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Non-extractable procyanidins and lignin are important factors in the bile acid binding and radical scavenging properties of cell wall material in some fruits

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Abstract The cell wall components and the food functions of alcohol-insoluble solids (AIS) of Chinese quince, quince, hawthorn, apple, pear and blueberry fruits were analyzed. Chinese quince contained characteristically high contents of cellulose, lignin, and non-extractable procyanidins (NEPCs). On the other hand, the quince AIS contained the highest proportion of NEPCs, the highest mean degree of polymerization (mDP), the strongest radical scavenging activity, and strong bile acid binding activity. In fruit AIS, the lignin and NEPC contents both showed positive correlations with the bile acid binding and radical scavenging activities. The value for mDP × NEPC content was a good index for the radical scavenging activity. The results suggest that highly polymerized NEPCs and lignin are important factors of cell wall components of fruits to having a high functionality, and Chinese quince and quince are interesting fruits from this view point.

Keywords Bile acid binding activity · Cell wall materials · Cell wall polysaccharides · Free radical scavenging activity · Lignin · Non-extractable procyanidins

Abbreviations

AIS, alcohol-insoluble solids; mDP, mean degree of polymerization; NEPCs, Non-extractable procyanidins;

Introduction

Polyphenols and fiber are regarded as important functional components of fruits because the high consumption of these components appears to be associated with a decreased risk of cardiovascular disease [1]. Among the polyphenols, procyanidins (a major group of proanthocyanidins) are found in numerous fruits and fruit products, in the form of polymerized (epi)catechins with varying levels of polymerization. They have been reported to show various biological activities including antioxidant, free radical-scavenging, and anti-carcinogenic activities, inhibition of platelet aggregation [2], and anti-inflammatory activity [3]. Because flavonoids (especially procyanidins) are poorly absorbed, their biological actions may be exerted mainly in the gastrointestinal tract [4], which is also the main site of action of dietary fiber. Moreover, it has been reported that some procyanidins are strongly bound to cell wall components [5] and thus act as part of the dietary fiber. Because of the various food functions of procyanidins, a fiber with associated procyanidins should have broader functions than a fiber without them. Therefore, consideration of the associated procyanidins (and other polyphenols) is thought to be important in assessing the functionality of fruits or fruit by-products [6]. The procyanidins associated with fiber are also called non-extractable procyanidins (NEPCs).

The fibers of fruits consist mainly of cell wall components that are resistant to digestion and absorption in the human small intestine. Some epidemiological studies have shown a significant inverse association between dietary fiber intake and risk of coronary heart disease [7]. The consumption of water-soluble fiber as a dietary supplement has been shown to decrease both total and low-density lipoprotein cholesterol while not affecting the levels of high-density lipoproteins [8]. The cholesterol lowering effect of soluble fiber might be due to result from its interference with the enterohepatic circulation of bile acid [9]. The consumption of soluble fiber accelerates the fecal excretion of bile acids, and this would cause the liver to convert more cholesterol to bile acids. The bile acid excreting effect of fiber should be related to its bile acid binding capacity. However, little is known about this capacity of fruit fiber with NEPCs.

The objective of this study was to compare the cell wall components (including polysaccharides, lignin, and NEPCs) and the relating functions such as the bile acid binding and free radical scavenging activities in some fruits. The beneficial impacts of fruit cell wall components (as dietary fiber) on the bile acid binding and radical scavenging activities, as well as the usability of underutilized fruits such as Chinese quince and quince, were discussed.

Materials and Methods

Plant Materials

Commercially ripe fruits of Chinese quince (*Pseudocydonia sinensis* Schneid. var. *Toukarin* 'Kegai'), quince (*Cydonia oblonga* Mill. 'Smyrna'), hawthorn (*Crataegus* spp.), apple (*Malus domestica* Borkh. 'Fuji'), pear (*Pyrus communis* L. 'Winter Nelis'), and blueberry (*Vaccinium corymbosum* L. 'Northland') were obtained at a local orchard in the Nagano Prefecture, Japan. Freshly harvested pear fruits were regarded as "immature fruit," while fruits that had been ripened for 16 days at 22.0 ± 0.3°C were considered to be "ripe fruit." Immature blueberries, which were still red, were also used. For each of the Chinese quince, quince, apple, and pear, the flesh of at least six fruits was pooled to create the samples used in the experiments. Blueberries were sampled using whole fruit, and hawthorn was sampled after removal of the seeds. The sampled fruit tissues were frozen in liquid nitrogen, lyophilized using a FD-5N freeze-dryer (EYELA, Tokyo, Japan), then powdered before use.

