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ARTICLE

Effect of Inhibitor Compounds on N^{ε}-(Carboxymethyl)lysine (CML) and N^{ε}-(Carboxyethyl)lysine (CEL) Formation in Model Foods

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The possible adverse effects on health of diet-derived advanced glycation endproducts (AGEs) and advanced lipoxidation endproducts (ALEs) is of current interest. This study had the objective of determining the effects of the addition of AGE/ALE inhibitors and different types of sugar and cooking oil on N^{ε}-(carboxymethyl)lysine (CML) and N^{ε}-(carboxyethyl)lysine (CEL) formation in model foods (sponge cakes). The cake baked using glucose produced the highest level of CML (2.07 ± 0.24 mmol/mol lysine), whereas the cake baked using fructose produced the highest concentration of CEL (25.1 ± 0.15 mmol/mol lysine). There were no significant differences between CML concentrations formed in the cakes prepared using different types of cooking oil, but significant differences ($P < 0.001$) were observed between the cakes prepared using different proportions of cooking oil. The cakes containing oil generated greater concentrations of CML than sucrose. α-Tocopherol and rutin did not inhibit CML and CEL formation. In contrast, ferulic acid and thiamin, thiamin monophosphate, and thiamin pyrophosphate reduced CML and CEL formation.

KEYWORDS: Advanced glycation endproducts (AGEs); advanced lipoxidation endproducts (ALEs); N^{ε}-(carboxymethyl)lysine (CML); N^{ε}-(carboxyethyl)lysine (CEL); AGE/ALE inhibitors

INTRODUCTION

Food composition and processing variables are the most important factors affecting food appearance, texture, taste, and nutritional value, such as loss of available lysine and formation of Maillard reaction products (MRPs). During food processing or storage, free amino groups on lysine residues of protein can react with the carbonyl group of reducing sugar or lipid oxidation products to form advanced glycation endproducts (AGEs) and advanced lipoxidation endproducts (ALEs) (*1*, *2*), including N^{ε}-(carboxymethyl)lysine (CML) and N^{ε}-(carboxyethyl)lysine (CEL) (**Figure 1**) (*3*−*6*). The formation of AGE/ALEs is of current interest to health due to their association with aging and various diseases associated with oxidative stress (*5*−*7*). Dietary AGE/ALEs are important with regard to consumer risk assessment due to their absorption through the gastrointestinal tract, which can possibly lead to an increase in the body's level of AGEs (*7*). CML can be formed through a number of different pathways. Condensation of glucose with the ε-amino group of lysine results in the formation of fructoselysine, an Amadori rearrangement product (ARP), which is oxidized to form CML. It can be also produced through an alternative route via a reaction of lysine with glyoxal (GO) (*3*−*6*). CEL, the analogue of CML, forms mainly by the reaction of lysine residues with methylglyoxal (MGO) or triose-phosphate (**Figure 1**). GO and MGO are produced during lipid peroxidation and sugar degradation (*3*−*5*). It is well-known that the rate of reaction and the concentration of AGE/ALE formation in food is affected by many factors including temperature, time, moisture content of the samples, the nature and amount of the reactants (e.g., sugar, lipid, and protein), and inhibitory compounds such as antioxidants (*2*, *8*−*10*). Antioxidant compounds have been reported to have beneficial and detrimental effects on AGE formation in vitro (*10*−*12*), in food (*13*, *14*), and in vivo (*15*). Therefore, research for glycation/lipoxidation inhibitors is of significant interest. Of particular value in food systems would be components that can selectively act on certain reaction pathways leading to inhibition of undesirable substances. Thus, inhibition of AGE/ALE formation might involve different mechanisms including reactive carbonyl trapping (*12*), antioxidant activity (*14*), sugar autoxidation inhibition (*16*), and amino group binding inhibition/competition (*17*, *18*).

