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Original article

Anti-glycative stress effect of yogurt whey

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Abstract

***Purpose*:** Glycation is a non-enzymatic reaction between reducing sugars and amino group proteins and forms advanced glycation end products (AGEs). AGEs are accumulated in diverse tissues and organs. Along with aging, AGEs induce inflammations, pigmentations and deuteration of physiological functions, and is involved in the onset and progression oflifestyle-related diseases. This study verified the inhibitory effects of yogurt whey on glycative stress.

***Methods*:** Plain yogurts were obtained in markets and whey was collected via centrifugal separation. Whey was then added to human serum albumin (HSA)-Glucose glycation models. After thermal reaction, inhibitory effects on glycation were measured: formations of fluorescent AGEs, pentosidine, N^{ε}-carboxymethyllysine (CML) and intermediates for AGEs. Inhibitory effects on protein carbonylation were examined after whey was added to HSA-Acrolein in reaction models and incubated at a high temperature. Antioxidative effects were measured employing the DPPH method. Inhibitory effects on AGE cross-linking were evaluated, using SDS-PAGE, in Lysozyme-Fructose glycation cross-linking models, in which whey was added and was thermally treated. Whey was added to pig skin glycation models and proteins were extracted. Inhibitory effects on skin glycation such as on AGEs formation and yellowish pigmentation were evaluated by glycation models using pig skin.

***Results*:** Yogurt whey had strong inhibitory effects on the formation of fluorescent AGEs, pentosidine, and CML by mean inhibitory ratios of, respectively, 50.0%, 82.3%, and 64.9%. Whey inhibited the formation of glyoxal and methylglyoxal, which are intermediates for AGEs, by 48.6% and 56.8%, respectively. Furthermore, whey had weak DPPH radical-scavenging activity and had inhibitory effects on skin protein glycations of AGE formation and yellowing. Polymerization of AGEs glycated lysozyme due to fructose was inhibited by whey.

***Conclusion*:** It was suggested that yogurt whey is a food that had inhibitory effects on glycative stress, having effects of anti-glycation, AGE cross-linking inhibition, and antioxidation.

***KEY WORDS*:** yogurt, whey, glycation, advanced glycation end products (AGEs)

Introduction

Reducing sugars, such as glucose and fructose, are indispensable nutrients for biological activities. These reducing sugars react non-enzymatically with amino group proteins in the body. This reaction is referred as glycation. Advanced glycation end products (AGEs) are formed via glycative intermediates [1]. AGEs are accumulated in diverse tissues and organs along with aging, which induces inflammations, pigmentations and deuteration of physiological function. Moreover, AGEs accumulation is involved in the onset and progression of lifestyle-related diseases, such as diabetic complications, ophthalmic diseases [2], skin aging [3], osteoporosis [4], Alzheimer's type dementia [5], and arteriosclerosis [6]. The concept of the series of risks of aging and diseases due to glycation is referred as glycative stress. Currently, glycative stress has been confirmed as a risk factor to promote aging.

Yogurt is a fermented dairy food produced by adding lactic bacteria and yeast to milk such as *lac vaccinum*. It has been reported that fermented foods produced with lactic bacteria have beneficial functions, such as improving effects on intestinal environments, cancer risk reduction, immunoregulatory activity, and antihypertensive effect [7].

To prevent glycative stress, the inhibition of hyperglycemia, the inhibition of glycation, and the degradation and

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discharge of glycation reaction products would be effective[8]. We have previously reported that yogurt whey, supernatant, had inhibitory effects on glycation [9]. The present study, focusing on inhibitory effects against glycative stress by yogurt whey, examined the inhibitory effects on glycation and protein carbonylation, radical-scavenging activities of DPPH (1,1-diphenyl-2-picrylhydrazyl,) α-glucosidase-inhibiting action, inhibitory effects on AGE formation and yellowish pigmentation evaluated by glycation models using pig skins, and inhibitory actions on protein cross-linking.

Methods

1. Samples

The proteins in glycation models were human serum albumins (HSA) (lyophilized powder, ≥ 96%, agarose gel electrophoresis), which were purchased from Sigma-Aldrich Japan (St. Louis, MO, USA). Other samples, which were at special or HPLC grade, were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan) or Nacalai Tesque, INC. (Kyoto, Japan).

2. Sample preparation

Table 1 shows a yogurt profile of 19 product names purchased in supermarkets in Kyoto, Japan. Each yogurt was sufficiently mixed in the product container and poured into a 50 mL tube. Subsequently, samples were centrifuged at 3,000 rpm for 15 minutes at 20°C and whey (supernatant liquid) was used for the experiments. A solid concentration (mg/mL) of whey was obtained after 3 mL of whey was placed on an aluminum tray and the weights were measured before and after dehydration at 120°C for 120 minutes. Samples were prepared for the experiments to dilute with purified water to

Table 1. Yogurt profile.

