



Comparative analysis of phenolic compounds in different thinned unripe kiwifruits and their biological functions

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ABSTRACT

Thinned unripe kiwifruits (TUK) are considered the major agro by-products in kiwifruit production. To promote their potential applications, polyphenols and biological effects of unripe fruits from nine commercial kiwifruit cultivars were compared. Our findings showed that TUK were rich in bioactive polyphenols, which varied greatly by different cultivars. Indeed, catechin, epicatechin, procyanidin B1, procyanidin B2, protocatechuic acid, neochlorogenic acid, and gallic acid were measured as the major phenolic components in most TUK, with the highest levels observed in 'Hongao' and 'Cuiyu' cultivars. Furthermore, TUK exerted strong *in vitro* antioxidant capacities, inhibitory effects on digestive enzymes, and anti-inflammatory activities. Particularly, their stronger antioxidant effects and inhibitory effects on digestive enzymes were probably attributed to their higher contents of phenolic compounds, especially procyanidin B2. Collectively, our findings reveal that TUK are potential resources of valuable polyphenols, which can be exploited as natural antioxidants and natural inhibitors of α -glucosidase and α -amylase.

1. Introduction

Kiwifruit, also known as the Chinese gooseberry, Macaque peach, and Mihoutau, is the fruit of a woody vine belonging to the genus *Actinidia* (Li et al., 2024; Wang et al., 2021). It is regarded as one of the most popular fruits for its delicious taste and outstanding health-promoting benefits (Li et al., 2024; Satpal et al., 2021; Wang et al., 2021). At present, there are more than seventy kiwifruit species around the world, of which the *A. deliciosa* and *A. chinensis* are the most

commercially significant species (Li et al., 2024; Wang et al., 2021). Furthermore, accumulating evidence has proven that the dietary consumption of kiwifruits and their processed products is beneficial to preventing and managing chronic metabolic syndromes, and is also beneficial for immune function and antioxidant defense (Mai et al., 2022; Sanz et al., 2021; Wang et al., 2021). That's because kiwifruits possess a plethora of valuable phytochemicals, e.g., phenolic acids, flavonoids, anthocyanins, and pectic polysaccharides, which contribute to their various pharmacological properties (Lai et al., 2024; Satpal et al.,

Abbreviations: ABTS, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid); CA, caffeic acid; Ca, catechin; CHL, chlorogenic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EC, epicatechin; FA, ferulic acid; FRAP, ferric-reducing antioxidant power; GA, gallic acid; GFKC, green-fleshed kiwifruit cultivars; HCA, hierarchical cluster analysis; HPLC, high-performance liquid chromatography; IL-6, Interleukin-6; LPS, lipopolysaccharide; mg/g DW, mg/g kiwifruit dry weight; mg CE/g DW, milligram catechin equivalent per gram kiwifruit dry weight; mg GAE/g DW, milligram gallic acid equivalent per gram kiwifruit dry weight; mg RE/g DW, milligram rutin equivalent per gram kiwifruit dry weight; NCHL, neochlorogenic acid; NO, nitric oxide; OH, hydroxyl; PA, protocatechuic acid; PB1, procyanidin B1; PB2, procyanidin B2; PC1, procyanidin C1; p-CA, p-coumaric acid; QGlc, quercetin 3-O-glucoside; QRha, quercetin 3-O-rhamnoside; RFKC, red-fleshed kiwifruit cultivars; T2D, type 2 diabetes; TFC, total flavonoids; TNF- α , tumor necrosis factor- α ; TPAC, total procyanidins; TPC, total phenolics; TUK, thinned unripe kiwifruits; UPLC-Q-TOF-MS, ultra-performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry; YFKC, yellow-fleshed kiwifruit cultivars..

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2021), such as antioxidant, anti-inflammatory, anti-diabetic, hypoglycemic, and hypolipidemic effects. As a consequence, kiwifruits have been widely utilized in the common food, functional food, medical, and cosmetic industries (Lai et al., 2024; Satpal et al., 2021).

China has the richest natural resources of kiwifruits around the world, and is the main producer of kiwifruits. In fact, China possesses the largest planted areas around the world, and accounts for more than fifty percentages of the world's kiwifruit production (Lai et al., 2024; Pinto et al., 2020). In China, there are various commercial cultivars of *A. deliciosa* (e.g., 'Miliang', 'Xuxiang', 'Qinmei', 'Hayward', 'Cuixiang', add 'Jinkui') and *A. chinensis* (e.g., 'Hongyang', 'Donghong', 'Jinyan', 'Jinshi', 'Cuiyu', and 'Qihong') with different appearances (Liang et al., 2021; Zhang et al., 2020). Indeed, these commercial cultivars can be classified into different categories according to their flesh colors, including green-fleshed cultivars (e.g., 'Hayward', 'Miliang', 'Xuxiang', and 'Cuixiang'), yellow-fleshed cultivars (e.g., 'Jinyan' and 'Jinshi'), and red-fleshed cultivars (e.g., 'Hongyang', 'Donghong', 'Qihong', and 'Hongao') (Liang et al., 2021; Zhang et al., 2020). Usually, to obtain commercial kiwifruits with high yield and superior quality, fruit thinning will be carried out by orchard workers for the first time at about 20 days after fruit-setting. Subsequently, approximate 30–50 % of unripe kiwifruits (about 20–60 days after fruit-setting) will be thinned for 2–3 times by orchard workers, and then discarded in the orchard (Jiao et al., 2019; Wu et al., 2023; Wu et al., 2024), causing serious environmental pollutions and resources wasting. Hence, these thinned unripe kiwifruits are required a suitable management or processing to promote their potential applications. Actually, compared with mature fruits, these thinned unripe kiwifruits (TUK) also possess abundantly bioactive ingredients, such as polyphenols and pectin molecules (Jiao et al., 2019; Wu et al., 2023; Wu et al., 2024), exhibiting various biological functions, including antioxidant, anti-glycosylation, anti-inflammatory, anti-diabetic, and immunostimulatory properties. Therefore, TUK possess good potentials to be exploited as healthy products. Previous studies have revealed that bioactive polyphenols and health benefits of mature kiwifruits vary by different species and cultivars (Gao et al., 2021; Leontowicz et al., 2016; Li et al., 2018; Liang et al., 2021; Park et al., 2014). Nevertheless, the knowledge about phenolic compounds and health-promoting benefits of TUK from different species and cultivars is still unclear, which ultimately restricts their food applications. To select suitable TUK for further processing in the food industry, it is extremely necessary to evaluate their bioactive compounds and biological properties.

Hence, to exploit the food applications of TUK, phenolic compounds and biological functions of thinned unripe fruits from nine commercial kiwifruit cultivars that cultivated in China, including three red-fleshed cultivars (*Actinidia chinensis* cv. 'Hongao', *Actinidia chinensis* cv. 'Hongshi', and *Actinidia chinensis* cv. 'Hongyang'), three green-fleshed cultivars (*Actinidia chinensis* cv. 'Cuiyu', *Actinidia deliciosa* cv. 'Xuxiang', and *Actinidia deliciosa* cv. 'Miliang'), and three yellow-fleshed cultivars (*Actinidia chinensis* cv. 'Xinzhong', *Actinidia chinensis* cv. 'Jinshi NO. 2', and *Actinidia chinensis* cv. 'Jinshi NO. 3'), were comprehensively compared. The findings can provide good evidence for the development and utilization of TUK in the functional food industry.

2. Material and methods

2.1. Materials and chemicals

Thinned unripe kiwifruits (about 20 days after fruit-setting, fruiting thinning for the first time) from three red-fleshed cultivars ('Hongao', 'Hongshi', and 'Hongyang'), three green-fleshed cultivars ('Cuiyu', 'Xuxiang', and 'Miliang'), and three yellow-fleshed cultivars ('Xinzhong', 'Jinshi NO. 2', and 'Jinshi NO. 3') were collected from the same kiwifruit breeding and cultivation base located in Deyang City, Sichuan Province, China. After washing, TUK were lyophilized and then pulverized into a fine powder with a 120-mesh sieve.

