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Original article

Effect of ceramic-treated water on food functionality of strawberries.

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Abstract

***Aim*:** Glycation is a non-enzymatic reaction between reducing sugars and proteins. It forms advanced glycation end products (AGEs) and is involved in multiple diseases. We have reported that strawberries are one of the plants that inhibit the formation of AGEs. It has been reported that water treated with ceramic chips (ceramic-treated water) enhanced the growth of agricultural crops. In this study, we examined the effect of ceramic-treated water on the food functionality of strawberries, especially the anti-glycative effect and antioxidant efficacy.

***Methods*:** Fourteen varieties of strawberry plants were cultivated using either normal water or ceramic-treated water, and harvested strawberries were sliced, dried, and ground. Extracts were prepared with hot water and introduced into the human serum albumin (HSA)-Glucose glycation model. Inhibitory effects on glycation were evaluated by measuring fluorescent AGEs, and antioxidative efficacy was measured by the DPPH method.

***Results*:** All the strawberry samples showed anti-glycative effects and antioxidant activity. There was no significant difference between the cultivation water in the paired t-test for either effect. However, one variety showed a significant increase in inhibition of AGEs formation, and 4 varieties showed a significant increase in antioxidant activity in the ceramic-treated water cultivation. No correlation between anti-glycative and antioxidative effects was found.

***Conclusion*:** In this study, we examined the effect of growing water on 14 strawberry varieties. Ceramic-treated water changed the feature of strawberries, but the change depends on the varieties. This indicates that the effect of ceramic-treated water may be limited to certain varieties. In addition, the lack of correlation between anti-glycative and antioxidative effects suggested that the substances contributing to each effect were different.

***KEY WORDS*:** special-glaze-applied ceramic chips, anti-glycative effect, antioxidative efficacy, *Fragaria* × *ananassa*

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Introduction

Reducing sugars, such as glucose and fructose, are essential nutrients for biological activities. However, an excess of reducing sugars in the body reacts with proteins non-enzymatically, forming advanced glycation end products (AGEs)[1]. This reaction is called glycation. AGEs accumulate in the body as we age and are involved in the development of lifestyle diseases such as cancer[2], diabetes[3], osteoporosis[4], atherosclerosis[5], and Alzheimer's disease[6,7]. The concept of glycative stress is a comprehensive view of the stress caused by glycation and the process leading to lifestyle diseases. The ways to inhibit glycative stress include preventing hyperglycemia, inhibiting AGE production, inhibiting AGE absorption, promoting AGE degradation and excretion, and inhibiting AGE-induced damage to cells and tissues. In our previous study, we found that strawberry (*Fragaria* × *ananassa*) is one of the plants that has the effect of inhibiting glycation (anti-glycative effect)[8]. Strawberry is a plant native to the Netherlands, and vitamin C and polyphenols included in its edible pseudo-fruits have been reported to have not only antioxidative and anti-glycative effects but also anti-inflammatory effects[8-11]. There are many varieties of strawberries produced by crossbreeding, and Japan is one of the countries with the largest number of strawberry varieties, with more than 300 varieties on the market[12]. The consumption of fruits has been reported to reduce the risk of developing type 2 diabetes[13]; however, the fructose contained in fruits is often considered to be related to obesity, hyperlipidemia, and diabetes.

Special-glaze-applied ceramic chips (referred to as ceramic chips) developed in 2000 have been reported to improve water quality by decomposing odor, sterilizing, and reducing biochemical oxygen demand (BOD)[14,15]. When water treated with ceramic chips (ceramic-treated water) is used for crops, it has been shown to increase weight, tighten flesh, and improve taste in peach cultivation; to increase the yield in rice cultivation[15]. However, the effect of ceramic-treated water on strawberries has not been reported.

In this study, we examined whether the use of ceramic-treated water for growing 14 strawberry varieties enhanced their food functions, specifically their anti-glycative and antioxidative effects.

Material and methods

Materials

Fourteen varieties of strawberry plants were provided from the Center for Clinical Pharmacy Education and Research, Faculty of Pharmacy, Doshisha Women's College of Liberal Arts (***Table 1***).

Strawberry seedlings of each variety were planted on cultivation devices about 1 m in height in the same arrangement and irrigated with either normal water or ceramic-treated water, which was prepared by passing normal water once through a metal pipe (10 cm in diameter and 100 cm in length) filled with special-glaze-applied ceramic chips. Each plant was drip irrigated four times a day with about 50 mL per plant (***Fig. 1***). The irrigation conditions were controlled to be the same.