No	Product name	Manufacturer / Seller	Characteristics
1	Bulgaria yogurt	Meiji (Tokyo, Japan)	LB81 (*Lactobacillus bulgaricus* 2038, *Streptococcus thermophilus* 1131)
2	Bifidus plain yogurt	Morinaga Milk Industry (Tokyo, Japan)	*Bifidobacterium longum* BB536
3	Bifidus plain yogurt fat zero	Morinaga Milk Industry (Tokyo, Japan)	*Bifidobacterium longum* BB536
4	Caspian sea yogurt	Fujicco (Kobe, Japan)	*Lactococcus lactis* subsp. *cremoris* FC
5	Koiwai namanyu 100% yogurt	Koiwai Dairy Products (Tokyo, Japan)	*Bifidobacterium lactis* BB12
6	Nature megumi plain yogurt	Megmilk Snow Brand (Tokyo, Japan)	*Lactobacillus gasseri* SP, Bifidobacteria SP
7	Nature megumi fat zero plain yogurt	Megmilk Snow Brand (Tokyo, Japan)	*Lactobacillus gasseri* SP, Bifidobacteria SP
8	BifiX yogurt	Ezaki Glico (Osaka, Japan)	*Bifidobacterium animalis* ssp. *lactis* GCL2505
9	Rakunou yogurt low fat plain	Nippon Dairy (Osaka, Japan)	*Bifidobacterium lactis* BB12
10	TOPVALU probiotics plain yogurt	AEON	Bifidobacteria, Casei bacteria, Acidophilus
11	Meiji probio yogurt LG21 sugar zero	Meiji (Tokyo, Japan)	*Lactobacillus gasseri* OLL2716
12	Meiji probio yogurt R-1 sugar zero	Meiji (Tokyo, Japan)	*Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1073R-1
13	TOPVALU greek yogurt plain	AEON	Thermophilus, Bulgaricus
14	TOPVALU plain yogurt	AEON	Thermophilus, Bulgaricus
15	TOPVALU low fat probiotics plain yogurt	AEON	Bifidobacteria, Casei bacteria, Acidophilus
16	TOPVALU raw milk 100% plain yogurt	AEON	Bifidobacteria, Acidophilus, Thermophilus, Bulgaricus
17	Rakuren plain yogurt	Shikoku Nyugyo (Ehime, Japan)	Thermophilus, Bulgaricus
18	Bulgaria yogurt luxury creamy raw milk 100 plain	Meiji (Tokyo, Japan)	LB81 (*Lactobacillus bulgaricus* 2038, *Streptococcus thermophilus* 1131)
19	Bulgaria yogurt fat zero	Meiji (Tokyo, Japan)	LB81 (*Lactobacillus bulgaricus* 2038, *Streptococcus thermophilus* 1131)

50 mg/mL of solid concentration. Lactate was diluted with purified water to 10 mg/mL. Similarly, aminoguanidine (AG) was diluted to 1 mg/mL and acarbose was diluted to 3.3 mg/mL.

3. Verification of inhibitory effects on glycation

The glycation HSA-Glucose model was employed to verify inhibitory effects on glycation [10]. A glycation solution was prepared with 0.1 mol/L phosphate buffer solution (pH 7.4), 2.0 mol/L glucose, and 40 mg/mL HSA. Furthermore, adjusting final concentrations, 0.05 mol/L, 0.2 mol/L and 8 mg/mL, respectively, the sample was added to obtain a 1/10 concentration. Subsequently, the glycation solution was reacted at 60°C for 40 hours and AGE-derived fluorescence was measured by microplate reader (excitation wavelength of 370 nm, emission wavelength of 440 nm). A reference (ref) was prepared with the same amount of purified water in place of whey and reacted under the same conditions. As a positive control for fluorescent AGE inhibitory effect, AG, which is known as an AGE formation inhibitor, was employed, adding the same amount of AG (1 mg/mL) in place of the sample. The fluorescence intensity was calculated as the relative value when the fluorescence intensity of 5 μg/mL quinine sulfate was 1,000.

The ratio of inhibitory effect on fluorescent AGE formation (%)

=100 – {(sample Glucose (+) – sample Glucose (–)) /
(ref Glucose (+) – ref Glucose (–)) x 100}

Pentosidine was hydrolyzed using hydrochloric acid after the termination of glycation and the quantity was determined, using reversed-phase high performance liquid chromatography (HPLC) [11, 12].

For the measurement of CML, whey was added to HSA-Glucose glycation models, obtaining 0.5 mg/mL final concentration of whey. The CML formed during glycation was measured after the termination of glycation reaction, employing enzyme-linked immunosorbent assay (ELISA) with a measurement kit (CircuLex CML / N^{ε}-(carboxymethyl) lysine, MBL, Aichi, Japan).

Glycation intermediates formed during glycation were measured after termination of the reaction. 3-deoxyglucosone (3DG), glyoxal (GO) and methylglyoxal (MGO) were measured, employing reversed-phase HPLC after pre-labeling with 2,3-diaminonaphthalen (DAN) [13].

4. Verification of inhibitory effects on protein carbonylation

Sample solutions were prepared with 0.1mol/L phosphate buffer solution, 0.1 mol/L acrolein, and 40 mg/mL HSA. HSA-Acrolein reaction models were prepared, adjusting the final concentration of phosphate buffer solution to 0.05 mol/L, acrolein to 0.01 mol/L, and HSA to 8 mg/mL. Whey was added to obtain a final concentration of 25 mg/mL. Similarly, AG was prepared as a positive control to the final concentration of 0.5 mg/mL. Incubation time for HSA-Acrolein reaction models were two hours at 37°C. The quantity of protein carbonyl formed in the reaction solution was measured with 450 nm of absorbance, employing DNPH Alkaline method [14, 15]. DNPH Alkaline method is a measurement where DNPH binds with carbonyl groups of a protein, protein-hydrazone is formed, the addition of NaOH induces alkalization, and the maximum absorption wavelength is shifted to 450 nm.

