FORMATION AND INHIBITION OF ADVANCED GLYCATION ENDPRODUCTS IN MEAT AND MODEL SYSTEMS

by

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B.S., China Agricultural University, 2006
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Food Science

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Abstract

Advanced glycation endproducts (AGEs) are formed in many cooked meat products via Maillard browning reactions. Current research suggests consumption of these compounds may be a contributor to chronic diseases such as diabetes and heart diseases. Thus, information on the prevalence and inhibition of these compounds in food is desirable.

The first objective was to determine the AGE content, as determined as N^{ε}-carboxymethyllysine (CML) level, in cooked meat and fish prepared by general cooking methods recommended by U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). We found AGE was detected in all the cooked samples, but the levels depended on the different cooking conditions. Broiling and frying at higher cooking temperatures produced higher levels of CML and broiled beef contained the highest CML content (21.84 μg/g). However, the baked salmon (8.59 μg/g) and baked tilapia (9.72 μg/g) contained less CML as compared to the other samples.

In order to investigate the inhibitory effect of selected natural antioxidant on AGEs formation in cooked meat, four cereal brans, wheat (Jagger, JA), triticale (Spring Triticale, ST; Thundercale, TH), and Rye (RY) bran were added to beef patties before cooking. RY (42.0% inhibition), ST (27.5% inhibition), and TH (21.4% inhibition) brans significantly decreased CML formation compared with the control. The inhibition of CML was correlated to the water-holding activity (WHC) of the samples, and the radical scavenging activity of the brans.

The effect of cereal bran extracts (JA, ST, TH, and RY), was studied in a bovine serum albumin and glucose (BSA-GLU) model system. The ST extract significantly (P <0.05) inhibited CML formation compared to the control group. ST particularly contained vanillic acid (VA),

chlorogenic acid (CHA), gentisic acid (GEA), and ferulic acid (FA), where GEA and CHA mitigated CML with an average percentage decrease of 29.6% for CHA and 51.1% for GEA. It therefore may be useful in preventing AGEs formation by using ST bran as a food addictive, which contains abundant phenolic acids.

In summary, current dietary AGEs database will provide important information for use in estimating AGEs exposure, and also these data demonstrate that a significantly reduced intake of dietary AGEs can be achieved by low heat AGE cooking methods such as baking, which can be used at home or in the meat industry. Cereal bran addition to meat products may reduce formation of AGEs that is a desired attribute for the processed meat products industry.

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In summary, current dietary AGEs data will provide important information for use in estimating AGEs exposure. These data demonstrate that a significantly reduced intake of dietary AGEs can be achieved by low heat AGE cooking methods such as baking, which can be used at home or in the meat industry. Cereal bran addition to meat products may reduce formation of AGEs that is a desired attribute for the processed meat industry.

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Chapter 1. REVIEW OF LITERATURE

INTRODUCTION

Research shows that the Maillard reaction occurs in food processing, which can change the taste, aroma, and color of roasted, grilled, and boiled foods. In the Maillard reaction, reducing sugars react with proteins in food to form a brown product that provides distinctly different food flavor. However, the reaction can also cause food safety/toxicology concern (Ames, 2009; Somoza, 2005). In the reaction, free amino groups of proteins can react with the carbonyl group of reducing sugars to form advanced glycation endproducts (AGEs), which are a group of complex and heterogeneous compounds that have pathogenic significance in metabolic diseases such as diabetes and heart diseases (Poulsen et al., 2013).

Excessive AGEs are linked to many diseases related to aging, including diabetes, nephropathy, renal disorders, and Alzheimer's (Ahmed, 2007; Brownlee, 1994; Kim, Reddy, Rahbar, Lanting, & Natarajan, 2002). AGEs have two main pathologic effects: (A) altering the chemical and biological properties of body protein by cross-link formation and (B) increasing oxidative stress, inflammation, and disorders in an organism through binding with receptors on the cell surface (Chuyen, 2006). Some epidemiological studies show that too much of certain dietary AGEs correlate with reactive oxygen species (ROSs), which may be important in disease pathologies (Uribarri et al., 2007). A number of clinical experiments have found excess accumulation of AGEs in diabetic patients (Vlassara et al., 2002).

According to numerous reports (Ahmed, 2005; Henle, 2005; Wu, Huang, Lin, & Yen, 2011), the common AGEs found in food are N^{ε}-carboxymethyl lysine (CML), glyoxal-lysine dimer

(GOLD), methylglyoxal-lysine dimers (MOLD), glyoxal (GO), methylglyoxal (MGO), 3-deoxyglucosone (3-DG), pentosidine, and pyrraline (Figure 1).

Although the mechanism of Maillard reaction is still not fully understood, research has established that the modern diet is a major source of AGEs. Their levels depend on such things as cooking method, temperature, and chemical precursors in foods. In particularly, heat-processed cookery leads to high levels of AGEs in cooked food (Forster, & Henle, 2003).

Consequently, an emerging question is how to decrease the formation of dietary AGEs. The answer depends on such changes as decreasing cooking temperatures and times (Goldberg, Cai, Peppa, Dardaine, Baliga, & Uribarri, 2004). Investigating and understanding AGE inhibitors is another effective way to reduce AGE formation (Reddy, & Beyaz, 2006), and research on these inhibitors has intensified in recent years. A variety of synthetic agents have been evaluated for the ability to reduce AGEs, but natural antioxidants from plants or foods may be more promising inhibitors; they are less toxic and may scavenge free radicals associated with the Maillard reaction (Cameron, & Cotter, 1993; Wu, & Yen, 2005).

Extensive studies on how dietary AGEs affect the population can be found in recent literature, inspiring the following questions: (1) Which kinds of dietary AGEs are toxic for humans? (2) What are the limits for toxic AGE consumption and how can people reduce their uptake? (3) What are the mechanisms of toxicity of dietary AGEs and how can people actually avoid cooking foods to the point where AGEs form? This review provides basic information on dietary AGEs, their formation mechanism, the relationship between AGEs and human health, the methods through which dietary AGEs can be detected in foods, the occurrence of AGEs in foods, what affects AGE formation in food, and how can dietary AGEs be mitigated.

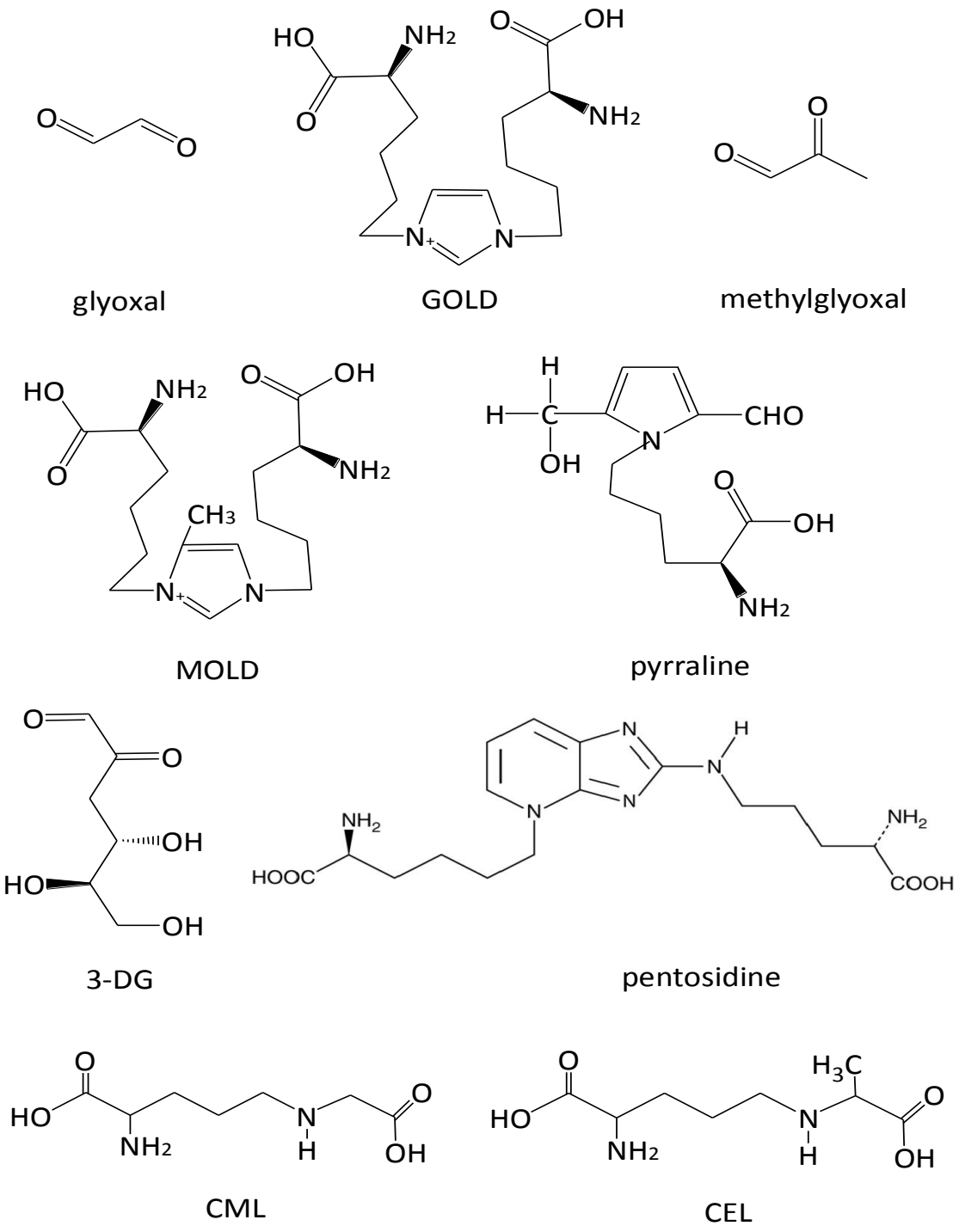

Figure 1. Chemical structure of AGEs (modified from Henle, 2005). Glyoxal-lysine dimer (GOLD), methylglyoxal-lysine dimers (MOLD), glyoxal (GO), methylglyoxal (MGO), 3-deoxyglucosone (3-DG), N^{ε}-carboxymethyl lysine (CEL), and N^{ε}-carboxymethyl lysine (CML).

AGES IN FOODS

Food processing induces glycation of protein through the Maillard reaction. In 1912, a French chemist Louis-Camille Maillard undertook his study on the reaction of amino acids and sugars (Wolfrom, Schlicht, Langer, & Rooney, 1953). In the late 1940s and early 1950s, a series of studies revealed how pigments were formed during the Maillard reaction. In 1953, an American chemist, John E. Hodge, explained a mechanism for the Maillard reaction (Everts, 2012). During the following two decades, research focused on the impact the Maillard reaction had on flavor of food, and how amino acids were modified during the Maillard reaction (Erbersdobler, & Somoza, 2007; Finot, Deutsch, & Rujard, 1981). The subject of AGEs was first raised by Brownlee, Vlassara, and Cerami (1984) when they studied the influence of non-enzymatic browning on plasmase. Neeper et al. (1992) first characterized one of the typical receptors for AGEs (RAGEs), which is an immunoglobulin with a mass of 42 kDa. RAGEs related glycation research remains an area of significant interest in glycation-linked physiological processes associated with chronic diseases. Clearly, food is a rich source of AGEs, but researchers still question the physiological significance of AGEs in foods and continue to study their formation mechanism.

Although how AGEs form in foods is still not clear, one proposed mechanism, which is called free radical formation, has been identified (Hayase, Shibuya, Sato, & Yamamoto, 1996). AGEs are generated through the Maillard reaction between reducing sugars and free amino groups (Figure 2). In the early stages of the mechanism, the carbonyl group of sugars reacts with the amino group to produce the Schiff base. Then, the stable ketosamines compounds

(Amadori products) take shape. They decompose to reactive carbonyls like GO, MGO, and 3-DG and free radicals. In addition, highly reactive carbonyls may form through severe oxidation of fatty acids (Fu et al., 1996). In the late stages of this mechanism, the carbonyls react with amino groups of compounds to generate AGEs, which also can form oxidatively from Amadori products (Nguyen, Van der Fels-Klerx, & Van Boekel, 2014; Ruttkat, & Erbersdobler, 1995).

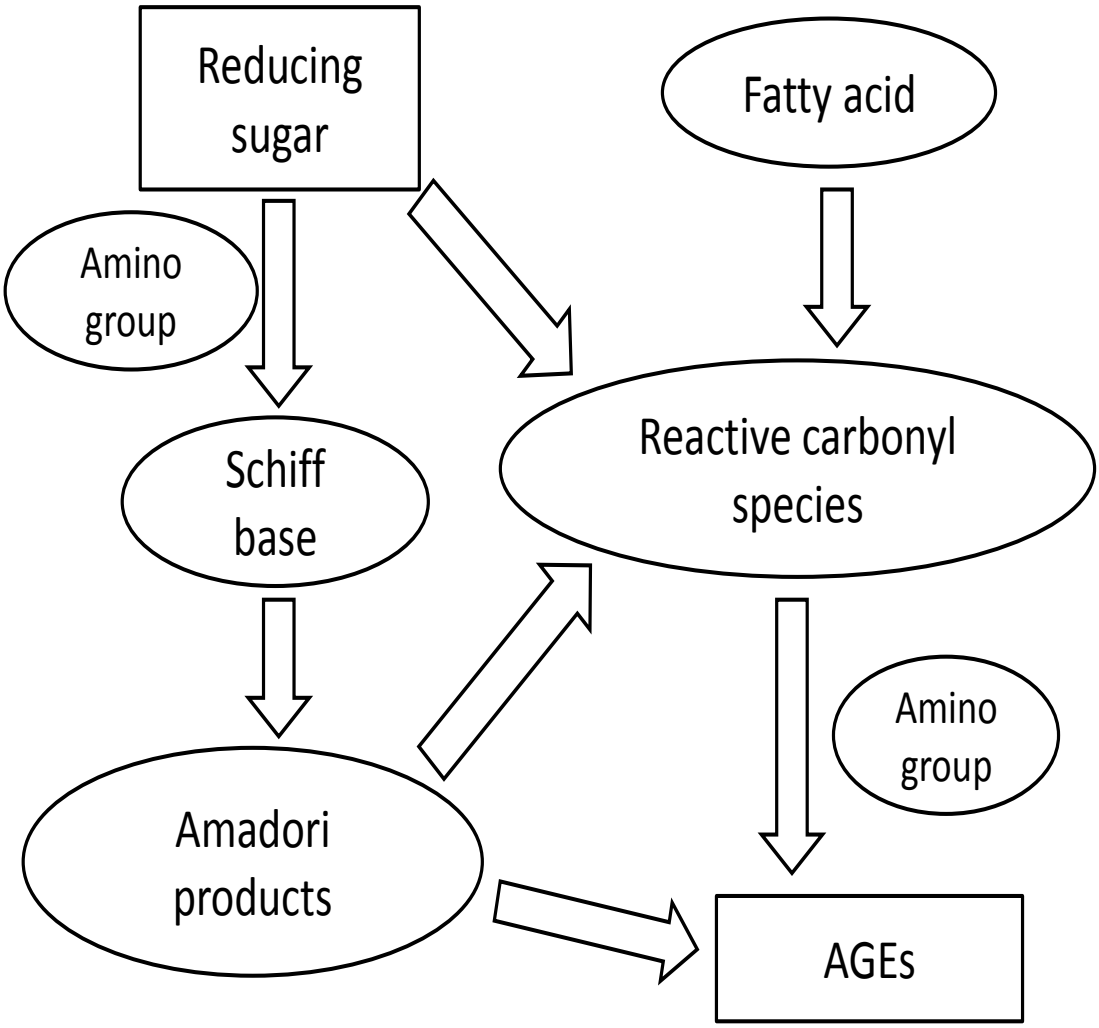

Figure 2. Formation pathway of AGEs (modified from Poulsen et al., 2013).

In CML formation (Figure 3), a reducing sugar (glucose) condenses with an amino group of lysine, to form a Schiff base/glucosamine. The glucosamine is rearranged to yield the Amadori product, which subsequently oxidizes to form CML. And CML can also form directly from the reaction between lysine and GO, which is produced by glucose autoxidative glycosylation or Schiff's base decomposition (Ahmed, Thorpe, & Baynes, 1986; Poulsen et al., 2013; Uribarri et al., 2007). In an analogous reaction, 3-DG reacts with lysine residues to form pyrraline, while pentosidine can be generated through the reaction of pentose with lysine and arginine as well as other types of AGEs like GOLD and MOLD, which can be derived from the Amadori products (Chuyen, 2006; Wolff, & Dean, 1987).

TOXICITY OF DIETARY AGES

Food-derived AGEs and their possible toxicity have become a topic of increasing interest in research. Hofmann et al. (2002), in an animal study using mice, revealed that consuming high-AGEs diets was associated with insulin resistance, which is a cause of type 2 diabetes. Vitek et al. (1994) found that reducing intake of food-derived AGEs could decrease significantly those diseases related to inflammation and oxidative stress, as well as significantly increase the lifespan of mice. Moreover, the levels of circulating AGEs correlated with the levels of food-derived AGEs in a study using human-derived endothelial cells *in vitro* (Goldberg et al., 2004). Furthermore, a low-AGE diet significantly reduced serum AGE levels as well as endothelial dysfunction in a group of diabetic subjects (Raj, Choudhury, Welbourne, & Levi, 2000). More clinical trials with an AGE-restricted diet are shown in Table 1. These studies suggest that dietary AGEs may be toxic, thus affecting human health.

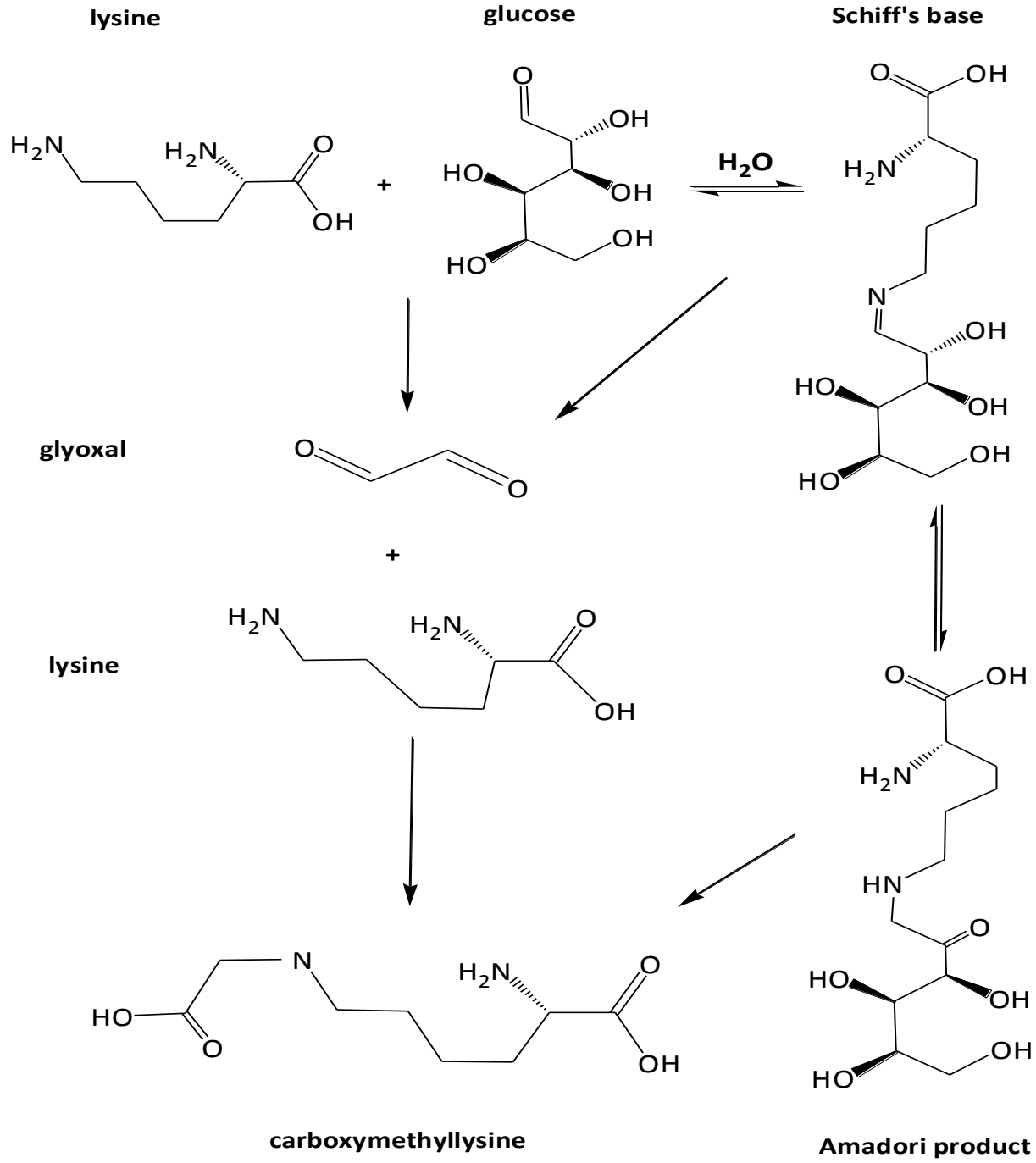

Figure 3. A free radical mechanism for CML formation derived from lysine and glucose (modified from Ahmed et al., 1986; Poulsen et al., 2013; Uribarri et al., 2007).

Table 1. Clinical trials with an AGE-restricted diet.

Study population	Trial design	Results	References
Diabetes (United States)	Crossover	Decrease AGEs and markers of oxidative stress and inflammation	Vlassara et al., 2002
End-stage renal disease no diabetes (United States)	2 parallel groups (high and low AGE)	Decrease AGEs and markers of inflammation	Uribarri et al., 2003
Healthy and chronic kidney disease no diabetes (United States)	2 parallel groups (high and low AGE)	Decrease AGEs and markers of oxidative stress and inflammation	Vlassara et al., 2009
Diabetes (United States)	2 parallel groups (high and low AGE)	Decrease AGEs, oxidative stress, inflammation, and homeostatic model assessment	Uribarri et al., 2010
Healthy (France)	2 parallel groups (high and low AGE)	Decrease AGEs and homeostatic model assessment	Birlouez-Aragon et al., 2010
Healthy (United States)	2 parallel groups (high and low AGE)	Decrease AGEs but no change in endothelial function and inflammation	Semba et al., 2014
Overweight women (Denmark)	2 parallel groups (high and low AGE)	Decrease urinary AGEs and homeostatic model assessment	Mark et al., 2014
Overweight or obese men (Mexico)	3 parallel groups (diet+exercise)	Decrease AGEs and weight	Macias-Cervantes et al., 2015
Type 2 diabetes (Mexico)	2 parallel groups (high and low AGE)	Decrease AGEs and weight	Luevano-Contreras et al., 2013

AGES AND HUMAN DISEASES

With the continued research on dietary AGEs, their effects on human health have become apparent, and a diet pattern with high levels of AGE intake may be a potential risk factor in a variety of diseases like diabetes, age-related diseases, atherosclerosis, cancer, and other diseases (Ahmed, 2005; Brownlee, 1994; Kim, et al., 2002).

Poulsen et al. (2013) suggested two possible AGE pathogenic pathways in the occurrence and development of chronic diseases: first, the cross-linking of AGEs and biomolecules like proteins, nucleic acids, may directly destroy the structure and function of the biomolecules; and second, the binding of AGEs to specific (RAGEs) may activate a series of signaling pathways that trigger expression of inflammatory mediators and cellular oxidative stress, thereby enhancing cellular activation and degradation, encouraging inflammation and even dysfunction or inflammatory disorders of cells and tissues (Heizmann, 2007). The RAGE-dependent pathway is an important pathogenic route (Thornalley, 2007). In particular, diseases like dementia, cardiovascular disease, and renal failure may be caused or enhanced by AGEs (Table 2).

Table 2. Major diseases possibly caused by AGE exposure[a].

Possible pathologies	**Failure of maintenance in cell or tissues**
Cardiovasular diseases, atherosclerosis	Blood vessels
Dementias, neuropathy	Neurones
Complications of diabetes	Insulin metabolism
Blindness, cataracts, retinopathy	Retina, lens
Renal failure, nephropathy	Glomeruli

[a]Adapted and modified from references (Brownlee, 1995; Palinski, et al., 1995).

Mechanism of action

RAGEs are a major signal transduction receptor for AGEs, mediating many chronic diseases. RAGEs belong to a multiligand group in the immunoglobulin super family that includes S100/calgranulin (Heizmann, 2007), high mobility group box 1 (HMGB1), b-amyloid peptide, and b-sheet fibril (Neeper et al., 1992). The binding of AGEs to RAGEs causes oxidative stress and activates the transcription factor called nuclear factor kappa B (NF-κB), which controls such genes as COX-2 (cyclo-oxygenase-2), TNFα (tumor necrosis factor α), inducible nitric oxide synthase (iNOS), and IL-6 (interleukin-6) that are expressed as infection and inflammation. Their synthesis and secretion, as regulated by NF-κB, may also create a vicious cycle that may lead to complications in chronic diseases like diabetes (An et al., 2011; Thornalley, 2007). However, we still need to describe a more precise molecular mechanism to explain how the interaction of AGEs with RAGEs controls the inflammation process.

AGEs and diabetes

The incidence of diabetes is rapidly increasing worldwide year by year, reaching 387 million in 2014, increasing from about 285 million in 2010 and 124 million in 1997 (Shi, & Hu, 2014). Complications are common. However, how AGEs affect the pathogenesis of diabetes is only partly understood; AGEs and their receptors on specific cell types may contribute to this increase in diabetes and its complications. A large number of studies have found that AGEs accumulated in patients suffering from type 2 diabetes mellitus, also identified as hyperglycemia-associated glycotoxins (Huebschmann, Regensteiner, Vlassara, & Reusch, 2006). According to Vlassara et al. (2009), the uptake of dietary AGEs correlates with serum AGE levels

in patients. A variety of studies show that dietary AGEs may be important in type 2 diabetes mellitus and its complications because it is involved in the development of insulin resistance and cell dysfunction (Vlassara, & Uribarri, 2014). Moreover, glycation intermediates, the reactive carbonyl species (RCSs) including GO, MGO, 3-DG, also contribute to complications in diabetic patients (Uribarri, et al., 2007).

Tanji et al. (2000) stated that deposition and accumulation of AGEs was significant to diabetic nephropathy, which may cause glomerulosclerosis. Nagai, Hayashi, Xia, Takeya, and Horiuchi (2002) have revealed AGE level in tissues not only correlates with the severity of atherosclerotic lesions but also the quantity of plasma proteins, so diabetic patients may also see significant atherosclerosis .

Blood vessel damage is a common complication in diabetics (Peppa, Uribarri, & Vlassara, 2004). Zhang et al. (2003) observed that AGEs could be involved in oxidative damage and accelerated coronary atherosclerosis in diabetics. In addition, Hughes et al. (2004) reported AGE concentration correlated with the severity of retinopathy in patients; protein glycation may restrict blood flow in retinal blood vessels.

Accumulation of AGEs occurs especially in diabetes and chronic renal failure, and that may play a major pathogenetic role. Plasma CML levels were found to increase in patients with chronic renal failure (Singh, Barden, Mori, & Beilin, 2001). CML accumulated in the tissues of uremic patients, which may be also related to impaired renal function (Schwenger, Zeier, Henle, & Ritz, 2001). Moreover, inflammatory markers like C-reactive protein were reduced in dialysis patients who followed an AGEs-free diet for 4 weeks (Uribarri et al., 2007).

Clinical studies show that for diabetic patients with cataracts, lens CML concentration was significantly higher than control patients (Hashim, & Zarina, 2011). Based on the results from Franke, Dawczynski, Strobel, Niwa, Stahl, and Stein (2003), the levels of serum CML generated by oxidation may contribute to cataract formation.

AGEs and age-related diseases

As AGEs and RAGEs form and interact, they can produce ROSs and elicit inflammation and then alter gene expressions, which can affect the development and progression of age-related diseases and induce age-related decline related to intracellular damage and apoptosis (Luevano-Contreras & Chapman-Novakofski, 2010). This has been implicated in Alzheimer's disease (Necula & Kuret, 2004), cardiovascular disease (Cuevas et al., 2011), coronary heart disease (Kanauchi, Tsujimoto, & Hashimoto, 2001), and other common age-related diseases.

It is well known that diabetes is a risk factor for Alzheimer's disease. In Necula et al. (2004), symptoms of Alzheimer's like neuronal cell death, amyloid plaque formation, and neuronal tangles can be caused by the glycation of τ-proteins. The reaction appears to occur in the early stages of Alzheimer's (Kimura, Takamatsu, & Araki, 1995). Vitek et al. (1994) suggested that the β-amyloid deposition and plaque formation in Alzheimer's patients accelerates significantly via crosslinking of AGEs *in vitro*. Moreover, over expression of RAGE in the brain may produce oxidative stress and inflammation through IL-1β, TNF-α, or NF-κβ up-regulation, which may accelerate the onset and progression of Alzheimer's (Cuevas et al., 2011).

Mitochondrial dysfunction contributes to the onset and development of cardiovascular disease, which may be activated by the interaction between AGEs and RAGE

(Nargund, Pellegrino, Fiorese, Baker, & Haynes, 2012). Extensive studies have shown an increase in cardiovascular risk as RAGEs increase, specifically in type 1 diabetes (Nin et al., 2011).

Kilhovd, Berg, Birkeland, Thorsby, and Hanssen (1999) reported levels of AGEs that were significantly higher in heart disease patients with type 2 diabetes mellitus than patients without diabetes. Furthermore, in coronary heart disease patients, the number of blood vessels with stenosis correlated with circulating AGEs (Kanauchi et al., 2001).

AGEs and cancer

Recently, more evidence has emerged implicating AGEs in the development of various types of cancers, through oxidation stress, proliferative effects, and inflammatory reactions. The interaction of AGEs with RAGE and their pathogenic signaling pathway may partly explain an increased risk of tumor growth and metastasis in patients with diabetes or other age-related diseases (Allmen, Koch, Fritz, & Legler, 2008).

Takino, Yamagishi, and Takeuchi (2010) noted, however, that glyceraldehyde-derived AGEs enhanced cancer malignancy, not proliferation, in their study of the influence of glyceraldehyde-derived AGEs on cultured lung cancer A549 cells. Moreover, by testing of the expression of CML and argpyrimidine in four different types of human tumors in immunohistochemistry, van Heijst, Niessen, Hoekman, and Schalkwijk (2005) found the AGEs have great influence on the expression of several kinds of human cancer tissues.

Although growing evidence shows that AGEs may affect the growth or progression of different types of cancer cells, the pathogenic mechanism remains unknown. Kang, Tang, Lotze, and Zeh (2012) found crucial evidence of the interaction of RAGE and the HMGB 1 protein in

the development of pancreatic cancer. Furthermore, AGEs-RAGE interactions induced the development of interleukin-6 and renal cell carcinoma (RCC) (Miki et al., 1993). Takino et al. (2010) found AGEs can active Rac 1 to enhance the migration of lung cancer A549 cells because they can induce generation of ROS. However, future studies must elucidate the pathogenic mechanism of AGE, as well as the physiological relevance of AGEs and the biology of cancer cells.

FACTORS AFFECTING AGES FORMATION

How quickly AGEs generate in food depends on composition of foods, availability of precursors or antioxidants added, processing temperature and time, availability of water, and other factors that influence the Maillard reaction (Sharma, Kaur, Thind, Singh, & Raina, 2015).

Composition of foods

Many foods provide a wide variety of precursors for the Maillard reaction and formation of AGEs, as among them, sugar and amino acids, intermediates-Amadori compounds, methylglyoxal, and other reactive carbonyl compounds present in foods. The type of sugar influences the reaction; for instance, Laroque et al. (2008) found reactions like ribose > fructose and glucose and aldose > ketose in model studies. Lysine is a long amino acid considered to have the strongest capacity for inducing the brown color in the reactions in glycose and amino acid systems (Ajandouz, & Puigserver, 1999). Uribarri et al. (2010) stated that the foods high in fat and protein contained more AGE content after cooking than vegetables, grains, and other carbohydrate-rich foods.

Time and temperature

Cooking time and temperature are important to the formation of Maillard reaction products (MRPs). As early as 1990, Ledl and Schleicher (1990) monitored the progress of Maillard reaction by measuring the degree of browning, finding similar results when heating samples for four weeks at 20°C, or 3 h at 100°C, or 5 min at 150°C. Augustin, Sanguansri, and Bode (2006) later found increasing temperature and time increased the rate of reactions as well as dark colored products in their model system. Uribarri et al. (2010) constructed a dietary AGE database of 249 foods used in modern cookery, showing that frying, broiling, and grilling at higher temperatures produced more AGEs than boiling, stewing, and steaming at lower temperatures. Based on their findings, high temperature cookery meant CML formation increased 10 to 200 times. Others had similar results (Chao, Hsu, & Yin, 2009), showing that in chicken, pork, beef, salmon, and cod samples that were boiled, fried, or baked, the CML levels were significantly higher than in raw foods ($P < 0.05$).

Moisture content

In food systems, water transports water-soluble precursors of the Maillard reaction during cooking, so water content of food is important. The Maillard reaction generally occurs when water activity is between 0.6-0.8 in some model systems (Ledl et al., 1990; Tanaka, Chiba, Ishizaki, Takai, & Taguchi, 1994). In foods with low water activity, if reactants are highly mobile, Maillard reaction products (MRPs) may form more quickly. Acevedo, Schebor, and Buera (2008) noted that the rate of reaction in a dried potato system increased dramatically as water activity increased. Some previous publications show that adding salt and phosphate greatly increased water-holding capacity (WHC) of foods, thus decreasing formation of some MRPs in meat

products. For instance, Persson, Sjõholm, and Skog (2003) showed that adding water-binding ingredients (1.5% sodium chloride and 0.3% sodium tripolyphosphate) to beef patties decreased the formation of heterocyclic amines like 2-amino-1-methyl-6-phenylimidazo (4,5-b) pyridine when patties were fried at 180°C and 220°C. Another article reveals that MRPs levels increased dramatically in meat systems, particularly at the meat surface (crust) when cooked at high temperatures as in grilling (Sinha et al., 2005). Moreover, Uribarri et al. (2010) demonstrated that dry heat cooking increases cooking loss and encourages dietary AGEs to form. However, direct information on moisture content of food and formation of individual dietary AGE remains rare.

pH

pH can greatly affect the formation of MRPs (Nursten, 2005a, b). The rate of the Maillard reaction is low at pH < 7.0, but increases as pH increases from 7.0 to 10.0. However, at that point, the rate of browning obviously decreases as pH goes past 11.0 (O'Brien, & Morrissey, 1989).

Metal ions

Metal ions can also influence the Maillard reaction at different stages depending on the type of metal ion. Kwak and Lim (2004) found that adding Fe^{2+} and Cu^{2+} could increase the rate of browning in a model system, which could be due to the water binding capacity of metal ions and subsequent reduction in water activity. On the contrary, Ca^{2+} and Mg^{2+} could decrease the rate of the Maillard reaction.

DETECTING AGES IN FOODS

AGEs contain a wide range of complex chemical structures, and their levels are low in foods, so it is difficult to identify AGEs in many types of food items. The most common methods of detecting AGEs use fluorescence spectroscopy, enzyme-linked immunosorbent assays (ELISA), and chromatography like high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), gas chromatography (GC), and gas chromatography-mass spectrometry (GC-MS). There are no commonly accepted methods like analysis kits for detecting AGEs. Most analytical techniques have so far focused on CML (Table 3). Standardized methods to detect AGEs are needed so research results from different laboratories and units can be compared (Smit, & Lutgers, 2004).

Table 3. Summary of methods for identifying CML in different foods.

