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Pancreatic Lipase Inhibition by Polyphenols in Persimmon Leaf Tea

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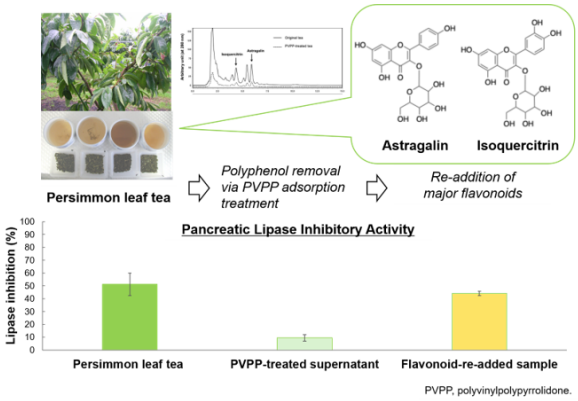
Abstract: Persimmon (*Diospyros kaki*) leaf tea is a traditional beverage rich in polyphenols and has been associated with various health benefits; however, its potential to inhibit dietary lipid digestion remains to be fully elucidated. This study evaluated the pancreatic lipase-inhibitory effect of hot-water extracts of persimmon leaves (persimmon leaf tea) using both a synthetic substrate (*p*-nitrophenyl palmitate, pNPP) and a physiologically relevant triacylglycerol substrate in an oil–in–water emulsion system. Persimmon leaf tea exhibited concentration-dependent inhibition in both assays, indicating lipase-inhibitory effects against both synthetic and physiologically relevant lipid substrates. The pNPP assay was therefore used for subsequent evaluations. High-performance liquid chromatography analysis identified astragalin and isoquercitrin as the predominant flavonol glycosides in the tea. Astragalin and isoquercitrin individually inhibited porcine pancreatic lipase activity in a concentration-dependent manner. Astragalin exhibited a slightly stronger lipase-inhibitory effect than isoquercitrin. When the two compounds were combined at concentrations corresponding to those in persimmon leaf tea, the mixture produced stronger inhibition than the tea itself, suggesting that matrix components may modulate flavonoid activity. Removal of polyphenols by polyvinylpolypyrrolidone treatment markedly reduced lipase-inhibitory effect and reconstitution with the two flavonoids substantially restored the effect, demonstrating that polyphenolic compounds are major contributors to the effect, although they may not fully account for the total inhibition. Although the tea exhibited lower maximal inhibition than the combined authentic standards, this difference is likely attributable to matrix effects from other constituents present in the tea. Compared with the pharmaceutical inhibitor orlistat, persimmon leaf tea showed milder inhibition, with IC_{50} values several hundred times higher, which may be advantageous for long-term dietary use. These findings indicate that persimmon leaf tea suppresses lipid digestion through the combined actions of its major flavonoids and suggest its potential as a functional beverage for moderating dietary fat absorption.



Keywords: persimmon leaf tea, pancreatic lipase, polyphenol, astragalin, isoquercitrin

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1 Introduction

Persimmon leaf tea prepared from the leaves of *Diospyros kaki* is widely consumed in Japan, China, and Korea as a traditional herbal tea. In addition to its pleasant flavor, it has long been appreciated for its potential health-promoting properties. Several literature reviews have demonstrated that persimmon leaf is rich in bioactive compounds, particularly flavonol glycosides such as quercetin-3-*O*-glucoside (isoquercitrin) and kaempferol-3-*O*-glucoside (astragalin), together with other phenolic constituents and vitamin C [1, 2]. The composition of these bioactive compounds can vary depending on processing and decoction conditions. Tsurunaga *et al.* reported that brewing methods significantly influenced the concentration of ascorbic acid, astragalin, total phenolic contents, and antioxidant levels in persimmon leaf tea, suggesting that preparation conditions may affect its biological activity [3, 4].

Recent nutritional and pharmacological studies have highlighted multiple beneficial effects of persimmon leaf extracts, including antioxidant [5], anti-inflammatory [6], anti-diabetic [7, 8], and improving lipid metabolism in animal models [9, 10]. These findings suggest that persimmon leaf may modulate lipid metabolism; however, the specific mechanisms underlying its anti-obesity effects are still not fully understood.

One important therapeutic target for reducing dietary fat absorption is pancreatic lipase, the principal enzyme responsible for hydrolysis of mostly ingested triglycerides into absorbable free fatty acids and monoacylglycerols in the small intestine [11]. Pharmacological inhibition of pancreatic lipase, exemplified by orlistat [12, 13], effectively reduces intestinal fat absorption and contributes to weight management. However, as synthetic inhibitors often produce gastrointestinal discomfort, research interest has prompted natural dietary inhibitors, even though they show mild inhibitory effect.

Dietary polyphenols, particularly flavonoids, have attracted attention as potential natural lipase inhibitors [14, 15]. Previous in vitro and mechanistic studies have demonstrated that flavonols such as quercetin [16] and kaempferol [17] and their derivatives, isoquercitrin [18] and astragalin [19], can inhibit pancreatic lipase through reversible interactions involving hydrogen bonding and hydrophobic contacts near the catalytic site. However, most previous investigations have focused on purified compounds or organic-solvent extracts. The hot-water extracts of persimmon leaf, which represents the actual tea drink consumed in daily life, have rarely been systematically evaluated for pancreatic lipase inhibition.

Furthermore, little is known about how the individual flavonoids in the tea contribute to lipase inhibition, whether synergistic effects occur between astragalin and isoquercitrin, or how food components such as carbohydrates influence their activity. Understanding these relationships is important for appreciating the functional relevance of persimmon leaf tea as a dietary beverage.

