Glycative Stress Research

Online edition : ISSN 2188-3610
Print edition : ISSN 2188-3602
Received : November 13, 2024
Accepted : December 2, 2024
Published online : December 31, 2024
doi:10.24659/gsr.11.4_184

Original article

Anti-glycation and anti-oxidation actions of soy sauce components: Involvement of melanoidins

Minaho Iida, Masayuki Yagi, Chieko Sakiyama, Yoshikazu Yonei

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

Abstract

***Subjects*:** Most of the biological effects of glycative stress are caused by the reaction of carbohydrate- and lipid-derived aldehydes with proteins in the body, resulting in the formation of advanced glycation end products (AGEs). These reactions are called glycation, and when AGEs are formed and accumulate in the body, they cause various disorders in tissues and cells. On the other hand, melanoidins have been reported to suppress elevated blood glucose levels, act as an antioxidant, and promote prebiotic activity (growth of bifidobacteria). Japanese people have a habit of consuming soy sauce, which is rich in AGEs and melanoidins. In this study, we examined the anti-glycation and anti-oxidation actions of ingredients in soy sauce.

***Methods*:** 13 commercial soy sauce products were used as samples. The soy sauce samples were filtered through an ultrafiltration filter with a molecular weight of 3,000 and divided into three fractions: unfiltered (fraction A), membrane filtrate (non-melanoidin fraction) (fraction B), and membrane residue (melanoidin fraction) (fraction C). After measuring fluorescent AGEs and pentosidine in the glycation reaction solution, the percentage inhibition of formation (%) or IC50 was calculated. The DPPH radical scavenging activity of the samples was determined. Glycine - Glucose melanoidin (GGM) was synthesized in the laboratory, and the melanoidin content in soy sauce was measured by absorbance (400 nm). The fluorescent AGE content in soy sauce was calculated as argpyrimidine equivalents.

***Results*:** All soy sauce samples inhibited the formation of fluorescent AGEs and pentosidine. Also, DPPH radical scavenging activity was observed. In fraction C, the IC50 of fluorescent AGEs was 3.4 times lower than that of fraction B. The DPPH radical scavenging activity of fraction C was greater than that of fractions A and B ($p < 0.05$). Fraction C was 2.51 times (0.36 mg/mL) more potent than GGM in inhibiting fluorescent AGE formation and 1.23 times (0.41 mg/mL) more potent in scavenging DPPH radicals.

***Conclusion*:** The components of soy sauce were found to have potentials of anti-glycation (inhibiting AGE formation) and anti-oxidation (scavenging DPPH radical). The melanoidin efficacy in soy sauce varies depending on the glycation time and raw ingredients (*i.e.*, sugars, amino acids), however, suggesting that it may contribute to both anti-glycation and anti-oxidation.

***KEY WORDS*:** Soy sauce, melanoidins, glycation, advanced glycation end products (AGEs)

Introduction

Due to differences in the conditions under which advanced glycation end products (AGEs) are produced, there are two types: endogenous AGEs, which are produced in the body, and exogenous AGEs (food-derived AGEs), which are produced outside the body.

Many of the effects of glycation stress on the body are caused by aldehydes derived from carbohydrates and lipids reacting with proteins in the body to produce endogenous AGEs[1]. Endogenous AGEs accumulate in various tissues and organs with age, causing inflammation, discoloration, and reduced physiological functions, and are involved in the onset and progression of lifestyle-related diseases such as diabetic complications, eye diseases[2], skin aging[3], osteoporosis[4], Alzheimer's dementia[5], and arteriosclerosis[6].

On the other hand, exogenous AGEs of food origin include substances that are considered beneficial (e.g., melanoidins) and substances that are considered harmful. The latter include acrylamide and argpyrimidine. When ingested as food, acrylamide is rapidly absorbed from the gastrointestinal tract

Contact Address: Professor Masayuki Yagi, PhD
Anti-Aging Medical Research Center / Glycative Stress Research Center,
Graduate School of Life and Medical Sciences, Doshisha University
1-3, Tatara Miyakodani, Kyotanabe-shi, Kyoto, 610-0394 Japan
TEL&FAX: +81-774-65-6394 e-mail: myagi@mail.doshisha.ac.jp
Co-authors; Iida M, ctuj2008@mail4.doshisha.ac.jp;
Sakiyama C, csakiyam@mail.doshisha.ac.jp; Yonei Y, yyonei@mail.doshisha.ac.jp

and distributed to the tissues. In the liver, activator metabolites of acrylamide (*e.g.*, glycidamide) bind to DNA and induce injury, which may lead to toxic and carcinogenic etiologies[7]. As for the toxicity of argpyrimidine, its mechanism of action is to induce inflammatory lesions in tissues via binding to RAGE [receptor for AGEs] and stimulation of inflammatory cytokine production[8, 9]. This is mainly a problem for routes of administration other than oral administration. In vivo, arginine residues of intracellular proteins combine with methylglyoxal to form argpyrimidine, which may be essential for neuronal differentiation[10]. The effects of AGEs in food after digestion and absorption are unclear. In individuals with normal renal function, approximately 10% of food-derived AGEs are transferred to the blood, of which only 3% are excreted in the urine within 48 hours. The remaining 7% is assumed to be either metabolized and eliminated or phagocytosed by the sinusoidal system of the liver or by the reticuloendothelial system of the spleen[11]. It is unlikely or to a minimal degree, if any, that food-derived AGEs are deposited or accumulated in tissues. Despite the fact that the effects of exogenous and endogenous AGEs on the body are not equivalent, some theories state that ingesting food-derived AGEs are "harmful" to health[12,13].

