JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Reddish Coloration of Chinese Quince (*Pseudocydonia sinensis*) Procyanidins during Heat Treatment and Effect on Antioxidant and Antiinfluenza Viral Activities

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To investigate the effects of processing Chinese quince fruit on the denaturation of phenolics and their food functions, fruit phenolic extracts were heated together with organic acid for up to 12 h. Chinese quince phenolic (mostly procyanidins) solution subjected to heat treatment changed from almost colorless, pale yellow, to a reddish color. Before heat treatment, the absorption spectra of polymeric procyanidins were observed only around 280 nm; after heat treatment, absorption occurred between 400 and 600 nm, which is related to the reddish color appearance. Thioacidolysis of denatured reddish phenolics showed that (−)-epicatechin subunits decreased during heat treatment and, in contrast, cyanidin increased. In addition, novel substances that could not be degraded by thioacidolysis were formed. Meanwhile, antioxidant activities, assessed by linoleic acid peroxidation, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin−Ciocalteu, and FRAP methods, increased during heat treatment. The antiinfluenza viral activity of denatured reddish phenolics was inferior to that of intact fruit phenolics; however, they retained moderate activity. These results indicate that red coloration of fruit products of Chinese quince was mainly due to the spectral (i.e. structural) changes of procyanidins accompanied with formation of cyanidin. Increasing the length of heat treatment increased the antioxidant capacity of phenolics, and the resultant reddish phenolics retained moderate antiinfluenza viral activity.

KEYWORDS: Polyphenol; flavan-3-ol; heating; coloration; denaturation; antioxidant activity; antiinfluenza viral activity

INTRODUCTION

Chinese quince (*Pseudocydonia sinensis* Schneider = *Chaenomeles sinensis* Koehne) is native to China, and the fruits have been used in traditional Chinese medicine. In Japan, there are few Chinese quince orchards for industry, but the trees have been planted in some Shinto shrine precincts or gardens of common houses. Chinese quince fruit has woody hard flesh and strong astringency; therefore, the raw fruit is not edible but has been consumed as products such as fruit liquor, jam, and glutinous starch syrup, etc. The fruit liquor or decocted syrup of the fruit has been used in folk medicine and claimed to have effects of antitussive, expectorant, and fortifying a weak constitution (*1*). In addition, the extracts have been used industrially in candies or beverages sold with health claims.

Phenolics are believed to be one of the bioactive compounds responsible for the medicinal effects of Chinese quince fruit and products of the fruit. Phenolics from the fruit have been reported to have an inhibitory effect on hyaluronidase and histamine release from mast cells, in vitro (*2*). It also has been reported that flavonoids from Chinese quince fruit have an antipruritic effect (*3*).

In our previous study, we found Chinese quince phenolics had strong antiinfluenza viral activity and antiulcerative activity and moderate antioxidant activity (*4*, *5*). However, these activities were the result of experiments using fresh fruit phenolics, and effects of processing were not considered. Since Chinese quince is normally consumed as processed products, it is important to investigate the effects of processing of functional components.

Products of Chinese quince fruit, such as fruit liquor, juice, and dried flesh, etc., often change their color into dark red or reddish brown (**Figure 1**). Some products, such as boiled down syrups, are subjected to heating for a certain time during processing and change to an intense reddish color. This phenomenon has been known empirically; however, there are no reports that address the cause of this reddish coloration during processing. Because the fruit is rich in procyanidins (**Figure 2**) as the major phenolics, it can be predicted that this reddish coloration results from the chemical conversion of procyanidins to cyanidin, a kind of red pigment. The conversion of procyanidins to cyanidin is a well-known reaction; however, the conversion is normally reported to be a result of a reaction with mineral acids such as HCl (*6*−*8*). It has been reported that when acetic acid was used instead of mineral acid, the conversion did not occur (*9*). Although the mechanism of the reddish

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10.1021/jf061836+ CCC: $37.00
Published on Web 01/20/2007

Figure 1. Red coloration observed in fruit liquor of Chinese quince fruit.

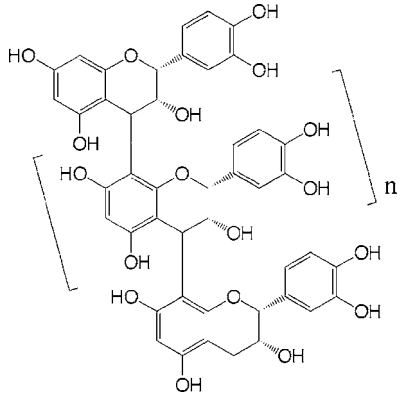

Figure 2. Structure of procyanidins as major phenolic compounds in Chinese quince fruit. $n \geq 0$.