Therefore, the present study aimed to evaluate the pancreatic lipase-inhibitory effect of persimmon leaf tea prepared by hot-water extraction and to elucidate the contributions of its principal flavonol glucosides, astragalin and isoquercitrin. Using both a *p*-nitrophenyl palmitate (pNPP) colorimetric assay and an oil-in-water emulsion system to determine fatty acid liberation by GC-MS, we compared the lipase-inhibitory effects of persimmon leaf tea, isolated flavonoids, their combinations, and flavonoid-removal-reconstituted systems. By clarifying the relative contributions of the major polyphenols and matrix effects, this study provides insight into the mechanisms by which persimmon leaf tea may naturally modulate dietary fat digestion.

2 Materials and methods

2.1 Chemicals and reagents

Porcine pancreatic lipase (EC 3.1.1.3; Type II, 314 units/mg protein) and orlistat were purchased from Sigma-Aldrich (St. Louis, MO, USA). *p*-Nitrophenyl palmitate (pNPP) was purchased from Fujifilm Wako Pure Chemical Co. (Osaka, Japan). Polyvinylpolypyrrolidone (PVPP) was obtained from Nacalai Tesque Inc. Isoquercitrin (quercetin-3-*O*-glucoside) was obtained from Extrasynthese S.A. (Genay, France), and astragalin (kaempferol-3-*O*-glucoside) was from Cayman Chemical Co. (Ann Arbor, MI, USA). All solvents utilized for HPLC and GC-MS analyses were of analytical or HPLC grade. The other chemicals were of biochemical or analytical grade.

2.2 Preparation of persimmon leaf tea

The tea leaves of dried persimmon (*Diospyros kaki)* leaf were kindly provided by Shimane Organic Farm Co., Ltd. (Gotsu, Shimane, Japan). An amount of the tea strips (4 g) was added to 50–150 mL of boiled distilled water, and the mixture was left to infuse for 5–15 min to obtain persimmon leaf tea, at varying concentrations. Following the extraction process, the sample was subjected to cooling to room temperature, filtered through Whatman No. 2 filter paper, and stored at −20 °C until subsequent analyses. For each experiment, the tea was newly prepared for each occasion.

2.3 Determination of total (poly)phenolic content and flavonoid glucosides

The total (poly)phenolic content (TPC) of the persimmon leaf tea was determined using the Folin–Ciocalteu colorimetric method [20] . Gallic acid was used as the standard, and results were expressed in units of mg of gallic acid equivalents (GAE).

A quantitative analysis of isoquercitrin and astragalin in the tea was performed using high-performance liquid chromatography (HPLC). The system comprised HPLC units (LC-20AD, Shimadzu Co., Kyoto, Japan) equipped with a photodiode array detector (SPD-M40, Shimadzu Co., Kyoto, Japan). The separation process was accomplished using a reversed-phase C18 column (150 mm × 4.6 mm, 5 µm particle size, TSK gel ODS-80Ts, Tosoh Co., Tokyo, Japan) maintained at 40 °C. The mobile phase consisted of methanol/water/acetic acid (43:55:2, v/v/v), and elution was carried out under isocratic conditions. The flow rate was set at 1.0 mL/min, and the injection volume was 20-50 µL. Prior to injection, the tea was diluted with methanol/water/acetic acid (43:55:2, v/v/v) at desired ratios, centrifuged at 15,000 rpm for 10 min at 4 °C, and the supernatants were applied as analytical samples. The overall pattern of polyphenols was observed at 280 nm. Standard solutions of isoquercitrin and astragalin were prepared at concentrations ranging from 20 to 100 µmol/L to construct quantitative calibration curves at 365 nm.

2.4 Pancreatic lipase inhibition assay using pNPP

The pNPP assay [21] was used to determine the lipase-inhibitory effect in a homogeneous solution as previously described [22] . Briefly, the reaction mixture contained 60 µL of persimmon leaf tea or flavonoid glycosides solution, 40 µL of pancreatic lipase solution (10 mg/mL), and 900 µL of Tris-HCl buffer (0.1 mol/L, pH 7.4) was preincubated at 37 °C for 15 min, and 100 µL of 20 mmol/L pNPP solution was added to initiate the reaction. The mixture was then incubated at 37 °C for 10 min, and the reaction was terminated by adding 500 µL of 95 % ethanol and the absorbance of the supernatants was measured at 405 nm. Orlistat was used as a positive control of lipase inhibitor. Enzyme activity without inhibitor was defined as 100 %, and percentage inhibition was calculated using the following formula:

Lipase Inhibition (%) = [1– (A sample–A blank) / (A test–A control)] × 100

Here,

A sample: Absorbance of the mixture of the tea, pNPP solution, buffer and enzyme solution.

A blank: Absorbance of the mixture of the tea, pNPP solution and buffer without enzyme.

A test: Absorbance of the mixture of buffer (instead of sample), pNPP solution, buffer and enzyme.

A control: Absorbance of the mixture of buffer, pNPP solution and buffer without enzyme.

All experiments were conducted in triplicate to ensure reproducibility and statistical reliability. The half-maximal inhibitory concentration (IC_{50}) was determined from dose–response curves.

2.5 Lipase inhibitory effects of individual flavonoids and combinations

Isoquercitrin and astragalin were dissolved in dimethylsulfoxide (DMSO) at concentrations of 10 mmol/L and diluted with Tris-HCl buffer (0.1 mol/L, pH 7.4) in the range of 10-100 µg/mL. Each compound was assessed in both individual and combination modes using the pNPP assay described above. The combined effect was evaluated by comparing the observed inhibition with the expected additive inhibition.

