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Research progress on ethyl carbamate in food

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Abstract. Ethyl Carbamate (EC) is a multi-site carcinogen produced in the fermentation and storage process of food, which is widely found in soy sauce, wine (liquor, rice wine, wine, etc.), cereal or bean fermented food, lactic acid bacteria fermented beverage and other products. EC is able to be quickly and completely absorbed by the gastrointestinal tract and skin, which poses a potential threat to the life and health of consumers. This paper systematically reviews the formation mechanism, limit standards and detection methods of EC in food in recent years, aiming to provide a scientific basis for the food industry to reduce the impact of EC on public health.

Keywords: food, ethyl carbamate, detection method

1. Introduction

Ethyl Carbamate (EC), molecular formula $H_2NCOOC_2H_5$, has the chemical structure shown in Figure 1. Also known as urethan/urethane, EC is a process contaminant that forms naturally during food fermentation and storage. In 2007, the World Health Organization's International Agency for Research on Cancer (IARC) re-evaluated EC as a Group 2A substance, "probably carcinogenic to humans" [2]. EC is widely present in soy sauce; alcoholic beverages (including baijiu, huangjiu, and wine); fermented foods made from grains or legumes; and lactic acid bacteria–fermented drinks [1]. Experimental data indicate that EC is rapidly and completely absorbed through the gastrointestinal tract and the skin; long-term exposure may cause neurological disorders and increase the risk of cancers at multiple sites (e.g., lung, lymphatic system, liver, and skin), posing a potential serious threat to human health [3, 4]. Accordingly, this paper systematically reviews, in recent years, the formation mechanisms of EC in foods, international limit standards, and detection techniques, with the aim of providing a scientific basis for the food industry to effectively reduce EC's impact on public health.

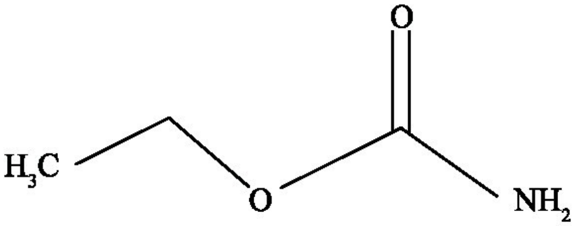

Figure 1. Chemical structure of EC

2. Formation mechanisms and limit standards for EC in foods

2.1. Formation mechanisms of EC in foods

Multiple precursors of EC have been identified in foods and alcoholic beverage systems, chiefly urea, citrulline, carbamyl phosphate, diethyl pyrocarbonate, and cyanide. Depending on the fermentation characteristics of different foods, EC forms through the following pathways: reaction of urea with ethanol; reaction of carbamyl phosphate with ethanol; reaction of citrulline with ethanol; reaction of diethyl pyrocarbonate with ammonia; and reaction of cyanide with ethanol. Notably, urea, carbamyl phosphate, and citrulline—typical carbamoyl compounds—are mainly found in fermented alcoholic beverages such as wine, huangjiu (Chinese yellow rice wine), and beer; in contrast, the dominant EC precursor in distilled spirits such as brandy and baijiu is cyanide, with EC formation occurring primarily during distillation and storage [5–7].

2.1.1. Urea reacting with ethanol to form EC

Urea is the most important precursor of EC. In fermented foods and alcoholic beverages, EC forms mainly via the reaction between urea and ethanol. On the one hand, urea largely originates from the agricultural use of nitrogen fertilizers; on the other hand, during food processing—such as in huangjiu production—yeast can generate urea by degrading arginine [8]. EC formation is also affected by storage and fermentation temperature, yeast strain, and other factors; temperature exerts a particularly strong influence, and studies show a linear relationship between EC formation rate and temperature [9]. Therefore, maintaining an appropriate storage temperature is critical for suppressing EC formation.

$$H_2NCONH_2 + C_2H_5OH \rightarrow H_2NCO_2C_2H_5 + NH_3 \tag{1}$$

2.1.2. Citrulline reacting with ethanol to form EC

Studies have shown that, in wine, citrulline is an EC precursor that can react directly with ethanol to form EC [10] as shown in Reaction (2). Citrulline arises from arginine metabolism during winemaking; however, the citrulline content in wine does not show a linear correlation with EC levels.