Numerous studies have investigated the effect of antiglycation/antilipoxidation agents on the level of formation of AGEs, but less attention has been paid to the formation of individual AGE/ALEs, such as CML and CEL. Furthermore, research has been limited to model systems and physiological studies. No research has been conducted on the activity of inhibitors on CML/CEL

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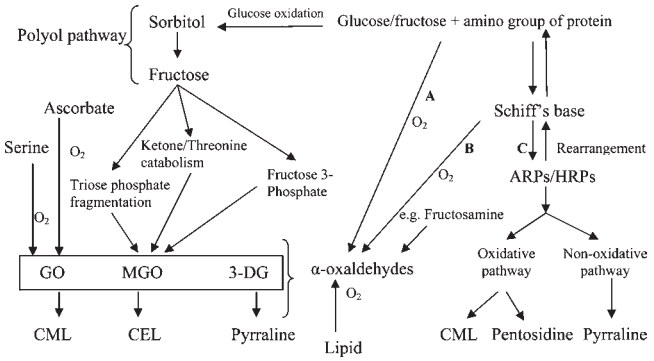

Figure 1. Formation pathways of α-dicarbonyl compounds, N^{ε}-(carboxymethyl)lysine (CML) and N^{ε}-(carboxyethyl)lysine (CEL) (modified from Singh et al., ref *4*): (A) Wolff pathway; (B) Namiki pathway; (C) Hodge pathway. GO, glyoxal; MGO, methylglyoxal; 3-DG, 3-deoxyglucosone.

formation in food (especially during processing). The goal of the present study was to assess the effect of food ingredients (types of sugar and oil) and AGE/ALE inhibitors (α-tocopherol, ferulic acid, rutin, thiamin, thiamin monophosphate, and thiamin pyrophosphate) on CML and CEL formation in model sponge cakes. The findings from the study may provide useful information concerning reduction of the level of AGE/ALEs in food systems, which may be beneficial to consumer health (*18*), especially consumers with diabetes (*7*).

MATERIALS AND METHODS

Reagents and Equipment. Wheat flour (self-rising flour), eggs, refined sucrose (white sucrose), unrefined sucrose (dark brown, Muscovado), margarine (vegetable fat spread, containing 59% fat), butter, olive oil, rice bran oil, sunflower oil, groundnut oil, and rapeseed oil were purchased from a local supermarket. CML, CEL, d_2-CML, and d_4-CEL were obtained from NeoPMS (Strasbourg, France). d_4-Lysine was purchased from Cambridge Isotopes (Andover, MA). Lysine, C_{18} (Supelclean LC-18) SPE tubes, inhibitor compounds (α-tocopherol, ferulic acid, rutin hydrate, thiamin hydrochloride, thiamin monophosphate, and thiamin pyrophosphate), glucose, fructose, and all other chemicals were of the highest grade available from Sigma (Gillingham, U.K.). The analytical mill was from IKA group (Staufen, Germany). A Genevac evaporator (EZ-2), robust system, was obtained from Genevac (Ipswich, U.K.). The Acquity UPLC-MS/MS was from Waters (Manchester, U.K.).

Preparation of Sponge Cake. *Model 1: Effect of Sugar*. The sugars used were glucose, fructose, refined sucrose (white sucrose), and unrefined sucrose (dark brown sucrose). Egg white (40 g) and sugar (40 g) were placed into a bowl and well mixed, and then wheat flour (40 g) and margarine (30 g) were added and mixed well to form a batter. The batter (40 g) was placed into a porcelain dish (d = 6.2 cm, h = 2.5 cm), which was then placed into a preheated oven (190 °C) and baked for 30 min.

Model 2: Effect of Oil. The cooking oils used were virgin olive, rice bran, sunflower, groundnut, and rapeseed. To examine if the pathway to CML formation via lipid oxidation or via sucrose oxidation was more important, three series of model cakes were prepared.

Series 1: Absence of Refined Sucrose. For the first series of cakes, sugar was omitted and margarine was replaced with oil.

