Glycative Stress Research

Online edition: ISSN 2188-3610
Print edition: ISSN 2188-3602
Received:October 29, 2019
Accepted: November 20, 2019
Published online: December 31, 2019
doi:10.24659/gsr.6.4_212

Review article

Glycative stress and anti-aging: 14. Regulation of Glycative stress. 2. Inhibition of the AGE production and accumulation

Masayuki Yagi, Yoshikazu Yonei

Anti-Aging Medical Research Center and Glycative Stress Research Center,
Faculty of Life and Medical Sciences, Doshisha University, Kyoto, Japan

Abstract

The lifestyle and dietary habits aimed at inhibiting glycative stress are known as anti-glycation. Specific measures for anti-glycation include the inhibition of postprandial glucose, inhibition of glycative reaction, and degradation and excretion of AGEs which are produced. Reducing sugars such as glucose, are mainly involved in the *in vivo* non-enzymatic reaction with amino acids or proteins and accumulate as advanced glycation end-products (AGEs) via glycated proteins. Since AGEs are also produced during the cooking of food by heating, the intake of large quantities of food cooked at high temperatures may lead to an increase in the *in vivo* accumulation of AGEs.

AGEs released in the blood due to protein metabolism and AGEs absorbed from the digestive tract following digestion after intake of food cooked by heating are excreted in the urine by the kidneys. Hence, if renal function declines, the accumulation of *in vivo* AGEs increases. Glycative stress and oxidative stress promotes protein alteration. The *in vivo* functions of altered proteins are reduced affecting cells and tissues. Usually, altered proteins are degraded by proteolytic enzymes. However, AGEs derived from proteins stiffen due to protein cross-linking and are difficult to degrade.

The inhibition action on the glycative reaction of natural products has been reported for many materials. These actions are presumed to be mainly due to polyphenols contained in plants. The *in vivo* production of AGEs has multiple bypasses and branching pathways, and glycative and oxidation reactions are complexly intertwined. For this reason, it is necessary to simultaneously inhibit multi-pathways using multiple components for obtaining a useful *in vivo* inhibition action on glycative reaction.

N-phenacylthiazolium bromide (PTB) is known as a substance with a degradation action on AGE-derived crosslinks of proteins. Plant ingredients with the same effect as PTB have been reported in many materials. The degradation action of natural products on AGE-derived crosslinks may contribute to the degradation and excretion of *in vivo* AGEs that have already accumulated.

Oxidized protein hydrolase (OPH) is a kind of serine protease and is widely present in living tissues. OPH acts on the degradation of N-terminal amino acids of proteins and aging proteins that have been modified by oxidation and glycation. Some herbs and health tea extracts also promote OPH activity. The increase in OPH activity through natural products may contribute to the promotion of degradation and excretion of *in vivo* AGEs. The inhibition action on the glycative reaction and AGE degradation and excretion action of natural products have also been verified in human clinical studies.

***KEY WORDS*:** inhibition of glycative reaction, AGE-derived crosslinks, oxidized protein hydrolase

1. Preface: Measures to Prevent Glycative Stress

The lifestyle and dietary habits aimed at inhibiting glycative reaction are known as anti-glycation[1)], and that include the inhibition of postprandial glucose, inhibition of glycative reaction, and degradation and excretion of AGEs, which are produced. In this paper, the mechanism of *in vivo* production, degradation and excretion of AGEs, and the possibility of contribution of materials inhibiting glycative reaction and materials degrading and excreting AGEs for anti-glycation will be explained.

Contact Address: Professor Masayuki Yagi, PhD
Anti-Aging Medical Research Center and Glycative Stress Research Center,
Faculty of Life and Medical Sciences, Doshisha University
1-3 TataraMiyakodani, Kyotanabe, Kyoto, 610-0394 Japan
Phone/Fax: +81-774-65-6394 E-mail: myagi@mail.doshisha.ac.jp
Co-author: Yonei Y, yyonei@mail.doshisha.ac.jp

2. In vivo Glycative Reaction Pathway

Reducing sugars such as glucose, are mainly involved in the *in vivo* non-enzymatic reactions with amino acids or proteins to form the Amadori product, which is a glycated protein, due to Amadori rearrangement through the formation of a Schiff base, which in turn creates an irreversible substance. Through the production of intermediates that mainly include carbonyl products such as 3-deoxyglucosone (3DG), glyoxal, methylglyoxal, glyceraldehyde and glutaraldehyde, the Amadori products result in advanced glycation end-products (AGEs). In a narrow sense, glycative reaction refers to these series of reaction processes.