$$H_2NCONH(CH_2)3CH(NH_2)COOH + C_2H_5OH \rightarrow H_2NCO_2C_2H_5 + H_2N(CH_2)3CH(NH_2)COOH \tag{2}$$

2.1.3. Carbamyl phosphate reacting with ethanol to form EC

Carbamyl phosphate is another EC precursor in fermented alcoholic beverages. Ough [11] elucidated this mechanism, as shown in Reaction (3).

$$H_2NCO_2PO_2H_2 + C_2H_5OH \rightarrow H_2NCO_2C_2H_5 + H_3PO_4 \tag{3}$$

Research indicates that, in the late stage of alcoholic fermentation during winemaking, citrulline, carbamyl phosphate, urea, and other metabolites from arginine degradation undergo slow chemical reactions with ethanol to generate EC, with urea contributing the most.

2.1.4. Cyanide reacting with ethanol to form EC

When cyanide serves as the precursor—typical in distilled spirits—cyanide is first oxidized to cyanate, which then reacts with ethanol to yield EC, as shown in Reactions (4) and (5):

$$HCN \rightarrow HOCN \tag{4}$$

$$HOCN + C_2H_5OH \rightarrow H_2NCO_2C_2H_5 \tag{5}$$

2.1.5. Diethyl Pyrocarbonate (DEPC) interacting with ammonia to form EC

In production, DEPC has been added to wine to remove bacteria and yeasts. In 1971, Lofroth et al. discovered that adding DEPC to white wine leads to EC formation in the presence of ammonia, as shown in Reaction (6); consequently, the addition of DEPC to wine is prohibited [10].

$$O(CO_2C_2H_5)_2 + NH_3 \rightarrow H_2NCO_2C_2H_5 + CO_2 + C_2H_5OH \tag{6}$$

2.2. Limit standards for EC in foods

Since the 1970s, researchers have successively detected Ethyl Carbamate (EC) in a variety of fermented foods and products—alcoholic beverages, soy sauce, pickles, yogurt, etc.—and its detection range has even been extended to non-fermented products such as bread and cigarettes [12–15]. Wang Lingli's research group [16], through a systematic study of beverage alcohols, fermented wheat products, and soybean products, reported a 100% detection rate of EC in huangjiu (Chinese yellow wine), a figure that convincingly confirms the widespread occurrence of EC contamination in alcoholic beverages. It is noteworthy that drinking has been identified as the primary route of human exposure to EC [2]. In response to this risk, regulatory authorities in various countries have progressively strengthened monitoring of EC levels in alcoholic beverages. Historically, developed countries such as Canada, the United States, and Japan were among the first (in 1985) to establish EC limit standards for alcoholic products [17, 18] (see Table 1). The 2002 international standard issued by the Food and Agriculture Organization of the United Nations (FAO) was a milestone, specifying that EC concentrations in foods should not exceed 20 μg/L [19]. However, it should be noted that, to date, China has not issued national EC limit standards for alcoholic beverages or foods.

Table 1. National limits for EC in beverage spirits (μg/L)

Country	Distilled spirits	Fruit brandy	Fortified wine	Sake (clear rice wine)	Wine
Canada	150	400	100	200	30
Czech Republic	150	400	100	200	30
Japan	150	400	100	100	30
France	150	1,000	-	-	-
United States	-	-	60	-	15
Germany	-	800	-	-	-
Switzerland	-	1,000	-	-	-
United Kingdom	-	1,000	-	-	-
Brazil	150	-	-	-	-
South Korea	-	-	-	-	30

Note: "-" indicates no regulation specified to date.

3. Research on detection methods for EC in foods

Since the International Agency for Research on Cancer (IARC) classified Ethyl Carbamate (EC) as a Group 2A carcinogen in 2007, research on analytical methods for EC has grown explosively. Method development faces two principal challenges: first, the typical EC concentrations in fermented foods are in the range of ng/mL to μg/mL; second, fermented-food matrices contain abundant interfering substances, and this complex chemical background requires analytical instruments with both high sensitivity and high selectivity. Against this background, modern analytical chemistry has developed several mature detection approaches, mainly including Gas Chromatography–Mass Spectrometry (GC/MS) [20], Gas Chromatography (GC) [21, 22], High-Performance Liquid Chromatography with Fluorescence Detection (HPLC-FLD) [23], gas chromatography–tandem mass spectrometry (GC/MS/MS) [24, 25], and Fourier-Transform Infrared Spectroscopy (FTIR) [26]. Each technique has its strengths; among them, chromatographic methods coupled with mass spectrometry—because of their superior separation capability and structural identification power—have become the mainstream approaches for EC detection.

