

Structural and Functional Characterization of Polyphenols Isolated from Acerola (*Malpighia emarginata* DC.) Fruit

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Two anthocyanins, cyanidin-3- α -O-rhamnoside (C3R) and pelargonidin-3- α -O-rhamnoside (P3R), and quercitrin (quercetin-3- α -O-rhamnoside), were isolated from acerola (*Malpighia emarginata* DC.) fruit. These polyphenols were evaluated based on the functional properties associated with diabetes mellitus or its complications, that is, on the radical scavenging activity and the inhibitory effect on both α -glucosidase and advanced glycation end product (AGE) formation. C3R and quercitrin revealed strong radical scavenging activity. While the inhibitory profiles of isolated polyphenols except quercitrin towards α -glucosidase activity were low, all polyphenols strongly inhibited AGE formation.

Key words: acerola; polyphenol; diabetes; α -glucosidase; advanced glycation end products

There is growing evidence supporting the observation that reactive oxygen species and free radicals are associated with certain diseases such as cancer, atherosclerosis, and coronary heart disease, which are so-called lifestyle-related diseases.^{1,2)} Hence intake of constituents from foods that have free radical scavenging activities is considered to be important in preventing such diseases. In this regard, recently, research on antioxidants in foods has gained renewed attention.³⁾

Diabetes mellitus, one of the lifestyle-related diseases, is characterized by chronic hyperglycemia, which is associated with increased risk of cardiovascular diseases.⁴⁾ Inhibiting glucose uptake in the intestines may help diabetic patients to control the blood glucose level in the postprandial state. Substances that inhibit amylase and glycosidases have thus been studied, and some of them have been developed as drugs to treat diabetes mellitus.^{5,6)}

Diabetes mellitus can lead to a number of microvascular and macrovascular complications. Recently,

many reports have suggested that these complications are caused by the formation of advanced glycation end products (AGEs), which are generally formed through Amadori-type compounds from proteins and glucose.^{7–11)} AGEs are postulated to be physiological markers of the Maillard reaction in a biological system. Formation of AGE has been observed *in vivo* in several long-living proteins such as lens crystalline and collagen affected by diabetes mellitus.^{9,11)} Some radical species including oxygen are considered to play an important role in these processes.¹⁰⁾ Hence compounds that scavenge radical species are expected to prevent the formation of AGEs.

Polyphenols, ubiquitous compounds in plants, are well known antioxidants, and are thus considered to be important constituents of the human diet.^{12–14)} There are many reports indicating that polyphenols are capable not only of preventing oxidative stress but also of inhibiting intestinal α -amylase or sucrase.^{5,15)} For these reasons, polyphenols are attractive compounds for the prevention of hyperglycemia and AGE formation.

Plants are generally the best natural sources for antioxidants such as ascorbic acid and/or polyphenols. In particular, tropical and subtropical plants are considered to be outstanding in qualities and quantities of antioxidants due to strong exposure of sunlight. Moreover, some tropical and subtropical foodstuffs have been shown to have a strong suppressing effect on carcinogenesis and liver injury.^{16,17)} Therefore, tropical and subtropical plants may be attractive sources of functional foods beneficial to human health.

Acerola is a fruit found from Central America to northern South America. It is well known to be one of the best natural sources of vitamin C, and has become extremely popular in daily life among those who are health-conscious. Since acerola is a tropical fruit, it is expected to contain various functional compounds other

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Abbreviations: C3R, cyanidin-3- α -O-rhamnoside; P3R, pelargonidin-3- α -O-rhamnoside; AGE, advanced glycation end product; APs, acerola polyphenols; HPX, Hypoxanthine; XOD, xanthine oxidase; BSA, bovine serum albumin; DMPO, 5,5-Dimethyl-1-pyrroline-*N*-oxide; TFA, trifluoroacetic acid

than vitamin C, such as polyphenols. As for acerola polyphenols (APs), however, only one compound has been reported so far. Santini and Huyke¹⁸⁾ found that acerola contains malvidin-3,5-diglucoside (malvin) as anthocyanin, but its characteristic properties were not described. Thus information on the structures and functions of APs are not yet sufficient.