Series 2: Presence of Refined Sucrose with High Level of Oil (*Molar Ratio Lysine/Oil = 1:50*). The second series of cakes contained refined sucrose as for model 1, but margarine was replaced by oil. The molar ratio of lysine/oil was 1:50.

Series 3: Presence of Refined Sucrose with Low Level of Oil (*Molar Ratio Lysine/Oil = 1:1*). The cakes were prepared as for series 2 but reducing the amount of oil with respect to the molar ratio of lysine/oil, which was 1:1. The molar ratio of lysine/oil is the molar ratio of lysine residues to the triglycerides content of the mixture containing egg white, flour, and oil.

Model 3: Effect of AGE/ALE Inhibitors. Refined sucrose was the sugar source, and butter was the oil source. The cakes were prepared with the addition of an AGE/ALE inhibitors (α-tocopherol, ferulic acid, rutin, thiamin, or thiamin derivatives). The amount of inhibitor used was either equimolar or a 5-fold molar excess with respect to the lysine content of flour and egg white. All of the inhibitors, with the exception of α-tocopherol, were added to the egg white and mixed. α-Tocopherol was added to the warm liquid butter (37 °C) before mixing with the other ingredients. The batter was baked as for model 1. Sponge cake baked without an AGE/ALE inhibitors was used as a positive control for CML and CEL formation.

Protein Hydrolysis. Samples were prepared for analysis based on that of Assar et al. (*19*). The cakes were cooled to room temperature and ground using an analytical mill. A quantity of sponge cake, equivalent to 5 mg of protein, was reduced overnight at 4 °C in sodium borohydride buffer (0.2 M, pH 9.2, 1 mL), fat was extracted using chloroform/methanol (2:1 v/v, 3 × 1 mL), and protein was hydrolyzed with 6 N HCl (1 mL) at 110 °C for 24 h. After the acid was removed under vacuum (Genevac EZ-2), the dried hydrolysates were reconstituted in distilled water (1 mL) and then solid phase extracted using C_{18} cartridges (200 μg of protein).

Determination of Equivalent Protein in Hydrolysates. The concentration of equivalent protein in the hydrolysates was determined according to the rapid fluorescamine method developed by Yaylayan et al. (*20*). Briefly, hydrolysate solution equivalent to 40 μg of protein was diluted with potassium borate buffer (0.2 M, pH 8.5, 1 mL). Diluted hydrolysate (150 μL, equivalent to 6 μg of protein), fluorescamine solution (0.3 mg/mL, 50 μL), and NaOH (1 M, 25 μL) were placed into the wells of a 96-well black plate. The plate was shaken for 3 min, and the fluorescence intensity was measured (λ_{ex} = 390 nm, λ_{em} = 475 nm) using a Tecan Safire microplate reader (Vector Scientific, Moneyrea, Northern Ireland). Hydrolysate BSA (0−10 μg of equivalent protein) was used to prepare a standard calibration curve.

UPLC-MS/MS Analysis. CML and CEL concentrations of hydrolysates were determined by UPLC-MS/MS (*19*). Briefly, protein hydrolysates (7.5 μg of protein, 7.5 μL) were injected onto a BEH C_{18} UPLC column (2.1 × 50 mm, 1.7 μm) housed in a column oven at 50 °C and operated in gradient elution mode. Solvent A was aqueous nonafluoropentanoic acid (NFPA, 5 mM), and solvent B was acetonitrile. The run time was 7.5 min. The analysis was performed using a Waters Acquity UPLC (Manchester, U.K.) coupled to a triple-quadruple MS operating in multiple reaction monitoring (MRM) mode. The flow rate was 0.2 mL/min. The MS was operated in electrospray ionization (ESI) positive mode using MRM. Data were analyzed using MassLynx (Waters) software. Lysine, CML, and CEL concentrations were determined by reference to the internal and external standards by plotting the amount ratio of the unlabeled to the labeled compounds. Data were reported as ± standard deviation of different runs, n = 3.

Statistical Analysis. Statistical analysis (ANOVA) was performed to determine if differences between two groups of means existed ($P < 0.05$).

