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Original article

Effects of varieties and cooking on the glycative stress inhibitory effect of eggplant

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Abstract

Accumulation of advanced glycation end products (AGEs) in the body due to glycative stress is a factor in the progression and development of aging and lifestyle-related diseases. Oxidation is one of the accelerators of glycative stress because it promotes the formation of AGEs. On the contrary, glycative stress suppression includes suppression of postprandial hyperglycemia, suppression of AGE generation, and degradation and excretion of AGEs. Various vegetables and herbs have already been reported to have antiglycative effects. In this study, for the purpose of verifying the usefulness of glycative stress suppression materials, we focused on eggplant as a vegetable that can be consumed as a familiar food, and verified its antiglycative effect and antioxidative effect. Seven commercial eggplant varieties were used as samples. Samples were tested for changes in effects among eggplant varieties and after, four types of cooking (baked, fried, boiled, and nukazuke [rice bran pickles]). In addition, the amounts of chlorogenic acid and anthocyanin, the major components of eggplant, were measured and their relationship to the antiglycative and antioxidative effects were verified. All eggplant varieties tested showed antiglycative and antioxidative effects. The difference between the varieties was 3.5-fold for antiglycation and 4.9-fold for antioxidative activity. The changes in the effects of eggplant after cooking were small for baked and fried eggplant, less than 5% change in antiglycation and less than 15% change in antioxidative activity. In contrast, after eggplant was boiled and nukazuke, the antiglycative and antioxidative effects decreased by more than 35% and 60%, respectively. It was estimated that the difference in the antiglycative and antioxidative effects of eggplant was largely influenced by the amount of chlorogenic acid. It was considered that the choice of cultivar and cooking method is important for the use of eggplant while focusing on glycative stress.

KEY WORDS: antiglycative effect, antioxidative effect, chlorogenic acid, anthocyanin, eggplant (*Solanum melongena*)

Introduction

The production and accumulation of advanced glycation end products (AGEs) caused by glycative stress is a risk factor that promotes aging, diabetic complications, osteoporosis, dementia, and other diseases [1)]. The formation of AGEs is also accelerated by oxidation and UV exposure [2)]. Therefore, oxidation is one of the accelerators of glycative stress. Suppression of glycative stress includes suppression of postprandial hyperglycemia, suppression of AGE generation, and degradation, and excretion of AGEs [3)]. We have reported that tea [4)], vegetables, herbs [5)], fruits [6)], and fermented foods [7)] are natural products that inhibit AGE formation. 187 varieties of vegetable and herb extracts were tested for their inhibitory effects on AGE formation, and the inhibitory effects on fluorescent AGE (F-AGE) formation were found to be significantly reduced by eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*), chile pepper (*Capsicum annuum*), paprika (*Capsicum annuum* var. 'grossum') and other vegetables of the Solanaceae family (Solanaceae) [5)].

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Eggplant is a crop which has been consumed in countries and regions around the world since ancient times. It is believed to have been introduced to Japan via China and cultivated in Japan around the 8th century (Nara Period)[8]. There are more than 170 varieties of eggplant in Japan, with different sizes, shapes, and colors, and distinctive varieties cultivated to suit local climates and customs[9]. The Ministry of Agriculture, Forestry and Fisheries has recognized eggplant as one of the designated crop vegetables with high consumption in Japan, and as a familiar vegetable with a close relationship to the diet[10].

Materials and Methods

1) Reagents

The reagents used were purchased from the following manufacturers. Human serum albumin (HSA, lyophilized powder, ≥ 96 %, agarose gel electrophoresis) was from Sigma-Aldrich Japan (Meguro-ku, Tokyo, Japan). Aminoguanidine hydrochloride (AG), (±)-6-Hydroxy-2,5,7,8-tetramethylchroman -2-carboxylic acid (trolox), chlorogenic acid, (-)-quinine sulfate dihydrate (-)-quinine sulfate dihydrate), and trifluoroacetic acid (TFA) are manufactured by Fujifilm Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 2-(N-morpholino) ethanesulfonic acid (MES) is from Dojin Chemical Laboratory (Kamimashiki-gun, Kumamoto, Japan). 1,1-diphenyl-2-picrylhydrazyl (DPPH) is from Cayman CHEMICAL (Ann Arbor, Michigan, USA). Delphinidin is from Tokiwa Phytochemical Laboratory (Sakura, Chiba, Japan). Other reagents were purchased from Fujifilm Wako Pure Chemical Industries or Nacalai Tesque (Kyoto, Japan).