3.1. Gas Chromatography-Mass Spectrometry (GC/MS)

Gas Chromatography-Mass Spectrometry (GC/MS) is regarded as the classical technique for detecting Ethyl Carbamate (EC) and has been included in China's National Food Safety Standard: Determination of Ethyl Carbamate in Foods (GB 5009.223–2014) as a standard method. This approach combines two technical advantages: it retains the speed and low operating cost of Gas Chromatography (GC) while harnessing the powerful structural identification capacity of Mass Spectrometry (MS), thereby enabling precise identification of target components from complex food matrices. However, it should be noted that GC/MS requires stringent sample pretreatment, and interference introduced during this process may significantly affect the accuracy of final results.

Currently, pretreatment methods used in GC/MS detection of EC mainly include Solid-Phase Extraction (SPE) [27], solid–liquid extraction, Liquid-Liquid Extraction (LLE) [28], and Solid-Phase Microextraction (SPME) [29]. Huang's research team [30] developed an optimized procedure for detecting EC in soy sauce and alcoholic beverages: samples were purified using diatomaceous earth SPE columns, with a mixed solution of ethyl acetate/ether (5:95, v/v) as the eluent. Validation data showed a quantitative detection limit of 5.0 μg/kg, an average recovery rate of 96.7%, and a Relative Standard Deviation (RSD) below 5%. Repeatability and reproducibility RSDs were 1.2-7.8% and 2.3-9.6%, respectively, demonstrating good reliability and reproducibility of the method.

Tan and colleagues [31] further optimized GC/MS detection protocols for fermented food matrices. Their method involved dissolving samples in saturated NaCl solution, ultrasonic extraction, purification with diatomaceous earth SPE columns, elution with dichloromethane, and the use of butyl carbamate as an internal standard. Qualitative and quantitative analyses were performed under GC/MS Selected Ion Monitoring (SIM) mode. Method validation indicated a good linear relationship within the 21-846 ng/mL concentration range ($r^2 > 0.99$), a detection limit of 5.0 ng/mL, and spiked recovery rates of 82.0%-109.7%, confirming the method's suitability for accurate detection of EC in a wide variety of fermented foods.

Although these pretreatment techniques are widely applied, they require large amounts of organic solvents, which not only pose health risks to laboratory personnel but also cause environmental pollution. To address this, Ma and colleagues [32] modified conventional solid-phase extraction by developing a novel liquid-liquid extraction Headspace Single-Drop Microextraction Technique (HS-SDME) for extracting EC from wine. The experimental setup is shown in Figure 2. On the basis of traditional headspace SDME, a matrix modifier was added to the analyte. This not only absorbs water in the sample—thus protecting the instrument from damage—but also adjusts the pH, enhances the ionic strength of EC, and shortens the enrichment time for the single droplet. Enrichment could be completed within 3 minutes, making it a simple, rapid, and accurate method for directly determining EC in food samples with high sensitivity.

