

Antioxidant and α -Glucosidase Inhibitory Activity of Polyphenols in Peel and Core from Five Different Pear Varieties

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Abstract This study was performed to compare polyphenol content, DPPH radical scavenging activity and α -glucosidase inhibitory activity in peel, core and pulp of five different pear cultivars. Oligomeric polyphenols were the main polyphenols in core, which exhibited greater polyphenols, DPPH radical scavenging activity, and α -glucosidase inhibitory activity, compared to peel and pulp. For the peel, Japanese pears including Yasato, 20-Seki and Shinko pear contained relatively more polyphenol content and α -glucosidase inhibitory activity, compared to European pears including Starkrimson and Purekosu pear. Monomeric polyphenols were the main polyphenols in the peel of European pears, in contrast, oligomeric polyphenols were the main polyphenols in those of Japanese pears. The peel of Yasato pear had high ratios of non-monomeric polyphenols, and showed highest DPPH radical scavenging activity and α -glucosidase inhibitory activity amongst five pear cultivars. Thus DPPH radical scavenging activity and α -glucosidase inhibitory activity may be related with non-monomeric polyphenols in pears. Moreover, as comparing with peel, pulp had lower polyphenol content, DPPH radical scavenging activity and α -glucosidase inhibitory activity. All of the observations suggested that peel and core of pear contained abundant polyphenols and possess potential the values of reusing.

Keywords: pear, peel, core, polyphenol, DPPH radical scavenging activity, α -glucosidase

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1. Introduction

Polyphenols are commonly found in plants and have been reported to have some biological effects, including antioxidant activity, α -glucosidase inhibitory activity, and anti-inflammatory [1,2,3]. Antioxidants are known to have health benefit, such as reducing the risk of many diseases that are related to oxidative stress. Many researchers have reported fruits such as grape, apple, cacao, and persimmon containing polyphenol compounds may act as antioxidant reagents [4,5]. α -Glucosidase is an intestinal cell membrane enzyme that can hydrolyze polysaccharides; hence, inhibiting the activity of α -glucosidase may be an effective way to treat pre-diabetes and slow the progression of diabetes [6]. Several researches have reported polyphenols from various fruits and vegetables had α -glucosidase inhibitory activity [7,8].

Pear is one of the most popular fruits, and it is commonly consumed as both fresh and fruit products. Pear contains

nutritional components such as minerals, dietary fiber, vitamin C and organic acids [9]. However, due to pear peel and core are lacking in good taste sensory, they are usually treated as fruit waste during consumption and processing. Although there are some reports on the chemical composition was detected, which had antioxidant activity and anti-inflammatory in pear peel [10,11]. There are less reports on the potential bioactive activity in pear core.

In the present study, we utilized five pear cultivars, which are the major varieties in Japan. We investigated and compared antioxidant activity and α -glucosidase activity of polyphenols in peel, core and pulp of five pear cultivars.

2. Materials and Methods

2.1. Reagents

All the reagents and chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), unless otherwise stated. Diaion HP-20 columns and Sephadex

LH-20 columns for chromatography were obtained from the Mitsubishi Chemical Corporation (Tokyo, Japan) and GE Healthcare Bio-Sciences AB (Uppsala, Sweden), respectively.

2.2. Samples

Five different pear cultivars were at their commercial ripening stage and obtained from supermarket (Obihiro, Japan). Yasato, 20-Seki, Shinko pear are *Pyrus pyrifolia* species, and those are named Japanese pears. The water content of Japanese pears is 88%. They taste very juicy and crunchy, and the shape looks like a round sphere. Purekosu and Starkrimson pear are *Pyrus Communis* species, and those are named European pears. The water content of European pears is 85%. They have a sweet flavor and a nice texture, and the shape looks like a gourd. The fresh weight of per pear ranged from 202.6 g to 481.5 g. The peel was hand-peeled approximately 3 mm thickness and core commonly as inedible parts was taken from pulp. The weight of peel, core and pulp for a fresh pear ranged from 19.9 to 72.8 g, 5.5 to 46.0 g and 166.4 to 376.3 g, respectively (Table 1).