2) Samples

The seven varieties of eggplant used as samples were Naga-nasu (A), Oonaga-nasu (B), Ko-nasu (C), Maru-nasu (D), Mizu-nasu (E), Bei-nasu (F), Shiro-nasu (G) (*Table 1*). Eggplants were used after removing the hull and calyx. Eggplants were purchased at a supermarket in Kyoto Prefecture.

3) Cooking method

Eggplant samples were cooked according to commonly practiced household methods.

a) Grilling

Eggplants were cut in half lengthwise and roasted in a frying pan over high heat (>100 °C) from the peel side. Once browned, then flipped and grilled from the pulp side for a total of about 5 minutes until cooked all the way through.

b) Deep frying

The eggplant was cut into round slices, a grid was cut on both sides, the moisture was removed with kitchen paper, 800 mL of canola oil (Ajinomoto, Chuo-ku, Tokyo) was poured into an electric deep fryer (Zojirushi Mahobin, Osaka, Japan), the eggplant was fried at 170 °C for 3 to 5 minutes, and then removed when they turned golden brown.

c) Boiling

Eggplants were cut into approximately 3 cm cubes and boiled in 800 mL of boiling water for 4 minutes.

d) Nukazuke (rice bran pickles)

After eggplants were cut in half lengthwise, they were soaked in fermented Nuka-Doko (Muji, Toshima-ku, Tokyo) for 24 hours at 4 °C according to the product's directions for use.

4) Preparation of sample extracts

Raw eggplants and cooked eggplants were sliced, dried at 65 °C for 100 hours using a warm-air dryer Petit Mini (Daikisangyo, Okayama, Japan), and powdered in a food processor. After deep-frying and cooking, the eggplant was defatted as follows. For deep-fried eggplant, 12 g of the powder after warm air drying was immersed in 30 mL butanol for 20 minutes, and the supernatant was removed by centrifugation. Then, 18 mL acetone was added and immersed for another 20 minutes, and the supernatant was removed by centrifugation. This defatting process was repeated twice. Finally, the defatted powder was then air-dried to dry powder.

The sample extract was obtained by placing 2 g of the dried powder in 40 mL of 70 % ethanol, extracting at 50 °C for 4 hours, and collecting the supernatant by centrifugation. The solid concentration (mg/mL) of the sample extract was calculated from the weight of 5 mL of the extract after drying at 120 °C for 2 hours.

5) Measurement of antiglycative effect

The antiglycative effect was verified using the HSA-glucose reaction model with reference to a previous report[11], and the inhibitory effect of fluorescent AGEs (F-AGEs) at a solid concentration of 0.3 mg/mL of the sample extract was verified. F-AGEs were evaluated by measuring AGE-derived fluorescence (excitation wavelength: 370 nm/fluorescence

Table 1. Characteristics of eggplant samples.

Sample	variety	general shape	color of skin	color of calyx
A	Naga-nasu	long ovoid	violet	violet
B	Oonaga-nasu	cylindrical	violet	violet
C	Ko-nasu	ovoid	violet	violet
D	Maru-nasu	globular	violet	violet
E	Mizu-nasu	pear shaped	violet	violet
F	Bei-nasu	obovate	violet	green
G	Shiro-nasu	pear shaped	white	green

Purchased at several supermarkets in Kyoto prefecture.

wavelength: 440 nm). F-AGEs were calculated as the concentration in the reaction solution after incubation, relative to the fluorescence value of 5 μg/mL quinine sulfate in 0.1 N sulfate solution, which was 1,000. AG was used as a positive control substance for inhibition of glycation reaction, and the inhibition rate (%) of F-AGE formation was calculated according to a previous report [11].

6) Measurement of antioxidative effect

For antioxidative activity, DPPH radical scavenging activity corresponding to the sample solution (solid concentration 1 mg/mL) was measured using the slope of the regression line created by Trolox, referring to a previous report [7].