Phenolic compound standards, including six phenolic acids (gallic acid, GA; caffeic acid, CA; chlorogenic acid, CHL; ferulic acid, FA; *p*-coumaric acid, *p*-CA; neochlorogenic acid, NCHL), two flavonols (quercetin 3-O-glucoside, QGL; quercetin 3-O-rhamnoside, QRha), and six flavanols (protocatechuic acid, PA; catechin, Ca; epicatechin, EC; procyanidin B1, PB1; procyanidin B2, PB2; procyanidin C1, PC1), were purchased from Shanghai Yuanye Biotechnology CO., Ltd. (Shanghai, China). α -Glucosidase and α -amylase were acquired from Solarbio® (Beijing, China). Acarbose tablets were obtained Bayer HealthCare Co., Ltd. (Beijing, China). Interleukin-6 (IL-6) ELISA kit and tumor necrosis factor- α (TNF- α) ELISA kit were acquired from Wuhan Elabscience Biotechnology Co., Ltd. (Wuhan, China).

2.2. Preparation of polyphenol-enriched extracts

Polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars were prepared according to a previously optimized approach (Wu et al., 2023). In brief, the extraction conditions were water content of 32 % (v/v) in deep eutectic solvent, liquid to solid ratio of 50: 1 mL/g, ultrasound extraction power of 450 W, and ultrasound extraction time of 23 min. After the extraction, the polyphenol-enriched extracts were centrifuged, and then the supernatants were collected for further analysis. The polyphenol-enriched extracts prepared from unripe fruits of different kiwifruit cultivars, including 'Hongao', 'Hongshi', 'Hongyang', 'Cuiyu', 'Xuxiang', 'Miliang', 'Xinzhong', 'Jinshi NO. 2', and 'Jinshi NO. 3', were coded as HA, HS, HY, CY, XX, ML, XZ, J2, and J3, respectively. The detailed procedure for the preparation of polyphenol-enriched extracts was supplied in the Supplementary Materials (Section S.1).

2.3. Determination of total polyphenols

Total polyphenols in polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars, including total phenolics (TPC), total flavonoids (TFC), and total procyanidins (TPAC), were measured by colorimetric methods (Wu et al., 2023), e.g., the Folin-Ciocalteu colorimetric assay, the aluminium trichloride-based colorimetric assay, and the vanillin-sulfuric acid colorimetric assay. The TPC, TFC, and TPAC were expressed as mg gallic acid equivalent per g kiwifruit dry weight (mg GAE/g DW), mg rutin equivalent per g kiwifruit dry weight (mg RE/g DW), and mg catechin equivalent per g kiwifruit dry weight (mg CE/g DW), respectively. The detailed procedures for colorimetric methods were also supplied in the Supplementary Materials (Section S.2).

2.4. Identification of individual phenolic compounds by UPLC-Q-TOF-MS

Individual phenolic compounds in polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars were analyzed by using ultra-performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (UPLC-Q-TOF-MS, Agilent 6545 Q-TOF-MS, Agilent Technologies, Santa Clara, CA, USA). The high-resolution Q-TOF-MS was operated in negative ion mode, and scanned in a mass range of m/z 100–1000. Data analysis was conducted using Agilent Qualitative Analysis 10.0 software. Parent ions were cross-referenced with database and literatures as well as several authentic standards to identify phenolic compounds in the polyphenol-enriched extracts from different kiwifruit cultivars. The PCDL Manager B. 08.00 software and TCM-database (Agilent Technologies, Santa Clara, CA, USA) were utilized for the characterization of individual phenolic compounds. Detailed procedures regarding the UPLC-Q-TOF-MS analysis were supplied in the Supplementary Materials (Section S.3).

2.5. Quantification of major phenolic compounds by HPLC analysis

The major phenolic components in polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars were detected by high-performance liquid chromatography (HPLC) analysis according to a

previously established method (Li et al., 2018). Hydroxybenzoic acid and flavanols were determined at the wavelength of 280 nm, hydroxycinnamic acids were measured at the wavelength of 320 nm, and flavonols were detected at the wavelength of 360 nm. In this study, fourteen commercially standard phenolic compounds, including six phenolic acids, six flavanols, and two flavonols were determined. The level of each standard compound in different unripe kiwifruit was presented as mg/g kiwifruit dry weight (mg/g DW). Detailed methods regarding the HPLC analysis were supplied in the Supplementary Materials (Section S.4).

2.6. Determination of *in vitro* antioxidant effects

To assess *in vitro* antioxidant effects of polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars, we conducted the 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, and hydroxyl (OH) free radical scavenging ability assays as well as ferric-reducing antioxidant power (FRAP) assay following our previously established methods (Li et al., 2018; Wu et al., 2023). Trolox was used as the standard in this study. The levels of FRAP of polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars were presented as micromolar Trolox equivalent per gram kiwifruit dry weight ($\mu\text{mol Trolox/g DW}$). The IC_{50} values of polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars against various free radicals were presented as milligram kiwifruit dry weight per milliliter (mg/mL). Detailed procedures for the determination of *in vitro* antioxidant capacities of polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars were supplied in Supplementary materials (Section S.5).

2.7. Determination of inhibitory effects on α -glucosidase and α -amylase

To assess the potential anti-diabetic effects of polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars, their inhibition rates against digestive enzymes, including α -glucosidase and α -amylase, were determined according to our previously established colorimetric methods (Wu et al., 2023; Yuan et al., 2019). The IC_{50} values of polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars against both α -glucosidase and α -amylase were expressed as microgram kiwifruit dry weight per milliliter ($\mu\text{g/mL}$). Detailed methods for the determination of inhibitory effects of different polyphenol-enriched extracts on α -glucosidase and α -amylase were supplied in Supplementary materials (Section S.6).

2.8. Determination of *in vitro* anti-inflammatory effects

To assess the *in vitro* anti-inflammatory activities of polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars, the lipopolysaccharide (LPS)-stimulated inflammatory cell model was carried out based on a previously described approach with minor modifications (Zhang et al., 2021). Firstly, the cytotoxic effects of different polyphenol-enriched extracts on cells were determined. Then, the inhibition rates of different polyphenol-enriched extracts against the release of proinflammatory factors (Nitric oxide (NO), IL-6 and TNF- α) from LPS-stimulated RAW 264.7 cells were measured, and their levels in the supernatant were measured by different kits following the manufacturers' guidelines. Detailed methods for the evaluation of *in vitro* anti-inflammatory effects of different polyphenol-enriched extracts were supplied in Supplementary materials (Section S.7).

2.9. Statistical analysis

One-way analysis of variance and two-tailed Student *t*-test were utilized for statistical analysis, and statistical significance for all tests was set at $p < 0.05$. Hierarchical cluster heatmap analysis was carried out to clarify differences and similarities among unripe fruits of different

kiwifruit cultivars. Furthermore, Pearson correlation was calculated using Origin 2022 software (OriginLab Corporation, Northampton, MA, USA) to explore potential correlations.

