

Original article

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

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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 AGEs¹. 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 diseases², skin aging³, osteoporosis⁴, Alzheimer's dementia⁵, and arteriosclerosis⁶.

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

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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 etiologies⁷⁾. 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 differentiation¹⁰⁾. 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 spleen¹¹⁾. 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-fermented¹⁴⁾, and clear¹⁵⁾. 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 Da¹⁶⁾. However, the structure of melanoidins has not been elucidated. Melanoidins have the effect of suppressing elevated blood glucose levels¹⁷⁾, strong antioxidant activity¹⁸⁾, 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 products²²⁾. In this study, we examined activities of anti-glycation (AGE formation inhibition) and anti-oxidation (DPPH [2,2-diphenyl-1-picrylhydrazyl] radical scavenging) of soy sauce ingredients *in vitro*.

Methods

(1) Reagents

Human serum albumins (HSA, lyophilized powder, $\geq 96\%$, 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 hours²³⁾.

(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 used²⁸. 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:

$$\text{AGE formation inhibition rate (\%)} = 100 - \{(\text{sample Glucose (+)} - \text{sample Glucose (-)}) / (\text{ref Glucose (+)} - \text{ref Glucose (-)}) \times 100\}$$

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 $18.84 \pm 3.53\%$ (mean ± SD) and $37.24 \pm 7.82\%$, 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-picrylhydrazyl.

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 μ mol-Trolox equivalent/mL, and No.13 - A was 14.12 ± 11.24 μ mol-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 report³⁴.

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

	Final Concentration (mg/mL)	Inhibition rate of fluorescent AGEs	IC ₅₀
Fraction A)	0.61	44.16 ± 5.56	0.73
	1.21	66.12 ± 8.05	
	2.42	85.34 ± 7.93	
Fraction B)	0.50	35.05 ± 3.92	0.98
	0.99	47.07 ± 6.70	
	1.98	68.93 ± 5.24	
Fraction C)	0.23	44.64 ± 2.10	0.27
	0.46	66.93 ± 1.93	
	0.70	82.15 ± 1.25	
AG			0.056

Data: mean \pm 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.

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 \pm SD), 26.7 ± 1.8 , and $60.1 \pm 3.2\%$ 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 μ mol-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

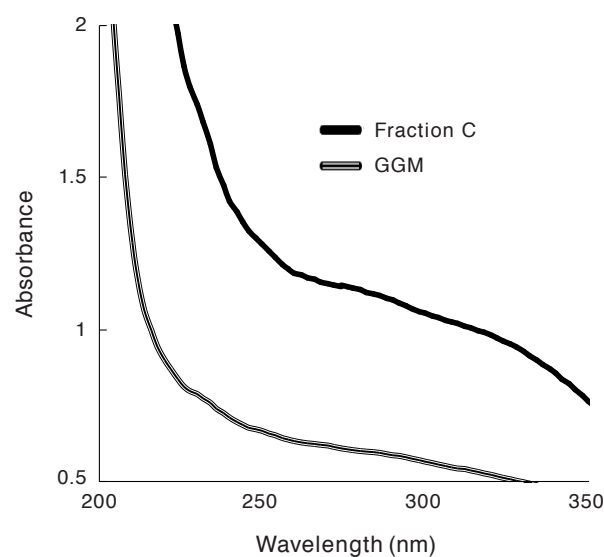


Fig. 1. Absorption spectrum of soy sauce fraction C and GGM.

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 women⁴⁰. 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] **] **
Fraction B)	26.38	±	12.17	
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 ($\mu\text{mol-Trolox equivalent/mL}$) in 2.0 mg/mL of each fraction. DPPH, 2, 2-diphenyl-1-picrylhydrazyl.

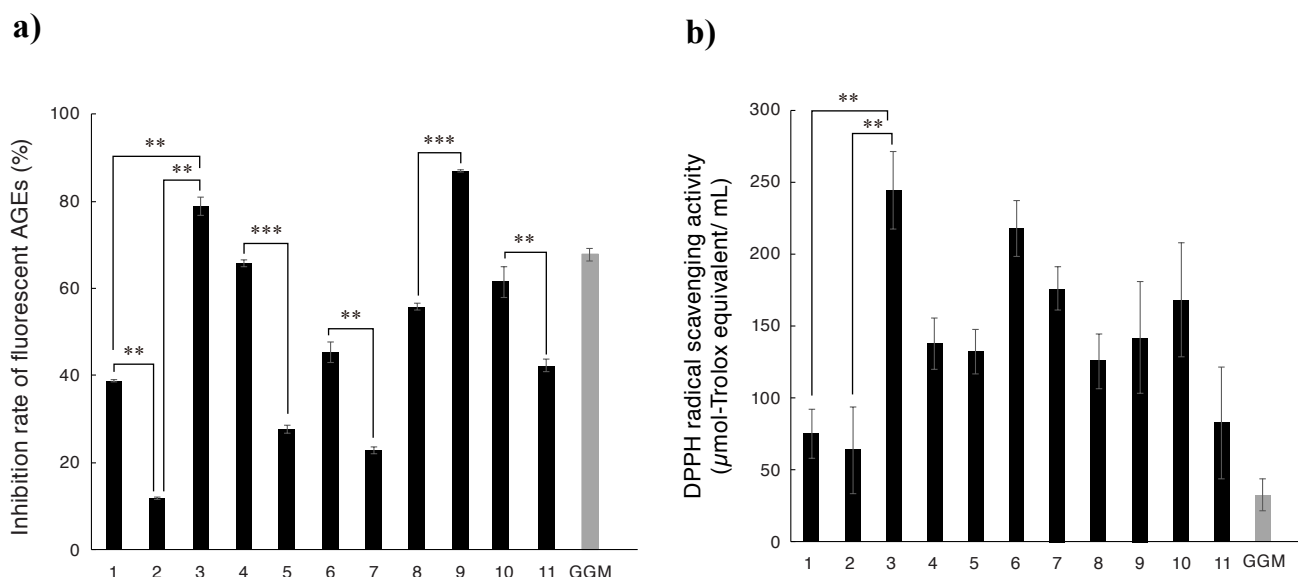


Fig. 2. Inhibition/scavenging effects of soy sauce melanoidins on AGEs and DPPH radicals.

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-picrylhydrazyl.

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 hyperglycemia¹⁷⁾ 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 hypertension⁴³⁾, one of the causes of hyperglycemia, varies depending on the fermentation time⁴⁴⁾. 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 products¹⁶⁾ 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.

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