

Glucosinolates and isothiocyanates from broccoli seed extract suppress protein glycation and carbonylation

Marina Hirano, Shogo Takeda, Shoketsu Hitoe, and Hiroshi Shimoda*

Research and Development Division, Oryza Oil & Fat Chemical Co., Ltd., 1 Numata, Kitagata-cho, Ichinomiya, Aichi 493-8001, Japan

Corresponding author: Hiroshi Shimoda, PhD., Research & Development Division, Oryza Oil & Fat Chemical Co., Ltd., 1 Numata, Kitagata-cho, Ichinomiya, Aichi 493-8001, Japan

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ABSTRACT

Background: Glucosinolates from brassica plants are hydrolyzed by internal or salivary myrosinase to produce isothiocyanates. Glucoraphanin, a major glucosinolate in broccoli, is hydrolyzed to sulforaphane (SFN), which exhibits antitumor and detoxification activities. Regarding the influence of broccoli and its constituents on the skin, a few studies have reported anti-inflammatory and antioxidant effects. Recently, advanced glycation end products (AGEs) and carbonyl proteins have been reported to accelerate skin aging.

Objective: To evaluate the effects of broccoli seed extract (BSE) and glucosinolates on protein glycation and carbonylation *in vitro*.

Methods: To evaluate the effects of BSE and its constituents, protein glycation and carbonylation were induced by mixing fructose with bovine serum albumin (BSA) and then

measuring production of AGEs, fructosamine, and carbonyl proteins (CP). Production of CP after mixing fatty acids with BSA was also assessed. Furthermore, the effect of BSE and its constituents on CP production by human fibroblasts (TIG103) was examined.

Results: BSE suppressed the production of AGEs, fructosamine, and CP after mixing fructose and BSA. BSE also suppressed production of CP when oxidized linoleic acid was mixed with BSA. Isothiocyanates, including SFN and iberin, suppressed fructose-based CP production, but SFN had no effect on CP production stimulated by oxidized linoleic acid. In contrast, glucosinolates from BSE did not suppress fructose-based CP production, but suppressed CP production due to oxidized linoleic acid. Among the glucosinolates in BSE, glucoberteroin showed the strongest suppression of CP production. CP production in fibroblasts was also suppressed by glucosinolates, including glucoiberin and glucoberteroin.

Conclusion: BSE demonstrated anti-glycation and anti-carbonylation effects on protein reactions with fructose and oxidized fatty acids. Isothiocyanates suppressed protein carbonylation induced by fructose, but not those induced by oxidized lipids. On the other hand, glucosinolates (precursors of isothiocyanates) did not suppress protein carbonylation induced by fructose, but suppressed carbonylation due to oxidized lipids. Moreover, glucoberteroin and glucoiberin suppressed CP production in a cell-based assay. These results suggest that glucosinolates in BSE could suppress protein carbonylation leading to aging of the skin.

Keywords: Broccoli, glucosinolate, sulforaphane, carbonylation, glycation, glucoberteroin, glucoiberin

BACKGROUND

Carbonyl proteins (CP) are produced by reactions between protein and various reactive aldehydes generated from peroxidation of fatty acids [1]. Reactive aldehydes are also synthesized by autoxidation of carbohydrates and subsequently induce protein glycation [2]. CP have been reported to be involved in many diseases, including dementia, chronic lung disease, chronic renal failure, diabetes, and sepsis [3]. In the skin, CP are mainly produced by the photoaging process [4] and generate reactive oxygen species (ROS) [5], which induce deterioration of dermal collagen. CP are involved in skin color changes during the aging process.

In the stratum corneum, production of CP leads to a darker skin color [4] and reduces water-holding capacity [6]. On the other hand, dermal production of CP is related to yellowing of skin [7] and reduced moisture content [8]. Accordingly, CP production due to photoaging is believed to result in deterioration of skin elasticity and changes of skin color.

Broccoli and sprouts contain ingredients with various health benefits due to anti-oxidant and detoxification activities [9]. Glucosinolates [10] are glucosides of isothiocyanates containing sulfur molecules, which are specifically found in brassica plants. The major glucosinolate in broccoli is glucoraphanin (GR), which has been reported to have several health benefits [11, 12]. Sulforaphane (SFN) is produced from GR by myrosinase [11] and has an active role in detoxification [13]. Besides GR, broccoli contains many other glucosinolates such as glucoiberin (GI) and sinigrin [9]. Regarding the effect of isothiocyanates and glucosinolates on the skin, SFN induces NF-E2 related factor 2 (Nrf2)-dependent anti-oxidant activity in UV-irradiated skin [14, 15] and also suppresses production of advanced glycation end products (AGEs) [16, 17]. Sinigrin, one of the glucosinolates in brassica plants, also suppresses production of AGEs [18]. However, the effects of isothiocyanates and glucosinolates on protein carbonylation have not been well studied. Consequently, we evaluated the effects of broccoli seed extract (BSE) and compounds isolated from BSE on protein carbonylation *in vitro*.