In addition to glucose in the blood, AGEs are also produced through carbonylation between proteins, and aldehydes and ketones produced by alcohol metabolism and lipid oxidation. Therefore, there are many types of AGEs substances with different production pathways [2].

AGEs include fluorescent and non-fluorescent substances. Pentosidine, crossline, pyrropyridine, *etc*. are fluorescent AGEs. N^{ε}-(carboxymethyl) lysine (CML), N^{ω}-(carboxymethyl) arginine (CMA), *etc*. are non-fluorescent AGEs. Also, pentosidine, crossline, *etc*. possess protein cross-linking properties. AGEs derived from proteins lead to a decrease in elasticity and flexibility of tissues. AGEs bind to the receptor RAGE (Receptor for AGEs) to activate intracellular signals and induce the production of inflammatory cytokines. For this reason, the *in vivo* production and accumulation of AGEs impairs various cells and tissues. AGEs are also produced when food is cooked by heating. Therefore, intake of large quantities of food cooked at high temperatures may lead to an increase in the *in vivo* accumulation of AGEs due to the digestion and absorption of food [3]. The concept that comprehensively captures biological stress caused by reducing sugar or aldehyde load and the subsequent reaction to it is called glycative stress.

3. In vivo Degradation and Excretion of AGEs

AGEs released in the blood due to protein metabolism and AGEs absorbed from the digestive tract following digestion after intake of food cooked by heating are excreted in the urine by the kidneys. The proximal tubule of the kidney has a membrane receptor called megalin that reabsorbs low molecular weight proteins filtered from urine. AGEs in the blood bind to the megalin in the kidneys and are then taken into the renal tubular cells by endocytosis, which is the action of cells taking in extracellular substances [4]. However, when large amounts of AGEs are taken in by megalin, lysosome, which is one of the intracellular organelles, is saturated with AGEs due to degradation, leading to the accumulation of AGEs in the renal tubular cells [5, 6]. Hence, renal function declines, and accumulation of *in vivo* AGEs increases.

Protein alteration plays a vital role in the decline of functions of the nervous, immune and endocrine systems, as well as cell and tissue functions associated with aging. The body removes altered proteins that are always produced inside the cells by repeated synthesis and degradation of proteins and reuses the amino acids produced by the degradation to maintain functions. However, in older animals, the protein synthesis ability of cells decreases with aging, and metabolic turnover slows down. Furthermore, degradation of protein becomes difficult due to the formation of AGEs with aging. Glycation stress and oxidative stress promotes protein alteration. The *in vivo* functions of altered proteins are reduced affecting cells and tissues [7]. β-Amyloid, which accumulates in the brains of patients with Alzheimer's disease, is a type of altered protein that damages nerve cells and causes neural functions such as memory and learning functions to decline. Also, crystalline in the lens of the eye is denatured with aging, aggregates and becomes turbid (protein alteration), causing cataracts. Furthermore, neurodegenerative diseases such as Parkinson's disease are caused by the accumulation of altered proteins with changed structure [8].

Usually, altered proteins are degraded by a proteolytic enzyme called proteasome. Proteasomes are of 2 types: 26S (molecular weight 2.5 million) and 20S (molecular weight 700,000). Many altered proteins are degraded by 26S proteasome after undergoing ubiquitination. Oxidized proteins are also degraded by 20S proteasome (***Fig. 2***) [8]. On the other hand, AGEs derived from proteins stiffen due to cross-linking of proteins, which makes them less susceptible to degradation with protease [9].

4. In vitro Inhibition Action on Glycative Reaction by Natural Products

One of the measures to prevent glycative stress is the inhibition of glycative reaction. The inhibition of glycative reaction by natural products is evaluated by calculating IC_{50} (50% inhibitory concentration) of the test substance from the amount of glycative reaction intermediates and various AGEs produced in the reaction solution obtained by adding extracts of multiple materials to the reaction system of various proteins and glucose such as human serum albumin (HSA) and collagen [10].

