Current Research in Food Science 7 (2023) 100610

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Response surface methodology-optimized extraction of flavonoids from pomelo peels and isolation of naringin with antioxidant activities by Sephadex LH20 gel chromatography

Yuchen Shangguan [a,b,1], Jing Ni [c,1], Lili Jiang [d], Yang Hu [a], Chuanbo He [a], Ying Ma [c], Guohong Wu [a,**], Hejian Xiong [a,*]

[a] College of Ocean Food and Biological Engineering, Fujian Provincial Engineering Technology Research Center of Marine Functional Food, Jimei University, Xiamen, 361021, China
[b] Jiangle County Agricultural Products Quality and Safety Inspection Station, Sanming, 353300, China
[c] Fisheries College of Jimei University, Xiamen, 361021, China
[d] Xiamen Municipal Southern Ocean Testing Co., L, Xiamen, 361021, China

ARTICLE INFO

Handling Editor: Professor Aiqian Ye

Keywords:
Flavonoids
Pomelo peels
Sephadex LH-20
Response surface methodology
Debittering

ABSTRACT

In this study, flavonoids were extracted from pomelo peels and naringin was isolated from the flavonoid extract. The effects of extraction parameters, namely, ethanol concentration, solid-to-liquid ratio, and extraction time, on the yield of flavonoids extracted from pomelo peels were analyzed according to the Box-Behnken design of response surface methodology. The experimental conditions for flavonoid extraction were optimized, and naringin was separated from the extracted flavonoids using Sephadex LH-20 column chromatography. Experimental results showed that the influence of factors on the extraction rate of flavonoids from pomelo peels was in the order of ethanol concentration > solid-to-liquid ratio > extraction time, and the optimal extraction parameters were 85% ethanol concentration, 1:20 solid-to-liquid ratio, and 4-h extraction time for extracting flavonoids from pomelo peels. Under these conditions, the yield of flavonoids was 6.07 ± 0.06 mg/g. After three times of extraction, the flavonoid extraction rate reached 96.55%, and the residual naringin in the pomelo peels was 0.017 mg/g, at which point the bitterness in the pomelo peels disappeared. Two components, namely, PF1 and PF2, were separated from the crude flavonoid of pomelo peels through Sephadex LH20 column chromatography. PF2 was identified as naringin by high-performance liquid chromatography tandem mass spectrometry, with a purity of 95.7 ± 0.23%. Both flavonoids and PF2 exhibited good in vitro radicals scavenging activities on DPPH, ABTS, superoxide anion and hydroxyl.

1. Introduction

Pomelo, a fruit belonging to the genus *Citrus* and the family Rutaceae, is widely cultivated and distributed in southern China (Reshmi et al., 2017). The global annual production of pomelo is approximately 9.34 million tons, of which 53.9% is produced in China (Yin et al., 2023), with Fujian Province alone having a production of more than 1.3 million tons (Zhang et al., 2021). As pomelo is the most often consumed fruit, a considerably large number of pomelo peels are generated every year, which are often carelessly discarded, resulting in wastage of naturally available resources and environmental pollution (Zhu et al., 2017). In fact, pomelo peels can be used to develop many functional products (Sang et al., 2022). Pomelo peels has a long edible history in the Fujian and Zhejiang provinces of China. According to the traditional Chinese medicine classic "Compendium of Materia Medica," pomelo peels has many beneficial effects, that is, it can soothe the diaphragm and aid in anger management. It can also be used for heat clearance, elimination of fire, alleviating phlegm, anti-inflammation, and detoxification. Pomelo peels contains a variety of important nutrients and compounds including pectin, naringin, limonin, and essential oil (Liu et al., 2017). Processing value-added products from pomelo peels have been a research focus in recent years.

* Corresponding author.
** Corresponding author.
E-mail addresses: storm_wgh@sina.com (G. Wu), hjxiong@jmu.edu.cn (H. Xiong).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.crfs.2023.100610
Received 27 June 2023; Received in revised form 31 August 2023; Accepted 2 October 2023
Available online 5 October 2023

Pomelo fruit is divided into flavedo (colored peripheral surface), albedo (white, soft center layer), pulp, and seed. The albedo accounts for approximately 30% of the fresh weight of pomelo fruit and is considered a potential source of dietary fiber and pectin (Wang et al., 2020). Although pomelo peels have abundance of flavonoids, naringin, the primary component of flavonoids, imparts a distinct bitter flavor to the pomelo peels (Kumar et al., 2021), which greatly limits the use of pomelo peel as a dietary ingredient (Sharma et al., 2022). However, studies have shown that flavonoids present in pomelo peels exhibit a variety of biological activities including reduction of blood lipids, anti-oxidation, and inhibition of digestive enzymes (Yun et al., 2020; Liu et al., 2021; Deng et al., 2022).

In recent years, much attention has been paid to the development of flavonoids from pomelo, and these studies included the extraction of flavonoids from most of the well-known varieties in China, such as Majiayu, Liangpingyu, Shatianyu, Guanxiyu (Yin et al., 2023). However, most of the studies were carried out with whole fruits or peels for extraction, rather than exclusively with albedo of the peel, and the processing methods, such as heating, fermentation, and enzymatic hydrolysis, destroyed the fiber structure of the pomelo peel and limited the further development of pomelo peel fiber (Tian et al., 2019; Deng et al., 2022). The yellow skin layer contains oil and pigment-like compounds, which will not only affect the extraction conditions of flavonoids, but also affect the quality of dietary fiber in the subsequent development of pomelo peels. In the present study, ethanol solvent was used to extract flavonoids from albedo of pomelo peels, which avoided the above disadvantages. The ratio of solid to liquid, temperature, ethanol concentration and extraction time are key parameters affecting the extract rate. Generally speaking, a higher liquid-solid ration and temperature, and multiple extractions will be beneficial for improving the extraction rate of flavonoids, but a higher temperature will lead to a decline in the activity of polyphenol extracts, and a higher liquid-solid ration and multiple extractions will lead to an increase of organic solvents, which is not conducive to the removal of organic solvents in the later stage (Song et al., 2019). Therefore, it is necessary to optimize the parameters for extracting flavonoids using response surface methodology (RSM).

In this study, flavonoids were extracted from discarded pomelo peels, and naringin was effectively isolated from the crude flavonoids through Sephadex LH20 gel chromatography. This approach not only utilized the flavonoids in pomelo peels but also eliminated the debittering effect caused by flavonoids in the peels, which makes it conducive for further development of the dietary fiber in pomelo peels and the comprehensive utilization of pomelo peels. The objectives of the study were as follows: (i) to optimize extraction conditions of flavonoids from pomelo peels, (ii) to isolate naringin from the crude extract of flavonoids, and (iii) to eliminate bitterness from pomelo peels so that it can be used as a dietary ingredient.