In this study, therefore, we first identified the structures of APs. Subsequently, we elucidated their functional properties. As described above, polyphenols possibly act as antioxidants and α -glucosidase inhibitors. For these reasons, the functional properties of APs associated with diabetes mellitus or its complications were evaluated *in vitro*; the antioxidant activities and the inhibitory effects on both α -glucosidase and AGE formation.

Materials and Methods

Chemicals. Hypoxanthine (HPX), xanthine oxidase (XOD), maltose, sucrose, and glucose were purchased from Wako Pure Chemical Industries (Osaka, Japan). Rat intestinal acetone powder, bovine serum albumin (BSA), and aminoguanidine were purchased from Sigma Chemical (St. Louis, MO). 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO) was purchased from LABOTEC (Tokyo, Japan). Quercetin-3- α -*O*-rhamnoside (quercitrin) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan).

Samples. Acerola (*Malpighia emarginata* DC.) was provided by Nichirei do Brazil Agricola (Recife, Brazil) and immediately frozen and stored at -35°C until use.

Isolation of APs. Frozen acerola fruits (5,000 g) were defrosted followed by removal of the seeds. Then the edible portion (3,547 g) was homogenized with methanol (7-liter) and extracted with stirring at room temperature for 1 h to obtain APs. The same procedure was repeated twice. The resulting extract was filtered, and the filtrate was concentrated and freeze-dried. The powder obtained (241.2 g) was dissolved in distilled water, and the suspended material was removed by filtration. The resulting extract was chromatographed on a C18 cartridge column (Sep-Pak Vac; Nihon Waters, Tokyo, Japan). The adsorbed extract was eluted successively with 0%, 10%, 20%, 30%, and 100% methanol containing 0.2% of trifluoroacetic acid (TFA), obtaining 5 fractions (total, 228.25 g; fr. 1, 221.6 g; fr. 2, 3.1 g; fr. 3, 2.4 g; fr. 4, 0.67 g; fr. 5, 0.48 g). Fr. 3 and fr. 4 containing APs were pooled by concentration to dryness and subjected to two further steps of column chromatography, as described below.

The pooled fractions, dissolved in 20% acetonitrile containing 0.1% TFA, were subjected to HPLC (CBM-10A system; Shimadzu, Kyoto, Japan) using a 10×250 mm C30 column (Develosil RPAQUEOUS-AR-5; Nomura Chemical, Aichi, Japan). The column oven was set at 40°C . The samples were eluted with

20% acetonitrile containing 0.1% TFA at a flow rate of 2.3 ml/min with detection at 280 nm. As a result, three major peaks were obtained, then these three compounds were isolated.

Finally, the three isolated peak fractions were separately dissolved in 43% methanol containing 0.1% TFA, and each was subjected to HPLC (SCL-10Avp system; Shimadzu, Kyoto, Japan) and purified further. Column (10×250 mm; phenomenex LUNA 5μ C18 (2) column; Phenomenex, Inc., Torrance, CA). The oven was set at 40°C . The samples were eluted with 43% methanol containing 0.1% TFA at a flow rate of 2.0 ml/min detected with a photodiode array detector.

Structural identification. NMR spectra were obtained on a JEOL λ -500 or a JEOL GSX-270 spectrometer (JEOL, Tokyo, Japan). Compounds 1 and 2 were measured in CD_3OD containing 3% TFA- d_1 ,¹⁹⁾ and compound 3 were in CD_3OD . FAB-MS was recorded on a JEOL DX-303HF spectrometer (JEOL, Tokyo, Japan).