Table 1. The fresh weight of peel, core and pulp for per pear in the five different pear cultivars

Parts	Weight (g/per pear)				
	Yasato	20-Seki	Shinko	Purekosu	Starkrimson
Peel	39.4 ^c ±0.7	63.1 ^b ±1.7	72.8 ^a ±2.8	19.9 ^d ±0.4	21.1 ^d ±1.4
Core	18.4 ^c ±1.8	46.0 ^a ±1.9	32.4 ^b ±1.4	16.3 ^c ±0.8	5.5 ^d ±0.4
Pulp	309.9 ^e ±1.3	334.7 ^b ±5.1	376.3 ^a ±6.1	166.4 ^c ±2.8	185.7 ^d ±1.8
Total	367.7 ^e ±2.6	443.8 ^b ±2.8	481.5 ^a ±5.4	202.6 ^c ±2.2	212.3 ^d ±1.3

Values represent mean ± S.E.M. Different superscript letters indicate significant differences ($p < 0.05$).

2.3. Extract Preparation and Fractionation

The peel, core and pulp of each pear cultivar was cut into pieces and homogenized using a Teflon homogenizer, respectively. The mixture was added with 20 mL of 80% ethanol and treated with ultrasound for 30 min. The mixture was centrifuged at 1,006 × g for 10 min to obtain the supernatant. The same extraction process was repeated twice more. The residues were subjected to another three rounds of extraction with 70% acetone–water, and the supernatant was obtained. Then, the supernatant was mixed, concentrated by rotary evaporation in a vacuum at 40°C, and purified by chromatography through a Diaion HP-20 column. The columns were washed with distilled water and then eluted with methanol. The methanol solution was concentrated by rotary evaporation in a vacuum at 40 °C and dissolved in 2 mL of methanol for the experiment. Part of the concentrate was dissolved in ethanol and fractionated by Sephadex LH-20 column chromatography. The column was successively eluted with ethanol, methanol, and 60% acetone to collect fraction I (Fra.I), fraction II (Fra.II), and fraction III (Fra.III), respectively.

2.4. Quantification of Polyphenols

Polyphenols were quantified using the Folin–Ciocalteu method [12]. 100 µL of methanol fraction (HP-20 column), Fra.I, Fra.II, Fra.III were treated with 300 µL of distilled water, 400 µL of Folin–Ciocalteu reagent, and 400 µL of a 10% Na₂CO₃ solution. The mixture was prepared in

triplicate, incubated at 30 °C for 30 min, and centrifuged at 1,006 ×g for 10 min. The absorbance of the mixed supernatant was measured at 760 nm. The polyphenol content is expressed in milligrams of catechin equivalents per gram of fresh pear.

2.5. Estimation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

DPPH radical scavenging activity was evaluated by the method described by Brand-Williams et al. [13] with some modifications. A 50-µL aliquot of the methanol fraction (HP-20 column) was mixed with 100 µL of ethanol, and the mixture was supplemented with 150 µL of 0.5 mM DPPH in ethanol. The absorbance of the mixture was measured using a microplate reader at 517 nm. The DPPH radical scavenging activity is expressed in micromoles of trolox equivalents per gram of fresh pear.

2.6. α-Glucosidase Inhibitory Activity

α-Glucosidase inhibition was analyzed following the methods of Matsumoto et al. [14] with modifications. Sucrose was broken down by α-glucosidase, and the amount of reducing sugar was calculated based on the α-glucose content. In total, 0.8 mL of enzyme reaction solution [50 µL of 0.4% sucrose, 625 µL of 0.1 mol/L sodium phosphate buffer (pH 6.8), and 125 µL of 1% NaCl] was pre-incubated at 37°C for 30 min. The methanol fraction (HP-20 column) was concentrated by rotary evaporation in a vacuum and dissolved in distilled water. 20 µg/mL of distilled water fraction was added to 0.1 U/mL α-glucosidase solution (EC3.2.1.20; Oriental Yeast Co., Ltd., Tokyo, Japan) at 37°C for 10 min. After pre-incubation, 200 µL of the mixture (polyphenol extract and α-glucosidase) was added to the enzyme reaction solution and incubated at 37°C for 30 min. The reaction was terminated by adding 125 mL of 2 M NaOH and 1% dinitrosalicylic acid in boiling water for 10 min. After incubation, the mixture was analyzed at 540 nm at 25°C. Enzyme inhibitory reactions for all polyphenol extract concentrations were replicated three times. The α-glucosidase inhibitory activity is expressed as the percent inhibition. The concentration of inhibitors required for the inhibition of 50% of the enzyme activity under the assay conditions was defined as the IC₅₀ value.

