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Short communication

A survey of ethyl carbamate in beer from Chinese market

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ABSTRACT

Result of a survey of ethyl carbamate (EC) in beer was carried out by gas chromatography-mass spectrometry (GC/MS). 217 samples were purchased from supermarkets in five cities of China. The concentrations of EC ranged from n.d. to 19.6 μg/kg with an average level of 2.2 μg/kg, and positive rate was 95.4%. Mean in domestic beer (N = 142, incidence 97.3%) was 1.8 μg/kg, lower than the 2.9 μg/kg in imported beer (N = 75, incidence 94.4%). The result of domestic beer showed that EC level in canned beer (N = 73, mean = 1.5 μg/kg) was lower than that in bottled beer (N = 69, mean = 2.1 μg/kg). Additionally, EC level of beer from different countries was varied. Furthermore, a strong positive correlation of EC content in beer with alcohol content, storage time, temperature and light exposure was observed.

1. Introduction

Ethyl carbamate (EC), a genotoxic and carcinogenic compound, has widespread occurrence in fermented foods and beverages, suggesting a potential carcinogenic risk to human (Beland et al., 2005; Lachenmeier et al., 2010; Park et al., 2009). It can be absorbed rapidly by the gastrointestinal tract and skin. (Cha et al., 2000; EFSA, 2007). Recently, EC was reclassified as a group 2A carcinogen by the World Health Organization's International Agency for Research on Cancer (IARC, 2010). Joint FAO/WHO Expert Committee on Food Additives (JECFA) estimated the Benchmark Dose Lower Limit (BMDL) of EC is 0.3 mg/kg bw per day, and the average daily dietary intake (ADI) of EC from food is approximately 15 ng/kg bw per day. However, with inclusion of alcohol beverages, the estimated intake would increase to 80 ng/kg bw per day (FAO/WHO, 2006). High consumption of alcohol beverages could lead to higher intakes of EC.

Alcoholic beverages are considered as a main part of EC intakes, that has attracted a great deal of attention in many countries, and several countries have set limits on their levels. Canada, America and Brazil have enacted limit values of EC in table wines, fortified wines, spirits, sugarcane spirit cachaça and other alcoholic beverages (Conacher & Page, 1986; Lachenmeier et al., 2010). By now, no limit values of EC has been set in beer. In China, large numbers of beer was produced, imported and consumed every year. For instance, about 4715.25 million and 53.8349 million liters beer were produced or imported during 2015 (Wang, 2016). However, few research about EC levels of beer in Chinese markets was reported.

In this study, the method of GC/MS with internal standard of d5-ethyl carbamate was applied for the quantification of EC levels in beer from Chinese market, and the alkaline diatomite solid-phase extraction column was adopted for the analyte extraction. It aimed to clarify the content of EC in beer and provide data for authorities to risk assessment and evaluate establishment of maximum limits for EC in China.

2. Materials and methods

2.1. Samples

217 Beer samples were collected at local super markets from five cities (Beijing, Shanghai, Guangzhou, Qingdao and Shijiazhuang) of China. The samples consisted of 142 domestic beer and 75 imported beer. Production date of total samples was the year of 2016 and the best before date were 180 days or 360 days.

2.2. Materials

Ethyl acetate, methanol, *n*-hexane and aether were chromatographic grade and anhydrous sodium sulfate was analytical reagent purchased from Merck Co. (Darmstadt, Germany). Standard ethyl

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Table 1
EC recovery and relative standard deviation (RSD) (n = 3).

Sample	EC content (μg/kg)	Spiked level (μg/kg)	Determined result (μg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)
Pale lager	1.54	0.5	1.93	95.33	98.86	3.64
		1	2.45	96.67		
		2	3.49	99.33		
		4	5.55	103.33		
Black beer	2.90	0.5	3.28	95.86		
		1	3.81	96.90		
		2	4.85	98.28		
		4	7.05	105.17		

carbamate and d5-ethyl carbamate were obtained from Sigma-Aldrich (Madrid, Spain). Alkaline diatomite solid-phase extraction column (2000 mg filler per 10 mL) were provided by Bonna-Agela Tec. Inc. (Tianjin, China).

