
UCDAVIS

ETHYL CARBAMATE

PREVENTATIVE ACTION MANUAL

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COOPERATIVE EXTENSION

PREAMBLE

Ethyl carbamate (EC, urethane) is a naturally occurring component of all fermented foods and beverages. Because EC has shown a potential for carcinogenicity when administered in high doses in animal tests, the wine industry is interested in reducing EC levels in their products. This advisory contains recommendations drawn from scientific research that are designed to help all winegrape growers, winemakers, and other industry members to minimize the levels of EC in wine.

These recommendations are advisory only, and not intended to restrict the freedom and diversity of winemaking styles.

FORMATION OF EC:

To better understand the possible actions we can take to minimize levels of EC in wine, it is necessary to review the basics of the major formation pathway and kinetics:

Arginine, usually one of the most abundant yeast available amino acids in grape juice, is taken up by wine yeast as a nutrient and may be metabolized yielding *urea* if present in excess amounts. If the urea can not be further metabolized and accumulates above a critical concentration, yeast strains release it from their cells into the wine during or at the end of fermentation. Urea can spontaneously react with the alcohol in wine to form EC. The chemical reaction between urea and ethanol is exponentially accelerated at elevated temperatures. To a lesser extent *citrulline*, an amino acid which is not incorporated into yeast protein, and is formed during arginine biosynthesis, can serve as an EC precursor. Lactic acid bacteria can also be a source of citrulline under winemaking conditions. However, the key reaction for EC formation in wine is between urea and ethanol.

PREVENTATIVE ACTIONS:

We have developed the following recommendations towards aspects of EC formation in wine:

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① VITICULTURE

① Vineyard Fertilization

- Nitrogen (N) fertilization in the vineyard has direct influence on the nitrogen contents of the grape berry and the resulting must. Excessive fertilization with urea, ammonia and other N-fertilizers in the past is considered partly responsible for generally higher EC levels found in wines from traditional wineproducing countries.
- The following OIV Expert Committee on Vine Physiology method for sampling of leaves to determine the nutritional status of grapevines in order to provide sufficient, but not excessive, nutrients for vine growth has been developed (Resolution Viti 4/95). There appears to be a growing opinion among viticulturists that nitrate-N in petioles at bloom has little if any relationship to growth, fruitset or general N status of the vine or N status of the berries and resulting juice.

OPERATION	LEAF BLADE	PETIOLE
Timing	- Berry set - Véraison	- Véraison
Minimum number of vines ¹	50-100	100
Position of the leaf on the shoot	Leaf blade opposite the first basal cluster	Petioles opposite the cluster
Position of the shoot on the cordon or cane ²	Fruitful shoot in the middle	Fruitful shoot in the middle
Minimum number of leaves	50-100 leaf blades	100 petioles
Treatment of the leaves	Wash quickly in distilled water ³ and then dry	Wash quickly in distilled water and then dry

¹ In relation to the size of the vineyard.

² The position according to the training system. The text of the Resolution includes illustrations of the correct sampling point for the major training systems in Europe.

³ Only for the determination of copper, zinc and manganese.

- In general, grapevines have a very low nitrogen requirement relative to most other crops. For example, 10 tons of grapes remove only about 25 lbs. of nitrogen from the vineyard.
- Growing grapes in soils previously used for vegetables, and thus, heavily fertilized, can result in excessive nitrogen contents of juice and EC levels in wine.
- The concentration of nitrogenous components such as arginine in juice and urea in wine increases proportionally with increasing nitrogen fertilization in the vineyard. If arginine concentrations in juice exceed 1000 mg/L, the vineyard must be considered over-fertilized.
- In a nitrogen deficient soil, an application of 100 lbs N/acre would achieve a level of about 150 mg of yeast available nitrogen/L of juice. This is considered sufficient for completion of fermentation. However, application of such high fertilizer rates can lead to excessive canopy growth and delayed fruit maturation. Potential nitrogen deficiencies in juices in regard to yeast nutrition should not be addressed through vineyard fertilization.