MATERIALS AND METHODS

Reagents

Bovine serum albumin, aminoguanidine hydrochloride (AG), 2,4-dinitrophenyl hydrazine (DNPH), *L*-(+)-ascorbic acid, linoleic acid, penicillin-streptomycin solution, and Dulbecco's minimum essential medium (DMEM) were purchased from Wako Pure Chemical Co. Ltd. (Osaka, Japan). D-(-)-fructose and trichloro acetic acid (TCA) were obtained from Kishida Chemical Co. Ltd. (Osaka, Japan). 1-Deoxy-1-morpholinofructose and 2-(*N*-morpholino) ethanesulfonic acid (MES) sodium salt were purchased from Sigma-Aldrich (St. Louis, USA). Fluorescein-5-thiosemicarbazide hydrochloride was obtained from CosmoBio Co. Ltd. (Tokyo, Japan). Fetal bovine serum (FBS) was obtained from Biosera (Rue, France). Sulforaphane was obtained from Toronto Research Chemicals Inc. (Toronto, Canada) and iberin was purchased from LKT LABS (St Paul, USA).

Preparation of BSE

Broccoli seeds (5 kg) were obtained from Suba Co. Ltd. (Longiano, Italy). Ground broccoli seeds were defatted twice with hexane (5 L) and then further defatted with chloroform (5 L). The

residue was extracted twice with 70% MeOH (10 and 5 L) at 70°C for 2 hr. The extracted solvent was evaporated to obtain a 70% MeOH extract, which was defined as BSE (445 g, yield 8.9%). The contents of glucoraphanin is 5.2%.

Isolation of constituents in broccoli seeds including glucosinolates

In light of published data [19, 20], glucosinolates and several other compounds (Fig. 1) were isolated from the 70% MeOH extract in the following way. The extract (200 g) was suspended in 20 mM acetate buffer (pH 4.2, 2 L) for 15 hr at 4°C to precipitate protein and the supernatant was evaporated to obtain deproteinized extract (188 g). This extract was applied to a DEAE Sephadex A25 (GE Health Care, UK) column (100 g as dry form) equilibrated with 20 mM acetate buffer. Next, the column was washed with water and was successively eluted with 1 L each of 0.1 M KNO₃, 0.2 M KNO₃, and 0.2 M KSO₄. Each eluted solution was evaporated, followed by extraction with hot MeOH (200 to 400 mL). Ice-cold EtOH (500-1000 mL) was added to the extracted MeOH solutions to obtain the recrystallized white 0.1 M KNO₃ fraction (Fr., 0.68 g), 0.2 M KNO₃ Fr. (3.2 g), and 0.2 M KSO₄ Fr. (3.8 g). The 0.2 M KNO₃ Fr. (2.0 g) was purified by reverse-phase HPLC (Inertsil ODS-SP, 20 φ × 250 mm; GL Sciences, Tokyo, Japan) with 7.5% MeOH containing 0.05% trifluoro acetic acid (TFA) to obtain GI [21] (142 mg, yield 0.11%), GR [22, 23], (8 mg, 0.0064%), glucoalyssin [24], (GA, 9 mg, 0.0072%), glucohesperin [22-24] (GH, 23 mg, 0.018%), glucosiberin [22-24] (GS, 46 mg, 0.037%), glucoerucin [25, 26] (GE, 16 mg, 0.013%), and glucoberteroin [25, 26] (GB, 12 mg, 0.0096%). Additionally, the 0.2 M KSO₄ Fr. (0.5 g) was purified by Inertsil ODS-SP with 7.5% MeOH containing 0.05% TFA to obtain GI (104 mg, 0.052%) and GR (493 mg, 0.25%).

To obtain other compounds, the 70% MeOH extract (85 g) was suspended in water (1 L), followed by extraction with ethyl acetate (1 L) and *n*-butanol (1 L). These solutions were evaporated to obtain an ethyl acetate Fr. (0.2 g), an *n*-butanol Fr. (3.8 g), and a water Fr. (80 g). The ethyl acetate fraction (0.2 g) was then purified by Inertsil ODS-SP with 70% MeOH to obtain sinapic acid [27] (SA, 79 mg, 0.093%), while the *n*-butanol fraction (1 g) was purified by Inertsil ODS-SP with 50% MeOH to obtain sinapine [23] (SP, 51 mg, 0.23%). A refractometer was used during purification of all compounds by HPLC. Isolated glucosinolates and other compounds were identified by comparing the ¹H and ¹³C-NMR spectra with reference values.