2. Materials and methods

2.1. Materials and chemicals

Guanxi pomelo peels were gathered from Pinghe County, Fujian Province, China. After removing flavedo, the sponge layer of the fruit was dried at 60 °C for 12 h, after which it was crushed, passed through an 80-mesh sieve, and stored in a desiccator.

Sephadex® LH-20 was purchased from General Electric Company (Sweden). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and rutin standard (purity greater than 99%) were bought from Aladdin Industrial Company (China). Acetonitrile, methanol, and formic acid were of high-performance liquid chromatography (HPLC) grade and purchased from Sigma Chemical Company (USA).

2.2. Extraction of flavonoids from pomelo peels

The fine powder of the pomelo sponge layer was extracted with a certain volume of ethanol solution at a certain solid-to-liquid ratio and for a certain time. The extracts were then centrifuged at 4500×*g* for 30 min, and the supernatant was made up to a final volume of 100 mL with ethanol solution for determining the flavonoid concentration as described in section 2.3. Based on single-factor experiment results, the extraction process of flavonoids from pomelo peel was optimized using Box-Behnken central experimental design of the response surface methodology (RSM), with three factors and three levels. The factors and levels of RSM are listed in Table 1.

2.3. Determination of flavonoid content

Flavonoid concentration was determined according to the method described by Hamed et al. (2019). The sample solution (1 mL) was taken in a 10-mL test tube with a glass stopper and adjusted to 2 mL with 1 mL of 60% ethanol. Then 0.5 mL of 5% $NaNO_2$ was added to the tube, and the contents were allowed to stand for 6 min, and 0.5 mL of 10% $AlCl_3$ (w/w) was added and mixed. Six minutes later, 4 mL of 4% NaOH was added to the mixture solution. After 15 min, the absorbance of the sample solution was read at 510 nm using an ultraviolet spectrophotometer (UV-3200, MAPADA, China). Total flavonoid content was determined by constructing rutin standard curves.

2.4. Components separation of pomelo peel flavonoids by sephadex LH-20

The extraction liquid derived from experiments described in section 2.2 was concentrated and freeze-dried, and the crude extract of flavonoids from pomelo peel was obtained, and the components were separated through Sephadex LH-20. Approximately 40 g of Sephadex LH-20 gel was weighed and completely saturated with 30% ethanol, after which it was transferred carefully to the column (16 mm × 60 cm). Subsequently, the column was eluted with 30% ethanol for at least 3 bed-volumes. Then, 5 mL of 85% ethanol containing 0.5 g of the crude flavonoid extract (solid-to-liquid ratio of 1:10) was loaded onto the column. The column was then eluted with 30% ethanol solution at a flow rate of 0.5 mL/min, and the eluates were collected using an automatic fraction collector (3.5 mL/tube).

2.5. Analysis of flavonoids by HPLC and HPLC-MS/MS

The crude and separated components of flavonoid extract samples were analyzed using the Agilent 1260 HPLC (Agilent Technologies, USA) and the Agilent ZORBAX SB-C18 column according to the method described by Yang et al. (2016). Ultrapure water and acetonitrile were used as mobile phases A and B, respectively. Sample solutions were filtered using a 0.22-μm-diameter filter, and 20 μL of the filtered solution was injected to the volume. The flow rate was set at 0.4 mL/min, and the gradient was set as follows: 0 min, 5% mobile phase B; 5 min, 25% mobile phase B; 10 min, 45% mobile phase B; 35 min, 50% mobile phase B.

For HPLC-tandem mass spectrometry (HPLC-MS/MS) analysis, the processing conditions were as follows: Mobile phase A was 0.3% formic acid in water (v/v), and mobile phase B was acetonitrile. The compounds were separated by gradient elution, which was programmed as follows: 10–100% mobile phase B (1–8 min), 100% mobile phase B

Table 1
Experimental design of response surface methodology with factors and their levels.

Independent factors	Units	Levels		
		Low (−1)	Medium (0)	High (1)
*X*1: Ethanol concentration	%	70	80	90
*X*2: Extraction time	h	2	4	6
*X*3: Solid-to-liquid ratio	g/v	1:15	1:18	1:21

(8–11 min), and 100–10% mobile phase B (11–11.1 min). The total flow rate was set at 0.3 mL/min, the oven temperature was set at 30 °C, and the injection volume was 10 μL. The MS conditions were set as positive sweeping mode: scan range 50–700 m/z, nitrogen gas (N_2) at 35 psi, ion source temperature of 600 °C, and source voltage of 3.5 KV (positive).

2.6. Analysis of antioxidant activity

The free radicals scavenging activities of DPPH and ABTS were measured according to the methods reported previously (Liu et al., 2023; Wang et al., 2022). The scavenging activities of superoxide anion ($O_2^{\cdot-}$) and hydroxyl radicals (·OH) were assessed using commercial diagnostic kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's protocol.

2.7. Bitterness evaluation

Fine powder (5 g) of the pomelo peel sponge layer was carefully weighed and extracted one to five times according to the method described in section 2.2. The residues after each extraction were dried at 60 °C for 12 h, and their bitterness was scored. Before scoring, 10 tasters (five men and five women) initially tasted the raw pomelo peel powder without extraction to achieve a more uniform sample sensation, which was scored as 5.0, and the residues after the extraction were scored. The bitterness was graded on a scale of 0–5 (0, no bitterness; 1, very weak; 2, weak; 3, moderate; 4, strong; 5, extremely strong) (Qiu et al., 2023). The scores were accurate to one decimal point.

2.8. Statistical analysis

All experiments were repeated three times, and data are presented as mean ± standard deviation (SD). Analysis of variance accompanied with Duncan test was performed to identify statistically significant differences between samples ($P < 0.05$). Data analysis was performed using SPSS 26 (Chicago, USA).

3. Results

3.1. Effects of extraction conditions on the extraction efficiency of flavonoids from pomelo peels

The effects of extraction parameters, namely, ethanol concentration, extraction time, and solid-to-liquid ratio on the extraction efficiency of flavonoids from pomelo peels are shown in Fig. 1. With an increase in the ethanol concentration, the yield of the extracted flavonoids increased significantly ($P < 0.05$). When the ethanol concentration reached 80%, the yield was 5.56 ± 0.05 mg/g, but when the ethanol concentration increased to 90%, the yield of flavonoids showed no significant change ($P > 0.05$) (Fig. 1A). The dissolution of flavonoids was affected by the amount of solvent used, and a low solid-to-liquid ratio prevented flavonoid dissolution from the pomelo peel (Xie et al., 2015). As shown in Fig. 1B, with an increase in the solid-to-liquid ratio, the yield of the extracted flavonoids from pomelo peel increased significantly ($P < 0.05$). When the solid-to-liquid ratio reached 1:21, the yield of the extracted flavonoids was the highest (5.810.03 mg/g). However, when the solid-to-liquid ratio continued to increase to 1:24, the yield of flavonoids no longer showed a significant increase. As shown in Fig. 1C, as the extraction time increased, the flavonoid yield initially increased and then decreased. When the extraction time was 4 h, the flavonoid yield was the highest (5.96 ± 0.19 mg/g). The reason for the decrease in the yield of flavonoids as the extraction time increased may be that some flavonoids were decomposed and destroyed as the extraction time prolonged (Cai et al., 2008).