① VITICULTURE

② Cover Crops

- Growers need to be aware that they may be adding a significant amount of nitrogen to their vineyards when they disk under winter legumes used as cover crops in the vineyard. The legumes include vetches (*Vicia* spp.), clover (*Trifolium* spp.), and pea (*Pisum* spp.). Use of these cover crops can increase vine nitrogen to excessively high levels. For example, clover tissue contains about 2.5% nitrogen, vetch tissue about 4%. Growing vetch can accumulate up to 75 lbs of nitrogen per acre, compared to an average of 25 -50 lbs. per acre applied through commercial fertilizer additions. If legumes are used as cover crops, soil and vine nitrogen status should be monitored in order to avoid excessive arginine levels in juices. If additional nitrogen fertilization of the soil is to be avoided, plowing-under of winter legumes should not be practiced.

③ Cultivars & Rootstocks

- Different grape cultivars exhibit variations in nitrogen uptake, with some varieties being generally lower in level of arginine than others. However, low nitrogen status of cultivars is largely related to own-root characteristics and will change with the use of different rootstocks. Total bloom petiole nitrogen can vary by more than 40% on average, while nitrate-nitrogen may even vary 10-12 fold depending on the rootstock-scion combination used. Rootstocks can therefore have a profound impact on vineyard nitrogen status and fertilizer management. Local viticulture farm advisors can provide information on nitrogen uptake by different rootstocks.

RECOMMENDATION:

Growers should be aware of vine and grape nitrogen status and modify vineyard procedures if juice arginine concentrations are significantly greater than 1000 mg/L.

② JUICE NUTRIENT STATUS/ADDITIONS

- To correctly determine the nutritional status of an individual grape juice, it may be necessary to measure the level of nitrogen compounds actually available to the yeast for its metabolic activity. Nitrogen status of grapes varies widely with vineyard site, soil, irrigation and fertilization practices, vintage weather, scion and rootstock, and grape maturity. The two major sources for nitrogen in must are ammonia and amino acids with the exception of proline. Proline cannot be used as a yeast nitrogen source without molecular oxygen, which is not present in an anaerobic grape juice fermentation.
Both available nitrogen sources can be analyzed in a winery lab or at contract laboratories. Ammonia assays include enzymatic/photometric tests or use of an ammonia probe. Yeast available amino acids may be measured rapidly by a colorimetric OPA/NAC test (Dukes & Butzke 1996), which requires a spectrophotometer. Analysis by HPLC determines all nitrogen sources simultaneously but requires highly skilled personnel and yields delayed results under winemaking conditions. It is necessary to obtain analytical results within a few hours after yeast inoculation in order to make decisions about nutrient additions to deficient musts.
It would also be desirable to determine critical arginine levels through a simple analytical procedure which can be applied in a winery lab. There is currently no rapid analysis for arginine in juice available to wineries today. Development of a procedure has been proposed by UC Davis.
- To avoid sluggish or stuck fermentations, it is permitted by BATF to add up to 8 lbs of diammonium phosphate (DAP) per 1,000 gal (960 mg/L) to a nitrogen deficient must, which translates into ca. 200 mg of nitrogen/L. However, excessive levels of nitrogen may contribute to urea formation and excretion by yeast. Although some nutrients are required to accomplish an optional malolactic fermentation, high nutrient levels at the end of fermentation can contribute to microbial instability of a wine (see lactic acid bacteria).
- *Yeast food* preparations may add an unidentified level of yeast available nitrogen to a juice. It is recommended that winemakers request the supplier to specify the different nitrogen sources.
- The use of urea as a fermentation supplement is prohibited. BATF has found that the use of urea is not considered acceptable in good commercial practice among wine producers and has rescinded the listing of urea as an authorized treatment (Federal Register, Vol. 55, No. 118, 24974-24982, 06/19/90).