2.7. Statistical Analysis

Values are presented as the means ± standard error. Statistical significance was evaluated by analysis of variance (ANOVA) and least significant difference (LSD) tests (SAS Enterprise Guide 5.1 system, Cary, NC, USA). Differences were considered significant at $p < 0.05$.

3. Results and Discussion

3.1. Polyphenol Content and Fractions

The polyphenol content of peel, pulp and core extracts of five pear cultivars is presented in Table 2. For the peel,

polyphenol content was 10.3 mg/g in Yasato pear, 9.3 mg/g in 20-Seki pear, 5.9 mg/g in Shinko pear, 4.7 mg/g in Purekosu pear, and 3.6 mg/g in Starkrimson pear. The Japanese pears including Yasato, 20-Seki and Shinko pear had significantly higher polyphenol content than that of European pears including Starkrimson and Purekosu pear. Polyphenol content of core ranged from 10.8 to 39.2 mg/g, and it was 2 to 7 times more than peel. For the core, the highest polyphenol content was in Yasato pear, and the lowest was in Purekosu pear. As comparing with peel, pulp exhibited lower polyphenol content. This agree with the research reporting concentrations of polyphenols in the peel of European pears were much greater than in the pulp [10,15]. The peel are commonly considered as a protective barrier for the pulp, and maybe had relatively higher polyphenols. The polyphenol content of pulp ranged from 0.4 to 4.2 mg/g being the highest in Purekosu pear and the lowest in Yasato pear. Moreover, we calculated polyphenol content for a fresh pear through using the weight of peel, core and pulp, and Japanese pears showed higher polyphenol content than European pears. Chen et al. [16] reported the polyphenol content may be attributed to the different species, geographical origin, climate factors, and cultural conditions of different pear cultivars. In this study, both Japanese pears and European pears grew and collected in Japan, so thus the species may be influence the polyphenol content.

Table 2. Polyphenol content in peel, core and pulp of the five different pear cultivars.

Parts	Polyphenol content (mg/g)				
	Yasato	20-Seki	Shinko	Purekosu	Starkrimson
Peel	10.3 ^a ±0.1	9.3 ^b ±0.2	5.9 ^c ±0.2	4.7 ^d ±0.1	3.6 ^e ±0.1
Core	39.2 ^a ±0.6	27.9 ^b ±0.7	15.9 ^d ±0.3	10.8 ^e ±0.5	24.7 ^f ±0.4
Pulp	0.4 ^c ±0.1	2.0 ^b ±0.1	1.2 ^d ±0.1	4.2 ^a ±0.2	1.4 ^c ±0.1

Values represent mean ± S.E.M. Different superscript letters indicate significant differences ($p < 0.05$).

Table 3. The ratio of polyphenols in Fra.I, Fra.II and Fra.III of peel and core from the five different pear cultivars

Parts	Peel polyphenols (%)			Core polyphenols (%)		
	Fra.I	Fra.II	Fra.III	Fra.I	Fra.II	Fra.III
Yasato	27.9	53.8	18.3	25.0	64.6	10.4
20-Seki	39.1	46.2	14.7	18.6	78.6	2.8
Shinko	32.9	59.4	7.7	9.1	63.5	27.4
Purekosu	79.6	18.9	1.5	36.1	50.8	13.1
Starkrimson	67.8	32.2	0.0	9.6	68.2	22.2

Polyphenols of peel and core from the five different pear cultivars were applied to Sephadex LH-20 column chromatograms for the successive fraction of ethanol, methanol and 60% acetone. Fra.I: ethanol fraction; fraction number, 1–20. Fra.II: methanol fraction; fraction number, 21–40. Fra.III: 60% acetone fraction; fraction number, 41–60. Abbreviations: Fra.I, fraction I; Fra.II, fraction II; Fra.III, fraction III; UD, undetected.