Soy sauce is a seasoning made by fermenting soybeans and wheat. It has been familiar to the Japanese for about 400 years and is an indispensable ingredient in cooking. There are several types of soy sauce, including dark, light, tamari (= product name, meaning puddle), white, re-fermented[14], and clear[15]. The characteristic brown color of soy sauce is formed by a brownish substance called melanoidin. Melanoidins are also produced during cooking and processing of foods, and affects the aroma, flavor, and color. Melanoidins are found not only in soy sauce, but also in miso, coffee, and other brown foods.

Melanoidins have a molecular weight of 3,000-55,000 Da or 10,000-140,000 Da[16]. However, the structure of melanoidins has not been elucidated. Melanoidins have the effect of suppressing elevated blood glucose levels[17], strong antioxidant activity[18], prebiotic activity (promotion of bifidobacterial growth)[19], and flavor formation[20, 21].

Suppression of glycative stress includes suppression of hyperglycemia, suppression of glycation reaction, and decomposition and excretion of glycation reaction products[22]. In this study, we examined activities of anti-glycation (AGE formation inhibition) and anti-oxidation (DPPH [2,2-diphenyl-1-pycrylhydrazyl] radical scavenging) of soy sauce ingredients *in vitro*.

Methods

(1) Reagents

Human serum albumins (HSA, lyophilized powder, ≥ 96%, agarose gel electrophoresis) were used as a model protein for glycation reactions. HSA was purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). Argpyrimidine (TFA salt) was purchased from IRS Iris Biotech GmbH (St. Adalbert-Zoellner, Marktredwitz, Germany). Other reagents were of special grade or HPLC grade. They were purchased from Fujifilm Wako Pure Chemical Industries, Ltd. (Osaka, Japan) or Nacalai Tesque, Inc. (Kyoto, Japan).

(2) Sample preparation

Soy sauce was purchased from supermarkets and online stores in Kyoto Prefecture, with 13 items and 6 varieties (3 Dark, 2 Light, 2 Tamari, 2 White, 2 Double fermented, 2 Clear) (***Table 1***). The soy sauce was filtered through an Amicon Ultra-0.5mL 3K filter and separated into three fractions: unfiltered (fraction A), membrane filtrate (non-melanoidin fraction: fraction B), and membrane residue (melanoidin fraction: fraction C). Each sample was weighed before and after drying, and the solid content [mg/mL] was calculated. Glycine-glucose melanoidin (GGM) was synthesized by reacting glycine and glucose at 90°C for 72 hours[23].

(3) Measurement of AGE content

The content of fluorescent AGEs was calculated as argpyrimidine equivalent by measuring the fluorescence value at an excitation wavelength of 370 nm and a fluorescence

Table 1. Soy sauce profile.

No.	Product name	Manufacturer / Seller	Characteristics
1	Special Whole Soy Soy Sauce	Kikkoman (Tokyo, Japan)	***Dark Soy Sauce***
2	Whole Soybean Soy Sauce	Daitoku Shoyu (Hyogo, Japan)	
3	Naturally brewed soy sauce, dark	Shokin Shoyu (Kanagawa, Japan)	
4	Light Whole Soybean Soy Sauce	Daitoku Shoyu (Hyogo, Japan)	***Light Soy Sauce***
5	Naturally brewed raw soy sauce, light	Shokin Shoyu (Kanagawa, Japan)	
6	Denemon Tamari	Ito Shoten (Aichi, Japan)	***Tamari Soy Sauce***
7	Umino Sei Japanese Organic Tamari Soy Sauce	Umi no Sei (Tokyo, Japan)	
8	Shichifuku Brewery Organic White Soy Sauce	Shichifuku Jozo (Aichi Japan)	***White Soy Sauce***
9	Yuasa Soy Sauce Kurasho White Squeezed	Marushin Honke (Wakayama, Japan)	
10	Kinbue Re-brewed raw soy sauce	Kinbue (Saitama, Japan)	***Double Fermented***
11	Domestic Organic Double Fermented Soy Sauce	Okanaosaburo Shoten (Gifu, Japan)	
12	Muku	Ichibiki (Aichi, Japan)	***Clear Soy Sauce***
13	Soy sauce-style seasoning	Fundodai (Kumamoto, Japan)	

13 items across 6 types (dark soy sauce: 3 items, light soy sauce: 2, tamari: 2, white soy sauce: 2, double-brewed soy sauce: 2, and clear soy sauce: 2), details of the soy sauce samples are shown in **Table 1**.

wavelength of 440 nm together with 0.025 to 0.50 mg/mL argpyrimidine solution [24,25]. Pentosidine was quantified by reversed-phase high-performance liquid chromatography (HPLC) after hydrolysis of the glycated reaction solution with hydrochloric acid [26,27].