RESULTS

Effect of Sugar on CML and CEL Concentration. The concentrations of CML and CEL found in sponge cake prepared using different types of sugar are shown in **Table 1**, parts **A** and **B**, respectively. The cakes made with glucose generated higher amounts of CML (81.7 ± 7.07 mg/kg protein) than those made with fructose (69.3 ± 6.16 mg/kg protein), refined sucrose (14.3 ± 1.59 mg/kg protein), or unrefined sucrose (10.5 ± 1.73 mg/kg protein). An up to 7.7-fold higher concentration of CML was observed in the cakes using monosaccharide (glucose or fructose) compared to the cakes using disaccharide (refined or unrefined sucrose). The highest concentration of CEL was found in the cake prepared with fructose (1059 ± 6.47 mg/kg protein), and the lowest concentration was in the cake prepared with unrefined sucrose (44.1 ± 1.62 mg/kg protein). The sponge cake containing fructose produced 6.8-, 14.5-, and 24-fold higher concentrations of CEL than the sponge cakes containing glucose, refined sucrose, and unrefined sucrose, respectively. In comparison to the levels of CML, CEL was 1.9-, 15.3-, 5.1-, and 4.2-fold higher in the cakes containing glucose, fructose, refined sucrose, and unrefined sucrose, respectively.

Table 1. CML (**A**) and CEL (**B**) Contents ± Standard Deviation of Different Runs (n = 3) in Sponge Cake Prepared Using Different Types of Sugar

sugar	mmol/mol lysine	mg/kg protein	mg/kg model cake
	(**A**) CML		
glucose	2.07 ± 0.24	81.7 ± 7.07	6.64 ± 0.57
fructose	1.75 ± 0.12	69.3 ± 6.16	5.63 ± 0.50
refined sucrose	0.36 ± 0.02	14.3 ± 1.59	1.16 ± 0.13
unrefined sucrose	0.27 ± 0.03	10.5 ± 1.73	0.86 ± 0.14
	(**B**) CEL		
glucose	3.69 ± 0.20	156 ± 8.30	12.7 ± 0.68
fructose	25.1 ± 0.15	1059 ± 6.47	86.2 ± 0.53
refined sucrose	1.73 ± 0.07	72.9 ± 2.83	5.93 ± 0.23
unrefined sucrose	1.04 ± 0.04	44.1 ± 1.62	3.59 ± 0.13

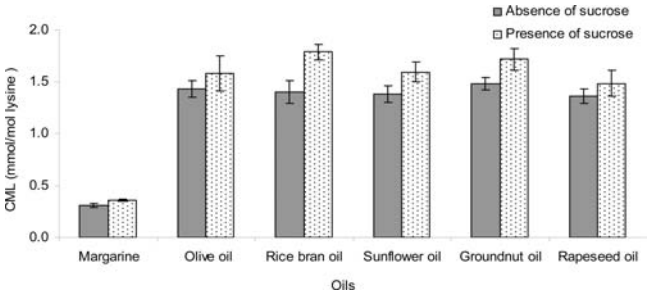

Figure 2. CML concentration in sponge cake containing different types of oil, in the presence and absence of added refined sucrose, ± standard deviation of different runs, n = 3 (P > 0.05).

Effect of Oil on CML Concentration. CML concentrations in sponge cake baked using different types of cooking oil are shown in **Figure 2**, and CML concentrations in sponge cake baked using high and low amounts of cooking oil are shown in **Figure 3**. In the presence of cooking oil, the concentration of CML found in the cakes baked using sucrose was between 1.48 ± 0.07 and 1.79 ± 0.11 mmol/mol lysine, whereas for the cake baked without sucrose, the CML level was between 1.36 ± 0.12 and 1.48 ± 0.10 mmol/mol lysine (**Figure 2**). There was no significant difference (P > 0.05) in CML concentration in the cakes produced using different types of oil, but a significant difference (3.8-fold different, P < 0.001) was observed in the cakes using the low level of oil (molar ratio lysine/oil = 1:1) compared to the high level of oil (molar ratio lysine/oil = 1:50) (**Figure 3**). Margarine produced a lower level of CML than oil (5-fold lower), in the presence or absence of sucrose (**Figure 2**). The sponge cake produced using margarine provided similar levels of CML regardless of the amount of margarine used (**Figure 3**).