Reagents and Standards

The sodium taurochenodeoxycholate (TCDC), sodium glycochenodeoxycholate (GCDC), sodium glycodeoxycholate (GDC), apple pectin (P8471), citrus pectin (P9135), cholestyramine, and (–)-epicatechin were purchased from Sigma-Aldrich, Ltd. (St. Louis, MO, USA). The *trans*-1,2-diaminocyclohexane-*N*,*N*,*N'*,*N'*-tetraacetic acid (CDTA), sodium taurocholate (TC), sodium glycocholate (GC), sodium taurodeoxycholate (TDC), porcine pancreatine, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2-(N-morpholino)-ethanesulfonic acid (MES), cellulose, and solvents were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). The toluene-α-thiol was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Methods

Preparation of Alcohol Insoluble Solids and the Sequential Extraction of Polysaccharides A portion of the lyophilized tissue powder (1.5 g) was boiled in 80% (v/v) EtOH (40 ml), homogenized, and filtered using Whatman No. 2 filter paper. The residues were washed sequentially with 40 ml each of 80% (v/v) EtOH, 70% (v/v) acetone, 99.5% EtOH, and diethylether, then dried under vacuum using the FD-5N freeze-dryer. The resultant powder was weighed and defined as the AIS. Triplicate AIS samples were prepared from each kind of fruit.

Cell wall polysaccharides were sequentially extracted from the AIS using water, chelating agent, and alkali solutions [10–11]. The extraction procedure was as follows: AIS (100 mg) were extracted with 20 ml of water (16 h at

room temperature), washed and the two aqueous solutions were combined and brought to a volume of 100 ml (water-soluble fraction). The water-insoluble residue was then extracted with 10 ml of 0.05 M CDTA (6 h at room temperature), washed twice with the same solution and all three extracts were combined (CDTA extract). The CDTA-insoluble residue was extracted with 10 ml of 0.1 M Na_2CO_3 solution containing 0.1% $NaBH_4$ (2°C for 20 h, followed by room temperature for 2 h), washed twice with the same solution and all three extracts were combined (Na_2CO_3 extract). The Na_2CO_3-insoluble residue was then extracted with 10 ml of 4% (w/v) KOH containing 0.1% $NaBH_4$ under an N_2 atmosphere (18 h at room temperature), washed twice with the same solution, and all three extract were combined, neutralized with acetic acid (4%KOH extract). The 4% KOH-insoluble residue was finally extracted with 10 ml of 24% (w/v) KOH containing 0.1% $NaBH_4$ under an N_2 atmosphere (2 h at room temperature), then treated in the same manner as the 4%KOH extraction. After each extraction, insoluble material was pelleted at 10,000*g* for 20 min. The CDTA, Na_2CO_3 and KOH extracts were dialyzed overnight against distilled water using Visking tubing (MWCO 12000–14000). All dialyzates containing the CDTA-, Na_2CO_3-, 4% KOH- and 24% KOH-soluble extracts were brought to a constant volume of 100 ml with water.

Determination of Cell Wall Components The cell wall polysaccharides in each fraction were analyzed colorimetrically for polyuronides using the 3,5-dimethylphenol method [12] and for total polysaccharides using the phenol-sulfuric acid method. α-D-Galacturonic acid and D-(+)-glucose were used as the standards for the polyuronide assay and the total polysaccharide assay, respectively. The neutral noncellulosic polysaccharide content was estimated by subtracting the interfering absorbance for uronic acid from the absorbance for total polysaccharides in the phenol-sulfuric acid analysis of each fraction.

The lignin (Klason lignin) and cellulose contents were measured according to de Escalada Pla et al. [13] with minor modifications. To determine the Klason lignin content, the AIS sample (100 mg) was dispersed in 72% sulfuric acid for 3 h at room temperature. The solution was then diluted to 1 mol/L sulfuric acid and hydrolyzed for 2.5 h at 100°C. The residue was recovered by centrifugation (11000×*g* for 20 min), washed three times with distilled water, and lyophilized. The Klason lignin was then quantified gravimetrically. The cellulose content was determined by subtracting the lignin content from the total lignin plus cellulose content. The lignin plus cellulose residue was obtained by centrifugation after hydrolysis of the AIS in 1 mol/L sulfuric acid at 100°C for 2.5 h.