Food products	Methods	Key points	Reference
Wide range of daily foods	ELISA	Monoclonal anti-CML antibody	Goldberg et al., 2004; Uribarri et al., 2010
Whole meals, milk products, cereal and meat products	HPLC	Fluorescence detection of OPA derivative	Dittrich et al., 2006; Delgado-Andrade et al., 2007; Hartkopt et al., 1994; Drusch et al., 1999
Infant formulas	GC	Heptafluorobutyryl isobutyl ester	Bueser et al., 1987
Meat and milk products, infant formulas	GC-MS	SIM of trifluoroacetyl methyl ester	Charissou et al., 2007
Milk products, beverages	LC-MS	Multiple reaction monitoring	Assar et al., 2009; Fenaille et al., 2006

Fluorescence spectrophotometry

Fluorescence spectrometry can quickly determine an analyte level in solution based on its fluorescence characteristics, which are directly proportional to the intensity of emission. Fluorescence spectrometry excites electrons in molecules of AGEs, which then emit light. These fluorescence values can be measured, with intensity reflecting the levels of AGEs, but the technique has poor specificity in identifying an individual AGE compound (Schmitt, Schmitt, Munch, & Gasic, 2005).

Enzyme-linked immunosorbent assay

ELISA uses the connection of an analyte to an enzyme, producing a specific antigen-antibody reaction, by which a substance can be identified through a change in color. The competitive ELISA method can measure AGEs levels in many food items but the antibody only reacts with certain AGEs, not with Amadori products or other non-glycosylated protein (Horiuchi, Araki, & Morino, 1991).

The ELISA technique has been used extensively to measure AGEs, including nonspecific AGEs (Koschinsky et al., 1997; Makita, Vlassara, Cerami, & Bucala, 1992), based on antibodies for CML or MGO derivatives (Uribarri et al., 2010). A competitive ELISA with an anti-CML monoclonal antibody (4G9) has also been developed and used to measure CML expressed as AGE units in a wide range of food products (Goldberg et al., 2004). Although the method is quick and effective, it relies on different anti-CML antibodies. Moreover, as Turk, Ljubic, Turk, and Benko (2001) have shown, as immunogens, AGEs may produce auto-antibodies that interfere with test results.

Chromatography

Chromatography separates and detects individual components from the matrix using chromatographic separation or color. The instrumental methods couple high performance liquid chromatography (HPLC) with diode array detectors (HPLC-DAD), fluorescence detectors (HPLC-FLD), mass spectrometer (HPLC-MS), or gas chromatography (GC) coupled with MS (GC-MS) (Ahmed, Argirov, Minhas, Cordeiro, & Thornalley, 2002; Charissou, Ait-Ameur, & Birlouez-Aragon, 2007; Hartkopt, Pahlke, Ludmann, & Erbersdobler, 1994; Rufian-Henares, Guerra-Henandez, & Garcia-Villanova, 2004). Although they may require either the acid or enzymatic hydrolysis of bound AGEs in food samples before analysis, the methods are sensitive and can provide accurate results. For instance, Rufian-Henares et al. (2004) first studied the pyrraline level in foods, and Charissou et al. (2007) determined how much CML occurred in different milk and meat samples. In addition, HPLC has been applied successfully after CML was derived using the *o*-phthaldialdehyde reagent (Drusch et al., 1999; Hartkopt et al., 1994) (Figure 4). Currently, ultra performance liquid chromatography (UPLC) has been used to test for AGEs and promises to be a universal method for identifying an extensive range of food-derived AGEs (Assar, Moloney, Lima, Magee, & Ames, 2009; Hull, Woodside, Ames, & Cuskelly, 2012).

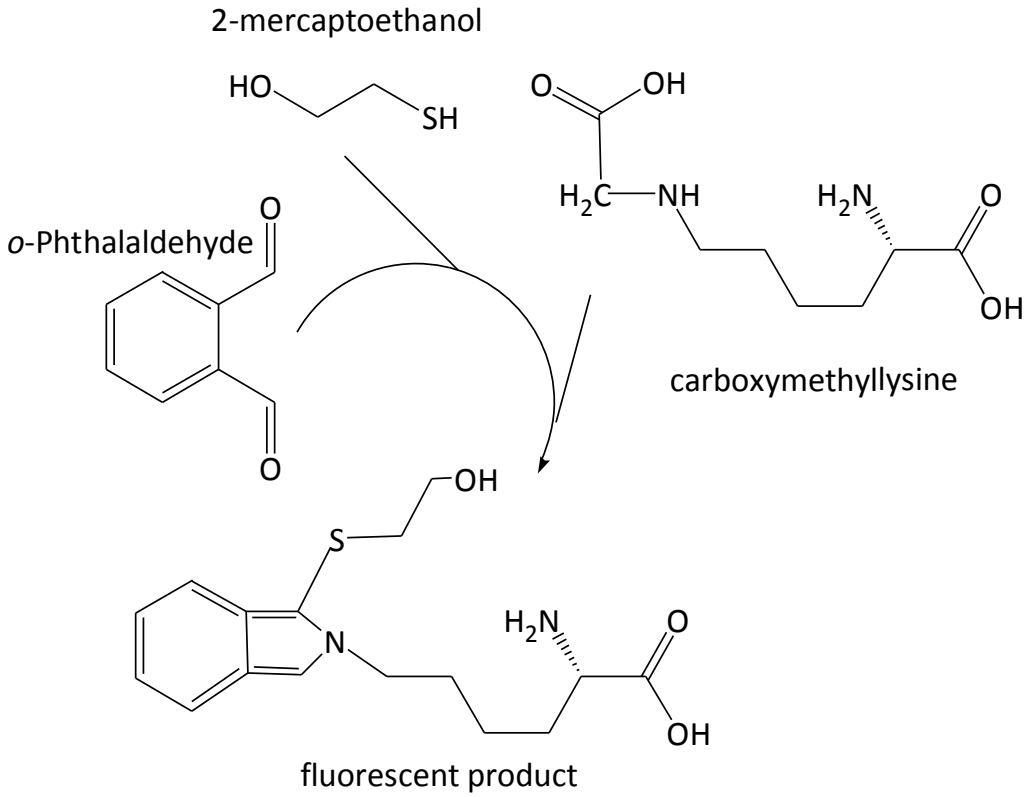

Figure 4. Phthaldehyde (OPA) derivatization reaction (modified from Drusch et al., 1999).

LEVELS OF AGES IN FOODS

AGEs can be generated under human physiological conditions, but diet is the major source of exogenous AGEs (Assar et al., 2009). As early as the 1990s, human studies confirmed that about 10% of dietary AGEs are absorbed in the body and correlate with circulating and tissue AGE levels (Koschinsky et al., 1997). Although AGEs may influence the progression of chronic diseases like diabetes and uremia, full information about the levels of AGEs in foods remains unknown. In addition, no regulations or industry standards exist for dietary AGEs. It is, therefore, desirable to estimate AGE levels in commonly consumed foods.

AGEs, including CML, pyrraline, and pentosidine, as well as the reaction intermediates,

occur in complicated food products. Fully quantifying these complicated compounds is generally difficult, but several types of analysis can currently find the level of CML and pentosidine in foods because their chemical structures are better understood in food and biology samples. CML was the first amino acid derivative identified during food processing and may form predominantly from the oxidation of Amadori products (Kasper & Schieberle, 2005). Pyrraline, as a pyrrole derivative of lysine, was successfully quantified in milk products using enzymatic hydrolysis (Hegele et al., 2008). Compared to the non cross-linked compounds, cross-linked AGEs were less easily identified in food samples; among these cross-linked AGEs, pentosidine was first detected in heated milk samples (Henle, Schwarzenbolz, & Klostermeyer, 1997). Ahmed et al. (2002) observed methyl-glyoxal-hydro-imidazolone (MG-H1) formation in bakery foods and in coffee, where it may generate by the reaction of arginine with carbonyls in the roasting process. Such reactive carbonyls as GO and 3-DG have been reported in several food products (Schwarzenbolz, Henle, Haebner, & Klostermeyer, 1997).

Levels of CML in foods

CML, as a marker and one of the more frequently studied AGEs, occurs in a wide range of foods among the different food categories. For the past 20 years, because CML is a relatively abundant AGE in biological or food systems, it has been extensively studied and used as a marker for total AGEs (Tauer, Hasenkof, Kislinger, Frey, & Pischetsrieder, 1999).

Both immunochemical and instrumental methods can identify CML levels in foods. Uribarri et al. (2010) tested CML levels in 549 foods using an ELISA, where AGE content was expressed in kilounits (kU) per serving size of different food items (Table 4), but according to Assar et al. (2009), instrumental methods like chromatograph analysis generally provide more

reliable data. These researcher evaluated CML levels in dairy, cereals, fruits, vegetables, meat and fish products; LC or GC data are provided in Table 5 and 6. Cooking method may speed up CML formation, particularly grilling, roasting, and frying. Furthermore, foods high in fat and protein generally contain larger amounts of CML. The food-derived CML database provides some information useful in estimating exposure of dietary AGEs, as well as serving as a practical guide for reducing their intake.

Table 4. The AGE content in food products.

Food product	AGE[a] kU/100g	Serving size	AGE kU/serving
Beef, steak, strips, fried without oil, 7 min	6973	90 g	6276
Beef, frankfurter, broiled 450°F, 5 min	11270	90 g	10143
Chicken, breast, broiled, 450°F, 15 min	5828	90 g	5245
Chicken, breast, fried without oil, 7 min	3554	90 g	3199
Cheese, American, low fat (Kraft)	4040	30 g	1212
Cheddar cheese	5523	30 g	1657
Pork, chop, pan fried, 7 min	4752	90 g	4277
Smoked deli ham	2349	90 g	2114
Salmon, fillet, broiled	3347	90 g	3012
Raw salmon	528	90 g	475
Egg, fried, one large	2749	45 g	1237
Egg yolk, large, 10 min	1193	15 g	179
Milk, whole (4% fat)	5	250 mL	12
Yogurt, vanilla, (Dannon)	3	250 mL	8
Vegetable juice, V8 (Campbell Soup Co)	2	250 mL	5
Milk, fat free (Tuscan Dairy Farms)	2	250 mL	4

[a]AGE content is expressed by CML level as assessed by competitive ELISA using monoclonal antibody (4G9) (Uribarri et al., 2010).

Table 5. Content of CML in dairy products, cereal-based foods, fruits, and vegetables.

Food products	CML (mg/kg protein)	CML (mg/100g food)	References
Dairy products			
UHT milk	29-46		Fenaile et al., 2006
UHT flavor milk	41-93		Drusch et al., 1999
Pasteurized milk	16.3		Fenaile et al., 2006
Infant formula	30		Hartkopf et al., 1995
Powdered infant formula	60.1		Fenaile et al., 2006
Pasteurized skimmed milk		0.02	Hull et al., 2012
UHT whole milk		0.22	Hull et al., 2012
Cheddar cheese		1.18	Hull et al., 2012
Cereal-based products			
Cornflakes	6.0-8.0		Charissou et al., 2007
Biscuit	5-35		Charissou et al., 2007
Toasted bread	0-13		Charissou et al., 2007
Potato bread	25.65		Hull et al., 2012
Fried white bread	80.79		Hull et al., 2012
White bread crust		0.35	Peng et al., 2010
Sponge cake		0.36	Srey et al., 2010
Fruit and vegetables			
Tomato	10.2		Hull et al., 2012
Apple	39.3		Hull et al., 2012
Raw carrot	not detectable		Charissou et al., 2007
Orange		0.08	Hull et al., 2012

Table 6. **Content of CML in meat and fish products.**

Food products	CML (mg/kg protein)	CML (mg/100g food)	References
Chicken			
Boiled chicken breast	17.2		Hull et al., 2012
Raw chicken		0.018	Chao et al., 2009
Baked chicken		0.07	Chao et al., 2009
Roasted chicken breast	17.4		Hull et al., 2012
Fried chicken breast	23.5		Hull et al., 2012
Pork			
Raw pork		0.02	Chao et al., 2009
Baked pork		0.076	Chao et al., 2009
Baked pork (leg joint)		0.27	Hull et al., 2012
Fried fillet strip		0.61	Hull et al., 2012
Grilled pork loin chop		0.39	Hull et al., 2012
Sausage	23		Hartkopf et al., 1995
Beef			
Raw beef	3.9		Assar et al., 2009
Boiled minced beef	27.3		Assar et al., 2009
Fried minced beef	61.1		Assar et al., 2009
Raw beef		0.013	Chao et al., 2009
Fried fillet beef steak		0.42	Hull et al., 2012
Grilled fillet beef steak		0.49	Hull et al., 2012
Fish			
Raw salmon		0.01	Chao et al., 2009
Baked salmon		0.068	Chao et al., 2009
Grilled/oven baked salmon	not detectable		Charissou et al., 2007
Tuna canned in brine		0.54	Hull et al., 2012
Raw cod		0.018	Chao et al., 2009

Baked cod		0.055	Chao et al., 2009
Grilled cod	3.7		Hull et al., 2012
Fried breaded cod		0.36	Hull et al., 2012

Levels of pentosidine in foods

When pentose links to both arginine and lysine residue, pentosidine is generated by Maillard reaction; pentosidine has been identified in a variety of foods. According to Henle et al. (1997), pentosidine concentrations were low in milk, coffee, bakery, and other products (Table 7). More studies are needed to evaluate how pentosidine affects the glycation of food proteins.

Table 7. Amounts of pentosidine in food products.

Food products	**Pentosidine concentration**
Raw milk	not detectable
Pasteurized milk	not detectable
Fresh UHT milk	(not detectable-0.05)[a]
Bread crust	(0.4-2.6)[a]
Roasted coffee	(10.8-39.9)[a]
UHT milk	(not detectable-0.01)[b]
Coffee	0.2[b]
Pasta	not detectable
Bakery products	(not detectable-0.4)[b]

[a]Pentosidine is in mg/kg protein (Henle et al., 1997); [b]Pentosidine is in mmol/mol arginine (Henle, 2008).

Levels of pyrraline in foods

Pyrraline, another acid labile AGE, results from a reaction of a lysine group-and 3-deoxyglucusulose. It is found in both food and model systems; protein-bound pyrraline was first detected among the alkaline hydrolysates of proteins and glucose (Hegele et al., 2008; Henle, 2008; Rufian-Henares et al., 2004). HPLC has been used to quantify the levels of pyrraline in a wide range of foods (Table 8).

Table 8. Amounts of pyrraline in food products.

Food products	Pyrraline concentration
Sterilized formula A	495[a]
Sterilized formula B	344[a]
UHT formula A	453[a]
UHT formula B	277[a]
Roasted meat	not detectable
Milk products	(not detectable-25)[b]
Pasta	(not detectable-13)[b]
Bakery products	(1-10)[b]

[a]Pyrraline is in mg/kg protein (Rufian-Henares et al., 2004); [b]Pyrraline is in mmol/mol lysine (Henle, 2008).

Levels of dicarbonyl compounds in foods

Dicarbonyl compounds like GO, MGO, and 3-DG form easily during the Maillard reaction (Yamaguchi, Ishida, Zhu, Nakamura, & Yoshitake, 1994). The literature does report quantitative data on MGO and GO (Bravo et al., 2008; De Revel, & Bertrand, 1993); research shows that

small amounts of carbonyl compounds were found in yogurt, beverages, and other food items (Table 9). Recently, research shows that average levels of MGO were 3.0 mg/kg in bread and 8.3 mg/kg in cookies (Degen, Hellwig, & Henle, 2012). Compared to GO and MGO, very little data is available on 3-DG in food, although honey was reported to contain large amounts of 3-DG, between 80 mg and 1450 mg/kg in tested samples (Weigel, Opitz, & Henle, 2004). This limited information on the dicarbonyl compounds in foods means more comprehensive quantitative data is needed to investigate dietary exposure to this compound.

Table 9. Ranges of dicarbonyl compounds in different food items.

Food products	**GO**	**MGO**	**3-DG**
Cheese[a]	4-6 mg/kg	4-11 mg/kg	not detectable
Yogurt[a]	0.6-0.9 mg/kg	0.6-1.3 mg/kg	not detectable
Wine[b]	not detectable	nd-4.5 mg/L	2.2-9.5 mg/L
Soy sauces[b]	not detectable	not detectable	32-832 mg/L
Brew Coffee[a]	not detectable	23-47 mg/L	not detectable
Honey[b]	not detectable	nd-436 mg/L	271-1641 mg/L
Cocoa[a]	0.9-3.4 mg/kg	0.02 mg/kg	0.5-3.6 mg/kg
Bread[b]	not detectable	nd-28 mg/kg	13-619 mg/kg
Roast bean coffee[a]	20-130 mg/L	20-220 mg/L	not detectable
Pasta[b]	not detectable	not detectable	nd-8.8 mg/kg
Cookies[b]	not detectable	1.8-68 mg/kg	8.5-385 mg/kg

[a]Data are from Henle (2008); [b]Data are from Degen et al. (2012).

BIOAVAILABILITY OF DIETARY AGES

To summarize animal experiments and pathological and clinical studies (Delgado-Andrade, Tessier, Niquet-Leridon, Seiquer, & Pilar, 2012; Somoza, Wenzel, Weiss, Clawin-Rädecker, Grübel, & Erbersdobler, 2006), consuming AGE-rich foods may lead to significantly higher AGEs in human plasma. Studies also indicated that the higher the intake of food-derived AGEs, the more AGEs the body excretes. However, how the intestines absorb AGEs remains as yet unclear. AGEs may be bound to specific receptors and subsequently decomposed by macrophages, or they may be broken down by the extracellular proteolytic system.

In addition, smaller molecules-soluble peptide AGEs can be excreted by the kidneys (Baumann, 2012), but any deterioration in the renal system like reduced renal function, can result in more accumulation of dietary AGEs in the body, which can cause oxidative, release pro-inflammatory molecules, cause abnormal signaling and gene expression and thus contribute to the pathology of chronic diseases like diabetes and add to medical complications (Bierhaus, Hofmann, Ziegler, & Nawroth, 1998; Singh, Barden, Mori, & Beilin, 2001). Therefore, accurately determining exposure to dietary AGEs is important for risk assessment.

HUMAN EXPOSURE TO DIETARY AGES

Only a few studies have examined human exposure to dietary AGEs. Delgado-Andrade, Seiquer, Navarro, and Morales (2007) showed, for children between 11 and 14 years old, 5.3 mg mean total CML intake/day indicated a diet with low MRPs, whereas 11.3 mg CML intake/day indicated a diet high in MRPs. A group of 18-24 year olds, according to Uribarri et al. (2007), consumed an average of 15000 kU/day of CML based on 3-day food records for 90

healthy subjects. In another study of a group of 18-24 year olds in France, the mean CML intake in a French diet was 5.4 mg CML/day when food was cooked at high temperatures, although that amount was significantly decreased when food was cooked using low temperature methods like steaming (Birlouez-Aragon et al., 2010), which adds to the evidence that high cooking temperatures can increase formation of dietary AGEs. Nevertheless, more studies are needed on dietary exposure to AGEs for other populations, including the kinds of food products that contribute more to daily AGE intake.

MITIGATING DIETARY AGES IN FOODS

A wide variety of epidemiological and animal studies have shown that dietary intake of AGEs increases health risks. Therefore, investigating and developing ways to decrease formation of AGEs in food products may delay or prevent chronic disease.

Some common mitigating methods include selecting different food types, modifying cooking methods, and adding inhibitors.

Food types

Overall, dietary AGEs form more easily in foods rich in fat and high in protein, such as red muscle meat products (beef and pork) with an average 43 kU/g CML. In contrast, vegetables and fruits contain relatively fewer dietary AGEs with an average 3.4 kU/g CML (Goldberg et al., 2004). Hull et al. (2012) provided additional results to support the data, showing the lowest average CML level in fruits and vegetables was 0.13 mg/100g. Uribarri et al. (2010) indicated animal-derived products contained the highest levels of AGEs; even uncooked cheeses and butter have large amounts of dietary AGEs. Based on current evidence, a diet with more fruits,

vegetables, and grains may reduce exposure to dietary AGEs.

Modifying cooking methods

Because food processing parameters like cooking time, cooking temperature, and cooking method are an important part of formation of dietary AGEs, they must be considered as part of the move to reduce dietary AGEs. However, certain types of cooking enhance the flavor and color of food, usually requiring higher cooking temperatures or longer cooking times, both of which can enhance formation of dietary AGEs (Chao et al., 2009). Some studies note that formation of dietary AGEs were significantly reduced by modifying culinary techniques, cooking for less time and at lower temperatures. Uribarri et al. (2010) found that AGE content was 5,828 kU/100 g in broiled chicken and 5,963 kU/100 g in broiled beef but dropped significantly to 1,123 kU/100 g when chicken was boiled in water, while in stewed beef, AGE content dropped to 2,230 kU/100 g. Goldberg et al. (2004) had already suggested daily AGE ingestion could be reduced by 50% by boiling and stewing foods. Assar et al. (2009) also showed the average CML content in boiled beef (5.0 mg/kg food) was much lower than in fried minced beef (11.2 mg/kg food). These research results show that cookery choice can reduce consumer intake of dietary AGEs.

AGE Inhibitors

Using AGE inhibitors during cooking can mitigate the formation of dietary AGEs. Generally speaking, inhibitors attenuate oxidative stress through scavenging AGE intermediates, the reactive dicarbonyls, or free radicals produced in the process of glycoxidation (Wu et al., 2011). Investigating and developing AGE inhibitors has been widely done, and inhibition may occur as part of the inhibitor's antioxidant activity or capacity to

scavenge carbonyl groups (Lo et al., 2006; Zieman, & Kass, 2004). To be specific, anti-AGE agents may act during the glycation process by trapping carbonyl groups on Amadori products and reactive dicarbonyl compounds (Tan, Wang, Lo, & Ho, 2008). However, antioxidants can actually prevent the formation of AGEs by inhibiting oxidation of sugar and subsequent formation of Amadori products, possibly because they can scavenge hydroxyl and superoxide radicals or chelate metal ions to alleviate oxidative stress. (Lee, Jang, Lee, Kim, & Kim, 2006; Yamaguchi, Ariga, Yoshimura, & Nakazawa, 2000)

A variety of synthetic and natural products have been evaluated as AGE inhibitors. Common synthetic AGE inhibitors include aminoguanidine (Nagai, Murray, Metz, & Baynes, 2012) and pyridoxamine (Culbertson, Enright, & Ingold, 2003), both of which are effective carbonyl trapping agents that decrease carbonyl stress (Figure 5). Even during cooking, adding aminoguanidine has prevented formation of new dietary AGEs in olive oil. Unfortunately, aminoguanidine is considered toxic if taken excessively; it may lead to hypercholesterolemia and hypertriglyceridemia (Cameron et al., 1993).

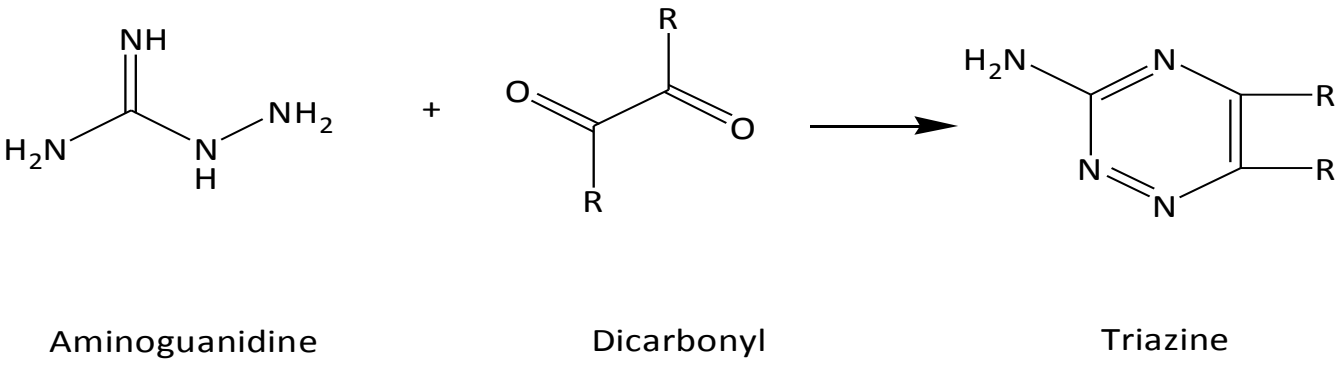

Figure 5. Proposed mechanism for reaction trapping dicarbonyl compounds with aminoguanidine (modified from Price, Rhett, Thorpe, & Baynes, 2001).

Natural anti-AGE agents, derived from plant or food products, have fewer side-effects and may be promising AGE inhibitors because they contain abundant antioxidants and may mitigate AGE formation by trapping free radicals. The inhibitory effect of cinnamon bark on AGE formations was demonstrated in a bovine serum albumin (BSA)-glucose model (Peng, Cheng, Ma, Chen, Ho, & Lo, 2008). Moreover, it effectively inhibited CML formation during *in vivo* glycation during oral administration of vitamin C (1 g/day) for four consecutive weeks (Vinson, & Howard, 1996). Nine main phenolic constituents in vaccinium ethanolic extracts inhibited CML formation as well (Beaulieu et al., 2010). In addition to anti-oxidants, anti-glycation in active compounds of natural products may also be related to their ability to trap carbonyls. For instance, phenolic compounds like chlorogenic acid are responsible for the properties that allow coffee silverskin extract to trap carbonyls (Mesias, Navarro, Martinez-Saez, Ullate, del Castillo, & Morales, 2014). Navarro and Morales (2015) noted that hydroxytyrosol (3,4-dihydroxyphenyl ethanol), one of the major phenolic compounds in olive oil, could trap the reactive dicarbonyl species MGO and therefore inhibit AGE formation (Figure 6). Based on the findings of previous studies, further research into anti-glycation agents is necessary because the use of such agents may delay the onset of chronic diseases like diabetes. The challenge of future research will be how to explain the direct structure and functional relationships in the anti-glycation process, as well as how to retain satisfactory flavor while keeping food safe.

Enhancing water-holding activity

Dry heat cooking actually encourages formation of dietary AGEs (Uribarri et al., 2010), perhaps due to increased cooking loss and mobility of water soluble precursors. Although we

still so not have enough definitive information on the correlation of water-holding activity (WHC) and dietary AGEs, we can assume that adding water-binding compounds may restrict transport of reaction precursors, leading to a decrease in the formation of dietary AGEs in food systems.

In conclusion, dietary AGEs may require changes in cooking methods for those foods with a high probability of forming AGEs. Lower cooking temperatures and less cooking time; steaming, stewing, and poaching instead of frying, grilling, and roasting; using natural antioxidants in foods to inhibit the formation of AGEs would all contribute to reducing AGEs in food.

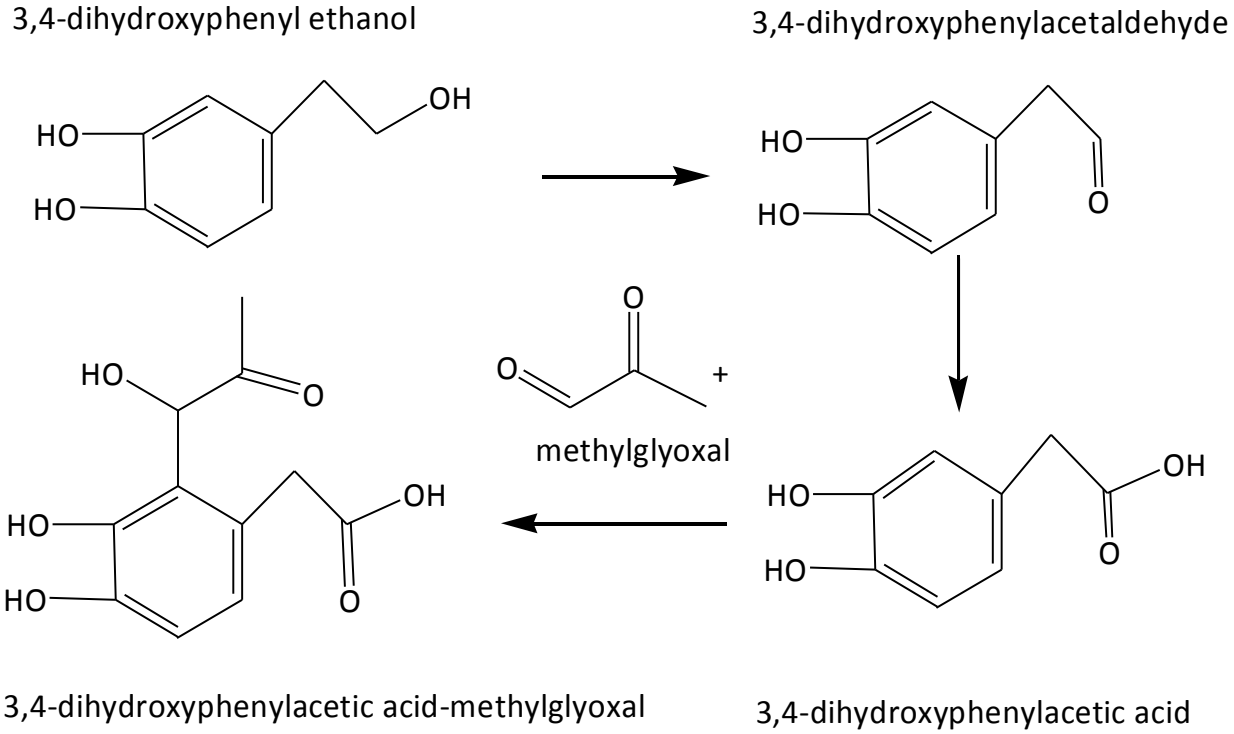

Figure 6. Proposed mechanism for reaction trapping methylglyoxal with 3,4-dihydroxyphenyl ethanol (modified from Navarro et al., 2015).

SUMMARY

The Maillard reaction results in the irreversible formation of advanced glycation endproducts (AGEs). Increasingly, AGEs are recognized as important in clinical science; they are associated with oxidation stress and inflammation, and thus with complications of diabetes and other chronic diseases.

AGEs are a large and heterogeneous group of compounds caused by the reaction between reducing sugars and amino groups from amino acids. Research points to dietary AGEs as important contributors to AGEs circulating in humans.

Dietary AGEs form because of the composition of food items, the cooking temperature and cooking time, humidity, pH, and the presence of antioxidants or antiglycation products. Generally speaking, animal-derived foods cooked at high temperatures and for a long time will show more AGEs than other foods.

Future research should focus on extending our database of levels of dietary AGEs in more foods, applying new methods of identifying dietary AGEs, understanding what contributes most to AGE exposure from cooked foods, and elucidating the pathogenic mechanisms of dietary AGEs.

REFERENCE

Acevedo, N. C., Schebor, C., & Buera, P. (2008). Non-enzymatic browning kinetics analysed through water-solids interactions and water mobility in dehydrated potato. *Food Chemistry*, *108*, 900-906.

Ahmed, N., Argirov, O. K., Minhas, H. S., Cordeiro, C. A. A., & Thornalley, P. J. (2002). Assay of advanced glycation endproducts (AGEs): surveying AGEs by chromatographic assay with derivatization by 6-aminoquinolyl-N-hydroxysuccinimidyl-carbamate and application to N-epsilon-carboxymethyl-lysine- and N-epsilon-(1-carboxyethyl)lysine-modified albumin. *Biochemical Journal*, *364*, 1–14.

Ahmed, N., Thorpe S., & Baynes J. (1986). Identification of N-epsilon-carboxymethyllysine as a degradation product of fructoselysine in glycated protein. *Journal of Biological Chemistry*, *261*(11), 4889–4894.

Ahmed, N. (2005). Advanced glycation endproducts-role in pathology of diabetic complications. *Diabetes Research and Clinical Practice*, *67*(1), 3–21.

Ajandouz, E. H., & Puigserver, A. (1999). Nonenzymatic browning reaction of essential amino acids: Effect of pH oncaramelization and Maillard reaction kinetics. *Journal of Agricultural and Food Chemistry, 47*, 1786-1793.

Allmen, E. U., Koch, M., Fritz, G., & Legler, D. F. (2008). V domain of RAGE interacts with AGEs on prostate carcinoma cells. *Prostate*, *68*(7), 748-758.

Ames, J. M. (2007). Evidence against dietary advanced glycation endproducts being a risk to human health. *Mololecular Nutrition and Food Research*, *51*, 1085–1090.

Ames, J. M. (2009). Dietary Maillard Reaction Products: Implications for Human Health and Disease. *Czech Journal of Food Science*, *27*, S66-S69.

An, X. F., Zhou, L., Jiang, P. J., Yan, M., Huang, Y. J., Zhang, S. N., Niu, Y. F., Ten, S. C., & Yu, J. Y. (2011). Advanced glycation end-products induce heparanase expression in endothelial cells by the receptor for advanced glycation end products and through activation of the FOXO4 transcription factor. *Molecular and Cellular Biochemistry*, *354*, 47–55.

Assar, S., Moloney, C., Lima, M., Magee, R., & Ames, J. (2009). Determination of N-epsilon-carboxymethyllysine in food systems by ultra performance liquid chromatography-mass spectrometry. *Amino Acids*, *36*(2), 317–326.

Augustin, M. A., Sanguansri, L., & Bode, O. (2006). Maillard Reaction Products as Encapsulants for Fish Oil Powders. *Journal of Food Science*. *71*(2), 25-32.

Baumann, M. (2012). Role of advanced glycation end products in hypertension and cardiovascular risk: Human studies. *Journal of the American Society of Hypertension*, *6*, 427–435.

Beaulieu, L. P., Harris, C. S., Saleem, A., Cuerrier, A., Haddad, P. S., Martineau L. C., Bennett S. A., & Arnason J. T. (2010). Inhibitory effect of the Cree traditional medicine wiishichimanaanh (Vaccinium vitis-idaea) on advanced glycation endproduct formation: identification of active principles. *Phytotherapy Research*, *24*(5), 741-747.

Bierhaus, A., Hofmann, M. A., Ziegler, R., & Nawroth, P. P. (1998). AGEs and their interaction with AGE-receptors in vascular disease and diabetes mellitus. I. The AGE concept. *Cardiovascular Research*, 37, 586–600.

Birlouez-Aragon, I., Saavedra, G., Tessier, F. J., Galinier, A., Ait-Ameur, L., Lacoste, F., Niamba C.

N., Alt, N., Somoza, V., & Lecerf, J. M. (2010). A diet based on high-heat-treated foods promotes risk factors for diabetes mellitus and cardiovascular diseases. *The American Journal of Clinical Nutrition*, *91*, 1220–1226.

Bravo, A., Herrera, J. C., Scherer, E., Ju-Nam, Y., Rübsam, H., Madrid, J., Zufall, C., & Rangel-Aldao, R. (2008). Formation of alpha-dicarbonyl compounds in beer during storage of Pilsner. *Journal of Agricultural and Food Chemistry*, *56*, 4134−4144.

Brownlee, M. (1994). Lilly lecture 1993-glycation and diabetic complications. *Diabetes*, *43*(6), 836–841.

Brownlee, M. (1995). Advanced protein glycosylation in diabetes and aging *Annual Review of Medicine*, *46*,223–234.

Brownlee, M. Vlassara, H. & Cerami, A. (1984). Nonenzymatic glycosylation and the pathogenesis of diabetic complications. *Annals of Internal Medicine*, *101*, 527–537.

Bueser, W., Erbersdobler, H. F., & Liardon, R. (1987). Identification and determination of N-ε-carboxymethyllysine by gas-liquid chromatography. *Journal of Chromatography A*, *387*, 515–519.

Cameron, N. E., & Cotter, M. A. (1993). Potential therapeutic approaches to the treatment or prevention of diabetic neuropathy-evidence from experimental studies. *Diabetic Medicine*, *10*(7), 593–605.