Compound 1. UV-vis λ_{max} : 281, 529 nm (MeOH + 0.2% TFA); FAB-MS, m/z 433 $[\text{M}]^+$; ^1H NMR (500 MHz, CD_3OD + 3% TFA- d_1) δ 8.87 (s, 1H), 7.91 (dd, $J = 8.5, 2.1$ Hz, 1H), 7.73 (d, $J = 2.1$ Hz, 1H), 6.93 (d, $J = 8.5$ Hz, 1H), 6.79 (d, $J = 1.8$ Hz, 1H), 6.60 (d, $J = 1.8$ Hz, 1H), 5.70 (d, $J = 1.2$ Hz, 1H), 4.22 (dd, $J = 3.4, 1.2$ Hz, 1H), 3.90 (dd, $J = 9.2, 3.4$ Hz, 1H), 3.63 (dd, $J = 9.5, 6.1$ Hz, 1H), 3.57 (dd, $J = 9.5, 9.2$ Hz, 1H), 1.29 (d, $J = 6.1$ Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD + 3% TFA- d_1) δ 170.5 (C), 163.6 (C), 159.0 (C), 157.4 (C), 155.6 (C), 147.6 (C), 144.6 (C), 135.4 (CH), 127.3 (CH), 121.0 (C), 117.8 (CH), 117.3 (CH), 113.3 (C), 103.4 (CH), 102.6 (CH), 95.1 (CH), 73.2 (CH), 72.3 (CH), 72.1 (CH), 71.6 (CH), 18.0 (CH₃).

Compound 2. UV-vis λ_{max} : 270, 335, 429, 511 nm (MeOH + 0.2% TFA); FAB-MS, m/z 417 $[\text{M}]^+$; ^1H NMR (500 MHz, CD_3OD + 3% TFA- d_1) δ 8.96 (s, 1H), 8.32 (d, $J = 8.8$ Hz, 2H), 7.03 (d, $J = 8.8$ Hz, 2H), 6.89 (s, 1H), 6.64 (s, 1H), 5.73 (d, $J = 1.5$ Hz, 1H), 4.20 (dd, $J = 3.4, 1.5$ Hz, 1H), 3.87 (dd, $J = 9.3, 3.4$ Hz, 1H), 3.62 (dd, $J = 9.4, 5.8$ Hz, 1H), 3.57 (dd, $J = 9.4, 9.3$ Hz, 1H), 1.28 (d, $J = 5.8$ Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD + 3% TFA- d_1) δ 170.7 (C), 166.5 (C), 164.1 (C), 159.1 (C), 157.6 (C), 144.5 (C), 135.9 (CH), 135.0 (CH), 120.7 (C), 118.0 (CH), 113.6 (C), 103.5 (CH), 102.6 (CH), 95.3 (CH), 73.2 (CH), 72.3 (CH), 72.2 (CH), 71.6 (CH), 18.0 (CH₃).

Compound 3. UV λ_{max} : 257, 351 nm (MeOH); FAB-MS, m/z 447 $[\text{M} - \text{H}]^-$; ^1H NMR (500 MHz, CD_3OD) δ 7.34 (d, $J = 2.1$ Hz, 1H), 7.31 (dd, $J = 8.2, 2.1$ Hz, 1H), 6.91 (d, $J = 8.2$ Hz, 1H), 6.37 (d, $J = 2.1$ Hz, 1H), 6.20 (d, $J = 2.1$ Hz, 1H), 5.35 (d, $J = 1.7$ Hz, 1H), 4.21 (dd, $J = 3.3, 1.7$ Hz, 1H), 3.74 (dd, $J = 9.3, 3.3$ Hz, 1H), 3.41 (dd, $J = 9.5, 9.3$ Hz, 1H), 3.33 (dd, $J = 9.5, 6.1$ Hz, 1H), 0.94 (d, $J = 6.1$ Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 179.6 (C), 165.9 (C), 163.2 (C), 159.3 (C), 158.5 (C), 149.8 (C), 146.4 (C), 136.2 (C), 123.0

(C), 122.9 (CH), 116.9 (CH), 116.4 (CH), 105.9 (C), 103.5 (CH), 99.8 (CH), 94.7 (CH), 73.2 (CH), 72.1 (CH), 72.0 (CH), 71.9 (CH), 17.7 (CH₃).

