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Review

Review of the Characteristics of Food-Derived and Endogenous N_ε-Carboxymethyllysine

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ABSTRACT

N_ε-Carboxymethyllysine (CML), a representative of advanced glycation end products (AGEs), is commonly found in food and is considered a potential hazard to human health. Food scientists have begun to investigate the formation of CML in food processes. As the understanding of CML is mainly based on that of endogenous CML from the fields of biology and medicine, this review summarizes the different characteristics of food-derived CML and endogenous CML with respect to food safety, detection methods, formation environment, formation mechanism, and methods for inhibiting the formation of CML. Additionally, future research directions for the study of food-derived CML are proposed, including understanding its digestion, absorption, and metabolism in human health, developing rapid, reliable, and inexpensive detection methods, revealing its relationship with food components and production processes, and controlling the formation of CML through the addition of inhibitors and/or modification of food processing conditions, so as to contribute to the methods for controlling food-derived AGEs.

The International Diabetes Federation estimates that the number of people with diabetes globally reached a staggering 366 million in 2011. Deaths due to diabetes have increased to around 4.6 million per year, and yearly health care spending on diabetes has reached $465 billion. Diabetes, with its complications, including atherosclerosis, nephropathy, neuropathy, and retinopathy, poses a threat to human health due to mortality, morbidity, and medical expenses. Consequently, diabetes is a world-wide problem. Advanced glycation end products (AGEs) play a role in diabetes pathogenesis, but the exact mechanism is still under debate. AGEs are generated in late stages of the Maillard reaction between carbonyl groups (carbohydrates and lipids) and amino groups (proteins and nucleic acids). There are several major kinds of AGEs, including N_ε-carboxymethyllysine (CML), N_ε-carboxyethyllysine, pentosidine, vesperlysine, pyrraline, and imidazolone. Presently, research on AGEs is mainly focused on endogenous AGEs in biology and medicine. It has been shown that AGEs exist in lesion tissue *(45),* plasma *(26, 30),* urine *(35),* and tears *(74)* of diabetic-complication patients, and the concentrations of AGEs in the human body increase with age *(24, 37, 50, 51, 69)*. Endogenously produced AGEs do harm to human health in four ways *(39)*: by changing protein function through modification *(13, 32),* by crosslinking proteins to stiffen tissue *(44, 70),* by inducing an inflammatory response by binding to the specific receptor for AGEs *(5, 56),* and by inducing radical formation, which accelerates body aging.

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Some endogenous AGEs have been isolated and their chemical structures identified (Fig. 1), which offers targets for food-derived AGE research. CML can be considered a representative of food-derived AGEs for two reasons. It is widely distributed in many varieties of food, and its safety is questioned. Therefore, investigating the mechanism of formation of food-derived CML in the food production process could contribute to a solution for controlling these potential hazards.

Therefore, the objective of this review is to summarize the characteristics of food-derived CML in comparison with those of endogenous CML as to food safety, detection methods, formation environment, formation mechanisms, and methods for inhibiting formation. Additionally, future directions for contributing to methods for controlling food-derived AGEs will be discussed.

FOOD SAFETY ASPECTS

Recently, food scientists were surprised to find that food-derived CML is widely distributed in the diet. Goldberg et al. *(23)* compared the amounts of CML in 250 kinds of food, classified by fat, protein, and carbohydrate, and found that CML existed in many foods (Table 1). Uribarri et al. *(67)* expanded the available CML database with 546 kinds of food to further confirm its wide distribution.