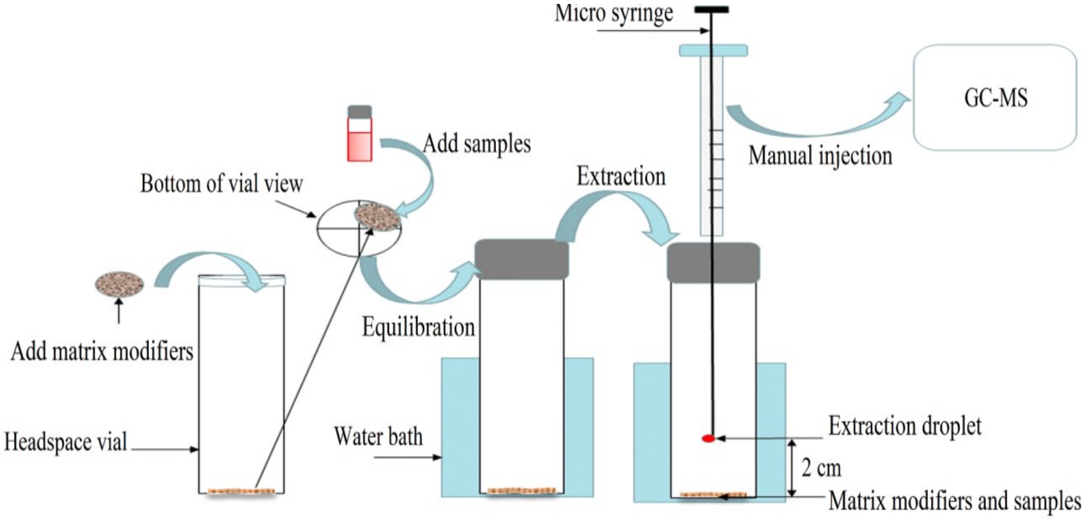

Figure 2. Experimental setup of HS-SDME

3.2. Gas Chromatography (GC)

Gas Chromatography (GC), as one of the foundational methods for EC detection, reflects the historical trajectory of methodological advancements in this field. Ma and colleagues [22] pioneered the use of two-dimensional chromatography for the direct quantitative analysis of EC in Chinese baijiu. Using ethyl n-propyl carbamate as the internal standard, the method required only a 2 μL injection to complete the detection. Technical validation indicated a detection limit of 13 μg/L and a Relative Standard Deviation (RSD) of 7.7%, thereby establishing its role as a routine method for EC determination in baijiu. In terms of technical evolution, Ma's team [21] further developed a multidimensional chromatography-Thermal Ionization Detector (TID) coupling technique, which enhanced sensitivity to 1 μg/L (RSD = 2%), significantly expanding the applicability of GC in monitoring fermented alcoholic beverages.

At the international level, a landmark study was conducted by Canas and colleagues [33], who organized a multinational collaborative effort involving 17 laboratories across six countries, including the United States, Japan, and the United Kingdom. This study employed GC/MS under Selected Ion Monitoring (SIM) mode, combined with diatomaceous earth extraction columns and a dichloromethane extraction system, using n-Propyl Carbamate (nPC) as the internal standard. Systematic analyses of distilled spirits, fortified wines, and other alcoholic beverages yielded stable recovery rates of 87%–93%, providing critical data support for the establishment of international standards for EC detection.

Nevertheless, it must be objectively noted that GC has significant limitations in application scope. According to Scopus database statistics, approximately 87% of GC-based EC studies focus on alcoholic beverages. Two primary technical bottlenecks account for this restriction: (1) the interference posed by the complex matrices of non-alcoholic fermented foods (e.g., soy sauce, fermented bean curd); and (2) the stringent qualitative requirements associated with trace-level EC detection (typically < 10 μg/kg). Since GC relies solely on retention time for qualitative identification, co-elution of multiple components can reduce qualitative accuracy by 23%-45% compared with LC-MS/MS methods. This explains why the application rate of GC in EC detection for complex-matrix foods such as soy sauce and vinegar remains below 15%.

3.3. High-Performance Liquid Chromatography with Fluorescence Detection (HPLC-FLD)

Abreu and colleagues [34] were the first to apply HPLC-FLD to EC detection in alcoholic beverages. However, the method demonstrated relatively poor accuracy, particularly in dry red and dry white wines, where standard deviations of detection accuracy ranged from 10.9% to 74.6%. Consequently, the method did not achieve widespread adoption. Subsequently, Gao and colleagues [19] applied HPLC-FLD to quantify EC in brandy. Their procedure involved mixing 500 μL of the sample with 9-hydroxyanthracene solution and 50 μL of 1.5 mol/L HCl. The mixture was allowed to react in the dark, generating fluorescent derivatives, which were then excited at 234 nm and detected at 600 nm after a 5-minute reaction time. The method showed excellent repeatability: intra-day RSD was 0.37%, inter-day RSD was 3.06%, and recovery rates ranged from 93.0% to 107%. Compared with GC/MS, HPLC-FLD is simpler, faster, and more cost-effective, making it particularly suitable for determining EC content in baijiu.