Aminoguanidine [11], which is a glycation inhibitor, is widely used as a positive control for the inhibition of glycative reaction. Anti-glycation activity has been reported in many materials such as mixed herbs [12], purple chrysanthemum [13], striped bamboo [14], herbal tea [15], fruits [16], vegetables [17], spices [18] and black galangal [19] using this evaluation system.

The inhibition of glycative reaction is presumed to be mainly due to polyphenols in plants. Substances that have an inhibition action on glycative reaction include phenolic acids such as cinnamic acid and benzoic acid analog and flavonoids (***Fig. 1***), isoflavones and procyanidins [20].

The distribution of polyphenols contained in plants is closely related to evolution and classification and is one of the indicators for chemotaxonomy. Plants belonging to the same taxonomic family or tribe are assumed to contain polyphenols with a similar structure [21]. On the other hand, the production of *in vivo* AGEs has multiple bypasses and branching pathways, and glycative and oxidation reactions are complexly intertwined. For this reason, it is necessary to simultaneously inhibit multi-pathways using multiple components for obtaining a useful *in vivo* inhibition action on glycative reaction. For achieving effective inhibition of glycative reaction, it is essential to consider the selection of plant materials and taxonomic knowledge of plants in combination.

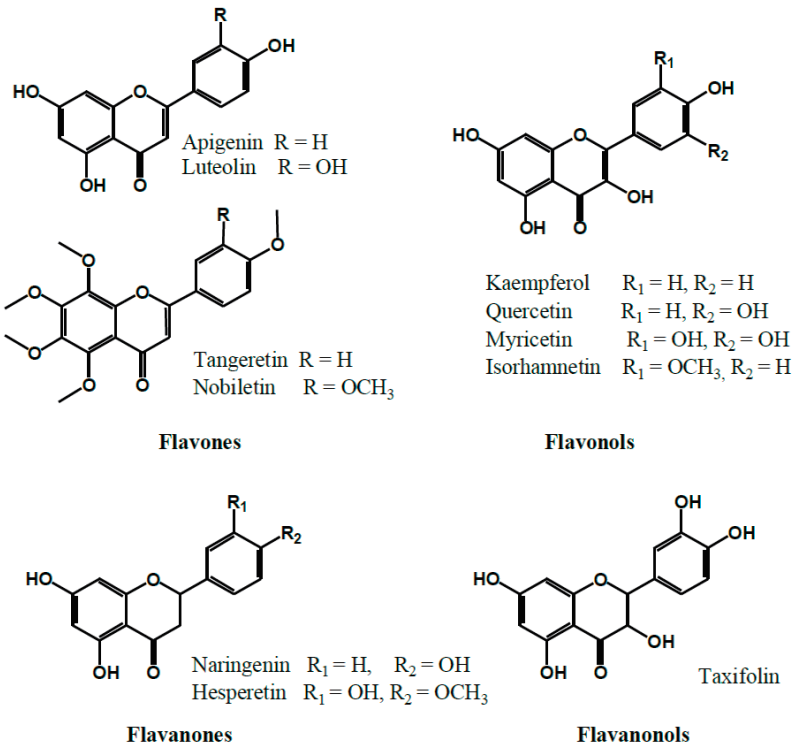

Fig. 1. **Common flavonoid in the plant**

The figure is adapted from Reference 20).

