ETHYL CARBAMATE FORMATION IN SUB-OPTIMAL WINE STORAGE CONDITIONS AND INFLUENCE OF THE YEAST STARTER

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Abstract

Aim : To evaluate the potential risk of ethyl carbamate (EC) formation in wine by studying its production kinetics at sub-optimal storage temperatures.

Methods and results : The kinetics of EC formation was investigated in 60 white wines obtained from 6 varietal juices fermented with 10 yeast strains. The wines were analysed for their urea content at bottling, then EC formation was monitored during in-bottle storage at < 12 °C for 150 days followed by 152 days at 40 °C. Storage at < 12 °C had no effect on EC formation, regardless of initial urea content; however, at 40 °C we found a positive correlation between initial urea content and final EC content.

Conclusion : Urea content higher than 20 mg/L in wine kept at 40 °C can produce from 15 up to 30 µg/L EC in less than 5 days. Two yeast strains, La Claire SP665 and Maurivin Platinum, minimised the urea in wine, reducing the risk of EC formation.

Significance and impact of the study : The temperatures used in this study can be accidentally - but easily - reached during sub-optimal wine storage and shipping, and in the presence of substantial amounts of urea, the EC level can exceed the warning levels established by some countries in just a few days. The paper confirms the importance of minimising urea production in wine and controlling temperature during storage and shipping.

Key words : urethane, urea, yeast, temperature, ageing

Résumé

Objectif : Évaluer le risque potentiel de formation de carbamate d'éthyle (CE) dans le vin par l'étude de la cinétique de production à des températures de stockage sous-optimales.

Méthodes et résultats : La cinétique de formation de CE a été étudiée chez 60 vins blancs issus de six moûts variétaux fermentés avec 10 souches de levure. Les vins ont été analysés pour leur teneur en urée à la mise en bouteilles, puis la formation de CE a été contrôlée au cours du stockage en bouteilles à < 12 °C pendant 150 jours, suivis par 152 jours à 40 °C. Le stockage à < 12 °C n'a eu aucun effet sur la formation de CE, quelle que soit la teneur en urée initiale. Cependant, à 40 °C, on a trouvé une corrélation positive entre la teneur initiale d'urée et la teneur finale en CE.

Conclusion : Une teneur en urée supérieure à 20 mg/L dans le vin conservé à 40 °C peut produire du CE de 15 - 30 mg/L, en moins de 5 jours. Deux souches de levures, La Claire SP665 et Platinum Maurivin ont minimisé l'urée dans le vin, réduisant ainsi le risque de formation du CE.

Importance et impact de l'étude : Les températures utilisées dans cette étude peuvent être atteintes accidentellement, mais facilement, pendant le stockage et l'expédition du vin en conditions sous-optimales. En présence de quantités importantes d'urée, le niveau du CE peut dépasser les niveaux d'alerte établis par certains pays en seulement quelques jours. Ces résultats confirment l'importance de la minimisation de l'urée dans la production de vin et de contrôle de la température pendant le stockage et l'expédition.

Mots clés : uréthane, urée, levure, température, élevage

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INTRODUCTION

Ethyl carbamate (EC), alias urethane, the ethyl ester of carbamic acid, has been recognised as "probably carcinogenic to humans" by the IARC (International Agency for Research on Cancer) since 2007. The mean intake of EC coming from fermented foods and drinks, excluding the contribution of alcoholic beverages in which EC concentration can reach several milligrams per litre, is estimated to be about 15 ng/kg b.w. per day. In wines EC comes mainly from the direct chemical reaction between urea and ethanol, the former being produced during fermentation from arginine, citrulline and ornithine, whereas in spirits the main precursors are cyanides (Stevens and Ough, 1993; Kodama *et al.*, 1994; Weber and Sharypov, 2009). The final amount of urea in wine is related to the genetic aspects of yeast, the grape variety and the fermentation conditions, and strategies for minimising the risk of EC synthesis have highlighted the role of temperature during ageing (Butzke and Bisson, 1997).

The aim of this work was to evaluate the potential risk of EC formation by studying its production kinetics at similar temperature conditions than those possible during sub-optimal storage and shipping in summer.

MATERIALS AND METHODS

1. Grape varieties and yeast strains

Sixty white wines were produced on a semi-industrial scale from 6 varietal white juices (Chardonnay, CH; Mueller-Thurgau, MT; Pinot Noir, PN; Sauvignon blanc, SB; aromatic Traminer, TRAM; and a blend of CH and PN juices, CHPN) fermented using 10 yeast strains inoculated at $1x10^7$ CFU/mL. Yeast strains were Laffort Zymaflore VL1 (VL1) and X5 (X5), Lallemand Lalvin R2 (R2) and RC212 (RC212), Pall La Claire EM2 (EM2) and SP665 (SP665), AWRI Maurivin Platinum (MP) and a pre-commercial strain (MD), Anchor Yeasts VIN13 (VIN13), and Red Star Montrachet (MRS). Wines were fermented at 20 ± 1 °C and sulphited (70 mg/L) at the end of alcoholic fermentation to avoid malolactic fermentation. Then they were subjected to batonnage at 12-15 °C three times a week under carbon dioxide blanketing for 1.5 month, sterile filtered (0.45 µm), and bottled (250 ml) 5 months after fermentation.