Chao, P., Hsu, C., & Yin, M. (2009). Analysis of glycative products in sauces and sauce-treated foods. *Food Chemistry*, *113*(1), 262–266.

Charissou, A., Ait-Ameur, L., & Birlouez-Aragon, I. (2007). Evaluation of a gas chromatography/mass spectrometry method for the quantification of

carboxymethyllysine in food samples. *Journal of Chromatography A*. *1140*, 189–194.

Chuyen, V. N. (2006). Toxicity of the AGEs generated from the Maillard reaction: On the relationship of food-AGEs and biological-AGEs. *Molecular Nutrition & Food Research*, *50*(12), 1140-1149.

Cuevas, M. E., Haensgen, H., Sepúlveda, F. J., Zegers, G., Roa, J., Opazo, C., & Aguayo, L. G. (2011). Soluble Aβ(1-40) peptide increases excitatory neurotransmission and induces epileptiform activity in hippocampal neurons. *Journal of Alzheimer's Disease, 23*(4), 673-687.

Culbertson, S. M., Enright, G. D., & Ingold, K, U. (2003). Synthesis of a novel radical trapping and carbonyl group trapping anti-AGE agent: A pyridoxamine analogue for inhibiting advanced glycation (AGE) and lipoxidation (ALE) end products. *Organic Letters*, *5*(15), 2659-2662.

Degen, J., Hellwig, M., & Henle, T. (2012). 1,2-dicarbonyl compounds in commonly consumed foods. *Journal of Agricultural and Food Chemistry, 2008*(56), 4134−4144.

Delgado-Andrade, C., Seiquer, I., Navarro, M. P., & Morales, F. J. (2007). Maillard reaction indicators in diets usually consumed by adolescent population. *Molecular Nutrition & Food Research, 51*, 341–351.

Delgado-Andrade, C., Tessier, F. E. J., Niquet-Leridon, C., Seiquer, I., & Pilar N. M. (2012). Study of the urinary and faecal excretion of N^{ε}-carboxymethyllysine in young human volunteers. *Amino Acids*, *43*, 595–602.

De Revel, G., & Bertrand, A. (1993). A method for the detection of carbonyl compounds in wine glyoxal and methylglyoxal. *Journal of the Science of Food and Agriculture*, *61*, 267−272.

Dittrich, R., Hoffmann, I., Stahl, P., Müller, A., Beckmann, M. W., & Pischetsrieder, M. (2006). Concentrations of Ne-carboxymethyllysine in human breast milk, infant formulas, and urine of infants. *Journal of Agricultural and Food Chemistry*, *54*(18), 6924–6928.

Drusch, S., Faist, V., & Erbersdobler, H. (1999). Determination of N[epsilon]-carboxymethyllysine in milk products by a modified reversed-phase HPLC method. *Food Chemistry*, *65*(4), 547–553.

Erbersdobler, H. F., & Somoza, V. (2007). Forty years of furosine-forty years of using Maillard reaction products as indicators of the nutritional quality of foods. *Molecular Nutrition & Food Research*, *51*(4), 423-430.

Everts, S. (2012). "The Maillard Reaction Turns 100". *Chemical & Engineering News*, *90*(40), 58–60.

Fenaille, F., Parisod, V., Visani, P., Populaire, S., Tabet, J. C., & Guy, P. A. (2006). Modifications of milk constituents during processing: a preliminary benchmarking study. *International Dairy Journal*, *16*, 728–739.

Finot, P. A., Deutsch, R., & Rujard, E. (1981). The extent of the Maillard reaction during the processing of milk. *Progress in Food Nutrition Sciences*, *5*, 345-355.

Forster, A., & Henle, T. (2003). Glycation in food and metabolic transit of dietary AGEs (advanced glycation end-products): studies on the urinary excretion of pyrraline. *Biochemical Society Transactions*, *31*, 1383–1385.

Franke, S., Dawczynski, J., Strobel, J., Niwa, T., Stahl, P., & Stein, G. (2003). Increased levels of advanced glycation end products in human cataractous lenses. *Journal of Cataract & Refractive Surgery*, *29*(5), 998-1004.

Fu, M. X., Requena, J. R., Jenkins, A. J., Lyons, T. J., Baynes, J. W., & Thorpe S. R. (1996). The advanced glycation end product, N^{ε}-(carboxymethyl)lysine, is a product of both lipid peroxidation and glycoxidation reactions. *Journal of Biological Chemistry*, *271*, 9982-9986.

Goldberg, T., Cai, W. J., Peppa, M., Dardaine, V., Baliga, B. S., & Uribarri, J. (2004). Advanced glycoxidation end products in commonly consumed foods. *Journal of the American Dietetic Association*, *104*(8), 1287–1291.

Hartkopf, J., & Erbersdobler, H. F. (1995). Model experiments with sausage meat on the formation of N^{ε}-carboxymethyllysine. *Zeitschrift für Lebensmitteluntersuchung und - Forschung A*, *201*, 27–29.

Hartkopt, J., Pahlke, C., Ludmann, G., & Erbersdobler, H. F. (1994). Determination of Ne-carboxymethyllysine by reversed-phase high performance liquid chromatography method. *Journal of Chromatography A*, *672*, 242–246.

Hashim, Z., & Zarina, S. (2011). Advanced glycation end products in diabetic and non-diabetic human subjects suffering from cataract. *Age (Dordrecht, Netherlands)*, *33*(3), 377-384.

Hayase F., Shibuya T., Sato J., & Yamamoto M. (1996). Effects of oxygen and transition metals on the advanced Maillard reaction of proteins with glucose. *Bioscience, Biotechnology, and Biochemistry*, *60*(11), 1820-5.

Heizmann, C. W. (2007). The mechanism by which dietary AGEs are a risk to human health is via their interaction with RAGE: arguing against the motion. *Molecular Nutrition & Food Research*, *51*, 1116–1119.

Hegele, J., Parisod, V., Richoz, J., Forster, A., Maurer, S., Krause, R.,Henle, T., Butler, T., & Delatour, T. (2008). Evaluating the extent of protein damage in dairy products-Simultaneous determination of early and advanced glycation-induced lysine modifications. *Annals of The New York Academy of Sciences*, *1126*, 300-306.

Henle, T. (2005). Protein-bound advanced glycation endproducts (AGEs) and bioactive amino acid derivatives in foods. *Amino Acids*, *29*, 313–322.

Henle, T. (2008). Maillard reaction of proteins and advanced glycation end products (AGEs) in food. *Process-induced food toxicants: occurrence, formation, mitigation and health risks*. 215-242.

Henle, T., Schwarzenbolz, U., & Klostermeyer, H. (1997). Detection and quantification of pentosidine in foods. *Zeitschrift für Lebensmitteluntersuchung und -Forschung A*, *204*, 95-98.

Hofmann, S. M., Dong, H. J., Li, Z., Cai, W., Altomonte, J., Thung, S. N., Zeng, F., Fisher, E. A., & Vlassara H. (2002). Improved insulin sensitivity is associated with restricted intake of dietary glycoxidation products in the db/db mouse. *Diabetes*. *51*, 2082–2089.

Horiuchi, S., Araki, N., Morino, Y. (1991). Immunochemical approach to characterize advanced glycation end products of the Maillard reaction. *Journal of Biological Chemistry*, *266*(12), 7329-7332.

Huebschmann, A. G., Regensteiner, J. G., Vlassara, H., & Reusch, J. E. (2006). Diabetes and advanced glycoxidation endproducts. *Diabetes Care*, *29*(6), 1420-1432.

Hughes, S. J., Wall, N., Scholfield, C. N., McGeown, J. G., Gardiner, T. A., Stitt, A. W., & Curtis, T. M. (2004). Advanced glycation endproduct modified basement membrane attenuates

endothelin-1 induced [Ca2+]i signalling and contraction in retinal microvascular pericytes. *Molecular Vision*, *10*, 996-1004.

Hull, G., Woodside, J., Ames, J., & Cuskelly, G. (2012). N-epsilon-carboxymethyllysine content of foods commonly consumed in a western style diet. *Food Chemistry*, *131*(1), 170–174.

Kanauchi, M., Tsujimoto, N., & Hashimoto, T. (2001). Advanced glycation end products in nondiabetic patients with coronary artery disease. *Diabetes Care*. *24*, 1620 –1623.

Kang, R., Tang, D., Lotze, M. T., & Zeh, H. J. 3rd. (2012). AGER/RAGE-mediated autophagy promotes pancreatic tumorigenesis and bioenergetics through the IL6-pSTAT3 pathway. *Autophagy*, *8*(6), 989-991.

Kasper, M., & Schieberle, P. (2005). Labeling studies on the formation pathway of N-epsilon-carboxymethyllysine in Maillard-type reactions. *Annals of The New York Academy of Sciences*, *1043*, 59-62.

Kilhovd, T. J., Berg, K. I., Birkeland, P., Thorsby, & Hanssen K. F. (1999). Serum levels of advanced glycation end products are increased in patients with type 2 diabetes and coronary heart disease. *Diabetes Care*, *22* (9), 1543-1548.

Kim, Y. S., Reddy, M. A., Rahbar, S., Lanting, L., & Natarajan, R. (2002). Effects of advanced glycation end products and RAGE ligation with S100B in mesangial and vascular smooth muscle cells. *Journal of the American Society of Nephrology*, *13*, 90A.

Kimura, T., Takamatsu, J., & Araki, K. (1995). Are advanced glycation end-products associated with amyloidosis in Alzheimer's disease? *NeuroReport*, *6*, 866–868.

Koschinsky, T., He, C. J., Mitsuhashi, T., Bucala, R., Liu, C., Buenting, C., Heitmann, K., & Vlassara, H. (1997). Orally absorbed reactive glycation products (glycotoxins): an environmental

risk factor in diabetic nephropathy. *Proceedings of the National Academy of Sciences*, *94*(12), 6474–6479.

Kwak, E. J., & Lim, S. I., (2004). The effect of sugar, amino acid, metal ion, and NaCl on model Maillard reaction under pH control. *Amino Acids*, *27*(1), 85-90.

Laroque, D., Inisan, C., Berger, C., Vouland, E., Dufosse, L., & Guerard, F. (2008). Kinetic study on the Maillard reaction. Consideration of sugar reactivity. *Food Chemistry*, *111*, 1032–1042.

Ledl, F., & Schleicher E. (1990). New aspects of the maillard reaction in foods and in the human body. *Angewandte Chemie International Edition*, *29*, 565–594.

Lee, G, Y., Jang, D. S., Lee, Y. M., Kim, J. M., Kim, J. S. (2006). Naphthopyrone glucosides from the seeds of cassia tora with inhibitory activity on advanced glycation end products (AGEs) formation. *Archives of Pharmacal Research*, *29*, 587–590.

Lo, C. Y., Li, S., Tan, D., Pan, M. H., Sang, S., & Ho, C. T. (2006). Trapping reactions of reactive carbonyl species with tea polyphenols in simulated physiological conditions. *Molecular Nutrition & Food Research*, *50*(12), 1118-1128.

Luevano-Contreras, C., & Chapman-Novakofski, K. (2010). Dietary advanced glycation end products and aging. *Nutrients*, *2*(12), 1247-1265.

Luevano-Contreras, C., Garay-Sevilla, M. E., Wrobel, K., Malacara, J. M., & Wrobel, K. (2013). Dietary advanced glycation end products restriction diminishes inflammation markers and oxidative stress in patients with type 2 diabetes mellitus. *Journal of Clinical Biochemistry and Nutrition*, *52*, 22–26.

Macias-Cervantes, M. H., Rodriguez-Soto, J. M. D., Uribarri, J., Diaz-Cisneros, F., Cai, W., Garay-Sevilla, M. E. (2015). Effect of a diet restricted in advanced glycation end products and exercise on metabolic parameters in adult overweight men. *Nutrition*, *31*(3), 446-451.

Makita, Z., Vlassara, H., Cerami, A., & Bucala, R. (1992). Immunochemical detection of advanced glycosylation end products *in vivo*. *Journal of Biological Chemistry*, *267*, 5133–5138.

Mark, A. B., Poulsen, M. W., Andersen, S., Andersen, J.M., Bak, M.J., Ritz, C., Holst, J.J., Nielsen, J., de Courten, B., Dragsted, L.O. & Bügel, S.G. (2014). Consumption of a diet low in advanced glycation end products for 4 weeks improvesinsulin sensitivity in overweight women. *Diabetes Care*, *37*, 88–95.

Mesias, M., Navarro, M., Martinez-Saez, N., Ullate, M., del Castillo, M. D., & Morales, F. J. (2014). Antiglycative and carbonyl trapping properties of the water soluble fraction of coffee silverskin. *Food Research International*, *62*, 1120-1126.

Miki, S., Kasayama, S., Miki, Y., Nakamura, Y., Yamamoto, M., Sato, B., & Kishimoto, T. (1993). Expression of receptors for advanced glycosylation end products on renal cell carcinoma cells *in vitro*. *Biochemical and Biophysical Research Communications*, *196*(2), 984-989.

Nagai, R., Hayashi, C. M., Xia, L., Takeya, M., & Horiuchi, S. (2002). Identification in human atherosclerotic lesions of GA-pyridine, a novel structure derived from glycolaldehyde-modified proteins. *Journal of Biological Chemistry*, *277*(50), 48905-48912.

Nagai, R., Murray, D. B., Metz, T. O., & Baynes, J. W. (2012). Chelation, a fundamental mechanism of action of AGE inhibitors, AGE breakers, and other inhibitors of diabetes complications. *Diabetes*, *61*, 549–559.

Nargund, A. M., Pellegrino, M. W., Fiorese, C. J., Baker, B. M., & Haynes, C. M. (2012). Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. *Science*, *337*, 587-590.

Navarro, M., & Morales, F. J. (2015). Mechanism of reactive carbonyl species trapping by hydroxytyrosol under simulated physiological conditions. *Food Chemistry*, *175*, 92-99.

Neeper, M., Schmidt, A. M., Brett, J., Yan, S. D., Wang, F., Pan, Y. C., Elliston, K., Stern, D., Shaw, A. (1992). Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *Journal of Biological Chemistry*, *267*, 14998–15004.

Necula, M., & Kuret, J. (2004). Pseudophosphorylation and glycation of tau protein enhance but do not trigger fibrillization *in vitro*. *Journal of Biological Chemistry*, *279*(48), 49694-49703.

Nguyen, H. T., van der Fels-Klerx, H. J., & van Boekel, M. A. J. S. (2014). N-epsilon-(carboxymethyl)lysine: A review on analytical methods, formation, and occurrence in processed food, and health impact. *Food Reviews International*, *30*(1), 36-52.

Nin, J. W., Jorsal, A., Ferreira, I., Schalkwijk, C. G., Prins, M. H., Parving H. H., Tarnow, L., Rossing, P., & Stehouwer, C. D. (2011). Higher plasma levels of advanced glycation end products are associated with incident cardiovascular disease and all-cause mortality in type 1 diabetes: a 12-year follow-up study. *Diabetes Care*, *34*, 442-447.

Nursten, H. (2005a). The Maillard Reaction Chemistry, Biochemistry and Implications. *Cambridge: The Royal Society of Chemistry*, (Chapter 1).

Nursten, H. (2005b). The Maillard Reaction Chemistry, Biochemistry and implications. *Cambridge: The Royal Society of Chemistry*, (Chapter 3).

O'Brien, J. M., & Morrissey, P. A. (1989). The Maillard reaction in milk products. *Bulletin of the International Dairy Federation*, *238*, 53-61.

Palinski, W., Koschinsky, T., Butler, S. W., Miller, E., Vlassara, H., Cerami, A. & Witztum, J. L. (1995). Immunological evidence for the presence of advanced glycosylation end products in atherosclerotic lesions of euglycemic rabbits. *Arteriosclerosis Thrombosis and Vascular Biology*, *15*, 571–582.

Peng, X., Cheng, K., Ma, J., Chen, B., Ho, C., & Lo, C. (2008). Cinnamon bark proanthocyanidins as reactive carbonyl scavengers to prevent the formation of advanced glycation endproducts. *Journal of Agricultural and Food Chemistry*, *56*(6), 1907-1911.

Peng, X. F., Ma, J. Y., Cheng K. W., Jiang, Y., Chen, F., & Wang, M. F. (2010). The effects of grape seed extract fortification on the antioxidant activity and quality attributes of bread. *Food Chemistry*, *119*(1), 49–53.

Peppa, M., Uribarri, J., & Vlassara, H. (2004). The role of advanced glycosylation end productsin the development of atherosclerosis. *Current Diabetes Reports*, *4*(1), 31-60.

Persson, E., Sjõholm, & Skog, K. (2003). Effect of high water-holding capacity on the formation of heterocyclic amines in fried beefburgers. *Journal of Agricultural and Food Chemistry*, *51*, 4472-4477.

Poulsen, M. W., Hedegaard, R. V., Andersen, J. M., de Courten, B., Bügel, S., Nielsen, J., Skibsted, L. H., & Dragsted, L. O. (2013). Advanced glycation endproducts in food and their effects on health. *Food and Chemical Toxicology*, *60*, 10-37.

Price, D. L., Rhett, P. M., Thorpe, S. R., & Baynes, J. W. (2001). Chelatng activity of advanced end products inhibitors. *Journal of Biological Chemistry*, *276*, 48967–48972.

Raj, D. S., Choudhury, D., Welbourne, T. C., & Levi, M. (2000). AGE: a nephrologist's perspective. *American Journal of* Kidney *Diseases*, *35*, 365–380.

Reddy, V. P., & Beyaz, A. (2006). Inhibitors of the maillard reaction and AGE breakers as therapeutics for multiple diseases. *Drug Discovery Today*, *11*, 646–654.

Rufian-Henares, J. A., Guerra-Henandez, E., & Garcia-Villanova, B. (2004). Pyralline content in enteral formula processing and storage and model systems. *European Food Research and Technology*, *219*, 42–47.

Ruttkat, A., & Erbersdobler, H. F. (1995). N-epsilon-carboxymethyllysine is formed during heating of lysine with ketoses. *Journal of the Science of Food and Agriculture*, *68*(2), 261-263.

Schmitt, A., Schmitt, J., Munch, G., & Gasic M. J. (2005). Characterization of advanced glycation end products for biochemical studies: Side chain modifications and fluorescence characteristics. *Analytical Biochemistry*, *338*(2), 201–215.

Schmidt, A. M., Vianna, M., Gerlach, M., Brett, J., Ryan, J., Kao, J., Esposito, C., Hegarty, H., Hurley, W., Clauss, M., Wang, F., Pan, Y. E., Tsang, T. E., & Stern, D. (1992). Isolation and characterization of two binding proteins for advanced glycosylation endproducts from bovine lung which are present on the endothelial cell surface. *Journal of Biological Chemistry*, *267*, 14987-14997.

Schwarzenbolz, U., Henle, T., Haebner, R., & Klostermeyer, A. (1997). On the reaction of glyoxal with proteins. *Zeitschrift für Lebensmitteluntersuchung und -Forschung A*, *205*, 121-124.

Schwenger, V., Zeier, M., Henle, T., & Ritz, E. (2001). Advanced glycation endproducts (AGEs) as uremic toxins. *Nahrung*, *45*(3), 172-176.

Semba, R. D., Gebauer, S. K., Baer, D. J., Sun, K., Turner, R., Silber, H.A., Talegawkar, S., Ferrucci, L., & Novotny, J.A. (2014). Dietary intake of advanced glycation end products did not affect endothelial function and inflammation in healthy adults in a randomized controlled trial. *Journal of Nutrition*, *144*, 1037–1042.

Sharma, C., Kaur, A., Thind, S. S., Singh, B., & Raina, S. (2015). Advanced glycation End-products (AGEs): an emerging concern for processed food industries. *Journal of Food Science and Technology*, *52*(12), 7561–7576.

Shi, Y. & Hu, F. B. (2014). The global implications of diabetes and cancer. *The Lancet*, *383* (9933), 1947–1948.

Singh, R., Barden, A., Mori, T., & Beilin, L. (2001). Advanced glycation end-products: a review. *Diabetologia*, *44*(2), 129-146.

Sinha, R, Peters, U, Cross, A. J., Kulldorff, M., Weissfeld, J. M., Pinsky, P. F., Rothman, N., & Hayes, R. B. (2005). Meat, meat cooking methods and perservation, and risk of colorectal adenoma. *Cancer Research*, *17*, 8034-8041.

Smit, A. J., & Lutgers, H. L. (2004). The clinical relevance of advanced glycation endproducts (AGE) and recent developments in pharmaceutics to reduce AGE accumulation. *Current Medicinal Chemistry*, *11*(20), 2767-2784.

Somoza, V. (2005). Five years of research on health risks and benefits of Maillard reaction products: An update. *Molecular Nutrition & Food Research*, *49*, 663-672.

Somoza, V., Wenzel, E., Weiss, C., Clawin-Rädecker, I., Grübel, N., & Erbersdobler, H. F. (2006). Dose-dependent utilisation of casein-linked lysinoalanine, N^{ε}-fructoselysine and N^{ε}-carboxymethyllysine in rats. *Molecular Nutrition & Food Research*, *50*, 833–841.

Srey, C., Hull, G. L. J., Connolly, L., Elliott, C. T., del Castillo, M. D., & Ames, J. M., (2010). Effect of inhibitor compounds on N-epsilon-(carboxymethyl)lysine (CML) and N-epsilon-(carboxyethyl)lysine (CEL) formation in model foods. *Journal of Agricultural and Food Chemistry*, *58*(22), 12036-12041.

Takino, J., Yamagishi, S., & Takeuchi, M. (2010). Cancer malignancy is enhanced by glyceraldehyde-derived advanced glycation end-products. *Journal of Oncology*, 739852.

Tan, D., Wang, Y., Lo, C. Y., & Ho, C. T. (2008). Methylglyoxal: its presence and potential scavengers. *Asia Pacific Journal of Clinical Nutrition*, *17*, 261-264.

Tanaka, M., Chiba, N., Ishizaki, S., Takai, R., & Taguchi, T. (1994). Influence of water activity and maillard reaction on the polymerization of myosin heavy chain in freeze-dried squid meat. *Fisheries Science*, *60*(5), 607-611.

Tanji, N., Markowitz, G. S., Fu, C., Kislinger, T., Taguchi, A., Pischetsrieder, M., Stern, D., Schmidt, A. M., & D'Agati, V. D. (2000). Expression of advanced glycation end products and their cellular receptor RAGE in diabetic nephropathy and nondiabetic renal disease. *Journal of the American Society of Nephrology*, *11*(9), 1656-66.

Tauer, A., Hasenkof, K., Kislinger, T., Frey, I., & Pischetsrieder, M. (1999). Determination of CML in heated milk products by immunochemical methods. *European Food Research and Technology*, *209*, 72–76.

Thornalley, P. J. (2007). Dietary AGEs and ALEs and risk to human health by their interaction with the receptor for advanced glycation endproducts (RAGE)–an introduction. *Molecular Nutrition & Food Research*, *51*, 1107–1110.

Turk, Z., Ljubic, S., Turk, N., & Benko, B. (2001). Detection of autoantibodies against advanced glycation endproducts and AGE-immune complexes in serum of patients with diabetes mellitus. *Clinica Chimica Acta*, *303*, 105-115.

Uribarri, J., Cai, W., Peppa, M., Goodman, S., Ferrucci, L., Striker, G., & Vlassara, H. (2007). Circulating glycotoxins and dietary advanced glycation endproducts: Two links to inflammatory response, oxidative stress, and aging. *The Journals of Gerontology Series A Biological Sciences and Medical Sciences*, *62*(4), 427-433.

Uribarri, J., Cai, W., Ramdas, M., Goodman, S., Pyzik, R., Chen, X., Zhu, L., Striker, G. E., & Vlassara, H. (2011). Restriction of advanced glycation end products improves insulin resistance in human type 2 diabetes: potential role of AGER1 and SIRT1. *Diabetes Care*. *34*, 1610–1616.

Uribarri, J., Peppa, M., Cai, W., Goldberg, T., Lu, M., He, C., & Vlassara, H. (2003). Restriction of dietary glycotoxin reduces excessive advanced glycation end products in renal failure patients. *Journal of the American Society of Nephrology*, *14*, 728–731.

Uribarri, J., Woodruff, S., Goodman, S., Cai, W., J., Chen, X., Pyzik, R., Yong, A., Striker, G., E., & Vlassara, H. (2010). Advanced glycation end products in foods and a practical guide to their reduction in the diet. *Journal of the American Dietetic Association*, *110*(6), 911-916.

van Heijst, J. W., Niessen, H. W., Hoekman, K., Schalkwijk, C. G. (2005). Advanced glycation end products in human cancer tissues: detection of Nepsilon-(carboxymethyl)lysine and argpyrimidine. *Annals of the New York Academy of Sciences*. *1043*, 725-733.

Vinson, J. A. & Howard, T. B. (1996). Inhibition of protein glycation and advanced glycation end products by ascorbic acid and other vitamins and nutrients. *Journal of Nurtirionnal Biochemitry*, 7(12), 659-663.

Vitek, M. P., Bhattacharya, K., Glendening, J. M., Stopa, E.,Vlassara, H., Bucala, R., Manogue, K. & Cerami, A. (1994). Advanced glycation end products contribute to amyloidosis in Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America*, *91*, 4766–4770.

Vlassara, H., Cai, W., Crandall, J., Goldberg, T., Oberstein, R., Dardaine, V., Peppa, M., & Rayfield, E. J. (2002). Inflammatory markers are induced by dietary glycotoxins: A pathway for accelerated atherosclerosis in diabetes. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 15596–15601.

Vlassara, H., Cai, W., Goodman, S., Pyzik, R., Yong, A., Zhu, L., Neade, T., Beeri, M., Silverman, J. M., Ferrucci, L., Tansman, L., Striker, G. E., & Uribarri, J. (2009). Protection against loss of innate defenses in adulthood by low AGE intake; role of a new anti-inflammatory AGE-receptor-1. *Journal of Clinical Endocrinology and Metabolism*, *94*, 4483–4491.

Vlassara, H., & Uribarri, J. (2014). Advanced Glycation End Products (AGE) and Diabetes: Cause, Effect, or Both? *Current Diabetes Reports*, *14*(1), 453-469.

Weigel, K. U., Opitz, T., & Henle, T. (2004). Studies on the occurrence and formation of 1,2-dicarbonyls in honey. *European Food Research and Technology*, *218*, 147-151.

Wolff, S. P., & Dean, R. T. (1987). Glucose autoxidation and protein modification. The potential role of 'autoxidative glycosylation' in diabetes. *Biochemistry Journal*, *245*(1), 243–250.

Wolfrom, M. L., Schlicht, R. C., Langer, A. W. Jr., & Rooney, C. S. (1953). Chemical Interactions of Amino Compounds and Sugars. VI. The Repeating Unit in Browning Polymers. *Journal of the American Chemical Society*, *75*, 1013.

Wu, C., Huang, S., Lin, J., & Yen, G. (2011). Inhibition of advanced glycation end product formation by food stuffs. *Food & Function*, *2*, 224–234.

Wu, C. H, & Yen G. C. (2005). Inhibitory effect of naturally occurring flavonoids on the formation of advanced glycation endproducts. *Journal of Agricultural and Food Chemistry, 53*(8), 3167-3173.

Yamaguchi, F., Ariga, T., Yoshimura, Y., & Nakazawa, H. (2000). Antioxidative and anti-glycation activity of garcinol from garcinia indica fruit rind. *Journal of Agricultural and Food Chemistry, 48*, 180–185.

Yamaguchi, M., Ishida, J., Zhu, X. X., Nakamura, M., & Yoshitake, T. (1994). Determination of glyoxal, methylglyoxal, diacetyl, and 2,3-pentanedione in fermented foods by high-performance liquid chromatography with fluorescence detection. *Journal of Liquid Chromatography*, *17*, 203−211.

Zieman, S. J., & Kass, D. A. (2004). Advanced glycation endproduct crosslinking in the cardiovascular system: potential therapeutic target for cardiovascular disease. *Drugs*, *64*(5), 459-470.

Zhang, L., Zalewski, A., Liu, Y., Mazurek, T., Cowan, S., Martin, J. L., Hofmann, S. M., Vlassara, H, & Shi, Y. (2003). Diabetes-induced oxidative stress and low-grade inflammation in porcine coronary arteries. *Circulation*, *108*(4), 472-478.

Chapter 2. DETERMINATION OF ADVANCED GLYCATION ENDPRODUCTS IN COOKED MEAT PRODUCTS[1,2]

ABSTRACT

Advanced glycation endproducts (AGEs), a pathogenic factor implicated in diabetes and other chronic diseases, are produced in cooked meat products. The objective of this study was to determine the AGE content, as measured by N^{ε}-carboxymethyllysine (CML) levels, in cooked chicken, pork, beef and fish (salmon and tilapia) prepared by three common cooking methods used by U.S. consumers: frying, baking, and broiling. The CML was detected in all the cooked samples, but the levels were dependent on types of meat, cooking conditions, and the final internal temperature. Broiling and frying at higher cooking temperature produced higher levels of CML, and broiled beef contained the highest CML content (21.8 μg/g). Baked salmon (8.6 μg/g) and baked tilapia (9.7 μg/g) contained less CML as compared to the other muscle food samples.

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INTRODUCTION

Advanced glycation endproducts (AGEs) are a group of complex and heterogeneous compounds that are formed through the Maillard reaction, a nonenzymatic reaction between reducing sugars and free amino groups (Ahmed, 2005). Although the mechanism of the Maillard reaction is still not fully known, the AGEs exist in the body as well as in food. The common AGEs found in food are N^{ε}-carboxymethyl lysine (CML), methylglyoxal-lysine dimers (MOLD), pentosidine and pyrraline (Wu, Huang, Lin and Yen, 2011). Current research suggests excessive consumption of these compounds may contribute to metabolic chronic diseases including diabetes, renal disorders, and Alzheimer's disease (Brownlee, 1994; Kim, Reddy, Rahbar, Lanting, & Natarajan, 2002; Koschinsk et al., 1997). Some epidemiological studies have shown that consumption of certain dietary AGEs are indicators of oxidative stress and inflammation such as 8-isoprostanes, which may play an important role in disease pathologies (Uribarri et al., 2007). Moreover, reductions of inflammatory mediators were also found in diabetic subjects by restricting their dietary AGEs (Vlassara et al., 2002). Based on some animal studies, AGE-rich diets fed to mice was associated with kidney disease and accumulation of AGEs in tissue (Hofmann et al., 2002; Vitek et al., 1994). Thus, information on the levels of dietary AGEs and the prevalence of these compounds in food items is desirable.

It is established that the diet is a significant source of exogenous AGEs. In addition, long-term storage and cooking procedures can increase AGEs content in foods (Forster & Henle, 2003). The concentrations and types of AGEs in cooked meat depend on several factors including cooking method, cooking temperature and time, and the presence of protein and fat (Goldberg et al., 2004). Traditional cooking methods may play a key role in AGEs consumption

and exposure. Compared to some Asian countries, it is estimated that broiling or grilling was used more to cook steak (34%), and pan frying was used more to cook chicken (56%) and fish (54%) in the U.S. (Keating & Bogen, 2004). All of these cooking methods have been reported to induce AGE formation (Ames, 2008; Delgado-Andrade et al., 2007). For example, Goldberg et al. (2004) found that higher levels of CML in meats cooked by broiling and frying with higher temperatures.

Although some previous studies have investigated AGEs levels in food (Dittrich et al., 2006; Drusch, Faist, & Erbersdobler, 1999; Goldberg, Cai, Peppa, Dardaine, Baliga, & Uribarri, 2004; Hull, Woodside, Ames & Cuskelly, 2012), direct comparison is of results is difficult because of the various preparation methods, meat types, and cooking conditions. For instance, meat samples have been cooked to different internal temperatures in past studies, which yielded inconsistent results. Therefore information on AGEs levels should include some standard parameters such as the internal temperature of the cooked samples.

CML has been studied extensively as an oxidation product, and is reported to be formed by numerous pathways in food systems (Ahmed et al., 1986). In the process of cooking meat products, CML may form though the oxidation of fructose lysine or the direct reaction of glyoxal and lysine. Many of the AGEs are not as stable to acids as CML is, so it is often used as an indicator in foods (Tauer, Hasenkof, Kislinger, Frey, & Pischetsrieder, 1999).

This study was performed to evaluate the AGEs content, as measured by CML levels, in meat and fish samples cooked to the internal temperatures recommended by the U.S. Department of Agriculture, Food Safety and Inspection Service (1998). The results can be used

as a guideline for evaluating the risk associated with AGE consumption and give some reasonable advice about dietary habits for consumers.

MATERIAL AND METHODS

Materials

The N^{ε}-carboxymethyl lysine (CML) standard was purchased from NeoMPS (Strasbourg, France), boric acid, sodium hydroxide, hydrochloric acid, 2-mercaptoethanol, sodium borohydride, and Na tetraborate decahydrate, were purchased from Sigma Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade), chloroform (HPLC grade), methanol (HPLC grade), and ortho-phthalaldehyde (OPA) reagent were obtained from Fisher Scientific (Fairlawn, NJ, USA).

Chemical analyses

Crude protein for uncooked meat samples was measured with a Leco FP-2000 protein analyzer (Leco Corp, St Joseph, MI, USA) according to AOAC Int method 992.15 (Kingbrink & Sebranek, 1993). Fat and moisture content were determined with a CEM Smart Trac system (CEM Corp., Matthews, NC, USA) using AOAC Int method 2008.06 (Leefler et al., 2008).

The pH of each sample was measured according to the method of Jang, et al. (2008). Five grams of fine ground tissue was added to 45 mL of distilled water, and mixed for 30 s at medium speed in a Waring blender (Waring Laboratory, Torrington, CT, USA) followed by measument with an Accumet AP115 pH meter (Fisher, Pittsburgh, PA, USA).

Preparation of meat samples

Fresh meat samples were purchased from regional supermarkets: beef rib round steak, pork top loin, skinless chicken breast, and fish fillet (tilapia and salmon). Fresh meat products were tempered to room temperature prior to cooking. A thermocouple temperature probe was inserted in the middle of each sample, and temperature was recorded with a data logger (USB-TC model, Measurement Computing, Norton, MA, USA).

The description of the cooking methods for the samples is presented in Table 10. The cooking methods preferred by U.S. meat consumers were used in the experiments. Meat samples were prepared by pan frying at different desired surface temperatures, oven broiling at 232°C (450°F), and oven baking at 177°C (350°F). To eliminate foodborne illness, the internal cooking temperature was used according to recommendation of USDA-FSIS (1998): 63°C (145°F) for fish, 71°C (160°F) for pork, 74°C (165°F) for chicken, and 71°C (160°F, well done) for beef. To compare the AGEs levels in cooked meat with different degrees of doneness, the pork samples were fried to 63°C (145°F, medium), and the beef steak samples were also fried to 54°C (130°F, very rare), 63°C (145°F, medium), 71°C (160°F, well done) and 77°C (170°F, over done). In order to compare the AGE contents in cooked meat by different frying methods, the beef samples were cooked to the same internal temperature of 71°C (160°F) by turning once (after 5 min) or multiple times (interval of one minutes). No salt, spice, and oil were used in the cooking procedures. Approximately 2 mm of the surface or 2 mm of the middle part of the meat was excised from the cooled samples with a motorized meat slicer (Cabela grade slicer, 1/3 hp, Sidney, NE, USA). The sample was then homogenized with a food processor (KitchenAid, model

KFP 750) and stored at 4°C overnight. The extraction of CML in each sample was performed the following day.