Preparation of crude AP fraction for the bioassay. Frozen acerola fruit (1,000 g) was defrosted and the seeds were removed. Then the edible portion was homogenized with ethanol (2-liter) and extracted with stirring at room temperature for 1 h to obtain APs. This procedure was repeated twice. The resulting suspension was filtered, and the filtrate was concentrated and freeze-dried under reduced pressure. The powder obtained was dissolved in distilled water again, and the suspension was removed by filtration. These extracts were subjected to a C18 cartridge column (Sep-Pak Vac; Nihon Waters, Tokyo, Japan), eluted with 100% ethanol containing 10% of acetic acid, and then freeze dried (451 mg). The freeze dried acerola powder was referred to as a crude AP fraction. It contained 40% of polyphenols as analyzed by the Folin-Denis method.²⁰⁾

Antioxidant assay. Antioxidant activity was evaluated by the scavenging activity of the superoxide anion radical (O₂⁻) with an electron spin resonance (ESR) spectrometer (JEOL JES-FR30; JEOL, Tokyo, Japan). For O₂⁻ analysis, the following reagents were added in a test tube in the following order: 65 μ l of 2.0 M of DMPO, 50 μ l of sample, 50 μ l of 2 mM HPX, and 50 μ l of 0.4 units/ml XOD. All reagents including purified compounds were dissolved in 50 mM sodium phosphate buffer (pH 7.4). The solution was mixed and placed in a flat ESR cell, and then, exactly 45 s after mixing, DMPO-O₂⁻ spin adduct was analyzed by ESR spectrometry. The ESR settings were as follows: microwave power 4 mW, modulation frequency 100 kHz, modulation width 0.1 mT, scan time 2 min, response 0.1 s, magnetic field intensity 335.9 \pm 5 mT, and amplitude 100. O₂⁻ scavenging activity was evaluated by calculating the percentage of signal intensity of DMPO-O₂⁻ with and without radical scavenger.

Enzyme assay. Rat intestinal acetone powder was suspended in 9 fold (w/v) of 56 mM maleate buffer (pH 6.0), and homogenized with glass homogenizer in an ice bath for 15 min. After centrifugation at 1000 \times g for 10 min, the resulting supernatant was diluted with an equal volume of 56 mM maleate buffer (pH 6.0) for sucrase assay, or in a ratio of 1:19 for maltase assay. The reaction was started by adding 300 μ l of 2% maltose or sucrose solution and 300 μ l of 2 mg/ml purified polyphenols or crude APs solution to 300 μ l of the intestinal extracts prepared above, and the mixture was kept at 37 °C for 120 min. The reaction was stopped by immersing the mixture into a boiling water bath for 10 min. After centrifugation at 3000 \times g for 10 min, the glucose content of the resulting supernatant was measured by HPLC (CBM-10A system; Shimadzu, Kyoto, Japan) on a 3.0 \times 150 mm NH₂ column (Inertsil NH₂,

GL Science, Tokyo, Japan) using an RI detector (Shimadzu RID-6A, Shimadzu). The column oven was set at room temperature. The samples were eluted with 75% acetonitrile at a flow rate of 1.5 ml/min. The inhibitory effect of α -glucosidase was evaluated by calculating the percentage of glucose content of the reacting supernatant with and without inhibitors. The glucose content was obtained by subtracting the initial glucose content before reaction.

