

## Green extraction of total flavonoids (TF) from Citrus pericarp using novel air nanobubbles (NBs) stabilized with biosurfactants assisted by ultrasonic disruption (UD)

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### ABSTRACT

In this study, air nanobubbles (NBs) generated with a new device were stabilised by a complex of arabic gum (AG) and stevioside (STE). Some basic metrics of NBs produced by biosurfactants at different concentrations were characterized to determine the stability of NBs. The mixture solution rich in NBs was assisted by ultrasonic disruption (UD) to extract total phenols (TP) and total flavonoids (TF) from 7 citrus pericarp. We evaluated four key factors affecting the TF extraction of '*Citrus reticulata 'Chachiensis'*' ('Chazhigan') pericarp by one-way test. The optimal extraction conditions were further predicted and validated by Box-Behnken response surface experiments. Under the conditions of 135 w capacity, 4.1 C mixture solution concentration, 30.5 °C water bath temperature, and 31 min ultrasonic time, the TF content of 'Chazhigan' pericarp ( $20.82 \pm 0.85$  mg/g DW) was significantly better than that extracted by 80 % ethanol ( $12.22 \pm 0.90$  mg/g DW). Finally, fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were utilised to provide a preliminary exploration of the potential mechanisms involved in the extraction process. The flavonoid extracts from the pericarp of 'Chazhigan' were qualitatively analysed. And the differences in the chemical antioxidant activity of their extracts were investigated before and after the application of traditional alcoholic extraction and novel green extraction. In conclusion, this study established a novel approach that is eco-friendly, which could effectively extract flavonoids from citrus peels.

### 1. Introduction

Citrus is a bulk fruit widely grown and processed globally. The processing of citrus juices and concentrate products accounts for one-third or more of total citrus consumption (Casquette et al., 2015; Durmus et al., 2024; Mahato et al., 2018, 2019), thus leaving a large amount of citrus peel underutilised. However, in recent years, studies have shown that citrus contains a large number of naturally occurring active substances, such as flavonoids and other polyphenols, citrus essential oils, organic acids, polysaccharides, dietary fibre and other phytochemicals with health-promoting properties (Teigiserova et al., 2021). Citrus peel, in its entirety, inclusive of oil cells and white peel layer, has been found to contain higher concentrations of flavonoids,

predominantly flavonoids and polymethoxyflavonoids, when compared with the pulp (Shah et al., 2024; Wang et al., 2017). Citrus flavonoids have a variety of activities, including antioxidant, anti-obesity, brain damage (Wang et al., 2020), antidepressant (Pannu et al., 2021), anti-aging, and other functions. They demonstrate significant potential in the domains of functional food, medicine, and healthcare. 'Chazhigan', a citrus species which is both emblematic of traditional Chinese medicine and food, has a profound relationship with the health industry. The dried peel of this species is the raw material of 'Chenpi', which is highly prized for utilisation and research purposes.

It has been demonstrated by preceding studies that the extraction methodologies employed significantly impact the yield, chemical structure and biological activity of citrus peel flavonoids (Zhu et al.,

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2023). Conventional flavonoid extraction methods are capable of extracting target compounds from raw materials, including solvent extraction and thermal reflux. However, these methods generally necessitate elevated temperatures or the utilisation of additional organic solvents. The traditional solvents most frequently employed, such as ethanol and methanol, have the potential to be toxic to human and may pose a health hazard in instances of prolonged use.

In recent years, extraction methods such as enzyme-assisted extraction (Nishad et al., 2019), steam blasting (Dorado et al., 2020; Yang et al., 2024), supercritical fluid extraction (Cheigh et al., 2012), microwave-assisted (Benassi et al., 2021) and ultrasound-assisted extraction (Chien et al., 2022) have been shown to be effective in damaging the plant cell wall and enhancing the release of active substances from citrus peels. Concurrently, a new generation of green solvents, based on the composition of hydrogen bond acceptors and hydrogen bond donors, is being explored as a more environmentally-friendly extraction process. These solvents are referred to as 'deep eutectic solvents (DES)'. DES under scrutiny is predominantly constituted of sugars, sugar alcohols, organic acids, urea, glycerol and choline chloride (Alchera et al., 2024). The components are able to form hydrogen bonds, and can also accept or donate external electrons or protons to form hydrogen bonds. This solvent has been used to promote the solubilisation of effective natural products in plants, such as polysaccharides (Wang and Li, 2022), lignin (Zheng et al., 2025), polyphenols (Mero et al., 2024; Wang et al., 2024) and so on. However, DES is also controversial in use due to its high viscosity and high density. Biosurfactants, particularly non-ionic surfactants, have been demonstrated to exhibit favourable properties in terms of mildness, stability and environmental friendliness, in addition to their solubilising capacity (Tang et al., 2023). In light of this, two non-ionic surfactants of natural origin were selected as green extraction solvents in this study, with the aim of reducing the utilisation of toxic solvents, such as methanol and ethanol, in conventional extraction methodologies. It has been demonstrated that AG and STE possess the capacity to stabilise air NBs. In addition, they contain hydroxyl and carboxyl groups, are weakly acidic and can be used as hydrogen bond donors. Flavonoids have been demonstrated to form hydrogen bonds under aqueous conditions due to the presence of multiple phenolic hydroxyl groups that can act as hydrogen bond acceptors. This provides a solid foundation for enhancing the efficiency of aqueous extraction processes.

The utilisation of NBs in extraction represents a novel methodology, whose rapid evolution is propelled by its interdisciplinary character, thereby establishing connections between the domains of physics, chemistry, life sciences and engineering (Ettoumi et al., 2022). In combination with enzyme-assisted extraction and ultrasound-assisted extraction, it has the potential to enhance the extraction efficiency of target products (Noroozi et al., 2021). In order to utilise NBs in a practical context, it is imperative to first prepare and stabilise them. The present study proposes a novel apparatus for the generation of airborne NBs, whilst concomitantly evaluating the capacity of a green solvent comprising two biosurfactants to stabilise the NBs at varying concentrations. In this paper, a novel approach of green extraction assisted by UD was developed using a mixture solvent consisting of AG and STE. The optimal extraction process of flavonoids from pericarp of 'Chazhigan' was derived through RSM. Furthermore, the antioxidant activity of NBs and extracts was partially evaluated, and the potential mechanisms involved in the extraction process were discussed.

## 2. Materials and methods

### 2.1. Fruit materials, chemicals, experimental devices

Seven citrus fruits were used in our study: *Citrus reticulata* 'Chachiensis', ('Chazhigan', Guangdong province, China), *Citrus reticulata* 'Unshiu', ('Gongchuan', 'Miyawa', Zhejiang province, China), *Citrus reticulata* 'Hong Mei Ren', ('Hongmeiren', Zhejiang province, China),

*Citrus reticulata* junos, ('Xiangcheng', Zhejiang province, China), *Citrus sinensis* 'Blood orange', ('Xuecheng', Sichuan province, China), *Citrus aurantium* 'Changshanhuoyou', ('Huyou', Zhejiang province, China), *Citrus maxima* (Burm.) Merr. cv. 'Yuhuanwendan', ('Wendan', Zhejiang province, China). The fruits were collected between the end of November (2023) and the end of February (2024) at the time of fruit ripening from different origins, transported to the laboratory via cold chain every other day, and selected to be photographed for uniformity of colour, size, etc., and freedom from pests and diseases. Citrus fruits were peeled and cut into small pieces measuring approximately 1 cm × 1 cm. The pieces were then stored in a -80 °C refrigerator. Approximately 50 g of each citrus peel was collected every other day and processed in a freeze dryer (SCIENTZ-18 N, Ningbo SCIENTZ Biotechnology Co., Ltd, China) for a period of three days (Jentzsch et al., 2022). The samples were then crushed into a very fine powder using a sample grinder and stored in a refrigerator at -40 °C for subsequent experiments.

