ARTICLE IN PRESS

Food Bioscience xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Evaluation of ethyl carbamate formation in Luzhou-flavor spirit during distillation and storage processes

Fang Fang[a,*], Yuying Qiu[a], Guocheng Du[a,b], Jian Chen[a,c]

[a] Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi 214122, China
[b] The Key Laboratory of Carbohydrate Chemistry and Biotechnology, Ministry of Education, Jiangnan University, Wuxi 214122, China
[c] National Engineering Laboratory for Cereal Fermentation Technology, Jiangnan University, Wuxi 214122, China

ARTICLE INFO

Keywords:
Ethyl carbamate
Urea
Citrulline, Luzhou-flavor spirit
Distillation
Storage

ABSTRACT

Ethyl carbamate (EC) is a toxic contaminant that poses hazard to humans due to its potential carcinogenicity. This compound has been widely detected in fermented foods and alcoholic beverages such as Chinese spirits. Clarifying the mechanism of EC formation is difficult but necessary for reducing EC levels in Chinese spirits because of their complex fermentation procedures. The formation of ethyl carbamate during distillation and spirit aging were evaluated in this study. All grains used as materials for producing Luzhou-flavor spirit contained ethyl carbamate precursors, urea and citrulline. The highest levels of urea (93 μg/kg) and citrulline (83 μg/kg) were detected in wheat and sorghum, respectively. EC and its precursors were introduced into the raw spirit of Luzhou-flavor spirit during distillation. EC, which was present at 92 μg/l concentration in raw spirit, came from the distillation process. EC content in the raw spirit increased to 66% during the first year of storage because of spontaneous formation of EC from its precursors mainly urea in the raw spirit.

1. Introduction

Ethyl carbamate (EC) is a genotoxic and carcinogenic compound that has been widely detected in fermented foods and alcoholic beverages (Jiao, Dong, & Chen, 2014; Weber & Sharypov, 2009). Among foods, alcoholic beverages are highly likely to contribute to EC intake. The presence of EC in beverages and the hazards it poses to humans have prompted research into the identification of EC precursors and sources, as well as research into the mechanism of EC formation, which is crucial to the development of approaches to eliminating EC in these products (Lachenmeier et al., 2010).

Ethyl carbamate precursors in materials used in the production of fermented foods and beverages form via microbial nitrogen metabolism or enzyme catalysis. Urea, one of the EC precursors, has been detected in grape wine, Chinese rice wine, and sake. It is mainly generated through the catalytic activity of arginase (CAR1) in *Saccharomyces cerevisiae* (Guo et al., 2016; Kitamoto, Oda, Gomi, & Takahashi, 1991; Wu et al., 2014). The accumulation of citrulline in wine and soy sauce may be attributed to arginine utilization through the arginine deiminase (ADI) pathway in bacteria (Azevedo, Couto, & Hogg, 2002; Mira de Orduna, Liu, Patchett, & Pilone, 2000; Zhang, Fang, Chen, & Du, 2014). Cyanic acid is involved in EC formation in spirits such as whiskey, stone fruit spirits and sugar cane spirits (Kobe, da Silva, Rocha, Godoy, & Franco, 2014; Lachenmeier et al., 2010; Riachi, Santos, Moreira, & De Maria, 2014), forming from cyanogenic glycosides (Alcarde, de Souza, & Bortoletto, 2012; Galinaro, Ohe, da Silva, da Silva, & Franco, 2015). Previous studies have shown that fermentation, distillation, and storage of alcoholic beverages induce EC formation during their production (Bruno, Vaitsman, Kunigami, & Brasil, 2007; Zhao et al., 2013). The mechanism of the metabolism of EC precursors such as urea and citrulline in fermented spirits has also been reported (Jiao et al., 2014). However, the origins of all EC precursors in spirits and their contribution to EC formation remain unclear. Chinese spirits produced from grains are well-known for their strongly aromatic flavor. This study aimed to determine EC precursors in all materials used for the production of Chinese spirits and to evaluate their contributions to EC formation during raw spirit distillation and storage.