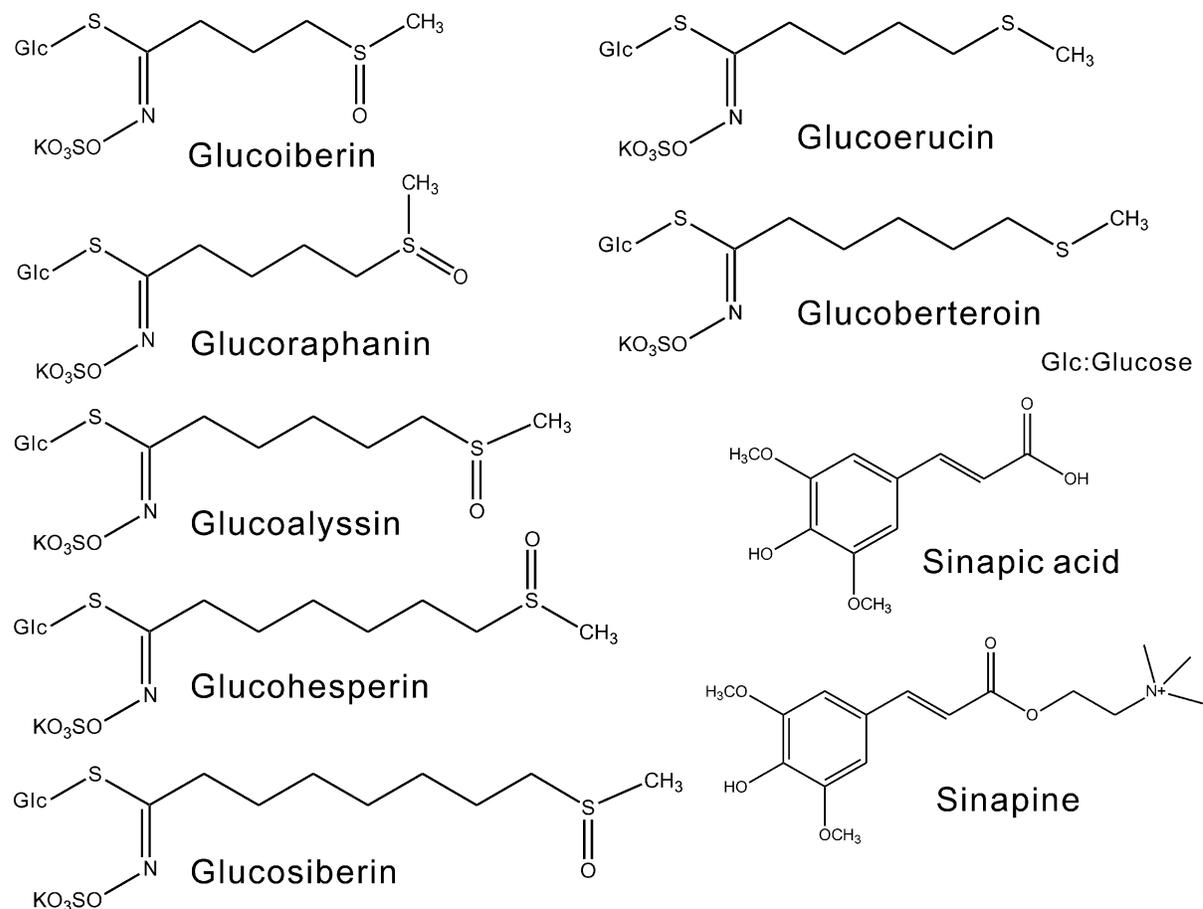


Figure 1. Compounds isolated from broccoli seeds

Glycation and carbonyl reaction in fructose/BSA mixtures

Mixtures (8 mL) containing BSA (10 mg/mL), 500 mM fructose, and a sample solution diluted in water (for BSE, glucosinolates, SP, and AG) or dimethyl sulfoxide (DMSO: for SA) were incubated at 37°C for 7 days. Mixtures without any sample solution were stored at 4°C as the un-reacted control (normal). For determination of fluorescent AGEs [28], the reaction mixture was diluted to 1/15 to 1/20 with water and fluorescence was measured at 460 nm (emission wavelength: 355 nm). For determination of fructosamine [28], the reaction mixture (10 μ L) was mixed with 90 μ L of 0.5 mM nitro blue tetrazolium (NBT) dissolved in 100 mM carbonate buffer (pH 10.4) and was incubated at 37°C for 10 min. Then the absorbance was measured at 530 nm using 1-deoxy-1-morpholino-fructose as the standard. For determination of CP [28], the reaction mixture (0.1 mL) was mixed with 10 mM DNPH solution in 2 M HCl (0.4 mL) and incubated at room temperature for 1 hr. Afterwards, 20% TFA (0.5 mL) was added, the mixture was let stand on ice for 5 min, and was centrifuged (4°C, 10 min, 11,000 \times g) to precipitate

protein. The precipitate was washed 3 times with 1 mL of a solvent consisting of ethyl acetate and ethanol (1:1). Then the precipitate was diluted in 6 M guanidine with 20 mM potassium phosphate dihydrogen (0.25 mL) and the absorbance was measured at 400 nm.

Carbonyl reaction in oxidized linoleic acid/BSA mixtures

To mixtures (900 μ L) consisting of a sample solution in water or DMSO, BSA (2 mg/mL), ascorbic acid (25 mM), and FeCl₃ (0.1 mM) in 50 mM HEPES buffer (pH 7.4), 100 mM oxidized linoleic acid (100 μ L) was added and incubation was performed for 15 hr at 37°C [29]. A mixture without a sample solution was stored at 4°C as the non-reacted control. CP was determined using a method of Levine et al. [30]. Briefly, 20% TFA (500 μ L) was added to the mixture after incubation and it was centrifuged (11,000 \times g) to precipitate protein. Then 10 mM DNPH in a 2% HCl solution (500 μ L) was added, the mixture was incubated (37°C, 1 hr), and then mixed several times. Next, 20% TFA (0.5 mL) was added and the mixture was centrifuged (11,000 \times g). The precipitate was washed 3 times with 1 mL of a mixture of ethyl acetate and ethanol (1:1), and was dissolved with 6 M guanidine in 20 mM potassium dihydrogenphosphate solution adjusted to a pH of 2.3 with TFA (0.6 mL) and incubated for 15 hr at 37°C. After debris in the mixture were sonicated and centrifuged, the absorbance was measured at 370 nm.