The NEPC content was determined by HPLC after thioacidolysis of the AIS. The AIS sample (4 mg) was added to 100 μL of 0.2 M HCl in MeOH and 100 μL of 5% (v/v) toluene-α-thiol in MeOH, and incubated for 1 h at 50°C. The mixture was added to 200 μL of water and filtered, then an aliquot of 20 μL was analyzed by HPLC-PDA (Shimadzu

Class-VP system). The chromatographic conditions were as described previously [14]. The separation was carried out on a Luna 5 μm C18 column (150 × 4.6 mm, Phenomenex Inc., Torrance, CA) at 40°C using two solvents: 0.1% TFA (solvent A) and 0.1% TFA in acetonitrile (solvent B). The gradient program was started with 5% B and changed to obtain 15%, 32%, 40% and 75% of B at 30, 35, 45 and 50 min, respectively. The NEPC content was calculated from the total amount of (epi)catechin units released from the AIS by thioacidolysis. The mean degree of polymerization of the procyanidins (mDP) was calculated based on the molar concentrations of (epi)catechin units derived from the terminal and extension units of the procyanidins, as described previously [15].

Bile Acid Binding and DPPH Radical Scavenging Activity For bile acid binding activity, each AIS or standard sample was incubated with 6 bile acids and pancreatine, after treatment with 0.01 M HCl, according to Kahlon and Smith [16] with minor modifications. Each AIS or standard sample (50 mg) was digested in 1 ml of 0.01 M HCl for 1 h at 37°C with shaking. After this simulated gastric digestion, the pH of the sample was adjusted to 6.3 with 0.1 ml of 0.1 M NaOH. The suspension was then added to 4 ml of the bile acid working solution (0.72 μmol bile acids/ml; GC:GCDC:GDC:TC:TCDC:TDC = 3:3:3:1:1:1) and 5 ml of porcine pancreatin solution (in a 0.1 M phosphate buffer, pH 6.3), and incubated for 1 h at 37°C. The mixture was centrifuged (4°C, 10,000 ×*g*, 10 min) and the unbound bile acids in the supernatant (in a 4 ml aliquot) were extracted using a Sep-Pak Plus C18 cartridge (Waters Co., Milford, MA) that had been preconditioned using 2 ml of MeOH and 2 ml of distilled water, sequentially. The bile acids were eluted from the cartridge with 2 ml of MeOH, after washing with 10 ml of water. The volume of the bile acid solution was increased to 4 ml with water, filtered, and then a 20 μL aliquot was analyzed using a HPLC-UV system (Shimadzu, Tokyo, Japan). Each sample was analyzed in triplicate.

The free radical scavenging activity of each AIS sample was measured using a DPPH radical solution as follows. First, 1 ml of 0.2 mM DPPH in ethanol was mixed with 1 ml of 200 mM MES buffer. An adequate amount of AIS was added to the DPPH/buffer solution, and the suspension was incubated for 5 h at room temperature, with agitation at 30 min intervals. The remaining DPPH radicals were measured by their absorbance at 517 nm. No physical bound of DPPH to the AIS was visually confirmed. The radical scavenging activity was expressed as the inverse of the EC_{50} value, which is the amount of each AIS (or standard) sample needed to decrease the initial DPPH concentration by 50%. Each sample was analyzed in triplicate.