2.3. Sample pretreatment

Analyze of EC was based on the pretreatment method from Huang et al. (2013) with some modifications. All samples were analyzed three times, and the data were presented as the average values. After degassing, 5.0 g of sample combined with 100 μL of 0.5 μg/mL d5-ethyl carbamate and 0.3 g sodium chloride were added into centrifuge tube. After 1 min vortex, the mixture was poured into alkaline diatomite solid-phase extraction column. 10 mL of *n*-hexane was used to wash the SPE column after 10 min static duration. The analyte was eluted with 10 mL of 5% ethyl acetate in ether. The collected elution was dehydrated by anhydrous sodium sulfate and concentrated via N_2 flow at 45 °C. Finally, the analyte was diluted with methanol to 1 mL for GC/MS analysis.

2.4. GC/MS analysis

The method of GC/MS was carried out by a Thermo Trace 1300 GC with an ultra-ISQ MS (Thermo Fisher Scientific Inc., Waltham, MA, USA) instrument. The column was of 30 m × 0.25 mm i.d. × 0.25 μm DB-Innowax capillary column. Procedure of GC/MS was based on the described by Huang et al. (2013). EC was quantified using calibration curves constructed from the ethyl carbamate/internal standard peak area ratios (m/z 62 vs. 64) for standards containing 2, 5, 10, 20, 40 and 80 μg/L ethyl carbamate.

2.5. Method validation

For the validation of the method, a pale lager and a black beer sample were extracted and analyzed for seven times. For the determination of the recovery, the samples were spiked with 0.5, 1, 2, 4 μg/L of EC. The limit of detection (LOD) was obtained using free-EC beer sample spiked with EC at low concentration levels at a signal-to-noise (S/N) ratio of 3.

3. Results and discussion

3.1. Quality control

In the validation studies, the EC exhibited good linearity from 2 to 80 μg/L with a high correlation coefficient of 0.9996. The recovery of the GC/MS method ranged from 95.33% to 105.17%, the mean recovery and its RSD were 98.86% and 3.64%, respectively (see in Table 1). The RSD of both the intraday (n = 7) and interday (n = 3, 7d) precision were 3.8% and 5.6%. The LOD and the limit of quantification (LOQ) (normally based on S/N = 10) were 0.1 and 0.3 μg/kg of the method, respectively. Regarding the validation date, the presented GC/MS method was sensitive, selective and reproducible.

3.2. General investigation

Results for the beer samples were summarized in Table 2. In all 217 beer samples, the detected EC level ranged from n.d. to 19.6 μg/kg, with a mean level of 2.2 μg/kg 207 (95.4%) samples were found to be positive for EC, which indicated a rather high incidence of EC contamination. Mean level of domestic beer (N = 142) was 1.8 μg/kg, lower than that of imported beer (N = 75, 2.9 μg/kg). Similarly, incidence of EC was 97.3% in imported beer slightly above the 94.4% in domestic beer. This result contrasts with the study of Hasnip et al. (2007), showed that no EC was detected in beer (N = 9) in the United Kingdom. However, our result was in close agreement with survey of Wu, Pan, Wang, Shen, and Yang (2012), whose research indicated that the average level of EC contamination was 2 μg/kg in beer (N = 20) from Zhejiang (city of China), and the EC content was range from 2 to 3 μg/kg. Likewise, research of Mo, He, Xu, Huang, and Ren (2014) demonstrated that the positive rate of EC was 58.3% in 12 beer from Zhejiang and the EC contents ranged from <2 to 6 μg/kg, with mean of 2.7 μg/kg. EFSA suggested an EC average was 0.005 mg/L in beer (EFSA, 2007), which was higher than our survey.

Table 3 summarizes the distribution of EC levels in beer tested and showed that EC levels of 85.7% samples (n = 186) were below 3.0 μg/kg, 90.8% (n = 129) and 76.0% (n = 57) in domestic and imported samples. Likewise, a majority of samples tested (n = 87, 40.1%) had EC levels between 1.0 and 2.0 μg/kg; in

Table 2
The levels of EC in beer from markets in China.

Category	No. of positives/total samples[a] (%)	Mean[b] (μg/kg)	Median[b] (μg/kg)	P_{95} (μg/kg)	Range[c] (μg/kg)
Total beer	207/217 (95.4)	2.2	1.7	5.8	n.d.–19.6
Domestic beer	134/142 (94.4)	1.8	1.5	4.3	n.d.–19.6
Imported beer	73/75 (97.3)	2.9	2.3	10.0	n.d.–10.7

Notes:
[a] In brackets: % percentage.
[b] Concentration of samples with no detectable EC is considered as LOD/2 (0.05 μg/kg) for the mean and median estimation.
[c] n.d. = not detected (<0.1 μg/kg).