Carbonyl protein production in fibroblasts

Human diploid fibroblasts (TIG103 or JCRB0528 cells) obtained from the JCRB Cell Bank (Osaka, Japan) were pre-cultured (37°C, 5% CO₂) in DMEM containing 10% FBS and 1% penicillin-streptomycin solution. Harvested cells (5.0×10^4 cells / 100 μ L) suspended in DMEM containing 10% FBS were seeded onto a 96-well culture plate and incubated for 24 hr. Then the medium was exchanged for a fresh medium containing the sample solution diluted in water or DMSO and 0.1 mM acrolein, followed by culture for a further 6 hr. The cells were washed with warmed phosphate-buffered saline (PBS) and then fixed with ice-cold MeOH (100 μ L) for 10 min. Subsequently, the cells were washed twice or more with PBS and incubated for 1 hr after the addition of 20 μ M fluorescein 5-thiosemicarbazide in 0.1 M MES-Na. Finally, the cells were washed 3 times with PBS and fresh PBS was added before measurement of fluorescence (excitation: 485 nm, emission: 530 nm).

RESULTS

BSE and isothiocyanates suppress production of fluorescent AGEs, fructosamine, and CP in fructose / BSA mixtures

As shown in Figure 2A, BSE (100 and 300 µg/mL) and SFN (10 - 100 µg/mL) significantly suppressed production of fluorescent AGEs in mixtures of fructose and BSA. The inhibition rate was 68% in the presence of 100 µg/mL SFN. BSE (100 - 1,000 µg/mL) and SFN (10 - 100 µg/mL) also significantly suppressed fructosamine production in a concentration-dependent fashion (Figure 2B). The inhibition rate was similar for 100 µg/mL SFN and 1,000 µg/mL AG.

BSE (300 and 1,000 µg/mL) revealed significant suppression of CP production (Figure 2C). Although glucosinolates found in BSE (GI and GR) did not suppress CP production, isothiocyanates from BSE (IB and SFN) significantly suppressed it at both 30 and 100 µg/mL (Figure 2D).