Effect of AGE/ALE Inhibitors on CML and CEL Concentration. The effect of AGE/ALE inhibitors on CML and CEL concentrations formed is shown in **Figure 4**, panels **a** and **b**, respectively. A comparison of the percent inhibition of CML and CEL formation in model sponge cake is shown in **Figure 5**. α-Tocopherol and rutin did not affect CML or CEL formation. In contrast, thiamin and its derivatives thiamin monophosphate and thiamin pyrophosphate showed a good inhibitory effect on both CML and CEL formation. The inhibition of CML and CEL was highest in the cakes made using thiamin, thiamin monophosphate, and thiamin pyrophosphate, and inhibition increased with increasing concentration of thiamin and thiamin derivatives. CML concentrations in the cake baked with ferulic acid were 0.34 ± 0.03 and 0.24 ± 0.02 mmol/mol lysine (molar ratio of lysine/ferulic acid = 1:1 and 1:5, respectively). CEL concentrations in the cake using α-tocopherol, rutin, and ferulic acid (molar ratio

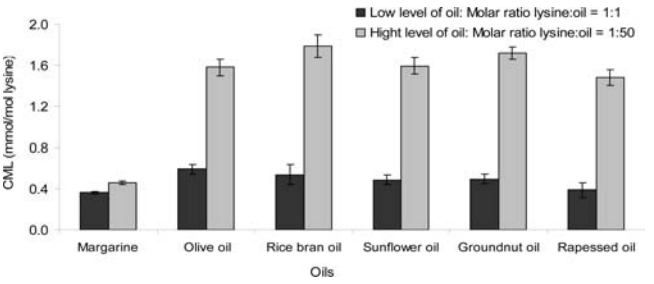

Figure 3. CML concentration in sponge cake using low level of oil (molar ratio lysine/oil = 1:1) and high level of oil (molar ratio lysine/oil = 1:50) ± standard deviation of different runs, n = 3 (P < 0.001).

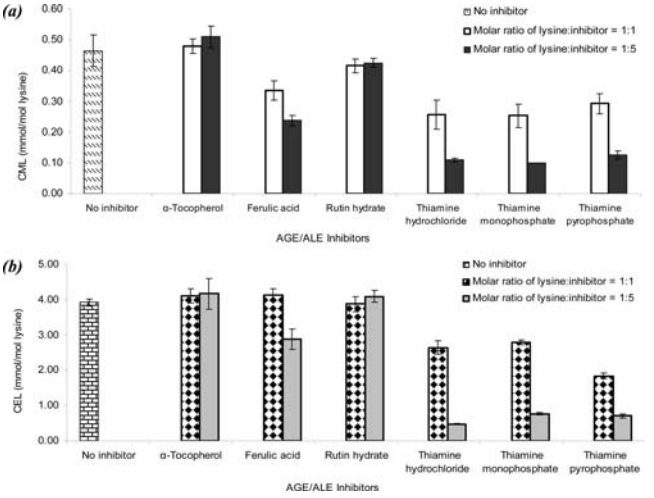

Figure 4. Effect of AGE/ALE inhibitors on CML and CEL formation ± standard deviation of different runs (n = 3): (**a**) effect of AGE/ALE inhibitors on CML formation; (**b**) effect of AGE/ALE inhibitors on CEL formation.