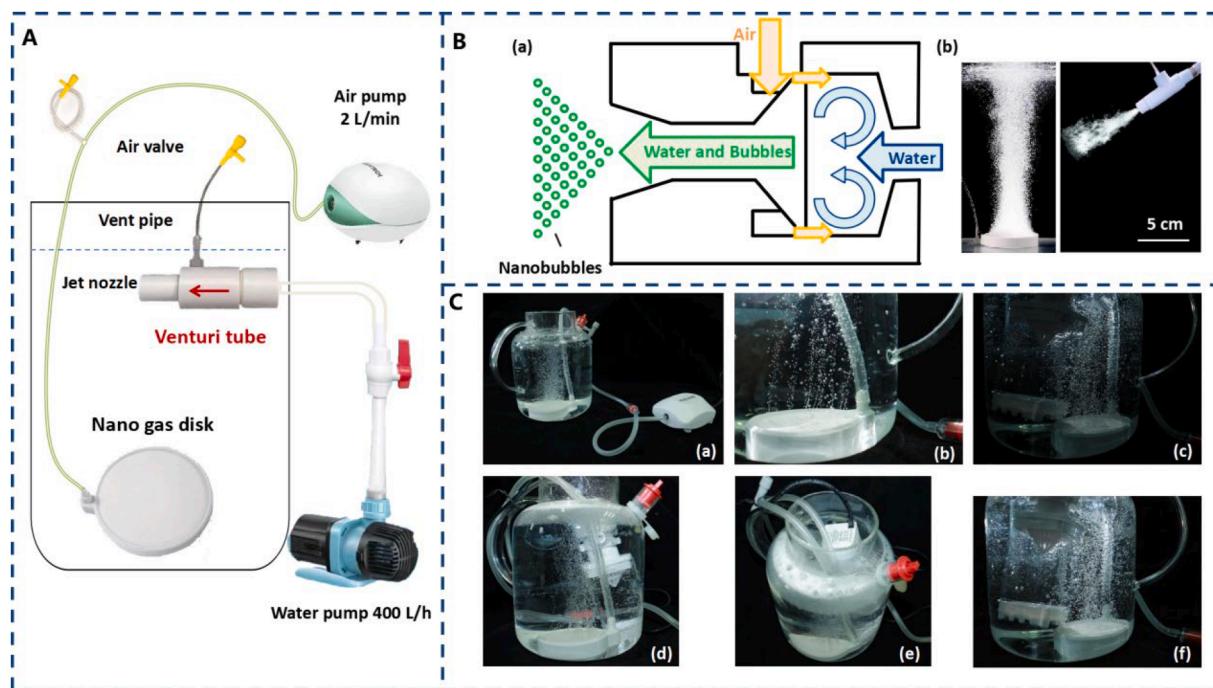
The pure water utilised in the experiment was 'Wahaha' (Hangzhou Wahaha Group Co., Ltd, China). Stevioside (100 g, ≥99 %) was procured from Jiangsu Ruikanglai Technology Co., Ltd, China, and Arabic gum (500 g, food grade) was obtained from Zhejiang Yinuo Biotechnology Co. Ltd, China. The following chemicals were procured from Aladdin Chemical Company (Shanghai, China): 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)1,3,5-triazine (TPTZ), ascorbic acid, hydrochloric acid, acetonitrile, methanol, ethanol (EtOH), and potassium persulphate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>). 2,2'-azino-bis (3-ethylbenzothiazole-6-sulfonic acid) (ABTS) was procured from Sigma Chemicals (Sigma-Aldrich, USA). Fourteen flavonoid standards were purchased from Shanghai Yuanye Bio-Technology Co., Ltd, China. Eriocitin (20 mg, ≥98 %), Neo-eriocitin (5 mg, ≥98 %), N arirutin (5 mg, ≥98 %), Naringin (100 mg, ≥98 %), Hesperidin (100 mg, ≥98 %), Neohesperidin (20 mg, ≥98 %), Didymin (20 mg, ≥98 %), Poncirus (10 mg, ≥98 %), Isosinensetin (10 mg, ≥98 %), Sinensetin (20 mg, ≥98 %), Nobletin (20 mg, ≥98 %), Heptamethoxyflavones (3,5,6,7,8,3',4'-heptemethylflavones, 10 mg, ≥98 %), Tangeretin (20 mg, ≥98 %), 5-demethylnobletin (Demethylnobletin, 20 mg, ≥98 %). All chemicals used were of analytical grade.

The raw materials used for the design and combination of the novel extraction device in this study were purchased from the T-Mall flagship shop (tmall.com): nano-atomised air disc (model C50, 5 cm diameter, with stop valve, plastic air tube, three-way valve, suction cup and regulating valve) purchased from Guangzhou Favourite Box, oxygen pump (model E01, 1.5 w), glass jar with handles (17 cm × 13 cm, 1.8 L, with (17 cm × 13 cm, 1.8 L, with exhaust valve and sealing lid), 3-in-1 water purification filter (3.6 cm × 3.6 cm × 12 cm, 4 w), water pump (the outlet diameter is 8 mm, 6 W).

### 2.2. Preparation of NBs

#### 2.2.1. Device and working principle

The device under consideration incorporates a ceramic nano gas disc, with a glass jar having been selected as the extraction vessel on account of their high temperature resistance and good densification (Fig. 1C). During operation of the nano air disc, the three-way valve is configured such that one end is connected to the oxygen pump, one end is in contact with the liquid, and one end is connected to the air. The twisting of the valve to adjust the air throughput can result in alterations to the instantaneous pressure in the micropores of the air disc. The subsequent extraction of flavonoids from citrus peels was accomplished by means of air passing through channels similar to the action of a venturi tube (Fig. 1A). Utilising Bernoulli's principle of fluid dynamics (Fig. 1B), this process was facilitated by the generation of energy through the bursting of NBs and the employment of ultrasonic cell-breaking technology in the context of a brief, high-speed pressure fluctuation. For specific parameter, the size of each part and component is presented above (2.1). The power and flow rate of the oxygen pump is 1.5 W and 2L/min respectively when in operation. The power of the water purification filter is 4



**Fig. 1.** Green Extraction Device and its operation.

(A. analogue schematic diagram B. schematic diagram C. physical diagram)

W and the flow rate is 300L/h during working. When turned on, the power of the water pump is 6 W and the flow rate is 400L/h.

### 2.2.2. Preparation of green extract mixture solution (*mix-sol*)

In order to stabilise the NBs generated in the device, it is necessary to prepare the mixture-sol. With regard to dosage, the ratio of AG:STE is 2:1. Based on the critical micelle concentration (1 CMC) of STE, which is 0.0025 mol/L (i.e. 2.0196 mg/mL), the STE 0.5 CMC is designated as the 1 C concentration, and the 1 CMC concentration (2.02 mg/mL) is the 2 C concentration, and so on, thus establishing the 0, 1, 2, 4, 5 C concentration gradient. The dose corresponding to the highest designed concentration (5 C) of each of the two biosurfactants was meticulously weighed on a balance on weighing paper and set aside. At ambient temperature ( $24 \pm 1^\circ\text{C}$ ), 1 L of purified water was added to a 2 L beaker. The beaker was placed on a magnetic stirrer (300 rpm). The biosurfactant was added in small quantities, several times and slowly. The process was continued until complete dissolution within 60 min. The plastic wrap was then sealed, and the stirring continued for a further 12 h. The configured extraction solvents were then diluted according to the concentration gradient, in order to carry out the subsequent experiments. The appearance of the mixture-sol can be seen in Supplementary Fig. 1.

### 2.3. Stabilisation and characterisation of NBs

#### 2.3.1. Determination of dynamic contact angle

Following the pouring of varying concentrations of green extraction solvent (mixture-sol) into the device, and subsequent energisation of the device for a duration of 0.5 h, a dense accumulation of bubbles was observed in the upper region of the device. This phenomenon was discernible to the unassisted eye. When the device was energised directly, the big bubbles were very easy to burst and escape. The liquid at the junction of the upper dense bubbles and the lower solution was then extracted using a burette, transferred to a glass vial with a volume of 20 mL, and the screw cap was tightened. Following the complete dissolution of the upper bubbles after approximately 30 min, the experiment was conducted. A volume of 100  $\mu\text{L}$  of the liquid under

investigation was collected using a needle suspender, fixed on the contact angle measuring instrument (Powereach, JC2000D1, Shanghai Zhongchen Digital Technology Equipment Co., Ltd, China), and a single drop of liquid was added in sequence. The dynamic photographs of the liquid droplets wetting the slides within 30 s were then captured. The measuring angle method was used to measure the liquid. The left contact angle was determined by the goniometric method and recorded. In order to avoid contamination, glass vials that had been cleaned and sterilised, as well as disposable cuvettes and syringes, were used for this experiment and for any subsequent experiments.

#### 2.3.2. Particle size distribution and Zeta potential measurement

The procedure for sample preparation was consistent with the method outlined in Section 2.3.1. The volume of solution was aspirated up to approximately two-thirds of the cuvette. The particle size distribution and Zeta potential of the NBs were then determined by means of a Nano particle size and Zeta potential analyser (BeNano 180 Zeta Pro, Dandong Ettersize Instrument Co., Ltd, China). This process was repeated on three separate occasions.

#### 2.3.3. Nanoparticle tracking analysis and mixture-sol pH change

To further investigate the stability of mixture-sol on NBs, different energisation times (0, 15, 30, 45, 60 min), resting times (0, 1, 3, 5 day) and storage ambient temperatures ( $-20, 4, 25, 37, 65^\circ\text{C}$ ) were set. The solutions at different resting temperatures were placed simultaneously in  $-20^\circ\text{C}$  refrigerator,  $4^\circ\text{C}$  refrigerator, room temperature incubator,  $37^\circ\text{C}$  incubator and  $65^\circ\text{C}$  oven, respectively, then were removed after 24 h. The NBs content was scanned and determined using a nanoparticle tracking analyser (Nanosight 300, Malvern Instruments Ltd, UK) at 640 nm. With the exception of the difference in experimental conditions, the sample collection method was the same as 2.3.1. In the test, the solution to be measured was aspirated with a 1 mL syringe and slowly injected into the sample tank, and the Brownian motion of NBs was observed. Three parallel sampling points were set up for each sample to be measured, and during the resting process, the solvent pH was measured every 12 h during the first 48 h and recorded (Supplementary Table 1).