(4) Verification of glycation reaction inhibition effect

To verify the glycation reaction inhibition effect, a human serum albumin-glucose (HSA-Glucose) glycation reaction model was used [28]. The glycation reaction solution was prepared by adding 0.1 mol/L phosphate buffer (pH 7.4), 2.0 mol/L glucose, and 40 mg/mL HSA to the sample. The glycation reaction solution was then reacted at 60 °C for 40 hours. The AGE-derived fluorescence value (excitation wavelength 370 nm / detection wavelength 440 nm) was measured using a microplate reader. Pentosidine was measured by HPLC [26,27]. As a control (reference: ref), an equal quantity of purified water was added instead of the sample and reacted under the same conditions. Aminoguanidine (AG), which is known as an AGE formation inhibitor, was used as a positive control for the fluorescent AGE formation inhibition effect. The fluorescent AGE formation inhibition rate (%) was calculated based on the following formula:

AGE formation inhibition rate (%) = 100 – {(sample Glucose (+) – sample Glucose (–)) / (ref Glucose (+) – ref Glucose (–)) x 100}

The 50 % inhibition concentration (IC50) value (mg/mL) was calculated from the fluorescent AGE formation inhibition rate (3 concentrations) for each sample. The smaller the IC50 value, the stronger the effect.

(5) Verification of DPPH radical scavenging activity

DPPH radical scavenging activity (μmol-Trolox equivalent/mL) was measured by calculating the equivalent quantity using Trolox as a standard substance [29,30].

(6) Measurement of melanoidin content

The melanoidin content in soy sauce was measured by measuring the absorption spectrum (200 - 800 nm) of GGM (0.296 mg/mL) and fraction C, and calculating the melanoidin content from the measured absorbance (400 nm) [31,32,33]. Fraction C was obtained from the soy sauce sample and measured.

(7) Analysis method

Measurements are shown as mean ± standard deviation (n = 3). For statistical analysis, Tukey's multiple comparison test was used for three or more groups, and T-test was used for two groups. Correlation was measured using Pearson's product moment correlation coefficient, with $0.4 < |r| < 1$ considered to be correlated. Results of statistical analysis were considered significant at a risk level (p value) of less than 5% (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

Results

AGE content in soy sauce

The content of AGEs and pentosidine in 11 types of soy sauce are shown in ***Table 2***. When comparing soy sauces by type, the content of AGEs and pentosidine was the highest in tamari soy sauce and the lowest in white soy sauce.

Inhibitory effect on AGE formation

The results for 13 types of soy sauce - A at 1.21 mg/mL are shown in ***Table 2***. All soy sauces inhibited the formation of fluorescent AGEs. When comparing the inhibition rate by individual, No. 11 had the highest inhibition rate and No. 5 had the lowest inhibition rate. In No. 12 and 13 - A at 1.21 mg/mL, the inhibition rates were 18.84 ± 3.53 % (mean ± SD) and 37.24 ± 7.82 %, respectively. No. 12 and 13 clear soy sauces also had anti-glycation effects.

Table 2. Content of glycation reaction products and effects.

No.	AGEs	Pentosidine	Melanoidin	Inhibition rate of fluorescent AGE production	Inhibition rate of pentosidine	DPPH radical scavenging activity
1	36.04	74.52	1.85	66.12 ± 8.05	55.89 ± 1.25	84.08
2	29.31	71.12	2.94	37.33 ± 1.09	50.34 ± 7.70	89.68 ± 26.35
3	43.67	83.57	1.02	13.89 ± 3.71	71.95 ± 1.78	77.80 ± 17.96
4	16.56	66.61	0.43	44.21 ± 1.15	63.31 ± 0.76	100.78 ± 15.77
5	20.46	114.35	1.80	0.60	75.34 ± 2.37	34.20 ± 9.25
6	80.95	103.35	7.52	72.81	64.39 ± 3.32	86.14 ± 5.88
7	63.95	105.13	2.68	77.97 ± 1.34	64.93 ± 2.00	124.19 ± 4.42
8	8.70	99.36	0.35	68.58 ± 0.56	69.60 ± 14.06	27.80 ± 10.39
9	13.10	24.98	0.21	72.40 ± 0.32	69.97 ± 4.42	1.47 ± 11.81
10	66.74	93.75	4.18	68.63 ± 3.32	71.39 ± 14.01	92.29 ± 16.74
11	54.29	101.84	8.85	83.64 ± 0.31	68.16 ± 3.57	138.91 ± 0.32

Data: mean ± standard deviation (n = 3), details of the soy sauce samples are shown in **Table 1**. AGE content (mg Argpyrimidine equivalent/mL), pentosidine content (ng/mL) and melanoidin content (mg/mL) in 11 types of soy sauce samples. Inhibition rate of fluorescent AGE formation and pentosidine at a final concentration of 1.21 mg/mL. DPPH radical scavenging activity at solid content 3.03 mg/mL. AGEs, advanced glycation end products; DPPH, 2, 2-diphenyl-1-pycrylhydrazyl.