3.2. Response surface optimization of the extraction conditions of flavonoids from pomelo peels

3.2.1. Response surface experiment results and analysis of variance

The experimental results of RSM are shown in Table 2. The quadratic polynomial regression equation for predicting the flavonoid extraction rate was obtained using Design Expert 8.0 software: $Y = 5.93 + 0.23 \times {}_1 + 0.030 \times {}_2 + 0.13 \times {}_3 + 0.023 \times 1 \times {}_2 + 0.10 \times 1 \times {}_3 + 0.007500 \times 2 \times {}_3 - 0.26 \times {}_1^2 - 0.29 \times {}_2^2 - 0.18 \times {}_3$. The regression model coefficient $R^2 = 0.9968$, R^2 adj $= 0.9911$, and coefficient of variation $= 0.50\%$ indicated that the regression model fitted well with the actual experimental results.

The results of analysis of variance are shown in Table 3. The P value

Table 2
Box-Behnken experimental design and results of the response surface methodology.

No.	Factors and levels			Y1 (mg/g)
	*X*1	*X*2	*X*3	
1	−1	−1	0	5.15
2	−1	1	0	5.18
3	−1	0	−1	5.20
4	−1	0	1	5.27
5	0	−1	−1	5.31
6	0	1	−1	5.34
7	0	−1	1	5.55
8	0	1	1	5.61
9	0	0	0	5.92
10	0	0	0	5.94
11	0	0	0	5.92
12	1	−1	0	5.52
13	1	1	0	5.64
14	1	0	−1	5.48
15	1	0	1	5.97

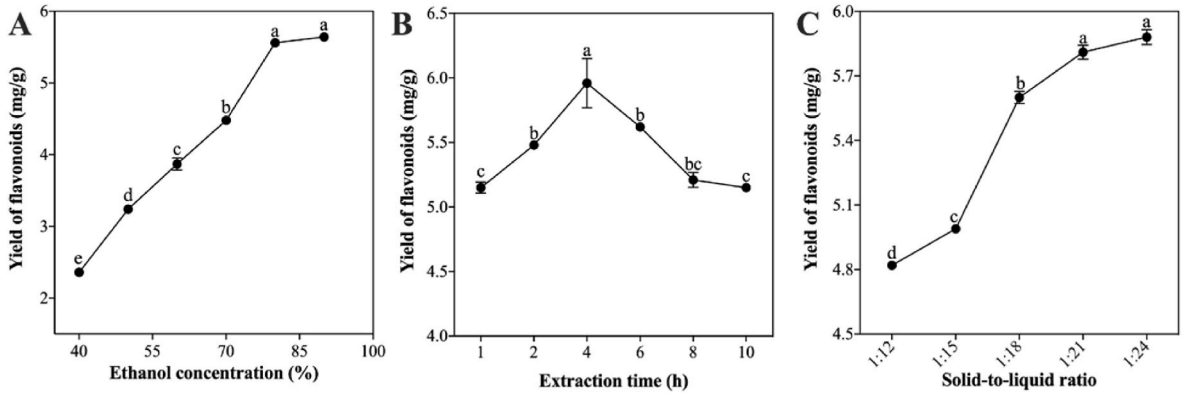

Fig. 1. Effects of ethanol concentration (A), extraction time (B), and solid-to-liquid ratio (C) on the yield of flavonoids from pomelo peels.

Table 3
ANOVA of experimental results of response surface methodology.

Source	Df	Sum of squares	Mean square	*F* value	*P* value (significant)
Model	9	1.22	0.13	175.17	<0.0001**
*X*1	1	0.41	0.41	532.99	<0.0001**
*X*2	1	0.0072	0.0072	9.37	0.0281*
*X*3	1	0.14	0.14	182.26	<0.0001**
X1X2	1	0.002025	0.002025	2.64	0.1654
X1X3	1	0.044	0.044	57.4	0.0006**
X2X3	1	0.000225	0.000225	0.29	0.6116
$X1^2$	1	0.26	0.26	333.24	<0.0001**
$X2^2$	1	0.31	0.31	406.48	<0.0001**
$X3^2$	1	0.12	0.12	161.52	<0.0001**
Lack of fit	3	0.003575	0.001192	8.94	0.1023
Pure error	2	0.0002667	0.0001333		
Cor total	14	1.22			

Notes: * and ** refer to differences that were significant at $P < 0.05$ and $P < 0.01$, respectively.
ANOVA: analysis of variance.

of the model was less than 0.0001, and the mismatch term P = 0.1023 > 0.05. These results indicated that the model was very significant and could be used for predicting the flavonoid extraction rate under different extraction conditions. The linear terms *X*1 (ethanol concentration) and *X*3 (solid-liquid ratio) had significant effects ($P < 0.01$) on the yields of the extracted flavonoids, and those *X*2 (extraction time) showed a significant effect ($P < 0.05$; Table 3). The larger the *F*-value was, the greater the factors had an influence on the extraction rate of flavonoids. According to the *F*-values of the linear terms, the influence of factors on the extraction rate of flavonoids from pomelo peels was in the order of ethanol concentration > solid-to-liquid ratio > extraction time.

3.2.2. Analysis of interactions between factors

Fig. 2 shows the response surfaces generated by the proposed models. The three-dimensional (3D) response surface plots A, B, and C demonstrated interactions between two independent variables, and the two-dimensional (2D) plots a, b, and c are contour plots corresponding to A, B, and C. A steeper slope in the 3D plot indicated that the extraction rate of flavonoids was more affected by the change in the independent variable. Conversely, a gentler slope indicated that the extraction rate was less affected by the change in the independent variable. When the 2D contour map was nearly a circular structure, the interaction between two independent variables had less influence, whereas when the contour map was nearly elliptical, the interaction had more influence. As shown in Fig. 2A and a, the 2D contour plot of the interaction between ethanol concentration and extraction time was elliptical, whereas the 3D plot was relatively flat, indicating that the interaction between the two factors had an insignificant effect on the flavonoid extraction rate. As shown in Fig. 2B and b, the 3D plot was steep, and the 2D plot was elliptical and densely packed, and as the ethanol concentration and solid-to-liquid ratio increased, the yield of the flavonoid content also increased. When the yield reached its peak, the increase slowed down, indicating that the interaction between the two independent variables had a significant effect on the flavonoid extraction rate. As shown in Fig. 2C and c, the 3D plot was flat and the 2D contour line was circular, indicating that the interaction between two factors (extraction time and solid-to-liquid ratio) had no significant effect on the flavonoid extraction