3. Results and discussion

3.1. Comparison of TPC, TFC, and TPAC in polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars

Generally, polyphenols, e.g., phenolic acids, flavonoids, and cyanidin, are considered the importantly bioactive components in kiwifruits (Lai et al., 2024; Satpal et al., 2021; Wang et al., 2021), and their contents usually vary greatly among different cultivars, maturity stages, fruit parts, and geographical regions (Wang et al., 2021). Although recent studies have revealed that TUK are also rich in bioactive polyphenols (Jiao et al., 2019; Wu et al., 2023), the knowledge about the variations of polyphenols in unripe fruits from different commercial kiwifruit cultivars remains unclear, which ultimately restrains their possible applications in the food industry. Therefore, the variations of TPC, TFC, and TPAC in polyphenol-enriched extracts from different kiwifruit cultivars, including three red-fleshed cultivars ('Hongao', 'Hongshi', and 'Hongyang'), three green-fleshed cultivars ('Cuiyu', 'Xuxiang', and 'Miliang'), and three yellow-fleshed cultivars ('Xinzhong', 'Jinshi NO. 2', and 'Jinshi NO. 3'), were investigated in this study. Fig. 1A showed that TUK were rich in polyphenols, similar to previous studies that unripe kiwifruits contained abundantly bioactive polyphenols (Huang et al., 2020; Jiao et al., 2019; Nie et al., 2020). Obviously, the TPC, TFC, and TPAC varied greatly by different kiwifruit cultivars, ranging from 25.34 mg GAE/g DW ('Xinzhong') to 119.23 mg GAE/g DW ('Hongao'), from 8.51 mg RE/g DW ('Xinzhong') to 41.67 mg RE/g DW ('Hongao'), and from 4.79 mg CE/g DW ('Xinzhong') to 32.61 mg CE/g DW ('Hongao'), respectively. This phenomenon was comparable to previous studies that total polyphenols varied by different species and cultivars of mature kiwifruits, and even different flesh colors (Liang et al., 2021; Liu et al., 2019; Ma et al., 2017; Zhang et al., 2020). In fact, several studies have also shown that the cultivar is the main factor influencing the quality and content of polyphenols in mature kiwifruits (Liang et al., 2021; Ma et al., 2017). Besides, the average levels of TPC, TFC, and TPAC in red-fleshed kiwifruit cultivars (RFKC), green-fleshed kiwifruit cultivars (GFKC), and yellow-fleshed kiwifruit cultivars (YFKC) ranged from 47.75 mg GAE/g DW (YFKC) to 100.76 mg GAE/g DW (RFKC), from 15.08 mg RE/g DW (YFKC) to 31.99 mg RE/g DW (GFKC), and from 9.52 mg CE/g DW (YFKC) to 22.55 mg CE/g DW (GFKC), respectively, indicating that the flesh colors of kiwifruits also had certain influences on the content of total polyphenols in TUK. Previous studies have also shown that the red-fleshed mature kiwifruits possess a higher level of total polyphenols than that of green and yellow-fleshed mature kiwifruits (Liu et al., 2019; Zhang et al., 2020). Furthermore, according to previous experimental results (Jiao et al., 2019; Li et al., 2018; Mai et al., 2022), the levels of TPC in different mature fruits of *A. chinensis* and *A. deliciosa* were in the range of 3.75–16.52 mg GAE/g DW, which were significantly lower than that of TUK. Overall, these results suggest that TUK, especially 'Hongao' and 'Cuiyu', are rich in natural polyphenols, exhibiting good potential applications in the food and functional food industries.

3.2. Comparison of major phenolic compounds in polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars

In the present study, the major phenolic components in polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars were analyzed by high-resolution UPLC-Q-TOF-MS analysis. As shown in Fig. 1B, 50 compounds were tentatively identified according to Agilent Qualitative Analysis 10.0 software, PCDL, TCM Database, literatures, and several authentic chemical standards. Their retention times, molecular formula, molar mass, observed *m/z*, error, and score are

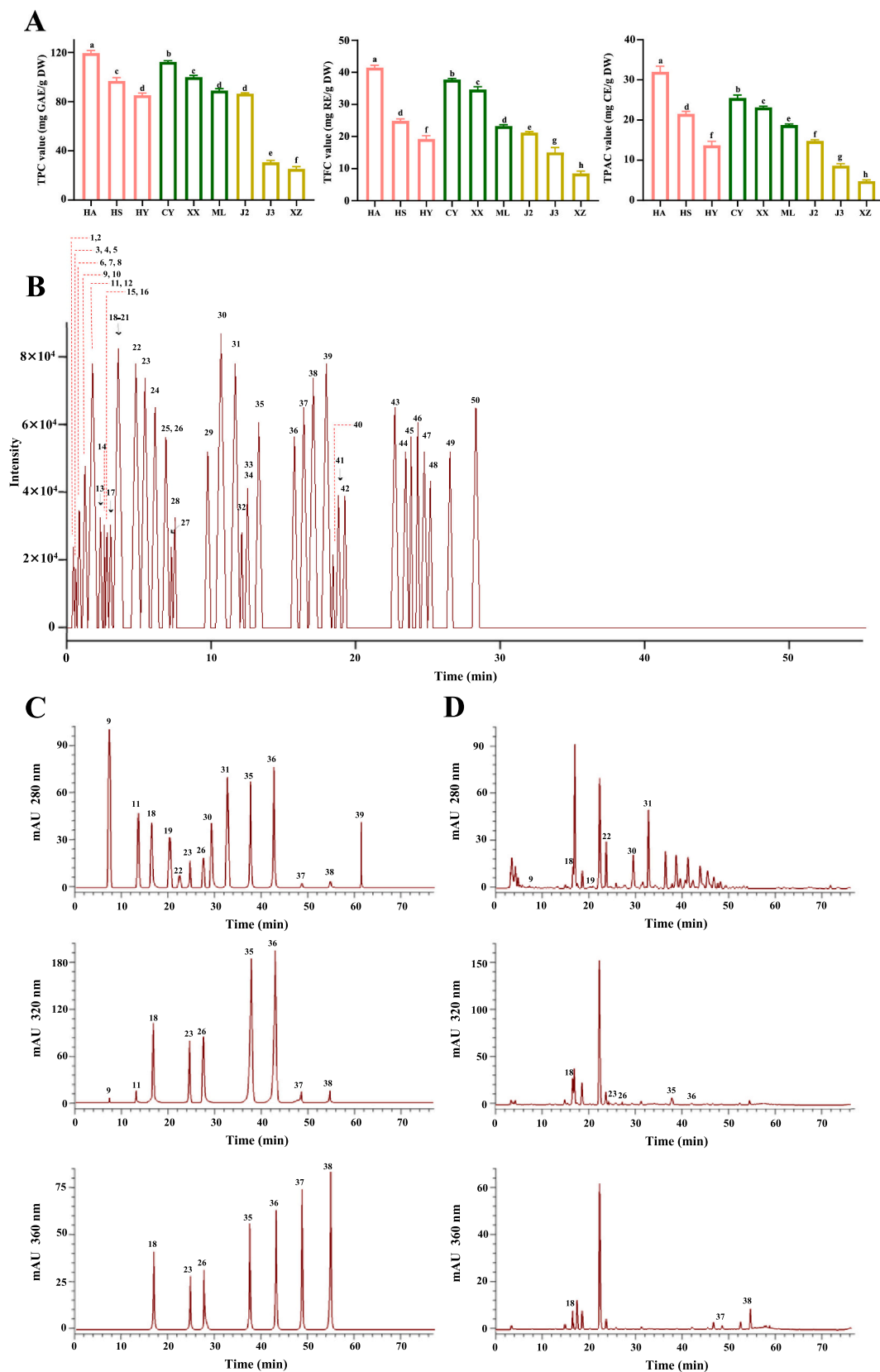


Fig. 1. Total polyphenols (A), UPLC-Q-TOF-MS extracted ions chromatogram (B), and HPLC profiles of mixed standards (C) and the presentative extract of thinned unripe kiwifruits (D).

HA, HS, HY, CY, XX, ML, XZ, J2, and J3 indicate polyphenol-enriched extracts from different kiwifruit cultivars, including ‘Hongao’, ‘Hongshi’, ‘Hongyang’, ‘Cuiyu’, ‘Xuxiang’, ‘Miliang’, ‘Xinzhong’, ‘Jinshi NO. 2’, and ‘Jinshi NO. 3’, respectively;

Different lowercase letters (a-h) indicate statistically significant differences among different kiwifruit cultivars ($p < 0.05$).

displayed in Table 1. These compounds also varied by different kiwifruit cultivars, similar to previous studies that different mature kiwifruits contained different types of polyphenols (Liang et al., 2021; Zhu et al., 2021). More specifically, most compounds could be observed in 'Hongao', 'Hongshi', 'Hongyang', 'Cuiyu', 'Xuxiang', 'Miliang', 'Jinshi No. 2', and 'Jinshi No. 3', while they were absent in 'Xinzhong'. In addition, several phenolic compounds, e.g., caffeic acid, caffeic acid methyl ester, *p*-coumaric acid, ferulic acid, and quercetin 3-O-glucoside, were absent in 'Hongyang'. In fact, among these 50 compounds, several polyphenols, including gallic acid, cinnamic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, chlorogenic acid, neochlorogenic acid, protocatechuic acid, catechin, epicatechin, catechin gallate, procyanidin B1, procyanidin B2, procyanidin C1, quercetin, kaempferol, rutin, quercetin 3-O-glucoside, and quercetin 3-O-rhamnoside, were commonly observed in various mature kiwifruits (He et al., 2019; Lai et al., 2024; Satpal et al., 2021; Wang et al., 2021).