#### 2.4. TP and TF content of seven citrus pericaps after traditional extraction

##### 2.4.1. Extraction by traditional methods

With minor modifications from our previous study (Wang et al., 2017), 0.5 g of lyophilised powder of citrus pericarp was accurately weighed and added to different extraction solvents (80 % ethanol, purified water, mixture-sol-no NBs, mixture-sol) at a material-liquid ratio of 1:20 and the extraction was repeated twice. After vortexing for 5 min, the extract was ultrasonicated in an ultrasonic cleaner at 53 kHz for 0.5 h. The extract was then centrifuged (8000 rpm) for 30 min, and the supernatant was summed up and centrifuged again to ensure that there were no impurities in the extract. The extracts were stored in a refrigerator at 4 °C for experimental analysis.

##### 2.4.2. Determination of TP and TF

The contents of the collected extracts were determined using the total phenol and total flavonoid content determination kit (microplate method, Beijing Biosharp Technology Co., Ltd), respectively, according to the instructions and repeated three times (Wang et al., 2017; Li et al., 2021).

#### 2.5. UD-assisted extraction of TF from 'Chazhigan' pericarp

##### 2.5.1. One-factor test

The extraction method was modified by incorporating 'Chazhigan' pericarp. Precisely measured 1~2 g of freeze-dried powder of citrus peel, and added different concentrations of mixture-sol (0, 1, 2, 3, 4, 5 C)

at a material-liquid ratio of 1:20, and repeated the extraction twice. After vortexing for 5 min, a constant temperature water bath was set for 0.5 h with different temperatures (0, 10, 20, 30, 40 °C). Different power (0, 65, 130, 195, 260 W) and time (0, 15, 30, 45, 60 min) were set using an ultrasonic cell crusher (X0-650D, Hangzhou Runmao Technology Co., Ltd, China), with 1 s on and 1 s off mode. The effects of varying solvent concentration, water bath temperature, ultrasonic crushing power and time on the extraction rate of total flavonoids from the peel of 'Chazhigan' were investigated separately.

##### 2.5.2. RSM optimisation and validation

Based on the results of the one-factor experiment, a four-factor three-level response surface experiment was designed (Table 1). Regression model ANOVA was performed using Design-Expert 13.0 software. The optimal extraction conditions of TF from 'Chazhigan' pericarp were predicted by response surface fitting (Supplementary Fig. 2). With the optimised conditions, the TF from 'Chazhigan' pericarp were extracted again as in 2.4.2.

##### 2.5.3. Measurement of antioxidant capacity

In the experiment, 15 mL of flavonoid extract from 'Chazhigan' pericarp, mixture-sol (4 C), aqueous-STE (4 C) and aqueous-AG (4 C) collected in 2.5.2 were pipetted out and placed in a cell culture dish with a diameter of 9 cm. The surface of the dish was then sealed with cling film, after which small holes were made. Then they were placed in a freeze dryer for 72 ~ 96 h. The powder was collected for testing. Traditional alcoholic extract of flavonoids from 'Chazhigan' pericarp was then dispensed into 10 mL centrifuge tubes and dried under vacuum

**Table 1**  
Box-Behnken response surface experiments.

Three levels	Four factor			
	A-Capacity (w)	B-Concentration (CMC, C)	C-Temperature (°C)	D-Time (min)
-1	65	3	20	15
0	130	4	30	30
1	195	5	40	45

BBD experimental design for the TF from *Citrus reticulata* 'Chachiensis' pericarp

Std	Run	Capacity	Concentration	Time	Temperature	Total flavonoids (mg /g DW)
1	16	65	3	30	30	8.94
2	25	195	3	30	30	9.28
3	9	65	5	30	30	8.91
4	14	195	5	30	30	12.96
5	22	130	4	20	15	9.63
6	21	130	4	40	15	11.25
7	15	130	4	20	45	10.39
8	7	130	4	40	45	11.65
9	11	65	4	30	15	7.62
10	19	195	4	30	15	9.17
11	26	65	4	30	45	9.14
12	8	195	4	30	45	9.28
13	28	130	3	20	30	10.03
14	5	130	5	20	30	11.14
15	24	130	3	40	30	10.11
16	12	130	5	40	30	12.98
17	17	65	4	20	30	8.25
18	23	195	4	20	30	10.16
19	18	65	4	40	30	9.13
20	27	195	4	40	30	10.46
21	10	130	3	30	15	9.84
22	2	130	5	30	15	10.69
23	20	130	3	30	45	12.38
24	3	130	5	30	45	13.04
25	13	130	4	30	30	20.84
26	1	130	4	30	30	19.65
27	6	130	4	30	30	20.99
28	29	130	4	30	30	21.81
29	4	130	4	30	30	21.53

Note: B-Concentration (CMC, C) : Based on the critical micelle concentration (1 CMC) of STE, which is 0.0025 mol/L (i.e. 2.0196 mg/mL), the STE 0.5 CMC is designated as the 1 C concentration, and the 1 CMC concentration (2.02 mg/mL) is the 2 C concentration.

in a rotary evaporator at a controlled temperature of 30 °C for 72 ~ 96 h. The products were collected for testing.

To assess the antioxidant capacity of biosurfactant, mixture-sol and extracts, two chemical antioxidant capacity evaluation tests were used in this study, namely DPPH method and ABTS method, which were slightly modified according to our previous papers (Chen et al., 2022).

## 2.6. Characterisation and identification of 'Chazhigan' pericarp flavonoids

### 2.6.1. Fourier infrared spectroscopy (FT-IR)

The sample powders prepared in 2.6 were used to determine the aqueous-STE, aqueous-AG, mixture-sol, and the flavonoid extracts of 'Chazhigan' pericarp by Fourier infrared spectroscopy (NICOLET iS50FT-IR, Thermo Scientific, Germany) in mid-infrared spectra. Main operating parameters of the instrument: resolution of 4cm<sup>-1</sup>, scanning frequency of 32 times, scanning range of 4000cm<sup>-1</sup>~400cm<sup>-1</sup>.

### 2.6.2. Scanning electron microscope (SEM) and transmission electron microscope (TEM)

In order to characterise the tissue morphology of the flavonoid extracts of 'Chazhigan' pericarp before and after UD, the samples were scanned with a thermal field emission scanning electron microscope (Zeiss G300, Carl Zeiss, Germany and Hitachi SU8010, Hitachi, Japan) and SEM images were taken at different magnifications. To observe the morphology of the green extraction mixture-sol stabilised NBs, mixture-sol (4 C) was configured and NBs were prepared by adding 1 drop to be measured directly onto 3 mm carbon supported membranes (Zhongjingkeyi Technology Co., Ltd, China) placed in the centre of the filter paper. After being placed under a yellow light to dry for 3–5 min, they were scanned and photographed under a transmission electron microscope (TEM). The diameters of individual bubbles stabilised at 0 C and 4 C concentrations were measured with Image-J and recorded. Three replicates were conducted.

### 2.6.3. Ultra performance liquid chromatography (UPLC) and high performance liquid chromatography (HPLC)

Fourteen flavonoid monomers were utilised as mixed standards for the material analysis of the flavonoids from 'Chazhigan' pericarp. A dried sample of 'Chazhigan' pericarp flavonoid extract weighing 0.05 g was dissolved in 1 mL of chromatographic-grade methanol. The solution was then mixed by vortexing and shaking, and subsequently diluted 10-fold to obtain a solution of 5 mg/mL. The solution was then subjected to centrifugation at 8000 rpm for a duration of 20 min. Thereafter, 150 microlitres of the solution was transferred into a liquid phase vial for the identification of flavonoids using UPLC-MS/MS.

The assay was performed on a Waters 2695–2996 UPLC system, utilising an ACQUITY UPLC®HSS T3 (1.8 μm, 2.1 × 150 mm) liquid chromatography column as the stationary phase. The mobile phases employed were water and acetonitrile, designated as phases A and B, respectively. The following elution program was used: 0/5,2/15,13/30,18/50,28/80,30/100,33/5,35/5 (min/B %). The sample injection volume was 2 μL, the detection wavelength was 280 nm, the column temperature was 30 °C and the flow rate was 0.3 mL/min. The sample was extracted from the column on an AB TripleTOF 6600plus System (AB SCIEX, Framingham, USA) system for LC-MS analysis, and mass spectrometry analysis was performed in positive and negative ion modes.