Human serum albumin (HSA) was purchased from Sigma-Aldrich (St. Louis, MO). DPPH, 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) free radical and 2-(N-morpholino) ethanesulfonic acid (MES) were obtained from Tokyo Chemical Industry (Tokyo, Japan). All other analytical-grade reagents were obtained from Fujifilm Wako Chemicals (Osaka, Japan).

Table 1. The cross-fertilization of starawberries

variety	the cross-fertilization of strawberry varieties
Ai-berry	undisclosed
Akihime	Nyohou × Kuno-wase
Asuka-ruby	Nyohou × Asuka-wave
Amaka	Amaou × Kiyoka
Kaorino	breeding lines (0028401) × breeding lines (0023001) (breeding among Nyohou × Ai-berry × Toyonoka × Houkou-wase × Akihime × Akasyanomitshuko × Tochiotome × Sanchigo)
Kiyoka	Nyohou × Ai-berry
Koiminori	breeding lines (03042-08) × Hinoshizuku
Nyohou	Harunoka × Dana × Reikou
Benihoppe	Akihime × Satinoka
Hoshinokirameki	undisclosed
Toukun	breeding lines (undisclosed) × Kurume-IHIgou
Yayoihime	(Tonehoppe × Tochiotome) × Tonehoppe
Yotsuboshi	Mie-bohon1gou × A8S4-147
Red-pearl	Ai-berry × Toyonoka

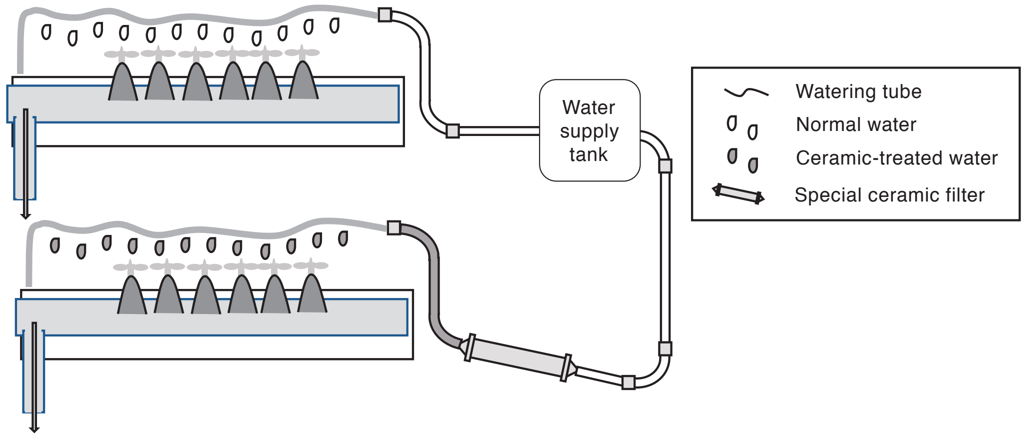

Fig. 1. **Special ceramic chip filtration system.**

Water was supplied from a common irrigation tank, one connected to normal water and the other to a special ceramic chip filtration system. Each plant was irrigated four times a day, about 50 mL each time.

Sample preparation

Strawberries were sliced, dried at 65 °C for 120 hours, and ground. To prepare the water extract, 2 g of dried powder was mixed with 40 mL of distilled water and was incubated at 80 °C for 75 minutes. Extracted samples were centrifuged at 3,350 × g for 10 minutes and filtered using filter paper. Five milliliters of the extracts were evaporated to measure the solid content, and then the extracts were adjusted to their appropriate concentrations with distilled water.

Preparation of Glycated Proteins

An HSA glycation model was used to evaluate the effect of strawberries on glycation as previously described [16]. HSA (8 mg/mL) and 0.2 mol/L glucose in 50 mmol/L phosphate buffer (PB, pH 7.4) were incubated at 60 °C for 40 hours (solution A). Simultaneously, heated proteins without glucose were also prepared (solution B). To determine the effects of strawberry extract, 30 mg/mL of the extract was added to reach 1/10 of volume concentration (final concentration; 3 mg/mL) with or without glucose (solutions C and D, respectively).

Measurement of AGE-derived fluorescence

After incubation, the fluorescence of the reaction mixture was measured as previously described [17]. Two hundred µL of the reaction mixture was used to measure fluorescence at an excitation wavelength of 370 nm and an emission wavelength of 440 nm by Infinite M1000 (Tecan Japan, Kanagawa, Japan) microplate reader. The ratio of inhibitory effect on fluorescent AGE formation (%) was calculated using the equation below.