DPPH radical scavenging activity was determined by adding 1 mg/mL of sample extract (25-100 μL), 50 μL of 200 mmol/L MES buffer (pH 6.0), 50 μL of 800 μmol/L DPPH solution and 50 % ethanol solution (0-75 μL) to the wells of a microplate, so that the mixture volume reached 200 μL. After 20 minutes of reaction at room temperature, absorbance was measured at 520 nm. The slope of the linear regression line between the amount of sample extract added and absorbance was then determined. The DPPH radical scavenging activity value was calculated as the Trolox equivalent (μmol-TE/L) of the sample extract with a solid concentration of 1 mg/mL by dividing the slope of the linear regression line of absorbance obtained from the 0-16 nmol/L Trolox solution reacted simultaneously with the sample by the slope of the regression line of the sample extract.

7) Determination of chlorogenic acid

Chlorogenic acid concentration in the extract was measured by UV absorbance method referring to a previous report [9]. For chlorogenic acid concentration, 200 μL of the sample extract was mixed with 50 μL of 1 % TFA, added to the wells of a microplate, mixed, and the absorbance at 330 nm was measured. The chlorogenic acid concentration (μg/mL) of the extract was calculated from the chlorogenic acid calibration curve.

8) Anthocyanin measurement

Anthocyanin concentration in the extract was measured by UV absorbance method with reference to a previous report [12]. Anthocyanin concentration was determined by mixing 200 μL of the sample extract with 50 μL of 1% TFA, adding the mixture to the wells of a microplate, and measuring the absorbance at 540 nm. Anthocyanin concentration was calculated as delphinidin equivalent (μg-DE/mL) from the calibration curve of delphinidin measured concurrently.

Statistical Analysis

Measurements were expressed as the mean ± standard deviation (SD) of triplicate measurements. Tukey's test or Dunnett's test was used to compare measurements. Pearson's product-rate correlation coefficient was used to analyze correlation. A high correlation was considered as $0.7 < |r| \leq 1.0$, from $0.4 < |r| \leq 0.7$ was correlated, and $0.2 < |r| \leq 0.4$ was not correlated. For statistical analysis results, a risk rate of less than 5 % ($p < 0.05$) was considered significant. Statistical analysis was performed using the statistical analysis software BellCurve for Excel (Shakai Joho Service, Shinjuku-ku, Tokyo).

Results

1) Antiglycative and antioxidative effects

The solid concentration of the extract was 25.2 ± 2.2 mg/mL in seven eggplant varieties (A-G). At a solid concentration of 0.3 mg/mL, C showed the greatest inhibition of F-AGE formation (88.7 ± 0.9 %), while B showed the least (25.4 ± 1.2 %, ***Fig. 1***). Similarly, DPPH radical scavenging activity showed a maximum value (165 μmol-TE/L) for C, while G and D exhibited the same minimum value (33.7 μmol-TE/L).

The inhibition of F-AGE formation in A showed a maximum value before cooking (74.6 ± 2.6 %) to fried (75.5 ± 1.4 %) and decreased to nukazuke (36.4 ± 3.4 %, ***Fig. 2***). Similarly, DPPH radical scavenging activity went from 117.8 μmol-TE/L before cooking (117.8 μmol-TE/L) to a maximum value at baking (134.7 μmol-TE/L) and a minimum value at boiling (47.1 μmol-TE/L).

The percentage inhibition of F-AGE formation and DPPH radical scavenging activity of the extract showed a positive high correlation ($y = 1.8413x - 13.598$, $r = 0.756$, $p < 0.05$, ***Fig. 3***).

2) Chlorogenic acid content and glycative stress suppression

The highest value of chlorogenic acid in 1 mg/mL of extract of seven eggplant varieties was 86.5 ± 1.8 μg (C) and the lowest value was 15.5 ± 0.2 μg (B), a 5.7-fold difference. Chlorogenic acid in the extract of A showed the highest value in grilled (51.2 ± 1.4 μg) and the lowest value in nukazuke (11.1 ± 0.1 μg) compared to before cooking (46.5 ± 0.4 μg). There was a 4.6-fold difference in chlorogenic acid due to cooking. Chlorogenic acid concentrations in all sample extracts were highly positively correlated with both antiglycative ($y = 0.2846x - 5.5247$, $r = 0.900$, $n = 11$, $p < 0.05$) and antioxidative ($y = 0.3393x + 4.8972$, $r = 0.784$, $n = 11$, $p < 0.05$) effects (***Fig. 4***).