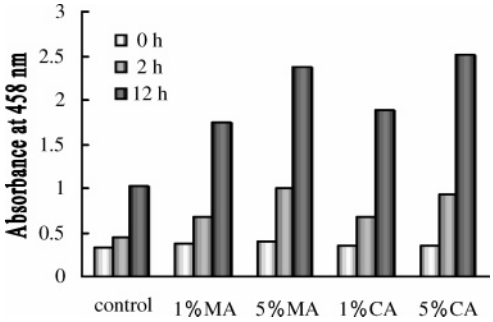

Figure 3. Red color intensity ($A = 458$ nm) of Chinese quince phenolic solution heated with organic acid: MA, malic acid; CA, citric acid.

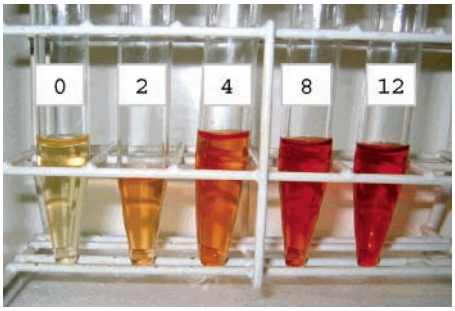

Figure 4. Reddish coloration in aqueous solution of Chinese quince phenolics during heating with citric acid. Numbers indicate heating time (h).

coloration of Chinese quince products has not been clarified, it is likely that procyanidins in the products are involved in the coloration and thus their structure and functions must be affected. Procyanidins seem to play an important role in antiinflammatory (*2*), antioxidant, antiinfluenza viral (*4*), and antiulcerative activities (*5*) of Chinese quince phenolics. Therefore, the effects of heating and the reddish coloration of Chinese quince extracts on the characteristics of phenolics, especially procyanidins, must be clarified.

The purpose of this research was, first, to confirm the cause of the reddish coloration of fruit extracts of Chinese quince and, second, to investigate the effect of heating and the reddish coloration (denaturation) of phenolics (especially procyanidins) on the antioxidant and antiinfluenza viral activities.

MATERIALS AND METHODS

Plant Materials. The ripe fruits of Chinese quince (*Pseudocydonia sinensis* Schneider var. Toukarin) were obtained from a local orchard in Nagano prefecture, Japan.

Chemicals and Reagents. (−)-Epicatechin and 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ) were purchased from Sigma−Aldrich, Ltd. (St. Louis, MO). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and citric and malic acids were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Solvents were purchased from Nacalai Tesque. Folin−Ciocalteu reagent, sodium dodecyl sulfate (SDS), linoleic acid, 2,2′-azobis(2-amidinopropane)-dihydrochloride (AAPH) and toluene-α-thiol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Preparation of Fruit Phenolic Extracts. The flesh part of the fruit (five to six fruits) was cut into small pieces, frozen in liquid nitrogen, freeze-dried using FD-5N (Tokyo Rikakikai Co., Ltd., Tokyo, Japan) and then homogenized. Before phenolic extraction, the powdered flesh (30 g) was mixed with 300 mL of petroleum ether in a beaker, stirred, and filtered to remove lipids; this was performed 5 times. The phenolics were then extracted from the residue with 60% aqueous acetone (300 mL, 3 times), and filtered as above. The 60% acetone extracts were evaporated under reduced pressure at 40 °C until all organic solvent was removed; they were then redissolved into acidified water (0.1% trifluoroacetic acid) and applied to a Sep-Pak Vac 20 cm^3 (5 g) C18 cartridge (Waters Co., Milford, MA), preconditioned with methanol and acidified water. The column was washed with 30 mL of acidified water, and the phenolics were recovered with 20 mL of methanol. The phenolics were redissolved into an aliquot of water, frozen and then freeze-dried to obtain dried phenolic powder (crude phenolic extracts). The overall yield of the dried phenolic extracts from 30 g of flesh powder was 3.02 g (containing 2022 mg of phenolics as (−)-epicatechin equivalent).

Total Phenolic Assay. The experimental procedure was adapted from that of Hamauzu et al. (*4*, *5*). A 2 mL aliquot of sample solution was mixed with 2 mL of diluted Folin−Ciocalteu reagent (1 N) in a test tube. After 3 min of reaction, 2 mL of Na_2CO_3 (10 g/(100 mL)) was added and the mixture was incubated for 60 min at room temperature. The absorbance was measured at 700 nm with a Shimadzu UV-1200 spectrophotometer (Tokyo, Japan) against a blank (2 mL of deionized water, plus reagents) in the reference cell. (−)-Epicatechin was used as the standard ($r = 0.999\,75$).