The ratios of inhibitory effect of fluorescence AGEs (%)
$= \{1 - (C - D) / (A - B)\} \times 100$

Evaluation of the antioxidative efficacy

The antioxidative efficacy of the strawberry extracts was determined as the free radical scavenging capacity using DPPH as described by Oki [18] with slight modification. Specifically, 0.5 mg/mL of the strawberry extract and 200 mol/L DPPH were reacted in 50 mmol/L 2-(N-morpholino) ethanesulfonic acid (MES) buffer (pH 6.0) for 20 minutes at room temperature. The absorbance of the solution was measured at 520 nm. Trolox, an analog of vitamin E, was used as a control.

Statistics

Data are expressed as mean ± standard deviation (SD) of at least three independent experiments. The statistical analyses performed by analysis of variance (ANOVA) were subjected to Dunnett's test for multiple comparisons between each of the samples and control groups. To compare the same varieties of samples that were cultivated using either the ceramic-treated water or normal water, we conducted paired t-tests. Differences were considered significant at p-values less than 0.05.

Results

Calculation of solid concentration of hot water extract of strawberry.

The solids concentration of the hot water extract of each strawberry variety is shown in ***Table 2***. There was no difference in the solid concentration among the varieties or the water used for cultivation (mean value of 36.3 mg/mL for ceramic-treated water cultivation, 36.4 mg/mL for normal water cultivation).

Table 2. Solid content of hot water extract of samples

variety	solid content [mg/mL]	
	Ceramic-treated water	Normal water
Ai-berry	36.5	35.7
Akihime	38.0	38.2
Asuka-ruby	34.4	35.2
Amaka	35.7	36.1
Kaorino	35.5	37.1
Kiyoka	36.0	36.3
Koiminori	36.1	36.9
Nyohou	36.6	36.8
Benihoppe	38.6	36.8
Hoshinokirameki	38.2	38.9
Toukun	33.5	31.1
Yayoihime	36.0	36.6
Yotsuboshi	37.8	39.2
Red-pearl	35.5	35.0
average	36.3	36.4

Effect of cultivation with ceramic-treated water on the inhibition of fluorescent AGE formation in hot water extracts of strawberry.

The results of the inhibition of fluorescent AGE formation by the hot water extract of strawberry at a final concentration of 3 mg/mL are shown in ***Table 3***. All 14 strawberry varieties significantly suppressed the formation of fluorescent AGEs regardless of the cultivation water. The highest suppression rate of fluorescent AGE formation was observed in Koiminori with ceramic-treated water cultivation (92.3 ± 1.77 %) and the lowest in Benihoppe with normal water cultivation (69.7 ± 3.80 %). Data were analyzed by a Student's t-test in each variety, only Koiminori showed a significant increase in the inhibition of fluorescent AGE formation by water cultivation with ceramic chips (92.3 ± 1.77 % for water cultivation with ceramic chips, 87.8 ± 1.37 % for water cultivation with normal water, $p = 0.024$). Student's paired t-tests showed no significant difference between water types (78.9 ± 6.47 % [ceramic-treated water cultivation], 79.8 ± 5.16 % [normal water cultivation], $p = 0.430$, ***Fig. 2***). Comparing 14 varieties of samples grown with normal water, 8 of them, namely, Yotsuboshi, Akihime, Ai-berry, Amaka, Nyohou, Toukun, Yayoihime, and Benihoppe, showed significantly lower levels of inhibition of fluorescent AGE formation than Koiminori (87.8 ± 1.37 %, ***Fig. 3***). This indicates that the effect of cultivation water on the inhibition of fluorescent AGE formation in strawberries is smaller than the effect of differences among the varieties.

Effect of cultivation with ceramic-treated water on antioxidant activity of strawberry.

As to antioxidative efficacy, we measured the DPPH radical scavenging activity of hot water extracts of strawberries cultivated with ceramic-treated water or normal water. DPPH radical scavenging activity was observed in all 14 strawberry varieties regardless of the water used (***Fig. 4***). The highest DPPH radical scavenging activity was observed in the normal water cultivation of Toukun (117.9 ± 0.81 nmol Trolox eq./mg-solid content), and the lowest was Amaka that cultivated with normal water (46.5 ± 1.47 nmol Trolox eq./mg-solid content). The Student's t-test in each variety showed that cultivation with ceramic-treated water significantly enhanced the DPPH radical scavenging activity in Amaka, Kiyoka, Nyohou, and Yotsuboshi, while it significantly decreased in Hoshinokirameki and Red-pearl. Paired t-test for each cultivation water showed no significant difference (72.1 ± 16.7 nmol Trolox eq./mg-solid content [ceramic-treated water cultivation], 69.9 ± 20.2 nmol Trolox eq./mg-solid content [normal water cultivation], $p = 0.540$, ***Fig. 5***). Comparing the 14 samples grown with normal water, Toukun showed significantly higher DPPH radical scavenging activity than all other 13 varieties (***Fig. 6***). This indicated that the effect of cultivation water on the antioxidative efficacy of strawberries was smaller than the effect of differences among the varieties.

