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Formation of ethyl carbamate and changes during fermentation and storage of yellow rice wine

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ABSTRACT

Ethyl carbamate (EC) was analyzed during yellow rice wine production and storage. EC increased slowly during fermentation and rapidly after frying and sterilization. Less amount of EC was formed when cooled rapidly to 30 °C than when cooled naturally. High temperature and long storage time increased EC formation. After 400 days storage, EC increased from 74.0 to 84.2, 131.8 and 509.4 μg/kg at 4 °C, room temperature and 37 °C, respectively, and there was significantly difference between the fried wine and the wine on sale from 2011 ($p < 0.01$). Urea increased during yellow rice wine fermentation and was above 20 mg/kg after the wine was fried; urea contributed to EC formation when the fried wine was cooled slowly. These results indicate that it is necessary for industry to optimize the wine frying conditions, such as temperature, time and cooling process in order to decrease EC formation.

1. Introduction

Ethyl carbamate (EC) is genotoxic and carcinogenic in animal species, such as mice, rats, hamsters, and monkeys (Beland et al., 2005). The World Health Organization's International Agency for Research on Cancer (IARC) re-classified EC as a group 2A carcinogen (IARC, 2010), which suggested a potential carcinogenic risk to human. EC has been detected in many alcoholic beverages (Alberts, Stander, & De Villiers, 2011; De Melo Abreu, Alves, Beatriz, & Herbert, 2005; Fu et al., 2010; Júnior, Mendonca, & Pereira, 2011; Lachenmeier, Kanteres, Kuballa, López, & Rehm, 2009; Lachenmeier, Schehl, Kuballa, Frank, & Senn, 2005; Liu et al., 2011; Wang, Ke, Wang, Yin, & Song, 2012; Wu & Chen, 2004; Wu, Pan, Wang, Shen, & Yang, 2012; Wu et al., 2012) and several countries such as Canada, USA, Brazil and others have set specific EC standard (Lachenmeier et al., 2010) to improve the safety of wines. Every year, large numbers of fermented foods and beverages are produced and consumed in China, and EC was detected in over 73% of the tested fermented samples, with a highest concentration of 650 μg/kg (Wu et al., 2012).

The yellow rice wine is a traditional fermented alcohol drink in China. Until now, two processes, the traditional one (human handing) and the mechanical one (using equipment) have been applied for yellow rice wine production. The traditional process (Fig. 1) is more time consuming than the mechanical method. The typical mechanical production process includes rice soaking, steaming, addition of starter culture, pre-fermentation, post-fermentation, squeezing, addition of caramel color, frying, package and storage; in this production process, the processes of rice steaming, addition of starter culture, fermentation, squeezing, frying and packaging are mechanically operated. The fermentation process lasts for 25–40 days, and then the yellow rice wines are stored for 6–12 months.

To understand the critical factors influencing EC formation during yellow rice wine fermentation and storage, a number of yellow rice wines from 3 factories in different cities of Zhejiang province were selected and the critical procedures and conditions influencing EC formation during yellow rice wine production and storage were studied.

2. Materials and methods

2.1. Samples

Yellow rice wines produced by the traditional and mechanical methods were collected and used for analysis in this experiment. The No. 1 and No. 2 yellow rice wines were from a Jiaxing factory produced by mechanical and traditional processes, respectively. The No. 3 yellow rice wine was from a Hangzhou factory produced by mechanical process. Some other yellow rice wines bought from a local supermarket in 2011 were also analyzed.

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Rice → Immerse and cooking → Cooked rice → Temperature drop → Addition of starter → Pre-fermentation (3-7 days) → Canned and Post-fermentation (30-60 days) → Wine squeezing → Caramel colored → Clarification (1-3 days) → Wine frying (86-92 °C) → Cool to room temperature → Storage (as semi-product for over 6 months) → Sterilization → Packaging and sale.

Fig. 1. Diagram of the traditional yellow rice wine production process.

2.2. EC analysis

EC was determined according to the AOAC (2000) first action method with minor modifications (Wu et al., 2012). In brief, the d_5-ethyl carbamate was used as an internal standard. A 2.0-g wine sample containing 100-μL 1.0 μg/mL d_5-ethyl carbamate was added to a centrifuge tube and vortexed for 1 min. Using a diatomite solid-phase extraction column, the analyte was eluted from the mixture with 10 mL of 5% ethyl acetate after 10 min of short static stretches. The collected elutate was dried by anhydrous sodium sulfate, and concentrated using N_2 flow at 30 °C. The analyte was further diluted with methanol to a final volume of 1 mL for GC/MS. All samples were measured three times and the data were presented as the average of the three measured values.

