



Incidence of nitrogenous compounds of must on ethyl carbamate formation induced by lactic acid bacteria

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- Ethyl carbamate (EC) is a toxic compound found in many fermented foods, such as bread, cheese and wine. EC can naturally form in wines during alcoholic and malolactic fermentations, and especially during aging. One of the known sources of EC is the metabolism of arginine by heterofermentative lactic acid bacteria (LAB), such as *Lactobacillus* and *Oenococcus*.
- There is a concern among winemakers that over-fertilized vines may produce juices with high residual levels of nitrogen compounds which, in turn, would contribute to high EC in the wines. Because of its toxicity, EC concentrations in wine have been regulated in Canada since 1985 (30 µl/L max), whereas in the USA, there is a 15 µl/L voluntary limit. To test whether high levels of nitrogen compounds in musts are a health risk, the current authors tested the effect of artificially enriching juices with high levels of four types of nitrogenous compounds: adenine, arginine, urea, and ammonium.
- The trial took place in 2002. A Tempranillo juice was divided into 5 treatments: adenine-enriched (1 mg/l), arginine-enriched (500 mg/l), urea-enriched (10 mg/l), ammonium phosphate-enriched (139 mg/l), and an un-enriched control. To test the ability of different LAB to form EC, microvinifications (75 ml) were inoculated with either *Lactobacillus hilgardii* or *Oenococcus oeni* after alcoholic fermentation (using *S. cerevisiae* Uvaferm). Finally, to test the influence of fermentation conditions on EC formation, fermentations were conducted at either 25°C or at 30°C, and within each temperature, at a pH of 3.2 or 3.8. There were a total of 40 treatments (5 enriching treatments x 2 bacteria x 2 temperatures x 2 pHs). All N-enriched fermentations were replicated 4 times (except for control treatments which were replicated 2 times).
- **Results.**
 - 1) **Urea** concentrations were low at the end of all of the fermentations, even in the urea-enriched juices. This is because yeast readily deplete the urea.
 - 2) **Ammonium** concentrations at the end of the fermentations were extremely low, showing remarkable depletion by the yeast. Ammonium, unlike urea, is not an EC precursor, but could still affect EC production indirectly.
 - 3) **Arginine** concentrations were higher in the treatments that had been arginine-enriched. Even though these high residual arginine wines showed a tendency to produce more EC, the difference was not significant. In fact, after incubation with either lactic bacteria, **no relationship could be established between higher arginine concentrations and higher EC.**
 - 4) EC accumulation was greater with *Lactobacillus hilgardii* than with *Oenococcus oeni*. EC production was greater at 30°C than at 25°C. Interestingly, maximum EC concentrations (38-44 µl/L) were found in controls that had been inoculated with *L. hilgardii* and incubated at 30°C at both pH 3.2 and 3.8. Therefore, **the readily-assimilable nitrogen sources that were added to the fermentations did not induce an increase in EC production in the wine.**

- In summary, even after the addition of large amounts of a variety of readily-available nitrogenous compounds to Tempranillo juices, the final wines did not contain high ethyl carbamate levels under the conditions of this study [*Maybe that's why – because the nitrogen sources were too available, and therefore, preferentially used by the yeast*]. Still, the authors warn that conducting malolactic fermentation over lees could lead to increased levels of residual arginine, thus providing a possible substrate for spoilage organisms. They recommend racking wines after alcoholic fermentation and conducting MLF in a clean vessel.

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