5. Verification of DPPH radical-scavenging activity

DPPH radical-scavenging activity was assessed with a calculation referring to Trolox equivalent antioxidant capacity [16, 17]. Dispensing 0～16 nmol/assay Trolox and 25～100 μL/assay sample into a 96-well plate, adding 200 mmol/LMES buffer solution and 800 μmol/LDPPH solution, they were reacted for 20 minutes. Absorbance with 520 nm was measured by a plate reader. DPPH radical-scavenging activity was evaluated with the gradient of regression line, which was obtained by Trolox. DPPH radical-scavenging activity was obtained corresponding to the quantity of Trolox for the equivalent of the added sample.

6. Verification of inhibitory effect on α-glucosidase

Inhibitory effects on α-glucosidase were measured using α-glucosidase derived from rat small intestine and *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG) [18]. Samples were prepared with 50 mmol/L phosphate buffer solution (including 100 mmol/L NaCl, pH 7.0), 7mmol/L *p*-NPG and 0.5 mol/L Tris. Small intestinal acetone powder from rats (Sasaki Chemical, Kyoto, Japan) was homogenized in 50 mmol/L phosphate buffer solution (including 100 mmol/L NaCl, pH 7.0) and centrifuged (13,000 x g, 20 minutes, 4°C) to obtain supernatant liquid. Consequently, α-glucosidase solution was prepared from this supernatant. The positive control was 3.3 mg/mL acarbose. The sample and α-glucosidase solution were dispensed into a tube and preliminary reaction was at 37°C for 5 minutes. Subsequently, 0.7 mmol/L *p*-NPG was added and reacted for 30 minutes. Subsequently, after adding 0.5 mol/L Tris, the reaction was stopped. Some of the reaction solution was dispensed into a 96-well plate and 400 nm of absorbance was measured. Thus, the inhibitory effect on α-glucosidase was calculated.

7. Verification of inhibitory effects on AGE formation and yellowish pigmentation evaluated by glycation model using pig skin

Glycation models using pig skin were prepared as follows [19]. Pet goods from a market (bone-shaped pork gum, Kohnan Shoji Co., Ltd., Osaka, Japan) were used for dried pig skin. After the dried pig skins were soaked in purified water for one night, they were reacted at 60°C for eight days, treated by immersion in a liquid mixture, which consisted of glycation liquid (0.1 mol/L phosphate buffer solution and 2 mol/L glucose) and the sample solutions (whey or purified water).

After the reaction, pig skins were cut into 1 cm square pieces, which were homogenized in 0.1 mol/L phosphate buffer solution of 800 μL. The homogenized solution of 50 μL was added by 5 N NaOH of 250 μL and was treated with ultrasonication (15 minutes) and then centrifugal separation (10,000 x g, 10 minutes). Collected supernatant liquid was used as pig skin extract. The protein content of the pig skin extraction liquid was measured by DC protein assay. Pig skin extract was evaluated for both the yellowness in the absorbance measurement (400 nm) and AGEs content in

fluorometric measurement (Ex 370 nm/Em 440 nm). The degree of yellowness and the AGEs content was calculated per 1 mg of protein content.

8. Verification of inhibitory action on protein cross-linking

Lysozyme-Glucose/Fructose glycation models were used for the examination of protein cross-linking [20, 21]. Preparing 0.2 mol/L of phosphate buffer solution, 3 mol/L glucose/fructose or 5 mol/L glucose/fructose, and 50 mg/mL lysozyme, final concentrations were 0.1 mol/L for phosphate buffer solution, 0.3 or 0.5 mol/L for glucose or fructose, and 5 mg/mL for lysozyme to produce Lysozyme-Glucose/Fructose glycation models. The sample was added at a concentration of 1/10. Subsequently, glycation models were incubated at 60°C for 40 hours and separated by SDS-PAGE (Sodium dodecyl sulfate-Polyacrylamide gel electrophoresis) with a separation gel concentration of 15%. A protein band was detected with CBB (Coomassie Brilliant Blue) staining. To quantify band intensity, image analysis was performed using Image J (NIH, Bethesda, MD, USA) [22, 23].

9. Analysis method

The measured values were expressed as the mean ± standard deviation. The measurement was conducted by using doublet specimens (n = 2) or triplet specimens (n = 3).

Results

Inhibitory effects on formation of AGEs and glycation intermediates

Inhibitory effects of yogurt whey and aminoguanidine (AG) on the formation of AGEs and glycation intermediates are shown in ***Fig. 1***. All of the 19 types of whey inhibited the formation of fluorescent AGEs and pentosidine. Yogurt of sample number 3 showed the highest inhibitory ratio on the formation of AGEs (60.5 ± 3.4%). Sample number 1 showed the highest ratio on the pentosidine formation (89.8 ± 14.8%, ***Fig. 1-a, b***). As for CML, inhibitory effects were observed excluding sample number 15. The whey of sample number 6 showed the highest ratio for inhibitory effect on the CML formation (99.1 ± 0.1%, ***Fig. 1-c***). Lactic acid inhibited the fluorescent AGE formation (29.5 ± 1.1%) and the pentosidine formation (49.0 ± 4.4%).

Regarding glycation intermediates, all of the 19 yogurt wheys inhibited the GO and MGO formation, except for 3DG. The mean ratios of inhibition on 3DG, GO and MGO formation were –18.3± 9.1%, 48.6 ± 15.9%, and 56.8 ± 11.0%, respectively (***Fig. 1-d***) Lactic acid inhibited only MGO formation (25.3 ± 3.3%).