2. Urea and EC analysis

The urea produced by yeast strains was measured after derivatization with xanthydrol 0.02 m (Sigma-Aldrich, Steinheim, Switzerland) dissolved in 1-propanol, using an Agilent 1200 HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a fluorometric detector set at 213 nm and 308 nm (excitation and emission wavelength, respectively). Separation was performed on an Eclipse XDB-C18 column (4.6 x 150 mm, 5 µm; Agilent Technologies) with pre-column set at a temperature of 35 °C.

EC formation was studied in the course of two consecutive periods of storage, for a total of 302 days. In the first period, the 60 bottled wines were stored at < 12 °C in the dark for 150 days. In the second, the same wines were subjected to "thermally accelerated ageing" in a oven set at 40 ± 1 °C in the dark for 152 days. The analytical controls were carried out by sampling one bottle per wine on day 0, 150, 170, 197, 240 and 302.

EC was determined according to Reg. (CE) N. 761/1999, using a GC-SIM Autosystem XL (Perkin-Elmer, Waltham, MA, USA) equipped with a capillary Innowax column (30 m, 0.32 mm, 0.50 µm; J & W Agilent Technologies). 20-mL wine samples, with the addition of 1 ml propyl carbamate (400 ng/mL in water) as an internal standard, were extracted with 20 g EXTRELUT® NT cartridge (Merck, Darmstadt, Germany). Elution was performed with 160 ml methylene chloride and the sample was concentrated to 1 ml at low temperature before injection under nitrogen stream.

3. Statistical analysis

Box plots and Wilcoxon matched pairs test were carried out using STATISTICA v. 8.0 (StatSoft Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

At the time of bottling the wines had the following composition (median, min, max): alcohol content 12.84, 10.75, 14.60 % vol.; pH 3.23, 3.17, 3.67; total acidity 6.80, 4.10, 8.70 g/L as tartaric acid; residual sugars 0.90, 0.25, 4.70 g/L; total SO_2 121, 89, 190 mg/L; and free SO_2 16, 5, 36 mg/L. Urea content ranged from 0.12 to 33.6 mg/L (median of 0.33 mg/L) and major differences were observed between the varietal wines (CH, median = 0.33 mg/L; CHPN 0.20; MT 3.18; PN 0.18; SB 0.27; and TRAM 3.37). Figure 1 shows the urea

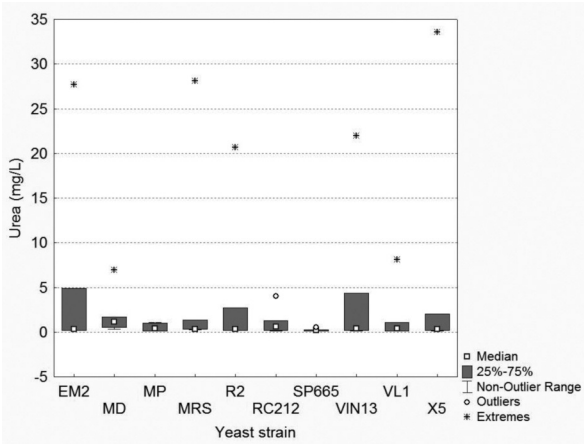

Figure 1 - Distribution of urea content in wine by yeast strain. Urea content was measured in 60 white wines (6 grape varieties using 10 different yeast strains) at bottling. See Materials and Methods for full names/description of yeast strains.

distribution per yeast strain. Though there were no statistical differences between strains, La Claire SP665 and Maurivin Platinum showed the lowest values of urea in wines. Indeed, no samples produced with these yeast strains exceeded 1.5 mg/L (i. e., well below the warning limit of 3 mg/L suggested by the OIV for urease treatment), a behaviour that confirms previous observations (Larcher *et al.*, 2007). Seven wines, all belonging to MT and TRAM, were above the level of 5 mg/L urea - considered as a limit of potential risk for EC formation - and these high values could be mainly traced back to the high arginine content originally available in the grape juice (823 mg/L for MT and 1047 mg/L for TRAM).

Figure 2 shows the evolution of EC content during the entire 302-day storage period. Before storage, the median EC content was 0.1 µg/L, ranging from below the detection limit (0.1 µg/L) to 13.5 µg/L, while after 150 days at < 12 °C the median was 0.1 µg/L and the content ranged from below the detection limit (0.1 µg/L) to 14.3 µg/L. Two samples were very close to the legal limit of 15 µg/L established for wine in some countries (Weber and Sharypov, 2009), while all the others were under 8 µg/L. During the "thermally accelerated ageing" (152 days/40 °C storage), the EC content increased linearly, without reaching a plateau, and ranged between 14.3 and 1136 µg/L - the median being 47.9 µg/L - and all the samples but one were above the aforementioned legal limit. The observed trends in EC formation suggest that roughly 10 % of the samples exceeded the legal limit by the 5th day of treatment at 40 °C. The Wilcoxon matched pairs test did not show significant differences ($p < 0.05$) between wines at 0 and 150 days (i. e., before and after storage at < 12 °C), whereas highly significant differences ($p < 0.001$) were found between wines at 150 and 302 days (i. e., before and after storage at 40 °C).