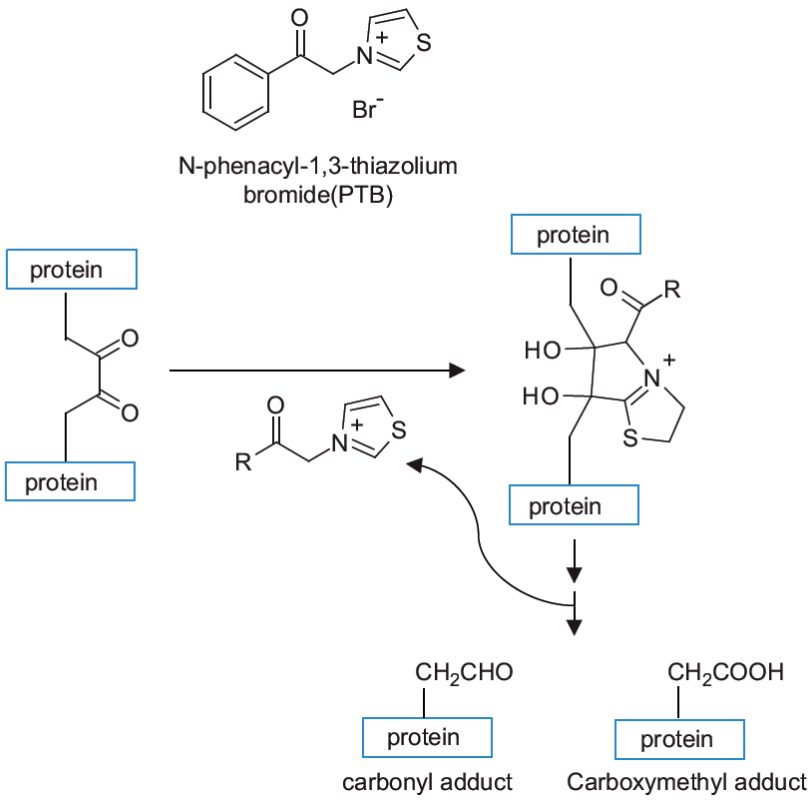

Fig. 2. **AGE breaking reaction mechanism of PTB**

The simplified reaction mechanism is referred to the dinucleophilic attack of the thiazolium ring toward the dicarbonyl AGE cross-link followed by internal rearrangement and hydrolysis. PTB, *N*-phenacylthiazolium bromide; AGE, advanced glycation end product. The figure is adapted from Reference 23).

5. Agents with Degradation Action on AGE-crosslinks

N-phenacylthiazolium bromide (PTB) is known as an agent with degradation action on AGE-derived crosslinks of proteins [22]. PTB recognizes the α-diketone structure of the cross-linking substance, Amadori-protein-ene-dion-derived protein, produced by glycative stress and degrades the C-C bonds and protein-protein crosslinks (***Fig. 2***) [23]. It has been suggested that this action may contribute to the inhibition of AGE accumulation in blood vessels and the treatment of vascular complications due to diabetes. In a study conducted with the oral administration of 10 mg/kg of PTB to diabetic rats for 4 weeks, inhibition of collagen AGE-derived crosslinks formation and degradation of AGEs in the blood vessels were observed [24]. Furthermore, vascular stiffening and inhibition action on the accumulation of AGEs was seen in a study conducted by the administration of 3-phenacyl-4,5-dimethylthiazorium chloride (ALT-711) with increased PTB water solubility to diabetic rats [25]. A clinical study conducted on the oral ingestion of ALT-711 for 8 weeks at 210 mg/day or 420 mg/day by humans, reported an improvement in vascular stiffening and uncontrolled systolic blood pressure [26, 27]. From these results, PTB is referred to as an "AGE breaker". On the other hand, PTB has been reported not to affect the degradation of AGE-derived crosslinks in the skin and tail collagen of diabetic rats [28]. For this reason, the effect of PTB is also viewed with skepticism.

Plant ingredients known to have the same effect as PTB are Japanese mugwort (*Artemisia indica*), rooibos (*Aspalathus linearis*), Chinese milk-vetch (*Astragalus sinicus*) [29], yuzu (*Citrus junos*) [30], pomegranate (*Punica granatum*) [31] and rosemary (*Rosmarinus officinalis*) [32].

Terpinen-4-ol, a kind of monoterpene alcohol contained in yuzu, a citrus fruit, has been shown to result in the hydrolysis of acid anhydride and degradation action on AGE-derived crosslinks through the production of carboxylate ester by the reaction type of Bayer-Villiger oxidation after nucleophilic substitution by hydroperoxide [30]. The trihydroxybenzene structure of ellagitannins is presumed to be involved in the degradation action of pomegranate extract and pomegranate-derived ingredients on AGE-derived crosslinks [31]. The degradation action of these natural products on AGE-derived crosslinks may contribute to the degradation and excretion of *in vivo* AGEs that have already accumulated.