Evaluation of the inhibitory effect on AGE production. One ml of 16 mg/ml BSA, 1 ml of 4 M glucose, 1 ml of 67 mM sodium phosphate buffer (pH 7.2), and 1 ml of 0.3 mg/ml purified polyphenols or crude APs were mixed. All reagents were dissolved in 67 mM phosphate buffer (pH 7.2). AGEs was quantified three and seven days after incubation of the reaction mixture at 60 °C, by using a fluorescence spectrometer (RF-5300PC, Shimadzu; excitation wavelength, 370 nm; emission wavelength, 440 nm).⁷⁾ The inhibitory effect of AGE production was evaluated by calculating the percentage of fluorescence of reaction mixture with and without inhibitors.

Statistical analysis. Statview version 5 was used for statistical analysis (Abacus Concepts, Berkeley, CA). The one-way ANOVA model fitted by using Tukey for enzyme assay and inhibition of AGEs production was used. Significance was determined at $P < 0.05$.

Results

Isolation and identification of APs

Acerola fruit was extracted with methanol, and the extracts were further fractionated into five fractions by C18 cartridge column out of which fr. 3 (eluted with 20% methanol containing 0.2% TFA) and fr. 4 (eluted with 30% methanol containing 0.2% TFA) were found to contain polyphenols. Therefore, fr. 3 and fr. 4 were pooled and further purified, as described under "Materials and Methods", resulting in three polyphenol compounds (compounds 1–3, Fig. 1). From ¹H and ¹³C NMR measurements, these three compounds were assumed to be glycosides that contain flavonoidal structures as aglycon, and the sugar moiety with a methyl group. From the J values of H1'' ($J = 1.2$ (1), 1.5 (2), 1.7 (3)), these compounds were assumed to be α -glycosides. Compound 1 contains 1, 2, 4 trisubstituted (δ 6.93, 7.73, 7.91 in the ¹H NMR) and 1, 2, 3, 5 tetrasubstituted (δ 6.60, 6.79 in the ¹H NMR) benzene. In the HMBC spectrum, long-range correlation between H1'' (δ 5.70) in the sugar moiety and C3 (δ 144.6) was observed, indicating that the sugar moiety was conjugated with aglycon at the C3 position. The ¹H and ¹³C NMR data of compound 2 were quite similar to that of compound 1, except for chemical shifts due to the B ring. Coupling between the proton signal at δ 8.32 (H2' and H6', $J = 8.8$ Hz) and that at δ 7.03 (H3' and H5',

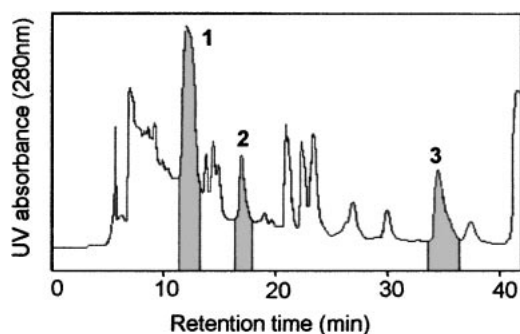


Fig. 1. HPLC Chromatogram for Fra. 3 (eluted with 20% methanol containing 0.2% TFA).

Acerola fruit was extracted with methanol and the extract was chromatographed on a C18 cartridge column by eluting with 10% and 20% methanol with 0.1% TFA successively. The 20% methanol eluted fraction was subjected to HPLC chromatography. Separation was made on a 10 × 250 mm Develosil RPAQUEOUS-AR-5 C30 HPLC column by eluting with 20% acetonitrile containing 0.1% TFA at a flow rate of 2.3 ml/min, with detection at 280 nm.

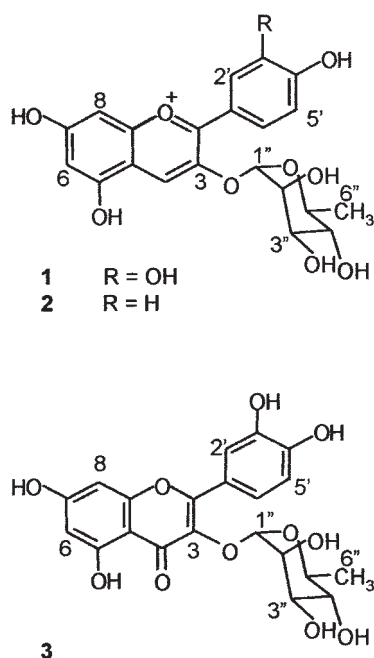


Fig. 2. Structures of the APs Isolated from Acerola Fruit.
1 cyanidin-3- α -O-rhamnoside, 2 pelargonidin-3- α -O-rhamnoside, 3 quercetin-3- α -O-rhamnoside.