3.4. Gas Chromatography-Tandem Mass Spectrometry (GC/MS/MS)

In the evolution of EC detection technologies, Gas Chromatography-Tandem Mass Spectrometry (GC/MS/MS) represents the most advanced analytical platform to date. Compared with conventional GC and GC/MS methods, its advantages are threefold: first, the Multi-Reaction Monitoring (MRM) mode provided by tandem mass spectrometry effectively eliminates matrix interferences; second, stronger ion selectivity improves quantitative accuracy by over 30%; and most importantly, its sensitivity to trace EC (< 0.1 μg/mL) is significantly superior to other methods.

A seminal study by the Lachenmeier team [24] provided critical evidence: by systematically comparing GC/MS and GC/MS/MS data across more than 70 alcoholic beverages, they demonstrated that when using *m/z* 44 as the quantification ion, the GC/MS/MS calibration curve achieved a regression coefficient of 1.000 (ideal value). Moreover, the method reduced the Limit of Detection (LOD) and Limit of Quantification (LOQ) to 0.01 μg/mL and 0.04 μg/mL, respectively—about two orders of magnitude lower than conventional GC/MS. This study was the first to confirm that GC/MS/MS could completely eliminate the common co-elution interference peaks observed in GC/MS for complex alcohol matrices.

In subsequent optimization, Lachenmeier and colleagues [25] achieved three breakthroughs: (1) incorporating Headspace Solid-Phase Microextraction (HS-SPME) as a pretreatment step, eliminating the need for time-consuming liquid extraction; (2) establishing an automated detection workflow that reduced single-sample analysis time by 60%; and (3) although the LOD slightly increased to 0.03 μg/mL (LOQ = 0.11 μg/mL), sample throughput was significantly improved. This "moderate sensitivity for high efficiency" approach is particularly suitable for large-scale screening of alcoholic beverages.

3.5. Fourier Transform Infrared Spectroscopy (FTIR)

Given that EC contains distinctive functional groups such as C=O and NH_3, it exhibits characteristic absorption peaks in infrared spectra. Manley et al. [35] employed Fourier Transform Near-Infrared Spectroscopy (FT-NIR) combined with principal component analysis (PCA) to detect EC in table wines, achieving a recognition rate of 88%. Similarly, Lachenmeier et al. [26] applied FTIR coupled with Partial Least Squares (PLS) regression for rapid screening of stone-fruit-based alcoholic beverages. Their method enabled detection within 2 minutes, without the need for reagent consumption or cumbersome sample pretreatment, highlighting its environmentally friendly advantages. However, the precision was limited, restricting the method to semi-quantitative screening applications.

3.6. Raman Spectroscopy

Surface-Enhanced Raman Spectroscopy (SERS), an emerging technique that integrates Raman spectroscopy with nanotechnology, has been widely applied to qualitative and quantitative detection in food safety [36]. Li et al. [37] used star-shaped silver nanoparticles (Ag NSs) as a novel SERS substrate and compared them with other plasmonic nanomaterials (Au NPs, Au NSs, and Ag NPs). Results indicated that Ag NSs exhibited the highest SERS activity, markedly enhancing the Raman signal of EC by several orders of magnitude at trace levels. Furthermore, the Raman intensity of EC showed a strong linear correlation with concentration. With its simplicity, rapidity, and low cost, this approach provides a feasible pathway for highly sensitive EC detection.

4. Conclusion and outlook

In summary, Ethyl Carbamate (EC) poses a potential threat to human health. This paper reviewed the formation mechanisms, regulatory limits, and analytical methods of EC in food, emphasizing the importance of monitoring its levels. Although current instruments and analytical techniques are sufficient for EC detection, conventional large-scale equipment still suffers from high costs, expensive operation, complex pretreatment procedures, and strict requirements for skilled personnel. Future research on EC should therefore focus on the development of green, efficient, and rapid detection technologies. In addition, more attention should be directed toward targeted removal of EC from fermented foods, as well as strategies to control or prevent its formation. A gradual improvement in risk assessment methods is also necessary, particularly through studies on the transformation relationships between EC and its precursors, in order to provide more scientific and reliable risk evaluations. Such research will offer a solid scientific basis and technical support for EC control in the food industry.

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