The safety of food-derived CML has been under investigation since confirmation of the presence of food-derived CML in the body's circulation. Koschinsky et al. *(31)* examined the oral absorption and renal clearance

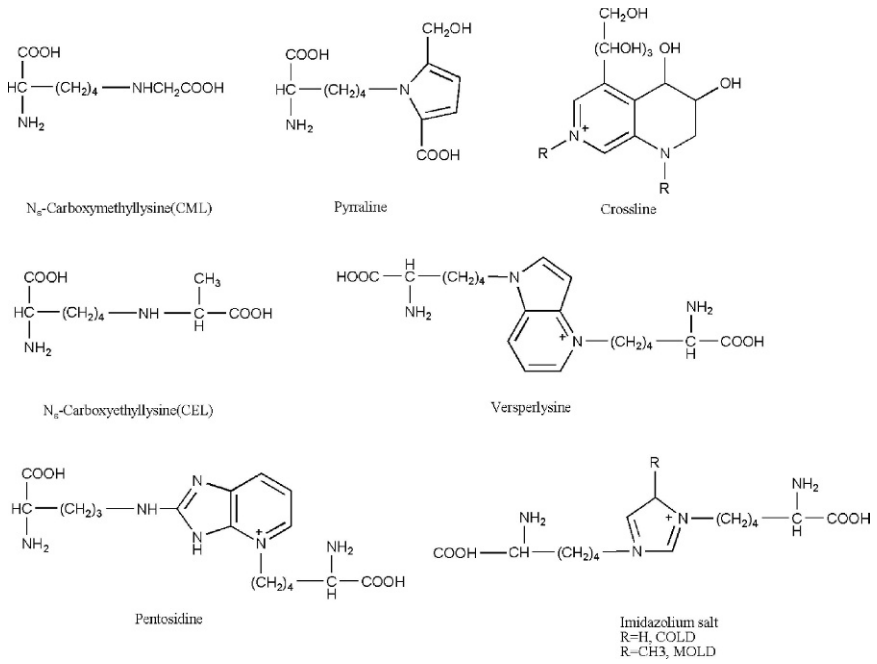

FIGURE 1. *Chemical structural formulas of some typical AGEs.*

TABLE 1. *AGE content of selected foods prepared by standard cooking methods (23)*

Food item	AGE (kU/g or ml of food)[a]
Fats	
Almonds, roasted	66.5 kU/g
Oil, olive	120 kU/ml
Butter	265 kU/g
Mayonnaise	94 kU/g
Proteins	
Chicken breast, broiled for 15 min	58 kU/g
Chicken breast, fried for 15 min	61 kU/g
Beef, broiled for 1 h	22 kU/g
Beef, broiled for 15 min	60 kU/g
Tuna, roasted for 40 min	6 kU/g
Tuna, broiled for 10 min	51 kU/g
Cheese, American	87 kU/g
Cheese, Brie	56 kU/g
Egg, fried	27 kU/g
Egg yolk, boiled	12 kU/g
Tofu, raw	8 kU/g
Tofu, broiled	41 kU/g
Carbohydrates	
Bread, whole-wheat center	0.54 kU/g
Pancake, homemade	10 kU/g
Milk, cow, whole	0.05 kU/ml
Milk, human, whole	0.05 kU/ml
Enfamil (infant formula)	4.86 kU/ml
Apple	0.13 kU/g
Banana	0.01 kU/g
Carrots	0.1 kU/g
Green beans	0.18 kU/g

[a] AGE, CML assessed by ELISA based on a monoclonal antibody and expressed as AGE units per ml or g of food.

kinetics of food-derived CML in diabetics and found that the renal excretion of orally absorbed CML was markedly suppressed in diabetic nephropathy patients. Somoza et al. *(54)* found that food-derived CML was bound to kidney cells but rapidly excreted after oral administration to rats. Šebeková et al. *(49)* analyzed the CML levels in plasma, urine, human breast milk, and commercial infant formulas and concluded that CML is absorbed into the circulatory system and rapidly excreted in the urine. Thus, food-derived CML probably has an effect on human health, as does endogenous CML. In reality, the effect of food-derived CML on human health is not fully understood, despite the animal and clinical experiments that have been conducted *(7, 8, 28, 53, 64, 73)*.