Heat Treatment. A 5 mL aliquot of crude phenolic solution (2 mg/mL as epicatechin equivalent) was mixed with 5 mL of 10% (w/v) citric acid in a screw-capped test tube and then heated for 0, 2, 4, 8, and 12 h in a dry bath incubator (GENIUS two block, Nippon Genetics Co., Ltd., Tokyo, Japan). The set temperature was 110 °C to obtain 95 °C of reaction mixture.

Measurement of Reddish Color Intensity. The reddish color formed by the heat treatment had an absorption maximum at 458 nm, so this wavelength was selected for measurement of reddish color intensity. The absorbance of heated solution was measured using a Shimadzu UV-1200 spectrophotometer after dilution with water. The reddish color intensity was defined as the absorbance at 458 nm by dilution factor.

Solid-Phase Extraction of Denatured (Reddish Colored) Phenolics. The reddish colored phenolics obtained by heat treatment were semipurified using a Sep-Pak plus C18 cartridge (Waters Co., Milford, MA) for the antiinfluenza viral assay. A three-cartridge (preconditioned with 5 mL of methanol and 0.1% trifluoroacetic acid) was used to

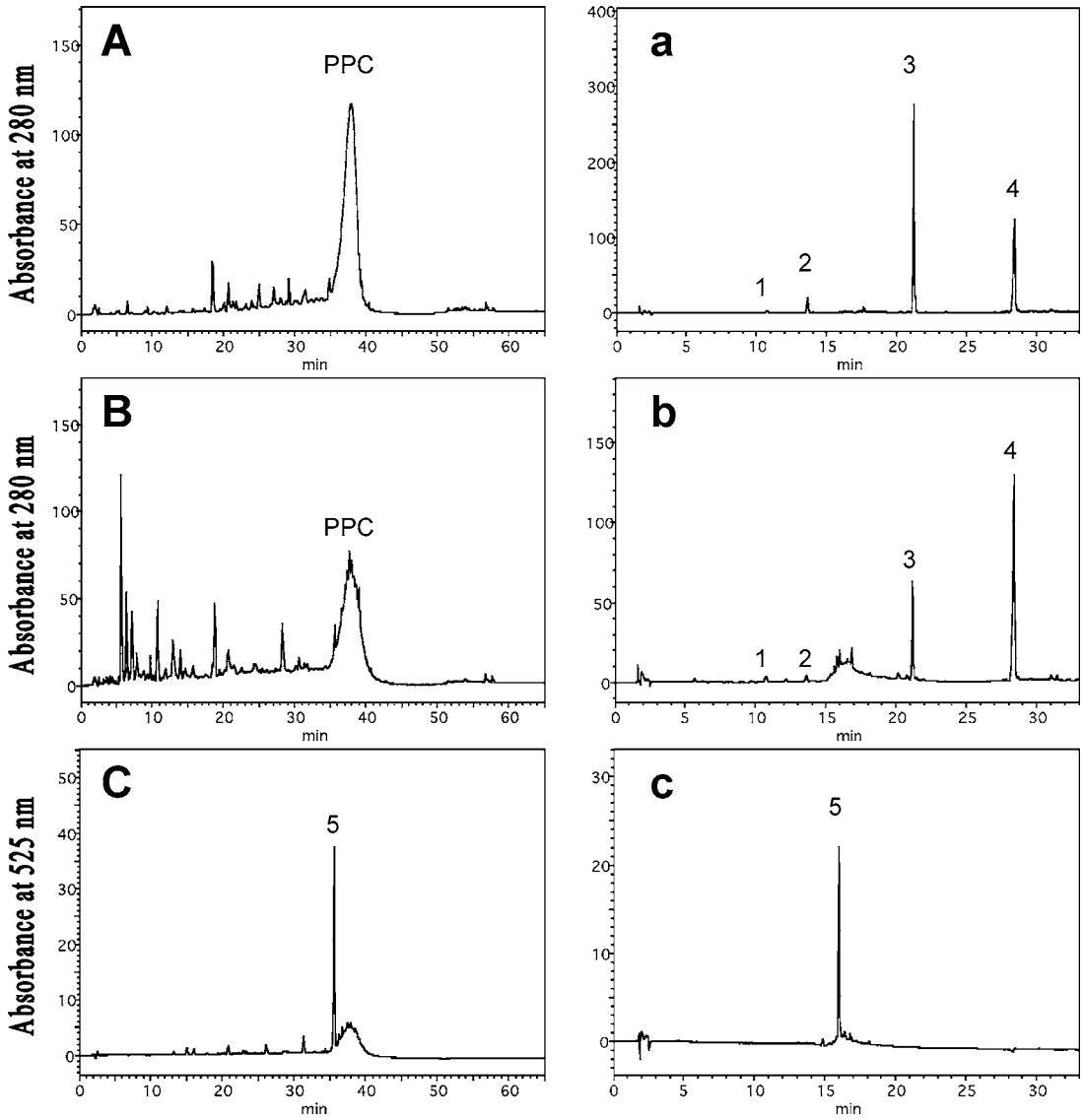

Figure 5. HPLC profiles of Chinese quince phenolics: (A) Chromatogram at 280 nm without heating and before thioacidolysis; (a) chromatogram at 280 nm without heating and after thioacidolysis; (B) chromatogram at 280 nm with heat treatment and before thioacidolysis; (b) chromatogram at 280 nm with heat treatment and after thioacidolysis; (C), chromatogram at 525 nm with heat treatment and before thioacidolysis; (c) chromatogram at 525 nm with heat treatment and after thioacidolysis. Heat treatment was conducted by heating of phenolics under existence of citric acid for 12 h. PPC, polymeric procyanidins; 1, (+)-catechin; 2, (–)-epicatechin; 3, (–)-epicatechin benzylthioether; 4, toluene-α-thiol; 5, cyanidin.

adsorb denatured reddish phenolics dissolved in 5 mL of reaction mixture. Phenolics were recovered with 1% perchloric acid−methanol, rotary evaporated, re-dissolved into an aliquot of water, and then freeze-dried. Reddish colored phenolics formed in Chinese quince fruit liquor during 2 years of storage were also collected using a Sep-Pak plus C18 cartridge, as above, and applied to the antiinfluenza viral assay.