2.6 Removal of polyphenols using PVPP and reconstitution

In order to verify whether the observed lipase-inhibitory effect was primarily attributable to polyphenolic compounds, polyvinylpolypyrrolidone (PVPP)—a specific adsorbent for phenolic constituents [23]—was used to selectively deplete these compounds from persimmon leaf tea. PVPP treatment was performed as previously described [24] with some modifications. Briefly, 100 µg/ml of PVPP was mixed with the tea (1:1, v/v) and kept at 4 °C for 4 h. After the first extraction, the supernatant was collected via centrifugation at 3,000 rpm for 10 min. Then, the first-extraction supernatant was mixed with a freshly prepared PVPP solution (1:1, v/v), and the mixture was subsequently incubated at 4 °C for 4 h. The resulting second-extraction supernatant obtained by centrifugation was used as the polyphenol-depleted extract. Subsequent reconstitution experiments were performed by supplementing the polyphenol-depleted sample with authentic astragalin and isoquercitrin to match their original concentrations.

2.7 Lipase inhibition in an oil-in-water emulsion system

To simulate physiological lipid digestion conditions, lipase activity was also evaluated using an oil-in-water emulsion, as previously described [22, 25]. Briefly, the emulsion was prepared by mixing 10 mg of triolein with an aqueous phase consisting of 1.0 mL of 1.1 % $CaCl_2$ solution, 0.8 mL of 0.5 mol/L Tris–HCl buffer (pH 7.0), and 0.2 mL of 2 % sodium taurocholate. The mixture was sonicated for 60 s to obtain a stable emulsion. The reaction mixture containing 0.2 mL of

the tea and 25 µL of pancreatic lipase solution (10 mg/mL, with approximately 20 units/assay) was vortexed for 20 s and incubated at 37 °C for 60 min. The reaction was terminated by boiling for 10 min. A 25 µL of aliquot was extracted with 250 µL of chloroform and 250 µL of water, and the liberated free fatty acids were derivatized using trimethylsilyldiazomethane (TMSD) at room temperature for 30 min, followed by termination with 10 % acetic acid in methanol. The resulting fatty acid methyl esters were quantified using GC–MS (GCMS-QP2010 Ultra, Shimadzu Co., Kyoto, Japan). Separation was performed on a DB-5ms column (30 m × 0.25 mm i.d., 0.25 µm film thickness) with helium as the carrier gas at a flow rate of 0.89 mL/min. The oven temperature was programmed from 40 °C (2 min) to 320 °C at 6 °C/min. Electron ionization was performed at 70 eV. Enzyme inhibition was calculated relative to a control without inhibitor, using an internal standard with margaric acid (C17:0).

2.8 Statistical analysis

The lipase-inhibitory effect was expressed as the mean ± standard deviation (SD). Statistical significance was set at $p < 0.05$. Statistically significant differences among three or more groups were tested using a non-repeated one-way ANOVA, and post-hoc tests were performed when significant differences were identified. All statistical analyses were performed using Microsoft Excel with the statistical program ystat (Igakutosho Shuppan, Tokyo, Japan).

3 Results

3.1 Pancreatic lipase inhibition by persimmon leaf tea in the pNPP assay and under oil-in-water emulsion conditions

To evaluate the inhibitory potential of the persimmon leaf tea, two distinct experimental systems were employed. Primarily, pNPP assay was employed to ascertain the intrinsic lipase-inhibitory effect within a homogeneous solution, thereby facilitating a rapid and standardized evaluation of enzyme-inhibitor interaction. Secondly, an oil-in-water emulsion system using triolein as a substrate was utilized to more effectively simulate the heterogeneous environment of physiological lipid digestion, wherein the enzyme acts at the lipid-water interface.

As shown in **Fig. 1**, the tea exhibited a clear concentration-dependent inhibition against lipase activity in the pNPP assay. The lipase inhibition increased progressively with increasing TPC, reaching a plateau at approximately 52 %. The IC_{50} value of the tea was estimated by the linear regression for the range of 143 to 1,062 µg GAE/mL as approximately 1,020 µg GAE/mL.

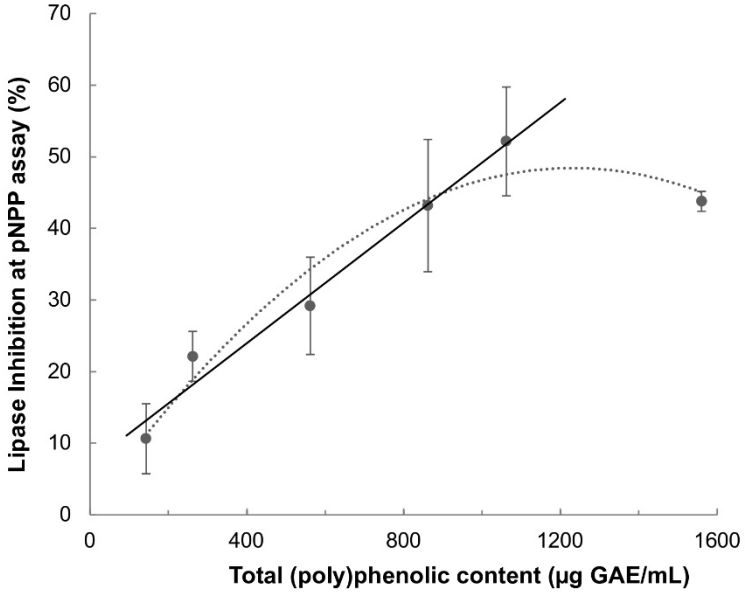

Fig. 1 Dose-dependent inhibition of pancreatic lipase by persimmon leaf tea in the pNPP assay. Lipase-inhibitory effect was measured at various TPCs of the tea using pNPP as a substrate. The dotted curve indicates the regression curve, plotted as a second-degree polynomial function, for the range of 143 to 1,560 µg GAE/mL ($R^2 = 0.9497$). The linear regression (solid line) was calculated for the range of 143 to 1,062 µg GAE /mL ($y = 0.042x + 7.1645$, $R^2 = 0.9772$), to determine the IC_{50} value. Data are expressed as mean ± SD ($n = 3$).