Determining of CML

The CML was extracted from meat samples according to Drusch, Faist, & Erbersdobler (1999) except that chloroform/methanol (2:1, v/v) solution was used as the defatting solvent. Each meat sample (0.20 gram) was defatted using two extractions of 20 mL chloroform/methanol (2:1, v/v) solution followed by centrifugation at 10,000 rpm (10,600 xg) at 4°C for 10 min with a Fisher MARATHON 21000(R) Centrifuge fitted with a 6-Place Fixed Angle rotor (9.5 cm radius, 04-976-011). The defatted samples were dried completely at 50°C, and reduced with 4 mL sodium borate buffer (0.2 M, pH 9.4) and 2 mL sodium borohydride (1 M in 0.1 M NaOH) for 4 h at room temperature. Hydrochloric acid (HCl) was added to the reduced samples to a final concentration of 6 M HCl. Each sample was flushed with a stream of nitrogen for 5 min followed by hydrolysis at 110°C for 20 h in screw-capped vials. Upon completion of hydrolysis, samples were dried by rotary evaporation, and transferred into a 10 mL volumetric flask with water and made to volume. After filtration, the filtrates were concentrated and dissolved in sodium borate buffer (0.2 M, pH 9.4). The sample was brought to 10 mL in a volumetric flask follow by a final membrane filtration (nylon, 0.45um) for later derivatization.

Table 10. Cooking conditions of the meat and fish samples.

Meat type[a]	Cooking method[b]	Internal temperature (°C)	Cooking temperature (°C)	Cooking time (min)	Cooking loss[c] (%)
Beef	Frying	71	204	20	25.23±1.80
	Broiling	71	232	16	30.81±4.10
	Baking	71	177	45	21.59±2.69
Pork	Frying	71	204	16	24.83±1.79
	Broiling	71	232	14	31.25±3.93
	Baking	71	177	35	22.50±3.03
Chicken	Frying	74	204	18	29.66±2.08
	Broiling	74	232	16	35.90±2.31
	Baking	74	177	35	27.24±1.45
Salmon	Frying	63	204	12	19.64±2.42
	Broiling	63	232	10	25.98±2.64
	Baking	63	177	14	16.71±1.24
Tilapia	Frying	63	204	12	18.12±1.89
	Broiling	63	232	8	25.26±2.54
	Baking	63	177	12	19.84±2.82

[a]Beef: rib round steak, 270-310g, 2.5 thickness; pork: top loin: 220-250g, 2.3 cm thickness; chicken: breast without skin, 230-250g, 2.3 thickness; salmon: 180-200g, 1.8 cm thickness; tilapia:140-160g, 1.5 thickness.
[b]Frying: meat was fried in a Teflon-coated frying pan; broiling: meat was prepared on a broiler pan to be out of the drippings and cooked in an oven; baking: the meat was prepared on a baking pan and baked in an oven.
[c]% cooking loss= (before cook weight−after cook weight)/before cook weight*100%.
Values are represented as mean ± standard deviation (n=3).

The hydrolysate (50 µL) was mixed with 200 µL of ortho-phthalaldehyde (OPA) derivatization reagent and reacted 5 min prior to HPLC analysis. The CML was analyzed with an HP1090A Series II HPLC (Agilent Technologies, Santa Clara, CA, USA) coupled with a HP 1046A

programmable fluorescence detector according to the method of Peng et al. (2010). The CML separation was achieved with a reversed-phase TSK gel ODS-80 TM column (25 cm×4.6 mm, 5 µm, 80 Å, Tosohass, Montgomeryville, PA, USA), and with the fluorescence settings of 340 nm (excitation) and 455 nm (emission) (Figure 7). The mobile phases were: (solvent A) sodium acetate buffer (20 mM, adjusted to pH 6.7 with acetic acid)/acetonitrile (90:10, v/v) and (solvent B) acetonitrile. The flow rate was 1.0 mL/min and the injection volume was 20 µL. The CML separation was achieved with a linear gradient that started with 5% B and changed to 70% B within 5 min and kept at 70% B till 17 min. The gradient was set back to 95% B in 1 min followed by a post run of 15 min to allow the column to equilibrate prior to the next injection. Data were analyzed using ChemStation software Rev A.06.00. The identities of CML peaks were achieved by comparison between the retention times and the standard. CML content of the samples was determined based on the peak areas of the corresponding derivatives.

Statistical Analyses

The experiment was a randomized complete block design. Analyses of variance (ANOVA) were performed on the data by using SAS version 9.1 (SAS Inst. Inc., Cary, NC, U.S.A.), and $P < 0.05$ was selected as the decision level for significant differences.

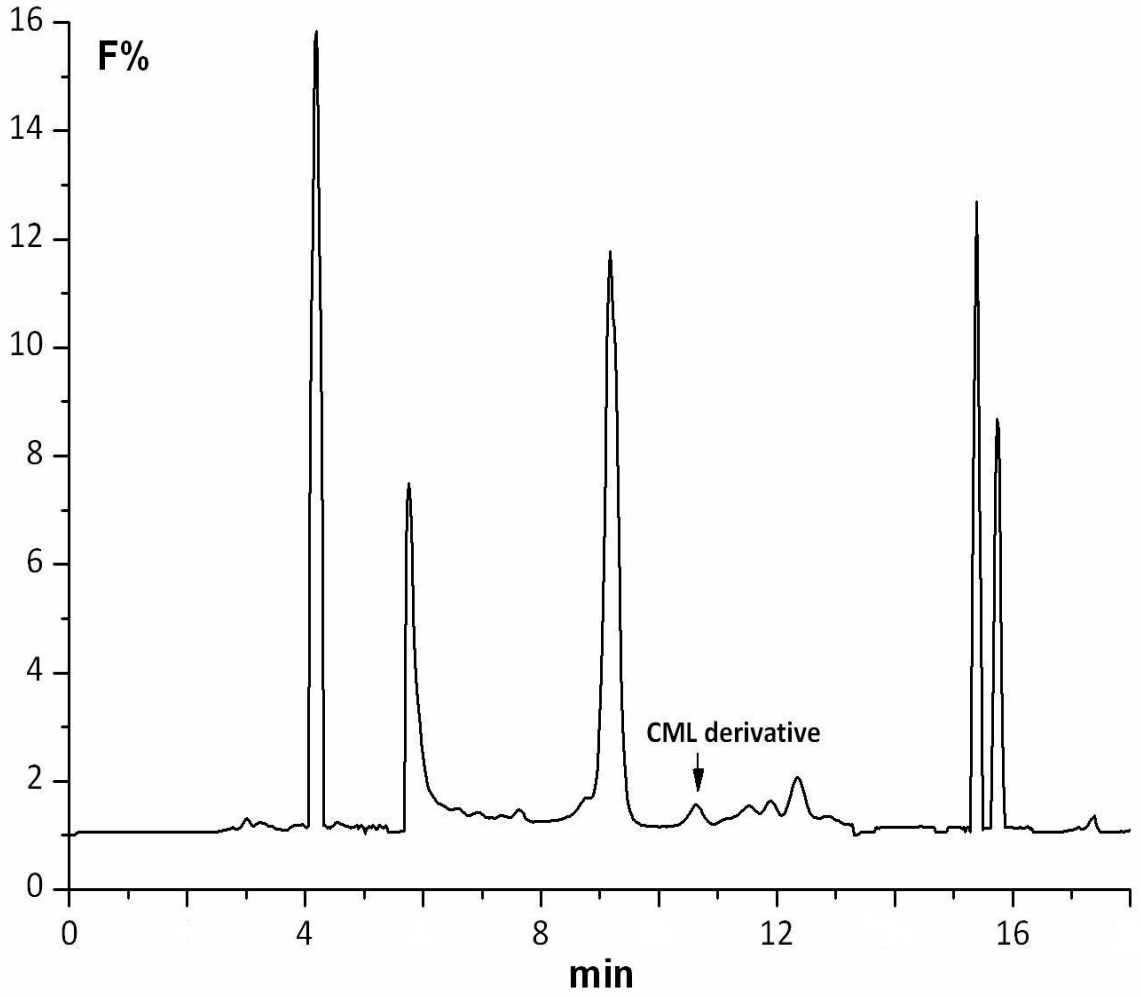

Figure 7. HPLC chromatograph of CML derivative peaks related to a raw beef sample using a TSK gel ODS-80 TM column (25 cm×4.6 mm, 5 µm) with a fluorescence intensity of settings at 340 nm (excitation) and 455 nm (emission). The flow rate was 1.0 mL/min and injection volume was 20 µL. The mobile phases were: (solvent A) sodium acetate buffer (20 mM, adjusted to pH 6.7 with acetic acid)/acetonitrile (90:10, v/v) and (solvent B) acetonitrile.

RESULTS AND DISCUSSION

Chemical analyses

Chemical analyses of the fresh meat products are summarized in Table 11. The pH of raw meat samples ranged from 5.56 to 7.95. The moisture level of the raw meat samples was between 69.25 and 77.21%. The fat levels of raw meat samples were from 1.68 to 7.18%. The protein levels of raw meat samples ranged from 17.98 to 21.53%.

Table 11. Chemical analyses of raw meat samples.

Meat type	Moisture (%)	Fat (%)	Protein (%)	pH
Beef	69.25±1.06	7.18±0.94	21.53±0.57	5.56±0.11
Pork	74.49±1.41	6.16±1.52	19.01±2.38	6.08±0.17
Chicken	73.92±2.99	4.25±1.06	21.88±0.59	6.16±0.10
Salmon	75.03±2.96	1.94±1.27	18.59±1.70	6.85±0.07
Tilapia	77.21±1.78	1.68±1.41	17.98±2.42	7.95±0.09

Each value is represented as mean ± standard deviation (n=3).

CML levels in cooked meat and fish

Meat and fish samples were extracted in triplicate and analyzed by HPLC. The limit of detection was 1.5 ng/mL and the limit of quantification was 5.0 ng/mL for CML. The CML quantitative determinations in raw, boiled, fried or baked meat are summarized in Figure 8 and Table 12, and the amount of each sample was expressed in μg/g food. In the present study, we first detected the CML levels in the outer layer (2 mm) among all the samples, and in the middle layer (2 mm) of fried samples. CML was found among all samples and the contents were in the range of 1.09 μg/g food (inside layer of fried pork) to 21.84 μg/g food (outer layer of broiled

beef steak). We found that all cooked meat items showed higher levels of CML in the outer layer as opposed to the untreated meat (P <0.05), such as in the fried beef (20.03 μg/g) compared to the raw samples (2.05 μg/g). The results are similar to those reported by Assar et al. (2009), who indicated CML content in fried minced beef (11.2 mg/kg food) was much higher than it was in raw minced beef (0.72 mg/kg food). As can be seen in Figure 8, all cooking methods (broiling, frying and baking) significantly increased the formation of CML in the outer layer of the meat samples.

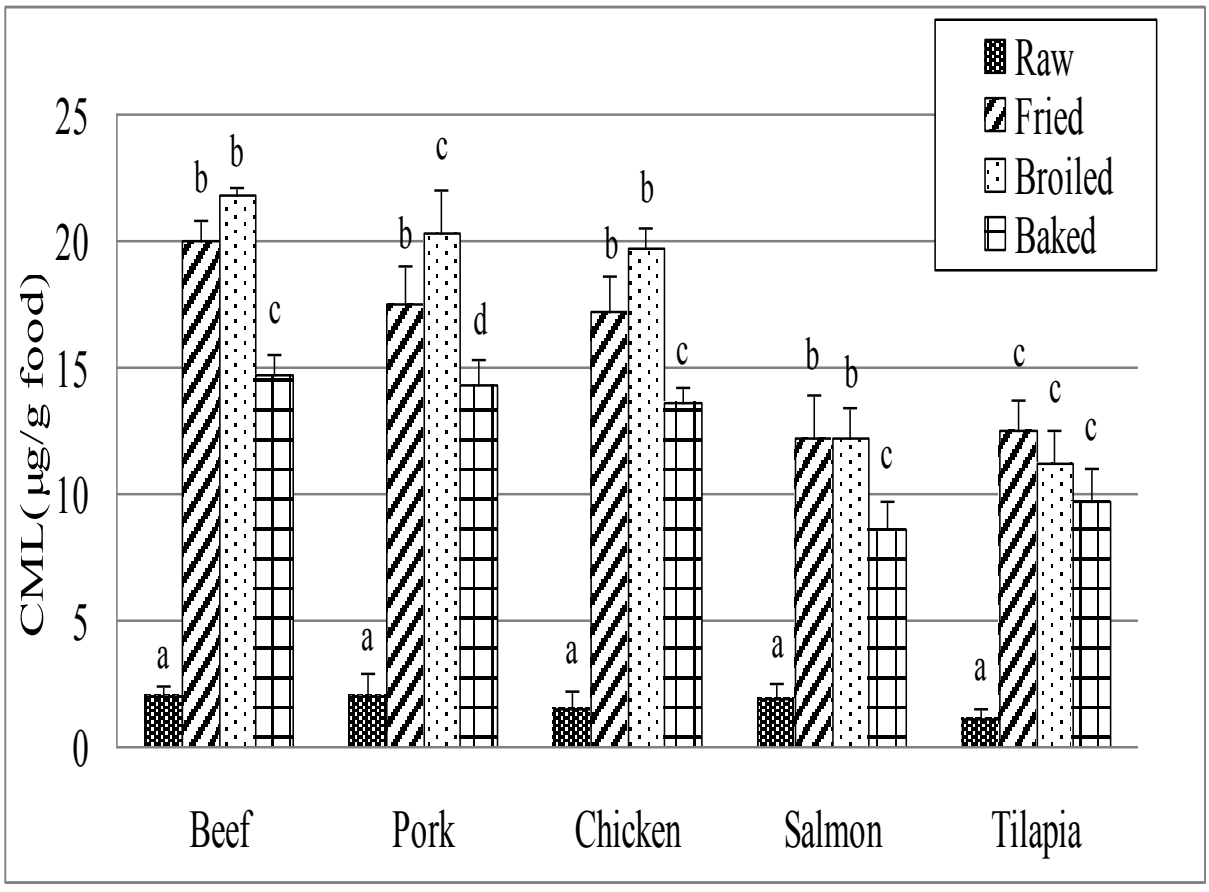

Figure 8. CML content (μg/g) of meat samples cooked by different methods.

[abcd]Bars with the different superscript letters differ significantly (P < 0.05).

Table 12. CML content (μg/g) of meat samples was identified in the outer layer (2 mm) among all the samples (out), and also in the middle layer (2 mm) of fried samples (in).

	Treatment				
Meat types	**Raw (control)**	**Frying (out)**	**Frying (in)**	**Broiling (out)**	**Baking (out)**
Beef steak	2.05±0.40[a]	20.03±0.83[a]	3.13±0.68[a]	21.84±0.28[a]	14.31±1.04[a]
Pork top loin	1.98±0.97[a]	17.53±1.48[b]	1.09±0.53[a]	20.35±1.64[a]	12.53±1.19[a]
Chicken breast	1.48±0.77[a]	17.16±1.43[b]	2.99±0.89[a]	19.69±0.78[a]	13.58±0.63[a]
Salmon	1.92±0.61[a]	12.20±1.68[c]	2.05±0.63[a]	12.23±1.13[b]	8.59±1.07[b]
Tilapia	1.07±0.38[a]	12.53±1.19[c]	3.43±1.10[a]	11.24±1.25[b]	9.72±1.33[b]

[abc]Means with different superscripts within the same column are significantly different at $P < 0.05$.
Values are represented as mean ± standard deviation (n=3).

As shown in Table 13, except for the tilapia samples, we did not find dramatic change of the CML contents in the inside layer of fried meat and fish ($P > 0.05$). All the levels of CML in the outer layer of fried meat were much higher than they were in the middle layers ($P < 0.05$). It is possible more water-soluble precursors are transferred to the surface of meat to form AGEs at cooking. Therefore CML was concentrated more in the outer layer of the fried meat, which raised an interesting issue related to AGE intake and etiology. Some consumers are fond of the aromatic outer crust of cooked meats, and it may enhance their exposure to the risk of dietary AGEs.

Table 13. CML content of raw and fried samples (μg/g).

	Meat type				
Treatment	**Beef steak**	**Pork top loin**	**Chicken breast**	**Salmon**	**Tilapia**
Raw (uncooked)	2.05±0.40[a]	1.98±0.97[a]	1.48±0.77[a]	1.92±0.61[a]	1.07±0.38[a]
Frying (inside)	3.13±0.68[a]	1.09±0.53[a]	2.99±0.89[a]	2.05±0.63[a]	3.43±1.10[b]
Frying (outside)	20.03±0.83[b]	17.53±1.48[b]	17.16±1.43[b]	12.20±1.68[b]	12.53±1.19[c]

[abc]Means with different superscripts within the same column are significantly different at $P < 0.05$.
Values are represented as mean ± standard deviation (n=3).

The CML levels of cooked chicken samples (internal temperature of 74°C) are summarized in Figure 8. The levels in the broiled and fried chicken breast were higher compared to the baked samples (P <0.05). The CML levels of beef samples cooked to well-done (internal temperature of 71°C) are also reported in Figure 8. There was an increase of CML amounts (~ 1.5-fold) in both fried and broiled beef as compared to the baked beef, following the increase in cooking temperature. In addition, CML contents in broiled pork (20.35 μg/g) and fried pork (17.53 μg/g) were both significantly higher than those in samples baked to an internal temperature 71°C (P <0.05). Frying meat in direct contact with a heat source may produce more CML than oven baking, in which the meat is cooked indirectly by hot air. The fact that our CML levels were higher in fried pork than in baked pork is inconsistent with the report of Chao et al. (2009), who stated that all baked chicken, pork and beef samples had more CML than fried samples. They found the CML contents in fried fish samples (salmon and cod) were higher than those were in baked samples, which was similar to our result, that the CML levels were significantly less in baked salmon (8.59 μg/g) than fried salmon (12.20 μg/g) and broiled

salmon (12.23 μg/g) samples (P <0.05). However, the amount of CML did not differ much among baked, fried, and broiled tilapia samples (P >0.05). The different cooking temperature or degrees of doneness of the cooked meat may count for the inconsistent results. It is notable that our baking temperature was 170°C rather than the 230°C used by Chao et al. (2009). In our study, cooking temperature may affect CML formation more since the temperature used for broiling (232°C) and frying (204°C) were higher than that used for baking (177°C).

As shown in Table 10, CML levels correlated well with the cooking temperature and cooking loss. For example, cooking loss of broiled chicken in the present study was higher (35.90%) along with the higher CML contents (19.69 μg/g). However, it was lower in baked chicken (27.24%). According to the studies by Skog and Jägerstad (1997) and Janoszka and Sajewicz (2009), water-soluble reactants can leach out of the product and collect on the surface enhancing Maillard endproduct formation. High cooking temperature accelerates this process, which may explain why high cooking loss correlates to the high levels of CML.

We investigated the occurrence of CML in broiled meats (Table 12). There was no significant difference among beef, pork and chicken (P >0.05). However, it is evident that there were lower levels of CML in the broiled salmon and tilapia as contrasted to the other meat items (P <0.05), which was in agreement with the results from Uribarri et al. (2010). The results of the baked samples are also in Table 12, and the CML contents in the fish (salmon and tilapia) ranked relatively lower compared to the muscle meat samples (P <0.05). Among all the fried samples, the highest level of CML was found in beef steak (20.03 μg/g), followed by pork (17.53 μg/g) and chicken (17.16 μg/g). They were all significantly higher than those in salmon (12.20

μg/g) and tilapia (12.53 μg/g). The different chemical ingredients in the raw meat products (Table 10) may be responsible for the differences of the CML levels to some extent in our study.

Although we found less CML in fried fish than in fried muscle meat, Chao et al. (2009) reported salmon had the highest level of CML in fried fish and meat samples. Moreover, there were no significant differences in CML between fried pork and chicken (P >0.05), which was consistent with the result from Uribarri et al. (2010). However, it was not in agreement with the report from Hull et al. (2012), who pointed out more CML was found in pork strips (0.61 mg/100g) than in chicken strips (0.51 mg/100g) and beef steak (0.42 mg/100g). The inconsistent results may be due to the different conditions of the raw samples (such as the thickness of meat), the cooking utensils, and the degree of doneness.

We compared the results in different degrees of doneness of fried beef and pork samples (Table 14). For the beef samples, the CML content increased significantly (P <0.05) with increasing degree-of-doneness from medium to well done (internal temperature from 63 to 71°C) both in cooking temperature of 204°C (from 10.15 to 20.03 μg/g) and 160°C (from 10.52 to 16.30 μg/g). We did not find any dramatic differences for other change of the degree of doneness of cooked samples (P >0.05). In pork samples, the CML levels increased significantly (P <0.05) with rising the internal temperature from 63 to 71°C both in cooking temperature of 204°C (from 13.29 to 17.53 μg/g) and 160°C (from 8.84 to 17.44 μg/g).

Table 14. CML content (μg/g) of meat fried to different degrees of doneness.

Cooked items	Internal temperature (°C)	Cooking temperature (°C)	
		204	**160**
Beef steak	54 (Very rare)	9.17±0.58[a]	9.87±0.71[a]
	63 (Medium)	10.15±1.43[a]	10.52±0.96[a]
	71 (Well done)	20.03±0.82[b]	16.30±1.03[b]
	77 (Over done)	21.01±1.92[b]	16.56±1.31[b]
Pork top loin	63 (Medium)	13.29 ±1.15[a]	8.84±0.66[a]
	71 (Well done)	17.53±1.48[b]	17.44±1.43[b]

[ab]Means with different superscripts within the same column are significantly different at $P < 0.05$.
Values are represented as mean ± standard deviation (n=3).

The turning frequency also affected the CML formation of meat when pan frying (Table 15). The beef steak was placed, one at a time, in a pan preheated from 160°C to 204°C. In the first session, these samples were turned at 1-minute intervals until the final temperature (71°C) were reached. In the second session, beef samples were also cooked to the same internal temperatures and flipped after 5 minutes. The CML levels increased significantly from 180°C to 204°C regardless of whether turning repeatedly ($P < 0.05$). At 204°C, the CML level (20.03 μg/g) by single turning was remarkable higher than it was (12.16 μg/g) by multiple turning when reaching the same internal temperature. Therefore, we believed multiple flipping of the meat reduced CML formation compared with single turning when frying, which may be as a result of accelerating the loss of water-soluble precursors.

Table 15. CML content of fried beef steak (71°C internal temperature) in frying.

Preheat temperature (°C)	CML content (μg/g)	
	Single turn	Multiple turn
160	16.30±1.03[a]	9.88±1.19[a]
170	17.64±0.91[a]	9.02±0.77[a]
180	17.81±0.48[a]	10.61±0.90[a]
204	20.03±0.82[b]	12.16±0.63[b]

[ab]Means with different superscripts within the same column are significantly different at $P < 0.05$.
Values are represented as mean ± standard deviation (n=3).

As mentioned earlier in Table 12, the CML contents of meat products cooked by standard methods can be ordered relatively from low to high. Low levels of total CML (less than 10 μg/g) were found in baked salmon (8.59 μg/g) and baked tilapia (9.72 μg/g). Intermediate levels of CML (10 to 15 μg/g) were found in fried salmon (12.20 μg/g), fried tilapia (12.53 μg/g), broiled salmon (12.23 μg/g), broiled tilapia (11.24 μg/g), baked beef (14.31 μg/g), baked pork (12.53 μg/g), and baked chicken breast (13.58 μg/g). High levels of CML (more than 15 μg/g) were found in fried beef (20.03 μg/g), fried pork (17.53 μg/g), fried chicken breast (17.16 μg/g), broiled beef (21.84 μg/g), broiled pork (20.35 μg/g), and broiled chicken breast (19.69 μg/g). In conclusion, due to the high or intermediate levels of CML detected in the fried or broiled muscle food, our study indicated people frequently consuming these muscle meat have a high exposure to dietary CML that may increase risk of diseases.

CONCLUSION

Dietary AGEs have been suggested as a factor in many chronic diseases such as diabetes, and their relationship continues to be developed. These results indicate that the levels of AGEs as monitored by CML levels in cooked meat depended on chemical composition of meat, cooking conditions and the final internal temperature. For example, among all the meat categories, exposure to higher temperatures coincided with higher CML levels in the outer layer of meat as compared to the samples prepared at lower temperatures. The data may provide researchers information for estimating dietary AGEs exposure, and help food professionals guide consumers to reduce the intake of AGEs formed in cooked meat, i.e., cooking at lower temperature, flipping meat more during pan frying, and removing the outside crusted layer of excessive cooked meat prior to consumption. Future studies are desirable to further expand the AGEs database, and investigate other methods to prevent AGEs formation at cooking, such as adding inhibitory compound such has antioxidants.

REFERENCES

Ahmed, N. (2005). Advanced glycation endproducts-role in pathology of diabetic complications. *Diabetes Research and Clinical Practice*, *67*(1), 3–21.

Ahmed, M., Thorpe S., & Baynes J. (1986). Identification of N-epsilon-carboxymethyllysine as a degradation product of fructoselysine in glycated protein. *Journal of Biological Chemistry*, *261*(11), 4889–4894.

Ames, J. M. (2008). Determination of N-epsilon-(carboxymethyllysine in foods and related systems. *Annals of the New York Academy of Sciences*, *1126*, 20–24.

Assar, S., Moloney, C., Lima, M., Magee, R., & Ames, J. (2009). Determination of N-epsilon-carboxymethyllysine in food systems by ultra performance liquid chromatography-mass spectrometry. *Amino Acids, 36*(2), 317–326.

Brownlee, M. (1994). Lilly lecture 1993-glycation and diabetic complications. *Diabetes, 43*(6), 836–841.

Chao, P., Hsu, C., & Yin, M. (2009). Analysis of glycative products in sauces and sauce-treated foods. *Food Chemistry, 113*(1), 262–6.

Delgado-Andrade, C., Seiquer, I., Pilar, N. M., & Morales, F. J. (2007). Maillard reaction indicators in diets usually consumed by adolescent population. *Molecular Nutrition & Food Research*, *51*(3), 341–351.

Dittrich, R., Hoffmann, I., Stahl, P., Müller, A., Beckmann, M. W., & Pischetsrieder, M. (2006). Concentrations of Ne-carboxymethyllysine in human breast milk, infant formulas, and urine of infants. *Journal of Agricultural and Food Chemistry*, *54*(18), 6924–6928.

Drusch, S., Faist, V., & Erbersdobler, H. (1999). Determination of N[epsilon]-carboxymethyllysine in milk products by a modified reversed-phase HPLC method. *Food Chemistry, 65*(4), 547–553.

Forster, A., & Henle, T. (2003). Glycation in food and metabolic transit of dietary AGEs (advanced glycation end-products): studies on the urinary excretion of pyrraline. *Biochemical Society Transactions*, *31*, 1383–1385.

Goldberg, T., Cai, W. J., Peppa, M., Dardaine, V., Baliga, B. S., & Uribarri, J. (2004). Advanced glycoxidation end products in commonly consumed foods. *Journal of the American Dietetic Association*, *104*(8), 1287–1291.

Hofmann, S. M., Dong, H. J., Li, Z., Cai, W., Altomonte, J., Thung, S. N., Zheng, F., Fisher, E., & Vlassara, H. (2002). Improved insulin sensitivity is associated with restricted intake of dietary glycoxidation products in the db/db mouse. *Diabete*, *51*, 2082–2089.

Hull, G., Woodside, J., Ames, J., & Cuskelly, G. (2012). N-epsilon-carboxymethyllysine content of foods commonly consumed in a western style diet. *Food Chemistry*, *131*(1), 170–174.

Jang, A., Liu, X. D., Shin, M. H., Lee, B. D., Lee, S. K., Lee, J. H., & Jo, C. (2008). Antioxidative potential of raw breast meat from broiler chicks fed a dietary medicinal herb extract mix. *Poultry Science*, *87*(11), 2382–2389.

Janoszka, B., Blaszczyk, U., Damasiewicz-Bodzek, A., & Sajewicz, M. (2009). Analysis of heterocyclic amines (HAs) in pan-fried pork meat and its gravy by liquid chromatography with diode array detection. *Food Chemistry*, *113*(4), 1188–1196.

Keating, G. A., & Bogen, K. T. (2004). Estimates of heterocyclic amine intake in the US population. *Journal of Chromatography B*, *802*(1), 127–133.

Kim, Y. S., Reddy, M. A., Rahbar, S., Lanting, L., & Natarajan, R. (2002). Effects of advanced glycation end products and RAGE ligation with S100B in mesangial and vascular smooth muscle cells. *Journal of the American Society of Nephrology*, *13*, 90A–90A.

Kingbrink, M., & Sebranek, J. (1993). Combustion method for determination of crude protein in meat and meat-products - collaborative study. *Journal of AOAC International,*, *76*(4), 787–793.

Koschinsk, T., He, C. J., Mitsuhashi, T., Bucala, R., Liu, C., Buenting, C., Heitmann, K., & Vlassara, H. (1997). Orally absorbed reactive glycation products (glycotoxins): An environmental risk factor in diabetic nephropathy. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(12), 6474.

Leefler, T. P., Moser, C. R., McManus, B. J., Urh, J. J., Keeton, J. T., & Claflin A. (2008). Determination of moisture and fat in meats by microwave and nuclear magnetic resonance analysis: Collaborative study. *Journal of AOAC International*, *91*, 802–810.

Peng, X. F., Ma, J. Y., Cheng K. W., Jiang, Y., Chen, F., & Wang, M. F. (2010). The effects of grape seed extract fortification on the antioxidant activity and quality attributes of bread. *Food Chemistry*, *119*(1), 49–53.

Skog, K., Augustsson, K., Steineck, G., Stenberg, M., & Jägerstad, M. (1997). Polar and non-polar heterocyclic amines in cooked fish and meat products and their corresponding pan residues. *Food Chemistry Toxicology*, *35*(6), 555–565.

Tauer, A., Hasenkof, K., Kislinger, T., Frey, I., & Pischetsrieder, M. (1999). Determination of CML in heated milk products by immunochemical methods. *European Food Research and Technology*, *209*, 72–76.

Uribarri, J., Cai, W., Peppa, M., Goodman, S., Ferrucci, L., Striker, G., & Vlassara, H. (2007). Circulating glycotoxins and dietary advanced glycation endproducts: Two links to inflammatory response, oxidative stress, and aging. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *62*(4), 427–433.

U.S. Department of Agriculture, Food Safety and Inspection Service. (1998). Why use a thermometer? Washington, DC. U.S. Department of Agriculture, Food Safety and Inspection Service. http://www.fsis.usda.gov/factsheets/Use_a_Food_Thermometer/index.asp. Retrieved May, 2014.

Vitek, P., Bhattacharya, K., Glendening, J. M., Stopa, E., Vlassara, H., Bucala, R., Manogue, K., & Cerami, A. (1994). Advanced glycation end products contribute to amyloidosis in Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America*, *91*, 4766–4770.

Vlassara, H., Cai, W., Crandall, J., Goldberg, T., Oberstein, R., Dardaine, V., Peppa, M., & Rayfield, E. J. (2002). Inflammatory markers are induced by dietary glycotoxins: A pathway for accelerated atherosclerosis in diabetes. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 15596–15601.

Wu, C., Huang, S., Lin, J., & Yen, G. (2011). Inhibition of advanced glycation end product formation by food stuffs. *Food & Function*, *2*, 224–234.

Chapter 3. INHIBITION OF ADVANCED GLYCATION ENDPRODUCTS IN COOKED BEEF PATTIES BY CEREAL BRAN ADDTION[1,2]

ABSTRACT

Advanced glycation endproducts (AGEs) are formed in cooked meat products via Maillard reaction, which are seen as a contributor to chronic diseases such as diabetes and heart diseases. A number of reports have shown that natural antioxidants such as phenolic acids in grains, herbs, and spices can inhibit their formation. The objective of the study was to determine the inhibitory effects of selected wheat (Jagger, JA), triticale (Spring Triticale, ST; Thundercale, TH), and Rye (RY) bran on AGEs levels in cooked beef patties, as measured by N^{ε}-carboxymethyl lysine (CML) contents. The CML was detected in all the cooked samples, whereas the patties to which RY (42.0% inhibition), ST (27.5% inhibition), and TH (21.4% inhibition) brans were added significantly decreased CML formation. RY and ST, were more abundant in total phenolics content (TPC) and exhibit higher properties as free radical scavengers. Using Pearson's correlation and multiple linear regression analysis, the inhibition of CML in patties was correlated to the water-holding activity (WHC) of the samples, and the radical scavenging activity of the brans as measured by the 2,2-diphenylpicrylhydrazyl (DPPH) assay. These results suggest that addition of bran may be a potential method of decreasing the formation of AGE in cooked patties.

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INTRODUCTION

Advanced glycation endproducts (AGEs) are formed from the Maillard reaction, which is a non-enzymatic reaction of proteins with reducing sugars (Ahmed, 2005). Well known AGEs in foods include N^{ε}-carboxymethyl lysine (CML), methylglyoxal (MGO), and pentosidine. The CML in food has been studied extensively and used as a marker since it is a relatively abundant AGE in food systems (Wu, Huang, Lin, & Yen, 2011). Formation and accumulation of AGEs are suspected to be involved in the pathogenesis of aging and several diseases such as diabetes (Poulsen et al., 2013). The pathogenic effect of AGEs might modify the chemical and biological properties of molecules, as their binding capacity to cellular receptors in a wide range of tissues resulting in functional changes of DNA, proteins, and lipids (Ahmed, 2005). In cell culture studies, AGEs were found to induce cellular oxidative stress and cell activation, and excess consumption of dietary AGEs was also considered to increase inflammation and oxidative stress in some epidemiological studies (Goldberg et al., 2004; Uribarri et al., 2007). Moreover, a series of animal studies found that consumption of AGE-rich diets by mice was associated with kidney disorders and damage (Hofmann et al., 2002). These findings suggest that dietary AGEs may be considered a chronic risk factor for human health. It therefore is desirable to acquire the information on the prevalence of dietary AGEs in food.

In view of previous research reports, limiting the formation of AGEs in foods depends on many factors, such as decreasing cooking temperature, cooking time, and increasing of water-holding capacity (WHC) of meat samples (Chen, & Smith, 2015; Goldberg et al., 2004; Persson, Sjöholm, & Skog, 2003). However, a potential treatment may involve use of various natural antioxidants, which may scavenge the generated free radicals accompanied with the formation

of AGEs though the Maillard reaction. Some reports have indicated that the phenolic extracts from spice (Ahmad, & Ahmed, 2006), microalgae (Sun, Peng, Liu, Fan, Wang, & Chen, 2010), buckwheat products (Szawara-Nowak, Koutsidis, Wiczkowski, & Zielinski, 2014), and wheat bran (Wang, Sun, Cao, & Tian, 2009) can inhibit AGE formation.

Adding cereal bran to meat products, with its significant amount of dietary fiber and antioxidant, may have a positive effect on health promotion (Liu, 2007; Reddy, Hirose, Cohen, Simi, Cooma, & Rao, 2000). Cereal is a good dietary source of natural antioxidants such as phenolic acids, which are concentrated more in the bran portion and include ferulic, vanillic, p-coumaric, and syringic acids (Mattila, Pihlava, & Hellstrom, 2005). Recently, the ability of certain phenolic acids such as vanillic acid, ferulic acid and *p*-coumaric has been shown to inhibit AGEs formation *in vitro* (Wu et al., 2011; Zhang et al., 2015). Huang, Chuang, Wu, and Yen (2008) reported vanillic acid can scavenge reactive carbonyl species in glycation process, thus decreasing AGE formation. During cooking, water is important for the transport of water-soluble precursors of Maillard reaction products (MRPs) from the center to the surface of meat (Persson et al., 2003). Some previous research has shown that additives, such as salt and phosphate with good WHC, can increase and decrease the formation of some MRPs in meat systems (Persson, Sjöholm, & Skog, 2002). Thus, it is possible that the bran addition has an inhibitory effect on the formation of AGEs in meat products due to its WHC.