3) Anthocyanin content and glycative stress suppression

The highest value of anthocyanins in 1 mg/mL of extracts of seven eggplant cultivars was 0.722 ± 0.015 μg-DE (B) and the lowest value was 0.106 ± 0.006 μg-DE (G), a 6.8-fold difference. Anthocyanins in the extract of A showed the highest value (1.374 ± 0.029 μg-DE) in fried and the lowest value (0.070 ± 0.002 μg-DE) in nukazuke, compared to before cooking (0.673 ± 0.017 μg-DE). There was a 19.6-fold difference in anthocyanin content by cooking. Anthocyanin concentration in all sample extracts was positively and weakly correlated ($y = 0.002x + 0.0236$, $r = 0.353$, $n = 11$, $p < 0.05$) with antiglycation and positively correlated ($y = 0.0033x + 0.1543$, $r = 0.429$, $n = 11$, $p < 0.05$) with antioxidative activity (***Fig. 5***).

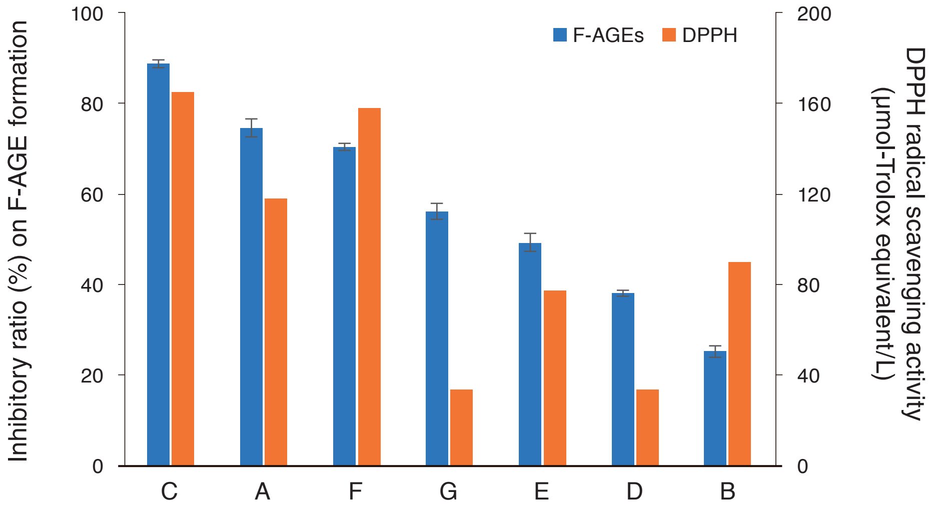

Fig. 1. **Antiglycative and antioxidative effects of eggplant varieties.**

Data are expressed as mean ± SD, n = 3 (F-AGEs) or mean, n = 2 (DPPH). The concentration of extract, 0.3 mg/mL (inhibitory ratio of F-AGE formation), 1 mg/mL (DPPH radical scavenging activity); A – F, Eggplant varieties are shown ***Table 1***. F-AGEs, fluorescent advanced glycation end products; DPPH, 1,1-diphenyl-2-picrylhydrazyl; SD, standard deviation.