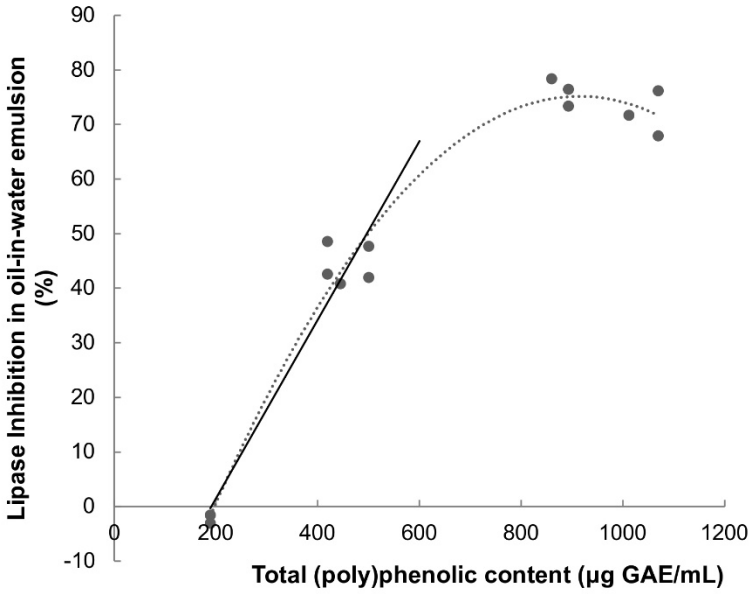

Fig. 2 Dose-dependent inhibition of pancreatic lipase by persimmon leaf tea in an oil-in-water emulsion system. Lipase-inhibitory effect was measured at various TPCs of the tea in an oil-in-water emulsion system using triolein as a substrate. The dotted curve indicates the regression curve, plotted as a second-degree polynomial function, for the range of 189 to 1,069 μg GAE/mL (R^2 = 0.9750). The solid line was plotted as a linear function for the range of 189 to 500 μg GAE /mL (y = 0.1636x + 31.227, R^2 = 0.9224), which was used to determine IC_{50} value.

The inhibitory effect of persimmon leaf tea on porcine pancreatic lipase was also examined in an oil-in-water emulsion system, which serves as a physiological lipid digestion model. As shown in **Fig. 2**, the lipase-inhibitory effect observed in the emulsion system exhibited a concentration-dependency, reaching a plateau at approximately 75 %. The IC_{50} value of the tea in the oil-in-water emulsion system was approximately 500 μg GAE/mL, indicating stronger lipase-inhibitory effect against emulsified triolein than against pNPP. Despite the variation in the intensity of the lipase-inhibitory effect observed between the two systems, a comparable pattern of inhibition was demonstrated, suggesting that persimmon leaf tea maintains its lipase-inhibitory effect under conditions that more accurately replicate physiological lipid digestion.

3.2 Estimation of major flavonoid contents in persimmon leaf tea

HPLC analysis confirmed the presence of isoquercitrin and astragalin, the predominant flavonol glycosides, in the tea. As shown in **Fig. 3**, the concentrations of these compounds increased in proportion to TPC of the tea. The content of astragalin was found to be 61.6 ± 1.4 μg/mL (137.3 ± 1.4 μmol/L) (**Fig. 3**A), while the content of isoquercitrin was determined to be 72.3 ± 1.7 μg/mL (155.7 ± 1.7 μmol/L) (**Fig. 3**B) in the tea with 860 μg GAE/mL, which showed approximately 45 % inhibition against pancreatic lipase and was slightly below the saturation point that was previously indicated (**Fig. 1**). These results demonstrate that persimmon leaf tea contains substantial amounts of flavonol glycosides, which vary in accordance with the total (poly)phenolic concentration.

3.3 Estimation of the lipase-inhibitory effect of major flavonoids in persimmon leaf tea

To evaluate the contribution of major flavonol glycosides to lipase inhibition, authentic astragalin and isoquercitrin were tested individually using the pNPP assay. As shown in **Fig. 4**, both compounds exhibited concentration-dependent inhibition of pancreatic lipase activity. Astragalin exhibited stronger lipase-inhibitory effect than isoquercitrin, reaching approximately 50 % inhibition. Furthermore, astragalin showed saturation within the concentrations corresponding to persimmon leaf tea concentrations tested in this study (**Fig. 4**A). Similarly, isoquercitrin demonstrated substantial inhibition, though it was slightly weaker than astragalin (**Fig. 4**B). The IC_{50} values were estimated to be approximately 51.2 μg/mL (114 μmol/L) for astragalin and 98.4 μg/mL (212 μmol/L) for isoquercitrin.