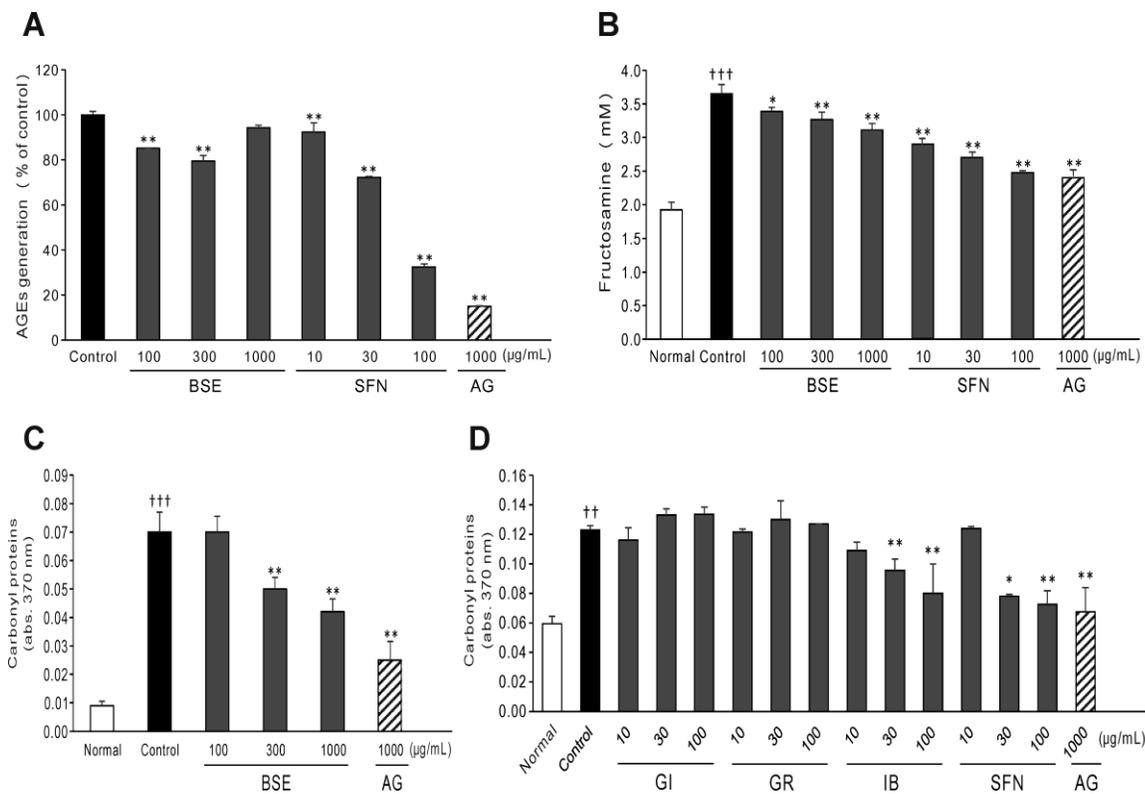


Figure 2. Suppressive effect of BSE, isothiocyanates (SFN and IB), and glucosinolates (GI and GR) on glycation and carbonylation products in fructose / BSA mixtures: Fluorescence of AGEs (A), fructosamine (B), and CP (C, D). Data are indicated as the mean ± S.E. (n = 3). Asterisks denote significant differences from the control by Dunnett’s test at *: $p < 0.05$, **: $p < 0.01$. Daggers indicate significant differences from the normal group by Student’s t -test at ††: $p < 0.005$, †††: $p < 0.001$.

BSE and several glucosinolates suppress production of CP in oxidized linoleic acid / BSA mixtures

BSE (100 and 1,000 µg/mL) significantly suppressed CP production in oxidized linoleic acid / BSA mixtures by approximately 49 and 66% respectively (Figure 3A). However, SFN did not suppress CP production even at 1,000 µg/mL (Figure 3B). Examination of the constituents of BSE (Figure 1) revealed that glucosiberin (GS) and glucoberteroin (GB) significantly suppressed CP production at both 100 and 1,000 µg/mL (Table 1). Additionally, CP production was significantly suppressed by glucoerucin (GE), glucoalyssin (GA), glucohesperin (GH), and glucoiberin (GI) at 1,000 µg/mL. However, glucoraphanin (GR) did not suppress CP production and SP actually enhanced CP production.

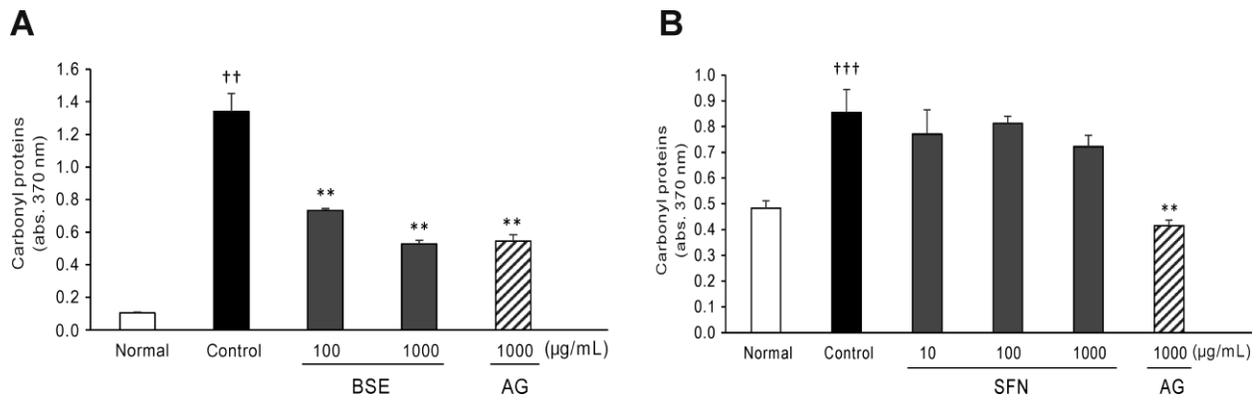


Figure 3. Suppressive effect of BSE (A) and SFN (B) on CP production in oxidized linoleic acid / BSA mixtures: Data are indicated as the mean ± S.E. (n = 3). Asterisks denote a significant difference from the control by Dunnett’s test at **: *p* < 0.01. Daggers indicate a significant difference from the normal group by Student’s *t*-test at ††: *p* < 0.005, †††: *p* < 0.001.

Table 1. Suppressive effect of glucosinolates and sinapne derivatives on CP production in oxidized linoleic acid/BSA mixtures.

Compounds	Inhibition ratio of CP (%)		
	0 µg/mL	100 µg/mL	1,000 µg/mL
GI	0.00±0.02	-1.1±0.02	33.0±0.99**
GR	0.00±0.04	17.2±1.00	31.7±1.76
GA	0.00±0.02	23.1±0.54	41.1±2.41**
GH	0.00±0.03	12.1±0.11	34.2±1.55**
GS	0.00±0.01	25.2±0.94**	66.0±1.24**
GE	0.00±0.03	-	79.7±0.58**
GB	0.00±0.04	36.9±0.96**	103.0±3.04*
SA	0.00±0.01	1.4±0.06	42.0±0.89
SP	0.00±0.04	-88.0±2.16**	-108.0±2.25**

Values represent the mean ± S.E. (n = 4). Asterisks denote significant differences from the untreated group (0 µg/mL) by Dunnett’s test at *: *p* < 0.05, **: *p* < 0.01

Suppression of CP production in human fibroblasts by glucosinolates derived from BSE

As shown in Table 2, BSE (10 µg/mL) barely suppressed the production of CP by cultured fibroblasts. Glucoiberin (GI) and glucoberteroin (GB) significantly suppressed CP production at 1 and 10 µg/mL. The other glucosinolates, SP, and sinapic acid had no suppressive effect on CP production. No toxicity of test sample was observed at 10 µM.