Results and Discussion

Composition of Cell Wall Materials

Among the fruit tested, Chinese quince had the highest AIS content (8.5%, w/w), followed by hawthorn (7.0%), and quince (4.1%) (Table 1). The AIS contents of immature blueberries and pears was moderate (3.4% for immature blueberries, 2.8% for immature pears, and 2.6% for ripe pears), and apple had the lowest AIS content (1.5%). The high levels of AIS in Chinese quince fruit were due to the high cellulose and lignin contents (1.9% and 2.1% (w/w) of flesh, respectively). The total amount of cellulose plus lignin was almost half (47%) of the total AIS content of the Chinese quince fruit, and this percentage was larger than that in European quince. This seems to be related to the woody, hard flesh of Chinese quince fruit, which is characterized by especially high lignin content. This high lignin content might be relating to the presence of stone cells, which are highly lignified [17]. Manabe and Kadowaki [18] reported that Chinese quince fruit contain many large stone cells (max. length 1 mm) and this contributes to the gritty feel of the pulp. In contrast apple fruit flesh, which has no stone cells, contained only 0.02% (w/w) lignin, which was about one hundred times lower than that of Chinese quince. On the other hand, hawthorn fruit also contained high levels of cell wall components, comparable to those in Chinese quince. However, hawthorn fruit had relatively low lignin concentrations (0.44%, w/w) and characteristically high concentrations of polyuronides (2.7%, w/w). Chinese quince and quince fruits also had relatively high amounts of polyuronides (approximately 0.8%, w/w of flesh), which were more than twice the amount in apple. This indicated that both Chinese quince and quince fruits are good sources of pectic polysaccharides (pectin). Pectin is one of functional ingredients in cell walls, and it is reported to have a number of pharmacological actions including hypoglycemic action, cholesterol reduction, and antiulcerative activity [10]. The pectic fraction of Chinese quince was reported to be moderately active against acute gastric ulcers induced by ethanol in rats [14].

Another characteristic of the cell wall components of Chinese quince and quince fruits was that they contained high amounts of NEPCs (267 mg and 190 mg per 100 g FW, respectively) (Table 2). These contents were almost 150 and 100 times higher than that of apple. The NEPC content of Chinese quince was 1.4 times higher than that of quince, however the percentage of NEPCs in the AIS and the mDP value of the NEPCs were higher in quince than in Chinese quince. The mDP values for Chinese quince and quince fruit (40 and 93) were much higher than those for apple and hawthorn (8 and 7) but lower than those for immature and ripe pear (136 and 147) although the pear mDP values may vary with cultivars. Among the other fruits tested, blueberries (both immature and ripe) also had relatively high levels of NEPCs (70 and 83 mg/100 g FW). However, these NEPCs may be mainly in the seeds, which were sampled along with the fruit. This possibility is supported by the observation that blueberries also contained high levels of lignin (Table 1), and seeds are known to contain certain amounts of NEPCs and lignin [19]. Because the mDP and the content

are both important factors relating to procyanidins, mDP × NEPCs (% in AIS) also shown in Table 2. The mDP × NEPCs values were ordered as follows: quince (437) >> pear (279) ≥ immature pear (204) > Chinese quince (120) > blueberry (89.1) ≥ immature blueberry (86.4) > hawthorn (1.26) ≥ apple (0.96).

Bile Acid Binding Activity of Fruit AIS and Related Food Factors

The total bile acid binding activity for the various fruit AIS ranged from 4.5 to 15.9 μmol/g (Table 3). Among the selected standards, cellulose had the lowest activity (2.9 μmol/g) and cholestylamine had the highest activity (462 μmol/g). The activity of apple pectin was lower (3.5 μmol/g) than those of the fruit AIS, and the activity of the citrus pectin (7.9 μmol/g) was within the range of the fruit AIS. Among the fruit AIS, the total bile acid binding activity (μmol/g) was ordered as follows: blueberry (15.9) > quince (11.2) > immature blueberry (10.2) > hawthorn (8.3) > pear (7.1) > immature pear (4.8) ≥ Chinese quince (4.6) ≥ apple (4.5). Thus on an AIS basis, blueberry and quince fruit had relatively strong bile acid binding activity, but apple and Chinese quince showed relatively weak activity. In addition, among the bile acids, TCDC, GCDC, and GDC tended to be more strongly bound to the fruit AIS than were TC and GC. It has been reported that dihydroxy-bile acids (such as GCDC and GDC) are more strongly bound by dietary fiber preparations than trihydroxy-bile acids (such as GC) [20]. The mechanism is not known in detail but it has been explained that hydrophobic interactions are involved with binding [21]. Our results using the AIS samples are in good agreement with those previous results.