6. Degradation Action on AGEs by Oxidative Proteolytic Enzymes

Oxidized protein hydrolase (OPH) is a kind of serine protease and is widely present in living tissues such as porcine liver, human blood and rat brain [33, 34]. OPH has also been reported to be present in the stratum corneum of human skin [35]. OPH is also known as acylamino-acid releasing enzyme (AARE) since it releases N-terminal acylated amino acids in proteins. In addition to acylation, OPH acts on the degradation of N-terminal amino acids in formyl, acetyl, butyl and propylated proteins [36] as well as aging proteins that have been modified by oxidation and glycation [37]. OPH also acts on the degradation of aging proteins together with proteasome [38]. In diabetic rats, serum OPH activity increases significantly and the amount of carbonyl-modified protein in the blood decreases [39].

Natural products that affect OPH activity are plant extracts such as tea, herbs and vegetables. Some herbs and health tea extracts promote OPH activity [40]. OPH has a degradation action on glycated proteins and AGEs widely present in living tissues. Therefore, the increase of OPH activity by natural products may contribute to the promotion of degradation and excretion of *in vivo* AGEs.

7. Inhibition Action on Glycative Reaction and Verification of the Effect of AGE Degrading Materials in Humans

The inhibition action on glycative reaction, and AGE degradation and excretion action of natural products have also been verified in human clinical studies. In a study conducted by the ingestion of extracts or foods containing the extracts that inhibit glycative reaction for 8 to 12 weeks, reduction of blood and skin AGEs, and increase in skin elasticity has been reported (***Table 1***) [41-53]. The degradation action on AGEs has been verified with extracts containing Japanese mugwort (yomogi) with degradation action on AGE-crosslinks produced using a collagen gel-glucose reaction system model. A study conducted for the 6-month use of skin lotion, milky lotion and cream containing Japanese mugwort extract, showed improvement in the elasticity and yellowness (b*) of skin [29]. In addition to *in vitro* studies, the anti-glycation activity of natural products has been verified in clinical studies with human subjects as well.

Conflict of Interest Statement

The authors claim no conflict of interest in this study.

Table 1. Clinical study of antiglycative food

Test food [Reference]	Test period	Study design	Screening test	Subjects	Significant differences	Subgroup	Significant differences in the subgroup analysis
Supplement containing Silybum marianum extract [41]	12 weeks	Double-blind	Skin AGEs	Healthy women n = 56 41 - 69 years old	Skin viscoelasticity Wrinkle area rate	–	–
Water chestnut extract [42]	12 weeks	Double-blind	Skin AGEs	Healthy men and women n = 30 30 - 60 years old	HbA1c Blood pentosidine Skin elasticity	Women	HbA1c Blood pentosidine
Black vineger drink containing mangosteen pericarp extract [43]	12 weeks	Double-blind	Skin AGEs	Postmenopausal women n = 24 45 - 65 years old	Skin AGEs Skin viscoelasticity	–	–
Mixed herb extract [44]	12 weeks	Double-blind	Skin AGEs	Healthy women n = 24 40 - 65 years old	Melanin index Brown spots Skin color	Excluding the subjects suspected of having diabetes	Blood 3DG
Soymilk bevarage containing rice bran / rice bran oil [45]	12 weeks	Single-blind	HbA1c Skin AGEs	Healthy women n = 23 35 - 60 years old	Immunoreactive insulin Blood CML Glycation age	BMI > 25	Fasting plasma glucose Blood CML Skin viscoelasticity
Vineger beverage containing mixed herb extract [46]	12 weeks	Double-blind	Postprandial blood glucose Skin AGEs	Postmenopausal women n = 23 50 - 65 years old	No significant difference	Postprandial blood glucose > 150 mg/dL	Corneum CML
Mixed herb extract [47]	8 weeks	Double-blind	Fasting plasma glucose HbA1c	Pre-diabetes mellitus men and women n = 26 20 - 65 years old	No significant difference	Fasting plasma glucose HbA1c	Blood 3DG, CML
Food containing lingonberry extract and cherry bloosom extract [48]	12 weeks	Single-blind	Fasting plasma glucose HbA1c	Healthy men 30 - 65 years old postmenopausal women n = 30	Fasting plasma glucose Skin AGEs	–	–
Mangosteen pericarp extract [49]	12 weeks	Double-blind	–	Healthy women n = 40 25 - 59 years old	Skin moisture Arterial pressure	–	–
Mixed herb extract [50]	12 weeks	Open	–	Diabetes mellitus men and woman n = 7	Blood 3DG, CML Skin viscoelasticity	–	–
Supplement containing mixed herb extract and two crude drugs [51]	12 weeks	Open	–	Healthy men and women n = 8 Diabetes mellitus men and women n = 4	Skin AGEs	–	–
Mangosteen pericarp extract [52]	12 weeks	Open	–	Healthy women n = 11 32 - 48 years old	Blood pentosidine Skin AGEs Skin viscoelasticity Skin moisture	–	–
Pomegrante extract [53]	12 weeks	Open	–	postmenopausal women n = 10 30 - 65 years old	HbA1c Glycoalbumin Blood 3DG, pentosidine	–	–