HPLC Conditions. Chromatographic separation was carried out on a Luna 5μ C18 column (150 × 4.6 mm, Phenomenex, Inc., Torrance, CA) at 40 °C. Solvents were 0.1% trifluoroacetic acid (A) and 0.1% trifluoroacetic acid in acetonitrile (B). The gradient program was started with 5% B and changed to obtain 15% B at 30 min, 32% B at 35 min, 40% B at 45 min, and 75% B at 50 min. The 75% B was maintained until 65 min. The flow rate was 1.0 mL/min, and the injection volume was 20 μL. Detection was performed at 280 nm for flavan-3-ols and 325 nm for hydroxycinnamic derivatives on a Shimadzu SPD-M10Avp photodiode array detector. For the detection of reddish colored substances, 458 and 525 nm were also used.

Identification and Quantification of Phenolics. Identification was achieved by comparing retention times and UV−visible spectra with those of standards. Procyanidins were identified by comparing the absorption spectra with (−)-epicatechin and also confirmed by chromatographic changes after thioacidolysis, described below.

Quantitative determinations were made from calibration curves obtained by injection of different volumes of commercial standards. (−)-Epicatechin and (+)-catechin subunits in procyanidins were determined after thioacidolytic degradation using toluene-α-thiol reagent (5% (v/v) in methanol), as described by Guyot et al. (*10*). Experimental conditions and the calculation method were as described in our previous report, Hamauzu et al. (*4*, *5*). HPLC conditions for determination of flavan-3-ol subunits were as described above except that the gradient program was shortened for rapid analysis.

Antioxidant Assay and Metal Reducing Ability Assay. The antioxidant capacity of phenolics was estimated using a linoleic acid peroxidation system and DPPH radical scavenging system. The experimental conditions were as described in our previous report (*5*). The activities were expressed as percentages of conjugated diene formation and of radical scavenging, respectively.

The metal reducing ability of phenolics was estimated using the Folin−Ciocalteu reagent and the ferric reducing antioxidant power (FRAP) method. Folin−Ciocalteu reagent reducing ability was measured as described in Total Phenolic Assay.

The experimental conditions for the FRAP method basically followed that of Benzie and Strain (*11*) with minor modification. The FRAP reagent was prepared as a mixture of 10 mL of 300 mM acetate buffer