In the area of animal experimental aspects, Šebeková et al. *(48)* investigated the dietary effect on nephrectomized rats and found that dietary CML induced an increase in proteinuria, indicating its detrimental effect on the kidney. Lin et al. *(34)* found an association between dietary CML and neointimal formation after arterial injury in hypercholesterolemic mice and that restricting dietary CML could prevent restenosis after angioplasty. Zheng et al. *(75)* found that excessive intake of dietary CML was a major contributor to diabetic nephropathy in mice. In the area of clinical experiments, Uribarri et al. *(66)* determined the CML levels in blood and urine of nondiabetic renal failure patients and found that low dietary AGE intake decreased serum CML. Uribarri et al. *(65)* also found that dietary CML was an important contributor to excess serum CML levels in patients with renal failure. Although food-derived CML is considered a potential hazard to human health, the molecular mechanisms of food-derived CML in human health still remain unclear. Therefore, understanding its digestion, absorption, and metabolism in human health will be one of the future research directions needed.

TABLE 2. *Summary of reports in the literature of CML determination methods for various samples*

Sample	Analytical method	Reference
Endogenous CML		
Human breast milk and serum	ELISA	*15*
Heart, renal, and lung tissues of diabetic patients	ELISA	*45*
Human skin, rat skin, and liver	ELISA	*27*
Human serum albumin	HPLC-fluorescence	*47*
Plasma proteins	HPLC-fluorescence	*68*
Urine	HPLC-fluorescence	*19*
Urine	GC-MS	*41*
Exhaled breath condensate	LC-MS/MS	*46*
Cellular and extracellular proteins	LC-MS/MS	*59*
Food-derived CML		
Heated milk products	ELISA	*57*
Sauces and sauce-treated food	HPLC-fluorescence	*11*
Milk products and cheese	HPLC-fluorescence	*16*
Milk and cookie	GC-MS	*12*
Bovine serum albumin, soy protein, casein, and gluten	GC-MS	*9*
Milk, butter, cheese, bread, beef, and olive oil	LC-MS/MS	*4*
Dairy products	LC-MS/MS	*14*
Beverages and food	LC-MS/MS	*2*

DETECTION METHODS

Table 2 summarizes the detection methods used for endogenous and food-derived CML in recent years. The enzyme-linked immunosorbent assay (ELISA) kit is widely used in biology and medicine for its good selectivity, low cost, and simple operation, as the determination of sample components from biological tissue, blood, and urine is relatively simple. Instrumental analysis methods provide more-reliable data for the determination of food-derived CML. They originated with the early high-performance liquid chromatography (HPLC)–UV and HPLC-fluorescence methods and evolved to the present-day gas chromatography–mass spectrometry (GC-MS) and HPLC–tandem mass spectrometry (MS/MS) methods. Because CML has no UV or fluorescence absorption, it needs to be derivatized first, but its content often changes in the derivatization process, so the direct levels in food are not reflected precisely. HPLC-MS/MS is a powerful tool to study CML. It has many advantages. It is applicable for heat-sensitive CML and its intermediates in the Maillard reaction system. It has a detection limit of nanograms per milliliter (lower than those of fluorescence and UV detectors). With multiple-reaction monitoring, it can simultaneously detect several parent ions and further reconfirm them by daughter ions. The latter advantage allows the determination of CML from hundreds of substances even if they are not isolated effectively by a chromatographic column, which gives less background and enhanced sensitivity. However, HPLC-MS/MS is expensive and requires highly trained personnel. Therefore, developing rapid, reliable, and inexpensive detection methods for CML will be another important future research direction needed.