Although cereal bran has been investigated for their antioxidant activity, there is no information regarding their inhibitory effect on the formation of AGEs in cooked meats. This study was conducted to determine the effects of selected wheat, triticale, and rye brans on AGEs levels in cooked beef patties as measured by CML contents. This study was also

performed to determine if AGEs inhibition is correlated with total phenolic content (TPC), antioxidant activity, or the WHC of samples.

MATERIAL AND METHODS

Materials

Fresh ground beef (10% fat) was purchased from a local supermarket. Cereal seed samples (wheat, rye, and triticale) were provided by a certified seed grower Vance Ehmke (Dighton, KS, USA). The samples included one wheat variety (Jagger 2010), two triticale varieties (Spring Triticale 2011, & Thundercale 2011), and one rye variety (Rye 2009). The N^{ε}-carboxymethyl lysine (CML) standard was purchased from NeoMPS (Strasbourg, France). High purity standards of phenolic acids, 2,2-diphenyl-1-picrylhydrazyl (DPPH), boric acid, hydrochloric acid, Folin–Ciocalteu's reagent, 2-mercaptoethanol, sodium chloride, sodium borohydride, anhydrous magnesium sulfate, and sodium tetraborate decahydrate were purchased from Sigma Aldrich (St. Louis, MO, USA). In addition, solvent and chemicals such as acetonitrile (HPLC grade), chloroform (HPLC grade), methanol (HPLC grade), ortho-phthalaldehyde (OPA) reagent, ACS grade acetone, petroleum ether, hexanes, sodium carbonate, sodium hydroxide, ethanol, ethyl acetate, ethyl ether, and methanol were obtained from Fisher Scientific (Fairlawn, NJ, USA).

Bran sample preparation

Before the milling of the cereal seed samples, moisture content was tested with a Single Kernel Characterization System (SKCS) (Perten Instruments, Hägersten, Sweden). The moisture content was adjusted to 15%, and the samples were equilibrated in the glass bottles for 24 h at

room temperature. A Quadrumat Junior mill system (Brabender, Duisburg, Germany) was used to mill the tempered seeds. The bran fractions were collected and sieved with a Ro-Tap sieve shaker (W.S. Tyler, Mentor, Ohio) over a 0.4 mm particle size screen for 3 min. Later the samples were collected, flushed with nitrogen, stored in the glass bottles, and refrigerated at 4°C.

Beef patty sample preparation

The selected wheat, triticale, and rye bran was added and homogenized with 100 g of fresh ground beef at a level of 5%, a concentration that does not cause noticeable change in flavors (Talukder, & Sharma, 2010). Control samples contained no bran. In order to ensure uniformity, a petri dish (10 cm × 1 cm) was utilized to form patties. Each patty was refrigerated overnight at 4°C, and cooked in a frying pan at surface temperature of 204°C (400°F), with a controller (Bernant, Barrington, USA). The patties were cooked until their internal temperature reached 71°C (160°F), which is recommended by the U.S. Department of Agriculture – Food Safety and Inspection Service (USDA-FSIS) (1998). After cooling for 30 min at room temperature, approximately 2 mm of the top of the patty samples was excised by a meat slicer (Cabela grade slicer, 1/3 hp, Sidney, NE, USA), and then was ground and homogenized by a processor (KitchenAid, model KFP 750) and refrigerated at 4°C. Determination of CML contents in samples was performed on the next day. Each sample was analyzed in triplicate.

Determination of CML

The CML of beef patty samples was extracted according to Drusch et al. (1999) except that chloroform/methanol (2:1, v/v) solution was used as the defatting solvent (Chen et al., 2015). Each sample (0.20 g) was defatted using 20 mL chloroform/methanol (2:1, v/v) solution

followed by centrifugation (10,600 x g at 4°C) for 10 min (Model 21000R Centrifuge, Fisher Scientific, Pittsburgh, PA, USA). Reducing reagent of 4 mL sodium borate buffer (0.2 M, pH 9.4) and 2 mL sodium borohydride (1 M in 0.1 M NaOH) were added to the dried sample for 4 h at room temperature. Subsequently, hydrochloric acid was mixed to a final concentration of 6 M HCl, and the sample was hydrolyzed for 20 h at 110°C. The final CML extracts were concentrated until dry with a rotary evaporator and dissolved in 10 mL of sodium borate buffer (0.2 M, pH 9.4), followed by a final membrane filtration (nylon, 0.45 mL). The extract (50 µL) was reacted with 200 µL of OPA derivatization reagent for 5 min prior to HPLC analysis.

According to the method of Peng, Ma, Cheng, Jiang, Chen, and Wang (2010), the CML was analyzed with an HP1090A Series II HPLC (Agilent Technologies, Santa Clara, CA, USA) coupled with a HP 1046A fluorescence detector programmed to excitation/emission wavelengths of 340nm and 455nm. A reversed-phase TSK gel ODS-80 TM column (25 cm x 4.6 mm, 5 µm, 80 Å, Tosohass, Montgomeryville, PA, USA) was utilized to separate CML with the mobile phases: (solvent A) acetate buffer (pH 6.7, 20 mM)/acetonitrile (90:10, v/v), and (solvent B) acetonitrile. The flow rate was 1.0 mL/min and the injection volume was 20 µL. The CML separation was achieved with a linear gradient program that started with 5% B and changed to 70% B within 5 min, and kept at 70% B till 17 min. The gradient was set back to 95% B in 1 min followed by a post run of 15 min for equilibration. The identity of CML was confirmed by comparing retention times between samples and standards in the fluorescence spectra, and levels were determined by the peak areas of their corresponding derivatives respectively.

Determination of WHC of beef patty samples

WHC of samples was determined by the method of Wardlaw, Mccaskil, and Acton (1973). Meat sample of 15 g was mixed with 22.5 mL of 0.6 M sodium chloride solution, and then was stirred for 1 min and refrigerated at 4°C for 15 min. The slurry was stirred again and centrifuged (12,000 x g) for 15 min (Fisher Scientific, Model 21000R Centrifuge, Pittsburgh, PA, USA). The supernatant was decanted and the volume recorded. The amount of solution retained by meat was reported as the WHC in mL per 100 g sample.

Determination of phenolic acid composition in bran

A modified extraction method was used to extract free phenolics from bran samples (Krygier, Sosulski, & Hogge, 1982). An extraction solvent A of methanol/acetone/water (7:7:6, v/v/v) was prepared, which was adjusted to pH 2 with concentrated hydrochloric acid. Bran sample of 1 g was de-fatted with 30 mL petroleum ether, and then homogenized with a mixer (Omni International, Kennesaw, Georgia, USA) using 7 mL of extraction solvent A. The sample was made up to final volume of 35 mL with the solvent A and shaken at room temperature for 2 h. The mixture was centrifuged (5000 x g) for 15 min (Sorvall RC-5C Plus Centrifuge, Kendro Laboratory Products, Newtown, CT, USA), and the supernatant was concentrated to about 15 mL by evaporation under vacuum at 40°C. Subsequently, the extract was subjected to a liquid-liquid extraction with 90 mL solvent B of ethyl ether/ethyl acetate (1:1 volume ratio). The supernatant layer was removed and evaporated to dryness under vacuum, and then reconstituted in methanol. It was clarified by a final membrane filtration (nylon, 0.45 mL), and stored at -20°C until analysis.

The composition of phenolic acids was identified by HPLC using a reverse-phase TSK gel ODS-80 TM column (25 cm×4.6 mm, 5 µm, 80 Å, Tosohass, Montgomeryville, PA, USA), according to an established method (Robbins, 2003). The extract or standard (10 µL) was injected into a HP1050 Series HPLC (Agilent Technologies, Santa Clara, CA, USA) coupled with a diode array detector (DAD). Eluent A was 1% acetic acid (v/v) while elute B was acetonitrile. At a flow rate of 1.0 mL/min, a solvent gradient was as follows: 0 min 100% A; 5 min 99% A; 9 min 90% A; 19 min 50% A; 20 min 0% A; and 30 min 100% A. The phenolic compounds were detected at 260 nm for p-hydroxybenzoic (4-OHBA), and vanillic (VA); at 320 nm for chlorogenic (CHA), caffeic (CA), gentisic (GEA), and ferulic acids (FA), by comparison of their retention times and spectra with standards, and quantitative analysis was based on their peak areas from the chromatograms. All calibration data showed good linearity ($R^2 > 0.998$) for the phenolic acids within the range of 3.1–125.0 µg/mL. The limit of detection (LOD), for all phenolic acids, was from 0.8 to 2.2 µg/mL, and the limit of quantification (LOQ) was from 2.8 to 7.3 µg/mL.

TPC assay in bran

TPC of the extracts was determined using a method of Singleton, and Rossi (1965). The phenolic extracts (200 µL) with ferulic acid solution, and methanol blank, were added to a 10 mL test tube, and then 1.5mL of 0.2N Folin-Ciocalteu reagent was added. The tube was vortexed in an analog vortex mixer (Fisher Scientific, Fairlawn, NJ, USA) and mixed with 1.5 mL of 6% sodium carbonate, and allowed to incubate in the dark at room temperature for 2h. Absorbance of the mixture was read at 725 nm, and TPC was expressed as mg ferulic acid equivalents (FAE) per gram of bran.

Radical DPPH scavenging activity assay in bran

The free radical scavenging capacity of each bran extract was estimated according to the DPPH assay by Singh, Chidambara-Murthy, and Jayaprakasha (2002). The phenolic extract samples (0.1 mL) or 0.1 mL methanol (blank) was mixed with 3.9 mL of DPPH solution (6×10^{-5} M) in a test tube. The samples were incubated at room temperature in the dark for 2 h, and the absorbance was measured at 517 nm.

DPPH scavenging activity (%) = $[(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$.

Statistical analyses

The experimental data were reported as means ± standard deviations for triplicate determinations. Analyses of variance (ANOVA) were performed on the data, and statistical significance was selected at $P < 0.05$. Pearson's correlation and regression analysis, and stepwise multiple linear regression (MLR) were conducted to evaluate relationships between CML inhibition and WHC, TPC, and DPPH scavenging activity. Stepwise MLR analysis was employed with the aim to explore the parameters related to the changes in the CML contents. An equation of MLR model takes the following form:

$$Y = \beta_0 + \beta_1 * X_1 + \beta_2 * X_2 + \beta_3 * X_3 + \ldots + \beta_p * X_p$$

Where Y is the dependant variable (inhibition of CML); X_1, X_2, X_3, …, X_p represent the independent variables (WHC, TPC , DPPH scavenging activity, and their interaction factors); β_0 is the intercept of this plane; β_1, β_2, β_3, …, and β_p are the standard partial regression coefficients of variables. The criteria for removal or selection of variables in the MLR model were based on the significance level of setting at 0.05. All statistical analyses were performed using SAS version 9.1 (SAS Inst. Inc., Cary, NC, USA).

RESULTS AND DISSCUSION

Method validation

The linearity of the standard calibration curve of CML was tested in a range from 0.025 to 0.50 μg/mL, and the correlation coefficient indicated acceptable linearity ($R^2 > 0.998$). The method showed good repeatability with a relative standard deviation (RSD) value of <8%. The limit of detection (LOD) was 0.007 μg/mL, whereas the limit of quantification (LOQ) was calculated to be 0.025 μg/mL. Recovery experiments were conducted by spiking the samples with 2.5, 6.5, and 20.0 μg/g CML (n=3). The average recovery rates were 81.39 ± 6.55, 86.38 ± 6.49, and 91.83 ± 8.71%. The recovery and linearity of CML from this study was comparable with other previous studies (Sun, Tang, Wang, Rasco, Lai, & Huang, 2015; Zhang, Huang, Xiao, & Mitchell, 2011).

CML content in beef patties

In the present study, four cereal brans (JA, RY, ST, and TH) were added to beef patties to investigate their inhibitory effects on AGE formation. The CML in each sample was extracted and analyzed by HPLC and the amount is expressed in μg/g sample. Although every raw and cooked sample contained CML, no significant differences ($P > 0.05$) in CML contents among the uncooked patties of five treatments were found: 2.83±0.43 μg/g in control, 4.21±1.35 μg/g in RY, 2.44±0.20 μg/g in ST, 4.52±1.62 μg/g in TH, and 3.55±1.67 μg/g in JA. The CML quantitative determinations in cooked samples are summarized in Table 16. All the cooked patties exhibited higher levels of CML as compared to the raw samples ($P < 0.05$). The result was consistent with values reported by Assar, Moloney, Lima, Magee, & Ames (2009), which indicated fried minced beef contained higher CML level than raw minced beef. Moreover, a similar finding was

demonstrated in our previous study when evaluating the influence of cooking methods on the dietary CML formation among a variety of meat products (Chen et al., 2015).

Table 16 also summarizes the effect of cereal bran on the CML content and inhibition in the cooked patties. Three cereal brans showed the capacity of reducing CML in the samples to different extents, which was consistent in the expression and report of data, for example, 10.69 μg/g in RY. The results indicated CML levels in the three treatments were significantly lower than in the control group ($P < 0.05$). Furthermore, adding RY (42.03%) and ST (27.52%) was effective in inhibiting of AGE in cooked patties, whereas the inhibitory effect of TH (21.35%) was lower. However, the addition of JA bran (17.33 μg/g, 6.08% inhibition) in the patties did not show a significant decrease in CML formation.

Table 16. Effect of bran source on CML concentrations (μg/g), percent inhibition, and WHC (mL/100g) in cooked patties samples.

Treatment/source	CML (μg/g)	% Inhibition	WHC (mL/100 g)
Control	18.45±3.50^{a}		14.22±4.07^{a}
Rye (RY)	10.69±2.51^{b}	42.03	27.55±3.36^{b}
Spring Triticale (ST)	13.37±1.12^{bc}	27.52	22.89±3.67^{bc}
Thundercale (TH)	14.51±1.06^{cd}	21.35	21.11±4.02^{ab}
Jagger (JA)	17.33±0.89^{ad}	6.08	18.89±4.34^{ac}

^{abcd}Means with different superscripts within the same column are significantly different at $P < 0.05$.
Value is represented as mean ± standard deviation (n=3).

A series of studies has demonstrated that adding natural plants or plants extracts reduced the formation of AGEs in foods or model systems. For example, Uribarri et al. (2010) showed that application of lemon juice on lean beef for 1 h before cooking significantly reduced AGE formation up to 50%. Grape seed ingredients inhibited CML formation in bread products probably due to the antioxidant activity of phenolic compounds in the extract, which performed in a dose-dependent way (Peng et al., 2010). According to the study reported by Farrar, Hartle, Hargrove, and Greenspan (2008), the AGE level in meat model system was reduced by approximately 60% with sorghum bran extract containing a high phenolic content. Moreover, buckwheat extracts showed an inhibitory effect on formation of AGEs in BSA-glucose model systems because of their radical scavenging activity (Szawara-Nowak et al., 2014).

WHC of beef patties

WHC of samples is also summarized in Table 16. RY and ST bran were effective in increasing the WHC in beef patties since both of them were with significant difference compared to the control ($P < 0.05$), whereas the value of JA was the lowest (18.89 mL/100g). The presence of different amounts of soluble dietary fiber in the bran might explain the result. In addition, the studies reported by Fernández-Ginés, Fernández-López, Sayas-Barberá, & Pérez-Alvarez (2005), and Talukder et al. (2010) both provided the similar results; that addition of cereal fiber or bran increased the WHC of meat products.

TPC of brans

TPC of the four brans ranged from 0.21 to 0.59 mg ferulic acid equivalents (FAE) per gram of bran (Table 17). ST possessed the highest level of 0.59 mg FAE/g bran, followed by RY (0.50 mg FAE/g bran), TH (0.30 mg FAE/g bran) and JA (0.21 mg FAE/g bran) with significant

differences between each other ($P < 0.05$). In this study, it seemed that the wheat variety (JA) bran had the lower level of TPC in comparison with the rye variety, which was consistent with other research findings (Povilaitis, Sulniute, Venskutonis, & Kraujaliene, 2015; Weidner, Amarowicz, Karamac, & Dabrowski, 1999).

Table 17. TPC and DPPH scavenging activity of selected bran sources.

Type	TPC (mg FAE/g bran)[A]	DPPH scavenging activity (%)[B]
Rye (RY)	0.50±0.06[a]	37.92±5.32[a]
Spring Triticale (ST)	0.59±0.03[b]	29.12±5.90[b]
Thundercale (TH)	0.30±0.03[c]	19.01±4.51[c]
Jagger (JA)	0.21±0.02[d]	12.37 ±0.78[c]

[abcd]Means with different superscripts within the same column are significantly different at $P < 0.05$. Value is represented as mean ± standard deviation (n=3).
[A]TPC was expressed as mg ferulic acid equivalents (FAE) per gram of bran.
[B]DPPH scavenging activity (%) = $[(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$.

Radical DPPH scavenging activity

As showed in Table 17, the DPPH scavenging activity of bran is listed as percent inhibition: RY (37.92%), ST (29.12%), TH (19.01%), and JA (12.37%); significant differences were observed between each other ($P < 0.05$). It is noticed that JA bran had the lowest DPPH scavenging activity of 12.37% while it also had the lowest amount of TPC in our study. However, cereal bran with a high DPPH scavenging activity did not always contain a high level of TPC, which was in agreement with some published results (Verma, Hucl, & Chibbar, 2009; Zhou, Laux, & Yu, 2004). For example, the ST bran had the highest TPC among the four brans, but

possessed a lower DPPH scavenging activity in comparison with the RY bran. The result may be due to other compounds in RY bran that could impact more on its antioxidant activity.

Individual phenolic acid

The major phenolic acids of each bran sample were determined in this study. Typical HPLC chromatograms of phenolic acids in cereal bran are shown in Figure 9, and their different levels in bran are reported in Table 18. As shown in Table 18, among all the brans samples FA was the prominent phenolic acid followed by VA while GEA, 4-OHBA, and CHA were present at the much lower contents, which was similar to previous reports (Hosseinian, & Mazza, 2009; Mattila et al., 2005). In relation to the different types of bran, GEA was specific in the triticale variety (ST and TH), but CHA had been identified in most of the cereal varieties other than in the wheat (JA). And it is worth mentioning that the caffeic acid was only found in Rye samples, which may contribute to its effective inhibition of AGEs, as illustrated in Table 16. Furthermore, the content of individual phenolic acid in RY (131.45 ug/g) and ST (120.79 ug/g) were higher than it were in JA (91.78 ug/g) and TH (88.85 ug/g). The result was not always consistent with the TPC data in our study. For example, there was significantly lower level of TPC in the JA than in the TH ($P < 0.05$). This may be possible because there may be other phenolic acids that were not identified, as shown in Figure 9. The different phenolic acids ingredients in bran may be responsible for the differences of their antioxidant capacity such as the free radical scavenging activity.

Table 18. The phenolic acids composition of the bran cereal cultivars[a].

Phenolic acids	Rye	Spring Triticale	Thundercale	Jagger
Vanillic acid (VA)	ND	18.92±0.34	ND	22.02±0.81
Caffeic acid (CA)	13.61±0.48	ND	ND	ND
Gentisic acid (GEA)	ND	4.39±0.11	4.14±0.42	ND
Ferulic acid (FA)	109.7±7.17	93.49±3.69	81.73±2.80	63.44±4.65
Chlorogenic acid (CHA)	3.52±0.49	3.99±0.18	2.97±0.80	ND
4-Hydroxybenzoic acid (4-OHBA)	4.65±0.11	ND	ND	6.32±0.19
Total content	131.45±7.23	120.79±3.45	88.85±1.75	91.78±4.02

[a]Results are expressed as μg per gram bran.
ND not detected.
Values are mean values ± standard deviation (n=3).

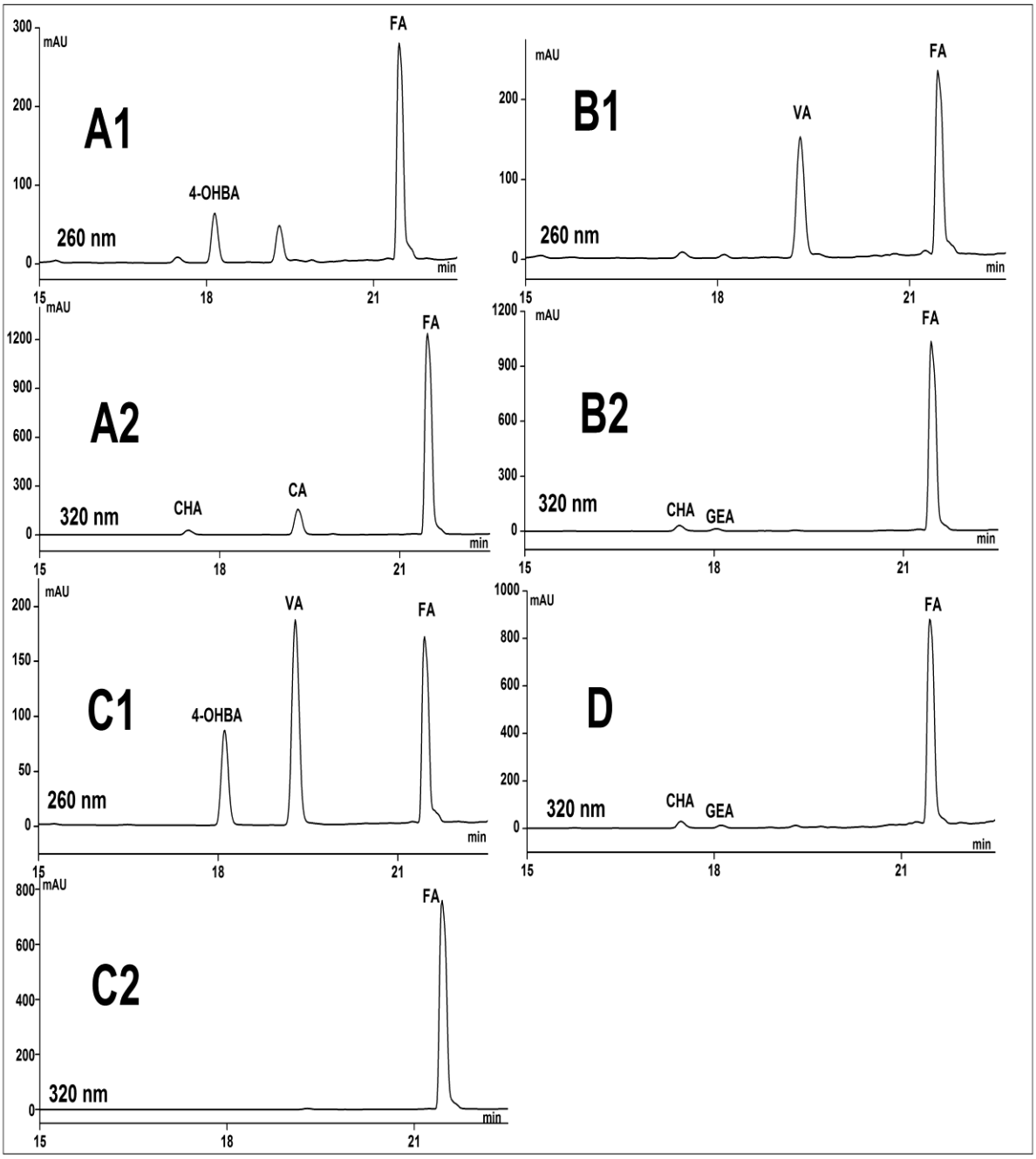

Figure 9. Representative HPLC chromatograms of phenolic acids in the cereal brans. (A1) Rye (260 nm); (A2) Rye (320 nm); (B1) Spring Triticale (260 nm); (B2) Spring Triticale (320 nm); (C1) Jagger (260 nm); (C2) Jagger (320 nm); and (D) Thundercale (320 nm).

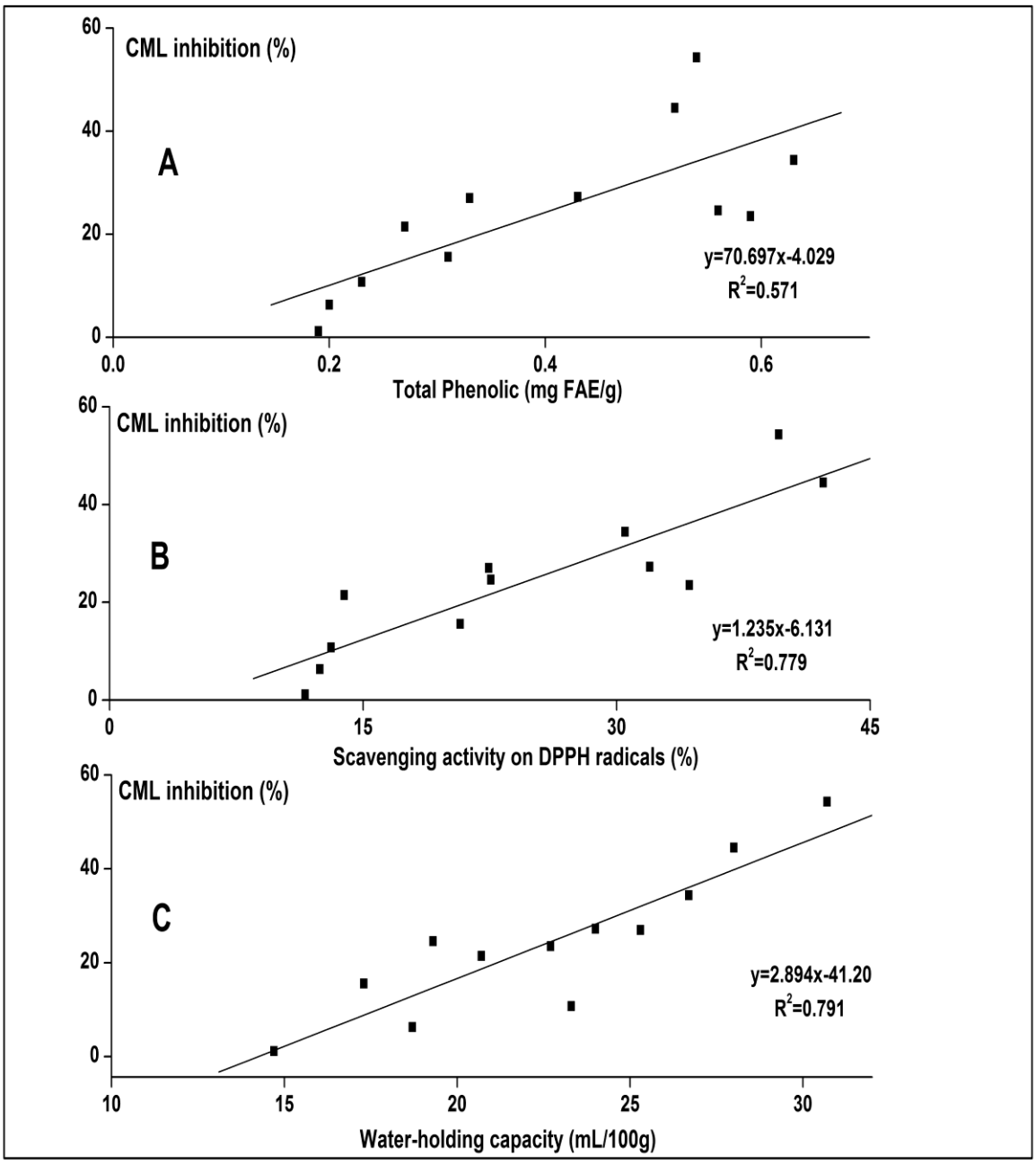

Figure 10. Regression analysis between the CML percent inhibition in samples which added and; (A) TPC of bran, (B) DPPH scavenging activity on bran, and (C) WHC of samples.

Correlation analysis of CML inhibition

Pearson's correlation analysis was utilized to evaluate the relationship between the CML percent inhibition and the TPC and DPPH scavenging capacity of bran, as well as the relationship to WHC. In this study, the correlation coefficients of WHC, DPPH and TPC scavenging capacity were 0.89, 0.88 and 0.75 respectively, which indicated the parameters positively correlated well with inhibition of CML ($P < 0.05$). However, regression coefficient of determination of TPC ($R^2 = 0.57$) was less than those of WHC ($R^2 = 0.79$) and DPPH scavenging capacity ($R^2 = 0.78$), which is illustrated in Figure 10.

The correlation analysis only dealt with a single variable which could be insufficient because the parameters may be interrelated. Therefore, MLR was also used to optimize the model for explaining the relationship between the CML percent inhibition and parameters. The first static treatment concluded inclusion of interaction caused no main effects since the *P*-value was much higher than the significance level in the *F*-test for the model including interaction factors. After excluding the interaction terms, it found the factor X_2 (TPC) and X_3 (scavenging activity on DPPH) were correlated to some extent by the diagnosis of collinearity. Nevertheless, in the stepwise selection process, the partial correlation was not significant for the variable X_2 (TPC) on the set significance level ($P > 0.05$), which led X_2 to be removed from the model. A final fitted regression model equation was constructed as following:

$$\hat{Y} = -30.24 + 1.68 * X_1 + 0.68 * X_3$$

In the equation, the coefficient of determination is 0.89, which indicated it could account for 89% of the variation in CML inhibition. The *F*-test with an associated *P*-value less than 0.0001 had also shown a significant linear relationship between the variables in the

analysis of variance. Moreover, the standard partial correlation coefficients of X_1 (WHC) and X_3 (scavenging activity on DPPH) were 0.51 and 0.48. According to the *P*-values, one of the most important variables affecting CML inhibition was WHC ($P < 0.001$) followed by scavenging activity on DPPH ($P = 0.025$). It seems that adding bran with high WHC and DPPH scavenging activity into patty samples is a good choice to decrease the AGE formation. This is because bran may restrict the transport of water-soluble precursors of AGEs, and antioxidants in bran could react or quench the free radicals generated in the Maillard reaction (Persson et al., 2003; Wu et al., 2011).

CONCLUSION

The four cereal brans included in this study, showed different TPC, phenolic acids composition, and DPPH antioxidant activity in which rye (42.0%) and spring triticale (27.5%) also exhibited good inhibitory effect on CML formation in beef patty samples. The added bran CML percent inhibition had a significant correlation to WHC of samples and DPPH scavenging activity of brans through stepwise MLR analysis. Hence, adding the natural cereal bran (rye or spring triticale) in meat patties would effectively inhibit AGE formation in cooking, which may be a healthier choice for meat consumers. Future studies are desirable to investigate the inhibitory mechanisms of natural phenolic compounds in cereal bran on AGE formation.

REFERENCES

Ahmed, N. (2005). Advanced glycation endproducts-role in pathology of diabetic complications. *Diabetes Research and Clinical Practice*, *67*(1), 3-21.

Ahmad, M. S., & Ahmed, N. (2006). Antiglycation properties of aged garlic extract: possible role in prevention of diabetic complications. *The Journal of Nutrition, 136*(3), 796S-799S.

Assar, S., Moloney, C., Lima, M., Magee, R., & Ames, J. (2009). Determination of N-epsilon-(carboxymethyl) lysine in food systems by ultra performance liquid chromatography-mass spectrometry. *Amino Acids*, *36*(2), 317-326.

Chen, G., & Smith, J. S. (2015). Determination of advanced glycation endproducts in cooked meat products. *Food Chemistry*, *168*, 190-195.

Drusch, S., Faist, V., & Erbersdobler, H. (1999). Determination of N[epsilon]-carboxymethyllysine in milk products by a modified reversed-phase HPLC method. *Food Chemistry*, *65*(4), 547-553.

Farrar, J. L., Hartle, D. K., Hargrove, J. L., & Greenspan, P. (2008). A novel nutraceutical property of select sorghum (Sorghum bicolor) brans: Inhibition of protein glycation. *Phytotherapy Research*, *22*(8), 1053-1058.

Fernández-Ginés, J. M., Fernández-López, J., Sayas-Barberá, E., & Pérez-Alvarez, J. A. (2005). Meat products as functional foods: A review. *Journal of Food Science*, *70*(2), 37-43.

Goldberg, T., Cai, W. J., Peppa, M., Dardaine, V., Baliga, B. S., & Uribarri, J. (2004). Advanced glycoxidation end products in commonly consumed foods. *Journal of the American Dietetic Association*, *104*(8), 1287-1291.

Hofmann, S., Dong, H., Li, Z., Cai, W., Altomonte, J., Thung, S., Zeng, F., Fisher, E., & Vlassara, H. (2002). Improved insulin sensitivity is associated with restricted intake of dietary glycoxidation products in the db/db mouse. *Diabetes*, *51*(7), 2082-2089.

Hosseinian, F. S., & Mazza, G. (2009). Triticale bran and straw: Potential new sources of phenolic acids, proanthocyanidins, and lignans. *Journal of Functional Foods*, *1*(1), 57-64.

Huang S., Chuang, H., Wu, C., & Yen, G. (2008). Cytoprotective effects of phenolic acids on methylglyoxal-induced apoptosis in Neuro-2A cells. *Molecular Nutrition & Food Research*, *52*(8), 940-949.

Krygier, K., Sosulski, F., & Hogge, L. (1982). Free, esterified, and insoluble-bound phenolic acids. 1. Extraction and purification procedure. *Journal of Agricultural and Food Chemistry*, *30*, 330-334.

Liu, R. H. (2007). Whole grain phytochemicals and health. *Journal of Cereal Science, 46,* 207.

Mattila, P., Pihlava, J. M., & Hellstrom, J. (2005). Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. *Journal of Agricultural and Food Chemistry*, *53*(21), 8290-8295.

Peng, X., Ma, J., Cheng K., Jiang, Y., Chen, F., & Wang, M. (2010). The effects of grape seed extract fortification on the antioxidant activity and quality attributes of bread. *Food Chemistry*, *119*(1), 49-53.

Persson, E., Sjöholm, I., & Skog, K. (2002). Heat and mass transfer in chicken breasts, effect on PhIP formation. *European Food Research and Technology, 214,* 455-459.

Persson E., Sjöholm, I., & Skog, K. (2003). Effect of high water-holding capacity on the formation of heterocyclic amines in fried beef burgers. *Journal of Agricultural and Food Chemistry*, *51,* 4472-4477.

Poulsen, M.W., Hedegaard, R. V., Andersen, J. M., de Courten, B., Bügel, S., Nielsen, J., Skibsted, L. H., & Dragsted, L. O. (2013). Advanced glycation endproducts in food and their effects on health. *Food and Chemical Toxicology*, 60, 10-37.

Povilaitis, D., Sulniute, V., Venskutonis, P. R., & Kraujaliene, V. (2015). Antioxidant properties of wheat and rye bran extracts obtained by pressurized liquid extraction with different solvents. *Journal of Cereal Science*, *6*2, 117-123.

Reddy, B. S., Hirose, Y., Cohen, L. A., Simi, B., Cooma, I., & Rao, C. V. (2000). Preventive potential of wheat bran fractions against experimental colon carcinogenesis: implications for human colon cancer prevention. *Cancer Research, 60,* 4792-4797.

Robbins, R. (2003). Phenolic acids in foods: An overview of analytical methodology. *Journal of Agricultural and Food Chemistry*, *51*(10), 2866-2887.

Singh, R. P., Chidambara-Murthy, K. N., & Jayaprakasha, G. K. (2002). Studies on the antioxidant activity of pomegranate (Punica granatum) and seed extracts using *in vitro* models. *Journal of Agricultural and Food Chemistry*, *50*(1), 81-86.

Singleton, V., Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144-158.

Sun, Z., Peng, X., F., Liu, J., Fan, K., W., Wang, M., F., & Chen, F. (2010). Inhibitory effects of microalgal extracts on the formation of advanced glycation endproducts (AGEs). *Food chemistry*, *120*(1), 261-267.

Sun, X., Tang, J., Wang, J., Rasco, B. A., Lai, K., & Huang, Y. (2015). Formation of advanced glycation endproducts in ground beef under pasteurization conditions. *Food Chemistry*, *172*, 802-807.