Reference

1) Yagi M, Yonei Y. Glycative stress and anti-aging: 13. Regulation of glycative stress. 1. Postprandial blood glucose regulation. Glycative Stress Res. 2019; 6: 175-180.
2) Ichihashi M, Yagi M, Nomoto K, et al. Glycation stress and photo-aging in skin. Anti-Aging Med. 2011; 8: 23-29.
3) Koschinsky T, He CJ, Mitsuhashi T, et al. Orally absorbed reactive glycation products (glycotoxins): An environmental risk factor in diabetic nephropathy. Proc Natl Acad Sci USA. 1997; 94: 6474-6479.
4) Christensen EI, Birn H. Megalin and cubilin: Multifunctional endocytic receptors. Nat Rev Mol Cell Biol. 2002; 3: 256-266.
5) Miyata T, Ueda Y, Horie K, et al. Renal catabolism of advanced glycation end products: The fate of pentosidine. Kidney Int. 1998; 53: 416-422.
6) Gugliucci A, Bendayan M. Renal fate of circulating advanced glycated end products (AGE): Evidence for reabsorption and catabolism of AGE-peptides by renal proximal tubular cells. Diabetologia. 1996; 39: 149-160.
7) Goto S, Takahashi R, Kumiyama A, et al. Implications of protein degradation in aging. Ann N Y Acad Sci. 2001; 928: 54-64.
8) Carrard G, Bulteau AL, Petropoulos I, et al. Impairment of proteasome structure and function in aging. Int J Biochem Cell Biol. 2002; 34: 1461-1474.
9) Schnider SL, Kohn RR. Effects of age and diabetes mellitus on the solubility and nonenzymatic glucosylation of human skin collagen. J Clin Invest. 1981; 67: 1630-1635.
10) Hori M, Yagi M, Nomoto K, et al. Experimental models for advanced glycation end product formation using albumin, collagen, elastin, keratin and proteoglycan. Anti-Aging Med. 2012; 9: 125-134.
11) Brownlee M, Vlassara H, Kooney A, et al. Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. Science. 1986; 232(4758): 1629-1632.
12) Yonei Y, Yagi M, Hibino S, et al. Herbal extracts inhibit Maillard reaction, and reduce chronic diabetic complications risk in streptozotocin-induced diabetic rats. Anti-Aging Med. 2008; 5: 93-98.
13) Yagi M, Nomoto K, Hori M, et al. The effect of edible purple Chrysanthemum extract on advanced glycation end products generation in skin: A randomized controlled clinical trial and *in vitro* study. Anti-Aging Med. 2012; 9: 61-74.
14) Yagi M, Nomoto K, Hori M, et al. Effect of Kumaizasa (*Sasa senanensis* Rehder) on the inhibition of advanced glycation end product (AGEs) formation. The Science and engineering review of Doshisha University. 2011; 52: 61-67. (in Japanese)
15) Hori M, Yagi M, Nomoto K, et al. Inhibition of advanced glycation end product formation by herbal teas and its relation to anti-skin aging. Anti-Aging Med. 2012; 9: 125-134.
16) Parengkuan P, Yagi M, Matsushima M, et al. Anti-glycation activity of various fruits. Anti-Aging Med. 2013; 10: 70-76.