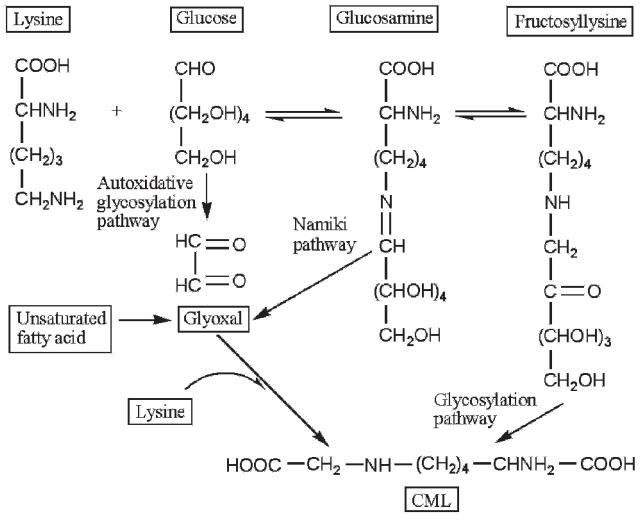

FIGURE 2. *Formation pathways of food-derived CML.*

Pretreatment of food samples prior to instrumental analysis is indispensable, because it is complicated and difficult to isolate components in Maillard reactions occurring at 100 to 250°C and using many raw materials, such as starch, protein, and fat. Pretreatments for CML samples include ultrafiltration to remove suspended particles, precipitation of protein in 20% trichloroacetic acid, removal of fat with a mixture of chloroform and methanol, hydrolyzation of bound CML in protein to free CML using 6 mol/liter hydrochloric acid or enzymes, and removal of impurities with solid-phase extraction *(3)*.

FORMATION ENVIRONMENT

There are three differences between endogenous CML and food-derived CML as to formation environment. First, endogenous CML is formed at the moderate temperatures (37°C) found in body fluid and tissue, while food-derived CML is formed at the more severe temperatures (100 to 250°C) encountered during cooking and thermal processing. Second, the composition of body fluid and tissue is relatively simple and stable, depending mainly on the enzymatic action of metabolism. In contrast, the composition of food-derived CML is complicated, mainly depending on the chemical action of thermal processing. Third, body fluid and tissue are in a relatively closed system that is not highly influenced by the external environment, while food production is in an open system that is influenced by external factors, such as oxygen and light. The difficulties in food-derived CML study are isolation of products and interactions between CML and unknown components of foods. Therefore, relating CML to food processes will be an important future research direction.

FORMATION MECHANISM

The mechanism of formation for food-derived CML is currently based on knowledge of endogenous CML

formation from biology and medicine. CML formation in vivo can be divided into carbohydrate oxidation and lipid oxidation (Fig. 2). In carbohydrate oxidation, there are three CML formation pathways *(17, 18, 61)*: (i) the autoxidative glycosylation pathway (glyoxal [GO] derived from glucose oxidation reacts with lysine to form CML *(72)*), (ii) the Namiki pathway (GO derived from Schiff base decomposition reacts with lysine to form CML *(22)*), and (iii) the glycosylation pathway (Schiff base undergoes Amadori rearrangement to form fructoselysine, which then is oxidized to form CML *(1)*). In the lipid oxidation aspect, Fu et al. *(20)* proposed that metal-catalyzed oxidation of polyunsaturated fatty acid leads to the formation of GO in the presence of protein in vivo, reacting with lysine to generate CML. In addition, free radicals produced in carbohydrate and lipid metabolism *(29, 36, 40, 58, 60)* may affect CML formation. Nagai et al. *(38)* showed that $OH^{\cdot}$ could promote fructoselysine conversion to CML in the endogenous CML formation process. However, there are few reports on food-derived CML formation pathways, which need to be investigated further.

Theories related to food-derived CML involve the Maillard reaction and lipid oxidation. The Maillard reaction consists of three stages. In the first stage, a reducing sugar reacts with a free amino group to form a Schiff base, which further forms Amadori rearrangement products. In the second stage, the Amadori product undergoes decomposition to generate active aldehyde and ketone intermediates. In the third stage, a series of reactions, including cyclization, dehydrogenation, rearrangement, isomerization, and condensation, occur to finally form AGEs containing CML. Because of the complexity of the Maillard reaction and diversity of food components, there may exist unknown pathways for the formation of food-derived CML that are different from those for the formation of endogenous CML.

The lipid oxidation theory specifies that dietary oil undergoes severe oxidation in high-temperature processes and produces highly reactive aldehydes and ketones, such as GO *(52)*. Fujioka and Shibamoto *(21)* treated dietary oils (tuna, salmon, cod liver, soybean, olive, and corn) under accelerated storage conditions and cooking conditions and found that fish oils with polyunsaturated fatty acids produced more GO than vegetable oils. Thus, it is supposed that GO derived from dietary oil oxidation probably participates in food-derived CML formation. Besides intermediate GO, free radicals produced from oil oxidation in food processes probably affect food-derived CML formation. The nonenzymatic free radical–mediated oxidation process for dietary oil is a free radical chain process consisting of chain initiation, propagation, and termination steps *(25)*. Few reports have investigated the effect of free radicals on CML formation in food processes.