Table 2. Suppressive effect of BSE, glucosinolates, SFN, and sinapine derivatives on CP production by TIG103 fibroblasts.

Sample	Inhibition ratio of CP (%)	
	1 µg / mL	10 µg / mL
BSE	17.6±0.41	11.6±0.21*
GI	28.6±0.63**	48.2±1.31**
GR	3.0±0.04	-5.3±0.10
GA	-	
GH	7.8±0.15	12.5±0.25
GS	10.5±0.19	15.9±0.42
GE	-1.1±0.03	-4.6±0.03
GB	49.2±0.84**	59.2±1.61**
SP	-1.2±0.06	0.7±0.13
SA	-2.0±0.03	3.6±0.01
SFN	-4.1±0.06	-3.3±0.08
AG	18.7±0.41**	12.4±0.24**

Values represent the mean ± S.E. (n = 4), Asterisks denote significant differences from the untreated group by Dunnett's test at *: $p < 0.05$, **: $p < \pm 0.01$.

DISCUSSION

We discovered the BSE and SFN suppressed production of both fluorescent AGEs and fructosamine during the glycation reaction between fructose and BSA. SFN has already been reported to suppress AGE production and related damage. For example, SFN inhibits expression of the receptor for advanced glycation end products (RAGE) in HUVECs [16] and human glomerular endothelial cells [17, 31]. It also suppresses inflammation caused by methylglyoxal, a precursor of AGEs, in SH-SY5Y human neuroblastoma cells [32]. Our results suggested that SFN could directly suppress the initial stages of the glycation reaction since it decreased the

levels of fructosamine and CP, which are produced in the early stage of AGE formation. On the other hand, the principal glucosinolates found in broccoli seeds (GI and GR) did not suppress CP production, unlike SFN and IB (isothiocyanates). As a result, the glucose moiety in glucosinolates seems to be unnecessary for a suppressive effect on sugar-derived CP production. SFN was reported to exhibit a strong antioxidant effect [14, 15, 33, 34] and oxidation is involved in the early stage of CP production. Therefore, SFN appeared to inhibit the oxidative reaction of sugars and lipids, which is the first step toward the glycation and carbonylation processes which result in production of AGEs and CP [35].

In contrast to the above, BSE suppressed CP production from oxidized linoleic acid and BSA, while SFN demonstrated no suppressive effect. Thus, isothiocyanates produced by hydrolyzation of glucosinolates did not suppress the production of CP by reaction between oxidized lipids and protein, in spite of inhibiting production of sugar-derived CP, but some glucosinolates inhibited production of lipid-derived CP. Structurally, GI, GR, GA, GH, and GS are classified as methylsulfinylalkyl glucosinolates, while GE and GB are methylthioalkyl glucosinolates. As GE and GB showed more potent suppression of CP production, a methylthioalkyl moiety appears to enhance this effect of glucosinolates. Moreover, comparing the different methylsulfinylalkyl glucosinolates suggested that the length of the branched chains is involved in enhancement of their suppressive activity. Glucosinolates with longer branched chains and with an uneven number of carbon atoms, such as GS and GA, showed stronger activity. In contrast to the suppressive effects of glucosinolates, SP appeared to enhance CP production. This may have occurred because the direct reaction of N^+ in the choline structure of SP and DNPH produced a fluorescent compound that affected the fluorescence due to CP; i.e., fluorescence from the SP-DNPH conjugate may have artificially enhanced CP fluorescence.

GI and GB exhibited a stronger suppressive effect on CP production in fibroblasts and both compounds were more potent than AG (positive control). As GI and GB also have an uneven number of carbon atoms in their branched chains, it seems that the structure of glucosinolates with uneven carbons is associated with suppression of CP production in living cells as well as in the test tube. GB seems to be the most promising active compound in BSE for suppression of lipid-derived CP production in skin cells.

Sinigrin was reported to have a strong anti-glycation effect in experiments using BSA [18, 36]. It was reported that sinigrin binds to BSA at Sudlow's site I due to a high affinity for anionic heterocyclic ligands. Sinigrin binds to amino acid residues by 4 hydrogen bonds and shows hydrophobic interaction. As a result, conformational changes of BSA are inhibited and this has an anti-glycation effect. Thus, glucosinolates in BSE (especially GI and GB) may show a

suppressive effect on CP production by binding to BSA and stabilizing its conformation through a similar mechanism to sinigrin. Moreover, the conformation of glucosinolates is likely to have an important influence on the inhibition of protein glycation and carbonylation. Accordingly, further studies are required to fully clarify the influence of the conformational features glucosinolates on the suppression of AGE and CP production.