The inhibition rates and IC50 of fluorescent AGE formation for No.1 - A, B, and C are shown in ***Table 3***. All fractions showed inhibitory effects on glycation reaction. The anti-glycation effect was in the order of fraction C, A, and B. The IC50 of melanoidin fraction C was 3.4 times lower than that of non-melanoidin fraction B.

DPPH radical scavenging activity

Table 2 shows the results of 13 types of soy sauce - A at 3.03 mg/mL. All soy sauces scavenged DPPH radicals. Comparing the scavenging activity by individual, No.11 was the highest, No. 9 was the lowest, No.12 – A was 9.14 ± 9.50 µmol-Trolox equivalent/mL, and No.13 – A was 14.12 ± 11.24 µmol-Trolox equivalent/mL. Antioxidant activity was also observed in clear soy sauce.

Table 3 shows the DPPH radical scavenging activity results at 2.0 mg/mL for No.1 – A, B, and C. Fraction C had a strong antioxidant effect ($p < 0.01$). The results for No.1 - B and C show that the melanoidin fraction was 5.8 times higher than the non-melanoidin fraction.

Melanoidin content in soy sauce

The melanoidin content is shown in ***Table 2***. When compared by type, the highest content was found in re-brewed soy sauce, followed by tamari, dark soy sauce, light soy sauce, and white soy sauce. The melanoidin content in No. 12 and 13 (both clear soy sauces) were below the detection limit (0.01 mg/mL).

The absorption spectra of GGM and No.1-C are shown in ***Fig 1***. No clear absorption maximum was observed in either sample. The two spectra were almost identical to the UV-visible absorption spectrum of melanoidin reported in a previous report [34].

Relationship between the content of glycation reaction products and the inhibitory effect on AGE formation/DPPH radical scavenging activity

No correlation was observed between the content of glycation reaction products and the inhibitory activity on AGE formation or the DPPH radical scavenging activity.

Anti-glycation and anti-oxidation potential of melanoidins

The inhibition of fluorescent AGE formation of GGM was 7.2 ± 3.2 (mean ± SD), 26.7 ± 1.8, and 60.1 ± 3.2% at 0.065, 0.163, and 0.244 mg/mL. GGM showed concentration-dependent inhibition of fluorescent AGEs formation. The contribution ratio of melanoidins in soy sauce was 16.0, 39.9, and 48.6% at melanoidin content of 0.146, 0.364, and 0.544 mg/mL. No. 1-C was 2.51 times more potent than GGM in inhibiting fluorescent AGE formation (at melanoidin concentration of 0.36 mg/mL). At three content measured, No.1-C had higher values than GGM ($p < 0.001$). The results of the fluorescent AGE formation inhibition rate of melanoidins contained in soy sauce fraction C are shown in ***Fig.2-a***. Even though the same melanoidin content was contained, there was a maximum difference of 2.09 times between the types. The p values differed between all 5 types of individuals (*** $p < 0.001$, ** $p < 0.01$). Sample No. 9 had the greatest anti-glycation effect by individual.

The DPPH radical scavenging activity of GGM was 29.4 ± 11.9, 72.8 ± 15.6, and 85.4 ± 14.3 µmol-Trolox equivalent/mL at solid concentrations of 0.131, 0.408, and 0.639 mg/mL. GGM showed concentration-dependent DPPH radical scavenging activity. The contribution ratio of melanoidins in soy sauce was 41.6, 81.0, and 73.8 % at melanoidin concentrations of 0.131, 0.408, and 0.639 mg/mL. Sample

Table 3. Inhibition rate of fluorescent AGE production of Dark Soy Sauce.

	Final Concentration (mg/mL)	Inhibition rate of fluorescent AGEs	IC50
Fraction A)	0.61	44.16 ± 5.56	
	1.21	66.12 ± 8.05	0.73
	2.42	85.34 ± 7.93	
Fraction B)	0.50	35.05 ± 3.92	
	0.99	47.07 ± 6.70	0.98
	1.98	68.93 ± 5.24	
Fraction C)	0.23	44.64 ± 2.10	
	0.46	66.93 ± 1.93	0.27
	0.70	82.15 ± 1.25	
AG			0.056

Data: mean ± standard deviation (n = 3), sample is shown is No.1 in **Table 1**. Inhibition rate of fluorescent AGEs (%) and IC50 (mg/mL) of each fraction and AG. AGEs, advanced glycation end products; AG, aminoguanidine.