$J = 8.8$ Hz) in compound 2 supports the presence of 1,4 disubstituted benzene structure in compound 2. The ^1H and ^{13}C NMR spectra of compound 3 showed good agreement with those of authentic quercitrin. Long-range correlation between $\text{H}1''$ ($\delta 5.35$) in the sugar moiety and C3 ($\delta 136.2$) in the HMBC spectrum indicated that the sugar moiety is conjugated with aglycon at the C3 position.

From these results, these three compounds were identified as follows: Compound 1, cyanidin-3- α -O-rhamnoside (C3R); Compound 2, pelargonidin-3- α -O-rhamnoside (P3R); and Compound 3, quercetin-3- α -O-rhamnoside (quercitrin, Fig. 2).

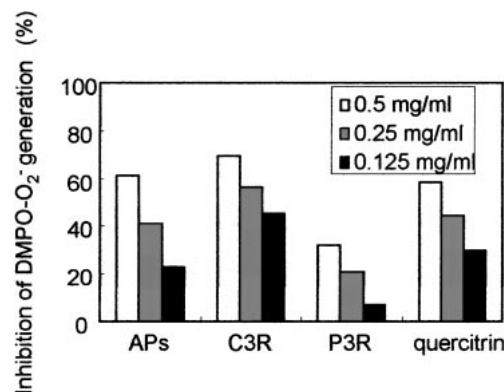


Fig. 3. Inhibitory Effect of Three Isolated Compounds and Crude AP Fraction on DMPO-O_2^- Generation Measured by ESR.

O_2^- was produced by reaction between HPX and XOD, and the O_2^- produced was trapped by DMPO (2.0M) to form DMPO-O_2^- spin-adduct. O_2^- scavenging activity was evaluated by calculating the percentage of signal intensity of DMPO-O_2^- with and without radical scavenger. The concentration of the sample was set at 0.125–0.5 mg/ml (C3R: 0.29–1.15 mM, P3R: 0.30–1.20 mM, quercitrin: 0.28–1.12 mM).

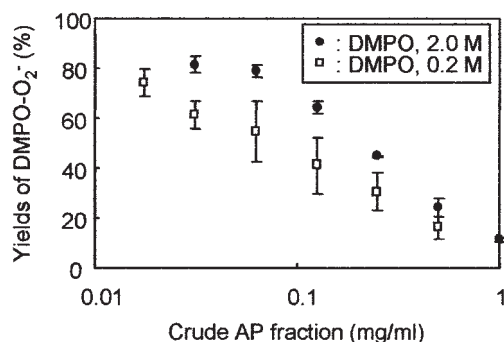


Fig. 4. Relationship between Signal Intensities of DMPO-O_2^- and Concentrations of Crude AP Fraction.

The relationship between generation of DMPO-O_2^- spin adduct and concentrations of the crude AP fraction was evaluated in the two different concentrations of DMPO (from 2.0 M to 0.2 M). Results are the means \pm SD from two separate experiments.

Antioxidant activity

The reactive oxygen scavenging activities of the three purified polyphenols as well as the crude AP fraction were assessed at concentrations of 0.125–0.5 mg/ml by ESR (Fig. 3). All samples inhibited the generation of DMPO-O_2^- spin adduct, and particularly, C3R and quercitrin showed strong inhibition on generation.