The optimal parameter settings that were determined through rigorous experimentation are outlined below: positive ion mode: source voltage set to +5.5 kV, source temperature set to 550 °C, nebuliser gas 1 (air) and nebuliser gas 2 (air) pressures set to 50 psi, and curtain gas (N2) pressure set to 35 psi. It is important to note that the maximum permissible error was set to ± 5 ppm. The de-cluster potential (DP) was set to 80 V, and the collision energy (CE) was set to 10 V. In the context of MS/MS acquisition mode, the parameters exhibited a high degree of

similarity, with the exception of the ion release delay (IRD), which was set to 67, and the ion release width (IRW), which was set to 25. In MS/MS acquisition mode, the parameters were nearly identical, except that the collision energy (CE) was set to 40 ± 20 V, the ion release delay (IRD) was set to 67, and the ion release width (IRW) was set to 25. The m/z scan ranges for precursor and product ions were set to 100–1500 Da and 50–1500 Da, respectively. Prior to each analysis, accurate mass number calibration was performed automatically using the auto-calibrated delivery system.

HPLC assays were performed using a Waters 0702503C HPLC system with an ACQUITY ®BEH C18 (1.7 μm, 2.1 × 150 mm) liquid chromatography column as the stationary phase. The mobile phases A and B were acetonitrile and 0.1 % formic acid (v/v) in water, respectively. The optimized elution program was as follows: 0/80, 10/73, 25/60, 35/40, 40/20, 42/0, 45/80, 52/80 (min/B %); sample injection volume of 8 μL; detection wavelengths of 280 nm and 330 nm; column temperature of 25 °C; flow rate of 1 mL/min.

## 2.7. Data analysis and graphing

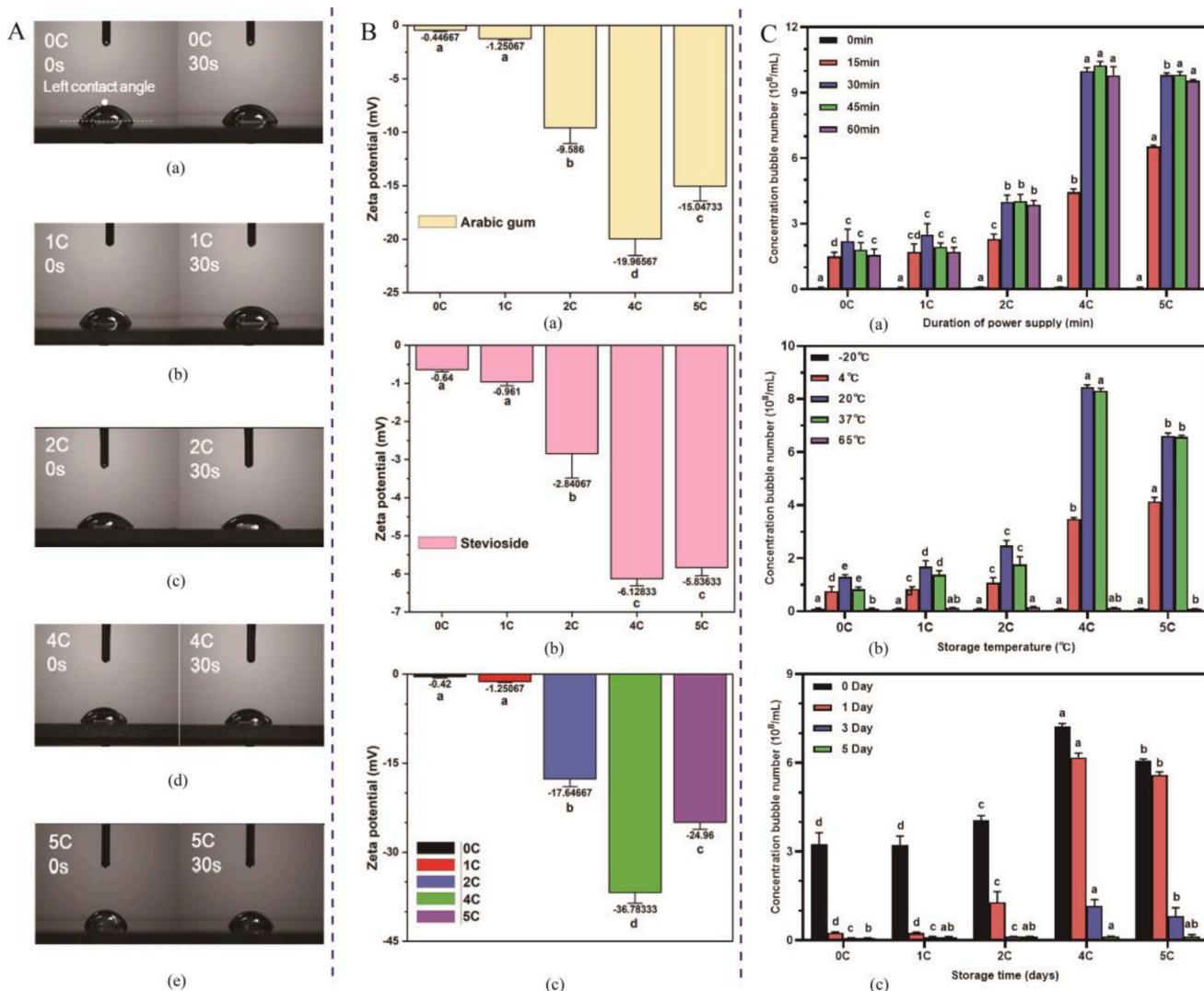
The subsequent smoothing and peak-labelling of the infrared spectral data was conducted utilising EZ OMNIC software. Chromatographic data were processed using PeakView software (version 1.2, AB SCIEX, Toronto, ON, Canada). Statistical analysis was performed using IBM SPSS Statistics 22 software (IBM, Chicago, IL, USA). The data were expressed as mean ± SEM. Multiple group comparisons were performed using one-way ANOVA with Turkey multiple comparisons. The difference was statistically significant. \*or #, p < 0.05; \*\* or ##, p < 0.01; \*\*\* or ###, p < 0.001; \*\*\*\* or ####, p < 0.0001. Different superscript letters on the same line indicates a significant difference (p < 0.05) and the same superscript letters indicate no significant difference (p > 0.05). The data were analysed using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA), OriginPro 2021b Learning Edition (Microcal Software Inc., Northampton, MA, USA), and biorender.com to create graphics.

## 3. Results

### 3.1. Characterisation of NBs

The dynamic contact angle is closely related to the surface tension of substance. The goniometric method was utilised to ascertain the dynamic contact angles of diverse concentrations of green extraction solvents employed for the generation of NBs, thereby facilitating the elucidation of their hydrophilicity and liquid surface state. A contact angle <90° was deemed to be more hydrophilic, and the lower the value, the more hydrophilic. As demonstrated in Fig. 2A and Fig. 3A, low concentrations of solvents exhibited enhanced infiltration and hydrophilicity from 0 to 30 s, as illustrated by 1 C and 2 C. Conversely, concentrations exceeding 4 C have been observed to introduce a measure of lipophilicity, thereby modulating the overall hydrophilicity. To examine whether the bubbles generated and stabilised by our new device and different concentrations of mixture-sol are in the nanoscale, the solution particle size distributions were measured and Gaussian fitting curves were made, and it was found that the particle size of the bubbles stabilised by 0C (Fig. 3B-a) averaged 422 ± 37.62 nm, and the green solvent of 1C concentration (Fig. 3B-c, 233 ± 28.91 nm). The nano-bubbles stabilised by solvent at concentrations ranging from 2C to 5C were within 100 nm, 61 ± 17.29 nm, 36 ± 3.07 nm, and 41 ± 1.45 nm, respectively (Fig. 3B-b,d,e).

The magnitude of the Zeta potential is indicative of the stability of a liquid, with higher absolute values corresponding to enhanced stability. In this study, it was observed that both biosurfactants, when dissolved individually or when mixed with water, exhibited a negative Zeta potential. The stability of both AG (Fig. 2B-a) and STE (Fig. 2B-b) was found to be significantly enhanced when dissolved alone or in mixture (Fig. 2B-c) at 4 °C in comparison to other concentrations. This



**Fig. 2. Characterisation of basic indexes of NBs in different concentrations of green extraction mixture-sol (A. Photographs of dynamic changes in contact angle over 30 s (a-e) B. Zeta potentials of AG (a), STE (b) and mixture-sol (c) C. Effect of duration of energisation (a), resting temperature (b) and resting time (c) on the amount of stabilised bubbles in different concentrations of green solvent).**