17) Ishioka Y, Yagi M, Ogura M, et al. Antiglycation effect of various vegetables: Inhibition of advanced glycation end product formation in glucose and human serum albumin reaction system. Glycative Stress Res. 2015; 2: 22-34.
18) Moniruzzaman M, Parengkuan L, Yagi M, et al. Effect of proteins, sugars and extraction methods on the anti-glycation activity of spices. Glycative Stress Res. 2015; 2: 129-139.
19) Yagi M, Tateiwa Y, Inoue K, et al. Antiglycative effect of *Kaempferia parviflora* Wall. Ex. Baker (Zingiberaceae): Prevention of advanced glycation end product formation. Glycative Stress Res. 2018; 5: 163-170.
20) Odjakova M, Popova E, Sharif MA, et al. Plant-derived agents with anti-glycation activity. InTechOpen Glycosylation. 2012: 223-256.
21) Robards K, Antolovich M. Analytical chemistry of fruit bioflavonoids. Analyst. 1997; 122: 11R-34R.
22) Vasan S, Zhang X, Zhang X, et al. An agent cleaving glucose-derived protein crosslinks *in vitro* and *in vivo*. Nature. 1996; 382(6588): 275-278.
23) Aldini G, Vistoli G, Stefek M, et al. Molecular strategies to prevent, inhibit, and degrade advanced glycoxidation and advanced lipoxidation end products. Free Radic Res. 2013; 47(Suppl 1): 93-137.
24) Cooper ME, Thallas V, Forbes J, et al. The cross-link breaker, *N*-phenacylthiazolium bromide prevents vascular advanced glycation end-product accumulation. Diabetologia. 2000; 43: 660-664.
25) Freidja ML, Vessières E, Toutain B, et al. AGEs breaking and antioxidant treatment improves endothelium-dependent dilation without effect on flow-mediated remodeling of resistance arteries in old Zucker diabetic rats. Cardiovasc Diabetol. 2014; 13: 55.
26) Bakris GL, Bank AJ, Kass DA, et al. Advanced glycation end-product cross-link breakers. A novel approach to cardiovascular pathologies related to the aging process. Am J Hypertens. 2004; 17: 23S-30S.
27) Kass DA, Shapiro EP, Kawaguchi M, et al. Improved arterial compliance by a novel advanced glycation end-product crosslink breaker. Circulation. 2001; 104: 1464-1470.
28) Yang S, Litchfield JE, Baynes JW, et al. AGE-breakers cleave model compounds, but do not break Maillard crosslinks in skin and tail collagen from diabetic rats. Arch Biochem Biophys. 2003; 412: 42-46.
29) Tada A. An evaluation and the material choice of the natural ingredient for anti-glycation cosmetics. Cosmetic Stage. 2011; 5: 33-38. (in Japanese)
30) Nagamatsu R, Mitsuhashi S, Shigetomi K, et al. Cleavage of α-dicarbonyl compounds by terpene hydroperoxide. Biosci Biotechnol Biochem. 2012; 76: 1904-1908.
31) Yagi M, Mitsuhashi R, Watanabe A, et al. Cleaving effect of pomegranate (*Punica granatum*) extract on crosslink derived from advanced glycation endproducts. Glycative Stress Res. 2015; 2: 58-66.
32) Jean D, Pouligon M, Dalle C, et al. Evaluation *in vitro* of AGE-crosslinks breaking ability of rosmarinic acid. Glycative Stress Res. 2015; 2: 204-207.