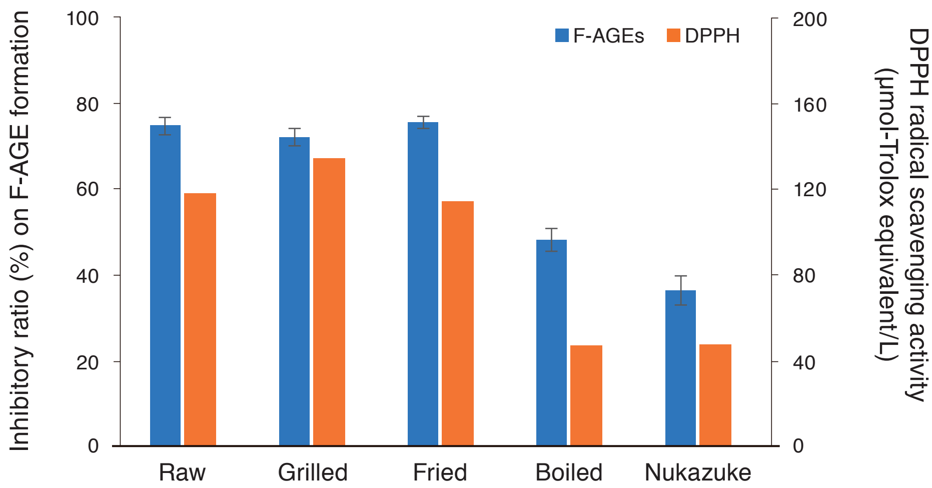

Fig. 2. **Post-cooking antiglycative and antioxidative effects of eggplant.**

Data are expressed as mean ± SD, n = 3 (F-AGEs) or mean, n = 2 (DPPH). The concentration of extract, 0.3 mg/mL (inhibitory ratio of F-AGE formation), 1 mg/mL (DPPH radical scavenging activity); Eggplant varieties; A. F-AGEs, fluorescent advanced glycation end products; DPPH, 1,1- diphenyl-2-picrylhydrazyl; SD, standard deviation.

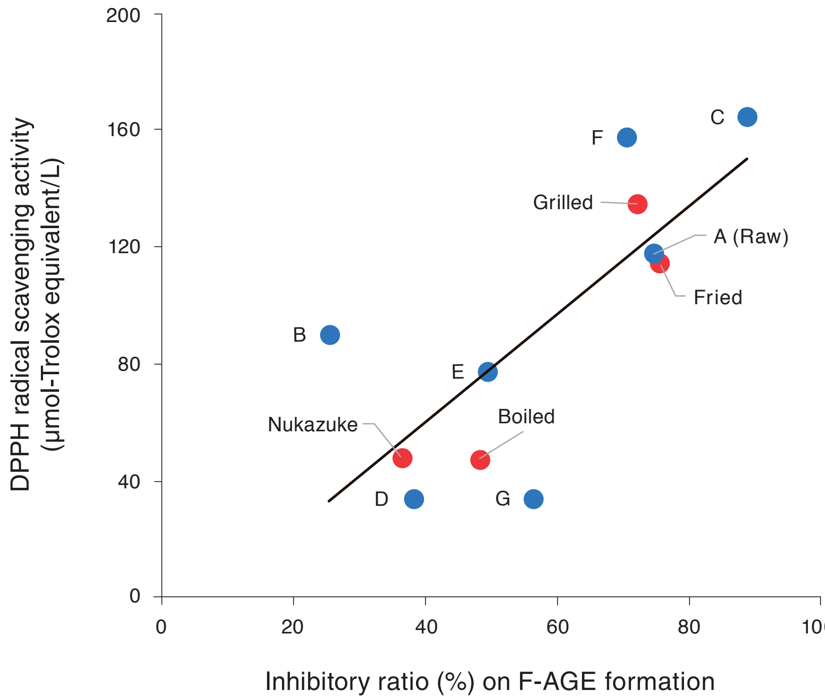

Fig. 3. **Relationship between antiglycative and antioxidative effects.**

$y = 1.8413x - 13.598$, $n = 11$, $r = 0.756$, $p < 0.05$. The concentration of extract, 0.3 mg/mL (inhibitory ratio of F-AGE formation), 1 mg/mL (DPPH radical scavenging activity); A – F, Eggplant varieties are shown ***Table 1***; Cooked eggplant varieties was A. F-AGE, fluorescent advanced glycation end product; DPPH, 1,1-diphenyl-2-picrylhydrazyl.