The correlation between anti-glycative and antioxidative effects of strawberries.

Since it is known that oxidation occurs during AGE production, we hypothesized that strawberries with strong anti-glycative activity would also have high antioxidant efficacy. However, as shown in ***Fig. 7***, the R^2 values were −0.0273 (ceramic-treated water cultivation) and 0.0278 (normal water cultivation), respectively; therefore, no correlation was observed.

Table 3. The ratio of inhibitory effect on fluorescent AGE formation (%)

variety	Ceramic-treated water	Normal water	p value
Ai-berry	76.8 ± 3.26	77.7 ± 4.10	0.782
Akihime	72.4 ± 2.91	79.7 ± 4.28	0.071
Asuka-ruby	84.8 ± 1.69	85.1 ± 1.59	0.845
Amaka	81.8 ± 2.53	77.4 ± 1.56	0.062
Kaorino	78.8 ± 3.09	80.9 ± 2.26	0.396
Kiyoka	86.9 ± 0.80	85.8 ± 0.45	0.118
Koiminori	92.3 ± 1.77	87.8 ± 1.37	0.024
Nyohou	78.5 ± 3.90	76.8 ± 4.97	0.664
Benihoppe	74.3 ± 4.56	69.7 ± 3.80	0.253
Hoshinokirameki	74.4 ± 4.91	80.8 ± 2.84	0.123
Toukun	70.3 ± 6.67	76.1 ± 3.57	0.255
Yayoihime	70.0 ± 5.74	73.1 ± 4.04	0.486
Yotsuboshi	80.8 ± 1.80	80.0 ± 2.70	0.690
Red-pearl	82.4 ± 1.93	85.8 ± 2.86	0.156

The results are expressed as mean ± SD of 3 experiments. AGE, advanced glycation end products; SD, standard deviation

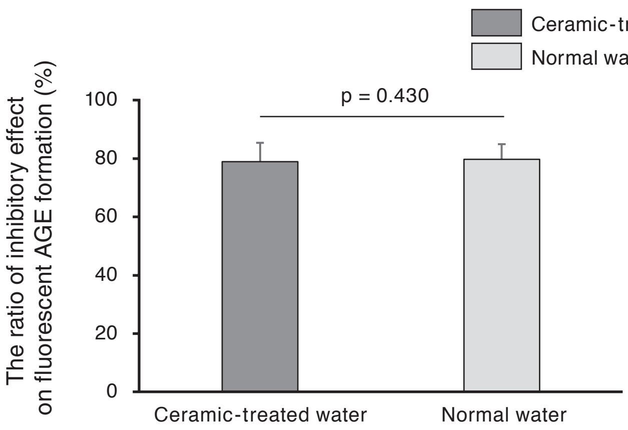

Fig. 2. **Effect of ceramic-treated water on inhibitory effect on fluorescent AGE formation of strawberry varieties.**

Fourteen variety of strawberry plants were cultivated either ceramic-treated water or normal water. Hot water extracts of each strawberry variety were prepared and used to determine the inhibitory effect of fluorescent AGE formation. Paired t-test was conducted to investigate the effect of cultivation water on anti-glycative effect of strawberries. All data were shown as the mean ± SD (n = 3). AGE, advanced glycation end product; SD, standard deviation.