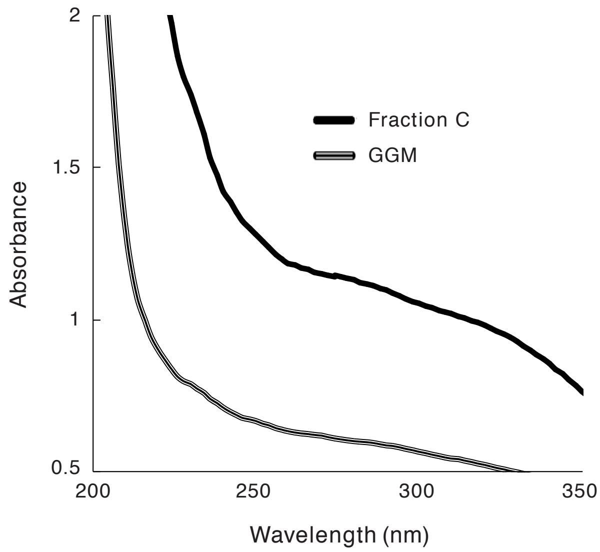

Fig. 1. **Absorption spectrum of soy sauce fraction C and GGM.**

Results shows absorption spectrums measured at 200 nm to 800 nm. Samples are "Fraction C of dark soy sauce (No.1 in **Table 1**)" and "GGM (Glycine - Glucose Melanoidin)".

No. 1-C had 1.23 times higher DPPH radical scavenging activity than GGM (melanoidin concentration 0.41 mg/mL). The DPPH radical scavenging activity of No. 1-C was higher than that of GGM only at the melanoidin content of 0.13 mg/mL ($p < 0.05$). The results of the DPPH radical scavenging activity of melanoidins are shown in ***Fig. 2-b***. Even with the same melanoidin content, the activity differed depending on the type of soy sauce (** $p < 0.01$). There was a maximum difference of 1.57 times between types. Differences were observed between samples only in dark soy sauce (** $p < 0.01$). Comparing the anti-oxidation effect of different plants, No. 3 was the most effective and No. 2 was the least effective.

Discussion

Soy sauce's glycative stress inhibitory effect

All 13 types of soy sauce tested had an AGE formation inhibitory effect and DPPH radical scavenging activity. In addition, all 11 types of soy sauce tested had a pentosidine inhibitory effect. From these results, soy sauce was considered to be a food that may suppress glycative stress.

The average fluorescent AGE formation inhibitory rate and DPPH radical scavenging activity of the commonly used dark soy sauce (No. 1) were highest in fraction C (***Tables 3, 4***). This suggests that components with molecular weights of 3,000 or more may have a strong inhibitory effect on glycative stress. Fraction C had a 3.4-fold higher fluorescent AGE formation inhibitory rate and 5.8-fold higher DPPH radical scavenging activity than fraction B. Melanoidins had anti-glycation and anti-oxidation effects. Fraction C contains melanoidins, but fraction B does not. From the above, melanoidins may be involved as a glycative stress inhibitor in soy sauce.

On the other hand, melanoidins were not detected in the clear soy sauce (No. 12, 13). However, the clear soy sauce had anti-glycation and anti-oxidation effects. Furthermore, No. 1-B also had anti-glycation and anti-oxidation effects (***Tables 3, 4***). Previous studies have reported that free amino acids such as L-arginine and L-cysteine [35,36] and soy isoflavones [37,38,39] have anti-glycation and anti-oxidation effects. Based on these findings, in addition to melanoidins, amino acids and isoflavones with molecular weights of less than 3,000 may also contribute to these effects. Therefore, even clear soy sauce without melanoidins may have anti-glycation and anti-oxidation effects.

In this study, the IC50 of aminoguanidine (AG) was 13.0 times lower than that of the original solution fraction A of dark soy sauce No. 1 (***Table 3***). Since the solid concentration of No. 1 was 327.87 mg/mL, the amount of soy sauce required to obtain the same effect as the glycation reaction inhibitor AG was approximately 0.0022 mL. Furthermore, the recommended intake of dark soy sauce for Japanese people is 45 mL (just under 3 tablespoons) for adult men and 37.5 mL (2.5 tablespoons) for adult women [40]. Therefore, soy sauce may have a glycation reaction inhibitory effect in small amounts.

Table 4. DPPH radical scavenging activity of Dark Soy Sauce.

	DPPH radical scavenging activity			
Fraction A)	33.04	±	3.51	] ** (A vs C)
Fraction B)	26.38	±	12.17	] ** (B vs C)
Fraction C)	153.13	±	13.42	

Data: mean ± standard deviation (n = 3), ** $p < 0.01$, Tukey test, sample is shown is No. 1 in **Table 1**. DPPH radical scavenging activity (μmol-Trolox equivalent/mL) in 2.0 mg/mL of each fraction. DPPH, 2, 2-diphenyl-1-pycrylhydrazyl.