Inhibitory effects on protein carbonylation

Figure 2 shows the inhibitory effects of whey and AG on protein carbonylation. Mean ratio of inhibition in protein carbonylation was 4.3 ± 15.3% among the 19 whey types.

DPPH radical-scavenging activity

Figure 3 shows DPPH radical-scavenging activity of whey. Mean ratio of DPPH radical-scavenging activity was 52.3 ± 19.3 μmol-Trolox equivalent /L among 19 whey types.

Inhibitory effect on α-glucosidase

Figure 4 shows inhibitory effects on α-glucosidase of yogurt whey as a positive control of acarbose (final concentration: 0.099 mg/mL). The whey that had the largest effect was sample number 4, showing 19.6% of α-glucosidase inhibition ratio. The other 18 whey types indicated a 10% or lower inhibition of α-glucosidase.

Inhibitory effects of pig skin glycation model on AGEs formation and yellowish pigmentation

Figure 5-a shows an image of the pig skin glycation models on the 5th day after the commencement of an incubation. Changes of AGE content are shown along with time in ***Fig. 5-b***. Its time changes of yellowing are shown in ***Fig. 5-c***. Time-dependent changes of Glucose (+)-pig skin extraction model increased in terms of both the amount of fluorescent AGEs and the degree of yellowness. In the pig skin Glucose (+)-purified water model, fluorescent AGE content increased by twice and yellowness increased by 1.6 times on the 8th day following commencement of incubation, compared with pig skin in Glucose (+)-yogurt whey. Furthermore, pig skin immersed in Glucose (+) purified water model became stiffer than that in Glucose (+)-whey.

Inhibitory action on protein cross-linking

Figure 6-a shows the analysis results of Lysozyme-Glucose/Fructose glycation model by SDS-PAGE. The relative strength of each band is shown, considering the strength of 0 mol/L Glucose/Fructose as 1 (***Table 2-a***). Monomer of lysozyme with 14,307 of molecular weight was detected with approximately 11 kDa. Dimer of lysozyme, due to a cross-linked polymer, was detected with approximately 25 kDa. Trimer was detected above 35 kDa. In comparison between Lane 2 – 3 and Lane 4 – 5, intensity of the band was higher in Lane 4 – 5 (fructose), as shown in ***Table 2-a***. In comparison between Lane 4 and Lane 5, Lane 5 (0.5 mol/L fructose) indicated a higher band intensity: 1.5 times in trimer and 1.2 times in dimer in ***Table 2-a***.

The results of SDS-PAGE of whey-added models of Lysozyme-Fructose glycation model are shown in ***Fig. 6-b***, ***Table 2-b*** represents relative intensity of bands of Whey-added glycation models, considering band intensity of Lysozyme-0.5 mol/L Fructose glycation model as 100. Yogurt whey inhibited protein cross-linking in trimer (93.1 ± 3.4%) and in dimer (68.5 ± 4.5%). These data suggested that yogurt whey inhibited polymerization due to protein cross-linking.