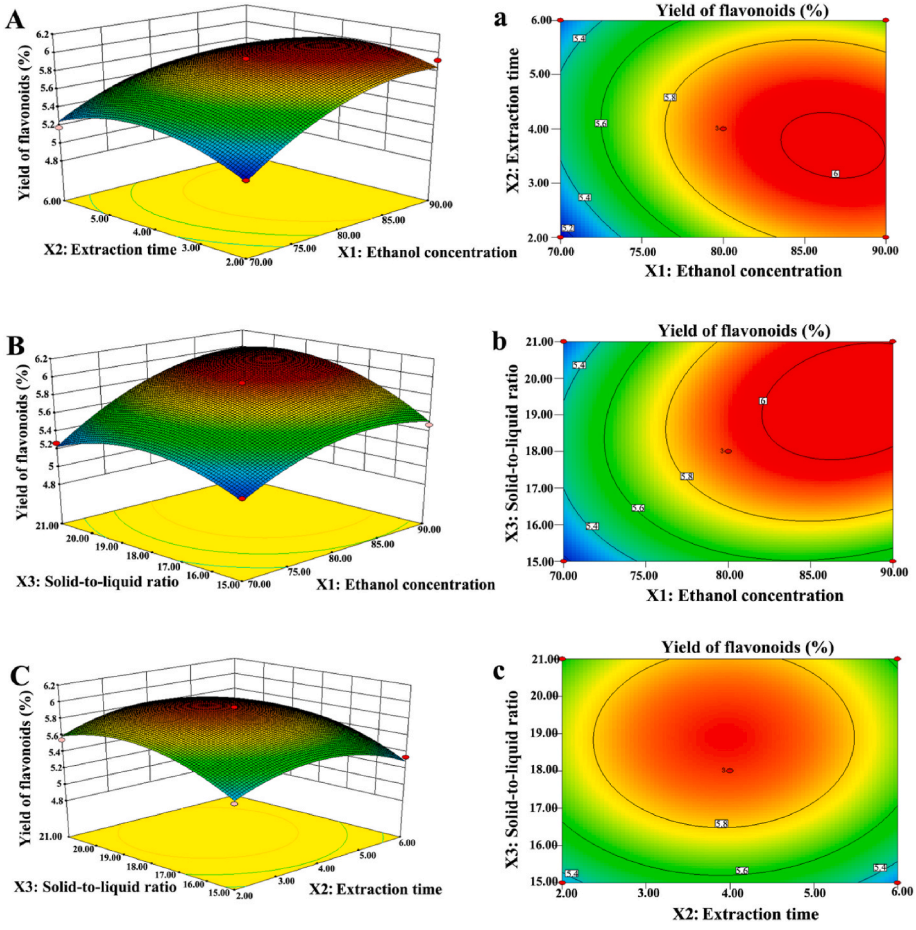

Fig. 2. Three-dimensional (A–C) and two-dimensional (a–c) response surface plots showing the effects of interactions between independent factors, namely, ethanol concentration, extraction time, and solid-to-liquid ratio, on the yield of flavonoids from pomelo peels.

rate. The results derived from the interaction analysis were consistent with those of ANOVA presented in Table 3.

3.2.3. Model validation

The results of RSM were processed using Design-Expert software, and the optimal process parameters for extracting flavonoids from pomelo peel were as follows: ethanol concentration of 85.37%, extraction time of 4.16 h, solid-to-liquid ratio of 1:19.56 (g:mL), and predicted flavonoid yield of 6.02 mg/g.

To facilitate the actual operation, the extraction parameters were adjusted to an ethanol concentration of 85%, extraction time of 4 h, and a solid-to-liquid ratio of 1:20 (g:mL), and the flavonoid yield under the adjusted conditions was 6.07 ± 0.06 mg/g, showing no significant difference with the predicted value (6.02 mg/g; $P > 0.05$), indicating that the model was stable and reliable.

3.2.4. Effects of multiple extraction on the flavonoid extraction rate and bitterness evaluation

Multiple extractions of flavonoids from pomelo peel raw materials were performed after adjusting the extraction parameters as mentioned in the previous section. The total extraction rate with five extractions was set as 100%, and the extraction rate for each extraction was calculated by accumulating the amount of each extraction (Fig. 3). The extraction rate of flavonoids from pomelo peel was 84.68% after the first extraction, and as the number of extractions increased, the yield of flavonoids increased significantly ($P < 0.05$). After three extractions, the total extraction rate reached 96.55%. The influence of number of extractions on the bitterness evaluation score is presented in Table 4. After three extractions, the bitterness of pomelo peel disappeared, the bitterness score was 0, and the content of naringin measured in pomelo peel was 0.017 mg/g.

3.3. Components separation of flavonoids extracted from pomelo peel

The crude extract of flavonoids from pomelo peel was obtained using ethanol. Purified flavonoids (PF1) and 2 (PF2) were obtained by separating the components from the crude extract of flavonoids through Sephadex LH20 column chromatography. The elution curve with 30% ethanol as the mobile phase is shown in Fig. 4. The figure shows two elution peaks with a large spacing, indicating that the separation effect was apparent.

Table 4
Effect of number of extraction times on bitterness scores of pomelo peels.

Extraction times	0	1	2	3	4	5
Sensory evaluation score	5	2.5	1.2	0	0	0

3.4. Component analysis of flavonoids in pomelo peel

Naringin is the main component among flavonoids in pomelo peel (Zhang et al., 2011; Yun et al., 2020). Fig. 5 shows the HPLC plots of crude extract of flavonoids, PF1, PF2, and naringin standard samples. PF1 had more peaks and complex components, which were primarily non-naringin components in the crude extract of flavonoids. PF2 contained a major peak with a retention time of 20.49 min, consistent with the peak time of the naringin standard sample; thus, PF2 was presumed to be naringin. The purity of naringin in PF2 was 95.78 ± 0.23% determined according to the external standard method.

HPLC-MS/MS analysis results of PF2 are shown in Fig. 6. All flavonoids identified showed strong signals corresponding to the [M+Na] or [M+H]. Briefly, in the positive ion mode of ionization, naringin (molecular weight [MW] = 580.53) yielded protonated adducts at m/z 581.2 [M+H] and m/z 603.1 [M+Na], and naringenin (MW = 272.25), the aglycone of naringin, yielded protonated adducts at m/z 273.2 [M+H]. Liu et al. (2017) observed an ion peak with m/z 603.2 [M+Na] in the mass spectrometry analysis of naringin. Moreover, weak [M+K] signals of naringin were observed at m/z 619.0. These results indicated that the main component of PF2 was naringin, consistent with the results of HPLC analysis shown in Fig. 5.