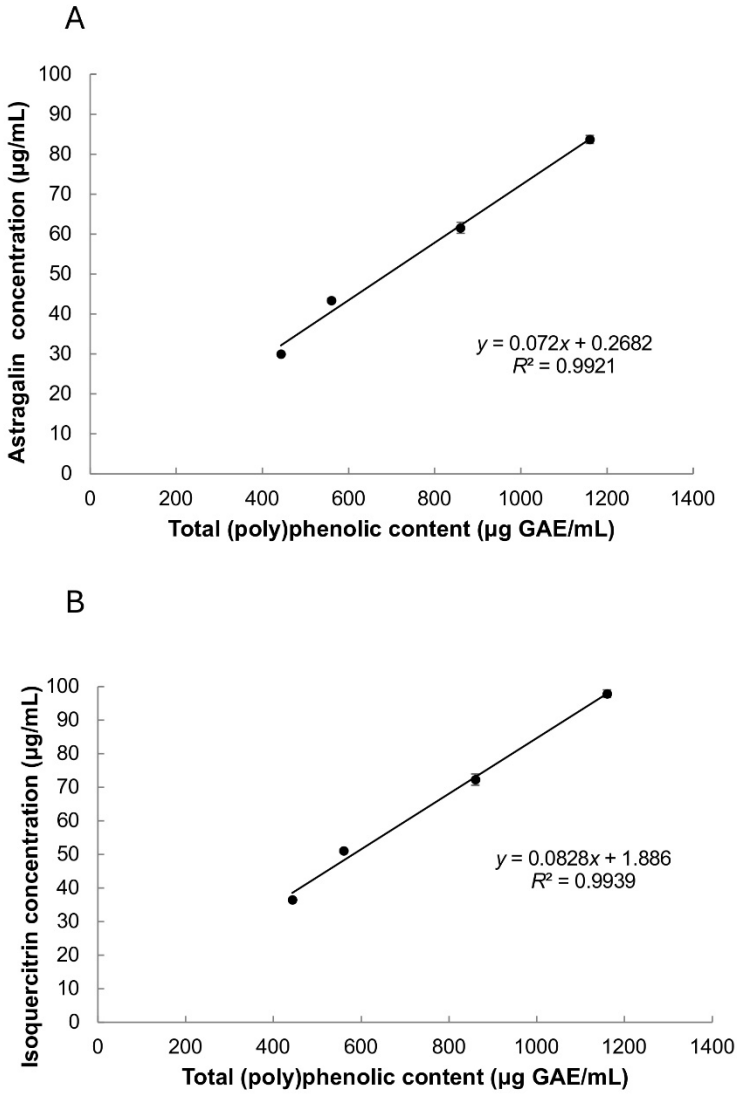

Fig. 3 Relationship between TPC and the contents of major flavonol glycosides in persimmon leaf tea. Persimmon leaf tea with 440–1,160 μg GAE/mL were analyzed to determine astragalin (A) and isoquercitrin (B) concentration by HPLC. Data represent measurements obtained at different TPCs (mean ± SD, $n = 3$).

Orlistat was used as a reference inhibitor to evaluate the relative potency of persimmon leaf tea and its major flavonol glycosides. As shown in **Fig. 5**, orlistat demonstrated a clear dose-dependent inhibition of pancreatic lipase activity in the pNPP assay. Its effect exhibited a biphasic pattern that varied between low and high concentrations. The lipase inhibition increased steadily with increasing orlistat concentration, reaching approximately 60 % inhibition at 0.25 μg/mL. The highest lipase-inhibitory effect was observed at 1.00 μg/mL, where pancreatic lipase activity was suppressed by 93.7 %. The IC_{50} value of orlistat was estimated to be 0.21 μg/mL, which was markedly lower than IC_{50} values of isoquercitrin and astragalin under the same experimental conditions.

3.4 Combination effects of major flavonoids in persimmon leaf tea

To simulate the mixed presence of flavonoids in the tea, astragalin and isoquercitrin were tested as a mixture at concentrations corresponding to their levels in the tea. As shown in **Table 1**, the tea containing approximately 140 μmol/L of astragalin and 155 μmol/L of isoquercitrin exhibited 43 % inhibition of pancreatic lipase activity at approximately 860 μg GAE/mL. Astragalin alone at 140 μmol/L (62.8 μg/mL) showed a comparable lipase-inhibitory effect (49.3 %), whereas isoquercitrin alone at 155 μmol/L (69.7 μg/mL) exhibited slightly lower inhibition (37.1 %). The combination of astragalin

and isoquercitrin at equivalent concentrations yielded considerably enhanced lipase-inhibitory effect (78.6 %) than either compound alone. Since the total concentration in the mixture group was approximately twice as high as that in each individual treatment, this increase may partly reflect a concentration-dependent effect rather than a strictly additive or synergistic interaction.