Moreover, the levels of major phenolic compounds in polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars

were measured by HPLC analysis. Based on UPLC-Q-TOF-MS analysis and previous studies (Wang et al., 2021; Waswa et al., 2024), fourteen commercially available phenolic compounds were assessed in different TUK. Six phenolic acids (GA, NCHL, CHL, CA, *p*-CA, and FA), six flavanols (Ca, PA, EC, PB1, PB2, and PC1), and two flavonols (QGlc and QRha) were quantified and their calibration curves were shown in Table S1 (Supplementary material). Fig. 1C and D showed the HPLC chromatograms of mixed standard phenolic compounds and polyphenol-enriched extracts from the presentative sample ('Cuiyu'), and their contents were summarized in Table 2. The findings showed that the levels of individual phenolic components varied greatly by different kiwifruit cultivars, which were comparable to previous studies that total phenolics varied greatly by various species and cultivars of mature kiwifruits (Liang et al., 2021; Ma et al., 2017). The levels of total phenolic compounds in polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars ranged from 10.304 mg/g DW to 31.548 mg/g DW, and the highest content was observed in 'Hongao', while the lowest content was found in 'Xinzhong'. In addition, the average content

Table 1

Tentative identification of compounds in polyphenol-enriched extracts from different kiwifruit cultivars by UPLC-Q-TOF-MS.

NO.	Formula	Proposed compounds	RT (min)	Molar mass	Observed (<i>m/z</i>)	Error (ppm)	Score	Kiwifruit cultivars
1	C ₆ H ₁₄ N ₂ O ₂	L-Lysine	0.406	146.1051	145.0978	-2.76	91.49	a-i**
2	C ₆ H ₉ N ₃ O ₂	L-Histidine	0.412	155.0692	154.0619	-1.84	86.94	a-i**
3	C ₇ H ₁₂ O ₆	Quinic acid	0.561	192.0634	191.0561	0.09	99.9	a-i**
4	C ₄ H ₆ O ₅	Malic acid	0.588	134.0215	133.0142	-0.11	87.46	a-i**
5	C ₆ H ₈ O ₇	Citric acid	0.671	192.0270	191.0196	-0.1	99.55	a-i**
6	C ₄ H ₄ O ₄	Fumaric acid	0.721	116.0110	115.0038	0.7	87.57	a-h**
7	C ₄ H ₆ O ₄	Succinic acid	0.796	118.0271	117.0198	3.76	98.34	a-h**
8	C ₇ H ₆ O ₂	Benzoic acid	0.812	122.0167	121.0094	1.41	86.73	a-i**
9	C ₇ H ₆ O ₅	Gallic acid	1.228	170.0233	169.0161	2.38	80.64	a-i***
10	C ₂₂ H ₁₈ O ₁₀	Catechin gallate	1.381	442.0887	441.0815	-2.93	92.78	a-h**
11	C ₇ H ₆ O ₄	Protocatechuic acid	1.658	154.0266	153.0188	-3.42	98.02	a-i***
12	C ₁₃ H ₁₆ O ₉	Protocatechuic acid-O-hexoside	1.770	316.0802	315.073	2.35	97.28	a-h**
13	C ₆ H ₆ O ₆	Aconitic acid	2.490	174.0169	173.0095	2.48	83.81	a-h*
14	C ₁₀ H ₈ O ₅	Fraxetin	2.578	208.0365	207.0293	-3.14	84.75	a-h**
15	C ₁₆ H ₁₈ O ₉	1-O-Caffeoylquinic acid	2.761	354.0953	353.0882	0.61	98.29	a-h**
16	C ₆ H ₈ O ₂	Sorbic acid	2.763	112.0519	111.0446	-4.67	98.11	a-h*
17	C ₉ H ₈ O ₂	Cinnamic acid	3.012	148.0527	147.0456	1.58	81.02	a-h**
18	C ₁₆ H ₁₈ O ₉	Neochlorogenic acid	3.371	354.0958	353.0886	2.13	98.2	a-i***
19	C ₃₀ H ₂₆ O ₁₂	Procyanidin B1	3.580	578.1437	577.137	2.21	94.68	a-i***
20	C ₉ H ₆ O ₄	Aesculetin	3.735	178.0260	177.0188	-3.43	84.13	a-g*
21	C ₁₅ H ₁₆ O ₉	Aesculin	3.747	340.0787	339.0715	-2.04	90.82	a-g*
22	C ₁₅ H ₁₄ O ₆	Catechin	4.821	290.0796	289.0723	2.04	98.65	a-i***
23	C ₁₆ H ₁₈ O ₉	Chlorogenic acid	5.472	354.0958	353.0886	2.13	98.2	a-i***
24	C ₈ H ₈ O ₄	Vanillic acid	6.211	168.0421	167.0348	-0.94	98.98	a-h**
25	C ₁₆ H ₁₄ O ₄	Alpinetin	6.820	270.0887	269.0814	-1.82	84.52	g, h, i *
26	C ₉ H ₈ O ₄	Caffeic acid	6.907	180.0423	179.035	0.45	99.77	a, b, d-i***
27	C ₁₀ H ₁₀ O ₄	Caffeic acid methyl ester	7.278	194.0571	193.0498	-4.29	96.61	a, b, d-i *
28	C ₉ H ₁₀ O ₅	Syringic acid	7.554	198.0532	197.0459	1.88	98.53	a-h**
29	C ₁₅ H ₁₈ O ₉	Caffeic acid-O-hexoside	10.230	342.0965	341.0894	4.16	88.78	a-h**
30	C ₃₀ H ₂₆ O ₁₂	Procyanidin B2	10.810	578.1437	577.1338	-2.54	93.31	a-i***
31	C ₁₅ H ₁₄ O ₆	Epicatechin	12.246	290.0796	289.0711	-2.35	97.06	a-i***
32	C ₈ H ₈ O ₃	Vanillin	12.406	152.0470	151.0398	-2.04	98.9	a-h**
33	C ₂₇ H ₂₂ O ₁₂	Lithospermic acid	12.611	538.1097	537.1024	-2.66	95.96	a-h*
34	C ₂₇ H ₃₀ O ₁₇	Quercetin 3-O-gentiobioside	12.618	626.1473	625.1407	-1.54	89.79	a-f*
35	C ₉ H ₈ O ₃	<i>p</i> -Coumaric acid	13.002	164.0479	163.0407	3.52	85.21	a, b, d-i***
36	C ₁₀ H ₁₀ O ₄	Ferulic acid	15.913	194.0581	193.0508	0.79	85.6	a, b, d-i***
37	C ₂₁ H ₂₀ O ₁₂	Quercetin 3-O-glucoside	16.570	464.0952	463.0883	0.75	84.92	a, b, d, e***
38	C ₂₁ H ₂₀ O ₁₁	Quercetin 3-O-rhamnoside	17.315	448.1011	447.0941	0.74	85.65	a-e***
39	C ₄₅ H ₃₈ O ₁₈	Procyanidin C1	18.251	866.2057	865.1971	-1.98	93.5	a-i***
40	C ₁₀ H ₁₀ O ₂	Methyl cinnamate	18.597	162.0674	161.0602	-4.19	84.16	a-h*
41	C ₁₈ H ₁₆ O ₇	Usnic acid	18.983	344.0886	343.0813	-3.04	96.63	a-h*
42	C ₈ H ₈ O ₂	4'-Hydroxyacetophenone	19.439	136.0520	135.0447	-3.16	98.67	a-h*
43	C ₉ H ₁₀ O ₄	Homovanillic acid	22.929	182.0577	181.0505	-0.94	87.22	a-i**
44	C ₁₅ H ₁₀ O ₅	Apigenin	23.880	270.0536	269.0462	2.83	80.85	a-g**
45	C ₂₇ H ₃₀ O ₁₆	Rutin	24.062	610.1523	609.1451	-1.79	92.91	a-g**
46	C ₂₇ H ₃₀ O ₁₅	Glucosyl-vitexin	24.531	594.1582	593.1512	-0.42	94.71	a-h*
47	C ₂₁ H ₂₀ O ₁₂	Hyperoside	24.973	464.0943	463.0873	-2.54	90.3	a-h**
48	C ₁₂ H ₁₄ O ₃	Ethyl- <i>p</i> -methoxycinnamate	25.415	206.0939	205.0866	-1.81	98.4	a-g*
49	C ₁₅ H ₁₀ O ₇	Quercetin	27.331	302.0414	301.0346	-2.71	96.91	a-h**
50	C ₁₅ H ₁₀ O ₆	Kaempferol	28.813	286.0480	285.0406	2.13	84.92	a-i**

* Compared with database; ** Compared with database and literatures; *** Compared with database, literatures, and authentic standards;

a-i stand for different kiwifruit cultivars, including 'Hongao', 'Hongshi', 'Hongyang', 'Cuiyu', 'Xuxiang', 'Miliang', 'Jinshi NO. 2', 'Jinshi NO. 3', and 'Xinzhong'.