Moreover, we performed Sephadex LH-20 column chromatography to obtain three polyphenol fractions, i.e., Fra.I, Fra.II, and Fra.III from five pear cultivars (Table 3). According to Saito et al. [17], Fra.I contains monomeric polyphenols, Fra.II contains oligomeric polyphenols, and Fra.III contains polymeric polyphenols. For the peel, monomeric polyphenols from European pears were the main polyphenols, in contrast, oligomeric polyphenols from Japanese pears were the main polyphenols. The

monomeric compounds arbutin and chlorogenic acid were the major compounds in the peel of European pears (*P. communis*) [10]. For the core, the ratios of oligomeric polyphenols were also higher than monomeric and polymeric polyphenols in five pear cultivars.

3.2. DPPH Radical Scavenging Activity

DPPH radical scavenging activity of peel, core and pulp extracts of five pear cultivars is presented in Table 4. DPPH radical scavenging activity of core ranged from 51.5 to 143.0 $\mu\text{mol/g}$, and it was higher than peel and pulp of five pear cultivars. The core of Yasato pear exhibited the highest DPPH radical scavenging activity, and Shinko pear exhibited the lowest. Moreover, DPPH radical scavenging activity in peel from Yasato pear was 33.6 $\mu\text{mol/g}$ also being the highest, and Shinko pear was 11.2 $\mu\text{mol/g}$ also being the lowest. As comparing with peel, pulp had lower DPPH radical scavenging activity, and ranged from 1.1 to 5.5 $\mu\text{mol/g}$. The pulp of Purekosu pear showed the highest DPPH radical scavenging activity, and Yasato pear showed the lowest. We found positive relationship between polyphenol content and DPPH radical scavenging activity in peel, core and pulp of five pear cultivars, and the correlation coefficient was 0.98. Many studies have demonstrated that polyphenols possessed strong antioxidant activity [18,19,20].

Table 4. DPPH radical scavenging activity in peel, core and pulp of the five different pear cultivars

Parts	DPPH radical scavenging activity ($\mu\text{mol/g}$)				
	Yasato	20-Seki	Shinko	Purekosu	Starkrimson
Peel	33.6 ^a ±0.6	22.1 ^b ±0.3	11.2 ^d ±0.1	15.1 ^c ±0.1	11.3 ^d ±0.2
Core	143.0 ^a ±0.8	94.5 ^b ±0.6	51.5 ^e ±1.5	63.4 ^d ±0.8	91.8 ^c ±0.7
Pulp	1.1 ^c ±0.1	2.4 ^d ±0.1	2.9 ^c ±0.1	5.5 ^a ±0.1	3.5 ^b ±0.1

Values represent mean ± S.E.M. Different superscript letters indicate significant differences ($p < 0.05$).

3.3. α -Glucosidase Inhibitory Activity

The effects of 20 $\mu\text{g/mL}$ of the polyphenols from five pear cultivars on α -glucosidase activity were investigated (Table 5). For the peel, Yasato pear exhibited the highest inhibitory activity, and Japanese pears both Purekosu and Starkrimson pear exhibited the lowest inhibitory activity on α -glucosidase. Moreover, we analyzed and compared the α -glucosidase inhibitory activity of 20 $\mu\text{g/mL}$ of polyphenols from Fra.I, Fra.II, and Fra.III of peel of five pear cultivars (Figure 1). The greatest α -glucosidase inhibitory activity was observed in non-monomeric (oligomeric and polymeric) polyphenols. Since Fra.III contains polyphenols with higher degree of polymerization compared to those of Fra.II, the fact that the former had a stronger α -glucosidase inhibitory activity compared to the latter indicates that more highly polymerized polyphenols have more potent inhibitory activities. So thus, the peel of Japanese pears contained mainly oligomeric polyphenols had greater inhibitory activity than that of European pears contained mainly monomeric polyphenols. Consistent with this, we also observed that non-monomeric polyphenols exhibited stronger α -glucosidase inhibitory activity than monomeric phenolic compounds in scarlet runner beans [3]. Fra.II, and Fra.III of peel from Yasato