3.5. Antioxidant activity of flavonoids in pomelo peel

As shown in Fig. 7, the scavenging activities of the crude extract of

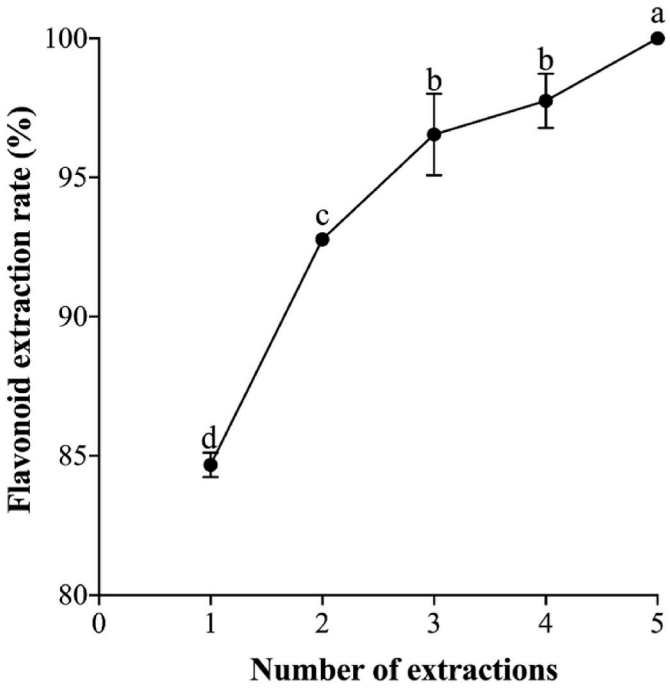

Fig. 3. Effect of number of extractions on the flavonoid extraction rate from pomelo peels.

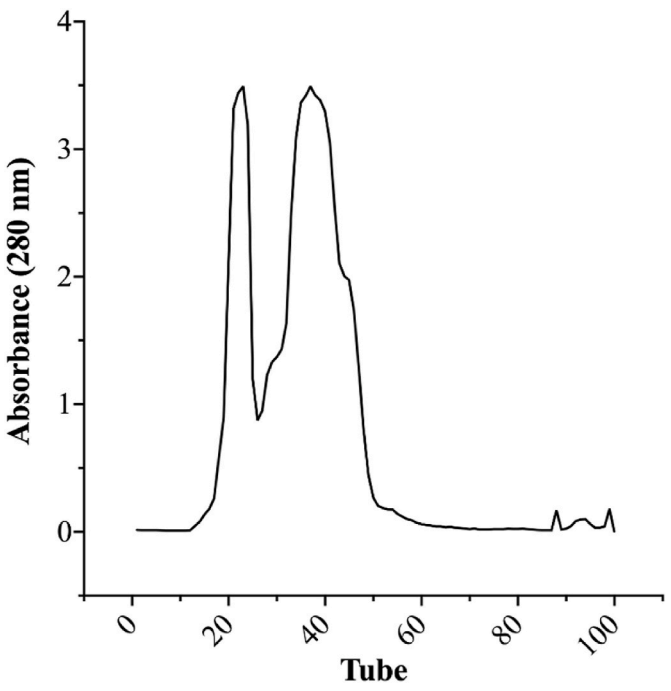

Fig. 4. Elution curve of the crude extract of flavonoids from pomelo peels using column chromatography.

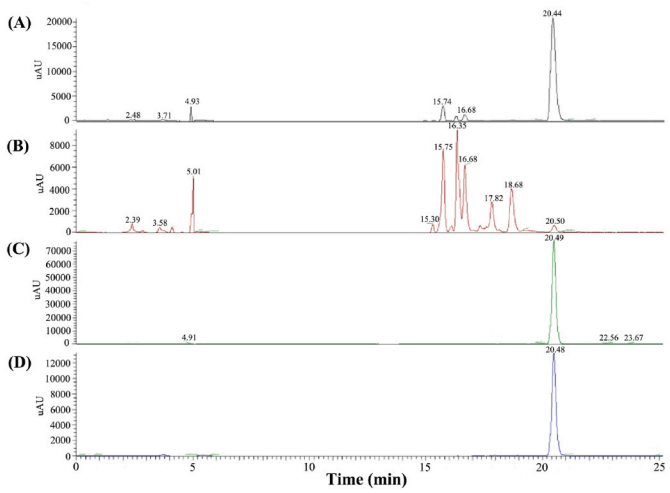

Fig. 5. High-performance liquid chromatography analysis of CF (A), PF1 (B), PF2 (C), and standard naringin sample (D). CF: crude extract of flavonoids; PF1: purified flavonoids 1; PF2: purified flavonoids 2.

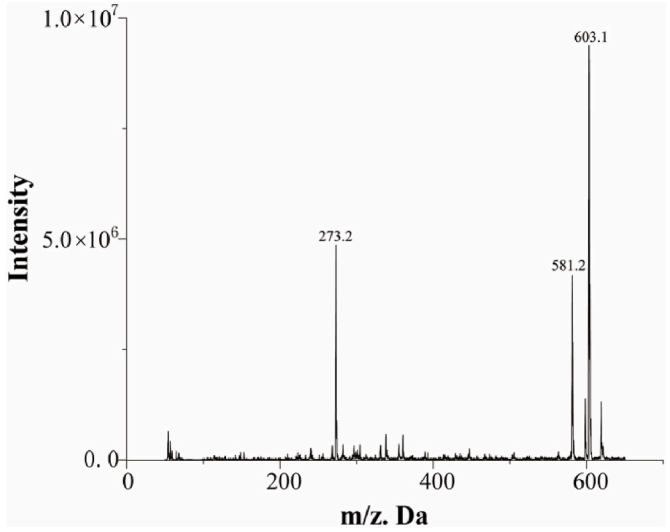

Fig. 6. High-performance liquid chromatography tandem mass spectrometry of purified flavonoids 2.

flavonoids (CF) and PF2 against DPPH·, $ABTS^{+}$, $O_2^{\cdot-}$ and ·OH increased with the increase of concentration. CF exhibited higher scavenging activities on DPPH·, $ABTS^{+}$ and ·OH than those of PF2. The calculation results showed that the IC_{50} values of CF and PF2 were 4.66 mg/mL and 6.72 mg/mL for DPPH·, 0.748 mg/mL and 2.54 mg/mL for $ABTS^{+}$, and 5.649 mg/mL and 8.329 mg/mL for ·OH, respectively (Fig. 7A, B and 7D). The IC50s of CF and PF2 for scavenging $O_2^{\cdot-}$ were similar, 3.62 mg/mL and 3.41 mg/mL, respectively, but PF2 showed better scavenging of $O_2^{\cdot-}$ than CF when the concentration exceeded 4 mg/mL (Fig. 7C).