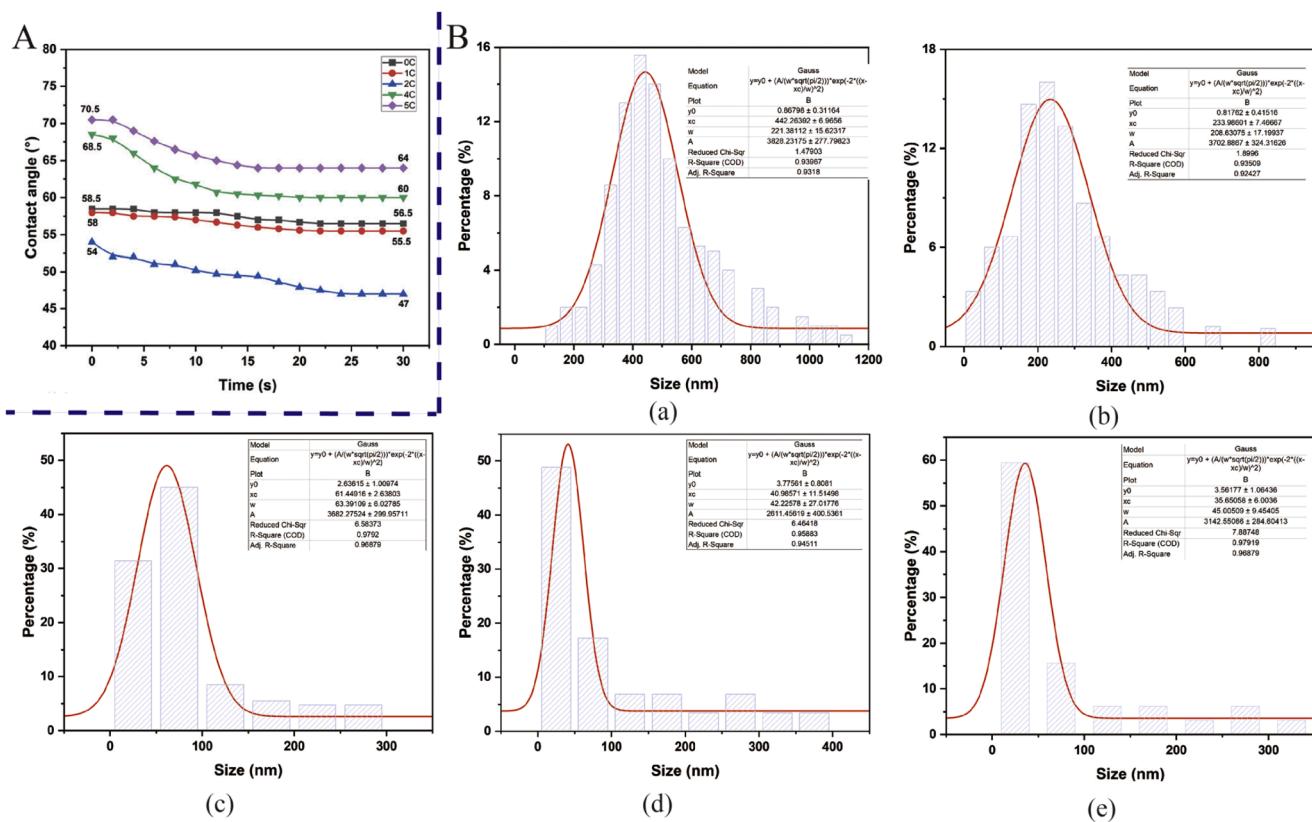
observation is in agreement with the earlier results obtained from the surface tension measurements. Furthermore, it was determined that varying energisation durations, resting times and ambient temperatures affect the density of NBs stabilised by the green solvent. The presence or absence of energisation in the novel device was found to determine the presence or absence of NBs production. The highest concentration of NBs was observed in the green solvent when sustained energisation was maintained for a duration of 0.5 h and above, which is significantly more than the 15-minute threshold. Conversely, an increase in energisation duration has been observed to potentially saturate the NBs in the solution, resulting in their escape and rupture (Fig. 2C-a). Furthermore, it was observed that both cryogenic freezing and elevated temperatures resulted in a substantial reduction of stabilised bubbles in the green solvent compared to ambient temperatures, with low temperatures of 4 °C also significantly reducing the amount of stabilised NBs. Conversely, warming up to around 37 °C had little effect on reducing the bubble concentration (Fig. 2C-b). After 5 days of standing at room temperature, different concentrations of green extraction solvents exhibited varying degrees of bubble stabilisation. It was observed that at 4 °C and 5 °C concentrations, there was a substantial reduction in nanobubbles after 3 days. At 5 °C, it became challenging to sustain nanobubbles in a stable state after 5 days (Fig. 2C-c). This suggests that, in order to make the best

use of NBs, it is better to prepare the green extraction solvents as they are, or use them within 1 day.

It was hypothesised that different concentrations of mixture-sol would show better maintenance potential for NBs at 4 °C concentration under these several different conditions. The hypothesis was formulated that this phenomenon may be attributable to the elevated 5 °C concentration, which exhibits a high surface tension and generates a greater level of bubble resistance in comparison to the 4 °C concentration. Furthermore, the pH of the mixture-sol was measured over a 48-hour period, and it was found to be stable, displaying neutral to weakly acidic characteristics with no significant change (Supplementary Table 1).

### 3.2. Comparison of TP and TF contents between traditional and novel green extraction

In order to compare the effects of different solvents on the yield of TP and TF extracts from citrus peels under the traditional process, the pericarp of seven citrus fruits was extracted by the traditional solvent extraction method using 80 % ethanol, water, and different concentrations of mixture-sol (with or without NBs), respectively. As demonstrated in Fig. 4A, it was observed that extraction solvents containing



**Fig. 3.** Characterisation of basic indexes of NBs in different concentrations of green extraction mixture-sol—continuation.

(A. Measured values of dynamic changes in contact angle over 30 s. B. Particle size distribution of NBs stabilised by mixture-sol at concentrations of 0 C, 1 C, 2 C, 4 C and 5 C (a-e).

NBs exhibited significantly higher extraction rates of TP from 'Gongchuan' pericarp in comparison to those lacking NBs. Conversely, in the extraction of TF from 'Gongchuan' pericarp, while NBs did play a role, their effect was not found to be statistically significant. Subsequent to this, a comparative and analytical approach was employed to assess the impact of green extraction solvents containing NBs on the extraction of TP and TF from several additional citrus pericarp under conventional extraction conditions.

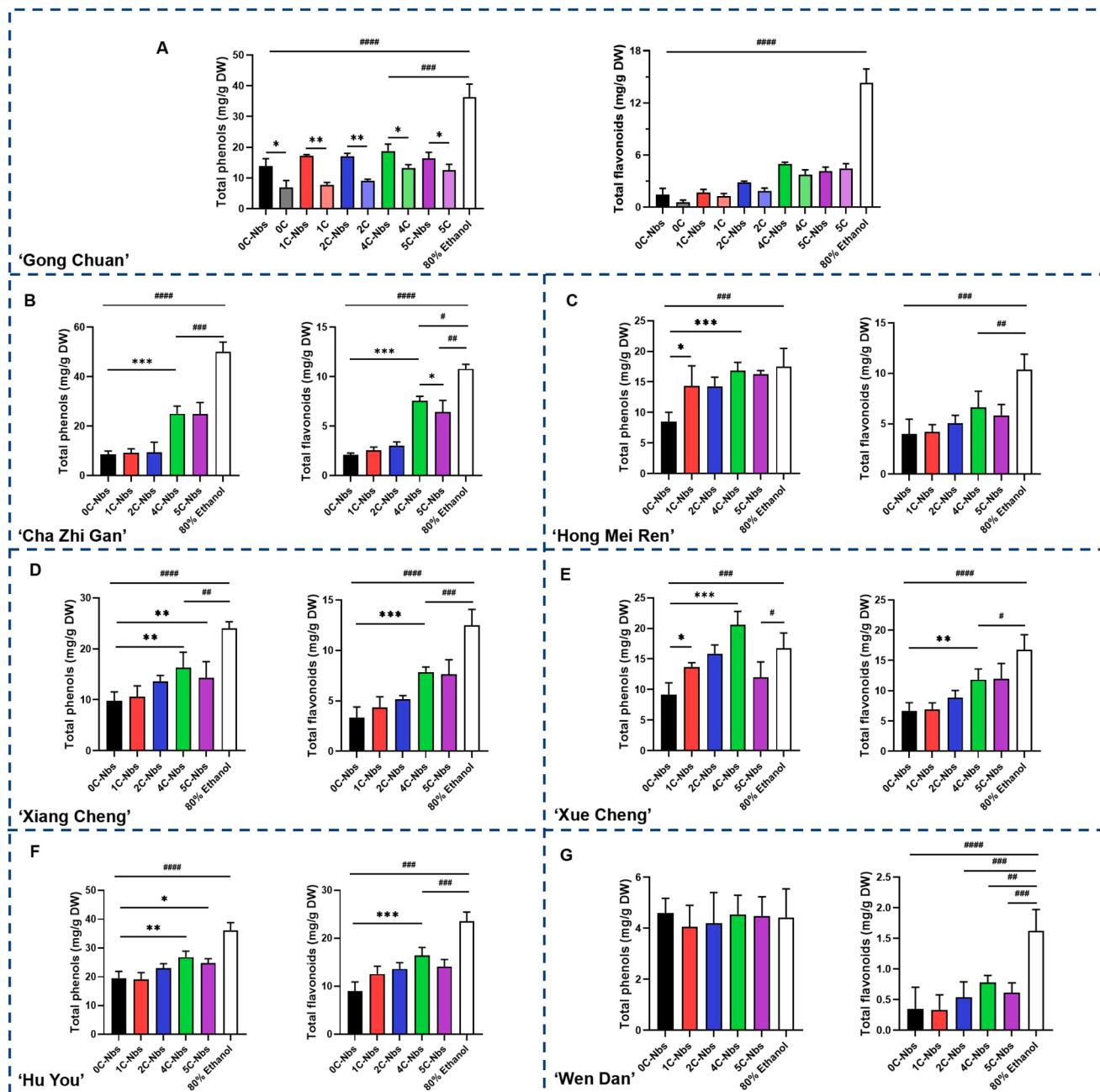
The results demonstrated that there was no significant difference in TP content of the 'Wendan' pericarp under different concentrations of the mixture-sol (Fig. 4B-G). In the extraction of TP and TF from the pericarp of several other citrus fruits, the green extraction solvent at the concentration of 4 C was the most effective, followed by 5 C. However, the yields of TP and TF from citrus fruit pericarp were significantly higher in 80 % ethanol solvent than in the mixture-sol under the extraction of the traditional extraction. Consequently, to enhance the extraction efficiency of the green solvent, further optimisation and redesign of the extraction process is necessary. Meanwhile, as a representative species of citrus, 'Chazhigan' peel is closely related to the health industry. Consequently, the present study focuses on 'Chazhigan' pericarp as the target for the subsequent optimised extraction of flavonoids.