2. Materials and methods

2.1. Luzhou-flavor spirit production and sampling

One ton of grains (a mixture of rice, glutinous rice, corn, wheat and sorghum) were ground and mixed with 3 t fermented grains, divided into three batches and steamed in a steel steaming bucket for 2.5 h, cooled to room temperature (10–25 °C), and mixed with the starter

* Corresponding author.
E-mail address: ffang@jiangnan.edu.cn (F. Fang).

https://doi.org/10.1016/j.fbio.2018.02.007
Received 29 August 2017; Received in revised form 29 January 2018; Accepted 15 February 2018

Please cite this article as: Fang, F., Food Bioscience (2018), https://doi.org/10.1016/j.fbio.2018.02.007

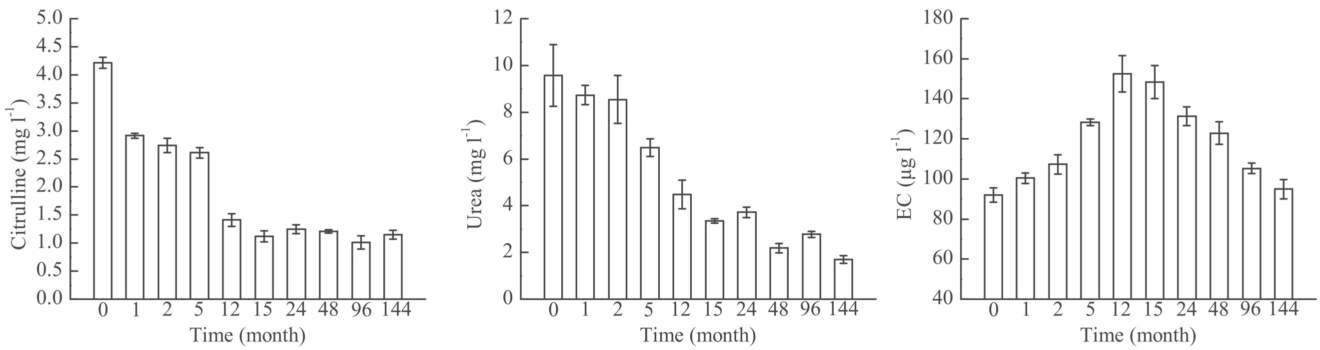

Fig. 1. Determination of EC and its precursors in the raw spirit of Luzhou-flavor spirit.

(*Daqu*), before underground anaerobic fermentation in a mud pit for about 60 days. The fermented grains were then mixed with fresh ground grains and transferred to the steel steaming bucket and steam-distilled. Spirits obtained from distillation, which comprise the raw spirit, were stored in appropriate containers at 20 °C for at least four years before blending and packaging.

Raw spirit of Luzhou-flavor spirit from different batches aged for 1 month to 12 years were collected from a spirits distillery located in eastern China. Samples of fresh spirits (i.e., spirits obtained from distillation) and fermented grains from the same batch were collected from the same spirits distillery.

2.2. *Evaluation of the contribution of precursors to EC formation*

Absolute ethanol was mixed with either urea or citrulline to a final concentration (65%, v/v) equal to that in the raw spirit. The pH of the solutions were adjusted to 4.5 ± 0.1 with 1.0 M hydrochloric acid (HCl) solution. The resulting solutions were heated at 85 °C for 40 min and then cooled at 4 °C before determination of EC.

The contribution of precursors in grains to EC formation during spirit distillation along with heat treatment was evaluated using a steaming model. To enhance the dissolution of water-soluble substances, 20 g of grains and 30 ml of water were combined and sonicated before mixing with ethanol (final concentration is 65%, v/w) and deionized water to a volume of 100 ml. These mixtures were heated with refluxing below 100 °C for 40 min in a water bath, and then the EC content was analyzed.