The relationships between the bile acid binding activities of the fruit AIS samples and their polyuronide contents, lignin contents, NEPC contents, and mDP × NEPC values are shown in Figure 1. With a single regression analysis, there was a moderate relationship between bile acid binding activity and lignin content (R^2 = 0.3407). NEPC content also had a moderate relationship (R^2 = 0.2074) but this was disrupted when the mDP was taken into account. Meanwhile, polyuronide content had a weak inverse relationship with bile acid binding activity (R^2 = 0.0469). Polyuronides are the major component of pectic polysaccharides, which have been reported previously to have bile acid binding activity. This discrepancy may be due to other components (such as lignin and NEPCs) that have stronger bile acid binding activities than pectin in selected fruits. Sayar et al. [22] reported that together with β-glucan, lignin had a great impact on the bile acid binding activity of oat flours, and that only the lignin concentrations of the flours significantly correlated with the activity. Also in alfalfa and wheat bran, lignin appears to be an important component in the interactions between dietary fiber and bile acids [23]. It has been speculated that lignin binds bile acids mainly via hydrophobic interactions [24]. Tannins present in the fiber, such as NEPCs, are also important bile acid binding agents.

According to Würsch [25], the removal of tannins from the carob fiber resulted in a sharp decrease of its bile acid binding activity. However, the bile acid binding activity of the fruit AIS samples in our current study could not be fully explained by their lignin and NEPC contents. A strong bile acid binding activity seems to require a proper balance of components, including pectin, lignin, and NEPCs. Although the actual nutritional impact of fruit fiber rich in polyphenol is unclear, the hypocholesterolemic effect of carob fiber rich in polyphenols has been recently confirmed in human study [26].

Radical Scavenging Activity of Fruit AIS and Related Food Factors

The AIS of selected fruits all showed radical scavenging activity in the DPPH assay, and the activities expressed as $1/EC_{50}$ values ranged from 0.34 (hawthorn) to 5.63 (quince)(Table 3). Commercial citrus pectin, α-D-galacturonic acid, and cellulose all showed almost no activity, while (–)-epicatechin showed very strong activity (236). It has been reported that some dietary fibers have antioxidant activity that is mainly due to the non-extractable polyphenols associated with the fiber matrix [27]. In case of ripe blueberry, there was slight remaining of anthocyanins on the AIS and it might affect to the radical scavenging activity. However, the contribution of the remaining anthocyanins seemed to be negligible because there was no difference between ripe blueberry and immature one. The relationships between the DPPH radical scavenging activities and the polyuronide contents, lignin contents, NEPC contents, and mDP × NEPC contents in the AIS samples from various fruits are shown in Figure 1. There was a strong relationship (R^2 = 0.7227) between the NEPC contents and the $1/EC_{50}$ values, which was enhanced when the mDP of the NEPCs was taken into account (R^2 = 0.9194). Thus, the mDP × NEPCs value is a good index of the radical scavenging capacity of the fruit AIS. Moreover, there was also a moderate relationship between lignin content in the AIS and DPPH radical scavenging activity (R^2 = 0.2324). These results suggest that both the NEPCs and the lignin contribute to the radical scavenging activity of the AIS, and in particular, NEPCs with high mDP values are important contributors to the radical scavenging activity.

With regard to the nutritional impact of the antioxidant function of the fruit AIS, this antioxidant function will be mainly exerted in the gastrointestinal tract, because most of the AIS components will behave as the dietary fiber. However, the polyphenols associated with fiber may be partly biologically available. Pérez-Jiménez et al. [28] reported that polyphenols present in a dietary fiber matrix, such as grape antioxidant dietary fiber, are at least partially absorbed by the human digestive tract. This was indicated by a significant increase in plasma antioxidant capacity. Therefore, fiber containing high amounts of NEPCs has a greater potential for biological activity (such as vascular protection) than

merely exerting a radical scavenging activity within the gastrointestinal tract.

Conclusions

NEPCs and lignin are important fruit cell wall components that contribute to the bile acid binding and radical scavenging activities of the fruit AIS. NEPCs appear to contribute a strong radical scavenging property to fruit cell walls, and the value for mDP × NEPC content is a good index of the radical scavenging activity. NEPCs may also contribute to the bile acid binding activity at a certain level. Meanwhile, lignin in fruit cell walls seems to contribute moderately to both the bile acid binding and radical scavenging activities of the AIS. Therefore, fruits that have large amounts of highly polymerized NEPCs and lignin seem to be good functional food materials with the potential for cholesterol lowering and antioxidant actions. From this viewpoint, the underutilized fruits such as Chinese quince and quince have the potential to be used as functional foods with bile acid binding and antioxidant properties. Further research is needed to determine how to make the best use of these properties of Chinese quince and quince fruits.