RECOMMENDATION:

Winemakers should know the nitrogen status of their juices and not over-supplement with diammonium phosphate.

③ YEAST STRAINS

- Wine yeast strains differ in their ability to rapidly catabolize urea during fermentation. When excess urea accumulates in the cell's cytoplasm, it is released into its environment, the must. High urea producing yeasts are those that have a high capacity to degrade arginine to urea, but a low urea metabolizing ability. Low urea metabolizing ability may result from low activity of urea amidolyase, inhibition of amidolyase activity by the presence of high levels of ammonia, deficiencies of cofactors required by amidolyase, or apparently low activity due to hyperactive arginase. Genetic as well as environmental factors influence the amount of urea released by the cells. Some commercial yeast strains such as Lallemand 71B[®], Red Star SC1120[®] and Premier Cuvée (PdM)[®] have been described as producing relatively low levels of urea. Yeast companies will be able to recommend their lowest urea excreting strains for each specific winemaking application, and it is suggested that they be consulted.
- Spontaneous fermentations with undefined yeast strains will necessitate monitoring of arginine in juices and urea and EC levels in every fermentation. It is not clear what impact natural fermentations will have on EC levels as this has not been thoroughly investigated. However, it is anticipated that indigenous yeast strains will display a similar variability in urea catabolism as observed in commercial strains.

RECOMMENDATION:

If juice is high in arginine content, fermentation should be inoculated with known low-urea producing strains of yeast appropriate for the winemaking application.

④ LACTIC ACID BACTERIA

- Certain wine lactic acid bacteria are capable of forming small amounts of citrulline, a precursor of ethyl carbamate, from the amino acid arginine, and excreting this precursor into the wine. Routine nitrogen supplement of juices with unknown nutritional status can increase the potential for bacteria available nitrogen after primary fermentation. Additionally, even strains not able to degrade arginine may produce small increases in ethyl carbamate, suggesting that nitrogenous precursors other than those derived from arginine may be involved. Research results indicate the need for caution in the selection of starter cultures for conducting malolactic fermentation in wine, since citrulline formation from arginine degradation could result in elevated levels of ethyl carbamate, even at normal temperatures, during prolonged storage. In addition, spontaneous malolactic fermentation by undefined strains should be avoided, as this may lead to formation of ethyl carbamate precursors.

RECOMMENDATION:

If malolactic fermentation is desired, winemakers should either use a commercial strain that does not produce high levels of citrulline or monitor juice for citrulline content post-fermentation.

5 UREASES

- Since urea is the major precursor for EC in wine, enzymatic hydrolysis of urea to ammonia and CO₂ appears to be a suitable way to eliminate formation from this source. Preparations of urease enzyme are commercially available and permitted by BATF for the treatment of wine. However, urease activity is severely limited under normal wine conditions, specifically with respect to low pH and ethanol. Urease is especially inhibited by high concentrations of malic acid, and fluoride residues (from cryolite[®] application in the vineyard) in excess of 1 mg/L. Any combination of these factors make can it practically impossible to reach the desired low urea levels in reasonable time, even at a very high enzyme dosage. A complete elimination of EC is not possible.

RECOMMENDATION:

If wine is high in residual urea, winemakers may be able to use a urease treatment to reduce urea levels. However, the effectiveness of the urease addition must be evaluated for each wine to confirm the enzyme is active.