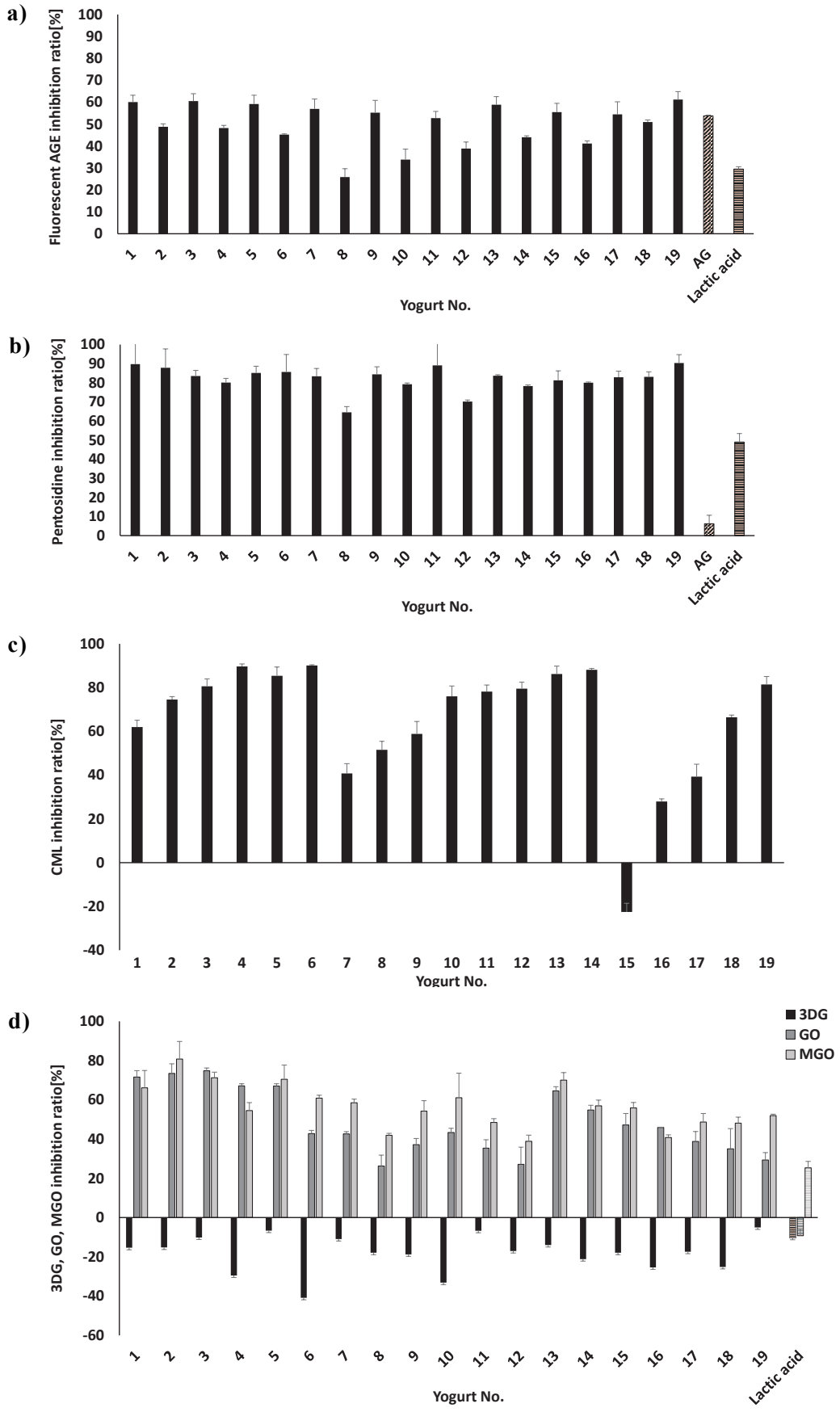

Fig. 1. **Inhibitory effect of yogurt whey on AGEs and intermediate formation in the HSA-Glucose reaction model.**

Results are expressed as mean ± SD. Yogurt whey were introduced into glycation models containing 8 mg/mL HSA and 0.2 mol/L glucose (n = 3). After 40-hour incubation at 60°C, **(a)** fluorescent AGEs were measured by Ex 370 nm / Em 440 nm, **(b)** pentosidine were measured by HPLC, **(c)** CML were measured by ELISA, **(d)** 3DG, GO, MGO were measured by HPLC. Final concentration of whey was 5 mg/mL (except for **(c)**: 0.5 mg/mL). Final concentration of AG was 0.1 mg/mL. Final concentration of Lactic acid was 1 mg/mL. AGEs, advanced glycation end products; HSA, human serum albumin; HPLC, high performance liquid chromatography; CML, N^{ε}-carboxymethyllysin; 3DG, 3-deoxyglucosone; GO, glyoxal; MGO, methylglyoxal; AG, aminoguanidine; SD, standard deviation.

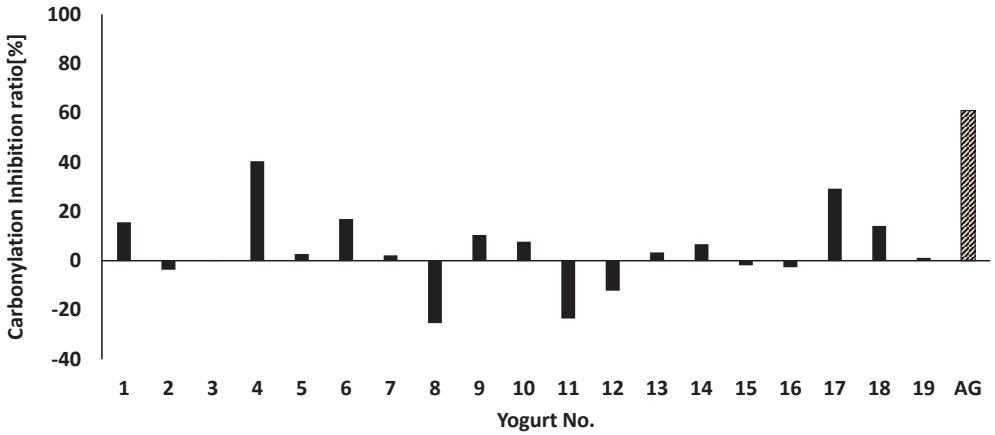

Fig. 2. **Inhibitory effect of yogurt whey on carbonylation in the HSA-Acrolein reaction models.**

Yogurt whey were introduced into carbonylation models containing 8 mg/mL HSA and 0.01 mol/L acrolein (n = 2). After 2-hour incubation at 37°C, measured by absorbance at 450 nm (final concentration of whey: 25 mg/mL, AG: 0.5 mg/mL). HSA, human serum albumin; AG, aminoguanidine.

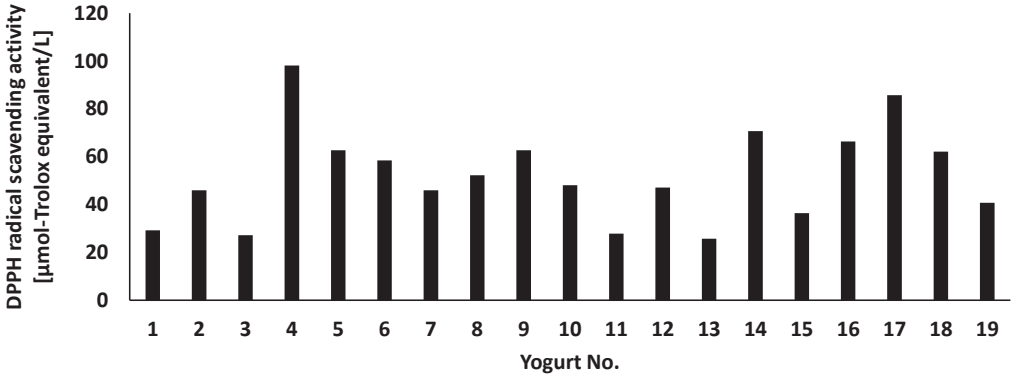

Fig. 3. **Anti-oxidative activity of yogurt whey.**

DPPH radical scavenging activity of whey was measured by a method of calculating the equivalent amount of Trolox using it as a standard substance (n = 2). DPPH, 1,1-diphenyl-2-picrylhydrazyl.