Szawara-Nowak, R., Koutsidis, G ., Wiczkowski, W., & Zielinski, H., (2014). Evaluation of the *in vitro* inhibitory effects of buckwheat enhanced wheat bread extracts on the formation of advanced glycation end-products (AGEs). *LWT - Food Science and Technology*, *58*(2), 327-334.

Talukder, S., & Sharma, D. P. (2010). Development of dietary fiber rich chicken meat patties using wheat and oat bran. *Journal of Food Science and Technology-Mysore*, *47*(2), 224-229.

Uribarri, J., Cai, W., Peppa, M., Goodman, S., Ferrucci, L., Striker, G., & Vlassara, H. (2007). Circulating glycotoxins and dietary advanced glycation endproducts: two links to inflammatory response, oxidative stress, and aging. *The Journals of Gerontology Series A Biological Sciences and Medical Sciences, 62*(4), 427-433.

Uribarri, J., Woodruff, S., Goodman, S., Cai, W., J., Chen, X., Pyzik, R., Yong, A., Striker, G., E., & Vlassara, H. (2010). Advanced glycation end products in foods and a practical guide to their reduction in the diet. *Journal of the American Dietetic Association*, *110*(6), 911-916.

Verma, B., Hucl, P., & Chibbar, R. N. (2009). Phenolic acid composition and antioxidant capacity of acid and alkali hydrolyzed wheat bran fractions. *Food Chemistry*, *116*(4), 947-954.

Wardlaw, F., B., Mccaskil L., H., & Acton J., C. (1973). Effect of postmortem muscle changes on poultry meat loaf properties. *Journal of Food Science*, *38*(3), 421-423.

Wang, J., Sun, B., G., Cao, Y., P., & Tian, Y. (2009). Protein glycation inhibitory activity of wheat bran feruloyl oligosaccharides. *Food Chemistry*, *112*(2), 350-353.

Weidner, S., Amarowicz, R., Karamac, M., & Dabrowski, G. (1999). Phenolic acids in caryopses of two cultivars of wheat, rye and triticale that display different resistance to pre-harvest sprouting. *European Food Research and Technology*, *210*(2), 109-113.

Wu, C., Huang, S., Lin, J., & Yen, G. (2011). Inhibition of advanced glycation end product formation by food stuffs. *Food Function*, 2, 224-234.

Zhang, G., Huang G., Xiao L., & Mitchell, A., E. (2011). Determination of advanced glycation endproducts by LC-MS/MS in raw and roasted almonds (*Prunus dulcis*). *Journal of Agricultural and Food Chemistry, 59*(22), 12037-12046.

Zhang, X., C., Tao, N., P., Wang, X., C., Chen, F., & Wang, M., F. (2015). The colorants, antioxidants, and toxicants from nonenzymatic browning reactions and the impacts of dietary polyphenols on their thermal formation. *Food & Function*, *6*(2), 345-355.

Zhou, K. Q., Laux, J. J., & Yu, L. L. (2004). Comparison of Swiss red wheat grain and fractions for their antioxidant properties. *Journal of Agricultural and Food Chemistry*, *52*(5), 1118-1123.

Chapter 4. INHIBITION EFFECTS OF CEREAL BRAN EXTRACT ON THE FORMATION OF ADVANCED GLYCATION ENDPRODUCTS[1,2]

ABSTRACT

The adverse health effects of advanced glycation endproducts (AGEs) is of current interest; some research have indicated that consuming these compounds may contribute to chronic diseases such as diabetes and heart diseases. The objective of this study was to determine the inhibitory effect of cereal bran extract from wheat (Jagger, JA), triticale (Spring Triticale, ST, and Thundercale, TH), and Rye (RY) on AGE formation in a bovine serum albumin-glucose (BSA-GLU) model system. The ST extract inhibited AGE formation as measured by N^{ε}-carboxymethyllysine (CML) levels. Subsequent HPLC analysis revealed four major phenolic acids that were present; vanillic (VA), chlorogenic (CHA), gentisic (GEA), and ferulic acids (FA). The present study also investigated the antioxidant and antiglycation properties of the phenolic acids, which showed that GEA and CHA were effective radical scavengers and acted against dicarbonyl compounds. The results indicated that using ST bran extract may be useful in preventing AGE formation because phenolic acids scavenge free radicals and trap carbonyl species.

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2- Presented in part at IFT Annual Meeting 2016. Toxicology and Safety Evaluation Division #155.

INTRODUCTION

During food processing or storage, free amino groups of protein can react with the carbonyl group of reducing sugar to form advanced glycation endproducts (AGEs) via Maillard browning reactions. An array of dietary AGE products has been detected *in vivo*, some of which are characterized as N^{ϵ}-carboxymethyllysine (CML), N^{ϵ}-carboxyethyllysine, pentosidine, and pyrralines (Ahmed, 2005; Sell et al., 1991). Several diseases, such as diabetes, are widely associated with the chemical processes that cause AGEs to form and accumulate (Poulsen et al., 2013; Rojas, & Morales, 2004). For example, cells interact with AGE-modified proteins, inducing several biological responses *in vivo*, among them the development of diabetic vascular complications (Dickinson, Carrington, Frost, & Boulton, 2002). The initial Maillard reaction yields a Schiff base, which then rearranges to form a stable Amadori product like fructosamine (Nagaraj, & Sady, 1996). Then, a series of irreversible reactions occur through the Amadori rearrangement step forming highly reactive dicarbonyl intermediates such as glyoxal, methylglyoxal, and 3-deoxyglucosone (Singh, Barden, Mori, & Beilin, 2001; Vlassara, 1996). Hence, inhibiting fructosamine adducts and dicarbonyls may reduce formation of AGEs and lower the risk of chronic disease development.

At present, treatment with AGE inhibitors may prevent clinical complications as a result of their antioxidant activity or reactive carbonyl species trapping activity. A variety of synthetic products like aminoguanidine have been evaluated as inhibitors of AGE formation. However, natural products from foods or plants may be more promising inhibitors because they are less toxic (Wu, Huang, Lin, & Yen, 2011). Cereal bran, containing most of the phenolic acids found in whole grain, has been a healthy source of dietary fiber and natural antioxidants (Liu, 2007).

Recent literature suggests the phenolic acids fractions in bran are compounds with high antioxidant capacity (Verma, Hucl, & Chibbar, 2009). Lo, Hsiao, and Chen (2011) demonstrated the carbonyl trapping activity of various phenolic acids. Buckwheat extracts inhibited the formation of AGEs in a BSA-GLU model system because of their ability to scavenge radicals (Szawara-Nowak, Koutsidis, Wiczkowski, & Zielinski, 2014). Farrar, Hartle, Hargrove, and Greenspan (2008) showed that sorghum bran extract with a high phenolic content reduced the AGE level approximately 60% in their model system. Nevertheless, to our knowledge, studies of the effect of bran extracts in preventing formation of AGEs in a model system are still rare. In most studies, fluorescence spectrometry or enzyme linked immunosorbant assay (ELISA) has been commonly used to determine the AGEs. Fluorescence spectrometry can determine the intensity to reflect the level of AGEs. However, it cannot easily identify an individual AGE compound (Schmitt, Gasic-Milenkovic, & Schmitt, 2005). On the other hand, AGEs may produce auto-antibodies that interfere with results from ELISA test (Turk, Ljubic, Turk, & Benko, 2001).

The formation of AGEs was estimated by chromatography method in model systems, as measured by CML formation, to see how well different bran extracts inhibited AGEs. The objectives thus were to compare AGE inhibition by bran extracts, determination of which phenolic acids were most efficacious.

MATERIAL AND METHODS

Materials

Vance Ehmke (Dighton, KS, USA), a certified seed grower, provided cereal seed samples (wheat, rye, and triticale). The N^{ε}-carboxymethyl lysine (CML) standard was purchased from

NeoMPS (Strasbourg, France). High purity phenolic acids, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-trinitrobenzenesulfonic acid (TNBS), BSA, glyoxal, nitro blue tetrazolium chloride (NBT), sodium azide, Girard-T reagent, glucose, boric acid, hydrochloric acid, 2-mercaptoethanol, sodium chloride, sodium borohydride, anhydrous magnesium sulfate, sodium sulfite, dibasic sodium phosphate, and sodium tetraborate decahydrate, were obtained from Sigma Aldrich (St. Louis, MO, USA). Solvent and other chemicals like acetonitrile, chloroform, methanol, ortho-phthalaldehyde (OPA) reagent, acetone, petroleum ether, ethyl acetate, ethyl ether, methanol, hexanes, sodium carbonate, sodium hydroxide, and ethanol were purchased from Fisher Scientific (Fairlawn, NJ, USA).

Preparing bran extracts

Moisture content of the cereal seed samples was tested using a Single Kernel Characterization System (Perten Intruments, Hägersten, Sweden), and moisture content was adjusted to 15% and equilibrated for 24 hours. A Quadrumat Junior mill system (Brabender, Duisburg, Germany) was used to mill the tempered seed. A Ro-Tap sieve shaker (W.S. Tyler, Mentor, OH) was used to collect the bran fraction and sieve it for 3 minutes over a 0.4 mm particle size screen. The sample was collected, flushed with nitrogen, and stored in glass bottles in a refrigerator held at 4°C.

A modified extraction method was used to extract free phenolics from bran samples (Krygier, Sosulsk, & Hogge, 1982). The sample of bran (10 g) was de-fatted with petroleum ether, then homogenized using a professional mixer (Omni International, Kennesaw, GA, USA), adding a solvent with a volume ratio of methanol/acetone/water (7:7:6, pH 2), adjusted with HCl. The sample was brought to 350mL with the solvent, and shaken at room temperature for 2

hours. The mixture was centrifuged (5000 x g) for 15 minutes (Sorvall RC-5C Plus Centrifuge, Kendro Laboratory Products, Newtown, CT, USA), and the supernatant was then concentrated to about 150 mL by evaporation under vacuum at 40°C. The extract was then subjected to a liquid-liquid extraction with ethyl ether/ethyl acetate (1:1 volume ratio). The supernatant layer was collected and evaporated to dryness and then reconstituted in methanol, and stored at -20°C until analysis.

Constructing BSA-GLU system

BSA (2 μg/μL), glucose (100 mM), and NaN_3 (0.1 g/mL) were incubated with bran extract (1μg/μL) or phenolic acid compounds (1 mM) in 100 mM phosphate buffer (pH 7.4) at 37°C for 7 days. The control group was the reagents without test samples. After the procedure, samples were stored at -20°C before analysis.

Measuring degree of glycation

Degree of glycation was determined by a TNBS method (Nissen, 1979) with some modification. The sample was dissolved in 0.1 M sodium borate containing 0.1 M sodium hydroxide; 0.5 mL was then reacted with 2 mL of 1 M TNBS for 5 min. To stop the reaction, 1.5% sodium sulfite (1.5 mM) and 98.5% of dibasic sodium phosphate (0.1 M) were added. The absorbance was measured at 420 nm using a Genesys 10vis spectrophotometer (Thermo Scientific Inc., Waltham, MA, USA). The degree of glycation was calculated according to the equation below.

$$\text{Degree of glycation} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

Identifying phenolic acids composition

An HPLC method (Robbins, 2003) was used with an HP1050 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) coupled with a diode array detector. A reverse-phase TSK gel ODS-80 TM column (25 cm×4.6 mm, 5 µm, 80 Å, Tosohass, Montgomeryville, PA, USA) was selected to analyze ST bran extract. The flow rate was 1.0 mL/min and injection volume was 15 µL. The mobile phases were 1% acetic acid (solvent A) and acetonitrile (solvent B). The solvent gradient was as follows: 0 min 100% A; 5min 99% A; 9 min 90% A; 19 min 50% A; 20 min 0% A; and 30 min 100% A. The phenolic acids were measured at 260 nm for VA and at 320 nm for CHA, GEA, and FA. Phenolic acids were identified by comparing retention times and spectra with standards, and quantitative analysis was based on peak areas from the chromatograms. All calibration data showed good linearity ($R^2 > 0.998$) for the studied phenolic acids within the range of 3.1–125.0 µg/mL. The limit of detection was 0.8 to 1.2 µg/mL, and the limit of quantification was 2.8 to 4.1 µg/mL.

Radical DPPH scavenging activity assay

Selected phenolic acids in bran extract were estimated using a previously reported DPPH assay (Singh, Chidambara-Murthy, & Jayaprakasha, 2002). Different levels of samples or control were mixed with the DPPH solution (6×10^{-5} M) in a test tube. Then samples were incubated for 1 hour in the dark, and absorbance was measured at 517 nm using a Genesys 10vis spectrophotometer (Thermo Scientific Inc., Waltham, MA, USA).

$$\text{DPPH scavenging activity (\%)} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

The activity was defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50% (IC_{50}).

Measuring fructosamine adduct

The fructosamine adduct was identified using an NBT method previously described by Baker, Zyzak, Thorpe, and Baynes (1994). Selected phenolic acid compounds (0.01 mM, 0.1 mM, 1 mM) were added to the BSA-GLU system and held at 37°C for 7 days. Quercetin was used as a positive control. The glycated samples (50 µL) were reacted with 150 µL of NBT reagent (300 µM) in sodium carbonate buffer (100 mM, pH 10.4) for 30 min at room temperature. The absorbance was measured at 530 nm with a Genesys 10vis spectrophotometer (Thermo Scientific Inc., Waltham, MA, USA).

$$\text{Inhibitory activity of fructosamine (\%)} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

where $Abs_{control}$ is the absorbance of the group in the absence of inhibition compounds. The result was calculated as IC_{50}, which is how much compound was needed to decrease fructosamine formation by 50%.

Determining dicarbonyl compounds

Dicarbonyl compounds were measured using an existing method with some modification (Mitchel, & Birnboim, 1977). A series of phenolic acids were added as the inhibition compounds, and the BSA-GLU system was held at 37°C for 7 days. Quercetin was used as a positive control. A total of 100 µL of sample was mixed with 50 µL Girard-T solution (500 mM) and 850 µL of sodium formate (500 mM, pH 2.9) for 1 h at room temperature. A Genesys 10vis spectrophotometer (Thermo Scientific Inc., Waltham, MA, USA) was used to measure the absorbance at 290 nm. The inhibitory activity of dicarbonyl compounds was calculated by the following equation.

$$\text{Activity (\%)} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

The $Abs_{control}$ is the absorbance value of the control group in the absence of inhibition compounds. The IC_{50} value represented the level of an individual compound required to decrease dicarbonyl compounds by 50%.

Constructing BSA-glyoxal system

BSA (2 μg/μL) and glyoxal (5 mM) were mixed with 0.1 g/L NaN_3 in 100 mM phosphate buffer (pH 7.4) at 37°C in the presence or absence of selected phenolic acids (1 mM). After incubating for 7 days, the samples were stored at -20°C prior to CML determination.

CML analysis

CML of the sample was extracted as described previously (Drusch et al., 1999). Prior to analysis, the samples was reduced with sodium borate buffer (0.2 M, pH 9.4) and sodium borohydride (1 M in 0.1 M NaOH), and was hydrolyzed by 6 N HCl for 20 h at 110°C (Chen, & Smith, 2015). The CML extract was concentrated by rotary evaporation, and dissolved in sodium borate buffer (0.2 M, pH 9.4), followed by membrane filtration (nylon, 0.45 mL). The OPA derivatization reagent was mixed with the samples for 5 min prior to HPLC analysis. The isolated CML were measured using an HP1050 Series II HPLC (Agilent Technologies, Santa Clara, CA, USA) coupled with a HP 1046A fluorescence detector programmed to excitation/emission wavelengths of 340 nm and 455 nm. Separations were achieved on a reversed-phase TSK gel ODS-80 TM column (25 cm x 4.6 mm, 5 μm, 80 Å, Tosohass, Montgomeryville, PA, USA). The mobile phase consisted of solvent A: acetate buffer (pH 6.7, 20 mM)/acetonitrile (90:10, v/v) and solvent B: acetonitrile. The gradient elution was performed as follows: start 5% B, change to 70% B within 5 min, then keep at 70% B for 17 min. The gradient was set back to 95% B in 1 min followed by a post run of 15 min for equilibration. The flow rate was 1.0 mL/min, and the

injection volume was 20 µL. CML was quantified by comparing retention times of samples and standards in the fluorescence spectra; levels were determined by the peak areas of their corresponding derivatives.

Statistical analyses

Data were subjected to analyses of variance (ANOVA). When significant differences were found, statistical presence was determined at a *P* value of 0.05. Data reported as means ± standard deviations for triplicate determinations, and all analyses were conducted using SAS version 9.1 (SAS Inst., Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Method validation

The limit of detection of CML was 0.007 µg/mL, and the limit of quantification was calculated to be 0.025 µg/mL. The linearity of the standard calibration curve ranged from 0.025 to 0.50 µg/mL, and the calculated correlation coefficient indicated a good linearity ($R^2 > 0.99$). Recovery experiments were conducted by spiking the samples with 0.25, 0.6, and 1.2 ng/µL of CML (n=3), for which the average recovery rates were 79.81, 80.52, and 88.87%. The recovery and linearity of CML in some previous reports compared favorably with our study (Sun, Tang, Wang, Rasco, Lai, & Huang, 2015; Zhang, Huang, Xiao, & Mitchell, 2011).

Degree of glycation

Glycation occurs in the model system because of the covalent reaction of the carbonyl group in glucose with the amino groups in proteins. Degree of glycation is often used to determine the degree of reaction by measuring the free amines with TNBS (Nissen, 1979).

Figure 11 shows the time dependency of the glycation process; degree of glycation increased from 4.17, 10.05, 18.15, and 33.10 to 35.39% during incubation. Although degree of glycation increased rapidly in the first five days, it did not change significantly ($P > 0.05$) later in incubation (days 5 to 7), which was consistent with the study of protein isolate-sugar system reported by Bu et al. (2015). Our results indicated that BSA and glucose reacted sufficiently during the last stage of incubation.

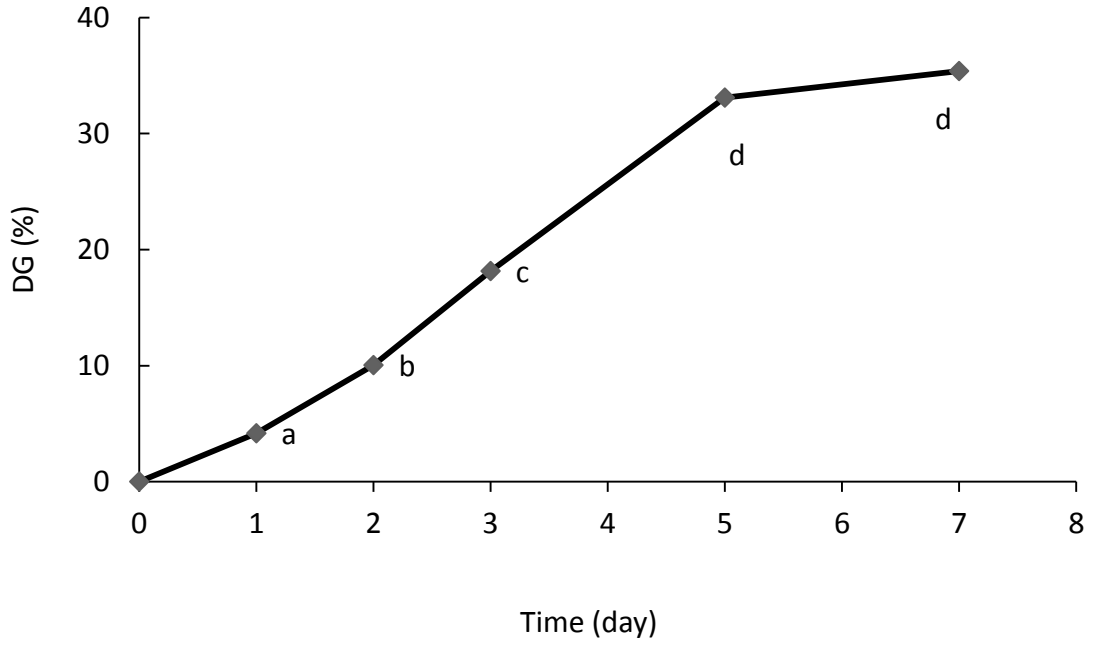


Figure 11. The degree of glycation in BSA with glucose after incubation.

[abcd]Values of degree of glycation without the same letter are significantly different ($P < 0.05$). The values shown are the mean ± SD (n=3).

Mitigating CML in BSA-GLU system

We first measured the inhibitory effect of cereal bran extracts on CML formation in the BSA-GLU system. Our results showed that incubating BSA with glucose in phosphate buffer (pH 7.4, 37°C) leads to CML formation; Table 19 shows the measured concentration in the system, and it exhibits no significant differences ($P > 0.05$) in average CML concentration between the control system (4.61 ng/10 µL) and any of the treatment groups of added RY (4.13 ng/10 µL), JA (3.98 ng/10 µL), and TH (5.52 ng/10 µL). However, we did find significant differences ($P < 0.05$) between the control and ST group (2.21 ng/10 µL), which suggests that ST bran extract can inhibit CML formation under assay conditions.

Table 19. Effect of brans extracts and phenolic acids on CML formation in BSA-GLU system.

Treatment	CML (ng/10 µL)	Treatment	CML (ng/10 µL)
Control	4.61±0.84^{a}	Control	4.61±0.84^{a}
Rye (RY)	4.13±0.87^{ac}	Gentisic acid	2.47±0.23^{b}
Thundercale (TH)	5.52±2.05^{a}	Ferulic acid	4.52±0.41^{ac}
Spring Triticale (ST)	2.21±0.27^{bc}	Vanillic acid	4.81±1.80^{a}
Jagger (JA)	3.98±1.01^{ac}	Chlorogenic acid	2.86±0.53^{bc}

^{abcd}Means with different superscripts within the same column are significantly different at $P < 0.05$.
Value is represented as mean ± standard deviation (n=3).

Phenolic acids in ST bran extract

The phenolic acids in ST bran extract may contribute to the inhibitory effect on CML formation. Figure 12 shows typical HPLC chromatograms of phenolic acids in ST bran extract. FA was the most common phenolic acid (82.98 ±8.55 µg/g), as can be seen in Table 20, with VA,

GEA, and CHA at lower concentrations which is in agreement with previously published research (Hosseinian, & Mazza, 2009; Mattila, Pihlava, & Hellstrom, 2005).

Table 20. The phenolic acids composition of ST extract and their scavenging effect on DPPH radicals expressed as $IC_{50.}$

Phenolic Acid	**Concentration (µg/g)**	**IC_{50} (µM)**
Gentisic acid	3.43±0.44[a]	9.34±1.82[a]
Ferulic acid	82.98±8.55[b]	42.68±1.51[b]
Chlorogenic acid	3.16±0.54[a]	16.10±0.84[c]
Vanillic acid	17.30±2.08[c]	ND[d]

[abc]Means with different superscripts within the same column are significantly different at $P < 0.05$.
[d]ND represents that the IC_{50} is not-detectable because the free radical scavenging activity was too low (the % inhibition was only 4.2% at 1 mM).
Value is represented as mean ± standard deviation (n=3).

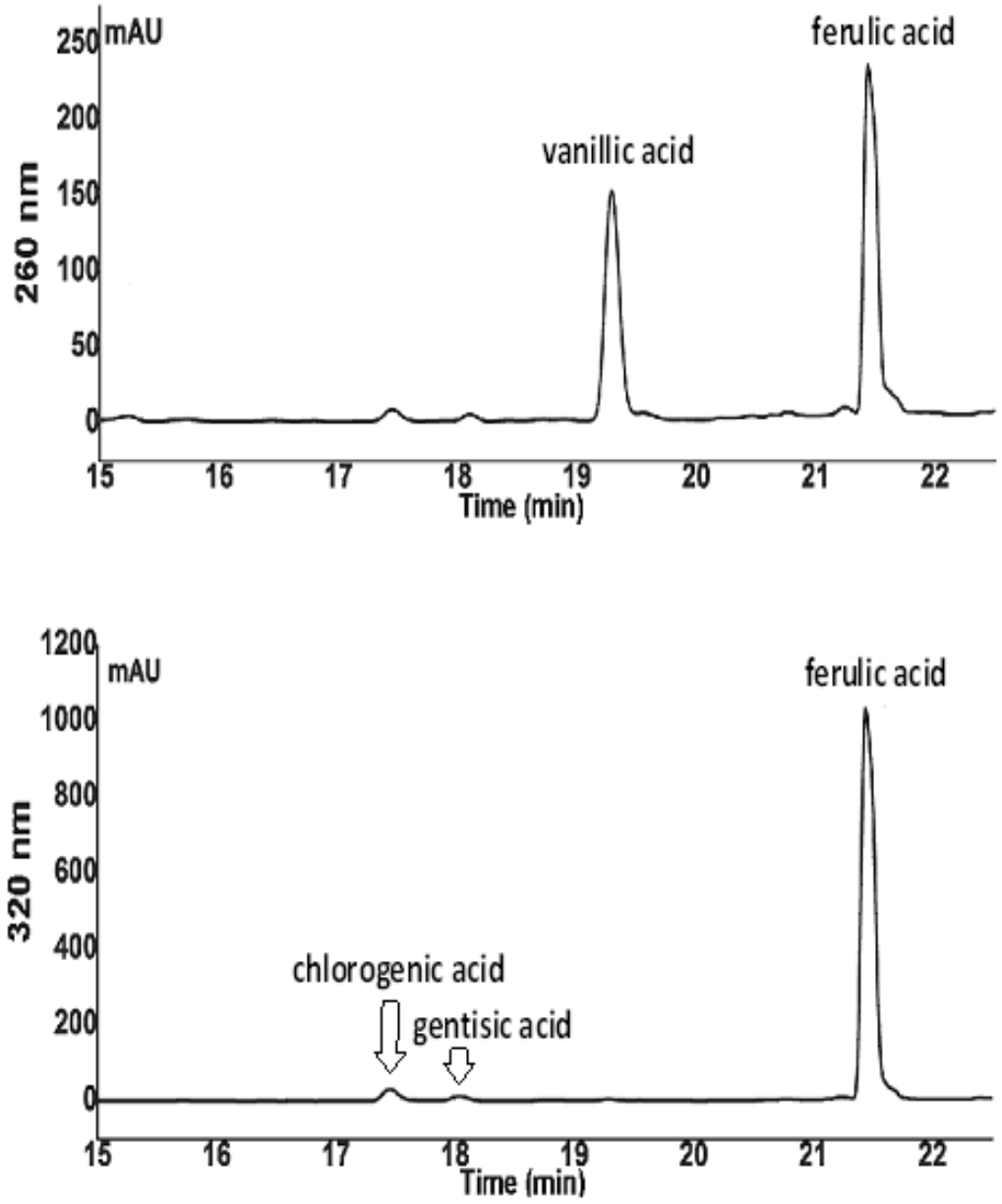


Figure 12. HPLC chromatographs of Spring Triticale bran extract using a TSK gel ODS-80 TM column (25 cm×4.6 mm, 5 µm) with ultraviolet detection at different wavelengths of 260 nm and 320 nm. The flow rate was 1.0 mL/min and injection volume was 15 µL. The mobile phases were 1% acetic acid (solvent A) and acetonitrile (solvent B).

Inhibiting CML formation by phenolic acids

The phenolic acids in brans have distinct antioxidant activities (Naczk, & Shahidi, 2006; Verma et al., 2009), but few have reported on their antiglycation activities. How they inhibited the formation of CML was first evaluated in the BSA-GLU model. Table 19 shows that among those compounds, CHA (37.9% inhibition) and GEA (46.4% inhibition) significantly influenced CML formation ($P < 0.05$). However, the control and the FA (4.52 ng/10 µL) and VA (4.81 ng/10 µL) treatment groups showed no significant differences ($P > 0.05$), which does not fully agree with previous reports (Gugliucci, Bastos, Schulze, & Souza, 2009; Mesías, Navarro, Martinez-Saez, Ullate, del Castillo, & Morales, 2014; Weerachat, Aramsri, Henrique & Sirichai, 2013; Yoo et al., 2010). This may be due to different reaction system conditions and detection methods. Gugliucci et al. (2009) indicated CHA was a major antiglycation compound in BSA-methylglyoxal system. And CHA was responsible for antiglycative properties of the in BSA-methylglyoxal and BSA-GLU model systems (Mesías et al., 2014). However, Weerachat et al. (2013) pointed out FA could slow the protein glycation process. In their glucose-glycated BSA systems, FA (1-5 mM) reduced the level of fluorescent AGEs by 12.61-36.49%, and CML by 33.61-66.51% using ELISA method. Moreover, VA also showed potent inhibitory activity on AGEs formation with an IC_{50} of 93.93 µM by testing the level of total fluorescen AGEs in a BSA model system containing added glucose and fructose solution (Yoo et al., 2010).

Antioxidant activity of phenolic acids

The mitigation effects of phenolic acids may be due to antioxidant activities, scavenging free radicals generated as AGEs form. Our results, because the DPPH assay was used to

measure the anti-radical effect of phenolic acids, showed decreases from GEA, CHA, FA, and VA, in that order (Table 20). The IC_{50} value indicates GEA as the lowest (9.34 μM), followed by CHA (16.10 μM), FA (42.68 μM), all of which are significantly different from each other ($P < 0.05$).

The diverse substituents on the aromatic ring of the phenolic acid are shown in Figure 13. Our study showed CHA and GEA, each with two hydroxyl groups, had higher radical scavenging activity than FA and VA with only one hydroxyl group each, which does agree with previous studies (Chen, & Ho, 1997; Pekkarinen, Stockmann, Schwarz, Heinonen, & Hopia, 1999). Brand-Williams, Cuvelier, and Berset (1995) noted that hydroxycinnamic acid derivatives were more active than hydroxybenzoic acid among the monohydroxyl phenolic acids. Our results agreed in that FA was more active than VA. The capacity of phenolic acids to trap radicals may be attributed to the reaction that forms resonance-stabilized phenoxyl radicals (D'Andrea, 2010; Sakihama, Cohen, Grace, & Yamasaki, 2002). For instance, compared to the carboxylic group of VA, the conjugated double bond of FA could stabilize radicals more through resonance. In addition, CHA and GEA exhibited more DPPH scavenging activity and inhibited CML formation in our study (Table 20). Therefore, radical scavenge activity may influence the antiglycation capacity of phenolic acids.

Antiglycation activity of phenolic acids

Not only free radicals, but also reactive intermediates like fructosamine or the dicarbonyl compounds, contribute to AGE formation during the Maillard reaction. Fructosamine is an Amadori product with a ketoamine structure, which could reduce the NBT reagent (Baker et al., 1994). Table 21 shows the inhibitory effect of phenolic acids on the fructosamine adduct. It seems that neither GEA nor CHA could inhibit fructosamine formation at the selected levels

of 0.01, 0.1, and 1 mM. Vlassopoulos, Lean, and Combet (2014) found otherwise in their study, indicating the presence of six phenolic acids during incubation could significantly reduce fructosamine formation in the BSA-GLU system. This difference in results may be due to different structures of the phenolic compounds and/or the model system conditions.

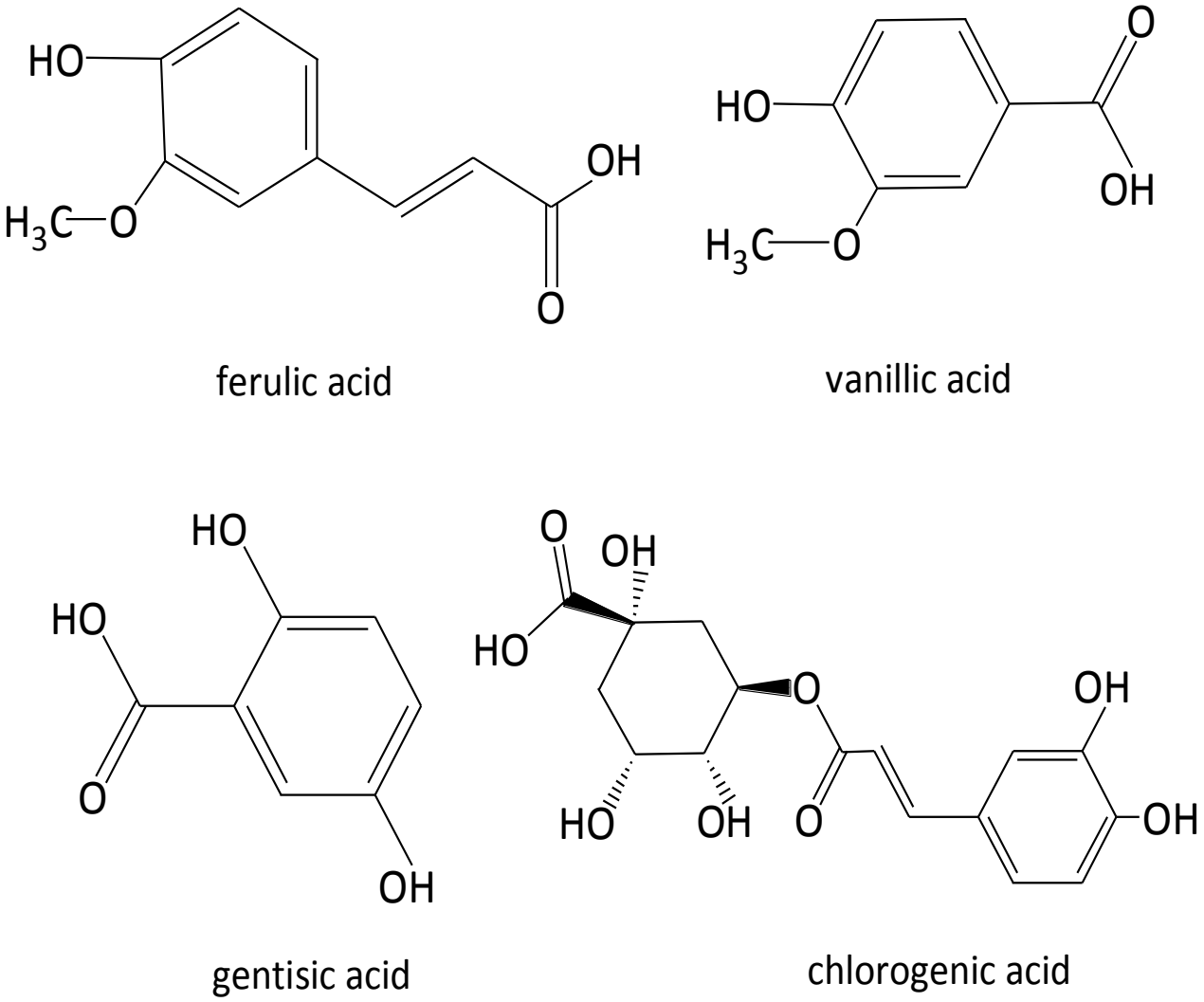


Figure 13. Chemical structures of phenolic acids in the Spring Triticale bran extracts**.**

Mitchel et al. (1977) found a reaction easily occurring between the Girard-T reagent and aldehyde or ketone compounds with dicarbonyl groups. We did observe GEA and CHA inhibited formation of dicarbonyl compounds (Figure 14). Moreover, GEA (IC_{50} = 13.7 μM) had more inhibitory capacity than CHA (IC_{50} = 17.3 μM) ($P < 0.05$). Wu, Hsieh, Wang, and Chen (2009) stated that some phenolic acids could prevent formation of dicarbonyl compounds in a glucose-mediated protein modification system and found that gallic acid had the strongest activity.

Table 21. Inhibition effect of phenolic acid on fructosamine adduct (%).

Compounds	Concentration			IC_{50}
	0.01 mM	0.1 mM	1 mM	
Chlorogenic acid (CHA)	0.58±1.85	1.53±1.48	2.39±1.89	ND[a]
Gentisic acid (GEA)	0.35±1.41	1.75±0.78	2.57±0.95	ND[a]
Quercetin	-	-	-	197.09±10.41[b]

[a]ND not-detectable.
[b]IC_{50} is the amount of quercetin (nM) to decrease the fructosamine by 50%.
All the results are expressed as mean values ± standard deviation (n=3).