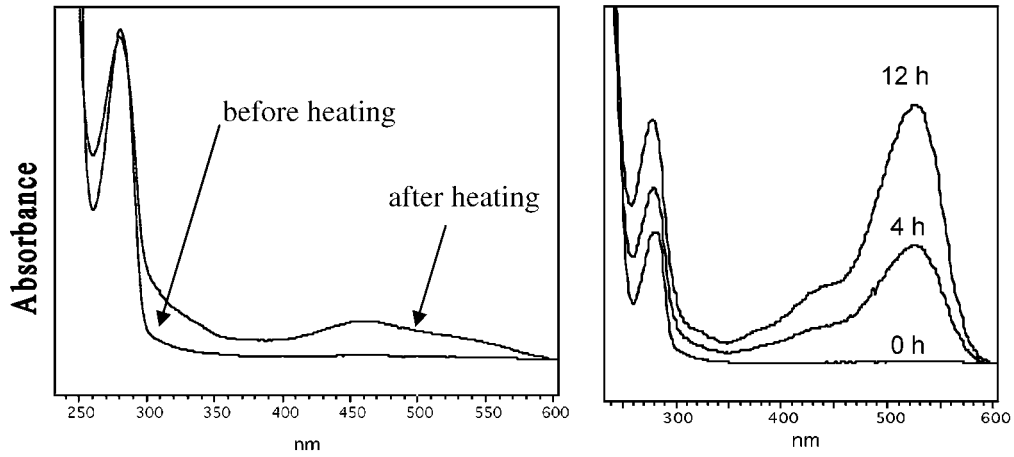

Figure 6. Change in absorption spectra of the polymeric procyanidins (left) and the increase of cyanidin (right; peak 5 in Figure 5) by heat treatment.

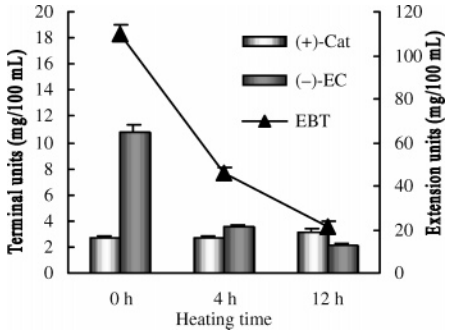

Figure 7. Change in (+)-catechin and (−)-epicatechin subunit content included in procyanidin structure during heating in the presence of citric acid. Bars indicate SE ($n = 3$).

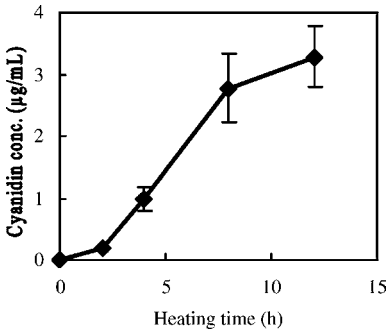

Figure 8. Change in cyanidin concentration during heating in the presence of citric acid. Bars indicate SE ($n = 3$).

(pH 3.6), 1 mL of 10 mM TPTZ−40 mM hydrochloric solution, and 1 mL of 20 mM $FeCl_3$ solution. The FRAP reagent, once prepared, was immediately incubated for 10 min at 37 °C, and then 1.5 mL of the reagent was added to 50 μL of sample solution previously diluted. The reaction mixture was incubated for 4 min at room temperature, and the absorbance at 593 nm was measured using a UV-1200 spectrophotometer.

Antiinfluenza Viral Activity. The hemagglutination inhibition test using chicken erythrocyte was used to measure the antiinfluenza viral activity. Before addition to the system, phenolics heated with citric acid were reextracted from the reaction mixture using a Sep-Pak plus C18 cartridge to remove the citric acid. The experimental procedure was as described in Hamauzu et al. (*4*).

RESULTS

Verification of Reddish Coloration of Phenolic Extracts from Chinese Quince Fruit. The intensity of reddish color and absorbance at 458 nm of Chinese quince phenolic solution increased during heating, and the increase was greater in solutions containing organic acid than without organic acid (**Figures 3** and **4**). The intensity of the reddish color was stronger with longer heating times (up to 12 h) or with higher concentrations of organic acids (up to 5%, v/v). There was no difference in coloration between the addition of malic acid and citric acid (**Figure 3**).

Effects of Heating on the Phenolic Profile of Chinese Quince Fruit Mixed with Citric Acid. Heating of the Chinese quince fruit phenolics in the presence of citric acid affected their HPLC profile. Upon examination of the HPLC chromatogram, an increase in the earlier peaks and a decrease in a lump of polymeric procyanidins (PPC) were observed at 280 nm (**Figure 5A**,**B**), whereas, at 525 nm, there was no peak before heating, but the appearance of a new large peak and PPC lump was observed (**Figure 5C**). Moreover, intact phenolics from Chinese quince were almost completely decomposed by thioacidolysis (**Figure 5a**), whereas complete decomposition was impossible after heat treatment (**Figure 5b**). The novel large peak detected at 525 nm was not affected by thioacydolysis (**Figure 5c**), whereas part of the PPC lump disappeared at 525 nm.