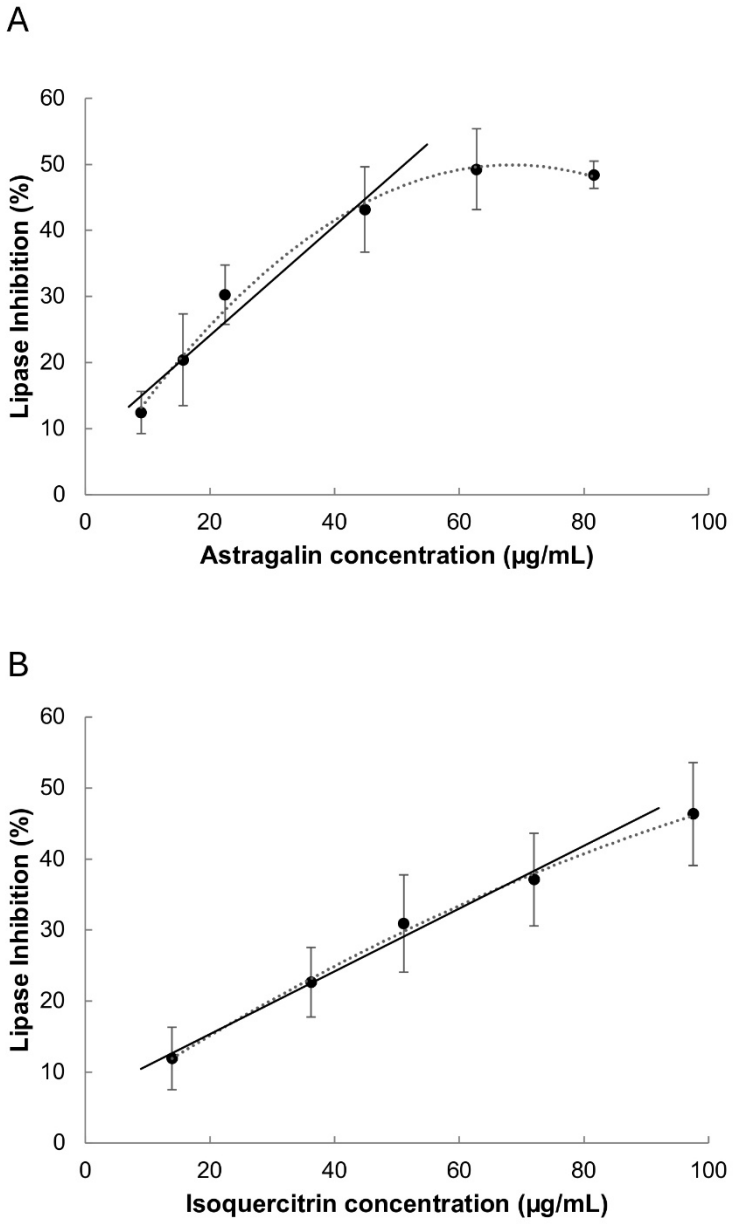

Fig. 4 Dose-dependent inhibition of pancreatic lipase by major flavonol glycosides in persimmon leaf tea in the pNPP assay. Lipase-inhibitory effect was measured at various concentrations of flavonoid glycosides using pNPP as a substrate: (A) Astragalin with 8.97–81.6 μg/mL (20–182 μmol/L) and (B) isoquercitrin with 13.9–97.5 μg /mL (30–210 μmol/L). The dotted curves indicate the regression curve, plotted as a second-degree polynomial function with R^2 = 0.9937 (A) and 0.9967 (B). The solid lines were plotted as a linear function: $y = 0.8294x + 7.527$, R^2 = 0.9507 (A) and $y = 0.4421x + 6.4996$, R^2 = 0.9847 (B), which were used to determine IC_{50} value. Data are expressed as mean ± SD (n = 3-9).

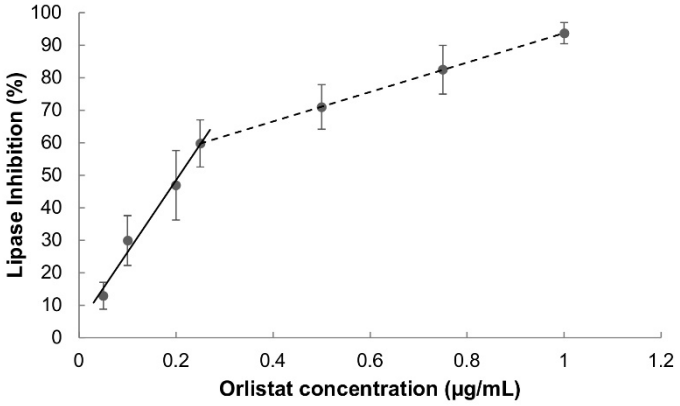

Fig. 5 Dose-dependent inhibition of pancreatic lipase by orlistat in the pNPP assay. Lipase-inhibitory effect was measured at various concentrations of orlistat using pNPP as a substrate. The dotted and solid lines indicate a biphasic pattern that differed between low and high concentrations. Data are expressed as mean ± SD (n = 3).

Table 1 Inhibitory effects of persimmon leaf tea and major flavonol glycosides on pancreatic lipase.

Sample	Astragalin (μg/mL)	Isoquercitrin (μg/mL)	Lipase inhibition (%)
Persimmon leaf tea (approx. 860 μg TPC/mL)	61.6 ± 1.4	72.3 ± 1.7	43.2 ± 9.2 [b]
Astragalin (140 μM)	62.8	–	49.3 ± 6.1 [b]
Isoquercitrin (155 μM)	–	69.7	37.1 ± 6.5 [a]
Astragalin + Isoquercitrin	62.8	69.7	78.6 ± 1.5 [c]

Data are expressed as mean ± SD (n = 3).
Different letters (a – c) within the Lipase inhibition (%) column indicate statistically significant differences between samples (one-way ANOVA, $p < 0.05$ or $p < 0.01$). Specifically, the difference between the tea and isoquercitrin was significant at $p < 0.05$, while all other comparisons were significant at $p < 0.01$.
Abbreviation: TPC, total (poly)phenolic content.