pear showed higher α -glucosidase inhibitory activity than those of other pear cultivars. Non-monomeric polyphenols are considered as proanthocyanidin. Proanthocyanidins belong to flavonoids and are oligomers or polymers of flavan-3-ols. They can be classified into two subgroups, namely B-type proanthocyanidins and A-type proanthocyanidins. We had reported that the degree of polymerization and the type of bond may be related with α -glucosidase inhibitory activity [3]. In this study, the type of bond or degree of polymerization from Yasato pear may be different with the other pear cultivars, and those influence α -glucosidase inhibitory activity.

Table 5. α -Glucosidase inhibitory activity of 20 μ g/mL of polyphenols in peel, core and pulp for the five different pear cultivars

Parts	α -Glucosidase inhibitory activity (%)				
	Yasato	20-Seki	Shinko	Purekosu	Starkrimson
Peel	14.2 ^a ±0.2	2.5 ^b ±0.1	1.3 ^c ±0.1	0.5 ^d ±0.1	0.5 ^d ±0.1
Core	11.0 ^d ±0.1	34.4 ^a ±0.6	7.8 ^e ±0.1	22.7 ^b ±0.8	14.2 ^c ±0.6
Pulp	<0.1	<0.1	<0.1	<0.1	<0.1

Values represent mean \pm S.E.M. Different superscript letters indicate significant differences ($p < 0.05$).

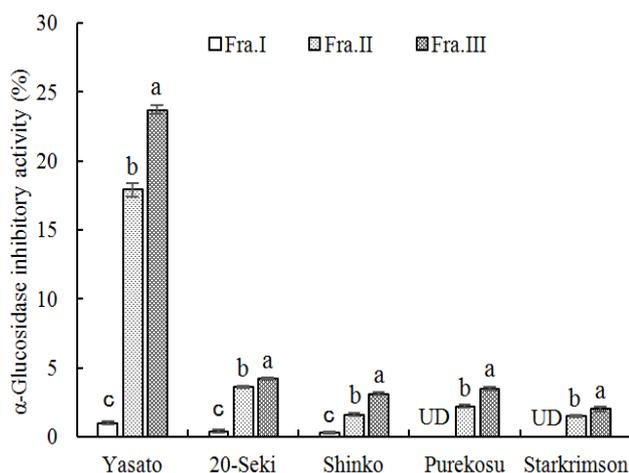


Figure 1. α -Glucosidase inhibitory activity of 20 μ g/mL of Fra.I, Fra.II, or Fra.III polyphenols in the peel from the five different cultivars. Values represent mean \pm S.E.M. Different superscript letters indicate significant differences ($p < 0.05$) for the same sample. Abbreviations: Fra.I, fraction I; Fra.II, fraction II; Fra.III, fraction III; UD, undetected

For the core, α -glucosidase inhibitory activity was 34.4% in 20-Seki, 22.7% in Purekosu, 14.2% in Starkrimson, 11% in Yasato, and 7.8% in Shinko at 20 μ g/mL of polyphenols. The core exhibited higher inhibitory activity, compared to peel and pulp. No inhibitory activity was detected in pulp at 20 μ g/mL of polyphenols for five different pear cultivars.

4. Conclusions

The peel and core of five different pear cultivars contain abundant oligomeric polyphenols, which had strong antioxidant activity and α -glucosidase inhibitory activity. These observations could provide the important information for the use of peel and core, which may serve as a source for development of nutraceuticals with antioxidant and anti-diabetes activity.

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Statement of Competing Interests

The authors have no competing interests.

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