### 3.3. Response surface optimisation and validation

The results of the one-way test (Fig. 5A) demonstrate that all four factors, including UD power (0–260 W), mixture-sol concentration (0–5

C), water bath temperature (0–40 °C), and UD duration time (0–60 min), have a significant impact on the extraction rate of TF from 'Chazhigan' pericarp. It was observed that the extraction rate of TF achieved  $13.69 \pm 1.11$  mg/g DW for 15 min at 130 w UD power, which was considerably higher than the extraction rate in the absence of UD treatment ( $2.06 \pm 0.21$  mg/g DW). Subsequent to placing the samples in a water bath at varying temperatures for 30 min, followed by 130 w UD for a further 15 min, it was observed that the extraction rate from the water bath at 30 °C was  $15.03 \pm 1.07$  mg/g DW, which was significantly higher than that from the non-water bath treatment ( $4.26 \pm 0.50$  mg/g DW). Subsequent to this, under 130 w ultrasonic crushing-assisted extraction for 15 min, it was determined that the extraction rate of TF by 4 C concentration of green solvent could reach  $18.25 \pm 0.75$  mg/g DW, which was significantly higher than that of water extraction ( $5.60 \pm 0.28$  mg/g DW). Finally, the UD time was prolonged, and it was found that the extraction rate of total flavonoids from 'Chazhigan' with 4 C concentration solvent, pre-treatment in water bath at 30 °C, followed by 130 w ultrasonic crushing for 30 min, was  $20.63 \pm 1.28$  mg/g DW, which was significantly higher than that of extraction without ultrasonic crushing ( $7.99 \pm 1.73$  mg/g DW).

RSM has been demonstrated to be a highly effective tool for optimising the process. To this aim, analysis of variance (ANOVA) was performed on the response surface optimisation regression equation model (Table 2) using the TF extraction rate from 'Chazhigan' pericarp as the response value (Table 1). The analysis yielded a *P*-value of <0.05, thereby indicating that all four independent variables were found to be the primary influencing factors. The model was deemed to be highly



**Fig. 4. Comparison of TP and TF contents of seven citrus pericarp extracted by traditional solvents and green solvents (A-G TP, TF content of 'Gongchuan', 'Chazhigan', 'Hongmeiren', 'Xiangcheng', 'Xuecheng', 'Huyou', 'Wendan' pericarp tissue). Each treatment was repeated 3 times, and the error bars were expressed as mean  $\pm$  SEM.**

significant, with no significant misfit term, indicating the feasibility of the optimisation model.

The optimisation equations are formulated as:

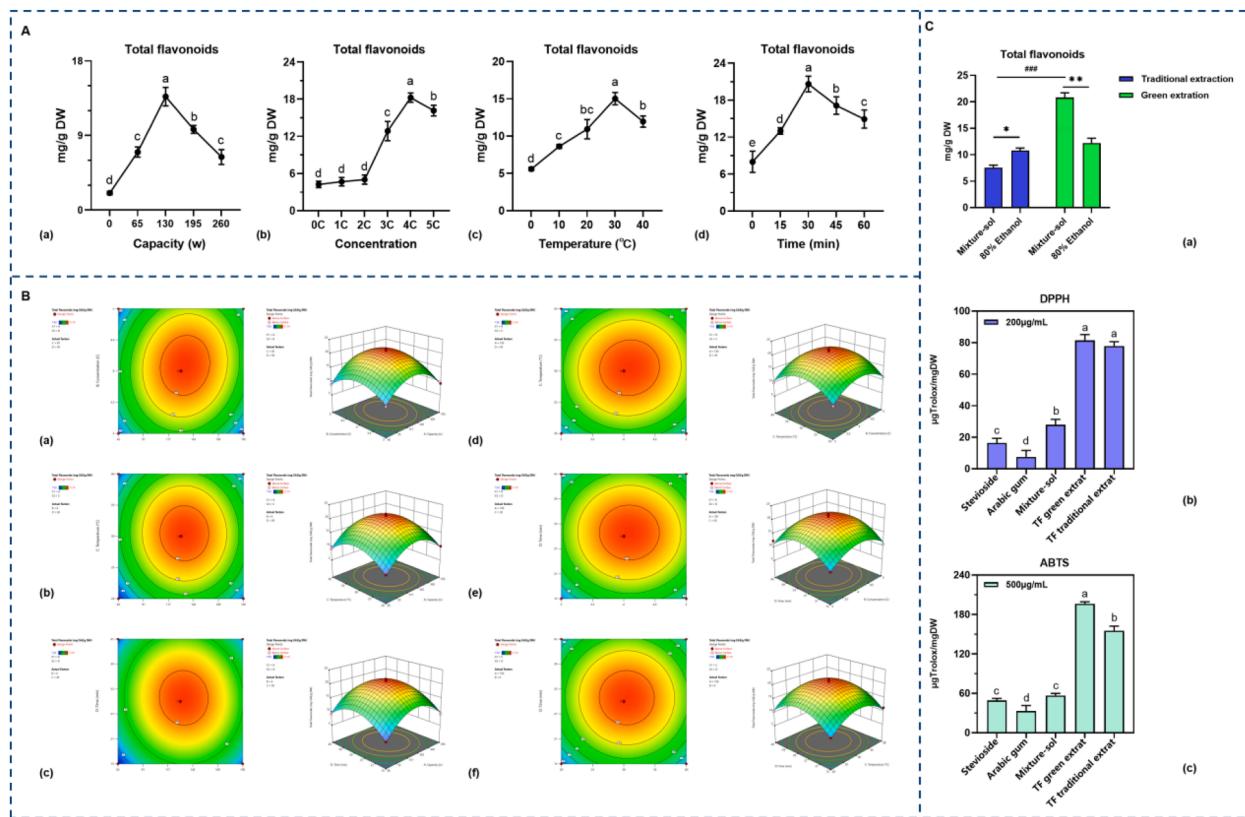
$$Y_{TF} = 20.96 + 0.7767 * A + 0.7617 * B + 0.4983 * C + 0.64 * D + 0.9275 * AB - 0.145 * AC - 0.3525 * AD + 0.44 * BC - 0.0475 * BD - 0.09 * CD - 6.59 * A^2 - 4.46 * B^2 - 5.1 * C^2 - 5.24 * D^2$$

[ $Y_{TF}$  is an abbreviation for "yield of total flavonoids". The four variable values represent UD power (A), extraction solvent concentration

(B), water bath temperature (C) and UD time (D).]

Following the computer prediction simulation, a three-dimensional response surface analysis was conducted (Fig. 5B). The degree of flattening of the contour map and the arching arc of the response surface

map were observed to judge the intersection effect among the four factors. The intersection effect between UD power and mixture-sol concentration was found to be significant, with the concentration



**Fig. 5.** Response surface experiment model analysis and chemical antioxidant results.

(A.) Effect of UD capacity (a), mixture-sol concentration (b), water bath temperature (c) and UD time (d) on the extraction of TF from ‘Chazhigan’ pericarp. B. Contour plots of two-by-two intersection effect of the influencing factors and 3D response surface plots (a) capacity intersected with concentration, (b) capacity intersected with water bath temperature (c) ultrasonic crushing power intersected with time (d) concentration intersected with temperature, (e) concentration intersected with time (f) temperature intersected with time. C. Validation of optimised method and chemical antioxidant evaluation of extracts (a) optimisation of the extraction scheme Extraction rates of TF from ‘Chazhigan’ pericarp by different solvents (b-c) DPPH and ABTS method to determine the antioxidant capacity of biosurfactants, solvents and extracts).

