors because the must is fermented in contact with the skins. In order to enhance flavor complexity and realize acid reduction, MLF is often encouraged in red wine production.

Another point to consider when choosing MLF is the acid profile of the must. The main acids in grapes (must) include tartaric, malic and citric. Tartaric acid is usually the predominant acid. However, in some varieties substantial amounts of malic acid can be present at harvest (for example Norton/Cynthiana). If high malate wines are subjected to MLF, they can have excess lactic acid giving wines a cheesy flavor. In such cases a winemaker needs to exercise caution in choosing MLF for acid reduction.

Flavor Modification

In addition to deacidification, flavor modification of the wine is an important aspect to be considered when choosing M LF. Malolactic fermentation involves growth and the metabolic activity of LAB. As a result of their metabolic activity many fruit and alcoholic fermentation derived aromas are modified, new compounds are formed and some substances are removed from the medium (wine). It is the concentration and interaction of metabolic end products that define the aroma of MLF wines.

Many descriptors have been used to describe the flavors of malolactic wines. The commonly used positive terms include buttery, nutty, toasty, sweaty, fruity, vegetative and spicy. The body of the MLF wine is usually noted as full, soft round and rich. The improvement in flavor of malolactic wines includes both the impact on body and the aroma of wine.

MLF can influence the wine's aroma in several ways. It can enhance fruitiness; it can reduce it or can leave it unchanged. It generally reduces the vegetative character; reduction in grassy, green pepper and green bean aromas has been noted in Cabel11et Sauvignon wines following MLF. Buttery, nutty, sweaty and yeasty aromas are typically associated with MLF wines. Diacetyl has been recognized as the main compound responsible for the buttery character. It is important to note that the flavor modification following MLF depends on several factors such as strains of LAB, variety of grapes, must composition and yeast bacteria interaction. In a sponta¬neous MLF several types of lactic acid bacteria can conduct the fermentation and some of these could be undesirable types producing unwanted flavors. Use of selected strains of LAB in conducting MLF can help in avoiding the undesirable flavors in wine.

Diacetyl

Diacetyl and related compounds such as acetoin and 2,3 -butanediol are the metabolic byproducts of citric acid metabolism, produced by LAB. The degradation of citric acid occurs soon after the malic acid is metabolized. It has been proposed that the breakdown of citric acid begins at the same time as the degradation of malic acid but the reaction is much slower; and consequently, some citric acid is left at the end of malic acid degradation. However, some recent evidence indicates the breakdown of malic and citric acid by LAB is not con¬comitant but sequential. It is believed that malic acid inhibits the activity of some enzymes involved in citric acid metabolism by the LAB.

The metabolic pathway of formation of diacetyl from citric acid is shown in
Fig 1. In the first step, in the bacterial cell, citric acid is broken down into acetic acid and oxaloacetic acid. In the next step oxaloacetic acid is decarboxylated to yield pyruvic acid. Other metabolic reactions involving pyruvic acid lead to the formation of alpha- acetolactic acid (ALA). This compound is not very stable. Decarboxylation of ALA produces acetoin, which is reduced to 2,3-butanediol. Diacetyl can also be produced from the oxidation of acetoin or the oxidative decarboxylation of alpha-acetolactic acid. See Fig 2.

A small amount of diacetyl, about 2-3mg/l in white and 4-5 mg/l in red, is believed to favorably enhance the aroma of the wine. At higher concentrations the aroma can be overwhelmingly buttery and thus constitute a defect. Several factors influence the final concentration of diacetyl in the wine; these include strains of LAB, conditions of MLF, amount of citric acid available and the oxygen content (Redox potential) of the wine.

Diacetyl is also produced by yeast during alcoholic fenl1entation but it is then remetabolised by the yeast yielding acetoin. A significant amount of diacetyl is also produced by LAB during MLF, which gives these wines their typical buttery character. During MLF, the concentration of diacetyl reaches a peak soon after the exhaustation of malic acid. Later on, its level is reduced due to the metabolic activity of LAB. This point is important in controlling diacetyl content and thus buttery aroma in wine. In order to preserve the buttery aroma in a wine, the winemaker should immediately stabilize the wine by sulfiting and filtration, when a desirable level of diacetyl is attained. It is important to remember that the degradation of diacetyl (buttery aroma) can be accomplished only by the living yeast and bacteria and stabilization is essential to stop their action. On the other hand if the buttery aroma is overwhelming and is bordering on a defect, the wine should be left in contact with the lees until the diacetyl level is reduced to an acceptable point.

There are two more factors that have been shown to influence diacetyl content in wine. First is the oxygen content or redox potential of wine. In an experiment Nielsen and Richelieu (1999) conducted on MLF under anaerobic (oxygen < O.2 mg/l) and semi-aerobic (oxygen 2-4 mg/l) conditions. The results of their experiment are given in Table 1 below. It is clear from their data that a higher concentration of diacetyl resulted when the MLF was conducted under semi-aerobic conditions and only a small amount of diacetyl was found under anaerobic conditions.

The second important factor affecting the level of diacetyl is the concentration of $S0_2$ in wine. Diacetyl is a carbonyl compound, which reacts strongly with $S0_2$. As a result of this reaction, after $S0_2$ addition, the diacetyl concentration of wine decreases (and bound $S0_2$ increases). The addition of sulfite to stabilize the wine can therefore, reduce the diacetyl content of the wine.

Table 1. MLF conducted under anaerobic and semi-aerobic conditions

Condition of MLF	Concentration of diacetyl mg/l	Concentration of acetonin mg/l
Semiaerobic	13	12
Anaerobic	2	20

This reaction is reversible, which means when the diacetyl-SO_2 complex dissociates, the diacetyl concentration will increase (free SO_2 will also increase). This point is an important consideration in controlling diacetyl content (buttery aroma) of the wine.

Suggested Readings

Henick-Kling T., T.E. Acree, M.H. Laurent and W. Edinger, Modification of wine flavor by malolactic fermentation. Wine East: 4:8-15 and 29-30, 1994.

Nielson J.C., and Marianne Richelieu. Control of flavor development in wine during and after malolactic fermentation by Oenococcus oeni. Applied and Environmental Microbiology 65(2): 740-745, 1999.

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