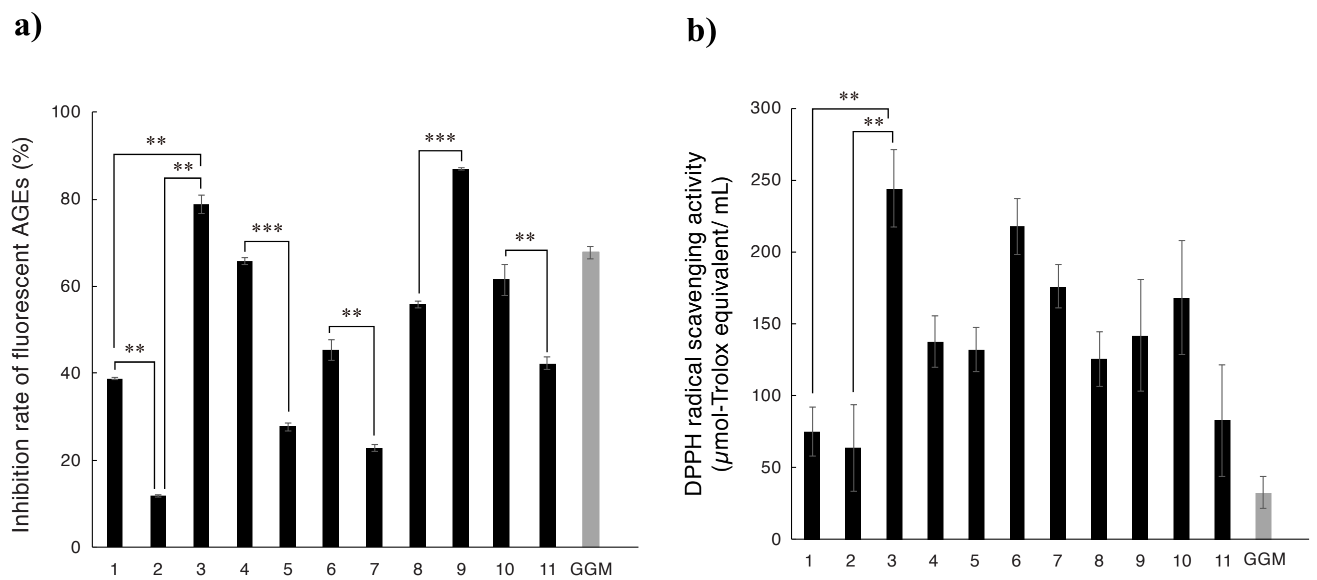

Fig. 2. **Inhibition/scavenging effects of soy sauce melanoidins on AGEs and DPPH radicals.**

Results are expressed as mean ± standard deviation (n = 3), *** $p < 0.001$, ** $p < 0.01$, Tukey test, Melanoidin content of the 11 types were standardized. **(a)** Inhibition of fluorescent AGE production. **(b)** DPPH radical scavenging activity. AGEs, advanced glycation end products; DPPH, 2, 2-diphenyl-1-pycrylhydrazyl.

From the above, soy sauce may suppress glycative stress. And melanoidin is likely to be one of the components of soy sauce that has anti-glycation and anti-oxidation effects.

Melanoidins suppress glycative stress

Melanoidins are found in brown foods such as soy sauce, miso, and coffee. Melanoidins have the ability to suppress postprandial hyperglycemia [17] and prebiotic activity (promotes the growth of bifidobacteria) [19]. In particular, there are many papers on their anti-oxidation effect [18, 41, 42]. Whereas, there are few research reports on their anti-glycative effect. In this study, we examined the anti-glycative and anti-oxidation effects of melanoidin (GGM) synthesized in the laboratory. As a result, GGM and the melanoidin fraction of soy sauce had anti-glycative and anti-oxidation effects. Melanoidins may be a component that suppresses glycative stress.

There was a difference between No. 1-C and GGM in the fluorescent AGE formation inhibition rate and DPPH radical scavenging activity. In addition, even with the same soy sauce, there was a difference in the above effects (***Fig. 2***). Furthermore, white soy sauce No. 9-C had a large anti-glycation effect despite a small melanoidin content, while dark soy sauce No. 2-C had the smallest anti-oxidation effect despite a relatively large melanoidin content (***Fig. 2***). From the above, there are various types of melanoidins, and the degree of the glycation inhibitory effect may differ depending on the type. There are differences in the composition of the sugars and amino acids that make up melanoidins and the manufacturing method depending on the type of soy sauce, as well as between soy sauce melanoidins and GGM, and also between types of soy sauce. Therefore, these may be the factors behind the difference in action.

The difference in action between soy sauce melanoidins and GGM, and between soy sauces themselves, was particularly large in the rate of inhibition of fluorescent AGE formation. This may be largely due to differences in production methods. The angiotensin converting enzyme (ACE) inhibitory effect, which suppresses hypertension [43], one of the causes of hyperglycemia, varies depending on the fermentation time [44]. For this reason, the fermentation time may affect the anti-glycation effect, resulting in differences in action. There is also the possibility that the reaction pathways for anti-glycation and anti-oxidation are different. These results suggest that the difference in action of melanoidins may be due to differences in raw materials and production methods.

Research limitations

The results obtained in this study indicate an inhibitory effect on the glycation. The present results were obtained *in vitro*, and verification of the anti-glycation activity in human clinical studies is also necessary. Further validation of the suppression of postprandial hyperglycemia and the degradation and excretion of glycation reaction products [16] by feeding studies is required in the future.