By changing the concentration of DMPO (from 2.0 M to 0.2 M) to vary the spin trapping rate of O_2^- radicals, the presence of a competitive reaction between crude APs fraction and O_2^- radicals was studied (Fig. 4).³⁾ By diluting DMPO concentration, the inhibition curve was shifted to the left, particularly in the low concentration range of the crude AP fraction, indicating that inhibition of the crude AP fraction on DMPO-O_2^- generation is due mainly to scavenging O_2^- radicals, not to direct inhibition of xanthine oxidase. The three isolated polyphenols also showed the same results (data not shown).

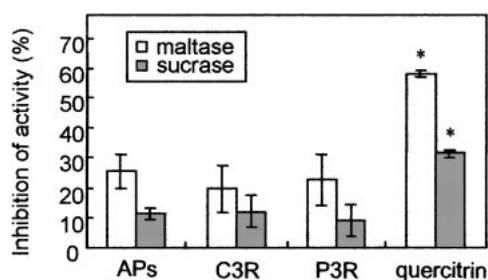


Fig. 5. Inhibitory Effects of Three Isolated Compounds and Crude AP Fraction on Maltase and Sucrase.

Maltase and sucrase were extracted from rat intestinal acetone powder, and the extracts were exposed to maltose or sucrose with and without inhibitors at 37°C for 120 min. Inhibition of α -glucosidase activity was evaluated by calculating the percentage of glucose content of the reacting supernatant with and without inhibitors. The concentrations of the inhibitors were set at 2 mg/ml (C3R: 4.62 mM, P3R: 4.79 mM, quercitrin: 4.47 mM). Results are the means \pm SD from five separate experiments. * $P < 0.01$ in comparison with the other three inhibitors.

Inhibitory effect on α -glucosidase

The inhibitory effects of the three purified polyphenols and crude AP fraction on maltase and sucrase were evaluated *in vitro*, and the data are shown in Fig. 5. Inhibitory effects were found in all samples, but inhibitory activities were not strong except for quercitrin. Quercitrin inhibited approximately 60% of maltase activity and thus was considered to be an effective α -glucosidase inhibitor.

Inhibitory effect on AGE formation

Figure 6 shows inhibition of AGE production by the purified polyphenols and crude AP fraction. Aminoguanidine, a hydrazine-like agent that blocks AGEs formation by interacting with Amadori-derived products,²¹⁾ was also used as a positive control. At a concentration of 0.3 mg/ml, all samples exhibited strong

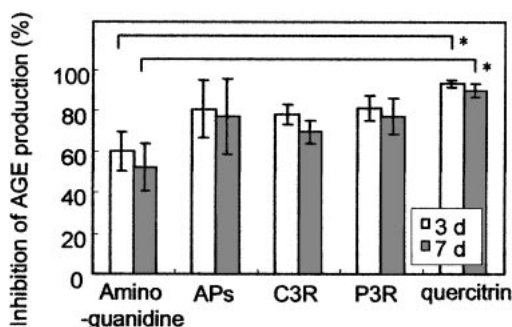


Fig. 6. Inhibitory Effects of Three Isolated Compounds and Crude AP Fraction on AGE Production.

Inhibition of AGE production was assessed by reacting BSA and glucose with and without inhibitors at 60°C for 3 and 7 d. AGE production was quantified by monitoring fluorescence at the excitation and emission wavelengths of 370 and 440 nm respectively. The concentration of the inhibitors was set at 0.3 mg/ml (C3R: 693 μ M, P3R: 719 μ M, quercitrin: 670 μ M). Results are the means \pm SD from five separate experiments. * $P < 0.05$ in comparison with aminoguanidine.

inhibitory effects, and especially, quercitrin showed higher inhibition than aminoguanidine.

Discussion

Recently, it has been reported that acerola fruit extract might have anticarcinogenic activity against lung cancer,²²⁾ and an inhibitory effect on NO production.²³⁾ These effects might have been due to components other than vitamin C, but the components responsible for these effects have not been identified. In addition to vitamin C, acerola is known to contain carotenoids as a food factor.²²⁾ This study presents new information on the functional components of acerola other than vitamin C and carotenoids.