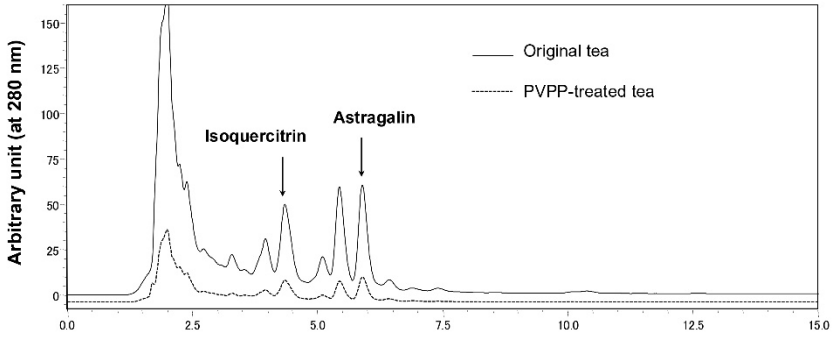

Fig. 6 HPLC chromatogram of persimmon leaf tea. The original tea and the supernatant after PVPP-treatment was analyzed by HPLC-PDA. The solid and dashed lines represent the chromatograms of the original tea and the PVPP-treated supernatant, respectively.

Table 2 Effect of PVPP treatment and flavonoid reconstitution on pancreatic lipase inhibition.

Sample	TPC (μg GAE/mL)	Astragalin (μg/mL)	Isoquercitrin (μg/mL)	Lipase inhibition (%)
Persimmon leaf tea	884.8 ± 13.7	56.8 ± 8.4	65.7 ± 1.3	51.2 ± 8.7 [c]
PVPP-treated supernatant	137.5 ± 2.4	12.1 ± 0.3	14.6 ± 0.3	9.55 ± 2.61 [a]
Flavonoid-reconstituted sample	Not determined	56.8	65.7	44.2 ± 1.6 [b]

Data are expressed as mean ± SD, $n = 3$.
Different letters (a–c) within the same column indicate statistically significant differences between samples at the $p < 0.01$. Abbreviations: GAE, gallic acid equivalents; PVPP, polyvinylpolypyrrolidone; TPC, total (poly)phenolic content.

3.5 Validation of flavonoid involvement using PVPP removal and reconstitution

To further verify the contribution of flavonoid glycosides responsible for the lipase-inhibitory effect, polyphenolic compounds were removed using PVPP treatment (**Fig. 6**) and subsequently reconstituted with authentic flavonoids. As shown in **Table 2**, the original tea at 885 μg/mL exhibited 51.2 ± 8.7 % inhibition of pancreatic lipase activity. After PVPP treatment, the TPC diminished to approximately 15 % of the original level, and the lipase-inhibitory effect decreased significantly to 9.55 ± 2.61 %, depending on the residual flavonoids, which were approximately 20 % of the original concentrations. When the PVPP-treated sample was supplemented with authentic astragalin and isoquercitrin to match the levels found in the original tea, the lipase-inhibitory effect was restored to 44.2 ± 1.6 %. This value was significantly lower than the initial effect but comparable to that of persimmon leaf tea, suggesting that additional phenolic components, which were simultaneously removed by PVPP treatment, or interactions within the tea matrix may also contribute to the overall lipase-inhibitory effect.

4 Discussion

The present study demonstrated that persimmon leaf tea exhibited clear pancreatic lipase-inhibitory effect thereby offering a plausible underlying mechanism for the reported health effects of persimmon leaf products on lipid metabolism. Pancreatic lipase is the principal enzyme responsible for the hydrolysis of dietary triglycerides in the small intestine, and its inhibition is an effective strategy for reducing fat absorption and postprandial lipid uptake [11, 26]. In the present study, persimmon leaf tea demonstrated concentration-dependent inhibition in both the pNPP assay and the oil–in–water emulsion system, indicating that the lipase-inhibitory effect is not limited to artificial substrates but is also effective under conditions that more closely mimic physiological lipid digestion.

Interestingly, the lower IC_{50} value observed in the oil-in-water emulsion system compared with the pNPP assay suggests that additional mechanisms beyond direct enzyme inhibition may be involved. In heterogeneous lipid systems, pancreatic lipase acts at the lipid–water interface, and therefore the accessibility of the enzyme to the substrate is a critical factor. It is plausible that flavonoids such as astragalin and isoquercitrin adsorb onto the surface of lipid droplets, thereby modifying interfacial properties and restricting enzyme access to the substrate. Indeed, flavonoids have been reported to accumulate at oil–water interfaces and stabilize emulsions through particle adsorption mechanisms [27]. In addition to interfacial effects, flavonoids are known to interact with lipid membranes, either by binding at the lipid–water interface or partitioning into the hydrophobic core of lipid bilayers [28]. Such interactions can alter membrane physicochemical properties, including fluidity and organization [29], and may influence the accessibility of lipase to its lipid substrates. Furthermore, flavonoid derivatives have been shown to localize within phospholipid bilayers and exert biological effects through their presence in membrane environments [30]. In addition to interfacial and membrane-related mechanisms, flavonoids have also been reported to directly interact with pancreatic lipase through reversible binding. Previous studies suggest that flavonol glycosides, including isoquercitrin and astragalin, may exhibit competitive or mixed-type inhibition depending on their structure and experimental conditions. However, the present study did not include detailed kinetic analysis; therefore, further investigation is required to clarify the precise mode of inhibition. Such dual mechanisms, involving both interfacial modulation and direct enzyme interaction, may explain the enhanced lipase-inhibitory effect observed under physiologically relevant conditions.

In our previous report, other types of herbal tea, perilla leaf tea, exhibited divergent results in these assays [22]. The lipase-inhibitory effect of perilla leaf tea was most pronounced at moderate concentrations and was only observed within the oil-in-water emulsion system. In contrast, no lipase-inhibitory effect was observed against the pNPP substrate. While

perilla and persimmon leaves contain different bioactive compounds, namely rosmarinic acid and the flavonol glycosides astragalin and isoquercitrin, respectively, it is also likely that the soluble components derived from these different leaves have varying effects on bioactive polyphenols.