33) Mitta M, Miyagi M, Kato I, et al. Identification of the catalytic triad residues of porcine liver acylamino acid-releasing enzyme. J Biochem. 1998; 123: 924-931.
34) Fujino T, Ando K, Beppu M, et al. Enzymatic removal of oxidized protein aggregates from erythrocyte membranes. J Biochem. 2000; 127: 1081-1086.
35) Yagi M, Ishigami M, Mori R, et al. Reduction effect of oxidized protein hydrolase (OPH) on advanced glycation end products and OPH-like activity in human stratum corneum. Glycative Stress Res. 2017; 4: 184-191.
36) Krishna RG, Wold F. Specificity determinants of acylaminoacyl-peptide hydrolase. Protein Sci. 1992; 1: 582-589.
37) Fujino T, Tada T, Beppu M, et al. Purification and characterization of a serine protease in erythrocyte cytosol that is adherent to oxidized membranes and preferentially degrades proteins modified by oxidation and glycation. J Biochem. 1998; 124: 1077-1085.
38) Shimizu K, Kiuchi Y, Ando K, et al. Coordination of oxidized protein hydrolase and the proteasome in the clearance of cytotoxic denatured proteins. Biochem Biophys Res Commun. 2004; 324: 140-146.
39) Shimizu K, Ikegami-Kawai M, Takahashi T, et al. Increased oxidized protein hydrolase activity in serum and urine of diabetic rat models. Biol Pharm Bull. 2009; 32: 1632-1635.
40) Yagi M, Ishizaki K, Sakiyama T, et al. The latest research of the aging mechanism resulting from a glycative stress, and application to product development. Cosmetic Stage. 2019; 13: 9-15. (in Japanese)
41) Ishii Y, Okada Y, Matsuoka S, et al. Effect of supplement containing *Silybum marianum* extract, soy extract, collagen peptide, bifidobacteria and apple extract on skin: A randomized placebo-controlled, double-blind, parallel group comparative clinical study. Glycative Stress Res. 2016; 3: 156-171.
42) Takeshita S, Ishioka Y, Uemura T, et al. Reducing effect of the long term intake of water chestnut (*Trapa bispinosa* Roxb.) pericap extract on glycative stress in the placebo-controlled double blinded clinical trial and *in vitro* inhibitory actions on low-density lipoprotein (LDL) glycation. Glycative Stress Res. 2017; 4: 299-316.
43) Takabe W, Yagi M, Ogura M, et al. Effect of mangosteen pericarp extract-containing black vinegar drink on skin quality through anti-glycative actions. Glycative Stress Res. 2017; 4: 158-171.
44) Kawai H, Shoshihara M, Kawakami H, et al. Anti-glycation and skin beautification properties from ingestion of mixed herb extract: A placebo-controlled, double-blind, randomized, parallel-group study. Glycative Stress Res. 2016; 3: 236-245.
45) Yonei Y, Yagi M, Hamada U, et al. A placebo-controlled, randomized, single-blind, parallel-group comparative study to evaluate the anti-glycation effect of a functional soymilk beverage supplemented with rice bran/rice bran oil. Glycative Stress Res. 2015; 2: 80-100.
46) Yagi M, Shimode A, Hamada U, et al. Evaluation of the anti-glycation effect and the safety of a vinegar beverage containing indigestible dextrin and a mixed herbal extract: A placebo-controlled, double-blind study. Glycative Stress Res. 2014; 1: 14-24.
47) Yonei Y, Miyazaki R, Takahashi Y, et al. Anti-glycation effect of mixed herbal extract in individuals with pre-diabetes mellitus: A double-blind, placebo-controlled, parallel group study. Anti-Aging Med. 2010; 7: 26-35.
48) Yonei Y, Yagi M, Ogura M, et al. Anti-glycation activity and safety of foods containing lingonberry extract and cherry blossom extract and chewable tablets containing citric acid and calcium: A placebo-controlled randomized single-blind parallel group comparative study. Anti-Aging Med. 2013; 10: 21-36.
49) Maejima K, Ohno R, Nagai R, et al. Effect of mangosteen pericarp extract on skin moisture and arterial stiffness: Placebo-controlled double-blinded randomized clinical trial. Glycative Stress Res. 2018; 5: 95-103.
50) Kubo M, Yagi M, Kawai H, et al. Anti-glycation effects of mixed-herb-extracts in diabetes and pre-diabetes. J Clin Biochem Nutr. 2008; 43(suppl.1): 66-69.
51) Tamura T, Yagi M, Nomoto K, et al. Anti-glycation effect of a novel herbal mixture made of mixed herbal extract and two crude drugs: Short and long term effect. The Science and Engineering Review of Doshisha University. 2012; 52: 243-251. (in Japanese)
52) Ohno R, Moroishi N, Sugawa H, et al. Mangosteen pericarp extract inhibits the formation of pentosidine and ameliorates skin elasticity. J Clin Biochem Nutr. 2015; 57: 27-32.
53) Yagi M, Parengkuan L, Sugimura H, et al. Anti-glycation effect of pomegranate (*Punica granatum* L.) extract: An open clinical study. Glycative Stress Res. 2014; 1: 60-67.