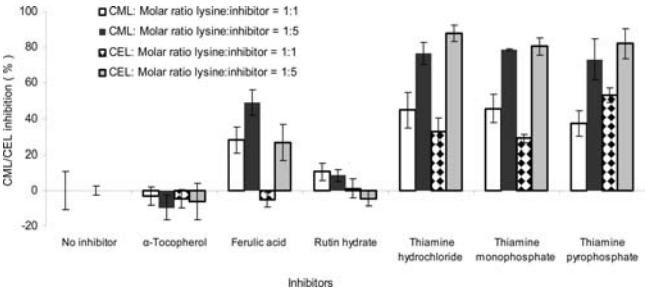

Figure 5. Comparison of percent inhibition of CML and CEL formation ± standard deviation of different runs in sponge cake produced using different type of inhibitors (n = 3).

of lysine/ferulic acid = 1:1) were not significantly different (P > 0.05) compared to those without addition of inhibitor (3.92 ± 0.10 mmol/mol lysine). In contrast, the cake containing ferulic acid (molar ratio of lysine/ferulic acid = 1:5) did show significantly lower levels of CML and CEL compared to the cake without addition of inhibitor (P < 0.005). The inhibitory effect of ferulic acid on CML formation was higher than that on CEL formation.

DISCUSSION

Effect of Sugar on CML and CEL Concentration. The concentrations of CML, found in the sponge cakes, were between 10.5 ± 1.73 and 81.7 ± 7.07 mg/kg protein depending upon the types of sugar present. This is in good agreement with the level of CML

reported in white bread crumb (31.4 ± 5.95 mg/kg protein), wholemeal bread crumb (40.5 ± 12.3 mg/kg protein), cookies (5−35 mg/kg protein), and toasted bread (13 mg/kg protein), but higher than in corn flakes (6−8 mg/kg protein) and lower than in white bread crust and wholemeal bread crust (382 ± 15.7 and 329 ± 15.3 mg/kg protein) (*8*, *9*, *19*). This is possibly because bread crust attained a higher temperature (about 230−250 °C) than sponge cake (temperature in the center of the sponge cake was about 100 °C). Goldberg et al. (*21*) reported the level of CML in a wide range of foods including desserts, cereals, and cake, but a meaningful comparison between the data sets is difficult due to their expression of data (kU/kg food). It should be noted that the appropriateness of the antibody used by Goldberg et al. (*21*) is disputed, due to matrix interference and lack of evidence of its validation in food systems (*1*, *9*). In the current study, the cake baked using glucose produced a greater level of CML than the cake baked using fructose (1.2-fold). This shows that CML formation from the ARP may be more important compared to CML formation from the HRP or that oxidation of glucose generated more glyoxal (GO, a precursor of CML) than oxidation of fructose (**Figure 1**).

CEL was found to be highest in the cakes baked using fructose, followed by glucose, refined sucrose, and unrefined sucrose. This is possibly due to fructose oxidation resulting in a greater yield of methylglyoxal (MGO, precursor of CEL), known as reactive carbonyl compound, than glucose (**Figure 1**). However, the importance of other precursors for CEL formation is still unknown. To date, the study of the pathways for the formation of CEL is limited when compared with those associated with CML, and it remains unclear. Furthermore, the levels of CEL formed were higher than those of CML. This might be due to the reaction of MGO, formed from lipid oxidation, with lysine occurring at a faster rate than the reaction of GO with lysine. Alternatively, MGO might be generated in a greater yield than GO (*3*, *22*) during sugar/fat oxidation. No direct comparison can be made between the value of CML and CEL of sponge cake with other published data, as this is the first time that the comparison between CML and CEL in bakery products using different types of sugar has been conducted. However, the concentration of CEL was reported to be higher than that of CML in rat chow, raw skimmed milk, and pasteurized bovine milk, but lower than in sterilized skimmed milk (*1*).