Chemical analysis of persimmon leaf tea revealed the predominant extraction of two flavonol glycosides: astragalin and isoquercitrin. The two compounds demonstrated clear dose-dependent lipase-inhibitory effect on pancreatic lipase when evaluated individually. Astragalin produced inhibition ranging from 12.5 % to 49.3 %, while isoquercitrin showed a comparable lipase-inhibitory effect, with values ranging from 11.9 % to 46.3 % across the tested concentrations. These results indicate that each flavonoid contributes substantially to the overall lipase-inhibitory effect observed in the tea, with astragalin showing slightly higher maximal inhibition under the conditions examined.

Interestingly, the combined authentic standards (astragalin and isoquercitrin) produced significantly stronger inhibition (approximately 79 %) than the tea (approximately 43 %) at equivalent flavonoid concentrations. This discrepancy suggests the presence of matrix effects inherent to the complex hot-water decoction. Non-phenolic constituents, such as polysaccharides, may interact with the active flavonoids through complex formation or steric hindrance [31, 32], which limits their ability to bind to the pancreatic lipase molecule. Consequently, while the major flavonol glycosides are the principal drivers of lipase-inhibitory effect their potency in the intact tea matrix is moderated by the presence of other food components.

Further evidence for the central role of polyphenols was obtained from PVPP treatment experiments. The elimination of polyphenolic constituents led to a significant reduction in lipase-inhibitory effect whereas the reconstitution of these constituents with astragalin and isoquercitrin substantially restored the effect. These findings demonstrate that these polyphenolic compounds are major contributors to pancreatic lipase inhibition, although minor components and interactions within the food matrix may also play supportive roles.

The inhibitory potency of persimmon leaf tea and its constituent flavonoids was lower than that of the pharmaceutical lipase inhibitor orlistat [33], which irreversibly inactivates the enzyme through covalent binding to the catalytic serine residue [12, 13] , with IC_{50} values being several hundred times higher. It should be noted that the comparison in this study is based on mass concentration (μg/mL). When expressed on a molar basis, the difference in inhibitory potency between the flavonoids and orlistat remains substantial, although the overall order of magnitude is not significantly altered, as their molecular weights are of similar magnitude (orlistat: 495.7; astragalin: 448.4; isoquercitrin: 464.4). However, this moderate effect may offer certain benefits in dietary applications. Strong pharmacological inhibition of fat digestion may cause gastrointestinal adverse effects [34], whereas milder suppression may allow for partial digestion while reducing overall lipid absorption. As a frequently consumed beverage, persimmon leaf tea can be ingested concurrently with meals, thereby enabling interaction with dietary lipids in the intestinal lumen without the necessity of precise dosing. It should be noted that the concentration points in the emulsion system were not evenly distributed, particularly around the IC_{50} region, and the number of data points at lower concentrations was limited. In addition, slight negative inhibition values were observed at low concentrations, which may reflect experimental variability inherent to the emulsion-based assay. Therefore, the IC_{50} value derived from this dataset should be interpreted with caution. For **Fig. 2**, IC_{50} values were also estimated using a four-parameter logistic (4PL) model, which yielded comparable results to those obtained by linear regression within the relevant concentration range. However, for the other datasets (**Figs. 1** and **4**), the limited number of data points and their sparse distribution around the IC_{50} region resulted in unstable parameter estimation when applying 4PL fitting. Therefore, linear regression within the approximately linear response range was considered to provide more robust and reliable IC_{50} estimates for these datasets.

Notably, compositional variability among commercial persimmon leaf tea products has been documented, with variations in flavonol glycoside content affecting biological activity [35]. Such variability may partly explain differences in lipase inhibitory efficacy among samples and highlights the importance of polyphenol composition as a determinant of functional properties.

The collective evidence from this study suggests that the functional efficacy of persimmon leaf tea does not derive from a single potent constituent, but rather from the harmonized action of its predominant flavonol glycosides. The complementary nature of astragalin and isoquercitrin in the tea matrix, potentially modulated by non-phenolic components such as polysaccharides, leads to a unique inhibitory profile. This observation indicates that the tea provides a more regulated modulation of lipid hydrolysis compared to the abrupt inhibition typically associated with synthetic agents. These characteristics position persimmon leaf tea as a sophisticated dietary strategy for managing postprandial lipid metabolism through regular, long-term consumption.

5 Conclusion

Persimmon leaf tea prepared by traditional hot-water decoction exhibited clear lipase-inhibitory effect against pancreatic lipase, indicating its potential to suppress dietary lipid digestion. The major flavonol glycosides astragalin and isoquercitrin were identified as key contributors to this effect and their combined presence produced stronger inhibition than either compound alone. Although there is a limitation in that most experiments of this study evaluate the effect of persimmon leaf tea using the synthetic ligand pNPP, the lipase-inhibitory effect was observed in both pNPP assays and oil-in-water emulsion systems with triolein as a substrate, suggesting relevance under physiological conditions. Although the tea showed lower potency than the combined authentic compounds, this effect is likely influenced by matrix interactions within the complex food system. Compared with the pharmaceutical inhibitor orlistat, persimmon leaf tea demonstrated a more moderate level of inhibition, with IC_{50} values being several hundred times higher, a property that may be advantageous for long-term dietary application. These findings support the potential of persimmon leaf tea as a functional beverage for moderating fat digestion and dietary lipid absorption.

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Conflict of interest statement

The authors report that there are no conflict of interests to declare.

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