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Table 1. Contents of alcoholic insoluble solids (AIS) and cell wall components in some fruits

	AIS[a]	Polyuronides[b]	nNCPs[c, b]	Cellulose	Lignin
Chinese quince	8.5 ± 0.04 (100%)	0.84 ± 0.04 (9.7%)	1.22 ± 0.14 (13.6%)	1.9 ± 0.03 (22.3%)	2.1 ± 0.02 (24.5%)
Quince	4.1 ± 0.02 (100%)	0.79 ± 0.02 (19.4%)	0.84 ± 0.02 (20.7%)	0.85 ± 0.002 (20.9%)	0.81 ± 0.01 (19.8%)
Hawthorn	7.0 ± 0.06 (100%)	2.7 ± 0.04 (36.1%)	1.51 ± 0.06 (20.1%)	1.8 ± 0.03 (23.5%)	0.44 ± 0.01 (5.9%)
Apple	1.5 ± 0.01 (100%)	0.37 ±0.004 (23.9%)	0.23 ± 0.01 (14.9%)	0.62 ± 0.003 (40.3%)	0.02 ± 0.002 (1.3%)
Pear (IM)[d]	2.8 ±0.02 (100%)	0.59 ± 0.01 (21.4%)	0.60 ± 0.005 (22.0%)	0.49 ± 0.006 (16.1%)	0.39 ± 0.004 (12.7%)
Pear (R)[d]	2.6 ± 0.02 (100%)	0.65 ± 0.01 (25.4%)	0.52 ± 0.01 (20.4%)	0.54 ± 0.02 (22.0%)	0.40 ± 0.01 (16.4%)
Blueberry (IM)	3.4 ± 0.01 (100%)	0.42 ± 0.01 (12.6%)	0.76 ± 0.02 (22.5%)	0.54 ± 0.007 (15.8%)	0.92 ± 0.004 (27.0%)
Blueberry (R)	2.4 ± 0.02 (100%)	0.34 ± 0.008 (13.7%)	0.41 ± 0.03 (16.6%)	0.41 ± 0.001 (16.0%)	0.73 ± 0.007 (28.4%)

[a] Data are mean (g/100g FW) ± SE (n=3).
Figures in parentheses represent percentages of the amounts in the AIS.
[b] Total amounts in 5 sub-fractions
[c] nNCPs, neutral noncellulosic polysaccharides
[d] IM, immature; R, ripe

Table 2. Characteristics of non-extractable procyanidins in the AIS of some fruits

Fruits	NEPCs[a] in fruit tissue (mg/100g FW)[b]	Amounts in AIS (%)	Distribution of catechin units (%)			mDP[d]	mDP × %[e]
			TU[c]		EU[c]		
			CAT[c]	EC[c]	EC		
Chinese quince	267 (9.5)	3.0 (0.1)	0.45	2.1	97.7	40 (0.1)	120
Quince	190 (6.3)	4.7 (0.2)	0.10	0.94	98.9	93 (1)	437
Hawthorn	12.6 (1.0)	0.18 (0.02)	1.2	12.8	83.3	7 (0.1)	1.26
Apple	1.80 (0.1)	0.12 (0.01)	2.4	9.2	83.3	8 (0.2)	0.96
Pear (IM)[f]	47.1 (1.6)	1.5 (0.06)	0.11	0.62	98.7	136 (3)	204
Pear (R)[f]	46.1 (1.1)	1.9 (0.04)	0.11	0.57	99.5	147 (3)	279
Blueberry (IM)	83.0 (5.0)	2.4 (0.2)	2.3	0.53	97.1	36 (1)	86.4
Blueberry (R)	70.5 (2.8)	2.7 (0.10)	2.3	0.73	97.1	33 (2)	89.1

[a] NEPCs, non-extractable procyanidins

[b] Data are means (SE) (n=3)

[c] TU, terminal units; EU, extension units; CAT, (+)-catechin; EC, (－)-epicatechin

[d] mDP, mean degree of polymerization of the procyanidins

[e] Amounts in AIS

[f] IM, immature; R, ripe.