4. Discussion

Flavonoids are important secondary plant metabolites, commonly found in abundance in fruits and vegetables, and are a class of bioactive polyphenolic compounds, so they attract researchers to extract and develop from various plants. The extraction and preparation of flavonoids are mainly carried out through three methods: supercritical fluid extraction, water extraction, and organic solvent extraction. According to sources, flavonoids from Xinjiang jujube (Song et al., 2019) and orange peel (Toledo-Guillén et al., 2010) were extracted using supercritical CO_2 fluid technology, and the main components have been identified. Subcritical water is used to extract flavonoids from citrus peel, and the extraction process has been optimized (Kim and Lim 2020). In the pressurized hot water method, flavonoids were isolated and identified from defatted *Camellia oleifera* seeds (Liau et al., 2017). Compared with the supercritical fluid extraction and water extraction processes, organic solvent extraction is the most widely used method because of its low cost and high efficiency (Wang et al., 2022). However, the organic solvent extraction process frequently requires various auxiliary measures such as high temperature (Puri et al., 2011), ultrasound (Barbhuiya et al., 2022), and microwave (Wang et al., 2021), among others. Although these physical supplementary approaches can save time, they increase equipment costs and process complexity. Furthermore, heat treatment easily leads to oxidation of flavonoids and causes browning of pomelo peel, thus posing a threat for the further development and utilization of pomelo peel fibers (Xiao et al., 2021). In the present study, efficient extraction of flavonoids from pomelo peel was successfully achieved by optimizing the extraction parameters and using ethanol solution at room temperature and under stirring. The single-time extraction rate of flavonoids from the raw materials could reach more than 80%, whereas the two-times extraction rate was close to 90%. Moreover, the bitterness of pomelo peel due to naringin disappeared, and the extraction or purification process did not cause deterioration in terms of the structure and color of the pomelo peel. Moreover, the procedure is simple and feasible.

The extraction of flavonoids from citrus peels has been reported in previous studies. The extraction rates of flavonoids were 10.87 ± 0.12 mg/g from orange peels (M'hiri et al., 2015), 4.0 ± 0.15 mg/g from Shatianyu (*Citrus grandis* L. Osbeck) (Deng et al., 2023), and 2.18–9.87 mg/g from Chinese local pummelo peels (*Citrus grandis Osbeck*.) (Xi et al., 2014). The flavonoid extraction rate (6.07 ± 0.06 mg/g) in the present study was similar to the above results.

Naringin is the main component of flavonoids in pomelo peel, accounting for more than 70% of the flavonoids in pomelo peel (Xi et al., 2014). It participates in many physiological activities, possessing anti-inflammatory (Tong et al., 2012), hypolipidemic (Yang et al., 2022), antioxidant (Liu et al., 2021), and anti-glycemic (Sarmah et al., 2022) effects. Naringin is usually purified by extraction or recrystallization (Kong et al., 2020), both of which are complex processes, and organic solvents used in the purification process, such as methanol or petroleum ether, have certain toxicity and can easily pollute the environment. Sephadex LH20 can be used for both gel filtration and reverse-phase partition chromatography, which have the advantages of high loading, easy regeneration, and low cost and can be used for the production of various natural products on a large scale and at low cost. In the present study, naringin was purified by Sephadex LH20 gel column chromatography with 30% ethanol solution as the eluent, and the purity of naringin was 95.78%. Naringin showed good free radicals scavenging activity. This method has the advantages of environmental protection, energy saving, simple process, safety, nontoxicity, and easy industrialization.

5. Conclusion

In conclusion, the flavonoids of pomelo peels were extracted effectively by optimizing three key parameters, namely, ethanol concentration, solid-to-liquid ratio, and extraction time, and the bitterness in pomelo peels was also removed, and high purity naringenin was isolated by Sephadex LH20 gel chromatography. Thus, high-value utilization of pomelo peels was realized and environmental pollution was reduced in the present study. The extracted flavonoids and naringin have a variety of activities such as antioxidative effects can be used to develop functional products, and the debitter pomelo peels can be used as dietary fiber.

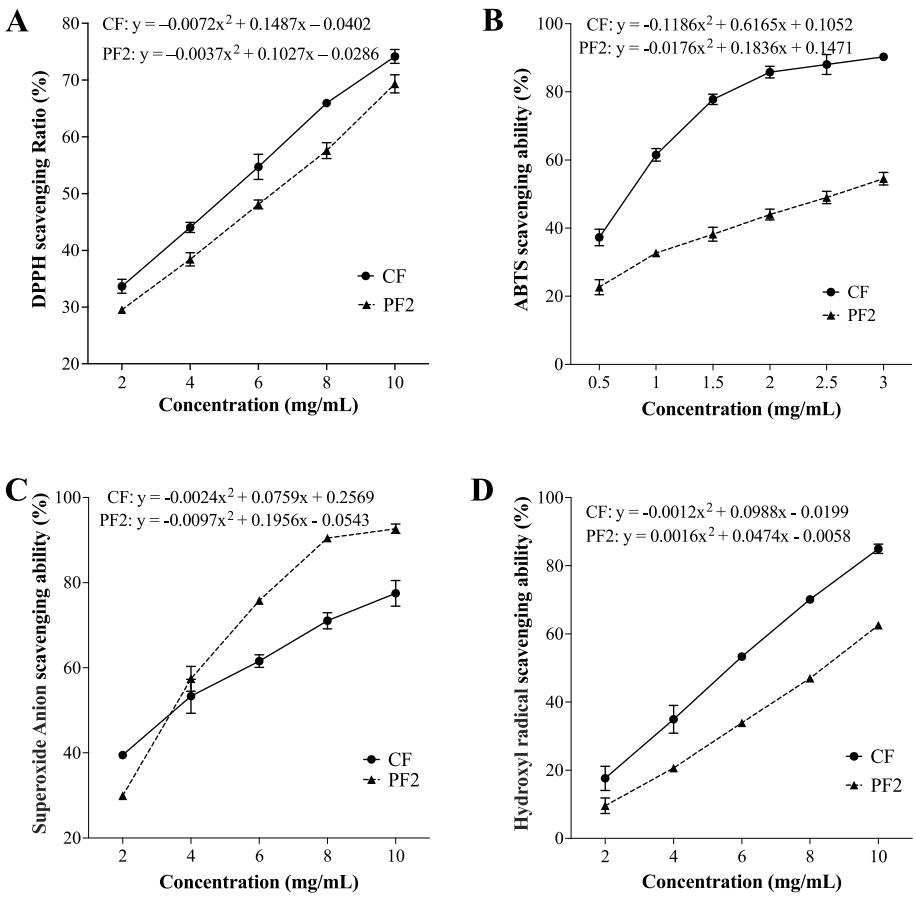

Fig. 7. Free radicals scavenging activities of DPPH (A), ABTS (B), Superoxide anion (C), and Hydroxyl (D) by CF and PF2. CF: crude extract of flavonoids; PF2: purified flavonoids 2.