Conclusion

Inhibitory effects on glycation (inhibition of AGE formation) and on oxidation (DPPH radical scavenging activity) have been observed in soy sauce-containing ingredients, of which the involvement of melanoidins is important. Although the efficacy of melanoidins depends on the glycation time and raw materials (*i.e.*, sugar, amino acids), it was suggested that melanoidins may contribute to both anti-glycation and anti-oxidation.

Declaration of Conflict of Interest

There are no companies or other entities with which we have COI relationships that should be disclosed in the course of conducting this research.

Funding

The study has not received any competitive research funding.

Reference

1) Negre-Salvayre A, Salvayre R, Augé N, et al. Hyperglycemia and glycation in diabetic complications. *Antioxid Redox Signal*. 2009; 11: 3071-3109.
2) Nagai R, Mori T, Yamamoto Y, et al. Significance of advanced glycation end products in aging-related disease. *Anti-Aging Med*. 2010; 7: 112-119.
3) Reiser KM. Nonenzymatic glycation of collagen in aging and diabetes. *Proc Soc Exp Biol Med*. 1998; 218: 23-37.
4) Saito M, Fujii K, Tanaka T. Reductions in degree of mineralization and enzymatic collagen cross-links and increases in glycation induced pentosidine in the femoral neck cortex in cases of femoral neck fracture. *Osteoporosis Int*. 2006; 17: 986-995.
5) Reddy VP, Obrenovich ME, Atwood CS, et al. Involvement of Maillard reactions in Alzheimer disease. *Neurotox Res*. 2002; 4: 191-209.
6) Brownlee M, Vlassara H, Kooney A, et al. Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. *Science*. 1986; 232(4758): 1629-1632.
7) Pedreschi F, Mariotti MS, Granby K. Current issues in dietary acrylamide: formation, mitigation and risk assessment. *J Sci Food Agric*. 2013; 94: 9-20
8) Cipollone F, Iezzi A, Fazia M, et al. The receptor RAGE as a progression factor amplifying arachidonate-dependent inflammatory and proteolytic response in humazn atherosclerotic plaques: Role of glycemic control. *Circulation*. 2003; 108: 1070-1077
9) Yoshimura K, Hosotani T, Yamamoto H. AGE specific receptor (RAGE). *Diabetes*. 2005; 48: 411-414. (in Japanese) https://doi.org/10.11213/tonyobyo.48.411
10) Nakadate Y, Uchida K, Shikata K, et al. The formation of argpyrimidine, a methylglyoxal-arginine adduct, in the nucleus of neural cells. *Biochem Biophys Res Commun*. 2009; 378: 209-212.
11) Koscinsky 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.
12) Ito R, Kusumoto M, Miyazawa S, et al. Acceleration of pro-inflammatory effects by dietary AGEs. *Japanese Journal of Food Chemistry and Safety*. 2023; 30, 54-67. (in Japanese) https://doi.org/10.18891/jjfcs.30.1_54
13) Takeuchi M, Takino J, Yamagishi S. Involvement of Toxic AGEs (TAGE) in the onset and progression of lifestyle-related diseases - A new prevention strategy - The truth about dietary AGEs and glucotoxicity. J Kanazawa Med Univ, 2015; 40: 95-103 (in Japanese) http://www.kanazawa-med.ac.jp/kouhou/pdf/PDF-2.pdf
14) Ministry of Agriculture, Forestry and Fisheries. Regarding the revision of the Japanese Agricultural Standards: Soy sauce (Shoyu). 2014. http://www.famic.go.jp/english/jas/_doc/jas1703.pdf
15) Kumamoto's transparent soy sauce has the advantage of not changing the color of ingredients - Supported by foreigners. Nihon Keizai Shimbun Digital. 2023-12-28. (in Japanese) https://www.nikkei.com/article/DGXZQOJC218NX0R21C23A2000000/, Viewed date: 2024-10-28
16) Yang S, Fan W, Xu Y. Melanoidins present in traditional fermented foods and beverages. *Compr Rev Food Sci Food Saf*. 2022; 21: 4164-4188.
17) Miura M. Physiological functions of melanoidins (brown pigments). *Journal of the Brewing Society of Japan*. 2002; 97: 253-256. (in Japanese) https://doi.org/10.6013/jbrewsocjapan1988.97.253
18) Borrelli RC, Fogliano V. Bread crust melanoidins as potential prebiotic ingredients. *Mol Nutr Food Res*. 2005; 49: 673-678.
19) Pérez-Burillo S, Rajakaruna S, Pastoriza S, et al. Bioactivity of food melanoidins is mediated by gut microbiota. *Food Chem*. 2020; 316: 126309.
20) Diaz-Morales N, Ortega-Heras M, Diez-Maté A, et al. Antimicrobial properties and volatile profile of bread and biscuits melanoidins. *Food Chem*. 2022; 373: 131648
21) Gigl M, Hofmann T, Frank O. NMR-based studies on odorant-melanoidin interactions in coffee beverages. *J Agric Food Chem*. 2021; 69: 15334-15344.
22) Ichihashi M, Yagi M, Nomoto K, et al. Glycation stress and photo aging in skin. *Anti-Aging Med*. 2011; 8: 23-29
23) Hirano M, Miura M, Gomyo T. A tentative measurement of brown pigments in various processed foods. *Biosci Biotechnol Biochem*. 1996; 60: 877-879.
24) Yagi M, Hara H, Mifuru R, et al. Suppression of glycated protein cross-linking formation and cross-linking cleavage reaction of edible purple Chrysanthemum flower extract. *Glycative Stress Res*. 2022; 9: 7-14.
25) Yagi M, Takabe W, Ishizaki K, et al. The evaluation of glycative stress and anti-glycation effect. *Oleoscience*. 2018; 18: 67-73. (in Japanese) https://doi.org/10.5650/oleoscience.18.67
26) Yagi M, Isshiki K, Takabe W, et al. Measurement of pentosidine in human plasma by the high-performance liquid chromatography. *Glycative Stress Res*. 2018; 5: 119-128.
27) Scheijen JLJM, van de Waarenburg MPH, Stehouwer CDA, et al. Measurement of pentosidine in human plasma protein by a single-column high-performance liquid chromatography method with fluorescence detection. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2009; 877: 610-614.
28) 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.
29) Oki T, Osame M, Masuda M, et al. Simple and rapid spectrophotometric method for selecting purple-fleshed sweet potato cultivars with a high radical-scavenging activity. *Breeding Science*. 2003; 53: 101-107.
30) Oki T, Masuda M, Furuta S, et al. Radical scavenging activity of fried chips made from purple-fleshed sweet potato. *Nippon Shokuhin Kagaku Kogaku Kaishi*. 2001; 48: 926-932. (in Japanese) https://doi.org/10.3136/nskkk.48.926
31) Martins SIFS, Van Boekel MA.JS. Kinetics of the glucose/ glycine Maillard reaction pathways: Influences of pH and reactant initial concentrations. *Food Chem*. 2005; 92: 437-448.