Figure 3 shows the correlation found between the urea content in wine before storage and the EC content at the end of the 40 °C/152-day treatment. In the conditions studied, an average amount of EC equal to roughly 3.4 % of the initial amount of urea was produced. On the contrary, no significant correlations were found between the EC formed (both as absolute content and per urea unit) and the alcohol content of wines.

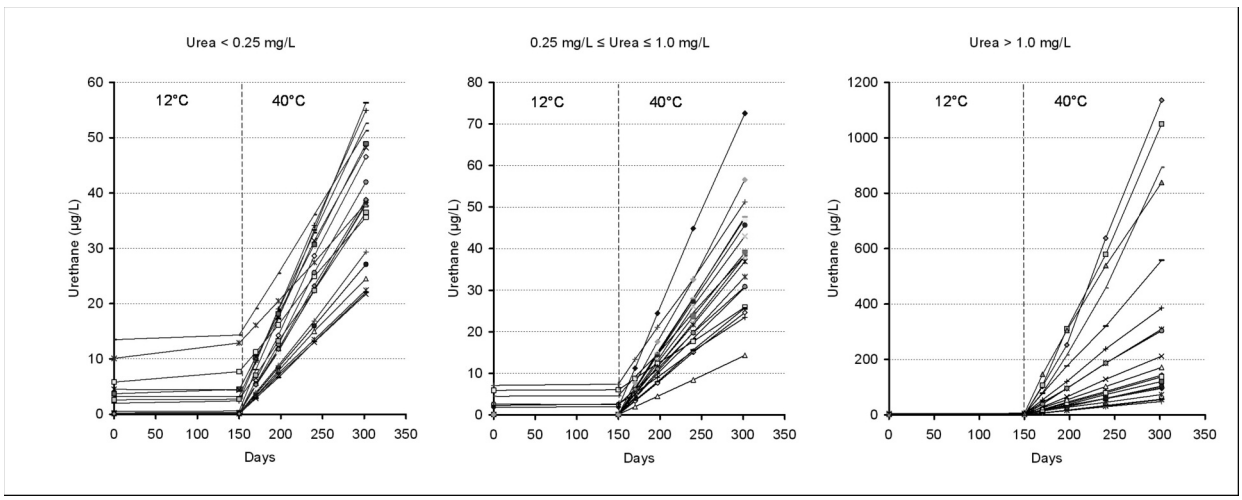

Figure 2 - Evolution of ethyl carbamate (urethane) in 60 wines (grouped by urea content at bottling) during storage for 150 days at < 12 °C followed by 152 days at 40 °C.

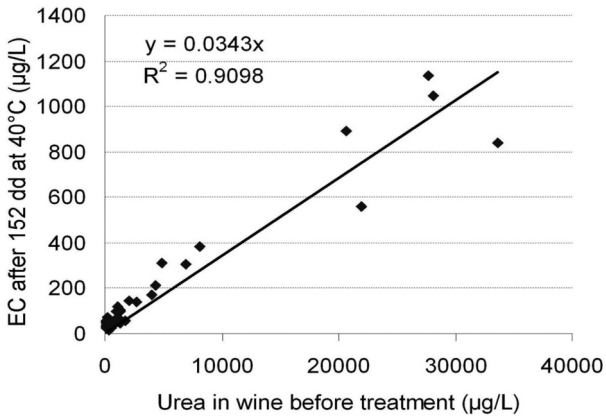

Figure 3 - Correlation between the initial urea content and the final ethyl carbamate (EC) content in 60 wines stored at 12 °C for 150 days and then at 40 °C for 152 days.

CONCLUSION

With roughly 5 months of storage at 40 °C, urea-ethanol reaction took place without reaching a plateau - confirming Hasnip *et al.* (2004) - and by the end a conspicuous amount of EC was produced, equivalent to 3.4 % of the initial urea content in wine. On the basis of our results, less than 5 days at 40 °C can be considered sufficient to produce from 15 to 30 µg/L EC, a warning level in many countries, in wines with more than 20 mg/L urea at bottling. These temperatures can easily be reached, mainly in summer, during wine shipping without temperature control or storage in non-specialised shops. In this experiment, two strains (La Claire SP665 and Maurivin Platinum) minimised the production of EC. Although this needs to be evaluated in other winemaking conditions, it suggests that the use of selected yeast strains could be an effective strategy to prevent the risk of EC formation, as far as their ability to produce urea was previously evaluated under conditions of white and red winemaking.

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