Table 2
Contents of major phenolic compounds in polyphenol-enriched extracts from different kiwifruit cultivars.

Phenolic compounds	Different cultivars								
	Red-fleshed kiwifruits			Green-fleshed kiwifruits			Yellow-fleshed kiwifruits		
	'Hongao'	'Hongshi'	'Hongyang'	'Cuiyu'	'Xuxiang'	'Miliang'	'Jinshi No. 2'	'Jinshi No. 3'	'Xinzhong'
GA	0.628 ± 0.037 ^{def}	0.660 ± 0.016 ^{def}	0.688 ± 0.011 ^c	0.594 ± 0.009 ^{fg}	0.664 ± 0.013 ^{de}	0.858 ± 0.025 ^b	0.580 ± 0.017 ^g	1.059 ± 0.025 ^a	0.808 ± 0.042 ^b
PA	0.773 ± 0.037 ^e	1.257 ± 0.051 ^b	0.911 ± 0.029 ^d	N.D.	0.669 ± 0.011 ^f	1.046 ± 0.029 ^c	0.601 ± 0.018 ^f	1.532 ± 0.065 ^a	1.16 ± 0.037 ^b
NCHL	6.210 ± 0.286 ^a	5.830 ± 0.193 ^a	1.054 ± 0.048 ^f	2.826 ± 0.122 ^c	4.282 ± 0.203 ^b	4.772 ± 0.151 ^b	1.860 ± 0.088 ^d	1.390 ± 0.110 ^e	1.346 ± 0.075 ^e
PB1	3.007 ± 0.104 ^{bc}	2.317 ± 0.117 ^d	3.931 ± 0.278 ^a	1.242 ± 0.054 ^e	2.867 ± 0.028 ^e	2.867 ± 0.141 ^{bc}	N.D.	3.222 ± 0.135 ^b	2.641 ± 0.125 ^c
Ca	1.038 ± 0.027 ^e	1.169 ± 0.075 ^d	N.D.	1.708 ± 0.092 ^a	N.D.	1.161 ± 0.092 ^d	1.271 ± 0.073 ^c	0.547 ± 0.018 ^f	1.631 ± 0.101 ^b
CHL	0.205 ± 0.008 ^b	0.171 ± 0.002 ^c	0.083 ± 0.007 ^d	0.09 ± 0.003 ^d	N.D.	0.210 ± 0.009 ^b	0.084 ± 0.007 ^d	N.D.	0.553 ± 0.019 ^a
CA	0.086 ± 0.004 ^a	0.077 ± 0.006 ^a	N.D.	0.081 ± 0.004 ^a	0.083 ± 0.004 ^a	0.073 ± 0.004 ^a	N.D.	N.D.	N.D.
PB2	11.957 ± 0.528 ^b	7.702 ± 0.247 ^c	7.309 ± 0.346 ^{cd}	13.185 ± 0.315 ^a	6.863 ± 0.197 ^{cd}	6.779 ± 0.285 ^d	7.679 ± 0.295 ^c	2.554 ± 0.133 ^e	1.693 ± 0.057 ^f
EC	5.726 ± 0.284 ^b	2.740 ± 0.153 ^d	0.284 ± 0.062 ^g	8.508 ± 0.253 ^a	4.366 ± 0.114 ^c	1.690 ± 0.134 ^e	0.795 ± 0.033 ^f	0.560 ± 0.015 ^f	N.D.
p-CA	0.176 ± 0.008 ^a	0.165 ± 0.006 ^a	N.D.	0.182 ± 0.009 ^a	0.163 ± 0.007 ^a	0.155 ± 0.041 ^a	0.166 ± 0.027 ^a	N.D.	0.142 ± 0.011 ^a
FA	0.013 ± 0.002 ^a	0.023 ± 0.004 ^a	N.D.	0.019 ± 0.001 ^a	N.D.	0.020 ± 0.003 ^a	0.018 ± 0.002 ^a	N.D.	N.D.
QGlc	0.838 ± 0.062 ^a	0.811 ± 0.017 ^a	N.D.	0.822 ± 0.063 ^a	0.815 ± 0.026 ^a	N.D.	N.D.	N.D.	N.D.
QRha	0.544 ± 0.023 ^c	0.682 ± 0.024 ^{bc}	0.704 ± 0.014 ^{bc}	0.833 ± 0.021 ^b	1.249 ± 0.25 ^a	N.D.	N.D.	N.D.	N.D.
PC1	0.340 ± 0.027 ^a	0.332 ± 0.019 ^a	0.408 ± 0.042 ^a	N.D.	0.322 ± 0.019 ^a	0.368 ± 0.033 ^a	N.D.	0.323 ± 0.019 ^a	0.330 ± 0.021 ^a
Total content (mg/g DW)	31.548 ± 1.487 ^a	23.931 ± 1.089 ^b	15.373 ± 0.371 ^d	30.09 ± 1.232 ^a	20.709 ± 0.233 ^c	18.839 ± 0.828 ^c	13.055 ± 0.562 ^{de}	11.189 ± 0.617 ^f	10.304 ± 0.619 ^f
Average of total contents (mg/g DW)	23.617 ± 5.496 ^a			23.213 ± 4.585 ^a			11.516 ± 1.026 ^b		

GA, gallic acid; PA, protocatechuic acid; NCHL, neochlorogenic acid; PB1, procyanidin B1; Ca, catechin; CHL, chlorogenic acid; CA, caffeic acid; PB2, procyanidin B2; EC, epicatechin; FA, ferulic acid; p-CA, p-coumaric acid; QGlc, quercetin 3-O-glucoside; QRha, quercetin 3-O-rhamnoside; PC1, procyanidin C1; Different letters (a–g) in the same column indicate significant differences at $p < 0.05$ determined by ANOVA; N.D. means not detected or the concentration is too low to be quantitated.

of total phenolic compounds in red-fleshed kiwifruit cultivars was determined to be 23.617 ± 5.496 mg/g DW, similar to that of green-fleshed kiwifruit cultivars (23.213 ± 4.585 mg/g DW), while notably higher than that of yellow-fleshed kiwifruit cultivars (11.516 ± 1.026 mg/g DW). These findings further proved that the flesh colors of kiwifruits could also affect the level of individual phenolic components in TUK, similar to the phenomenon that found in mature kiwifruits with different fleshed colors (Liu et al., 2019). Moreover, the levels of individual phenolic components in different TUK were notably higher than those of different mature *A. deliciosa* and *A. chinensis* (Jiao et al., 2019; Li et al., 2018; Mai et al., 2022).

Six phenolic acids, including GA, NCHL, CHL, CA, p-CA, and FA, were quantified in TUK, and their contents varied greatly by different cultivars (Table 2). Obviously, the dominant phenolic acid in TUK was NCHL, ranging from 1.054 mg/g DW ('Hongyang') to 6.210 mg/g DW ('Hongao'). Earlier studies have shown that mature kiwifruits are also rich in NCHL and CHL (Gao et al., 2021; Li et al., 2018; Ma et al., 2017; Mai et al., 2022; Zhou et al., 2023). Besides, the levels of GA also varied dramatically, ranging from 0.580 mg/g DW ('Jinshi No. 2') to 1.059 mg/g DW ('Jinshi No. 3'). In fact, GA has also been found as the dominant phenolic acid in the pulp of different mature kiwifruits (Liang et al., 2021). The levels of CHL ranged from 0.083 mg/g DW to 0.553 mg/g DW, which were obviously lower than those of NCHL and GA. The highest level of CHL was observed in 'Xinzhong', but it was not detectable in 'Xuxiang' and 'Jinshi No. 3'. In addition, compared with the level of NCHL and GA, only minor CA, p-CA, and FA were found in TUK, ranging from 0.073 mg/g DW ('Miliang') to 0.086 mg/g DW ('Hongao'), from 0.142 mg/g DW ('Xinzhong') to 0.182 mg/g DW ('Cuiyu'), and

from 0.013 mg/g DW ('Hongao') to 0.023 mg/g DW ('Hongshi'), respectively. Previous studies have also revealed that the levels of CA, p-CA, and FA in different mature kiwifruits are extremely lower than that of GA (Liang et al., 2021; Ma et al., 2017).