Table 3. Bile acid binding and DPPH scavenging activities of AIS prepared from various fruits, commercially available pectins, and selected standards

AIS/ Compounds	% Bile acid binding[a]						Total BBA		RSA
	TC	GC	TCDC	GCDC	TDC	GDC	(%) [b]	(μmol/g) [c]	$(1/EC_{50})$ [d]
Chinese quince	4.4 (1.0)	4.0 (0.5)	8.2 (1.9)	10.8 (1.8)	6.9 (1.3)	10.5 (1.3)	7.5 (1.0)	4.6 c [e] (0.5)	3.20 bc (0.05)
Quince	13.0 (2.4)	13.5 (1.9)	17.8 (2.2)	24.7 (2.0)	17.4 (1.7)	23.6 (2.2)	18.3 (2.0)	11.2 ab (1.0)	5.63 a (0.04)
Hawthorn	14.8 (2.0)	12.3 (2.5)	15.6 (1.5)	18.2 (1.9)	13.5 (2.3)	16.1 (2.7)	15.1 (2.1)	8.3 bc (1.0)	0.34 d (0.02)
Apple	5.6 (2.4)	7.9 (2.1)	13.6 (2.4)	2.5 (0.5)	10.0 (3.4)	11.1 (0.5)	8.5 (0.8)	4.5 c (0.4)	0.49 d (0.01)
Pear (IM)	3.7 (0.7)	2.1 (0.7)	10.9 (1.9)	16.0 (1.6)	5.5 (0.4)	8.5 (1.0)	7.8 (0.8)	4.8 c (0.5)	3.48 b (0.5)
Pear (R)	10.3 (0.8)	8.4 (2.2)	14.2 (0.9)	14.3 (1.4)	12.5 (0.3)	14.3 (0.6)	12.3 (0.7)	7.1 bc (0.5)	3.79 b (0.2)
Blueberry (IM)	10.3 (1.4)	12.1 (1.7)	20.4 (1.4)	21.2 (2.2)	16.4 (0.9)	22.0 (1.3)	17.1 (1.2)	10.2 b (0.8)	2.21 c (0.08)
Blueberry (R)	18.3 (3.3)	21.9 (4.0)	30.6 (2.8)	31.8 (3.1)	27.4 (3.7)	32.9 (2.8)	27.1 (3.2)	15.9 a (2.0)	2.22 c (0.08)
Apple pectin	0 (0)	5.9 (0.9)	4.9 (0.9)	10.1 (1.3)	7.8 (0.9)	4.8 (0.4)	5.6 (0.5)	3.5 (0.3)	0.14 (0.004)
Citrus pectin	11.5 (1.3)	12.5 (2.0)	14.2 (1.1)	15.9 (1.2)	16.3 (1.7)	12.2 (1.8)	13.8 (1.2)	7.9 (0.8)	0.07 (0.001)
α-D-GUA	NT	NT	NT	NT	NT	NT	NT	NT	0.017 (0.001)
Cellulose	3.4 (1.0)	4.8 (0.5)	5.0 (1.0)	5.7 (1.9)	6.7 (0.6)	4.3 (1.0)	5.0 (0.7)	2.9 (0.5)	0.003 (0.0002)
(–)-Epicatechin	NT	NT	NT	NT	NT	NT	NT	NT	236 (5.7)
Cholestyramine	30 (0.9)	23 (1.2)	69 (0.9)	58 (1.5)	66 (1.5)	57 (1.9)	50 (1.2)	462 (13)	NT

[a] Data are presented as means (SE) (n=3).

[b] Percentage of total bile acid that was bound by 50 mg of each material or compound (except for cholestyramine which was tested using 3 mg).

[c] Total amount (μmol) of bile acids adsorbed by 1 g of each material or compound.

[d] Inverse of the EC_{50} value, which represents the amount (mg) of each material or compound needed to decrease the initial DPPH concentration by 50%.

[e] Different letters within the same column show a significant difference by the Tukey-Kramer's test at the 5% level.