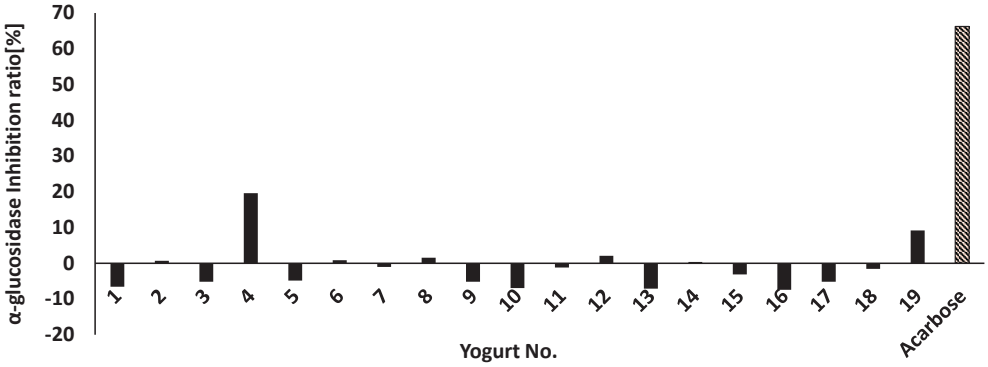

Fig. 4. **α-glucosidase inhibitory action of yogurt whey.**

After adding whey to 0.9 U/mL α-glucosidase solution, 0.7 mol/L p-nitrophenyl-α- D-glucopyranoside (p-NPG) was added and reacted (n = 2). After 30 min incubation at 37°C, measured by absorbance at 400 nm (final concentration of whey: 1.5 mg/mL, acarbose: 0.099 mg/mL).