Mitigating CML in BSA-glyoxal system

To further evaluate the inhibitory mechanism of phenolic acids, we measured their influence on CML formation in the BSA-glyoxal system. As illustrated in Figure 14, the two phenolic acids of CHA and GEA both inhibited CML formation with an average percentage decrease of 29.6% for CHA and 51.1% for GEA.

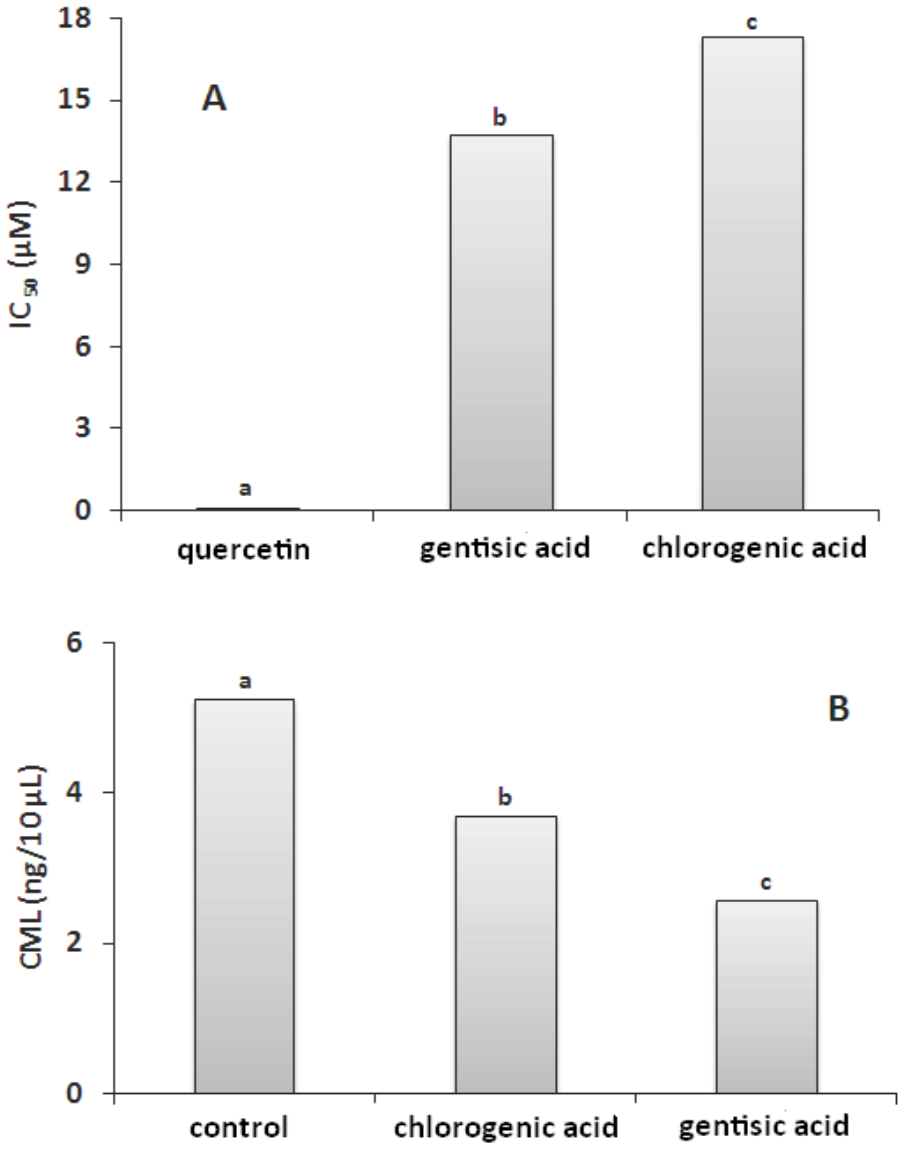


Figure 14. Inhibition effect on (A) dicarbonyl compounds formation in BSA-GLU model; and (B) CML formation in BSA-glyoxal model.

[abc]Means with the different letters on the bars are significantly different ($P < 0.05$).

The bars represented the mean ± SD (n=3).

To our knowledge, oxidation of Amadori products like fructosamine, and the sugar-glyoxal pathway, leads to CML formation in the Maillard reaction (Ahmed, 2005). In our study, the GEA and CHA scavenged reactive dicarbonyl compounds and inhibited, to some extent, CML formation in the BSA-glyoxal system (Figure 14), whereas neither exhibited inhibition activity on the fructosamine adduct (Table 21). From our results, we could infer that phenolic acids mitigated CML formation by inhibiting the glyoxal pathway, not by oxidizing Amadori products.

CONCLUSION

Cereal brans have long been recommended to consumers because of their high fiber and natural antioxidant content. This study investigated the effect of bran extract on AGE formation in model systems. Among the bran extracts examined, ST inhibited CML formation the most, which could be due to the phenolic acids in it, CHA and GEA, both of which are effective scavengers of free radicals and even, to some extent, antiglycation inhibitors. It also suggested that phenolic acids mitigated CML formation by inhibiting the glyoxal pathway in our systems. Thus, ST bran, which is rich in phenolic acids, could be a potentially useful food addictive for inhibiting AGEs.

In the present study, the Inhibitory effects on the CML formation by bran extract was demonstrated *in vitro*, but it cannot be assured the effect also could occur in *vivo*. Therefore, that should be taken into account for future research.

REFERENCES

Ahmed, N. (2005). Advanced glycation endproducts-role in pathology of diabetic complications. *Diabetes Research and Clinical Practice*, *67*(1), 3-21.

Baker, J. R., Zyzak, D. V., Thorpe, S. R., & Baynes, J. W. (1994). Chemistry of the fructosamine assay: D-glucosone is the product of oxidation of Amadori compounds. *Clinical Chemistry*, *40*, 1950–1955.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft and Technologie*, *28*(1), 25-30.

Bu, G. H., Zhu, T. W., Chen, F. S., Zhang, N., Liu, K. L., Zhang, L. F., & Yang, H. (2015). Effects of saccharide on the structure and antigenicity of β-conglycinin in soybean protein isolate by glycation. *European Food Research and Technology*, *240*, 285–293.

Chen, G., & Smith, J. S. (2015). Determination of advanced glycation endproducts in cooked meat products. *Food Chemistry*, *168*, 190-195.

Chen, J., & Ho, C. (1997). Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *Journal of Agricultural and Food Chemistry*, *45*(7), 2374-2378.

D'Andrea, G. (2010). Pycnogenol: A blend of procyanidins with multifaceted therapeutic applications? *Fitoterapia*, *81*(7), 724-736.

Dickinson P. J., Carrington, A. L., Frost, G. S., & Boulton A. J. (2002). Neurovascular disease, antioxidants and glycation in diabetes. *Diabetes/Metabolism Research and Reviews, 18*(4), 260-272.

Drusch, S., Faist, V., & Erbersdobler, H. (1999). Determination of N[epsilon]-carboxymethyllysine in milk products by a modified reversed-phase HPLC method. *Food Chemistry*, *65*(4), 547-553.

Farrar, J. L., Hartle, D. K., Hargrove, J. L., & Greenspan, P. (2008). A novel nutraceutical property of select sorghum (Sorghum bicolor) brans: Inhibition of protein glycation. *Phytotherapy Research*, *22*(8), 1053-1058.

Gugliucci, A., Bastos, D. H., Schulze, J., & Souza, M. F. (2009). Caffeic and chlorogenic acids in Ilex paraguariensis extracts are the main inhibitors of AGE generation by methylglyoxal in model proteins. *Fitoterapia*, *80*(6), 339-344.

Halliwell B. (1995). Antioxidant characterization: Methodology and mechanism. *Biochemical Pharmacology*, *49*(10), 1341–1348.

Hosseinian, F. S., & Mazza, G. (2009). Triticale bran and straw: Potential new sources of phenolic acids, proanthocyanidins, and lignans. *Journal of Functional Foods*, *1*(1), 57-64.

Krygier, K., Sosulsk, F., & Hogge, L. (1982). Free, esterified, and insoluble-bound phenolic acids. 1. Extraction and purification procedure. *Journal of Agricultural and Food Chemistry*, *30*, 330-334.

Liu, R. H. (2007). Whole grain phytochemicals and health. *Journal of Cereal Science*, *46*, 207.

Lo, C., Hsiao W., & Chen, X. (2011). Efficiency of trapping methylglyoxal by phenols and phenolic acids. *Journal of Food Science*. *76*(3), 90-96.

Mattila, P., Pihlava, J. M., & Hellstrom, J. (2005). Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. *Journal of Agricultural and Food Chemistry*, *53*(21), 8290-8295.

Mesias, M., Navarro, M., Martinez-Saez, N., Ullate, M., del Castillo, M. D. & Morales, F. J. (2013). Antiglycative and carbonyl trapping properties of the water soluble fraction of coffee silverskin. *Food Research International*. *62*, 1120-1126.

Mitchel, R. E. J., & Birnboim, H. C. (1977). The use of Girard-T reagent in a rapid and sensitive method for measuring glyoxal and certain other a-dicarbonyl compounds. *Analytical Biochemistry*, *81*, 47–56.

Naczk, M., & Shahidi. F. (2006). Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis, 41*(5), 1523-1542.

Nagaraj, R. H., & Sady, C. (1996). The presence of a glucose-derived Maillard reaction product in the human lens. *FEBS Letters*, *382*, 234–238.

Nissen J. N. (1979). Determination of the digress of hydrolysis of food protein hydrolysates by trinitribenzenesulfonic acid. *Journal of Agricultural and Food Chemistry*, *27*, 1256–1263.

Pekkarinen, S. S., Stockmann, H., Schwarz, K., Heinonen, I. M., & Hopia, A. I. (1999). Antioxidant activity and partitioning of phenolic acids in bulk and emulsified methyl linoleate. *Journal of Agricultural and Food Chemistry*, *47*(8), 3036-3043.

Poulsen, M.W., Hedegaard, R. V., Andersen, J. M., de Courten, B., Bügel, S., Nielsen, J., Skibsted, L. H., & Dragsted, L. O. (2013). Advanced glycation endproducts in food and their effects on health. *Food and Chemical Toxicology*, *60*, 10-37.

Robbins, R. (2003). Phenolic acids in foods: An overview of analytical methodology. *Journal of Agricultural and Food Chemistry*, *51*(10), 2866-2887.

Rojas, A., & Morales, M. A. (2004). Advanced glycation and endothelial functions: A link towards vascular complications in diabetes. *Life Science*, *76*(7), 715-730.

Sakihama, Y., Cohen, M. F., Grace, S. C., & Yamasaki, H. (2002). Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. *Toxicology*, *177* (1), 67-80.

Schmitt, A., Gasic-Milenkovic, J., & Schmitt, J. (2005). Characterization of advanced glycation end products: Mass changes in correlation to side chain modifications. *Analytical Biochemistry*, *346*(1), 101-106.

Sell, D. R. & Monnier, V. M. (1989). Structure elucidation of a senescence cross-link from human extracellular matrix. Implication of pentoses in the aging process. *Journal of Biological Chemistry*, *264*, 21597–21602.

Sell, D. R., Nagaraj, R. H., Grandhee, S. K., Odetti, P., Lapolla, A., Fogarty, J. and Monnier, V. M. (1991). Pentosidine: a molecular marker for the cumulative damage to proteins in diabetes, aging, and uremia. *Diabetes/Metabolism Research and Reviews*, *7*, 239–251.

Shimoi, K., Okitsu, A., Green, M. H., Lowe, J. E., Ohta, T., Kaji, K., Terato, H., Ide, H., & Kinae, N. (2001). Oxidative DNA damage induced by high glucose and its suppression in human umbilical vein endothelial cells. *Mutation Research*, *480*, 371-378.

Singh, R., Barden, A., Mori, T., & Beilin, L. (2001). Advanced glycation end-products: A review. *Diabetologia*, *44*, 129–146.

Singh, R. P., Chidambara-Murthy, K. N., & Jayaprakasha, G. K. (2002). Studies on the antioxidant activity of pomegranate (Punica granatum) and seed extracts using *in vitro* models. *Journal of Agricultural and Food Chemistry*, *50*(1), 81-86.

Sun, X., Tang, J., Wang, J., Rasco, B. A., Lai, K., & Huang, Y. (2015). Formation of advanced glycation endproducts in ground beef under pasteurisation conditions. *Food Chemistry*, *172*, 802-807.

Szawara-Nowak, R., Koutsidis, G ., Wiczkowski, W., & Zielinski, H., (2014). Evaluation of the *in vitro* inhibitory effects of buckwheatenhanced wheat bread extracts on the formation of advanced glycation end-products (AGEs*). LWT - Food Science and Technology*, *58*(2), 327-334.

Turk, Z., Ljubic, S., Turk, N., & Benko, B. (2001). Detection of autoantibodies against advanced glycation endproducts and AGE-immune complexes in serum of patients with diabetes mellitus. *Clinica Chimica Acta*, *303*, 105-115.

Verma, B., Hucl, P., & Chibbar, R. N. (2009). Phenolic acid composition and antioxidant capacity of acid and alkali hydrolysed wheat bran fractions. *Food Chemistry*, *116*(4), 947-954.

Vlassara, H. (1996). Advanced glycation end-products and atherosclerosis. *Annals of Medicine*, *28*, 419–426.

Vlassopoulos, A., Lean, M. E. J., & Combet, E. (2014). Protein-phenolic interactions and inhibition of glycation - combining a systematic review and experimental models for enhanced physiological relevance. *Food & Function*, *5*(10), 2646-2655.

Weerachat S., Aramsri M., Henrique C., & Sirichai A. (2013). A comparative study of ferulic acid on different monosaccharide-mediated protein glycation and oxidative damage in bovine serum albumin. *Molecules. 18*, 13886-13903.

Wen, Y., Skidmore, J. C., Porter-Turner, M. M., Rea, C. A., Khokher, M. A., & Singh, B. M. (2002). Relationship of glycation, antioxidant status and oxidative stress to vascular endothelial damage in diabetes. *Diabetes, Obesity and Metabolism*, *4*, 305-308.

Wu, J., Hsieh, C., Wang, H., & Chen, H. (2009). Inhibitory effects of guava (Psidium guajava L.) leaf extracts and its active compounds on the glycation process of protein *Food Chemistry*, *113*, 78-84.

Wu, C., Huang, S., Lin, J., & Yen, G. (2011). Inhibition of advanced glycation end product formation by food stuffs. *Food & Function*, *2*, 224-234.

Yoo, N. H., Jang, D. S., Lee, Y. M., Jeong, I. H., Cho, J. H., Kim, J. H., & Kim, J. S. (2010). Anthraquinones from the roots of knoxia valerianoides inhibit the formation of advanced glycation end products and rat lens aldose reductase *in vitro*. *Archives of Pharmacal Research. 33*(2), 209-214.

Zhang, G., Huang G., Xiao L., & Mitchell, A., E. (2011). Determination of advanced glycation endproducts by LC-MS/MS in raw and roasted almonds (Prunus dulcis). *Journal of Agricultural and Food Chemistry, 59*(22), 12037-12046.

SUMMARY

AGEs are a large and heterogeneous group of compounds caused by the reaction between reducing sugars and amino groups in amino acids. Dietary AGEs, a potent factor of chronic diseases such as diabetes, are formed during the cooking of meat and fish, and their levels were monitored by CML levels. The chemical composition of meat, cooking conditions and internal temperature can influence CML levels in cooked meat and fish samples. High levels of CML were found in beef, pork, and chicken prepared by frying and broiling methods. The CML content in cooked fish was less when the appropriate cooking methods were selected like baking at a lower temperature.

Because of some evidence of the risk of chronic disease, it is necessary to investigate how to prevent CML formation at cooking. Cereal brans have been seen as dietary foods due to their high fiber and natural antioxidant concentration. Addition of cereal brans (Spring Triticale and Rye) in beef patty inhibited the formation of CML, which may be a healthier choice for meat consumers. The CML percent inhibition had a significant correlation to WHC of samples and DPPH scavenging activity of brans.

The study to investigate the inhibitory mechanisms of cereal bran extract on AGEs formation was conducted in the model systems. ST extract had the highest inhibition activity on CML formation, and the phenolic acids in it, CHA and GEA, exhibited as the effective antioxidants and antiglycation compounds. Moreover, it was found that the mitigation mechanism of phenolic acids on CML formation was by inhibiting the glyoxal pathway in our systems. Therefore, ST bran, was rich in phenolic acids, which could be considered as a potentially useful AGE inhibitor as a food addictive.

Our research data can give researchers information for estimating dietary AGEs exposure in the general population of the U.S. Furthermore, these data can provide a reference by the food industry to modify the process conditions to reduce formation of dietary AGES.

Overall, more research is needed to better understand the role of dietary AGEs in health at the molecular and biological level. The standard methods for measuring the specific dietary AGEs are necessary. Future research should be conducted on optimizing food additives to mitigate dietary AGEs while maintaining food safety and flavor.

APPENDIX

LIST OF FIGURES

LIST OF TABLES

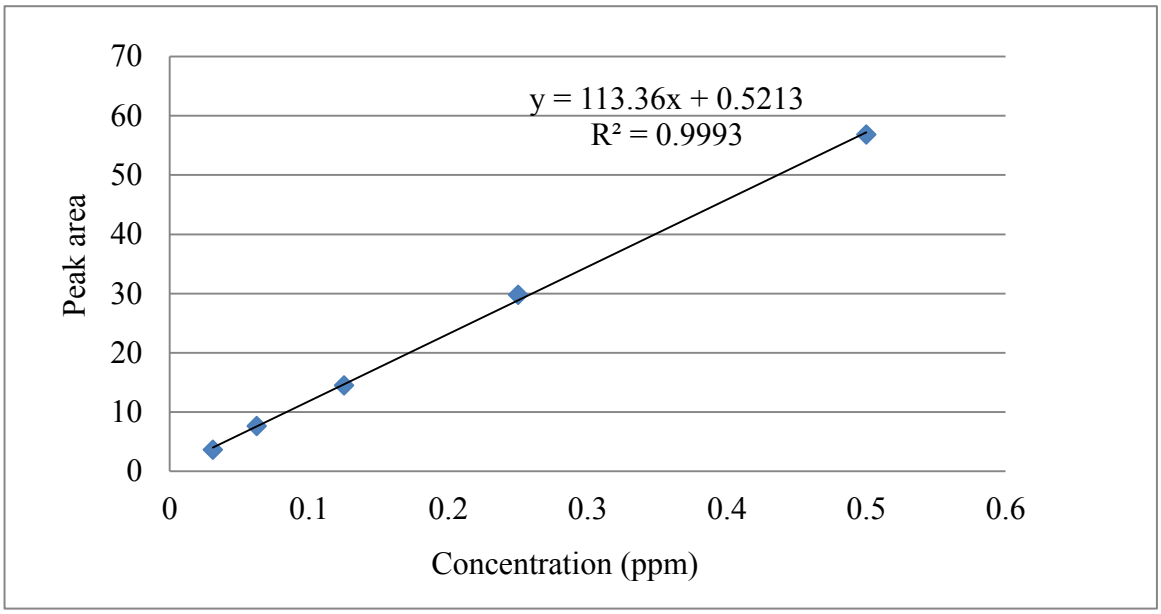


Figure A-1. Standard curve of CML by fluorescence detection settings of 340 nm (excitation) and 455 nm (emission).

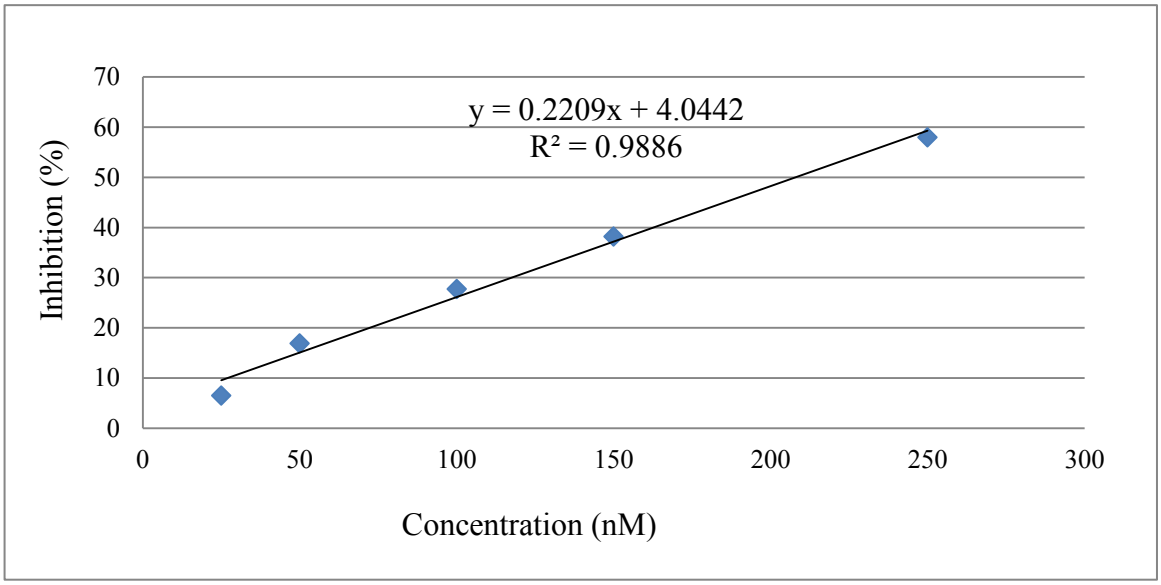


Figure A-2. Standard curve of inhibition activity of quercetin on fructosamine adduct for IC_{50} determination.

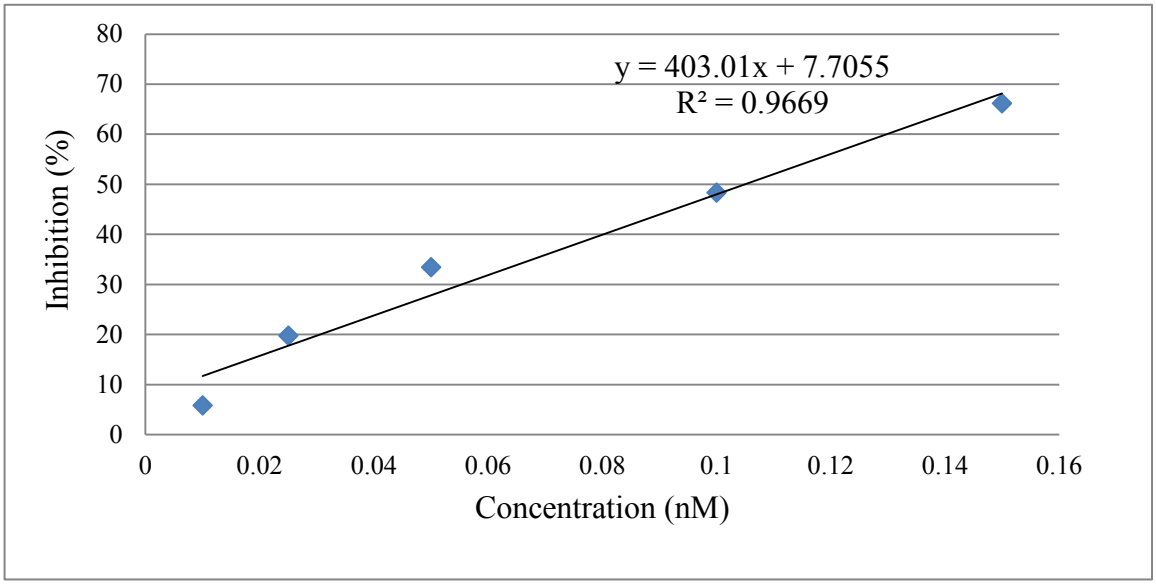


Figure A-3. Standard curve of inhibition activity of quercetin on dicarbonyl compounds for IC_{50} determination.

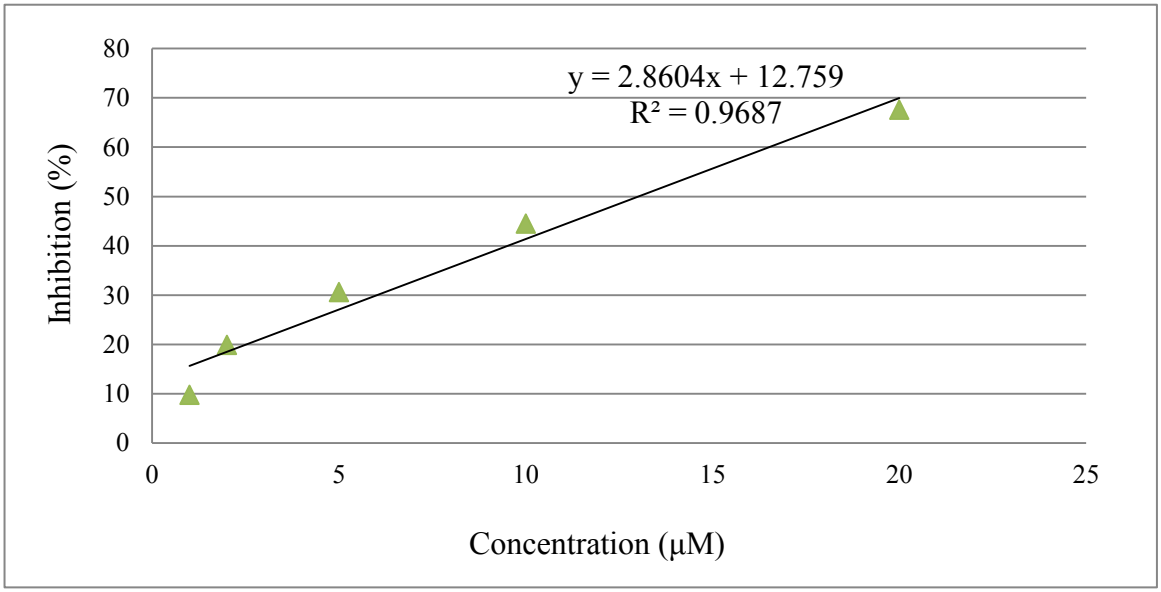


Figure A-4. Standard curve of inhibition activity of gentisic acid on dicarbonyl compounds for IC_{50} determination.

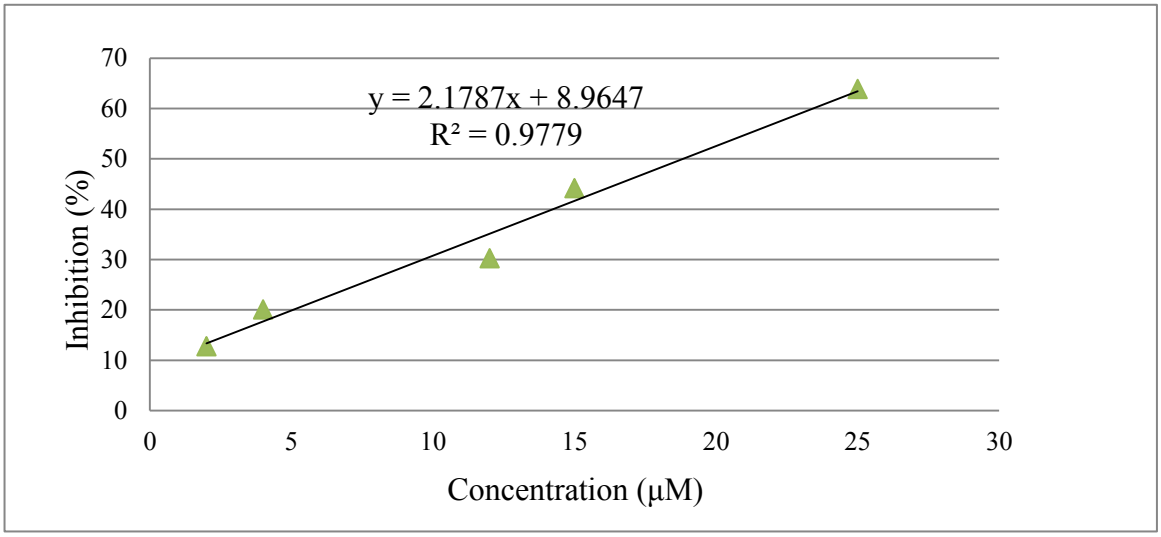


Figure A-5. Standard curve of inhibition activity of chlorogenic acid on dicarbonyl compounds for IC_{50} determination.

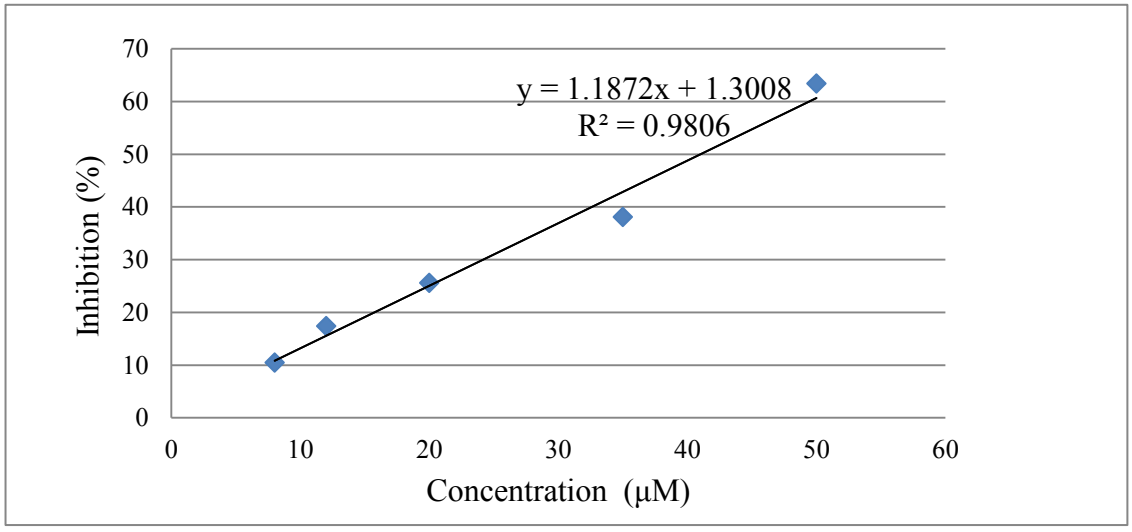


Figure A-6. Standard curve of inhibition activity of feruilic acid on scavenging activity on DPPH radicals for IC_{50} determination.

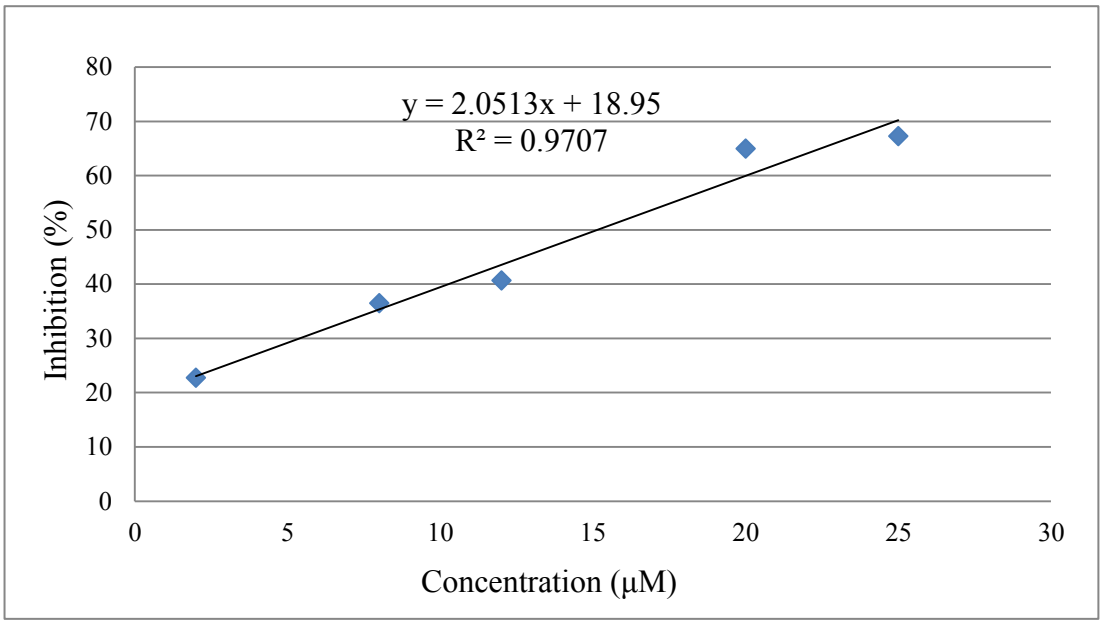


Figure A-7. Standard curve of inhibition activity of gentisic acid on scavenging activity on DPPH radicals for IC_{50} determination.