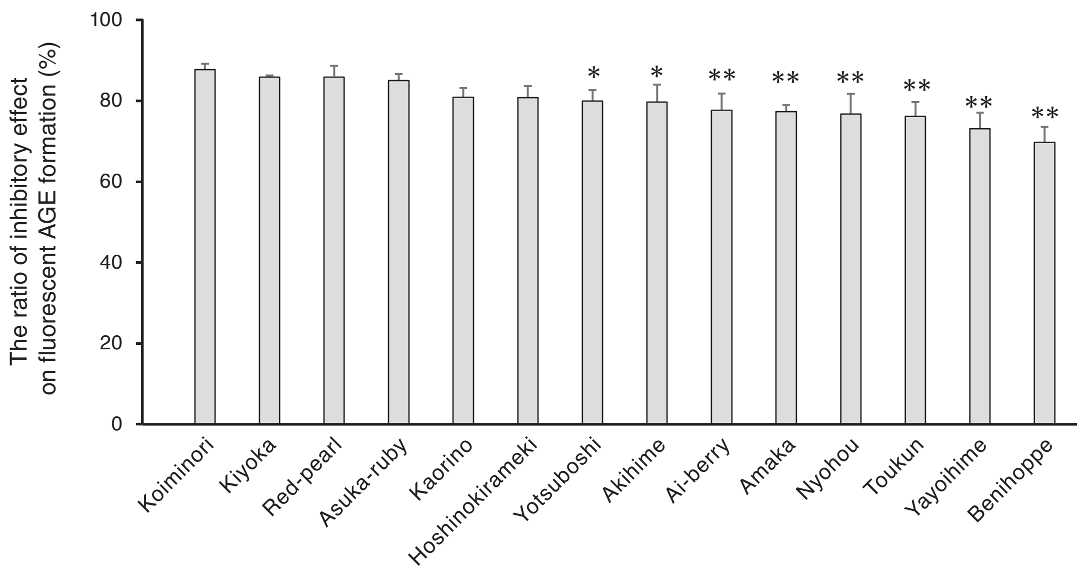

Fig. 3. **Differences in inhibitory effect on fluorescent AGE formation between strawberry varieties.**

Fourteen variety of strawberry plants were cultivated using normal water. Hot water extracts of each strawberry variety were prepared and used to determine the inhibitory effect of fluorescent AGE formation. All data were shown as the mean ± SD (n = 3). * $p < 0.05$ and ** $p < 0.01$ vs. Koiminori. AGE, advanced glycation end product; SD, standard deviation.

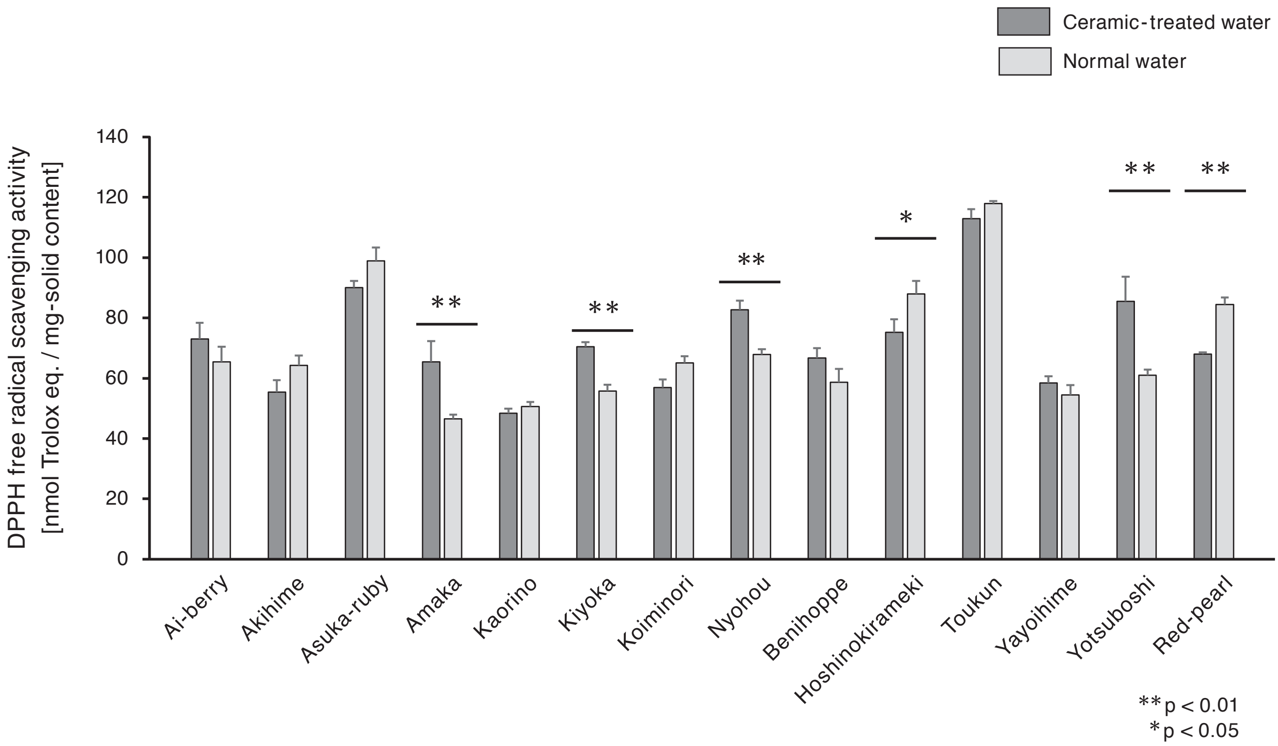

Fig. 4. **Differences in antioxidative efficacy between strawberry varieties.**

Fourteen variety of strawberry plants were cultivated either ceramic-treated water or normal water. Hot water extracts of each strawberry variety were prepared and used to examine the DPPH radical scavenging activity. All data were shown as the mean ± SD (n = 6). * $p < 0.05$ and ** $p < 0.01$ vs. normal water in each variety. DPPH, 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl; SD, standard deviation.