CRediT authorship contribution statement

Yuchen Shangguan: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **Jing Ni:** Formal analysis, Visualization, Writing – review & editing. **Lili Jiang:** Conceptualization, Formal analysis. **Yang Hu:** Conceptualization, Project administration, Supervision. **Chuanbo He:** Conceptualization, Project administration, Supervision. **Ying Ma:** Methodology, Formal analysis, Writing – review & editing. **Guohong Wu:** Methodology, Project administration, Funding acquisition, Supervision. **Hejian Xiong:** Conceptualization, Validation, Formal analysis, Writing – review & editing, Visualization, Supervision, Funding acquisition.

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

No data was used for the research described in the article.

Acknowledgments

This work was supported by the Science and Technology Project of Fujian Province (2023I0020) and the Science and Technology Project of Xiamen (2022CXY0307).

References

Barbhuiya, R.I., Singha, P., Asaithambi, N., Singh, S.K., 2022. Ultrasound-assisted rapid biological synthesis and characterization of silver nanoparticles using pomelo peel waste. Food Chem. 385, 132602 https://doi.org/10.1016/j.foodchem.2022.132602.

Cai, W., Gu, X., Tang, J., 2008. Extraction, purification, and characterization of the polysaccharides from Opuntia milpa alta. Carbohydr. Polym. 71, 403–410. https://doi.org/10.1016/j.carbpol.2007.06.008.

Deng, M., Dong, L., Jia, X., Huang, F., Chi, J., Muhammad, Z., Ma, Q., Zhao, D., Zhang, M., Zhang, R., 2022. The flavonoid profiles in the pulp of different pomelo (Citrus grandis L. Osbeck) and grapefruit (Citrus paradisi Mcfad) cultivars and their in vitro bioactivity. Food Chem. X. 15, 100368 https://doi.org/10.1016/j.fochx.2022.100368.

Deng, M., Zhang, R., Zhang, S., Lu, Q., Dong, L., Huang, F., Jia, X., Ma, Q., Chi, J., Zhao, D., 2023. The flavonoid profiles in different tissue parts of Shatianyu (Citrus grandis L. Osbeck) and their in vitro bioactivity. LWT (Lebensm.-Wiss. & Technol.) 180, 114712. https://doi.org/10.1016/j.lwt.2023.114712.

Hamed, Y.S., Abdin, M., Akhtar, H.M.S., Chen, D., Wan, P., Chen, G., Zeng, X., 2019. Extraction, purification by macrospores resin and in vitro antioxidant activity of flavonoids from Moringa oliefera leaves. South Afr. J. Bot. 124, 270–279. https://doi.org/10.1016/j.sajb.2019.05.006.

Kim, D., Lim, S., 2020. Kinetic study of subcritical water extraction of flavonoids from citrus unshiu peel. Separ. Purif. Technol. 250, 117259 https://doi.org/10.1016/j.seppur.2020.117259.

Kong, F., Ding, Z., Zhang, K., Duan, W., Qin, Y., Su, Z., Bi, Y., 2020. Optimization of extraction flavonoids from Exocarpium Citri Grandis and evaluation its hypoglycemic and hypolipidemic activities. J. Ethnopharmacol. (15), 262. https://doi.org/10.1016/j.jep.2020.113178, 113178.

Kumar, G.A., Partha, P.S., Poonam, M., 2021. Ultrasound aided debittering of bitter variety of citrus fruit juice: effect on chemical, volatile profile and antioxidative potential. Ultrason. Sonochem. 81, 105839 https://doi.org/10.1016/j.ultsonch.2021.105839.

Liau, B., Ponnusamy, V.K., Lee, M., Jong, T., Chen, J., 2017. Development of pressurized hot water extraction for five flavonoid glycosides from defatted Camellia oleifera seeds (byproducts). Ind. Crop. Prod. 95, 296–304. https://doi.org/10.1016/j.indcrop.2016.10.034.

Liu, H., Liang, C., Ma, L., Liu, J., Wang, Q., 2021. Analysis of antioxidant response in pomelo fruitlets subjected to external injury stress: significance of naringin

biosynthesis. LWT (Lebensm.-Wiss. & Technol.) 150, 111908. https://doi.org/10.1016/j.lwt.2021.111908.

Liu, J., Wu, G., Yang, J., He, C., Xiong, H., Ma, Y., 2023. Abalone visceral peptides containing Cys and Tyr exhibit strong in vitro antioxidant activity and cytoprotective effects against oxidative damage. Food Chem. X 17, 100582. https://doi.org/10.1016/j.fochx.2023.100582.

Liu, Z., Zu, Y., Yang, W., 2017. A process to preserve valuable compounds and acquire essential oils from pomelo flavedo using a microwave irradiation treatment. Food Chem. 224, 172–180. https://doi.org/10.1016/j.foodchem.2016.12.027.

M'hiri, N., Ioannou, I., Boudhrioua, N.M., Ghoul, M., 2015. Effect of different operating conditions on the extraction of phenolic compounds in orange peel. Food Bioprod. Process. 96, 161–170. https://doi.org/10.1016/j.fbp.2015.07.010.

Puri, M., Kaur, A., Schwarz, W.H., Singh, S., Kennedy, J.F., 2011. Molecular characterization and enzymatic hydrolysis of naringin extracted from kinnow peel waste. Int. J. Biol. Macromol. 48, 58–62. https://doi.org/10.1016/j.ijbiomac.2010.09.012.

Qiu, J., Cai, L., Xiong, S., You, J., Yin, T., An, Y., 2023. Characterization of aroma profiles and volatile organic compounds in silver carp surimi gel by GC-IMS, SPME-GC-MS, and sensory evaluation: affected by okara insoluble dietary fiber with varied particle sizes. Food Chem. Adv. 2, 100224 https://doi.org/10.1016/j.focha.2023.100224.

Reshmi, S.K., Sudha, M.L., Shashirekha, M.N., 2017. Starch digestibility and predicted glycemic index in the bread fortified with pomelo (Citrus maxima) fruit segments. Food Chem. 237, 957–965. https://doi.org/10.1016/j.foodchem.2017.05.138.

Sang, J., Li, L., Wen, J., Liu, H., Wu, J., Yu, Y., Xu, Y., Gu, Q., Fu, M., Lin, X., 2022. Chemical composition, structural and functional properties of insoluble dietary fiber obtained from the Shatian pomelo peel sponge layer using different modification methods. LWT (Lebensm.-Wiss. & Technol.) 165, 113737. https://doi.org/10.1016/j.lwt.2022.113737.