Six flavanols, including three monomers (PA, Ca, and EC), two dimers (PB1 and PB2), and one trimer (PC1), were quantified. Notably, PB2 was observed as the predominant flavanol in TUK, ranging from 1.693 mg/g DW ('Xinzhong') to 13.185 mg/g DW ('Cuiyu'). In addition, TUK were also rich in PB1, ranging from 1.233 mg/g DW ('Xuxiang') to 3.931 mg/g DW ('Hongyang'), while the level only in 'Jinshi No. 2' was undetectable. Similarly, previous studies have also shown that both unripe and mature kiwifruits are rich in PB1 and PB2, which are also considered as major phenolic compounds (Li et al., 2018; Liu et al., 2019; Wu et al., 2023). In fact, compared with mature kiwifruits, TUK contained higher levels of PB1 and PB2. Compared with PB1 and PB2, only minor PC1 was found in different TUK, ranging from 0.322 mg/g DW to 0.408 mg/g DW, while the levels in 'Cuiyu' and 'Jinshi No. 2' were undetectable. Furthermore, the contents of PA, Ca, and EC varied greatly by different kiwifruit cultivars, similar to previous studies (Jiao et al., 2019; Liang et al., 2021; Liu et al., 2019). More specifically, the contents of EC varied drastically, with the highest content observed in 'Cuiyu' (8.508 mg/g DW) and the lowest content observed in 'Hongyang' (0.284 mg/g DW), while the content in 'Xinzhong' was undetectable. Besides, the contents of PA ranged from 0.601 mg/g DW ('Jinshi No. 2') to 1.532 mg/g DW ('Jinshi No. 3'), while the level in 'Cuiyu' was undetectable. In addition, the contents of Ca ranged from 0.547 mg/g DW ('Jinshi No. 3') to 1.708 mg/g DW ('Cuiyu'), while the levels in 'Hongyang' and 'Xuxiang' were undetectable. Nevertheless,

compared with different mature kiwifruits (Jiao et al., 2019; Li et al., 2018; Liang et al., 2021), the levels of EC, PA, and Ca in most TUK were obviously higher.

Two flavonols (QGlc and QRha) were quantified in this study. Both QGlc and QRha were undetectable in all yellow-fleshed unripe kiwifruits ('Jinshi No. 2', 'Jinshi No. 3', and 'Xinzhong') and one green-fleshed kiwifruit ('Miling'). Compared with NCHL and PB2, the contents of QGlc in 'Hongao', 'Hongshi', 'Cuiyu', and 'Xuxiang' were extremely lower, ranging from 0.811 mg/g DW to 0.838 mg/g DW, similar to a recent study (Wu et al., 2023). In addition, the contents of QRha in TUK (e.g., 'Hongao', 'Hongshi', 'Hongyang', 'Cuiyu', and 'Xuxiang') ranged from 0.544 mg/g DW to 1.249 mg/g DW, with the highest level observed in 'Xuxiang' (1.249 mg/g DW).

Furthermore, to illustrate the potential differences and similarities among different TUK in terms of their individual phenolic compounds, the hierarchical cluster analysis (HCA) was carried out. Fig. 2 showed the heatmap constructed from individual phenolic compounds in polyphenol-enriched extracts from different kiwifruit cultivars. According to the variable analysis results, two distinctive clusters (cluster 1 and cluster 2) could be identified by HCA. 'Hongao' and 'Cuiyu' were in cluster 1, which were characterized by extremely higher contents of PB2, EC, and NCHL. Cluster 2 was composed of 'Jinshi No. 2', 'Jinshi No. 3', 'Miliang', 'Hongshi', 'Xuxiang', 'Hongyang', and 'Xinzhong'. Indeed, cluster 2 could be further clustered into two sub-groups (group A and group B). Group A was composed of 'Jinshi No. 2' and 'Jinshi No. 3', which were characterized by lower contents of individual phenolic compounds. 'Miliang', 'Hongshi', 'Xuxiang', 'Hongyang', and 'Xinzhong' were in group B, which had relatively higher contents of PB2 and NCHL. These results indicated that the compositions and contents of individual phenolic compounds in different kiwifruit cultivars were different. Among these unripe kiwifruits, 'Hongao' and 'Cuiyu' are better for the development of functional foods or functional food ingredients owing to their higher contents of valuable polyphenols.

3.3. *In vitro* antioxidant capacities of polyphenol-enriched extracts from different kiwifruit cultivars

Many studies have proven that kiwifruits and their extracts possess excellent antioxidant capacities, which vary greatly among different

species, cultivars, parts, or maturity stages (Wang et al., 2021; Waswa et al., 2024). Nevertheless, the variations in different TUK are still unclear. Therefore, to select potential varieties suitable for further food processing, the antioxidant capacities of polyphenol-enriched extracts from different kiwifruit cultivars were systematically investigated and compared in the present study. As shown in Figs. 3A–3D, polyphenol-enriched extracts from different unripe kiwifruits exerted remarkable scavenging abilities against various free radicals and high levels of FRAP. Obviously, their antioxidant capacities varied greatly by different cultivars, which were similar to the trends that observed in their TPC, TFC, and TPAC, indicating that polyphenols were the potential contributors to their antioxidant capacities (Du et al., 2009; Jiao et al., 2019; Liu et al., 2019; Wu et al., 2023). The highest antioxidant capacities were observed in 'Hongao' among all different cultivars, while the weakest was found in 'Xinzhong'. In fact, the IC₅₀ values of polyphenol-enriched extracts from different kiwifruit cultivars against ABTS, OH, and DPPH radicals ranged from 0.211 mg/mL ('Hongao') to 0.586 mg/mL ('Xinzhong'), from 0.234 mg/mL ('Hongao') to 0.522 mg/mL ('Xinzhong'), and from 0.265 mg/mL ('Hongao') to 0.644 mg/mL ('Xinzhong'), respectively. Besides, the values of FRAP of polyphenol-enriched extracts from different kiwifruit cultivars ranged from 20.41 μmol Trolox/g DW ('Xinzhong') to 77.45 μmol Trolox/g DW ('Hongao'). Notably, TUK showed similar variation trends for various free radicals scavenging abilities and FRAP antioxidant capacities, with the highest antioxidant capacities observed in 'Hongao', followed by 'Cuiyu', 'Xuxiang', 'Hongshi', 'Hongyang' / 'Miliang' / 'Jinshi No. 2', and 'Jinshi No. 3', and the lowest levels observed in 'Xinzhong'. In addition, the antioxidant capacities of most red-fleshed and green-fleshed kiwifruit cultivars were stronger than those of yellow-fleshed kiwifruit cultivars.

It has been revealed that the antioxidant capacity of kiwifruit is mainly attributed to its polyphenols (Wang et al., 2021; Waswa et al., 2024). Therefore, the correlations among different polyphenols and antioxidant capacities were measured to unveil the major contributors to the antioxidant activity of TUK. As displayed in Fig. 4, the IC₅₀ values of various free radicals scavenging activities exerted significantly negative relevance to the contents of TPC (r, -0.977–0.828), TFC (r, -0.955–0.7), and TPAC (r, -0.976–0.8), respectively. Besides, the levels of FRAP showed significantly positive relevance to the contents of TPC (r, 0.991), TFC (r, 0.892), and TPAC (r, 0.945), respectively. These

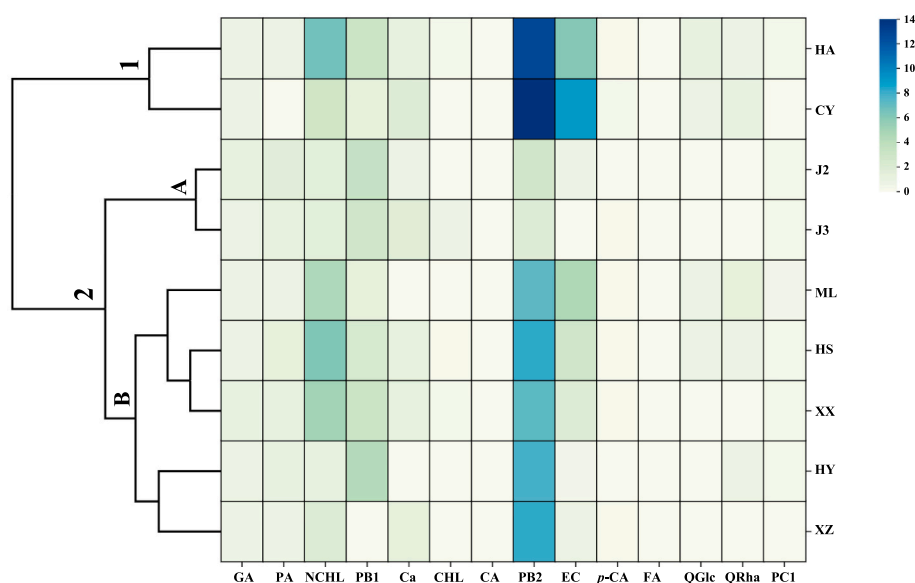


Fig. 2. Hierarchical cluster analysis of phenolic compounds in polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars. The sample codes were as the same as shown in Fig. 1.