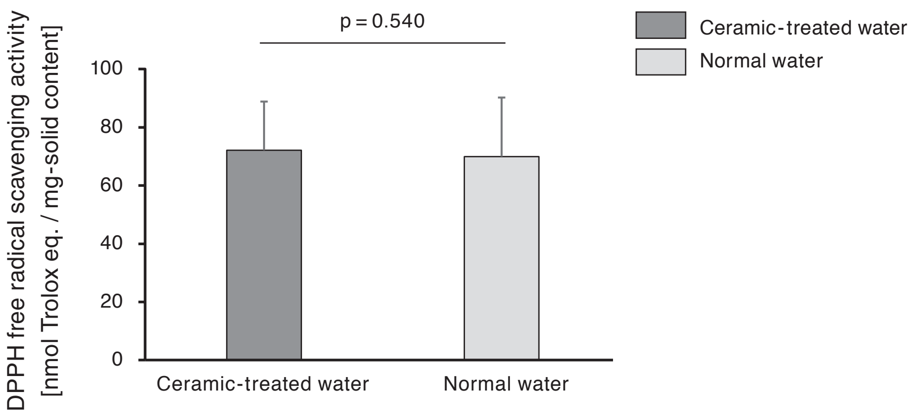

Fig. 5. **Effect of ceramic-treated water on antioxidative efficacy of strawberry varieties.**

Fourteen variety of strawberry plants were cultivated either ceramic-treated water or normal water. Hot water extracts of each strawberry variety were prepared and used to examine the DPPH radical scavenging activity. Paired t-test was conducted to investigate the effect of cultivation water on antioxidative efficacy of strawberries. All data were shown as the mean ± SD (n = 6). DPPH, 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl; SD, standard deviation.

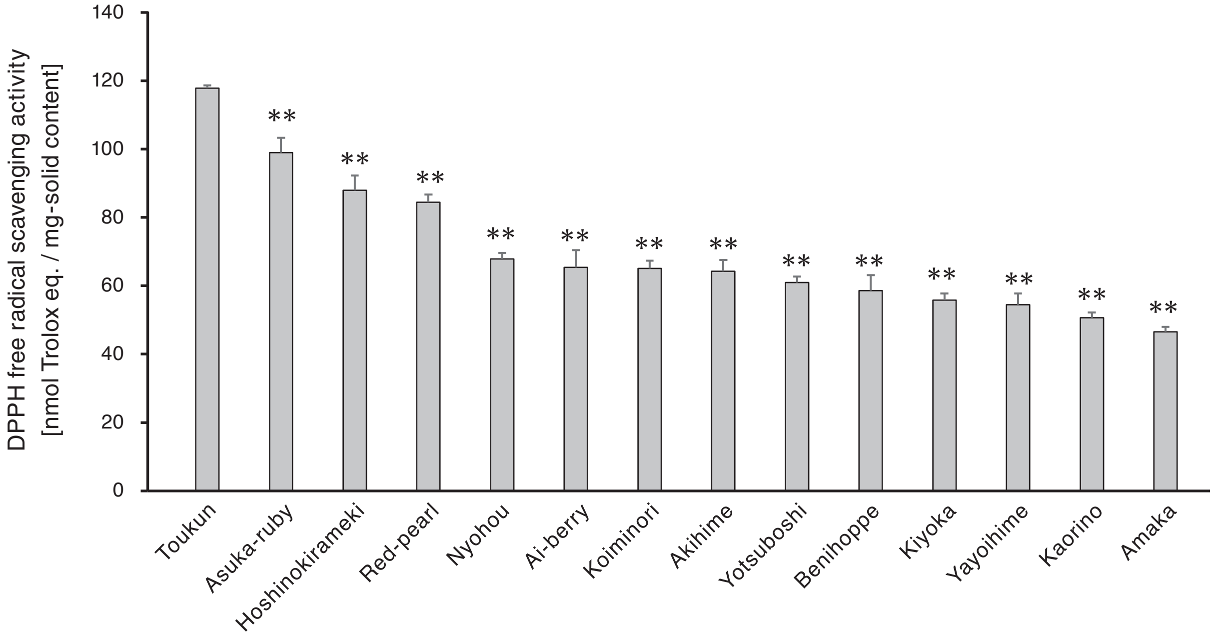

Fig. 6. **Differences in antioxidative efficacy between strawberry varieties.**

Fourteen variety of strawberry plants were cultivated using normal water. Hot water extracts of each strawberry variety were prepared and used to examine the DPPH radical scavenging activity. All data were shown as the mean ± SD (n = 6). ** $p < 0.01$ vs. Toukun. SD, standard deviation.