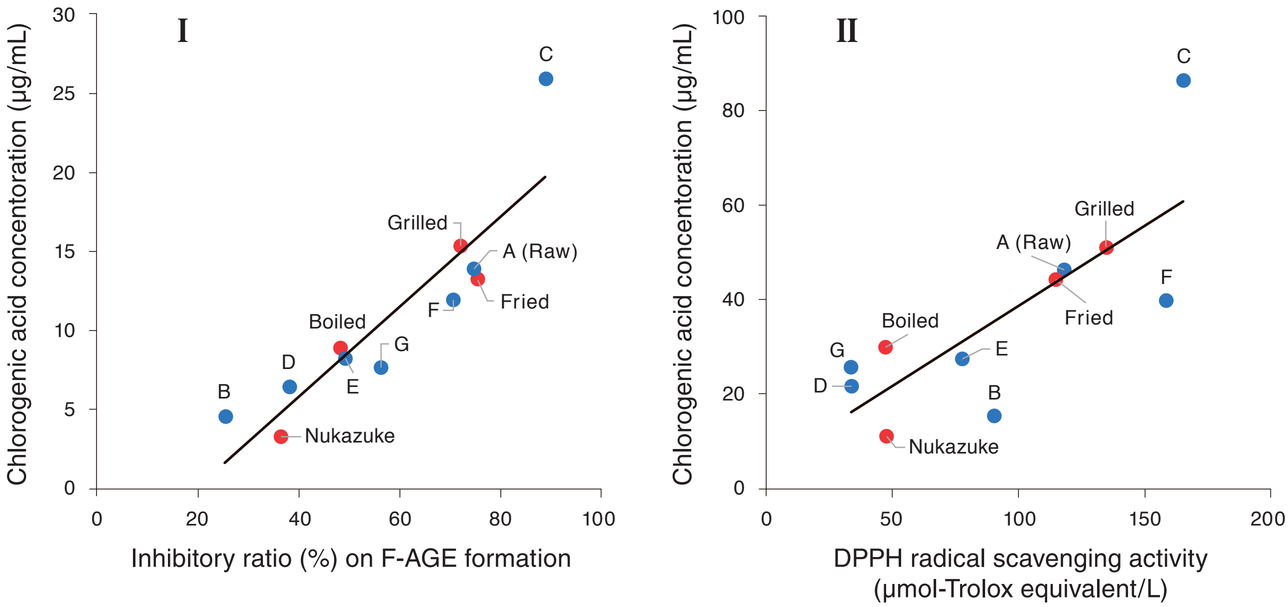

Fig. 4. **Relationship between chlorogenic acid concentration and antiglycative or antioxidative effect of eggplant extract.**

I, Antiglycative effect, $y = 0.2846x - 5.5247$, $n = 11$, $r = 0.900$, $p < 0.05$;
II, Antioxidative effect, $y = 0.3393x + 4.8972$, $n = 11$, $r = 0.784$. $p < 0.05$;

The concentration of extract, 0.3 mg/mL (inhibitory ratio of F-AGE formation), 1 mg/mL (DPPH radical scavenging activity); A – F, Eggplant varieties are shown ***Table 1***; Cooked eggplant varieties was A. F-AGE, fluorescent advanced glycation end product; DPPH, 1,1-diphenyl-2-picrylhydrazyl.

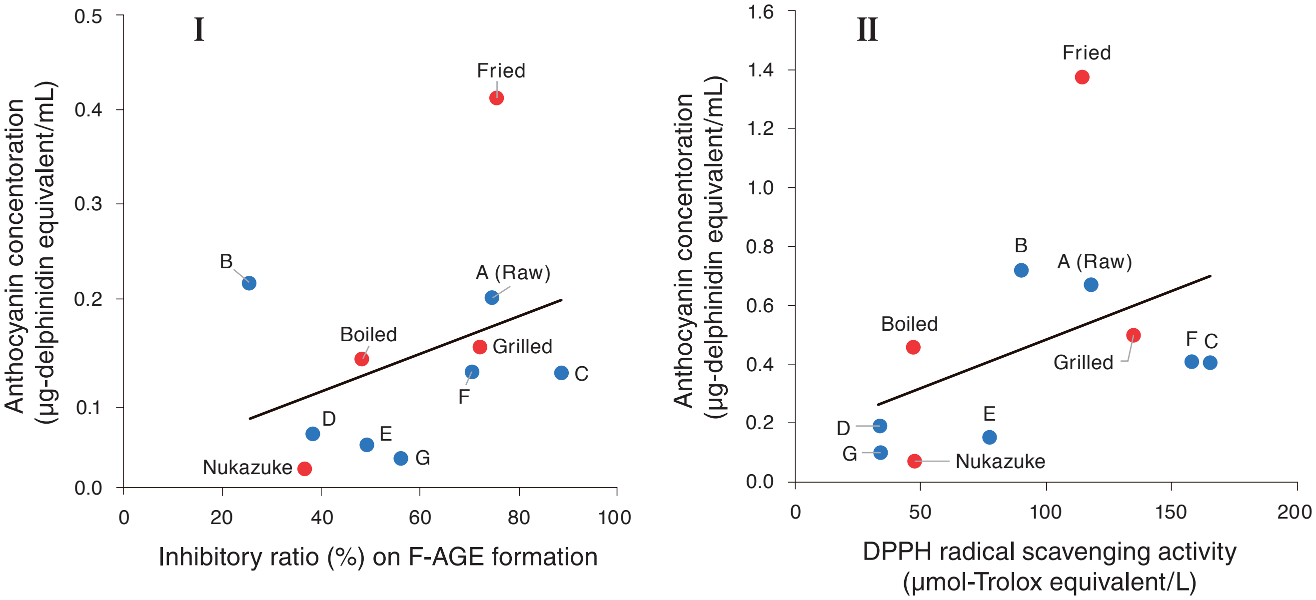

Fig. 5. **Relationship between anthocyanin concentration and antiglycative or antioxidative effect of eggplant extract.**