The absorption spectra of polymeric procyanidins changed after heat treatment, and absorption in the area of wavelength relating to the appearance of the red color increased (**Figure 6**, left). The absorption spectra of the novel large peak detected at 525 nm after heat treatment was a similar shape to that of cyanidin (**Figure 6**, right). Retention time of the novel peak was also the same as cyanidin after butanol−HCl degradation of procyanidins.

Thioacidolytic products of procyanidins decreased with an increase in heating time (**Figure 7**). The decrease of (−)-epicatechin benzylthioether as "extension units" was larger than that of (−)-epicatechin as "terminal units", although the percentage of the decrease was almost the same (80.3% of (−)-epicatechin subunits were lost), whereas (+)-catechin subunits as terminal units seemed to be constant. In contrast to the decrease of (−)-epicatechin subunits, cyanidin increased remarkably during heat treatment (**Figure 8**).

Effects of Heating on the Antioxidant and Antiinfluenza Viral Activities. Antioxidant activities assessed by linoleic acid peroxidation, DPPH radical scavenging, Folin−Ciocalteu reducing, and FRAP methods all increased during heat treatment and with an increase in the intensity of the red color (**Figure 9**).

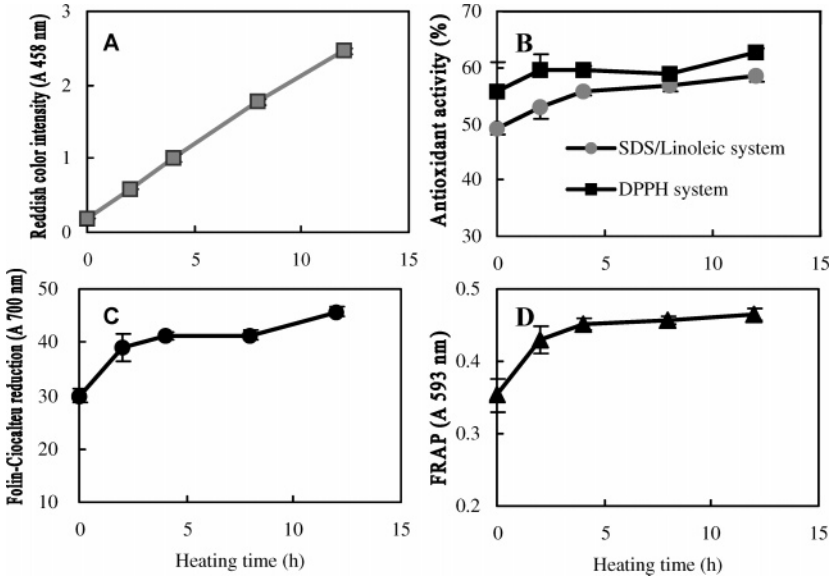

Figure 9. Changes in red color intensity (A), antioxidant capacity (B), and metal reducing ability (C and D) of Chinese quince phenolics during heating in the presence of citric acid.

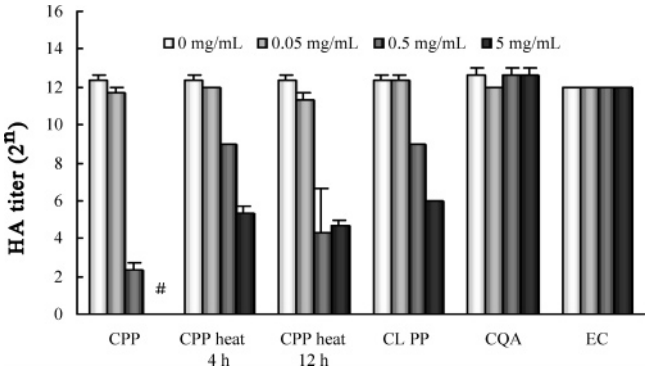

Figure 10. Antiinfluenza viral activity of Chinese quince polyphenolics (CPP), reddish colored CPP after heating (CPP heat), and reddish colored CPP from long-term stored fruit liquor (CL PP): HA, hemagglutination; CQA, caffeoylquinic acid (chlorogenic acid); EC, (–)-epicatechin. #, 5 mg/mL of CPP was impossible to determine. Bars indicate SE ($n = 3$).

The main increase occurred after 4 h of heating, and slight increases were observed thereafter.

The antiinfluenza viral activity of the reddish colored phenolics was inferior to the activity of intact fruit phenolics. The intact phenolics decreased the HA titer of influenza virus from $10^{12.3}$ into $10^{2.3}$ at a concentration of 0.5 mg/mL, whereas phenolics heated for 4 and 12 h decreased the HA titer into 10^{9} and $10^{4.3}$, respectively, at the same concentration (**Figure 10**). Reddish denatured phenolics collected from Chinese quince fruit liquor also showed moderate activity, and the effect was similar to that of the 4 h heated phenolics. However, chlorogenic acid and (−)-epicatechin standard had no antiinfluenza viral activity.