The Maillard reaction and lipid oxidation may also have interactive influences on food-derived CML formation. Srey et al. *(55)* studied the effect of cooking oil on CML formation in model foods. They found no significant differences in CML concentrations in cakes prepared with different types of cooking oil, while cakes prepared with more cooking oil generated more CML than sucrose. On the contrary, Lima et al. *(33)* compared casein-glucose and casein-glucose-arachidonic acid model systems heated at 95°C for 8 h. They found that less CML was generated in the latter system, suggesting a negative effect of oil on CML formation. There are few reports on the molecular mechanism of interaction between the Maillard reaction and lipid oxidation in food-derived CML formation. Therefore, studying the relationship of these reactions with food components and production processes will be an important future direction.

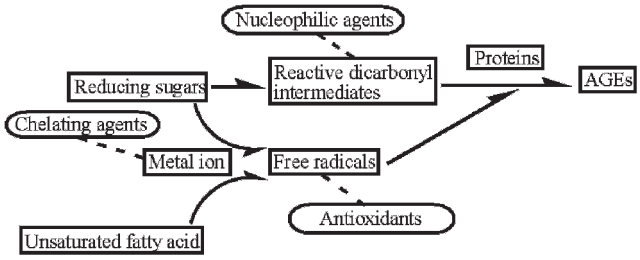

FIGURE 3. *Medicinal mechanisms for inhibiting endogenous AGEs.*

INHIBITION OF FORMATION

Two ways to inhibit the formation of food-derived CML can be considered for food processing: adding inhibitors studied for endogenous CML and modifying food processing parameters to decrease food-derived CML.

For inhibitors, medicinal mechanisms for inhibiting endogenous AGEs can be explored (Fig. 3). Antioxidants can scavenge free radicals. For example, orally administering vitamin C and vitamin E have an inhibitory effect on CML *(43)*. Chelating agents can shield copper, iron, and zinc ion to inhibit their catalysis of reducing sugars and polyunsaturated fatty acids so as to alleviate diabetic symptoms *(71)*. Nucleophilic reagents in medicine, such as aminoguanidine *(42),* pyridoxamine *(10),* and hydrazinophthalazine *(63),* can react with active dicarbonyls to inhibit CML. In any case, ideal CML inhibitors in food processing should have two desired features. First, the inhibitors must be widely distributed in nature with low cost due to their potential involvement in three meals a day. Second, the inhibitors must be contained in food raw materials to be used as preventive measures, not used as medicine to treat existing disease. Since chelating and nucleophilic reagents are rare in food materials and not economical, adding nucleophilic reagents as is done in medicine is not economical. Adding antioxidants to scavenge free radicals, however, is a practical method for food processing. For example, flavonoids and phenolic compounds have good potential for inhibiting food-derived CML and they are widely distributed in vegetables and fruits. Beaulieu et al. *(6)* identified nine main phenolic constituents from ethanolic extracts of *Vaccinium vitisidaea* with HPLC-MS and demonstrated that the flavonoid components of the berry extract could inhibit CML. Tsuji-Naito et al. *(62)* proved that the corolla of chrysanthemum species could inhibit the formation of CML in glycation

model reactions and characterized the structure as a flavonoid.

MODIFYING FOOD PROCESSING PARAMETERS

The human body itself is a CML-generating system whose reaction conditions are relatively steady and cannot be adjusted. In contrast, food processing conditions can be modified. This is a more suitable method for commercially produced foods, such as milk and beverages, to control food-derived CML. It is possible that some substances generated in food processes may convert CML to other safe substances that no longer pose a threat to human health. However, there are few reports on this aspect. Furthermore, controlling food-derived CML may affect traditional human food processing methods and even dietary culture. Therefore, controlling CML through inhibitors and modifying food processing parameters will constitute highly important future research.

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