I, Antiglycative effect, $y = 0.002x + 0.0236$, $n = 11$, $r = 0.353$, $p < 0.05$;
II, Antioxidative effect, $y = 0.0033x + 0.1543$, $n = 11$, $r = 0.429$, $p < 0.05$;
The concentration of extract, 0.3 mg/mL (inhibitory ratio of F-AGE formation), 1 mg/mL (DPPH radical scavenging activity); A – F, Eggplant varieties are shown *Table 1*; Cooked eggplant varieties was A. F-AGE, fluorescent advanced glycation end product; DPPH, 1,1-diphenyl-2-picrylhydrazyl.

Discussion

1) Inhibition of glycative stress in eggplant

Chlorogenic acid and anthocyanins are characteristic components in eggplants[13]. Chlorogenic acid is a polyphenol found in many plants and is an ester compound of caffeic acid and quinic acid. Chlorogenic acid has been reported to have antioxidative and antiglycative effects[14]. The purple component of eggplant peel is anthocyanin, which is also known as nasunin (delphinidin 3-(p-coumaroylrutinoside)-5-glucoside), delphinidin 3-rutinoside, delphinidin 3-(p-coumaroylrutinoside)-5-glucoside) , and delphinidin 3-rutinoside. rutinoside-5-glucoside, and their content and amounts vary by variety[15]. These anthocyanins have antioxidant properties[16, 17].

The 70 % ethanol extracts of the seven eggplant varieties tested showed both antiglycative and antioxidative effects. However, the intensity of the effects varied among the varieties, with 5.7- and 6.8-fold differences in chlorogenic acid and anthocyanin concentrations in the extracts among the seven varieties, respectively. The chlorogenic acid concentration in the extract was highly correlated with the antiglycative and antioxidative effects. However, the anthocyanin concentration in the extracts correlated better with antioxidative activity than with antiglycative activity. On the other hand, the chlorogenic acid concentration in each extract was 21 to 242 times higher than that of anthocyanins, suggesting that the chlorogenic acid content may be responsible for the differences in the antiglycative and antioxidative effects.

Vegetables and herbs contain a variety of polyphenolic components; a study of the relationship between polyphenol concentrations in 187 vegetable and herbal extracts and their antiglycative activity showed that the strength of the antiglycative effect did not correlate with total polyphenol concentration[18]. This result indicates that components other than polyphenols are also involved in the antiglycative effect of plant extracts. It has also been reported that the antiglycative activity of catechins depends on the number and position of hydroxyl groups in the molecule[19]. Although the types and amounts of components contained in the eggplants vary from one cultivar to another, the effects of chlorogenic acid content were considered to be significant in eggplant's antiglycative and antioxidative effects.

2) Effect of eggplant cooking on glycative stress inhibition

All extracts of eggplant variety A after cooking by four different methods showed antiglycative and antioxidative effects. The change in antiglycative activity during cooking was less than 5% increase or decrease in baked and fried eggplant compared to before cooking. However, in the case of boiled and nukazuke, the antiglycative effect decreased by more than 35%. Similarly, antioxidative activity increased by less than 15% in the baked and deep-fried samples and decreased by 60 % in the boiled and nukazuke samples. The decrease in antiglycative and antioxidative effects in the boiled and nukazuke were thought to be due to the leaching of eggplant components into the boiling water and the bed of bran.

In nukazuke, the fading of the rind was observed, suggesting that the decomposition of eggplant components by lactic acid bacteria and other bacteria in the bed was also present. Chlorogenic acid and anthocyanins may be affected by heat during cooking. However, chlorogenic acid in eggplant increased by 10% when grilled and decreased by 5% when fried compared to raw eggplant. Anthocyanins decreased by 26% when grilled and increased by 104% when fried compared to raw. Increases in chlorogenic acid and polyphenols due to cooking have been reported for potatoes [20] and kale [21]. The increase in components in the extract due to cooking may be attributed to a change in the extraction rate due to the breakdown of the eggplant tissue by heating [22].