2.3. *Quantification of ethyl carbamate*

Ethyl carbamate was detected by GC–MS using a previously described method (the LOD for detection of EC using this method is 10 μg/l with the precision of 5 μg/l) (Kim, Park, Choi, & Kim, 2013; Xia et al., 2014) with minor modifications. Samples were mixed with *n*-propyl carbamate (2 mg/l), passed through a Celite545/Na_2SO_4 SPE column (CNW Technologies), and eluted with 5% (v/v) ethyl acetate–ether solution before quantification. An Rtx-wax capillary column (30 m × 0.25 mm, 0.25 μm; Restek, USA) was used for GC, and highly purified helium at a flow rate of 1 ml/min was used as the carrier gas. The oven program used consisted of holding at 50 °C for 5 min, an increase to 180 °C at 8 °C/min and then to 220 °C at 10 °C/min, and holding at 220 °C for 10 min. Major ions at *m/z* 62, 74, and 89 were monitored in SIM mode; *m/z* 62 was used as the quantitative ion. A 0.5 μl sample was injected for GC–MS analysis.

2.4. *Determination of urea*

Urea was analyzed using HPLC (the LOD for detection of urea using this method is 2 mg/l with the precision of 0.5 mg/l) after pre-column derivatization with 9-xanthydrol and hydrochloric acid in the dark (Wang, Wu, Zhou, & Chen, 2014). Assays were performed on an Agilent 1260 series instrument equipped with an Aquasil-C18 column (250 × 4.6 mm, 5 μm; Thermo, Germany) and a fluorescence detector (FLD, Agilent, USA) under the following conditions: mobile phase, 20 mM sodium acetate; flow rate, 1 ml/min; injection volume, 10 μl; temperature, 35 °C; excitation wavelength, 213 nm; emission wavelength, 308 nm.

2.5. *Determination of cyanide*

2.5.1. *Determination of cyanide*

Cyanide in raw spirit was determined using a packtest free cyanide WAK-CN kit (the LOD for detection of cyanide using this kit is 0.05 mg/l with the precision of 0.1 mg/l) (Kyoritsu, Japan) by following the instructions according to the manufacturer.

2.6. *Quantification of citrulline*

Citrulline was analyzed using HPLC (the LOD for detection of cyanide using this kit is 0.5 mg/l with the precision of 0.1 mg/l) after pre-column derivatization with *o*-phthaldialdehyde-3-mercaptopropionic acid (OPA) and 9-fluorenylmethyl-chloroformate (FMOC) (Araque, Bordons, & Reguant, 2013). Raw materials were ultrasonically dissolved using 10% trichloroacetic acid before analysis.

3. Results and discussion

3.1. *Variation of ethyl carbamate and its precursors in raw spirit during storage*

Urea and citrulline are often considered as common EC precursors in fermented beverages and foods (Ough, 1976). Raw spirit of Chinese spirits after the distillation process and those stored for 1 month to 12 years were analyzed to determine EC formation during storage. Both EC and precursors (urea and citrulline) were detected in raw spirit after distillation. We found that the EC content of raw spirit of Chinese spirits after the distillation process is 92 μg/l (Fig. 1). Urea and citrulline in raw spirit detected right after the distillation process were respectively present at 11.37 mg/l and 4.37 mg/l concentrations. In the first year of storage (the aging process), EC concentration of the raw spirit increased drastically (61 μg/l) and then slightly decreased with further aging. Urea and citrulline concentrations showed a decreasing trend during raw spirit storage because of their natural decomposition and EC formation. EC is not the only product that can form from urea and citrulline; urea and citrulline can undergo decomposition through other reactions (Hasnip, Caputi, Crews, & Brereton, 2004). The ethanol content of the raw spirit showed a slight decrease due to slow evaporation, which could slightly influence the rate of EC formation (Baffa, Mendonca, Pereira, Pereira, & Soares, 2011). The presence of EC and its precursors in the raw spirit before the aging process indicates that EC precursors come from the grains or fermented grains.