**Table 2**

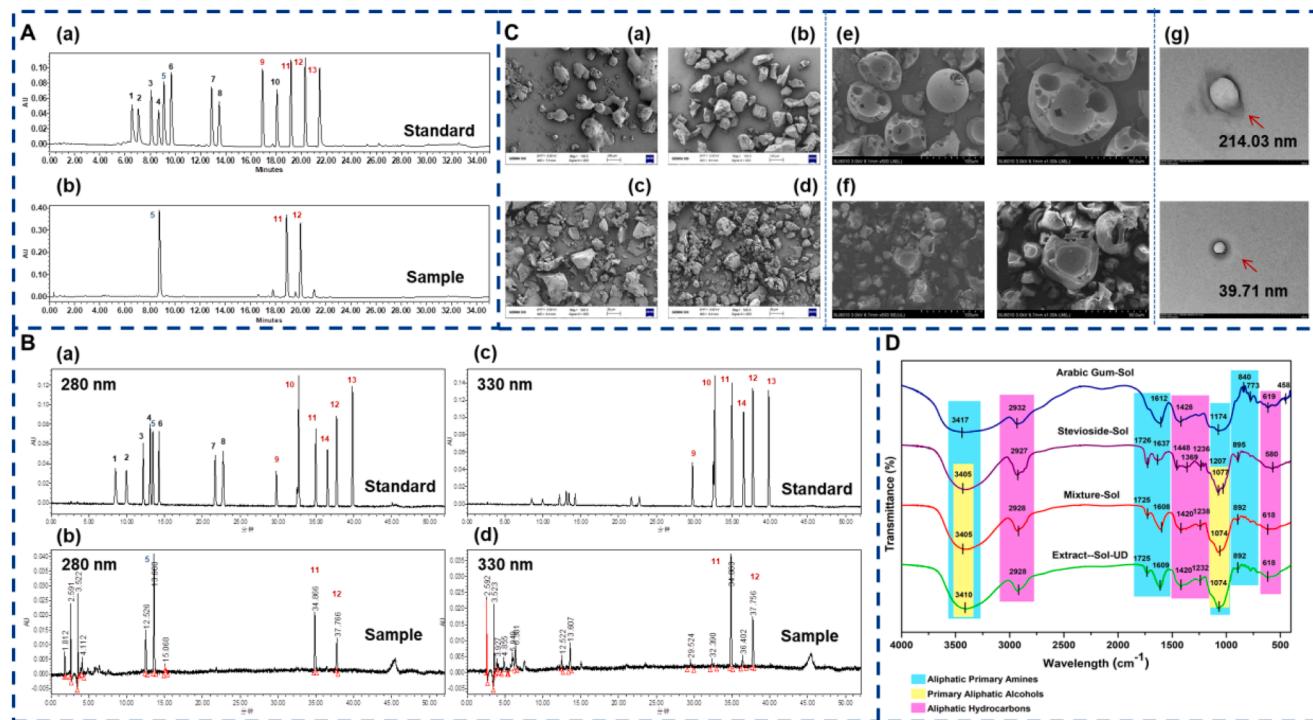
Response surface experiment optimisation regression model ANOVA results.

Source	Sum of squares	df	Mean Square	F-value	P-value	Significance
Model	519.48	14	37.11	61.71	< 0.0001	**
A-Capacity	7.24	1	7.24	12.04	0.0038	**
B-Concentration	6.96	1	6.96	11.58	0.0043	**
C-Temperature	2.98	1	2.98	4.96	0.0429	*
D-Time	4.92	1	4.92	8.17	0.0126	*
AB	3.44	1	3.44	5.72	0.0313	*
AC	0.0841	1	0.0841	0.1399	0.714	
AD	0.497	1	0.497	0.8266	0.3786	
BC	0.7744	1	0.7744	1.29	0.2755	
BD	0.009	1	0.009	0.015	0.9042	
CD	0.0324	1	0.0324	0.0539	0.8198	
A <sup>2</sup>	281.47	1	281.47	468.14	< 0.0001	**
B <sup>2</sup>	129.17	1	129.17	214.82	< 0.0001	**
C <sup>2</sup>	168.87	1	168.87	280.86	< 0.0001	**
D <sup>2</sup>	178.1	1	178.1	296.21	< 0.0001	**
Residual	8.42	14	0.6013			
Lack of fit	5.64	10	0.5639	0.8117	0.6424	
Pure error	2.78	4	0.6947			
Cor total	527.9	28				
R <sup>2</sup>	0.9841					
R <sup>2</sup> <sub>Adj</sub>	0.9681					

Note: ANOVA analysis, \* is significant ( $p < 0.05$ ) and \*\* is highly significant ( $p < 0.01$ ).

exhibiting a more pronounced effect. The optimal conditions for extracting TF from ‘Chazhigan’ pericarp were determined as follows: UD power of 135 W, mixyure-sol concentration of 4.1 C, water bath temperature of 30.5 °C, and UD time of 31 min.

Subsequently, the flavonoid yield from ‘Chazhigan’ pericarp was examined under optimal extraction conditions. The result (Fig. 5C-a) indicated that the novel green solvent extraction approach yielded a significantly higher TF concentration ( $20.82 \pm 0.85$  mg DW) when



**Fig. 6. Characterisation related substances in extraction process** (A) 280 nm UPLC spectra of (a) 13 flavonoid standards (b) Flavonoids extract B. 280 nm and 330 nm HPLC spectra (a,c) 14 flavonoid standards (b,d) Flavonoids extract. The corresponding substances were as follows: 1, Eriocitrin; 2, Neoeriocitrin; 3, Narirutin; 4, Naringin; 5, Hesperidin; 6, Neohesperidin; 7, Didymin; 8, Poncirin; 9, Isosinensetin; 10, Sinensetin; 11, Nobletin; 12, Heptamethoxyflavonoids; 13, Tangeretin; 14, Demethylnobletin. C. SEM and TEM of extracts and individual NBs (a) traditional aqueous extracts (b) green solvent extracts (c) green solvent extraction without UD (d) novel green solvent extraction (e,f) Morphology of STE and AG at magnification of 500  $\times$  and 1k  $\times$  in SEM (g) TEM of single NBs stabilised in 0C and 4C green solvents D. Fourier infrared spectroscopy of lyophilised extracts of flavonoids from different green extraction solvents.

compared to the 80 % ethanol extraction ( $12.22 \pm 0.90$  mg/g DW). The in vitro antioxidant assay demonstrated that both biosurfactants exhibited antioxidant activity, and the mixture-sol enhanced the antioxidant activity of the bioactive agents (Fig. 5C-b,c). The antioxidant activity of the TF extract of ‘Chazhigan’ pericarp extracted by green solvent was found to be higher than that of the ethanol extract.

#### 3.4. Characterisation of related substances in extraction process

TF extracted from ‘Chazhigan’ pericarp following the novel green extraction process were qualitatively analysed by UPLC (Fig. 6A) and HPLC (Fig. 6B). The spectra of 13 flavonoid standards (280 nm) and the corresponding spectra of the flavonoid extract are shown in Fig. 6A-a, b, respectively. The UPLC substance identification and HPLC peak times are shown in supplementary Tables 2 and 3. The substances labelled in blue in Fig. 6 are Hesperidin, and the substances labelled in red are polymethoxyflavones. The analysis of the standard calibration revealed the presence of the following main substances in ‘Chazhigan’ pericarp: No 5 Hesperidin, No 11 Nobletin and No 12 Tangeretin. Fig. 6B-a,c illustrates the spectra of 14 flavonoid standards at 280 nm and 330 nm, respectively. It was determined that the analysis of polymethoxyflavonoids was more efficiently conducted using the 330 nm spectrum. The corresponding HPLC spectra of the extracts are shown in Fig. 6B-b,d, and it was found that Hesperidin and two polymethoxyflavonoids could also be extracted and identified. The other peaks could be some potential other substances, but at very low levels.

A comparison of the SEM of the extracts obtained by different extraction methods (Fig. 6C-a-d) reveals that the green extraction solvent can more uniformly encapsulate the extracts to be extracted, while UD-assisted extraction can facilitate the disruption of the surface of the extracts to be extracted. Observation of the two biosurfactants provides further insight into their morphology and structure. As demonstrated in

Fig. 6C-e,f, both STE and AG are found to contain pores and possess internal cavities. At equivalent magnifications, individual STE manifest as slightly larger than AG and are more regular in shape, while AG exhibits a slightly wrinkled surface. The cavity theory posits that this internal structure facilitates the binding of solute molecules into the cavities of the molecular network through the formation of intermolecular hydrogen bonds, thereby enhancing solubility. Furthermore, the TEM observations of the morphology and size of the bubbles generated by water and the green extraction solvent demonstrate that the NBs generated by the green extraction solvent possess a homogeneous ‘shell’ on the exterior, which is more stable.

