ask the AWRI

Time, temperature and urea – the ethyl carbamate connection

Ethyl carbamate is a naturally occurring colourless and odourless chemical found in wine. It has been identified as a possible human carcinogen based on animal studies (JECFA 2006). In this article, AWRI Oenologist **Ben Cordingley** addresses winemaker questions relating to its occurrence and formation, as well as practices that result in lower ethyl carbamate concentrations.

How high is the risk of consuming ethyl carbamate in food and beverages?

The risk level of ethyl carbamate from dietary sources depends both on the concentrations present in a food or beverage type and average dietary consumption. This information is often used by regulatory bodies to limit total ethyl carbamate consumption from all food and beverage sources that comprise a typical diet, considering an acceptable range of consumption patterns. This means that higher ethyl carbamate concentrations can be acceptable in product types that are consumed less. Some markets have imposed maximum permitted levels for ethyl carbamate in wine and other alcoholic beverages.

Which food and beverages contain ethyl carbamate?

Ethyl carbamate occurs naturally in many fermented food and beverages, including wine, sprits, beer, bread and soy sauce. While foods typically present a low risk, alcoholic beverages often contain higher concentrations and therefore pose a slightly higher exposure risk depending on consumption levels. Multiple surveys have found that most table wines contain less than 15 µg/L ethyl carbamate, with many not containing any detectable levels (FSANZ 2007, FDA 2021). Sake, whisky, brandy and fortified wines typically contain higher concentrations compared to table wine. FSANZ (2007) reported that the exposure risk of ethyl carbamate from alcoholic beverages was negligible, even to consumers of alcohol at the 95th percentile level.

How is ethyl carbamate formed in wine?

The major pathway begins with the conversion of arginine to urea by yeast. Arginine is a naturally occurring amino acid found in grapes and is a major component of yeast assimilable nitrogen (YAN). The metabolism of arginine occurs during the early stages of fermentation as yeast biomass increases exponentially. Some of the urea produced is exported from the yeast cell but can be reabsorbed and used as a nitrogen source towards the end of fermentation. Residual urea can react with ethanol to form ethyl carbamate. A minor production pathway can be mediated by wine bacteria where arginine is converted to the amino acid citrulline. Reaction of citrulline with ethanol produces ethyl carbamate.

What are the factors that encourage ethyl carbamate formation?

There are several factors that affect the rate of formation and the total amount formed. These include the concentrations of urea and (to a lesser extent) citrulline precursors, storage time and temperature. Higher concentrations of urea at the conclusion of fermentation may be affected by the timing of urea or other nitrogen-based fertiliser applications in the vineyard, and excessive YAN additions to ferments. Less urea is required to be reabsorbed by fermentation yeasts when there is a surplus of other nitrogen sources. Different yeast strains also vary in the extent of urea production, the amount released from the cell, and the amount that is reabsorbed and used. Breaking down excess urea with urease enzymes has been proposed as a potential future treatment to lower

concentrations of ethyl carbamate. Higher temperatures have been shown to increase the formation of ethyl carbamate in wine. Avoiding elevated temperatures during wine storage and transport is therefore one tactic to avoid high concentrations. Wine temperature during transport is influenced by factors including the shipping route, format (refrigerated vs insulated vs non-insulated) and positioning of containers on ships (Nordestgaard 2016).

Why do fortified wines contain more ethyl carbamate than table wines?

Fortified wine production often involves arresting primary fermentation to leave unfermented sugars. This often occurs after urea has been exported from yeast cells, but before urea has been reabsorbed to be used as a nitrogen source toward the end of fermentation. This leaves a greater amount of residual urea available to react with ethanol and form ethyl carbamate. Export markets with limits for ethyl carbamate often have higher limits in fortified wines based on both the potential for higher concentrations and the generally lower dietary intake of these beverages.

How much can time and temperature increase ethyl carbamate concentration?

The AWRI helpdesk recently investigated this question for an Australian winemaker exporting wine to Canada, where there is maximum permitted level for ethyl carbamate of 30 µg/L in table wine. A wine contained no detectable ethyl carbamate when it departed Australia in a flexi-tank container. On arriving at its northern hemisphere destination, it was determined to contain 27 µg/L of ethyl carbamate. The winemaker asked the helpdesk what temperatures are expected during transit across the equator, and if this factor alone could explain the increase in ethyl carbamate. A six-week trial was set up using samples of a similar wine. Three storage temperatures were selected, with the middle being 27°C. This storage temperature is consistent with wine temperatures logged during an AWRI bulk wine transit study (Nordestgaard 2016).

The ethyl carbamate concentrations analysed at the conclusion of the trial are presented below.

Starting ethyl carbamate concentration	Storage temperature for the six-week trial	Final ethyl carbamate concentration
17 µg/L	20°C	23 µg/L
	27°C	28 µg/L
	35°C	44 µg/L

The trial results are consistent with reported impacts of time and temperature on ethyl carbamate formation. Hasnip *et al.* (2004) suggest that ethyl carbamate concentration can approach a maximum value for a given temperature. This trial confirmed that typical shipping times and temperatures can explain increases in ethyl carbamate concentration in overseas wine shipments. Results may vary based on precursor levels.

For further information on ethyl carbamate or any other technical winemaking or viticulture topic, contact the AWRI helpdesk on (08) 8313 6600 or helpdesk@awri.com.au

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