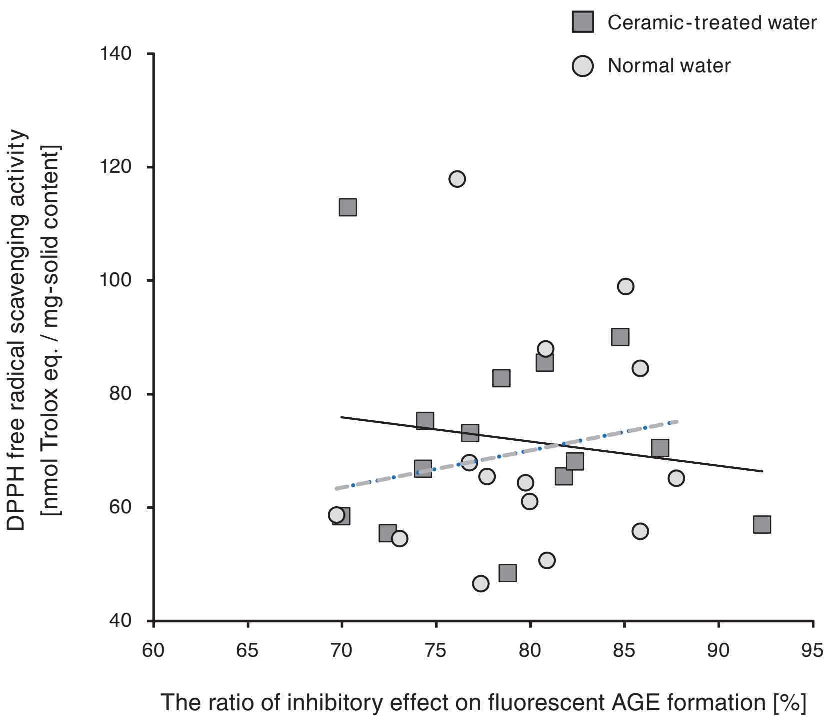

Fig. 7. **Correlation between anti-glycative efficacy and antioxidative activity.**

The inhibitory effect of fluorescent AGE formation was plotted in X-axis and DPPH radical scavenging activity was plotted in Y-axis. Black line was regression line of ceramic-treated water and gray break line was regression line of normal water. AGE, advanced glycation end product; DPPH, 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl.

Discussion

AGEs are produced in vivo by glycation, a non-enzymatic binding of reducing sugars to proteins, and accumulate in the body with age. Increased AGE production and accumulation are associated with increased concentrations of glucose and its metabolites in the blood as well as decreased activity of metabolic enzymes. In fact, accumulation of AGEs has been observed in the blood and tissues of diabetic patients [19, 20]. We have examined the anti-glycation effects of more than 500 kinds of plants and foods [8, 17, 21-23]. We have also examined fruits that are generally considered to have high fructose content and reported that various fruits including strawberries have anti-glycative effects [8]. In addition, we have recently reported that the anti-glycative effects vary among varieties in the same plants [24]. This is thought to be due to differences in the components contained in different varieties, but detailed verification has not been done yet.

Effect of strawberry varieties on food functionality.

Based on the results of the anti-glycative and antioxidative effects of 14 strawberry varieties grown in normal water, the differences among varieties were examined. All strawberry varieties were shown to have antioxidative and anti-glycative effects (***Table 3, Fig. 4***); however, focusing on each variety revealed that both anti-glycative and antioxidative effects vary depending on varieties. For example, Koiminori (87.8 ± 1.37 %) had the highest inhibition rate of fluorescent AGE formation which was significantly higher than that of the 8 varieties: Yotsuboshi, Akihime, Ai-berry, Amaka, Nyohou, Toukun, and Yayoihime and the lowest, Benihoppe (69.7 ± 3.80 %, ***Fig. 3***). In terms of antioxidant activity, Toukun showed significantly higher DPPH radical scavenging activity than the other 13 varieties (***Fig. 6***). This difference may be due to differences in the components of each variety as a result of crossbreeding. Although Nyohou was selected as one of the hybrids for most of the varieties, there were no common hybrids among the varieties that showed strong anti-glycative and antioxidative effects.

Anthocyanins and ascorbic acid are known as antioxidant compounds in strawberries [8-10]. Anthocyanins are glycosides with an anthocyanidin backbone, and appropriate amounts of anthocyanins intakes may reduce the risk of cardiovascular disease and type 2 diabetes, improve weight maintenance and neuroprotection, and have effects on vascular and glucose regulation [25]. Among anthocyanins, pelargonidin, cyanidin, and malvidin have been reported as aglycones in strawberry [26]. However, anthocyanins are easily denatured by heat. Even though we measured anthocyanins using a colorimetric method based on the previous report [27], we did not detect anthocyanins in the hot water extracts used in our study (data not shown). Since only monomeric anthocyanins can be measured by this method, these results indicate that the components which showed anti-glycative and antioxidative effects in the extracts are considered to be something other than monomeric anthocyanins. However, the details are not known. Further studies will be needed to identify the anti-glycative components of strawberries and measure their amounts in each variety.

