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Review

Ethyl carbamate: An emerging food and environmental toxicant

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ABSTRACT

Ethyl carbamate (EC), a chemical substance widely present in fermented food products and alcoholic beverages, has been classified as a Group 2A carcinogen by the International Agency for Research on Cancer (IARC). New evidence indicates that long-term exposure to EC may cause neurological disorders. Formation of EC in food and its metabolism have therefore been studied extensively and analytical methods for EC in various food matrices have been established. Due to the potential threat of EC to human health, mitigation strategies for EC in food products by physical, chemical, enzymatic, and genetic engineering methods have been developed. Natural products are suggested to provide protection against EC-induced toxicity through the modulation of oxidative stress. This review summarizes knowledge on the formation and metabolism of EC, detection of EC in food products, toxic effects of EC on various organs, and mitigation strategies including prevention of EC-induced tumorigenesis and genotoxicity by natural products.

1. Introduction

Ethyl carbamate (EC), also referred to as urethane, is an ethyl ester of carbamic acid. EC has been found in many fermented food products and alcoholic beverages such as cheese, bread, yoghurt, wine, whisky, soya sauce etc. (Li, Zhong, Wang, & Gao, 2017; Weber & Sharypov, 2009). An *in vitro* study indicated that EC has a potential to inhibit the growth of bacteria, plant tissue, and rat carcinoma. EC has been used for many years as an antineoplastic agent for medical purposes (Zhao et al., 2013). Since EC can produce long lasting anaesthesia without affecting blood gases or blood pressure, it has been used in acute studies (Thompson & Wasterlain, 2001). In earlier studies, EC was also used as a co-solvent for water-insoluble analgesic and sedative drugs in Japan (Nomura, 1975). Since EC was found to be involved in the development of benign and malignant tumours, especially lung tumours, EC-induced adenocarcinoma became an accepted model to study human adinocarcinomas (Narayan & Kumar, 2012).

Humans can be exposed to EC through fermented foods, and alcoholic beverages. EC is a by-product in food industries where fermentation, distillation, and long-term storage are involved (Hasnip, Caputi, Crews, & Brereton, 2004). EC has been considered as a most likely possible carcinogenic substance and a probable health risk for certain types of regular alcoholic beverage drinkers (Lachenmeier et al., 2010).

Metabolism studies using rodents have shown that approximately 90% of EC is hydrolyzed to ethanol, ammonia, and carbon dioxide by liver microsomal esterases, with only 5% of EC excreted as unchanged. Only very small amounts of EC are converted by cytochrome p-450 to form vinyl carbamate (approximately up to 0.5%), α-hydroxy ethyl carbamate, and ethyl-*N*-hydroxycarbamate (approximately 0.1%) (Weber & Sharypov, 2009). Cellular metabolism of EC is associated with oxidative stress (OS) and DNA damage. Formation of covalent bonds between EC metabolites and DNA, RNA and proteins have been described (Zhao et al., 2013).

Urea, citrulline, cyanogen, carbamyl phosphate and diethyl pyrocarbonate are the major EC precursors (Zhao et al., 2013). Thermal decomposition of urea forms cyanic acid, which further reacts with ethanol to form EC (Weber & Sharypov, 2009). Cyanide is formed in spirits by enzymatic action and thermal cleavage of cyanogenic glycosides. The reaction can be catalyzed by Cu^{II} ions (which are commonly found in fermented beverages). Complexation of cyanide with Cu^{II} forms cyanogen followed by oxidation. Subsequent disproportionation leads to cyanate and cyanide, then the cyanate reacts with ethanol to form EC (Weber & Sharypov, 2009). Furthermore, excessive amounts of nitrogen fertilizers on cropland can increase the concentration of EC precursors in raw materials, which increase the chances of EC production during fermentation (Jiao, Dong, & Chen, 2014). Human exposure to EC is low but long-term exposure can threaten human health. Efforts have therefore been made to decrease EC production during

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fermentation by the use of enzymes that degrade precursors of EC and by genetic modification of fermentative strains.

In this review, we describe the formation of EC in food, analytical methods for the detection and quantification of EC and strategies preventing EC-induced toxicity. Because EC-induced lung cancer served as a model for human lung cancer, a detailed account on EC-induced lung cancer is provided. We also describe the toxicity of EC to other organs and its modulation by natural products.

2. Ethyl carbamate in fermented food and spirits, precursors and mechanisms of formation

A large number of studies revealed the presence of EC in alcoholic beverages and fermented food products. Concerning the high amount of EC found in wines, for the first time, the Canadian government established a maximum residue level (MRL) for EC in alcoholic beverages as follows: wine 30 μg/l, fortified wine 100 μg/l, distilled spirits 150 μg/l, sake 200 μg/l and fruit brandies 400 μg/l (Conacher et al., 1987). US government set the MRL for EC in table wines and strong spirits as 15 μg/l and 100 μg/l, respectively. Brazilian legislation set up the MRL for EC as 150 μg/l for cachaça (Lachenmeier et al., 2010). The MRL for EC in Korea was limited to grape wine (30 μg/l). Japan, Germany, Australia and Argentina also set up the MRL for EC in alcoholic beverages. In China, a large number of fermented food products and alcoholic beverages were produced annually. Although a high level EC was found in regularly consumed Chinese rice wines, up to now no regulations for EC is implemented (Chen et al., 2017). According to Joint FAO/WHO Expert Committee on Food Additives (JECFA), intake of EC from other food sources is lower than that from alcoholic beverages. However, combined exposure to EC should not be neglected (European Food Safety Authority, 2007).