Abbreviations: BBA, bile acid binding activity; RSA, radical scavenging activity; TC, taurocholic acid; GC, glycocholic acid; TCDC, taurocheno-deoxycholic acid; GCDC, glycocheno-deoxycholic acid; TDC, taurodeoxycholic acid; GDC, glycodeoxycholic acid; IM, immature; R, ripe; GUA, galacturonic acid; NT, not tested

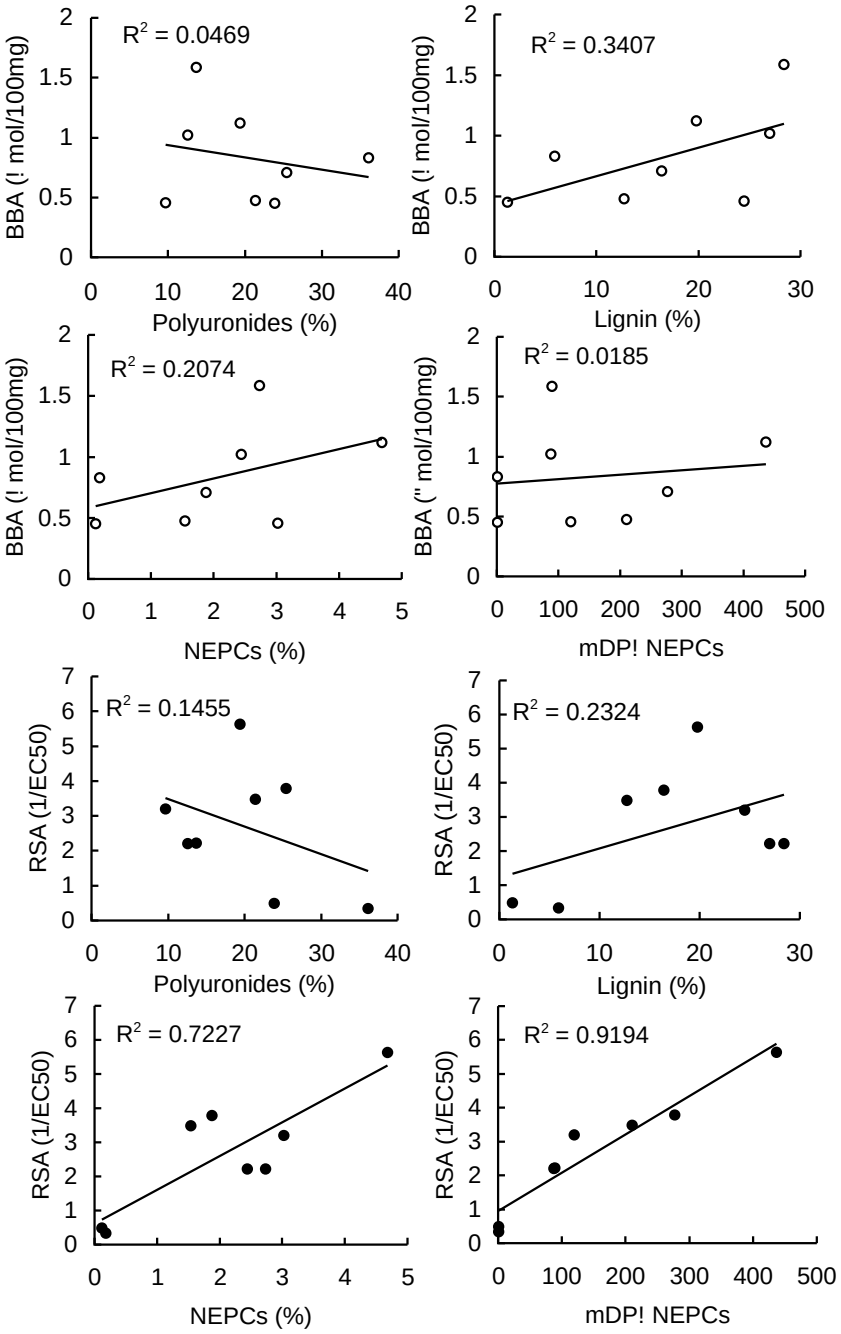

Fig. 1. Relationships between some food factors of fruit AIS and bile acid binding activities (open circles) or radical scavenging activities (filled circles). BBA, bile acid binding activity; RSA, radical scavenging activity; NEPCs, non-extractable procyanidins; mDP, mean degree of polymerization of the NEPCs.