The application of Fourier infrared (FT-IR) spectroscopy to the analysis of biosurfactants and flavonoid extracts revealed the presence of characteristic infrared peaks ( $\text{cm}^{-1}$ ) in the range of 3400 (O—H), 2880–2932 (C—H on the tetrameric ring), 1725 and 1608–1637 (C=O, C=C), 1074 (C—O), and 895 (glycosidic bond). It was established that the hydrolysed product of STE and AG complexed with pyranoside structural properties, with a change in the peak intensity of the telescoping vibration around 3400 (O—H) accompanied by a slight shift of the peaks, which may have occurred as a result of hydrogen bonding complexation. The IR spectra of TF extracts were found to be consistent across different solvents, suggesting that they may be encapsulated and transported within the biosurfactant cavity. The absence of chemical reactions between the flavonoid extracts and the green solvent was further confirmed, with only a change in their own dispersion state and intermolecular forces being observed.

#### 4. Discussion

As a significant by-product of the citrus processing industry, citrus peel is susceptible to wastage of resources and environmental contamination (Han et al., 2022). Nevertheless, the peel of citrus fruits is

abundant in polyphenols and flavonoids, which exhibit a wide range of bioactivities, with the highest concentrations being found in the peel. Consequently, the potential of citrus peel as a resource base for further exploration and development is significant, particularly in relation to its health benefits. For instance, the 'Chazhigan' pericarp is a well-known raw material in traditional Chinese medicine, specifically in the formulation of 'Chenpi', a medicinal food product. This study focuses on the selection of this species for the optimisation of the extraction process.

The present study encompasses a total of seven representative citrus species. The traditional solvent method for extracting citrus peel flavonoids predominantly employs organic solvents, such as methanol and ethanol, which are detrimental and toxic to the human body. Conversely, previous researchers opted for natural low eutectic solvents, such as choline chloride. However, it is important to note that non-food grade organic reagents may still be toxic to the human body (Jung et al., 2021; Marchel et al., 2022). Consequently, if they are to be used for trans-oral experiments, it may be necessary to find a way to remove solvent effects. In this study, two natural biosurfactants, food-grade STE and AG, were selected to ensure a safer and more environmentally-friendly approach. Furthermore, the chemical antioxidant assay revealed that both reagents possess inherent antioxidant properties, with the compounding process enhancing their antioxidant capacity. The anti-inflammatory, hypoglycaemic of STE have been previously demonstrated (Alavala et al., 2020; Dutta et al., 2024), while AG, the gold standard of food-grade emulsifiers, has been shown to have activities such as the prevention of obesity (Ahmed et al., 2022), the provision of prebiotics (Avelino et al., 2022) and soluble fibre (Suresh et al., 2022), and the ability to solubilise active substances *in vivo*. Consequently, it has been demonstrated that the combination of these two agents not only facilitates the solubilisation of bioactive components found in fruits and vegetables, but also functions as structural materials such as encapsulants, nano-self-assembled particles (Zhong et al., 2024), nano-micelles, biofilms (Huang et al., 2025) and analogous entities, thereby enabling the sustained release of active principles within the living organism. The extracted product in this study was also a mixture of solvents (AG, STE) and flavonoids, which can be explored in depth in the future in two ways. Firstly, a method for removing the solvent and purifying the flavonoid monomer must be established. Alternatively, the potential for the mixture of extracts to synergise antioxidant or other beneficial effects *in vivo* merits exploration.

Both inspiration and materials utilised in the design of the new device are drawn from life. In the process of raising pet fish in daily life, the authors found that the accumulation of organic matter in the water column with the nano air disc waterfall oxygen results in the production of a substantial number of dense micro-bubbles that are not easily to burst. These micro-bubbles exhibit excellent stability and can be retained for a period of 24 h. The extraction container of this device is a glass jar that is utilised in the brewing fermentation process on a daily basis. The glass jar not only ensures the retention of the micro-bubbles, but also provides a high-temperature-resistant material that facilitates the execution of the design and experimental processes. Conducting. In recent years, NBs and ultrasonic technology have emerged as prominent green technologies in the food processing industry (Cerdá-Bernard et al., 2023; Javed et al., 2024). Previous applications of NBs in extraction primarily utilised the gas cylinder depressurisation method (Javed et al., 2022; Nishad et al., 2019). This approach has two notable drawbacks. Firstly, the gas cylinder must be replaced when depleted. Secondly, the process entails a certain degree of risk and increases operational costs. In contrast, the device under scrutiny in this study can be directly energised and fed with a constant supply of air without the need to replace the gas cylinder, which is simple to operate and can reduce the cost of gas. However, in the optimised extraction process, during the ultrasonic crushing process, it is imperative for the experimenter to maintain constant vigilance to ensure that the probe does not come into contact with the inner wall of the beaker. It is also noteworthy that the heat

generation during ultrasonic crushing is significant, necessitating the placement of ice cubes on the exterior of the beaker containing the liquid to be measured, thereby safeguarding the instrument. This aspect can be further optimised in future studies. Furthermore, the solubilisation of total phenols and flavonoids in aqueous solvents was not achieved in the peel of all citrus varieties in this study, e.g. 'Wendan'. Consequently, it may be worthwhile to persist in the future in the comparison of the types, contents and structures of the flavonoid monomer components in different citrus pericarp, with a view to exploring the relationship between different structures and solubilising effects.

Combined with previous studies (Silva-Espinoza et al., 2021), AG is a kind of high molecular polysaccharide with proteins, which is easy to decompose into rhamnose, arabinose, and glucuronic acid in water. AG and STE contain a large number of hydroxyl and carboxyl groups, which are capable of acting as hydrogen-bonding acceptors or donors in water, thereby altering the interactions between the forces and, in turn, affecting the dispersion state of flavonoids in the pericarp, thus enhancing the solubility of flavonoids in water-based solvents. The zeta potential of NBs generated from the green solvent was found to be stable and electronegative.

The results of the SEM and TEM analysis demonstrate that the stable NBs can increase the contact area with the samples to be extracted, prolong the contact time between the samples and the biosurfactant, and rupture at a high speed to produce a 'burst'-like energy under UD-assisted extraction. This is conducive to the binding of solvents to the samples. Concurrently, the structural characteristics of AG and STE indicate their amphiphilic nature, signifying the presence of a hydrophilic exterior and a hydrophobic interior. Moreover, given that their molecular weights exceed those of flavonoids commonly found in citrus, it is conceivable that the flavonoids may be encapsulated into their hydrophobic cavities, thereby enhancing their solubility in water. In previous studies, we found that there are other amphiphilic substances with similar structures that are applied in the encapsulation and slow release of active substances. For instance,  $\beta$ -cyclodextrin (Sun et al., 2021) and zeinolysin (Bautista et al., 2019). While  $\beta$ -cyclodextrin has been more extensively studied for its interactions with Naringenin (Liang et al., 2022) and Quercetin (Chien et al., 2022) in citrus due to its comparatively low molecular weight, the interactions of polymethoxy-flavonoids are less well understood. This paper presents a discussion of potential mechanisms based on the analysis of results. The modes and sites of interactions between AG, STE and specific citrus peel flavonoids need to be further verified and explored.

## 5. Conclusion

In our study, aim to provide some hints for improving the bioavailability of citrus pericarp, a novel apparatus was designed to establish a green extraction approach for UD assisted biosurfactant stabilisation of novel airborne NBs. The fundamental indexes of the solvents of NBs stabilised by biosurfactants at different concentrations were characterised. The differences in the extraction rates of TP and TF from the peels of seven citrus fruits between different extraction methods were compared. The optimal green extraction conditions of flavonoids from 'Chazhigan' pericarp was also optimised by combining RSM. Finally, the possible mechanisms in the extraction process were discussed.

## Ethical statement

This study does not involve any experiments on humans or animals. All research conducted complies with the relevant ethical guidelines and academic integrity standards of Zhejiang University and the publishing policies of Elsevier. No ethical approval or informed consent was required for this study.

## CRediT authorship contribution statement

**Jiaojiao Liang:** Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Xinyu Shen:** Writing – review & editing, Resources, Formal analysis, Data curation, Conceptualization. **Ziyou Liu:** Validation, Methodology, Investigation, Data curation. **Yixin Hu:** Project administration, Methodology, Investigation, Data curation. **Hui Xu:** Investigation, Formal analysis, Data curation, Conceptualization. **Zimeng Yang:** Writing – review & editing, Visualization, Validation, Resources. **Fei Xie:** Visualization, Software, Resources. **Jinping Cao:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Yue Wang:** Writing – review & editing, Validation, Project administration, Methodology, Funding acquisition. **Chongde Sun:** Supervision, Resources, Project administration, 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.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.fufo.2025.100814](https://doi.org/10.1016/j.fufo.2025.100814).

## Data availability

The data that has been used is confidential.

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