A, gallic acid; PA, protocatechuic acid; NCHL, neochlorogenic acid; PB1, procyanidin B1; Ca, catechin; CHL, chlorogenic acid; CA, caffeic acid; PB2, procyanidin B2; EC, epicatechin; FA, ferulic acid; p-CA, p-coumaric acid; QGlc, quercetin 3-O-glucoside; QRha, quercetin 3-O-rhamnoside; PC1, procyanidin C1.

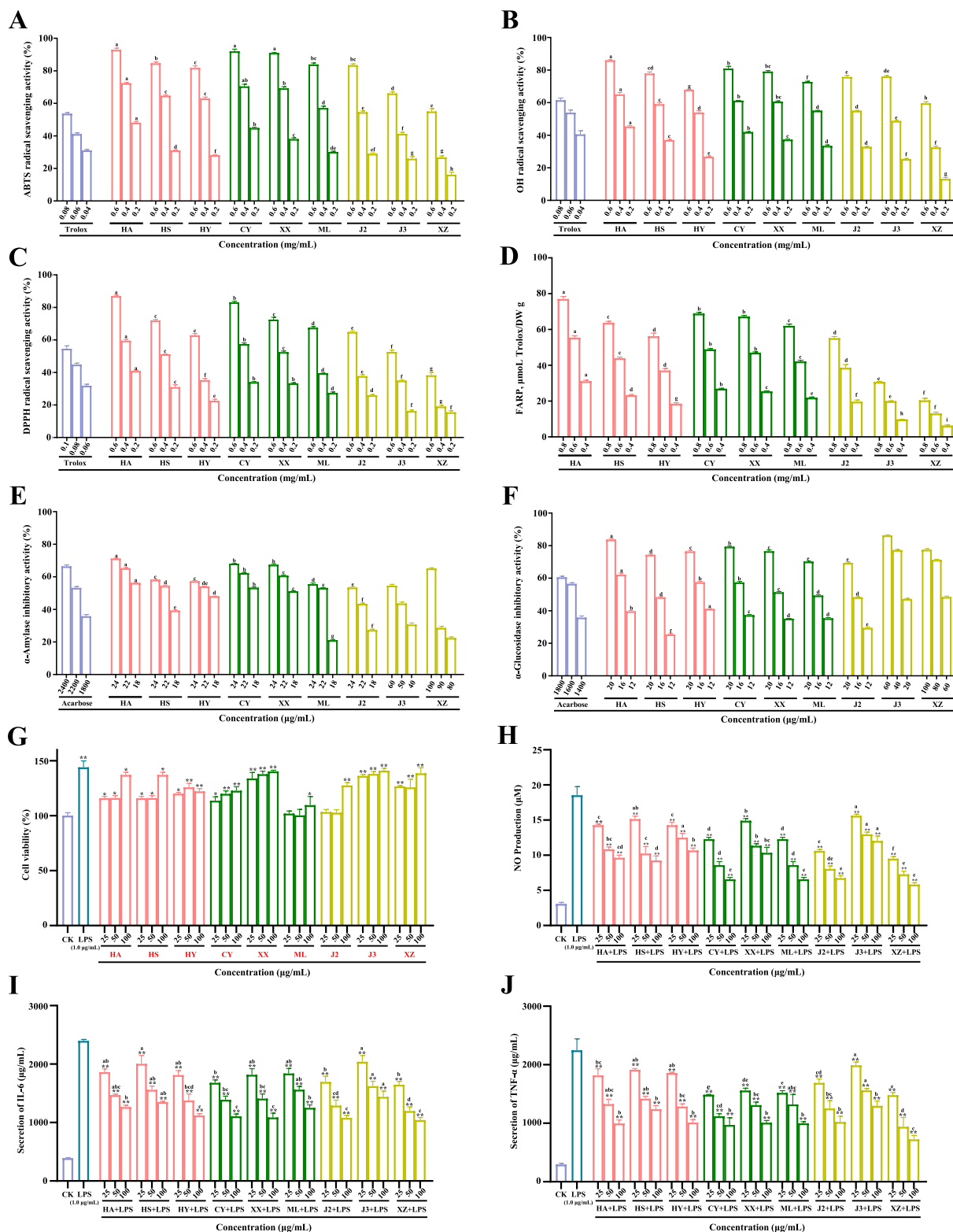


Fig. 3. Antioxidant capacities (A-D), anti-diabetic effects (E-F), and anti-inflammatory activities (G-J) of polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars.

A, ABTS radical scavenging ability; B, OH radical scavenging ability; C, DPPH radical scavenging ability; D, ferric-reducing antioxidant power; E, inhibitory effects on α -amylase; F, inhibitory effects on α -glucosidase; G, cell viability of RAW 264.7 cells; H, NO production from LPS-stimulated RAW 264.7 cells; I, secretion of IL-6 from LPS-stimulated RAW 264.7 cells; J, secretion of TNF- α from LPS-stimulated RAW 264.7 cells;

The sample codes were as the same as shown in Fig. 1;

Different letters (a-h) indicate statistically significant differences ($p < 0.05$) among different kiwifruit cultivars; Significant differences in cell viability of LPS and kiwifruit extracts vs. control are shown by * $p < 0.05$ and ** $p < 0.01$. Significant differences in NO production, secretion of IL-6, and secretion of TNF- α in kiwifruit extracts vs. LPS are shown by ** $p < 0.01$.



Fig. 4. Pearson correlation matrix among total polyphenols, individual phenolic compounds, *in vitro* antioxidant capacities, inhibitory effects on α -glucosidase and α -amylase, and *in vitro* anti-inflammatory activities.

GA, gallic acid; PA, protocatechuic acid; NCHL, neochlorogenic acid; PB1, procyanidin B1; Ca, catechin; CHL, chlorogenic acid; CA, caffeic acid; PB2, procyanidin B2; EC, epicatechin; FA, ferulic acid; p-CA, p-coumaric acid; QGlc, quercetin 3-O-glucoside; QRha, quercetin 3-O-rhamnoside; PC1, procyanidin C1; TPC, total phenolic content; TFC, total flavonoid content; TPAC, total procyanidin content; ABTS IC₅₀, IC₅₀ values of ABTS scavenging ability; DPPH IC₅₀, IC₅₀ values of DPPH scavenging ability; OH IC₅₀, IC₅₀ values of OH scavenging ability; FRAP, ferric-reducing antioxidant power; α -Glc IC₅₀, IC₅₀ values for the inhibition of α -glucosidase; α -Amy IC₅₀, IC₅₀ values for the inhibition of α -amylase; NO, NO production from LPS-stimulated RAW 264.7 cells; IL-6, IL-6 secretion from LPS-stimulated RAW 264.7 cells; TNF- α , TNF- α secretion from LPS-stimulated RAW 264.7 cells;

The positive and negative correlations are displayed in orange and green, respectively. Significant correlations are shown by * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

results confirmed that the polyphenols in different TUK were the major contributors to their antioxidant capacities, similar to previous studies (Du et al., 2009; Jiao et al., 2019; Liu et al., 2019; Wu et al., 2023; Zhang et al., 2020). Notably, PB2, EC, CA, NCHL, and QGlc showed notably negative relevance to the DPPH radical scavenging abilities, with the r values of -0.932 , -0.804 , -0.806 , -0.692 , and -0.750 , respectively. Additionally, PB2, EC, and CA also showed significantly negative relevance to the ABTS radical scavenging abilities, with the r values of

-0.877 , -0.701 , and -0.708 , respectively. Moreover, PB2, EC, CA, NCHL, and QGlc showed significantly positive relevance to the FRAP values, with the r values of 0.887 , 0.691 , 0.771 , 0.701 , and 0.682 , respectively. These results indicated that PB2 was one of the most important contributors to the antioxidant activities of different TUK, which was probably attributed to its highest content among all tested phenolic compounds in TUK. Collectively, the results revealed that TUK, especially 'Hongao', 'Cuiyu', 'Xuxiang', and 'Hongshi', had great

potentials to be utilized as natural antioxidants for the management of oxidative damages.