Effect of Oil on CML Concentration. CML has been quantified in a range of foods including bakery products (*8*, *9*, *19*), but whether sugar or lipid is the most important precursor has not yet been established. In the current study, a 3-fold lower level of CML was observed in the model cake prepared using the low level of oil (molar ratio lysine/oil = 1:1) compared to the high level of oil (molar ratio lysine/oil = 1:50). In comparison to the cake produced with refined sucrose, the cake produced without refined sucrose generated a 1.3-fold lower level of CML. This showed that sucrose affected CML formation to a lesser degree than oil in the model sponge cake system. This may be due to the generation of GO via lipid oxidation being greater than that produced via sucrose oxidation. Fu et al. (*3*) also suggested that GO may be a common intermediate in the formation of CML during oxidation of both carbohydrate and lipid and demonstrated that the reaction of lipid (fatty acids) with lysine produced a 23-fold higher level of CML than the reaction of sugar (glucose) with lysine, in an RNase-arachidonate/glucose model system (*3*). In the current study, the cake produced using margarine gave a lower concentration of CML than the cake baked using cooking oil. This is possibly due to margarine containing a lower percentage of lipid (59%) than oil (100%), or less reactive (more hydrogenated) lipid in margarine, or the fortification of margarine with vitamins, some of which have an antioxidant capacity (e.g., vitamins A and E). Among the oils examined, olive oil contains the highest level of monounsaturated fatty acids (73%) but the lowest level polyunsaturated fatty acids (8.2%), whereas sunflower oil contains the lowest level of monounsaturated fatty acids (23%) but the highest level of polyunsaturated fatty acids (65%, based on the information provided on the packaging). It is well-known that polyunsaturated fatty acids are more vulnerable to lipid oxidation than monounsaturated fatty acids, but no significant difference was observed in the level of CML produced using the different oils. This may be due to a complex matrix or because an excess amount of oil was used.

Effect of AGE/ALE Inhibitors on CML and CEL Formation. Oxidation of glucose/fatty acids and the formation of ARPs and α-oxoaldehydes produce superoxide and free anion radicals (*23*, *24*). AGE/ALE inhibitors used in the current study may inhibit these oxidative processes by different mechanisms such as chelation of transition metals, free radical scavenging, sugar autoxidation inhibition, and amino group binding inhibition/competition (*10*−*18*, *25*−*29*). Various AGE/ALE inhibitors have come to light in recent years. However, the ability of components possessing antioxidant activity to inhibit the AGE/ALE formation depends not only on the free radical scavenging activity of the samples but also on other factors such as type and concentration of ingredients and heating time and temperature (*10*−*14*). This may be why some authors found antioxidant compounds, such as ferulic acid, to have antiglycation properties (*10*), whereas others could not find any antiglycation activity (*11*). Many papers have reported the inhibition of AGE/ALE formation; however, a direct comparison between the effects of antiglycation/antilipoxidation agents on CML and CEL in processed foods is difficult and sometimes impossible to make.

α-Tocopherol is a lipid-soluble phenolic antioxidant compound that contains one hydroxyl group. It provides free radical scavenging activity and can react directly with free radicals by donation of a hydrogen atom (*24*, *27*). However, α-tocopherol did not inhibit CML/CEL formation in a glucose−BSA model system (*28*) and in the current study. This may be attributed to the insolubility of tocopherol in the buffer system, as well as thermal baking instability. For example, the antioxidant activity of tocopherol decreased by up to 100% when pork lard was heated to 150 °C (*27*).

Rutin contains a vicinyl dihydroxyl group that contributes to free radical scavenging activity and has been shown to provide a great inhibitory effect toward all stages of AGE formation, in a BSA−glucose model system, and to be even more effective than aminoguanidine (*26*). It has also been reported that rutin strongly inhibits the formation of both fluorescent (pentosidine) and nonfluorescent (CML) AGEs (*16*). However, both studies (*16*, *26*) were undertaken at moderate temperature (37 °C) and in liquid systems, whereas an intermediate-moisture food heated under baking conditions (190 °C) was studied in the present investigation and no inhibition of CML or CEL was observed. Inhibitor solubility may be one of the multiple factors that may affect the inhibitory power of this chemical in our particular system. Also, thermal (190 °C, 30 min) stability should be taken into account, as in the case of tocopherol; however, other factors are also very relevant.