Effect of Ceramic-treated Water Cultivation on Strawberries.

At the time of the development of special-glaze-applied ceramic chips, it was thought to be an environment in which cyanobacteria could grow, and it was assumed that the water purification effect of ceramic-treated water was due to the reactive oxygen species derived from the oxygen produced by cyanobacteria and the plant growth-promoting effect was due to the adaptive response of the antioxidant system, in which mild oxidative stress enhances the antioxidant system [15]. However, the redox potential of ceramic-treated water was reported to be lower than that of normal water, suggesting that the oxygen concentration was not increased. We now hypothesize that the lower redox potential is due to an increase in the concentration of dissolved hydrogen in the ceramic-treated water. As for the source of hydrogen, we are considering the possibility of vibration of the ceramic chips due to water flow and the catalytic effect of the glaze applied to the ceramic chips, but the details are still unknown. Another possibility is that hydrogen-producing bacteria may be growing on the special ceramic chips, but since hydrogen-producing bacteria are generally considered to be anaerobic [28], further studies are needed to determine whether they can grow under the conditions of this study. Hydrogen molecules have a strong reducing ability and not only directly scavenge hydroxyl radicals, but also induce antioxidants and antioxidative enzyme activity in cells [29, 30]. It has also been reported that hydrogen gas administration inhibits the injuries caused by reactive oxygen species-derived inflammation such as ischemia-reperfusion [31] and cellular senescence [32]. As for plant growth, it has been reported that hydrogen-rich water promotes the growth of roots of komatsuna (Japanese mustard spinach) [33] as well as leaf and root growth promotion in cucumber by induction of plant growth hormones such as gibberellins and auxins [34].
We have already shown that the use of ceramic-treated water induces growth promotion in peaches and rice as well as increases the sugar content of fruits [15]. In this study, we focused on the food functionality of strawberries rather than growth promotion, especially the anti-glycative and antioxidative effects.

When a paired t-test was performed on 14 strawberry varieties, cultivation with ceramic-treated water did not increase anti-glycative and antioxidative effects (***Fig. 2, 5***). However, when each variety was examined by t-test, Koiminori ($p = 0.024$) was the only variety that showed a significant increase in anti-glycative effect by the ceramic-treated water cultivation. Other than that, Amaka showed a trend of an increase ($p = 0.062$), but Akihime showed higher effects in the normal water cultivation ($p = 0.071$, ***Table 3***). No effect was observed for the other 11 varieties. In terms of antioxidant activity, four varieties (Amaka, Kiyoka, Nyohou, and Yotsuboshi) showed a significant enhancement of DPPH radical scavenging activity when cultivated with ceramic-treated water, while Hoshinokirameki and Red-pearl had significantly lower activity level when grown with the ceramic-treated water (***Fig. 4***). These results indicate that the effects of ceramic-treated water on the varieties are limited. We suspected that the difference is due to different levels of responsiveness against oxidative stress in each variety.

It is possible that the dissolved hydrogen concentration in the ceramic-treated water used in this study was not sufficient for varieties susceptible to oxidative stress, or the hydrogen concentration of the water was not sufficient to enhance the expression of genes involved in growth promotion and antioxidant enzymes. Because of that, the effects of the ceramic-treated water were not reflected in the promotion of food functions. In addition, the special ceramic chip filtration system could not be installed vertically in this study, so some of the water did not fully contact the ceramic chips, which may have resulted in a lower dissolved hydrogen concentration than originally expected. In the future, it will be necessary to verify the differences in the expression of growth hormones and antioxidant enzymes in different varieties when the ceramic chip treatment device is fixed vertically, and the plants are grown with ceramic-treated water. Moreover, identification and verification of changes in the amounts of anti-glycative and anti-oxidative components in strawberries is also a future task. It will also be necessary to verify whether the continuous use of ceramic-treated water enhances the food functions of the varieties that were not affected in this study.

Conclusion

Anti-glycative and antioxidative effects were observed in all 14 strawberry varieties used in this study regardless of the water used. However, the effect of ceramic-treated water on the anti-glycative effect and antioxidative efficacy of strawberries differed depending on the varieties, suggesting that the effects of ceramic-treated water may be limited to certain varieties.

Acknowledgment

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Conflict of interest

The authors claim no conflict of interest in this study.

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