3.4. Inhibitory effects of polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars on α -glucosidase and α -amylase

Type 2 diabetes (T2D) is a chronic metabolic disorder characterized by abnormal glucose metabolism, and the inhibition of α -glucosidase and α -amylase is one of the major approaches to ameliorate metabolic alterations related to T2D (Chen et al., 2022; Rutkowska & Olszewska, 2023). Accumulating evidence has verified that the dietary intake of polyphenols from fruits and vegetables can reduce the risk of T2D (Chen et al., 2022; Rutkowska et al., 2023). Actually, previous studies have shown that different species of kiwifruits possess excellent inhibitory effects against α -glucosidase and α -amylase (Li et al., 2018; Wojdyło et al., 2017; Wu et al., 2023). Nevertheless, the inhibitory effects of polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars on α -glucosidase and α -amylase are still unclear. As shown in Fig. 3E and F, TUK exhibited excellent inhibitory effects against both α -glucosidase and α -amylase when compared with the positive control. Obviously, their inhibitory effects against α -glucosidase and α -amylase also varied significantly by different cultivars. In detail, the IC_{50} values of polyphenol-enriched extracts from different unripe kiwifruits against α -amylase and α -glucosidase ranged from 16.15 μ g/mL to 95.40 μ g/mL and from 13.22 μ g/mL to 60.42 μ g/mL, respectively. The strongest inhibitor effect against α -amylase was observed in 'Hongao', followed by 'Cuiyu', 'Xuxiang', 'Hongyang', 'Hongshi', 'Miliang', 'Jinshi No. 2', and 'Jinshi No. 3', and the weakest level was observed in 'Xinzhong', similar to the variation trend of their TPC (Fig. 1A). Besides, the strongest inhibitory effect against α -glucosidase was also found in 'Hongao', and the weakest level was observed in 'Xinzhong'. Furthermore, it could be clearly found that polyphenol-enriched extracts from red-fleshed and green-fleshed cultivars inhibited α -amylase and α -glucosidase more effectively than those of yellow-fleshed cultivars.

Moreover, as displayed in Fig. 4, both TPC and TPAC showed significantly negative relevance to the IC_{50} values of α -amylase and α -glucosidase inhibitory effects, with the r values of -0.899 and -0.758 (TPC), and -0.771 and -0.675 (TPAC), respectively, similar to previous studies that phenolic acids and polymeric procyanidins in kiwifruits are major contributors to their inhibitory effects against digestive enzymes (Li et al., 2018; Wojdyło et al., 2017; Wu et al., 2023). Additionally, PB2 exhibited closely negative relevance to the IC_{50} values of α -amylase and α -glucosidase inhibitory effects, with the r values of -0.774 and -0.651 , respectively, suggesting that PB2 was one of the most important phenolic compounds in TUK in terms of their inhibitory effects against digestive enzymes. In fact, several studies have revealed that PB2 is a strong inhibitor towards α -amylase and α -glucosidase (Dai et al., 2018; Fernandes et al., 2020; Han et al., 2018; Lai et al., 2024; Siegień et al., 2021). Collectively, these results provided clear evidence that TUK, especially 'Hongao', 'Cuiyu', and 'Xuxiang', had great potentials to be applied as functional ingredients for management of T2D.

3.5. 3.4 *In vitro* anti-inflammatory effects of polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars

A growing body of evidence has revealed that kiwifruits and their extracts possess *in vitro* and *in vivo* anti-inflammatory effects (Pinto et al., 2020; Wang et al., 2021; Waswa et al., 2024). Therefore, *in vitro* anti-inflammatory effects of polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars were assessed in the present study. Figs. 3G-3J showed the *in vitro* anti-inflammatory effects of polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars on LPS-stimulated RAW 264.7 macrophages. As displayed in Fig. 3G, all polyphenol-enriched extracts from unripe fruits of different kiwifruit cultivars exerted no cytotoxicity effects on RAW 264.7 cells. Besides, as displayed in Figs. 3H-3J, the polyphenol-enriched extracts from

different unripe kiwifruits could notably reduce proinflammatory factors (NO, IL-6, and TNF- α) in LPS-stimulated RAW 264.7 cells, thereby exerting potential *in vitro* anti-inflammatory effects, similar to previous studies that kiwifruits and their extracts possess notable anti-inflammatory effects (Ahn et al., 2020; Deng et al., 2016; Goya-Jorge et al., 2023; Lian et al., 2019). Obviously, their *in vitro* anti-inflammatory effects also varied significantly by different kiwifruit cultivars. In detail, at the concentration of 100.00 μ g/mL, the inhibitory rates of different TUK on the production of NO were in the range of 32.99–69.30%. 'Cuiyu', 'Miliang', 'Jinshi No. 2', and 'Xinzhong' showed the greatest inhibitory effects on the NO production from LPS-stimulated RAW 264.7 cells, followed by 'Hongao', 'Honghi', 'Xuxiang', and 'Hongyang', and the weakest activity was observed in 'Jinshi No. 3'. In addition, the inhibitory rates of different unripe kiwifruits on the production of IL-6 ranged from 40.01% to 56.58%, with the highest levels observed in 'Xingzhong', 'Jinshi No. 2', 'Xuxiang', 'Cuiyu', and 'Hongyang', and the lowest levels observed in 'Jinshi No. 3' and 'Hongshi'. Furthermore, the inhibitory rates of different TUK on the production of TNF- α ranged from 42.36% to 67.81%, with the greatest level measured in 'Xinzhong' and the lowest level observed in 'Jinshi No. 3'. According to the correlation analysis, only Ca, CHL, p -CA, and FA in TUK showed close correlations with their *in vitro* anti-inflammatory effects (inhibitory effects on NO production), with the r values of 0.805, 0.603, 0.657, and 0.475, respectively, suggesting that *in vitro* anti-inflammatory effects of TUK were also contributed by other compounds, e.g., quinic acid conjugated phenolic compounds (Ahn et al., 2020). Collectively, the above findings revealed that TUK, especially 'Xinzhong', 'Jinshi No. 2', and 'Cuiyu', could be utilized as functional ingredients for the management of chronic inflammatory diseases.

4. Conclusions

In the present study, our findings showed that TUK were extremely rich in bioactive polyphenols, which varied greatly among different kiwifruit cultivars. Indeed, EC, Ca, PA, PB1, PB2, NCHL, and GA were measured as the major phenolic compounds in most TUK, with the highest levels observed in 'Hongao' and 'Cuiyu' cultivars. Furthermore, both 'Hongao' and 'Cuiyu' cultivars exhibited stronger *in vitro* antioxidant effects and inhibition potentials on digestive enzymes than those of others, which were mainly attributed to their higher contents of polyphenols, especially procyanidin B2. In addition, the higher *in vitro* anti-inflammatory effects were observed in 'Xinzhong', 'Jinshi No. 2', and 'Cuiyu' compared with others. Collectively, our findings demonstrate that TUK, especially 'Hongao', 'Cuiyu', and 'Xuxiang', possess great potentials to be developed as natural antioxidants or functional ingredients for the management and prevention of T2D. Besides, 'Xinzhong', 'Jinshi No. 2', and 'Cuiyu' could be also exploited as healthy products for the prevention of chronic inflammatory diseases.

CRedit authorship contribution statement

Wen Deng: Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Qian-Ni Yang:** Validation, Investigation, Formal analysis. **Hong-Yan Liu:** Writing – review & editing, Supervision, Resources, Funding acquisition, Formal analysis. **Yu Xia:** Validation, Investigation. **Huiling Yan:** Writing – review & editing, Funding acquisition, Formal analysis. **Jing-Wei Huang:** Writing – review & editing, Formal analysis. **Yi-Chen Hu:** Writing – review & editing, Formal analysis. **Liang Zou:** Writing – review & editing, Formal analysis. **Ren-You Gan:** Writing – review & editing. **Ding-Tao Wu:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2024.101815>.

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