CONCLUSION

We demonstrated that BSE suppressed glycation and carbonylation when protein (BSA) was reacted with fructose or oxidized linoleic acid. Isothiocyanates suppressed protein carbonylation in reactions with fructose, but not oxidized lipid. While glucosinolates (precursors of isothiocyanates) did not suppress protein carbonylation in reactions with fructose, carbonylation was inhibited in reactions with oxidized lipid. Furthermore, glucobrassicin and glucoiberin suppressed CP production in a cell-based assay. These results suggest that glucosinolates in BSE may potentially suppress carbonylation leading to skin aging.

List of Abbreviations: AGEs, advanced glycation end products; AG, aminoguanidine hydrochloride; BSA, bovine serum albumin; BSE, broccoli sprout extract; CP, carbonylated protein; D-MEM, Dulbecco's minimum essential medium; DNPH, 2,4-dinitrophenylhydrazine; FBS, fetal bovine serum; IB, iberin; GA, glucoalyssin; GB, glucobrassicin; GE, glucoerucin; GH, glucohesperin; GI, glucoiberin; GR, glucoraphanin; GS, glucosiberin; MES, 2-(*N*-morpholino)ethanesulfonic acid; NBT, nitro blue tetrazolium; Nrf, NF-E2 related factor; PBS, phosphate buffered saline; RI, refractive index; RAGE, receptor of advanced glycation end products; ROS, reactive oxygen species; SA, sinapic acid; SP, sinapine; SFN, sulforaphane; TCA, trichloro acetic acid; TFA, trifluoro acetic acid

Competing Interests: The authors declare no conflicts of interest associated with this manuscript.

Author's Contributions: Mrs. Hirano performed *in vitro* and cell experiments and wrote manuscript. Dr. Hitoie performed some carbonylation experiments. Dr. Shimoda isolated compounds in BSE and is responsible for the study. Mr. Takeda identified chemical structure of BSE compounds.

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REFERENCES

1. Fritz KS, Petersen DR: Exploring the biology of lipid peroxidation-derived protein carbonylation. *Chem Res Toxicol* 2011, 24:1411–1419.
2. Inagi R, Miyata T: Oxidative protein damage with carbohydrates and lipids in uremia: Carbonyl stress. *Blood Purif* 1999, 17: 95–98.
3. Dalle-Donne I, Giustarini D, Colombo R, Rossi R, Milzani A: Protein carbonylation in human diseases. *Trends Mol Med* 2003, 9: 169–176.
4. Yamashita Y, Okano Y, Ngo T, Buche P, Sirvent A, Girard F, Masaki H: Differences in susceptibility to oxidative stress in the skin of Japanese and French subjects and physiological characteristics of their skin. *Skin Pharmacol Physiol* 2012, 25: 78–85.
5. Mizutani T, Sumida H, Sagawa Y, Okano Y, Masaki H: Carbonylated proteins exposed to UVA and to blue light generate reactive oxygen species through a type I photosensitizing reaction. *J Dermatol Sci* 2016, 84: 314–321.
6. Iwai I, Hirao T: Protein carbonyls damage the water-holding capacity of the stratum corneum. *Skin Pharmacol Physiol* 2008, 21: 269–273.
7. Ogura Y, Kuwahara T, Akiyama M, Tajima S, Hattori K, Okamoto K, Okawa S, et al.: Dermal carbonyl modification is related to the yellowish color change of photo-aged Japanese facial skin. *J Dermatol Sci* 2011, 64: 45–52.
8. Yokota M, Shimizu K, Kyotani D, Yahagi S, Hashimoto S, Masaki H: The possible involvement of skin dryness on alterations of the dermal matrix. *Exp Dermatol* 2014, 23 Suppl. 1: 27–31.
9. Vale AP, Santos J, Brito NV, Fernandes D, Rosa E, Oliveira MB: Evaluating the impact of sprouting conditions on the glucosinolate content of *Brassica oleracea* sprouts. *Phytochemistry* 2015, 115: 252–260.
10. Vicas SI, Teusdea AC, Carbuнар M, Socaci SA, Socaciu C: Glucosinolates profile and antioxidant capacity of Romanian Brassica vegetables obtained by organic and conventional agricultural practices. *Plant Foods Hum Nutr* 2013, 68: 313–321.
11. Fahey JW, Holtzclaw WD, Wehage SL, Wade KL, Stephenson KK, Talalay P: Sulforaphane bioavailability from glucoraphanin-rich broccoli: Control by active endogenous myrosinase. *PLoS One* 2015, 10: e0140963.
12. Armah CN, Derdemezis C, Traka MH, Dainty JR, Doleman JF, Saha S, Leung W, et al.: Diet rich in high glucoraphanin broccoli reduces plasma LDL cholesterol: Evidence from randomized controlled trials. *Mol Nutr Food Res* 2015, 59: 918–926.