2.3. Urea determination

Urea was analyzed by the diacetyl monoxime method (Wang, Feng, Wu, Zhang, & Pan, 2010). In brief, 10.0 mL of the yellow rice wine was eluted using the Carb solid-phase micro-extraction column (200 mg/6 mL). The primary 1 mL elution fraction was discarded and the following 2 mL eluate was collected for further analysis. Subsequently, 2-mL water, 0.5-mL 2% diacetyl monoxime water solution, and 3-mL acid reagents (see below) were added to 2 mL of the yellow rice wine eluate. The fraction was allowed to boil in water for 15 min, and the reaction mixture was cooled in ice water and analyzed using an ultraviolet–visible spectrophotometer at 525 nm. Water (2 mL) was used as a control instead of the yellow rice wine. The aforementioned acid reagent was prepared by adding 1 mL of 98% sulfuric acid and 16.5 mL of 85% phosphoric acid to 50 mL of water and further cooled to room temperature, to which 0.5 g cadmium sulfate and 12.5 mg thiosemicarbazide were added to a final volume of 250 mL with water. Urea concentration was calculated by using a standard curve.

2.4. Statistical analysis

Analysis of variance (anova) was used to detect significant differences in the EC level of the yellow rice wine samples. The level of statistical significance was determined at confidence intervals of $p < 0.05$ and $p < 0.01$.

3. Results and discussion

3.1. Change in EC concentration during the process of yellow rice wine fermentation

EC was initially present in the starter fractions of the yellow rice wine production, and was less than 10.0 μg/kg. During the processes of pre-fermentation, post-fermentation, and squeezing, EC increased slightly and was less than 30 μg/kg (Fig. 2). The wines produced by the mechanical process contained 11.0 (Fig. 2A) and 13.7 μg/kg (Fig. 2C) of EC respectively, which was lower than that by the traditional process (31.6 μg/kg) (Fig. 2B). This may be due to the difference in factors such as change in fermentation time and temperature between the two conditions.

Both the traditional and the mechanical processes of yellow rice wine production included the steps of rice soaking, steaming, starter addition, pre-fermentation, post-fermentation, squeezing, caramel color addition, frying, packaging, and storage. In the mechanical process, steaming, addition of starter, fermentation, squeezing, frying, and packaging were all mechanically operated. The entire fermentation process was required approximately 25–40 days, which was lesser than that for the traditional process.

3.2. Changes in EC concentration during the wine frying process

Frying is a process that sterilizes and matures the yellow rice wine. The fried wine exhibits better quality, stability, and flavor. During the frying process, high temperature may promote EC formation because of the reaction between urea and ethanol. In the traditional yellow rice wine production process, wine frying was normally performed at 86–92 °C, the wine was sterilized at 85 °C, and cooled to room temperature or the wine was sterilized by steaming process post-squeezing and then allowed to cool under natural conditions. In the mechanical method, wine frying was performed by sterilization and canning and then the wine was cooled to room temperature after 2–3 days, or the wine was sterilized by pasteurization at 88–92 °C and then rapidly cooled to 30 °C.

The yellow rice wines from the different factories were fried; post-frying, EC concentration rapidly increased from 13.7 to 51.8 μg/kg (Fig. 2A), 31.6 to 88.6 μg/kg (Fig. 2B), and 11.0 to 25.3 μg/kg (Fig. 2C), respectively. The results validate that increase in EC concentration was rapid and occurred within a short period of time during wine frying. Wine frying significantly influenced EC formation, which was identical to other studies; the fried yellow rice wine had higher EC concentration than the fermented wine pre-frying (Wu, Hong, Ma, & Xu, 2011; Wu et al., 2012).

The No. 1 and No. 2 wine samples were fried at over 90 °C; post-frying, the temperature of the wine samples remained stable until the sterilization process was complete, after which the wine samples were allowed to cool to room temperature. EC concentration increased from 51.8 to 81.6 μg/kg (Fig. 2A) and from 88.6 to 121.2 μg/kg (Fig. 2B), respectively, which validated that EC was formed continuously post-frying. To study the influence of the cooling process on EC formation, the No. 1 wine sample was cooled rapidly to 30 °C post-frying, EC concentration was 34.6 μg/kg, which was lower than the naturally cooled wine of 51.8 μg/kg (Fig. 2A). This result validates that rapid cooling process decreased