Ferulic acid possesses a phenolic nucleus and conjugated side chain; it readily forms a resonance-stabilized phenoxy radical, which accounts for its potent antioxidant potential (*10*, *25*). It inhibits CML and CEL formation in the current study. The anti-CML/CEL effect of ferulic acid is attributed to its free radical scavenging activity (*10*, *13*) in the second phase of glycation, that is, GO and MGO production from sugar/lipid

or Amadori product oxidation. The data agree with an earlier study (*13*) suggesting that ferulic acid reduced the generation of some MRPs in a low-moisture glucose/glycine model (10% moisture) system heated at 200 °C. Ferulic acid has been used as a food additive to inhibit lipid peroxidation and subsequent oxidative spoilage and as a therapeutic agent against inflammatory disease and cancer (*15*).

Thiamin consists of substituted pyrimidine and thiazole rings linked by a methylene bridge. Thiamin occurs in free and phosphorylated forms such as thiamin monophosphate and thiamin pyrophosphate. These compounds contain an amino group, which can compete with the amino group of lysine during the Maillard reaction. Thiamin and its derivatives have a strong antiglycation effect, greater than that of aminoguanidine, in vitro (AGE−BSA/RNase system) (*11*), in vivo (*18*), and in the current investigation. Their inhibitory effects on CML/CEL formation, in the model cakes, is possibly through the inhibition of the intermediate product, for example, GO and MGO (*29*). Thus, the inhibitory effect may be through a competitive mechanism between the amino group of thiamin/thiamin derivatives with the ε-amino group of lysine residues within protein during glycation/lipoxidation, including reaction with GO and MGO. These findings agree with the study of Karachalias et al. (*18*) suggesting that thiamin administered orally reduces CML (74%) and CEL (118%) concentration in the plasma of diabetic rats.

From the AGE/ALE inhibitors examined in this investigation, only ferulic acid, thiamin, and thiamin derivative inhibited CML and CEL formation. Tocopherol and especially rutin, which are known as powerful antioxidants and antiglycation agents, had no effect on AGE/ALEs formation, in the current system. This is possibly due to the acid, HCl salt, and phosphate group contained within ferulic acid, thiamin hydrochloride, and thiamin monophosphate/pyrophosphate, respectively, interfering in the reaction of AGE/ALEs and formation during the baking process. However, the other possible mechanisms are still unclear and must be studied.

Although this current study did not examine the effect of ingredients (e.g., oils, sugars, and AGE/ALE inhibitors) on the foods' organoleptic/sensory qualities, our results agreed with those recently reported by Peng et al. (*14*) indicating that foods supplemented with glycation/lipoxidation inhibitors produce low levels of CML, but may adversely affect food sensorial properties.

In conclusion, the ingredient profile of foods, for example, sugar, lipid, and protein composition, has a substantial effect on AGE/ALE formation. Sponge cake made using glucose produced the highest level of CML, and sponge cake made with fructose produced the highest level of CEL. Levels of CEL were higher than those of CML in the cake baked using the same sugars. Sucrose produced lower levels of CML and CEL than glucose or fructose. Lipid has a greater influence on CML formation than sucrose. Addition of antiglycation/antilipoxidation compounds, such as ferulic acid, thiamin, thiamin monophosphate, and thiamin pyrophosphate may be an effective means of reducing CML/CEL formation in food systems. However, one disadvantage is that some inhibitors may adversely affect food flavor.

ABBREVIATIONS USED

CML, N^{ε}-(carboxymethyl)lysine; CEL, N^{ε}-(carboxyethyl)lysine; GO, glyoxal; MGO, methylglyoxal; ARP, Amadori rearrangement product; HRP, Heyns rearrangement product; AGEs, advanced glycation endproducts; MR, Maillard reaction; MRPs, Maillard reaction products; UPLC-MS/MS, ultraperformance liquid chromatography−tandem mass spectrometry.

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