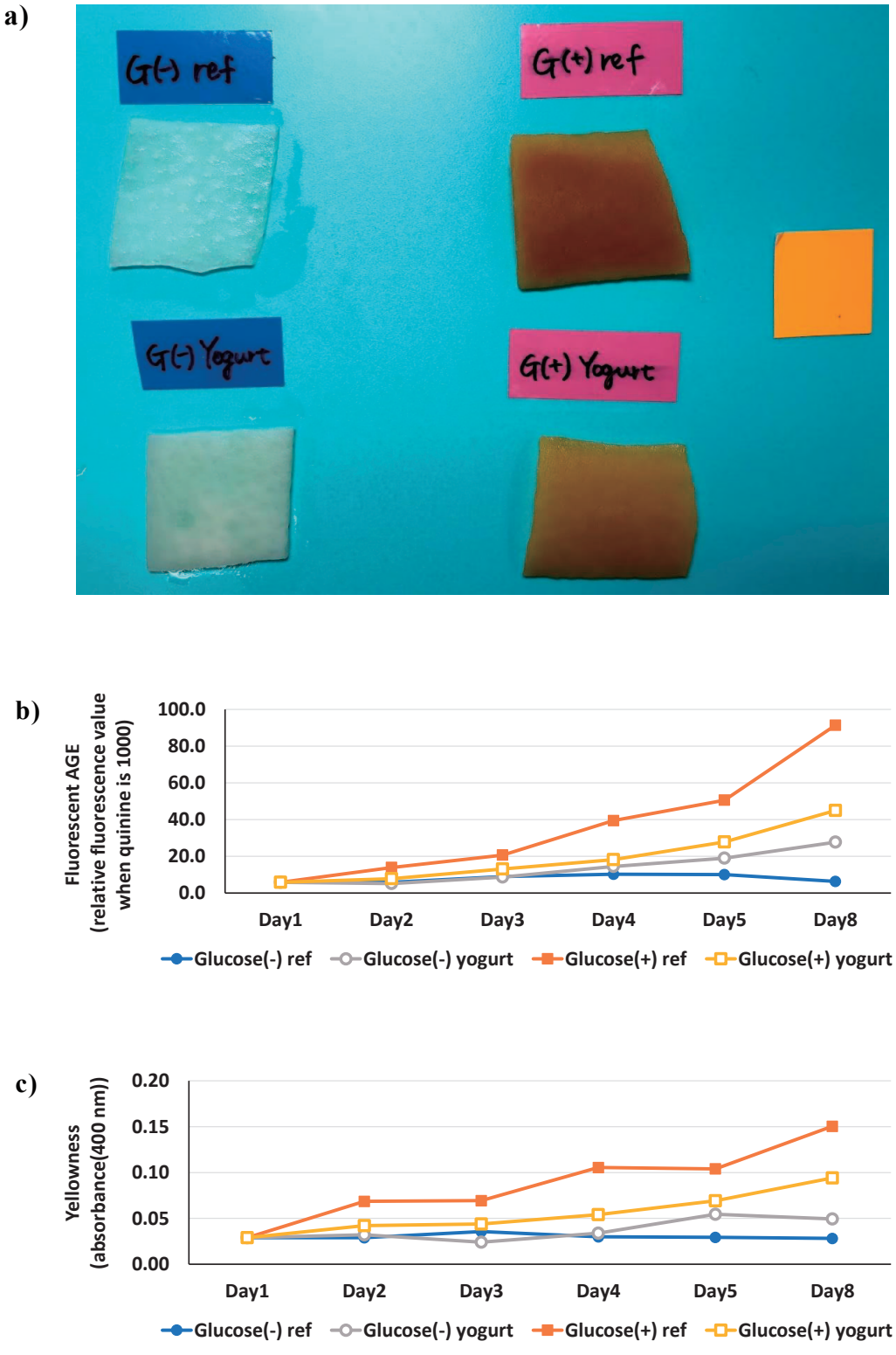

Fig. 5. **Glycative models of pig skin.**

The pig skin was immersed in 2 mol/L glucose solution and reacted at 60°C for 8 days. **(a)** Representative images fifth day. **(b, c)** Inhibitory effect of Yogurt whey (No.1) on AGEs and yellowness in the glycative pig skin model. Whole protein was extracted from pig skin, fluorescent AGEs were measured by 370 / 440 nm and yellowness were measured by absorbance at 400 nm (n = 2). AGEs, advanced glycation end products.

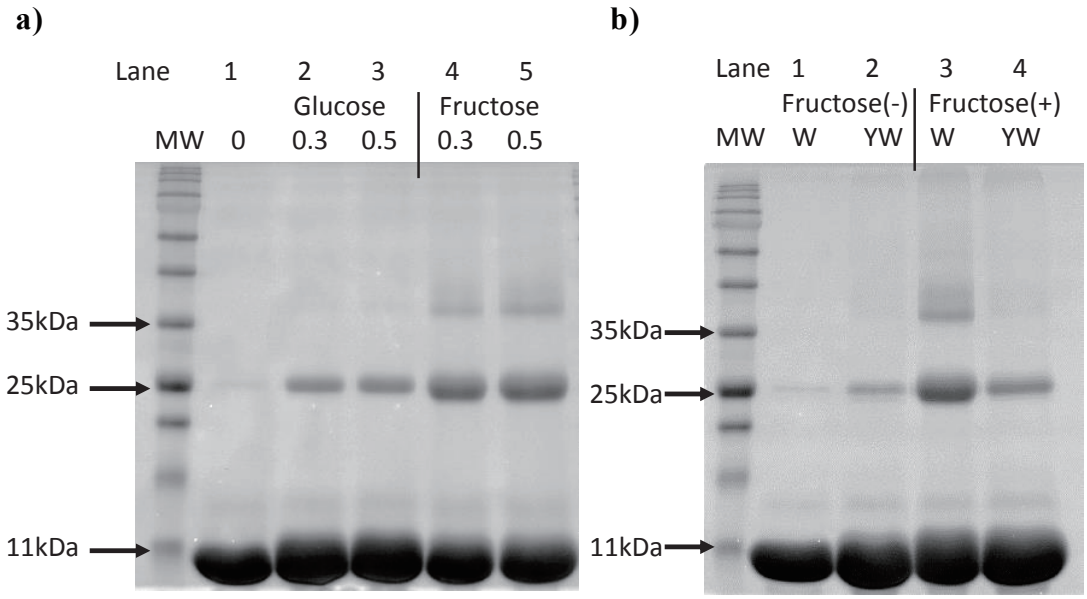

Fig. 6. **(a) Effect of glucose and fructose on cross-linking of proteins. (b) Inhibitory effect of yogurt whey on cross-linking of lysozyme.**

5 mg/mL lysozyme were incubated at 60°C for 40 hours **(a)** with glucose or fructose at concentrations of 0, 0.3 and 0.5 mol/L and **(b)** with or without 0.5 mol/L fructose in the presence or absence of whey (No.1). SDS-PAGE was conducted using 15% acrylamide gels. MW, molecular weight markers; W, incubation without whey (with water); YW, incubation with whey (No.1).

Table 2-a. Band relative strength (Lane 1 = 1).

Lane	1	2	3	4	5
Glucose / Fructose	0	0.3 Glc	0.5 Glc	0.3 Fru	0.5 Fru
Trimer	1	3.9 ± 2.3	5.5 ± 4.5	30.0 ± 17.7	46.3 ± 27.6
Dimer	1	16.5 ± 5.5	18.8 ± 5.6	37.6 ± 11.6	44.7 ± 17.3

Band intensity was analyzed by ImageJ. Glu, glucose; Fru, fructose; 0.3, 0.3 mol/L; 0.5, 0.5 mol/L.

Table 2-b. Cross-linking formation ratio (W=100).

Lane	W	YW
Trimer	100	6.9 ± 3.4
Dimer	100	31.5 ± 4.5

Band intensity was analyzed by ImageJ. Method of calculation: cross-linking ratio = (Fru(+)YW – Fru(–)YW)/ (Fru(+)W – Fru(–)W) x 100. W, incubation without whey (with water); YW, incubation with whey (No.1).

Discussion

Inhibitory effects of yogurt whey on glycation

Inhibitory effects on AGE formation were recognized in all of 19 types of whey. They had strong effects, particularly, on pentosidine and the inhibition ratio was 82.3 ± 6.2%. In terms of the formation of glycation intermediates, all of the 19 products had inhibitory effects on GO and MGO formation, although 3DG formation was not inhibited. Inhibitory activities on protein carbonylation were minor overall and the inhibition ratio was 4.3 ± 15.3%.