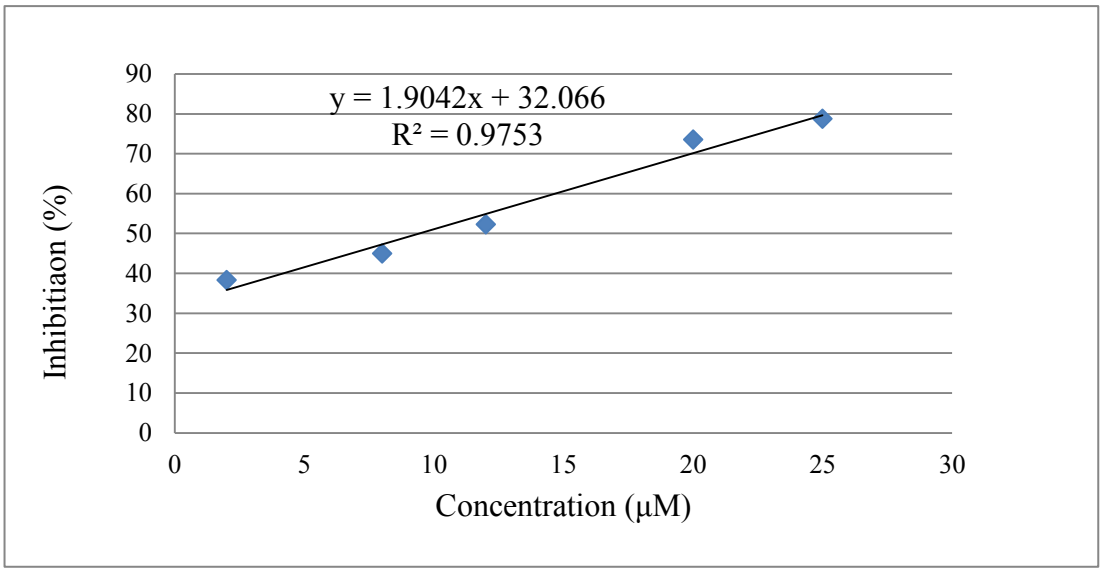
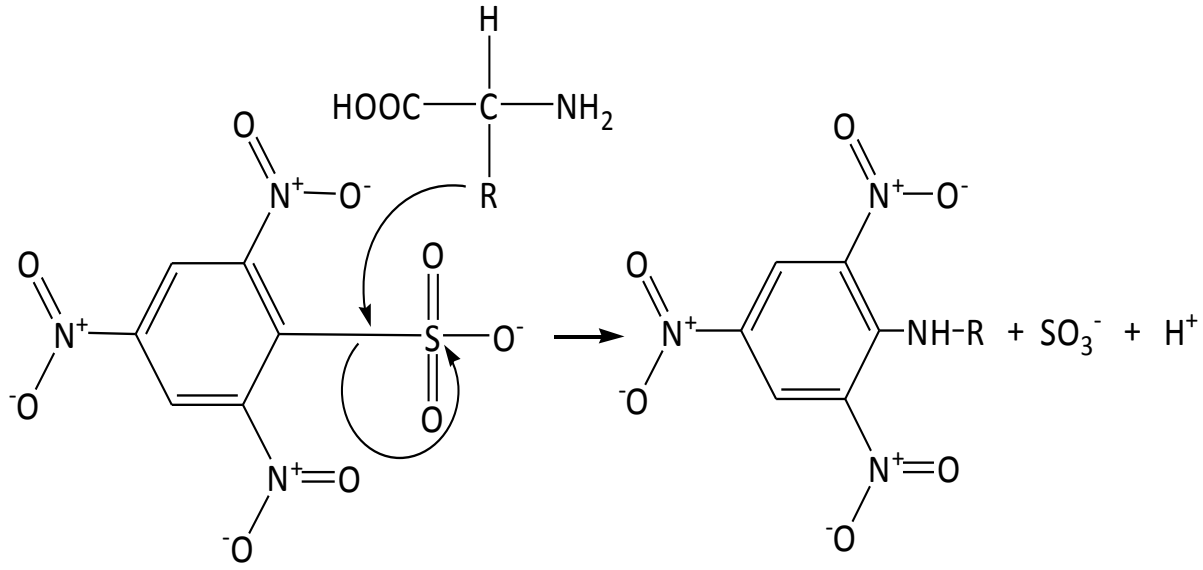
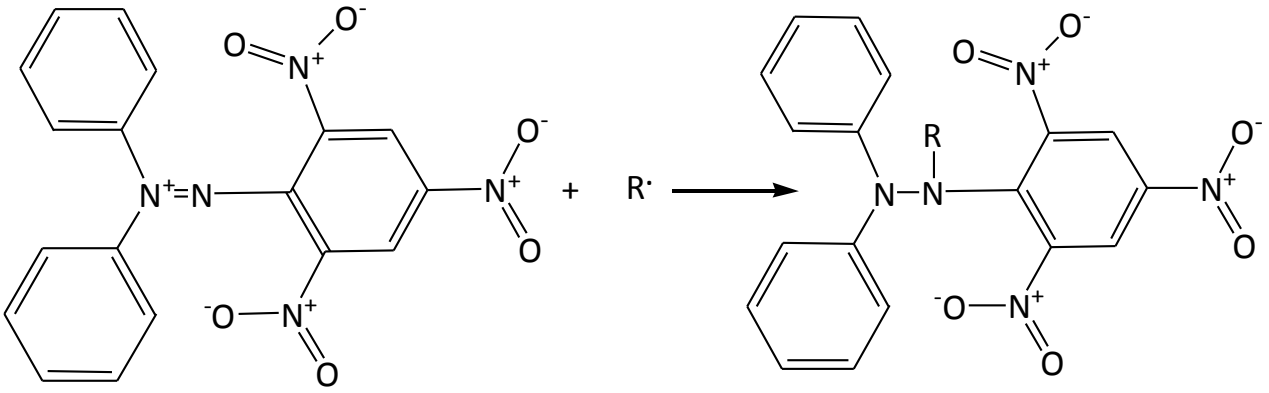


Figure A-8. Standard curve of inhibition activity of chlorogenic acid on scavenging activity on DPPH radicals for IC_{50} determination.



2,4,6-trinitrobenzene sulfonate

Figure A-9. Degree of glycation was determined by a TNBS method.



2,2-diphenyl-1-picrylhydrazyl

Figure A-10. The reaction between reactive radical and DPPH reagent.

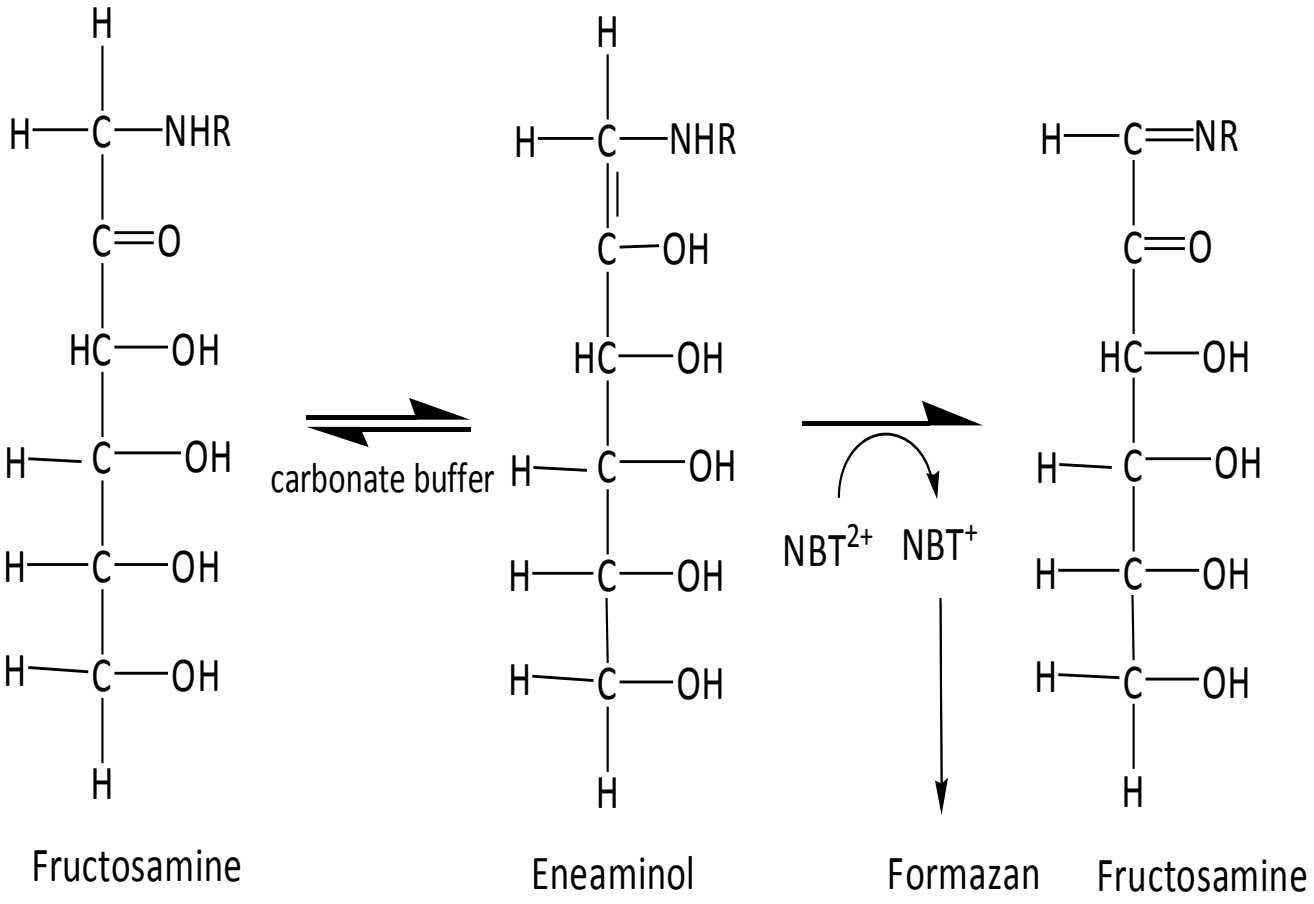


Figure A-11. Fructosamine adduct is identified using an NBT reagent method.

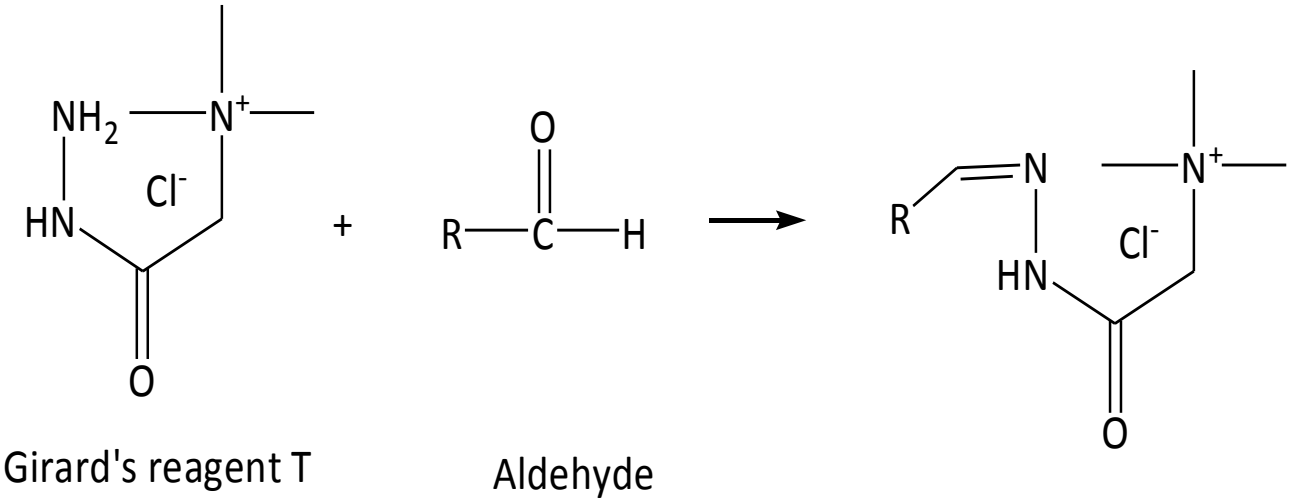
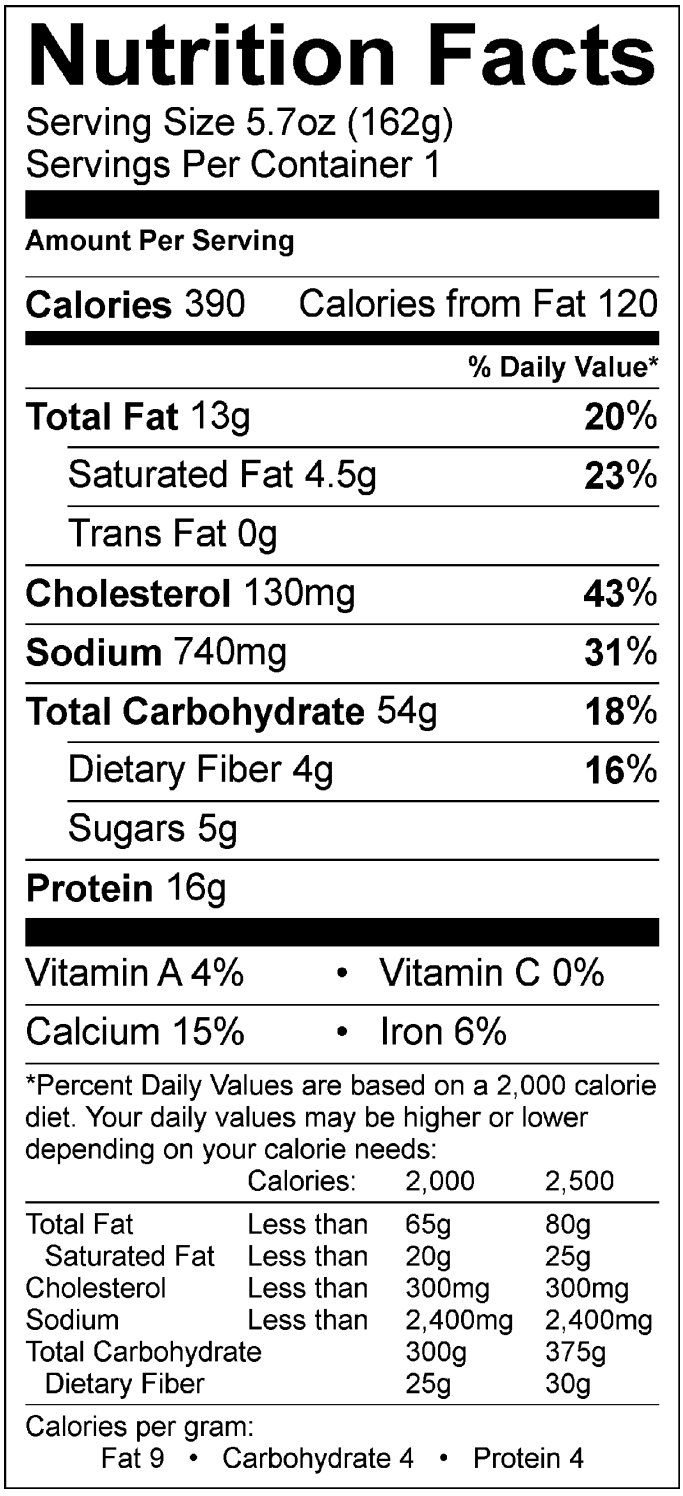


Figure A-12. The reaction between aldehyde and Girard's reagent T.



Nutrition Facts

Serving Size 5.7oz (162g)
Servings Per Container 1

Amount Per Serving

Calories 390	Calories from Fat 120
	% Daily Value*
Total Fat 13g	**20%**
Saturated Fat 4.5g	**23%**
Trans Fat 0g	
Cholesterol 130mg	**43%**
Sodium 740mg	**31%**
Total Carbohydrate 54g	**18%**
Dietary Fiber 4g	**16%**
Sugars 5g	
Protein 16g	

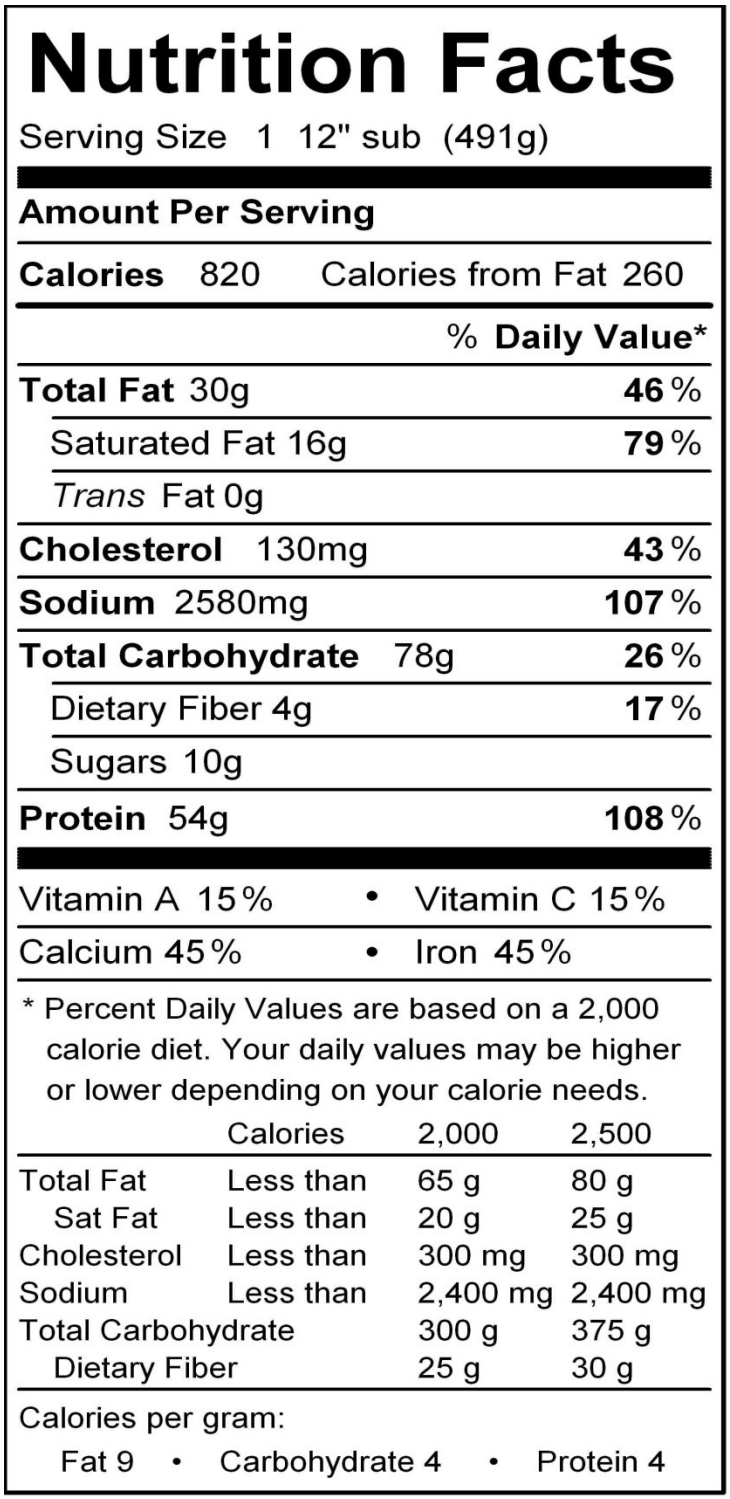
Vitamin A 4%	• Vitamin C 0%
Calcium 15%	• Iron 6%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Figure A-13. Nutritional information of breakfast honey wheat bagel. Available at: http://prgmichigan.com/2020-breakfast.php.



Nutrition Facts

Serving Size 1 12" sub (491g)

Amount Per Serving

Calories 820	Calories from Fat 260
	% **Daily Value***
Total Fat 30g	**46** %
Saturated Fat 16g	**79** %
Trans Fat 0g	
Cholesterol 130mg	**43** %
Sodium 2580mg	**107** %
Total Carbohydrate 78g	**26** %
Dietary Fiber 4g	**17** %
Sugars 10g	
Protein 54g	**108** %

Vitamin A 15% • Vitamin C 15%

Calcium 45% • Iron 45%

* Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

	Calories	2,000	2,500
Total Fat	Less than	65 g	80 g
Sat Fat	Less than	20 g	25 g
Cholesterol	Less than	300 mg	300 mg
Sodium	Less than	2,400 mg	2,400 mg
Total Carbohydrate		300 g	375 g
Dietary Fiber		25 g	30 g

Calories per gram:

Fat 9 • Carbohydrate 4 • Protein 4

Figure A-14. Nutritional information of 12" sub on roasted beef sandwich (wheat with American cheese, lettuce & tomatoes). Available at: http://www.pcpizza.com/items/roast-beef.

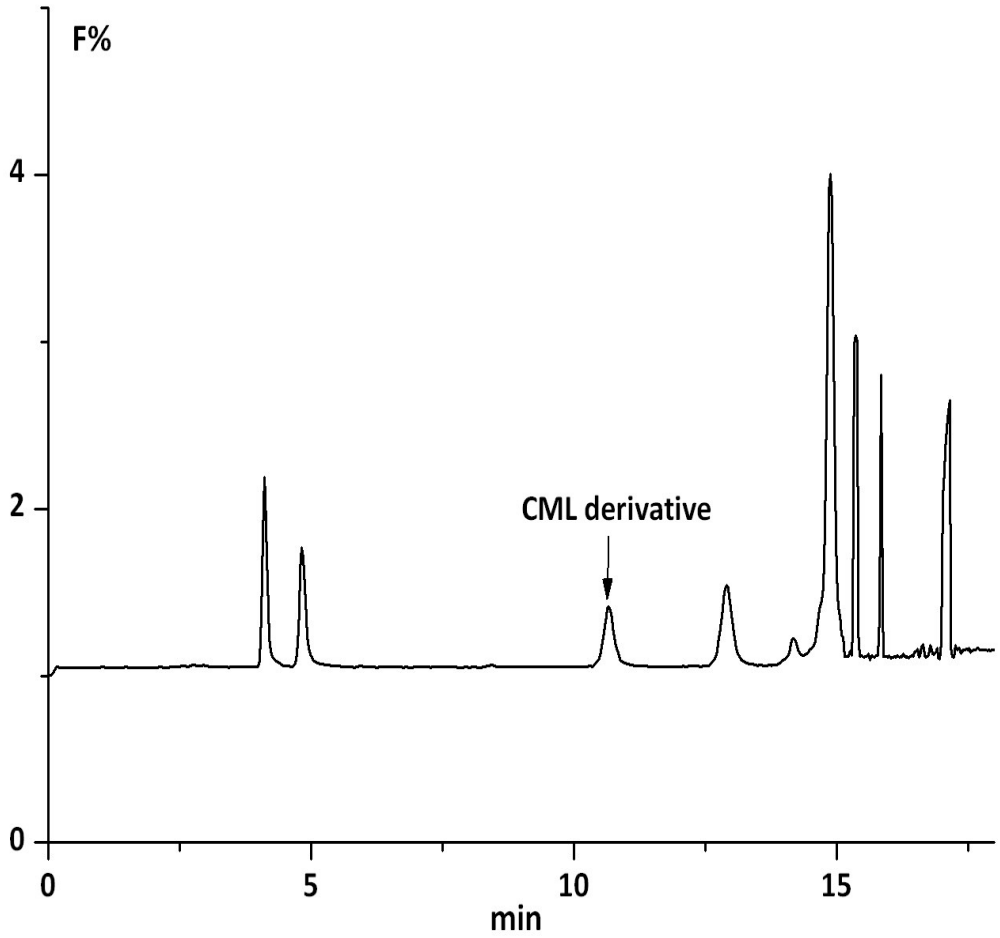


Figure A-15. HPLC chromatograph of CML derivative peaks related to the CML standard (0.025 µg/mL) using a TSK gel ODS-80 TM column (25 cm×4.6 mm, 5 µm) with a fluorescence intensity of settings at 340 nm (excitation) and 455 nm (emission). The flow rate was 1.0 mL/min and injection volume was 20 µL. The mobile phases were: (solvent A) sodium acetate buffer (20 mM, adjusted to pH 6.7 with acetic acid)/acetonitrile (90:10, v/v) and (solvent B) acetonitrile.

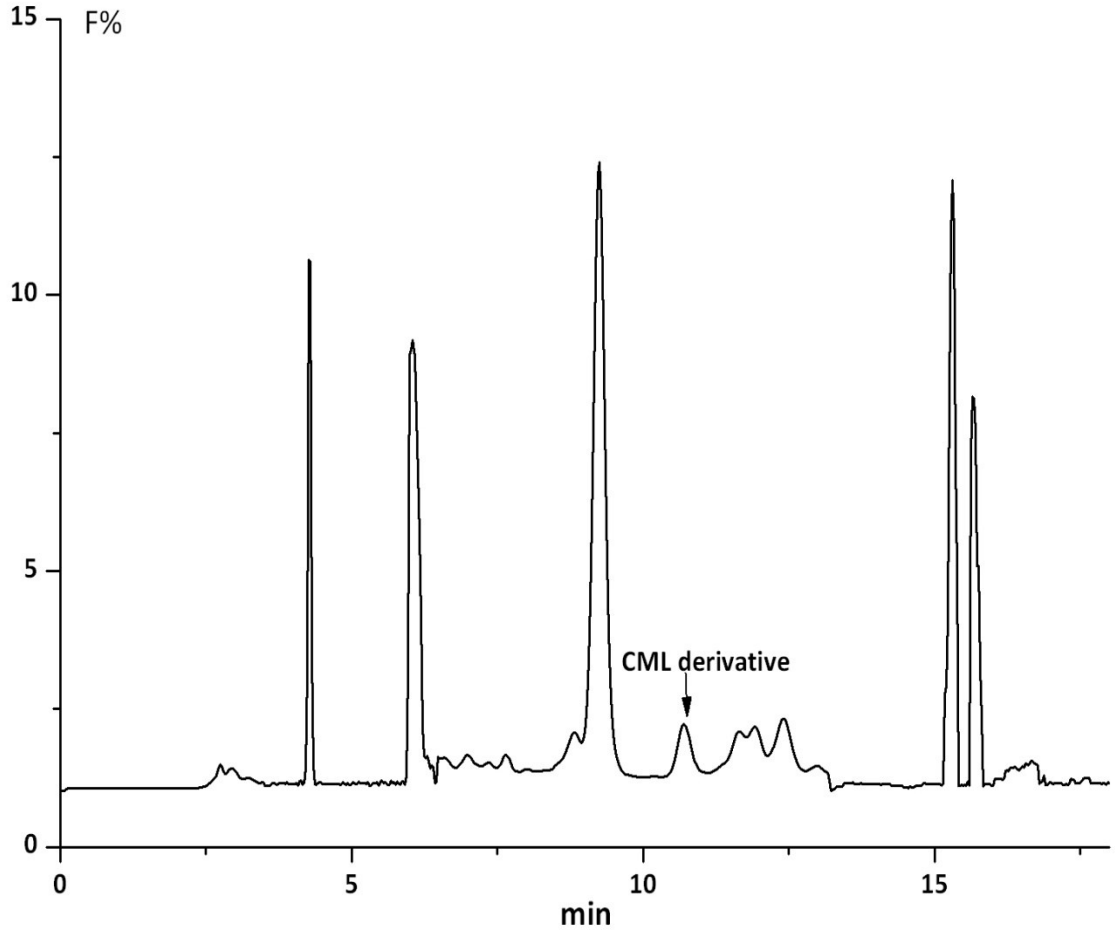


Figure A-16. HPLC chromatograph of CML derivative peaks related to a baked salmon sample using a TSK gel ODS-80 TM column (25 cm×4.6 mm, 5 µm) with a fluorescence intensity of settings at 340 nm (excitation) and 455 nm (emission). The flow rate was 1.0 mL/min and injection volume was 20 µL. The mobile phases were: (solvent A) sodium acetate buffer (20 mM, adjusted to pH 6.7 with acetic acid)/acetonitrile (90:10, v/v) and (solvent B) acetonitrile.

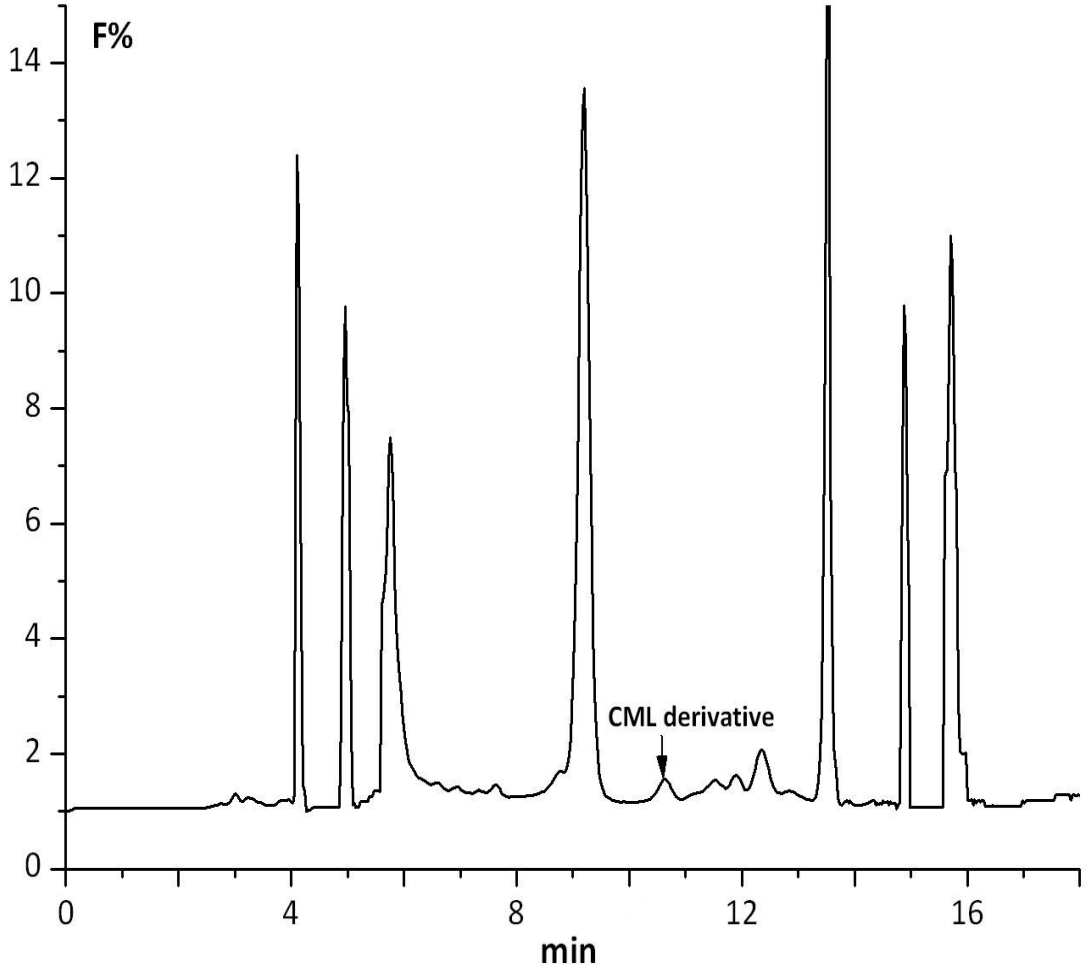


Figure A-17. HPLC chromatograph of CML derivative peaks related to a fried chicken sample (inside) using a TSK gel ODS-80 TM column (25 cm×4.6 mm, 5 µm) with a fluorescence intensity of settings at 340 nm (excitation) and 455 nm (emission). The flow rate was 1.0 mL/min and injection volume was 20 µL. The mobile phases were: (solvent A) sodium acetate buffer (20 mM, adjusted to pH 6.7 with acetic acid)/acetonitrile (90:10, v/v) and (solvent B) acetonitrile.

Table A-1. Basic information of dietary fiber.

Definition of dietary fiber	**Function of dietary fiber**	**Average daily intake (AI) recommendation**
Non-digestible form of carbo-hydrates and lignin. Naturally occurs in plants, helps provide a feeling of fullness. Best sources are whole grains, brans, beans and peas, additional sources of dietary fiber include nut, and other vegetables, and fruits.	May help reduce the risk of cardiovascular disease, obesity, and Type 2 diabetes. May promote healthy lipid profiles and glucose tolerance, and ensure normal gastrointestinal function.	The AI for fiber is 14 g per 1,000 calories, or 25 g per day for women and 38 g per day for men; Most Americans underconsume dietary fiber, and usual intake averages only 15 g per day. Children and adults should consume foods naturally high in dietary fiber in order to increase nutrient density.

[a]Modified by source: U.S. Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. 2009. Available at: http://www.ars.usda.gov/ba/bhnrc/ndl.

Table A-2. Selected food sources ranked by amounts of dietary fiber per standard food portion.

Food	Standard Portion Size	Dietary Fiber in Portion (g)[a]
Beans (navy, pinto, black, kidney, white, great northern, lima), cooked	½ cup	6.2–9.6
Bran ready-to-eat cereal (100%)	1/3 cup (about 1 ounce)	9.1
Split peas, lentils, chickpeas, or cowpeas, cooked	½ cup	5.6–8.1
Artichoke, cooked	½ cup hearts	7.2
Pear	1 medium	5.5
Soybeans, mature, cooked	½ cup	5.2
Plain rye wafer crackers	2 wafers	5.0
Bran ready-to-eat cereals (various)	1/3–¾ cup (about 1 ounce)	2.6–5.0
Asian pear	1 small	4.4
Green peas, cooked	½ cup	3.5–4.4
Whole-wheat English muffin	1 muffin	4.4
Bulgur, cooked	½ cup	4.1
Mixed vegetables, cooked	½ cup	4.0
Raspberries	½ cup	4.0
Sweet potato, baked in skin	1 medium	3.8
Blackberries	½ cup	3.8
Soybeans, green, cooked	½ cup	3.8
Prunes, stewed	½ cup	3.8
Shredded wheat ready-to-eat cereal	½ cup (about 1 ounce)	2.7–3.8
Figs, dried	¼ cup	3.7
Apple, with skin	1 small	3.6
Pumpkin, canned	½ cup	3.6

Greens (spinach, collards, turnip greens), cooked	½ cup	2.5–3.5
Almonds	1 ounce	3.5
Sauerkraut, canned	½ cup	3.4
Whole wheat spaghetti, cooked	½ cup	3.1
Banana	1 medium	3.1
Orange	1 medium	3.1
Guava	1 fruit	3.0
Potato, baked, with skin	1 small	3.0
Oat bran muffin	1 small	3.0
Pearled barley, cooked	½ cup	3.0
Dates	¼ cup	2.9
Winter squash, cooked	½ cup	2.9
Parsnips, cooked	½ cup	2.8
Tomato paste	¼ cup	2.7
Broccoli, cooked	½ cup	2.6–2.8
Okra, cooked from frozen	½ cup	2.6

[a]Modified by source: U.S. Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. 2009. Available at: http://www.ars.usda.gov/ba/bhnrc/ndl.

Table A-3. Cereal bran food recipes**.**

Food	Ingredients	How to make it	Source
Bran Beef Burger	500g lean beef mince 60g All-Bran Original 40g reduced fat mozzarella cheese 4 tablespoons spicy tomato chutney or tomato sauce Large handful rocket leaves 4 small wholegrain rolls	Combine the beef and All-Bran Original. Form into 4 patties and refrigerate for 30 – 45 minutes. Heat a heavy based fry pan over medium heat. Sprinkle the patties with salt and cook on one side for 4 minutes. Turn and place ¼ of the cheese on top of each patty. Cover and cook for 4 minutes. Lightly toast the rolls. Place the bases of the rolls on serving plates and top with the patty, sauce or tomato chutney, rocket leaves and tomato. Cover with the top of the roll.	https://www.allbran.co.uk/recipes/fibre-health/lunch/beef-burger-recipe.html
Meaty Meat Balls	1 ½ lb lean ground beef ¾ cup oat bran cereal, uncooked whole eggs coarse salt	Mix thoroughly; Form meatballs approx 2 inches, and place on ungreased baking sheet; Sprinkle with coarse salt. Bake at 350°F until nicely browned. Add to the chunky sauce OR place on top of finished spaghetti or other pasta dish. Sprinkle grated old cheddar on top.	http://grouprecipes.com/77936/elaines-meaty-meat-balls.html

Chili	1 pound lean ground beef 1 large onion, sliced 1/2 cup chopped green bell pepper 1 cup Kellogg's All-Bran Original cereal	In large saucepan, cook ground beef, onion and green pepper until meat is browned, stirring frequently. Stir in remaining ingredients, cutting tomatoes into pieces with spoon. Cover. Cook over low heat about 1 hour. Stir occasionally. Remove bay leaf before serving.	http://www.all-bran.com/recipes/chili.html
Turkey Bran Burger	2/3 cup Kellogg's All-Bran Original cereal 1/2 cup finely chopped onions and 1 egg white 2tablespoon worceste-rshire sauce 1 teaspoon garlic salt 1 pound ground turkey	Combine KELLOGG'S All-Bran cereal, onions, Worcestershire sauce, garlic salt and egg white. Let stand about 3 minutes or until cereal softens. Gently stir in ground turkey. Portion into six and shape into 4-inch patties. Grill, broil or pan fry about 4 minutes on each side or until browned and meat is no longer pink (160 degrees F).	https://www.kelloggs.com/en_US/recipes/turkey-bran-burgers-recipe.html

Table A-4. Stepwise selection process by SAS software in Chapter 3.

```
NOTE: SAS initialization used:
      real time          10.89 seconds
      cpu time           1.01 seconds

1     data AGE;
2     input inhibition TPC DPPH WHC;
3     datalines;

NOTE: SAS went to a new line when INPUT statement reached past the end of a line.
NOTE: DATA statement used (Total process time):
      real time          0.65 seconds
      cpu time           0.01 seconds


19    proc reg;
20    model inhibition=TPC DPPH WHC / collin
21              collinoint;
22
23    run;


NOTE: PROCEDURE REG used (Total process time):
      real time          1:20.89
      cpu time           2.81 seconds


24    data AGE2;
25    input inhibition TPC DPPH WHC;
26    datalines;

NOTE: SAS went to a new line when INPUT statement reached past the end of a line.
NOTE: DATA statement used (Total process time):
      real time          0.01 seconds
      cpu time           0.01 seconds

42    proc reg;
43    model inhibition=TPC DPPH WHC /
44    selection= stepwise
45    sle=0.30 sls=0.05 stb;
46    run;
```