Many precursors including cyanogenic glycosides, urea, arginine, and citrulline can generate EC during fermentation (Weber & Sharypov, 2009) (Fig. 1). Factors such as temperature, acidity, properties of the microorganisms used, affect the production of EC during fermentation (Zhao et al., 2013). The availability of EC precursors depends on the nature of the microorganism used in fermentation. *Saccharomyces cerevisiae* degrades arginine to urea and *Lactobacilli* convert arginine into citrulline (Fig. 1) (Benucci, Fiorelli, Lombardelli, Liburdi, & Esti, 2017; Jiao et al., 2014), both of which react with ethanol producing EC.

EC can be formed from cyanogenic glycosides at various stages of fermentation and distillation. Stone fruits are a rich source of cyanogenic glycosides. Enzymatic degradation of cyanogenic glycosides forms hydrocyanic acid which further oxidized to form cyanate. Cyanate reacts with ethanol to form EC (Fig. 1) (Zhao et al., 2013).

Urea can be found as an EC precursor in raw materials and it can also be formed by the degradation of arginine by yeasts (Fig. 1). Degradation of arginine by yeasts results in urea, which reacts with ethanol producing EC (Weber & Sharypov, 2009).

Citrulline is a major precursor for the synthesis of EC (Fig. 1). Arginine deiminase pathway mediates the EC production from citrulline. Three enzymes such as arginine deiminase (ADI), ornithine transcarbamylase (OTC) and carbamate kinase (CK) catalyze the degradation of L-arginine. EC is produced from two intermediates: citrulline and carbamyl phosphate (Jiao et al., 2014).

3. Determination of ethyl carbamate

3.1. Sample preparation and extraction

Sample preparation prior to EC determination includes various strategies based on the nature of the sample and its composition such as non-fatty liquid, alcoholic beverage, proteinous liquid and non-fatty solid. In addition, the concentration of the internal standard used in the sample preparation prior to EC extraction is varied according to the food composition (Ryu et al., 2015).

Liquid-liquid extraction (LLE), solid phase extraction (SPE) and solid phase microextraction (SPME) are used for the extraction of EC from food matrices. Dichloromethane (methylene chloride) was the most commonly used organic solvent for extraction in LLE along with other solvents. However, LLE method is time-consuming and requires a large amount of organic solvent with low reproducibility (Jiao et al., 2014). SPE was developed in which Extrelut® chromatography sorbent (wide pore diatomaceous earth) was most prevalently used by various

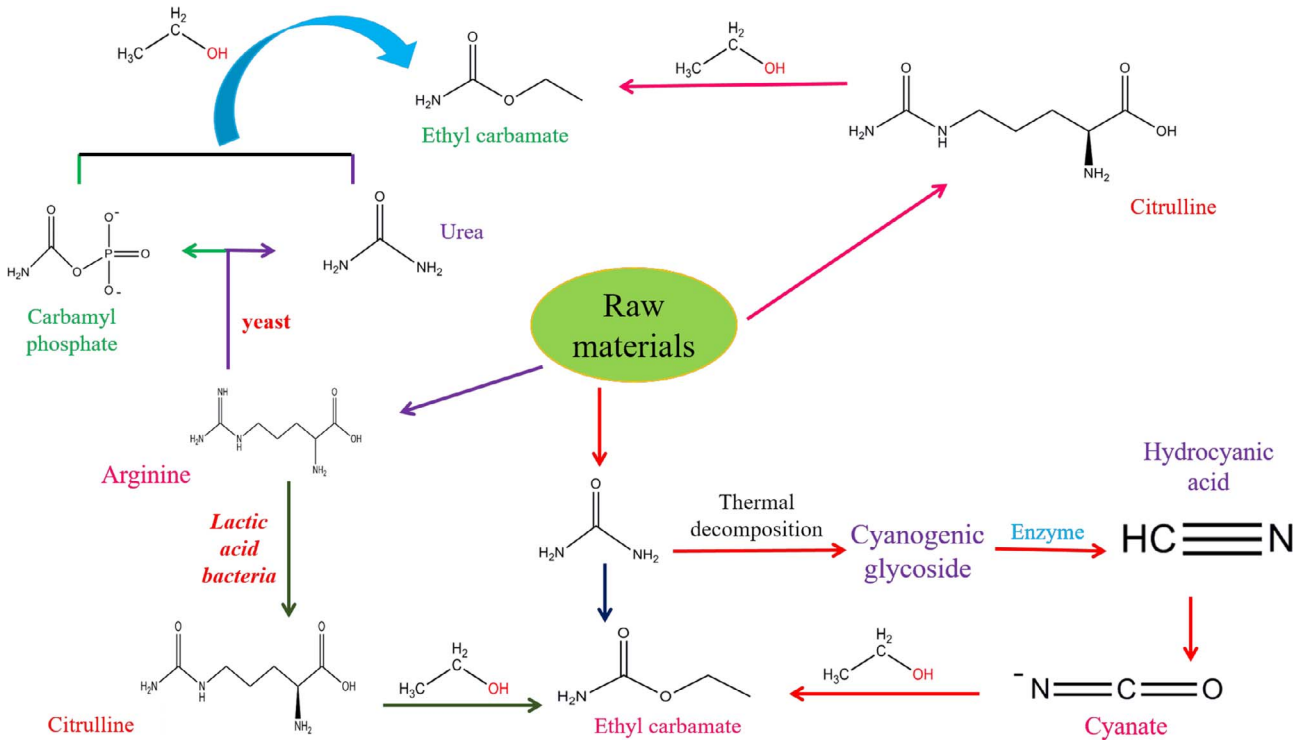

Fig. 1. Precursors and mechanism of EC formation.