13. Juge N, Mithen RF, Traka M: Molecular basis for chemoprevention by sulforaphane: a comprehensive review. *Cell Mol Life Sci* 2007, 64: 1105–1127.
14. Kleszczynski K, Ernst IM, Wagner AE, Kruse N, Zillikens D, Rimbach G, Fischer TW: Sulforaphane and phenylethyl isothiocyanate protect human skin against UVR-induced oxidative stress and apoptosis: role of Nrf2-dependent gene expression and antioxidant enzymes. *Pharmacol Res*, 2013, 78: 28–40.
15. Saw CL, Huang MT, Liu Y, Khor TO, Conney AH, Kong AN: Impact of Nrf2 on UVB-induced skin inflammation/photoprotection and photoprotective effect of sulforaphane. *Mol Carcinog* 2011, 50: 479–486.
16. Matsui T, Nakamura N, Ojima A, Nishino Y, Yamagishi SI: Sulforaphane reduces advanced glycation end products (AGEs)-induced inflammation in endothelial cells and rat aorta. *Nutr Metab Cardiovasc Dis* 2016, 26: 797–807.
17. Maeda S, Matsui T, Ojima A, Takeuchi M, Yamagishi S: Sulforaphane inhibits advanced glycation end product-induced pericyte damage by reducing expression of receptor for advanced glycation end products. *Nutr Res* 2014, 34: 807–813.
18. Awasthi S, Saraswathi NT: Sinigrin, a major glucosinolate from cruciferous vegetables restrains non-enzymatic glycation of albumin. *Int J Biol Macromol* 2016, 83: 410–415.
19. Song L, Iori R, Thornalley PJ: Purification of major glucosinolates from Brassicaceae seeds and preparation of isothiocyanate and amine metabolites. *J Sci Food Agric* 2006, 86: 1271–1280.
20. De Nicola GR, Rollin P, Mazzon E, Iori R: Novel gram-scale production of enantiopure *E*-sulforaphane from Tuscan black kale seeds. *Molecules* 2014, 19: 6975–6986.
21. Jaki B, Sticher O, Veit M, Frohlich R, Pauli GF: Evaluation of glucoiberin reference material from *Iberis amara* by spectroscopic fingerprinting. *J Nat Prod* 2002, 65: 517–522.
22. Simone R, Domenico C, Melanie S, Trenerry VC, Rod J: The isolation and purification of glucoraphanin from broccoli seeds by solid phase extraction and preparative high performance liquid chromatography. *J Chromato A*, 2006, 1120: 205–210.
23. Mark AB, Karl V, Gulab NJ, Brent T, Steven FV: Purification of a sinapine-glucoraphanin salt from broccoli seeds. *Am J Plant Sci* 2010, 1: 113–118.
24. Lijiang S, Renato I, Paul JT: Purification of major glucosinolates from Brassicaceae seeds and preparation of isothiocyanate and amine metabolites. *J Sci Food Agric*, 2006, 86: 1271–1280.
25. Leoni O, Iori R, Palmieri S, Esposito E, Menegatti E, Cortesi R, Nastruzzi C: Myrosinase-generated isothiocyanate from glucosinolates: isolation characterization and

- in vitro* antiproliferative studies. *Bioorg Med Chem* 1997, 5: 1799–1806.
26. Guy K, Richard NB, Nigel PB, Nicola ED, Avril ABR, Roger MW: High-performance liquid chromatographic separation of natural and synthetic desulphoglucosinolates and their chemical validation by UV, NMR and chemical ionisation-MS methods. *Phytochemical Anal* 2001, 12: 226–242.
 27. Caia R, Arntfielda SD, Charltonb JL: Structural changes of sinapic acid and sinapine bisulfate during autoclaving with respect to the development of colored substances. *JAOCS* 1999, 76: 433–441.
 28. Adisakwattana S, Thilavech T, Chusak C: *Mesona chinensis benth* extract prevents AGE formation and protein oxidation against fructose-induced protein glycation *in vitro*. *BMC Complementary Altern Med* 2014, 14: 130.
 29. Refsgaard H, Tsai L, Stadtman E: Modifications of proteins by polyunsaturated fatty acid peroxidation products. *PNAS* 2000, 97: 611–616.
 30. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, et al.: Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* 1990, 186: 464–478.
 31. Yamagishi S, Matsui T: Protective role of sulforaphane against vascular complications in diabetes. *Pharm Biol* 2016, 54: 2329–2339.
 32. Angeloni C, Malaguti M, Rizzo B, Barbalace MC, Fabbri D, Hrelia S: Neuroprotective effect of sulforaphane against methylglyoxal cytotoxicity. *Chem Res Toxicol* 2015, 28: 1234–1245.
 33. De Figueiredo SM, Binda NS, Nofueira-Machado JA, Vieira-Filho SA, Caligiorne RB: The antioxidant properties of organosulfur compounds (sulforaphane). *Recent Pat Endocrinol Metab Immune Drug Discov* 2015, 9: 24–39.
 34. Fahey JW, Talalay P: Antioxidant functions of sulforaphane: a potent inducer of phase II detoxication enzymes. *Food Chem Toxicol* 1999, 37: 973–979.
 35. Qin WS, Deng YH, Cui FC: Sulforaphane protects against acrolein-induced oxidative stress and inflammatory responses: modulation of Nrf-2 and COX-2 expression. *Arch Med Sci* 2016, 12: 871–880.
 36. Awasthi S, Saraswathi NT: Elucidating the molecular interaction of sinigrin, a potent anticancer glucosinolate from cruciferous vegetables with bovine serum albumin: effect of methylglyoxal modification. *J Biol Structure and Dynamics* 2016, 34: 2224–2232.