There are no reports examining changes in the antiglycative effects of vegetables and herbs due to cooking. The results of antioxidative activity before and after cooking, measured by the β-carotene fading method, showed increased activity with steaming spinach, microwaving eggplant, and baking carrots compared to raw [23]. Alternatively, polyphenol content decreased with boiling spinach, eggplant, and leafy green onions compared to raw, and increased with microwaving eggplant and baking carrots compared to raw. When onions were cooked, microwaving resulted in small changes in quercetin and ascorbic acid content [24]. The change in quercetin content was also small in fried onions. While in boiling, 30% of the quercetin in the onion leached into the boiling water.

In nukazuke of four eggplant varieties, the moisture content decreased and the amount of fructose increased [25]. The amounts of chlorogenic acid and anthocyanins in eggplant varied with cooking. However, baked and fried cooking methods of eggplant may have been less likely to attenuate the antiglycative and antioxidative effects than other cooking methods.

3) Use of eggplant as a countermeasure against glycative stress

Eggplant is a crop that is produced in more than 50 million tons worldwide and is a vegetable that is consumed in many different countries and regions [13]. Eggplants have a water content of approximately 90% and contain vitamins, phenols, and carotenoids [26]. Eggplant is considered a phytochemical food due to its antioxidant properties. The eggplants used as samples in this study were seven varieties available in supermarkets. Eggplant was found to have antiglycative and antioxidative effects. However, these effects differed among the varieties.

Eggplant varieties C, A, and F showed high antiglycative and antioxidative activity with F-AGE formation inhibition of more than 70% and DPPH radical scavenging activity of 120 µmol-TE/L. On the other hand, B showed the lowest inhibition of F-AGE formation (25.4%), while G and D showed the lowest DPPH radical scavenging activity (33.7 µmol-TE/L). It was considered that the variety was important in selecting eggplants for glycation stress inhibition.

The changes in antiglycative and antioxidative effects of cooking eggplant variety A were smaller for baked and fried than for raw. In particular, fried eggplant showed the smallest change in F-AGE formation inhibition rate (1.1%) and DPPH radical scavenging activity (2.9%). Chlorogenic acid and polyphenols in eggplant are one of the astringent components. It has been reported that the intensity of astringency of eggplant and chlorogenic acid is reduced by the addition of cooking oil [27].

It is estimated that factors that reduce astringency include the transfer of astringent components to cooking oil and the suppression of contact with the tongue surface by cooking oil. In addition, an increase in guanylic acid, an umami component, has been reported in eggplant when cooked [28]. In contrast, in boiling cooking, it was highly likely that the components leached into the boiling water. Therefore, miso soup or soup in which the whole boiled broth is consumed was considered preferable as a way of eating eggplant to compensate for its reduced antiglycative and antioxidative effects.

Nukazuke showed more than 50% less antioxidative and antiglycative activity, 76% less chlorogenic acid, and 90% less anthocyanins than raw. Therefore, it may not be suitable for cooking focused on glycative stress inhibition. In contrast, nukazuke (pickles in rice bran) is considered to be a useful cooking method that provides vitamin B1, γ-oryzanol, and ferulic acid contained in rice bran [29] and improves the balance of intestinal flora by lactic acid bacteria, and thus provides effects other than glycation stress suppression.

Research limitations

The antiglycative and antioxidative effects of different eggplant varieties and cooking methods tested in this study were in vitro results. Further validation is needed for their usefulness when consumed by humans on a continuous basis.

Conclusion

Antiglycative and antioxidative effects were observed in eggplants. These effects differed among eggplant varieties. The antiglycative and antioxidative effects of eggplant changed less after baking and frying compared to raw eggplant. Whereas, these effects decreased after boiling and nukazuke. Changes in the amount of chlorogenic acid were presumed to be responsible for the antiglycative and antioxidative effects of eggplant. Selection of cultivars and cooking methods is important for the use of eggplant with a focus on glycation stress.

Conflict of Interest Declaration

There are no conflicts of interest in conducting this research.

Acknowledgments

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