We have already evaluated anti-glycative effects of 500 types or more plant-derived materials [13, 24-27]. We have also reported that dairy products have inhibitory effects on the formation of fluorescent AGEs [9]. The present study demonstrated that yogurt whey had inhibitory activities on the formation of glycation intermediates for AGEs and pentosidine as well as fluorescent AGEs. The inhibitory effects that whey had on glycation were distinctive in some respects, compared with plant-derived materials. There is a tendency that plant-derived materials have a strong inhibiting effect on 3DG formation and DPPH radical-scavenging activities [28-30]. The reason is that plants contain polyphenol that has antioxidative effects, which scavenge free radicals and trap carbonyl [31, 32], as has been recognized. Yogurt whey contains no polyphenol so 3DG formation might not be inhibited and DPPH radical activity was sluggish.

In the present study, we explored components in yogurt whey to inhibit glycation. In 50 mg/mL of whey, lactic acid level was 10.9 ± 4.6 mg/mL, determined by Lactate Pro 2 (Arkray Inc., Kyoto, Japan) (data not shown). Lactate contained in yogurt whey was examined for inhibitory effects on glycation, referring the final concentration 1 mg/mL lactate, which was equivalent lactate amount with a final concentration of 5 mg/mL whey during the HSA-Glucose glycation model experiment. The lactate inhibited fluorescent AGEs (29.5 ± 1.1%), pentosidine (49.0 ± 4.4%) and MGO (25.3% ± 3.3%) formation. From these analysis results, lactate was considered to be one of components that inhibited glycation reaction. However, the contribution ratios of whey regarding fluorescent AGEs, pentosidine, and MGO were 59.0%, 59.4% and 44.6%, respectively (mean whey values of the 19 products). These findings suggested that there would be components in whey for glycation inhibitory effects other than lactate.

Inhibitory effects of Yogurt whey on glycation from a viewpoint of skin aging prevention

Yogurt whey has been recognized to have beneficial effects on the skin such as brightening effect, moisturizing effect, and prevention of rough skin. Whey application on the skin has been treated as a folk remedy. We verified anti-glycation effects of yogurt whey from a viewpoint of skin aging prevention in the present study. As a result, yogurt whey inhibited yellowing of skin models (***Fig. 5***). It has been reported that dermal carbonyl modification induces the yellowish color changes of the skin due to aging [33]. However, the present study showed that the effectiveness of yogurt whey was marginal for inhibition of protein carbonylation and the mean value of inhibition ratio was 4.3 ± 15.3% (***Fig. 2***). Contrarily, whey inhibited AGE glycated protein of the skin models (***Fig. 5***). These findings suggested that there were other causes of skin yellowing besides protein carbonylation, and whey had the preventing effects on skin yellowing caused by glycation.

In experiments using skin models, yogurt whey inhibited hardening of skin due to glycation. It has been reported that glycation induces collagen protein cross-linking and cross-linked protein causes skin to lose elasticity [34, 35]. As a result of the experiments of Lysozyme-Fructose glycation models, inhibitory effects of whey on protein cross-linking were shown to be present (***Fig. 6***). These findings suggested that inhibitory effects, which whey had on protein cross-linking, would be related to the inhibition of lowered elasticity in the skin models.

Inhibitory effects of yogurt whey on glycative stress

Furthermore, we investigated the correlations of inhibitory effects on glycative stress of whey. Strong positive correlations regarding inhibitory effects on formation were observed between fluorescent AGEs and pentosidine ($r = 0.79$, $y = 0.5092x + 56.789$), fluorescent AGEs and CML ($r = 0.92$, $y = 0.305x - 4.6222$), GO and MGO ($r = 0.82$, $y = 0.5681x + 29.168$). To name fluorescent AGEs, pentosidine, crossline, and pyrropyridine are mentioned [36, 37]. It was recognized that inhibition on formations of these substances and CML could be induced by the same component. The same components could induce inhibitory effects on GO and MGO formation.

Afterward, relations were examined between the fermentative bacteria of yogurt and the inhibitory effects on glycative stress. There existed varied genus, species, and strains in fermentative bacteria of 19 yogurt types (***Table 1***). However, no relations were observed between inhibitory effects on AGEs formation and genus, species, and strains in fermentative bacteria. No distinctions were observed between Lactobacillus and Bifidobacterium. Therefore, there was a possibility that raw materials and production methods such as fermentation time could be involved in the differences on inhibitions of AGE formation depended on yogurt whey types.

To prevent glycative stress, the inhibition of postprandial hyperglycemia (blood glucose spike), the inhibition on glycative products formation, and the degradation and discharge of glycation products would be effective [8]. It has been reported that intake of yogurt before eating cooked rice inhibited postprandial hyperglycemia [38]. The present study suggested that whey had marginal inhibiting effects on α-glucosidase so that the impact of α-glucosidase inhibiting effect by yogurt whey was marginal for the inhibition of postprandial hyperglycemia, as was considered.

The present study has suggested that yogurt inhibits the formation of AGEs. That is, it has been clarified *in vitro* and *semi-invitro*, yogurt inhibits glycative stress. Yogurt is a significant food, by oral ingestion, for inhibiting glycation reactions in the body and to maintain and promote skin health. Further investigation requires analyzing AGEs degradation *in vitro* and conducting animal and human experiments of yogurt ingestion to verify effectiveness on the skin for improvement in yellowing and elasticity.

Conclusion

The present study suggests that yogurt whey was a food with an anti-glycative stress effect by inhibiting glycative reaction, the inhibition of AGE cross-linking, and with an antioxidative effect.

Declaration of Conflict of Interest

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