Sarmah, S., Goswami, A., Belwal, V.K., Roy, A.S., 2022. Mitigation of ribose and glyoxal induced glycation, AGEs formation and aggregation of human serum albumin by citrus fruit phytochemicals naringin and naringenin: an insight into their mechanism of action. Food Res. Int. 157, 111358 https://doi.org/10.1016/j.foodres.

Sharma, P., Vishvakarma, R., Gautam, K., Vimal, A., Kumar, G.V., Farooqui, A., Varjani, S., Younis, K., 2022. Valorization of citrus peel waste for the sustainable production of value-added products. Bioresour. Technol. 351, 127064 https://doi.org/10.1016/j.foodres.2022.111358.

Song, L., Liu, P., Yan, Y., Huang, Y., Bai, B., Hou, X., Zhang, L., 2019. Supercritical CO_2 fluid extraction of flavonoid compounds from Xinjiang jujube (*Ziziphus jujuba Mill.*) leaves and associated biological activities and flavonoid compositions. Ind. Crop. Prod. 139, 111508 https://doi.org/10.1016/j.indcrop.2019.111508.

Tian, D., Wang, F., Duan, M., Cao, L., Zhang, Y., Yao, X., Tang, J., 2019. Coumarin analogues from the *Citrus grandis* (L.) Osbeck and their hepatoprotective activity. J. Agric. Food Chem. 67, 1937–1947. https://doi.org/10.1021/acs.jafc.8b06489.

Toledo-Guillén, A.R., Higuera-Ciapara, I., García-Navarrete, G., de la Fuente, J.C., 2010. Extraction of bioactive flavonoid compounds from orange (citrus sinensis) peel using supercritical CO_2. J. Biotechnol. 150, 314. https://doi.org/10.1016/j.jbiotec.2010.09.292.

Tong, L., Zhou, D., Gao, J., Zhu, Y., Sun, H., Kaishun, B., 2012. Simultaneous determination of naringin, hesperidin, neohesperidin, naringenin and hesperetin of Fractus aurantii extract in rat plasma by liquid chromatography tandem mass spectrometry. J. Pharmaceut. Biomed. Anal. 58, 58–64. https://doi.org/10.1016/j.jpba.2011.05.001.

Wang, X., Xu, R., Wang, Y., Ma, L., Nie, S., Xie, M., Yin, J., 2020. Physicochemical and rheological properties of pomelo albedo pectin and its interaction with konjac glucomannan. Int. J. Biol. Macromol. 151, 1205–1212. https://doi.org/10.1016/j.ijbiomac.2019.10.167.

Wang, Y., Peng, C., Zhang, Y., Wang, Z., Chen, Y., Dong, J., Xiao, M., Li, D., Li, W., Zou, Q., Zhang, K., Wei, P., 2022. Optimization, identification and bioactivity of flavonoids extracted from Moringa oleifera leaves by deep eutectic solvent. Food Biosci. 47, 101687 https://doi.org/10.1016/j.fbio.2022.101687.

Wang, Z., Zhao, X., Zhao, D., Zu, Y., Wang, L., Wu, M., Wang, L., Liu, Y., Zhang, Q., 2021. Ultrasonic microwave-assisted micellar extraction and purification of flavonoids from licorice by metal complex and antisolvent recrystallization. LWT (Lebensm.-Wiss. & Technol.) 147, 111501. https://doi.org/10.1016/j.lwt.2021.111501.

Xiao, L., Ye, F., Zhou, Y., Zhao, G., 2021. Utilization of pomelo peels to manufacture. value-added products: a review. Food Chem. 351, 129247 https://doi.org/10.1016/j.foodchem.2021.129247.

Xi, W., Fang, B., Zhao, Q., Jiao, B., Zhou, Z., 2014. Flavonoid composition and antioxidant activities of Chinese. local pummelo (Citrus grandis Osbeck.) varieties. Food Chem. 161, 230–238. https://doi.org/10.1016/j.foodchem.2014.04.001.

Xie, J., Dong, C., Nie, S., Li, F., Wang, Z., Shen, M., Xie, M., 2015. Extraction, chemical composition and antioxidant activity of flavonoids from Cyclocarya paliurus (Batal.) Iljinskaja leaves. Food Chem. 186, 97–105. https://doi.org/10.1016/j.foodchem.2014.06.106.

Yang, Y., Trevethan, M., Wang, S., Zhao, L., 2022. Beneficial effects of citrus flavanones naringin and naringenin and their food sources on lipid metabolism: an update on bioavailability, pharmacokinetics, and mechanisms. J. Nutr. Biochem. 104, 108967 https://doi.org/10.1016/j.jnutbio.2022.108967.

Yang, Y., Lai, X., Lai, G., Jiang, Z., Ni, H., Chen, F., 2016. Purification and characterization of a tyrosinase inhibitor from camellia pollen. J. Funct.Foods 27, 140–149. https://doi.org/10.1016/j.jff.2016.08.056.

Yin, J., Hu, X., Hou, Y., Liu, S., Jia, S., Gan, C., Ou, Y., Zhang, X., 2023. Comparative analysis of chemical compositions and antioxidant activities of different pomelo varieties from China. Food Chem. Adv. 2, 100180 https://doi.org/10.1016/j.focha.2022.100180.

Yun, L., Shi, Z., Yang, Z., Cai, Z., Wang, L., Wu, X., Ye, A., Jiang, J., 2020. Hypolipidemic effect of pure total flavonoids from peel of Citrus (PTFC) on hamsters of hyperlipidemia and its potential mechanism. Exp. Gerontol. 130, 110786 https://doi.org/10.1016/j.exger.2019.110786.

Zhang, M., Duan, C., Zang, Y., Huang, Z., Liu, G., 2011. The flavonoid composition of flavedo and juice from the pummelo cultivar (Citrus grandis (L.) Osbeck) and the grapefruit cultivar (Citrus paradisi) from China. Food Chem. 129, 1530–1536. https://doi.org/10.1016/j.foodchem.2011.05.136.

Zhang, S., Yang, W., Muneer, M.A., Ji, Z., Tong, L., Zhang, X., Li, X., Wang, W., Zhang, F., Wu, L., 2021. Integrated use of lime with Mg fertilizer significantly improves the pomelo yield, quality, economic returns and soil physicochemical properties under acidic soil of southern China. Sci. Hortic. 290, 110502 https://doi.org/10.1016/j.scienta.2021.110502.

Zhu, L., Wang, Y., Wang, Y., You, L., Shen, X., Li, S., 2017. An environmentally friendly carbon aerogels derived from waste pomelo peels for the removal of organic pollutants/oils. Microporous Mesoporous Mater. 241, 285–292. https://doi.org/10.1016/j.micromeso.2016.12.033.