6 SUR LIE AGING

- It is a common winemaking style to age wine *sur lie* (on the yeast lees) after primary fermentation in order to impact the wine's organoleptic properties. Aging on the lees leads to the liberation of nitrogenous compounds, amino acids and protein, into wine: a rapid excretion from the intracellular pool of the yeast cells during the first weeks of storage, and a slow increase during further storage due to autolysis of the yeast. However, it has been reported that, in wines made from grapes of low amino acid concentration, after extended lees contact, no increase in ethyl carbamate concentration was found, and that no additional ethyl carbamate precursors were released from the yeast during extended lees contact. Therefore, under above conditions, the practice of extended yeast lees contact appears not to raise ethyl carbamate potentials. No data are available to document an influence of sur lie aging on urea concentrations of wine made from grapes with excessive concentrations of yeast assimilable nitrogen.
- Similarly, no data are available regarding the production of sparkling wine and the evolution of levels of urea and other EC precursors during yeast autolysis, e.g. during long-term aging of tirage-bottled wines.

RECOMMENDATION:

Sur lie aging has not been shown to dramatically impact ethyl carbamate levels, but this has not been thoroughly tested.

⑦ DISTILLATION/FORTIFICATION

- Although urea is not volatile and EC itself possesses a poor volatility, EC may still be found in wine distillates. It can be formed post-distillation via the reaction of a volatile precursor, *isocyanate*, and ethanol both at ambient and elevated temperatures.
- Producers of fruit wine distillates have to be aware of another precursor for ethyl carbamate, in the form of *cyanides*. Stone-fruits, especially such as cherries, apricots or plums, contain sugar-bound cyanides in their seeds, which can be released during fermentation. Removal of stones prior to fermentation, and a secondary distillation are essential to avoid high concentrations of volatile EC precursors in this type of spirit.
- Producers of fortified wines have to take the same considerations into account as table winemakers, since fortification itself may aggravate the problem of urea excretion by yeast. Urea is often formed during the early and middle stages of fermentation with subsequent yeast generations utilizing it during the later stages. Maximum excretion occurs frequently, but with exceptions, at about 12° to 16° Brix. Arresting fermentation at this stage will lead to high urea concentrations in the fortified wine. It is recommended to perform a test fortification in the winery lab and to analyze both the fermenting wine and the resulting dessert wine for urea.
- In addition, the fortifying grape spirit or brandy can serve as the primary source for EC taint in fortified wines, and should be monitored for EC and potential EC.

RECOMMENDATION:

Since isocyanate is formed by break-down of urea, the same recommendation as for table wine production applies. No recommendation can be given at this point regarding the fractionation of distillates due to lack of data regarding the distillation behavior of volatile EC precursors.

⑧ SHIPMENT AND STORAGE

- The chemical reaction between urea and ethanol increases exponentially with temperature. It is therefore essential that a wine containing elevated levels of urea is not exposed to elevated temperatures (above 100°F) during storage or shipment.

RECOMMENDATION:

Since long-term exposure of wine to heat is also detrimental to its sensory properties and visual stability, wineries should educate and encourage the shipper, distributor, wholesaler, and retailer to minimize heat exposure by use of appropriate insulated containers, shipping schedules and storage facilities.

SUMMARY

- ⇒ Avoid excessive nitrogen fertilization in the vineyard.
- ⇒ Monitor soil nitrogen status.
- ⇒ Monitor vine nitrogen status.
- ⇒ Do not use winter legumes as cover crops if soil nitrogen status is already high.
- ⇒ Be aware that nitrogen uptake varies strongly with different cultivars and especially rootstocks.
- ⇒ Monitor juice nitrogen status.
- ⇒ Do not add excessive nitrogen supplements.
- ⇒ Do not add nitrogen supplements routinely.
- ⇒ Do not add urea as nitrogen supplement.
- ⇒ Avoid juice arginine levels greater than 1000 mg/L.
- ⇒ When choosing among wine yeast strains, avoid those with high urea excretion characteristics.
- ⇒ Use malo-lactic bacteria with known characteristics.
- ⇒ Be aware that use of urease preparations cannot completely eliminate EC formation.
- ⇒ Be aware that must fortification may aggravate the problem of urea excretion by yeast.
- ⇒ Monitor EC levels of fortification spirit.
- ⇒ Avoid exposure of wine to elevated temperatures during storage and transport.

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