DISCUSSION

In this study, we revealed that reddish coloration of Chinese quince fruit products was due to denaturation of procyanidins, an abundant constituent in the fruit products. An increase in absorption at 400−600 nm of polymeric procyanidins indicated their contribution to the reddish coloring in the fruit products, since orange to reddish compounds normally absorb at around this area of wavelengths. The coloration of fruit liquor or glutinous starch syrup made from Chinese quince fruit may be due to the reaction of procyanidins and organic acids derived from their flesh. It has been reported that Chinese quince fruit contains malic, citric, and quinic acids at high concentrations (*12*), and it may be one of a few fruits containing a high content of phenolics, organic acids, and ascorbic acid.

The mechanism of the reddish color change of the fruit products must at least involve a structural change in (−)-epicatechin subunits, which are abundant in Chinese quince procyanidins: a part of the (−)-epicatechin subunits are then converted into cyanidin. A decrease in (−)-epicatechin subunits in procyanidins with an increase in cyanidin indicated this conversion. This reaction is well-known and well-characterized; however, this reaction has normally been reported as a result of decomposition with mineral acids (*6*−*8*). In the current experiment, organic acids might have promoted the structural changes in the (−)-epicatechin subunits to form the cyanidin structure. However, cyanidin formation did not seem to be the main factor in the reddish coloration because the main spectral changes of the whole phenolic solution occurred at around 458 nm in the visible light area and were due to the spectral changes in polymeric procyanidins. This indicates that polymeric procyanidins had been partially rearranged during the heat treatment, and therefore novel substances formed from procyanidins had not been degraded by thioacidolysis. Although further studies are needed to elucidate the structure of the newly formed substances, there is a possibility that they include xanthylium cation structures generated by the reaction between flavan-3-ols and organic acids (*13*, *14*). It has been shown (*9*) that procyanidins can be rearranged in acidic aqueous media to form xanthylium salts that exhibit a λ_{max} at around 440 nm. The UV−vis spectrum of heated procyanidins in this study was similar to that reported for xanthylium pigments, although there were some differences. The xanthylium pigments reported exhibit a λ_{max} at around 440 nm, and there was almost no absorption at 550 nm (*14*), whereas, in heated procyanidins, the maximum absorbance was at 458 nm and there was a wide range of absorption up to nearly 600 nm. This suggests that cyanidin structure may also be included in heated procyanidins as a subunit.

Increased heating times did not decrease the antioxidant activity of the phenolics; in fact, activity increased as the reddish coloration increased with the duration of heating. Formation of lower molecular weight compounds may be involved in the

increase in activity because oligomeric procyanidins were reported to have stronger activity in the DPPH radical scavenging system than polymeric procyanidins (*15*). Moreover, low molecular weight compounds such as (−)-epicatechin or procyanidin B2 have been shown to have stronger antioxidant activity in the linoleic acid peroxidation system than polymeric procyanidins (*4*). This suggests a possibility to improve the beneficial health functions of phenolics by artificial modification. Further investigation is needed to verify whether the increase in antioxidant activity by heat treatment improves the antioxidant effect in vivo after ingestion of heat-treated phenolics.

In this study, Folin−Ciocalteu reagent was used to assess the metallic ion reducing ability of phenolics. Since heat treatment improved reduction of Folin−Ciocalteu reagent (and it is unlikely that the total phenolic concentration was increased by heating), the reducing ability of phenolics might increase by heating together with citric acid. This suggests estimation of total phenolic concentration using the Folin−Ciocalteu method should be taken cautiously when other treatments are involved.

In the experiment for antiinfluenza viral activity, the activity of denatured reddish phenolics produced after increased heating times were inferior to that of intact fruit phenolics. The reddish colored phenolics seemed to have a lower content of procyanidin structure than the original phenolics, which is consistent with the observation that the proportion of procyanidin is important in the strong antiinfluenza viral activity of phenolic extracts of fruits (*4*). However, the denatured phenolics did have moderate activity, and the reddish colored phenolics collected from Chinese quince fruit liquor also had moderate activity. These results indicate Chinese quince phenolics may inactivate the influenza virus even after certain processing and modifications.