32) Tamaki M, Ukai M, Honma S. Effect of onion juice on the polymerization of melanoidin in a model system. *Nippon Shokuhin Kagaku Kogaku Kaishi*. 1998; 45: 52-57. (in Japanese)
https://doi.org/10.3136/nskkk.45.52
33) Murata M. Various pigments formed by Maillard reaction. Journal of the Brewing Society of Japan. 2022; 117: 66-75. (in Japanese)
https://doi.org/10.6013/jbrewsocjapan.117.66
34) Murata M. Maillard reaction and coloring/browning Chemistry that turns brown when sugar and amino acids react. *Kagaku To Seibutsu (Japan Society for Bioscience, Biotechnology, and Agrochemistry)*. 2019; 57: 213-220. (in Japanese)
https://doi.org/10.1271/kagakutoseibutsu.57.213
35) Sugihara K, Kato S. Anti-glycation effects of free amino acids. *Trace Nutrients Research*. 2023; 40: 103-106. (in Japanese)
https://doi.org/10.51029/jtnrs.40.0_103
36) Fujishiro T. Development of an artificial enzyme to establish a production system for a new antioxidant functional amino acid "L-cysteine persulfide". NEDO Young researcher industry collaboration platform. (in Japanese)
https://wakasapo.nedo.go.jp/seeds/seeds-3147/, Viewed date: 2024-10-28
37) Esaki H, Kawagishi S. Formation of *o*-dihydroxyisoflavones in fermented soybean foods and their antioxidant role. 2002; 97(1): 39-45. (in Japanese)
https://doi.org/10.6013/jbrewsocjapan1988.97.39
38) Lv L, Shao X, Chen H, et al. Genistein inhibits advanced glycation end product formation by trapping methylglyoxal. *Chem Res Toxicol*. 2011; 24: 579-586.
39) Silvan JM, Srey C, Ames JM, et al. Glycation is regulated by isoflavones. *Food Funct*. 2014; 5: 2036-2042.
40) Ministry of Health, Labour and Welfare. Dietary intake standards for Japanese people report of the Study Group for Formulation of the Dietary Intake Standards for Japanese People. 2020.
https://www.mhlw.go.jp/content/001151422.pdf
41) Wang H, Qian H, Yao W. Melanoidins produced by the Maillard reaction: Structure and biological activity. *Food Chem*. 2011; 128: 573-584.
42) Yamamoto Y, Munesue S. Effects of glycation reaction on lifestyle-related diseases and our health. *Journal for the Integrated Study of Dietary Habits*. 2015; 25: 237-240. (in Japanese)
43) Inoue T. About white soy sauce. The Japan Soy Sauce Technology Center. 2007; 102: 24-30. (in Japanese)
https://doi.org/10.6013/jbrewsocjapan1988.102.24
44) Ibe S, Yoshida K, Kumada K. Angiotensin I-converting enzyme inhibitory activity of natto, a traditional Japanese fermented food. *Nippon Shokuhin Kagaku Kogaku Kaishi*. 2006. 53: 189-192. (in Japanese)
https://doi.org/10.3136/nskkk.53.189