As mentioned earlier, it has been reported that acerola contains malvin as anthocyanin.¹⁸⁾ This is inconsistent with the results of the present study, in where we did not detect any other anthocyanin but C3R and P3R. This might be due to the fact that those researchers identified the pigment only by comparison of physical properties and the UV/VIS spectra of several pigments.

It should be mentioned that the structures of the three polyphenols are already known. In particular, quercitrin, one of the isolated polyphenols, is a well-known flavonol, and has frequently been implicated in various physiological effects such as anti-inflammatory activity²⁴⁾ and antidiarrheic activity.²⁵⁾ Quercitrin has also been reported to inhibit aldose reductase²⁶⁾ and monoamine oxidase.²⁷⁾ Hence quercitrin might be beneficial for the prevention of diabetic complications such as cataracts. This study also found that quercitrin showed high activities in all assays tested. Taken together, it is reasonable to expect that acerola quercitrin can be used as an excellent antidiabetic agent.

Anthocyanin pigments are widespread in the plant kingdom. C3R and P3R have also been reported in several plants,^{28–31)} but studies on their functional properties appear to have drawn little attention. In our study, while C3R showed a strong O_2^- scavenging activity, of the same level as that of quercitrin, the activity of P3R was lower than that of C3R or quercitrin (Fig. 3). This result indicates that O_2^- scavenging activity is strongly correlated with the number of OH-bonds of B-ring in polyphenol.³²⁾ On the other hand, although inhibitory effects on α -glucosidase were observed in both C3R and P3R, they were much weaker than that of quercitrin (Fig. 5). In this regard, recently Matsui *et al.*^{33,34)} found that α -glucosidase inhibition of anthocyanins was due to acylated structures, and similarly, that α -glucosidase inhibition by deacylated anthocyanins was weaker than that by acylated ones. Since C3R and P3R are also deacylated anthocyanins, these inhibitory effects were not expected.

The results concerning the inhibitory effects on AGE formation, however, showed different tendencies from O_2^- scavenging activity or α -glucosidase inhibition (Fig. 6). C3R and P3R were both strong inhibitors of

AGE formation as well as quercitrin. Despite not being a good scavenger of the O_2^- radical, P3R strongly blocked AGE formation. These facts suggest that the production of radical species like O_2^- radical is not involved in the formation of AGEs. In addition, it is remarkable that inhibition of AGE formation by the three isolated compounds, especially quercitrin, was higher than that by aminoguanidine. In this study, AGEs were quantified by monitoring fluorescence at the excitation and emission wavelengths of 370 and 440 nm, and this is probably derived from glycated lysine-like compounds.⁷⁾ So the production of these types of AGEs might be strongly inhibited by isolated compounds. Nevertheless, AGE is a complicated product and it is not possible to monitor all types of AGEs by a single fluorescent wavelength. Hence it is necessary to examine the inhibitory effect on the production of the other types of AGEs further. Moreover, these results are restricted to data derived from *in vitro* study, and further detailed investigations *in vivo* are needed to substantiate these observations.

The functional properties of the crude AP fraction were almost the same level as those of C3R (Fig. 3–6). These results are probably due to the proportion of polyphenols in the crude AP fraction. The content of C3R was about 5–10 fold higher than the other polyphenols in acerola fruit (data not shown). Therefore, the functional properties of the crude AP fraction are perhaps close to those of C3R.

In conclusion, C3R, P3R, and quercitrin were identified as the constituents of acerola polyphenols for the first time. This study also showed that the three isolated purified polyphenols as well as the crude AP fraction from the fruit possessed O_2^- scavenging activity and an inhibitory effect on both α -glucosidase and AGE formation by *in vitro* study. Thus, compounds 1, 2, and 3 are expected to be beneficial constituents to prevent diabetes mellitus and its complications.

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