In conclusion, our results indicate that the red coloration of fruit products of Chinese quince was mainly due to a spectral change in procyanidins, of which absorbance between 400 and 600 nm, corresponding to the red color, increased. The spectral change seemed to be caused by a structural change of procyanidins to colored substances that might include xanthylium structure and cyanidin structure. Heating, especially in the presence of organic acid, accelerated these changes. Meanwhile, increased heating times of the fruit procyanidins in the presence of citric acid increased their antioxidant capacity, and the resultant reddish phenolics retained moderate antiinfluenza viral activity. Therefore, the denatured reddish procyanidins contained in the processed products of Chinese quince fruit may have health benefits.

ABBREVIATIONS USED

DPPH, 1,1-diphenyl-2-picrylhydrazyl; FRAP, ferric reducing antioxidant power; TPTZ, 2,4,6-tri(2-pyridyl)-s-triazine; SDS, sodium dodecyl sulfate; AAPH, 2,2′-azobis(2-amidinopropane)-dihydrochloride; PPC, polymeric procyanidins.

LITERATURE CITED

(1) Suzuki, H. *Encyclopedia of Traditional Oriental Drugs*; Ishiyaku: Tokyo, Japan, 1994; p 400 (in Japanese).

(2) Osawa, K.; Miyazaki, K.; Imai, H.; Arakawa, T.; Yasuda, H.; Takeya, K. Inhibitory effects of Chinese quince (*Chaenomeles sinensis*) on hyaluronidase and histamine release from rat mast cells (in Japanese with English summary). *Nat. Med.* **1999**, *53*, 188−193.

(3) Oku, H.; Ueda, Y.; Ishiguro, K. Antipruritic effects of the fruits of *Chaenomeles sinensis*. *Biol. Pharm. Bull.* **2003**, *26*, 1031−1034.

(4) Hamauzu, Y.; Yasui, H.; Inno, T.; Kume, C.; Omanyuda, M. Phenolic profile, antioxidant property, and anti-influenza viral activity of Chinese quince (*Pseudocydonia sinensis* Schneid.), quince (*Cydonia oblonga* Mill.), and apple (*Malus domestica* Mill.) fruits. *J. Agric. Food Chem.* **2005**, *53*, 928−934.

(5) Hamauzu, Y.; Inno, T.; Kume, C.; Irie, M.; Hiramatsu, K. Antioxidant and antiulcerative properties of phenolics from Chinese quince, quince, and apple fruits. *J. Agric. Food Chem.* **2006**, *54*, 765−772.

(6) Bate-Smith, E. C. Leuco-anthocyanins. 1. Detection and identification of anthocyanidins formed from leuco-anthocyanins in plant tissues. *Biochem. J.* **1954**, *58*, 122−125.

(7) Porter, L. J.; Hrstich, L. N.; Chan, B. G. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* **1986**, *25*, 223−230.

(8) Waterman, P. G.; Mole, S. *Analysis of Phenolic Plant Metabolites*; Blackwell: Oxford, U. K., 1994; pp 66−103.

(9) Jurd, L.; Somers, T. C. The formation of xanthylium salts from proanthocyanidins. *Phytochemistry* **1970**, *9*, 419−427.

(10) Guyot, S.; Marnet, N.; Laraba, D.; Sanoner, P.; Drilleau, J.-F. Reversed-phase HPLC following thiolysis for quantitative estimation and characterization of the four main classes of phenolic compounds in different tissue zones of a french cider apple variety (*Malus domestica* var. Kermerrien). *J. Agric. Food Chem.* **1998**, *46*, 1698−1705.

(11) Benzie, I. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of ''antioxidant power": The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70−76.

(12) Manabe, T.; Kadowaki, R. Some physico-chemical properties for non edibility of Chinese quince (*Chaenomeles sinensis* Koehne) (in Japanese with English summary). *Bull. Hiroshima Pref. Univ.* **1998**, *9*, 103−109.

(13) Es-Safi, N.-E.; Le Guernevé, C.; Fulcrand, H.; Cheynier, V.; Moutounet, M. New polyphenolic compounds with xanthylium skeletons formed through reaction between (+)-catechin and glyoxylic acid. *J. Agric. Food Chem.* **1999**, *47*, 5211−5217.

(14) Labrouche, F.; Clark, A. C.; Prenzler, P. D.; Scollary, G. R. Isomeric influence on the oxidative coloration of phenolic compounds in a model white wine: Comparison of (+)-catechin and (−)-epicatechin. *J. Agric. Food Chem.* **2005**, *53*, 9993−9998.

(15) Lu, Y.; Foo, L. Y. Antioxidant and radical scavenging activities of polyphenols from apple pomace. *Food Chem.* **2000**, *68*, 81−85.

Received for review June 30, 2006. Revised manuscript received November 27, 2006. Accepted December 4, 2006.

JF061836+