

Flavonoids extracts from Zhenghe white tea

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Article history:

Received: 23 February 2023

Received in revised form: 15 April 2023

Accepted: 23 May 2023

Available Online: 25 January 2024

Keywords:

Zhenghe white tea,
Flavonoids,
Ultrasound-assisted
extraction,
Optimisation

DOI:

[https://doi.org/10.26656/fr.2017.8\(1\).083](https://doi.org/10.26656/fr.2017.8(1).083)

Abstract

This study aimed to investigate the ultrasound-assisted extraction technique for extracting flavonoids from four different grades of Zhenghe white tea (arranged from lowest to highest): *Shou Mei* (SMT), *Gong Mei* (GMT), White Peony (WPT) and Silver Needle (SNT). The ultrasound-assisted extraction parameters (ethanol concentration, liquid-solid ratio, ultrasonic power and extraction time) were optimised to produce crude flavonoid extracts. The optimal parameters were as follows: SMT (60%, 42:1 mL/g, 130 W and 45 mins); GMT (67%, 40:1 mL/g, 100 W and 43 mins); WPT (47%, 31:1 mL/g, 110 W and 52 mins); and SNT (61%, 33:1 mL/g, 100 W and 65 mins). The extraction yields obtained under their respective optimal extraction parameters were as follows: 12.9 (SMT), 12.2 (GMT), 15.0 (WPT) and 16.5% (SNT). It is interesting for industries to note that different ultrasound-assisted extraction parameters are required when different grades of white tea are used as the source of flavonoids.

1. Introduction

White tea, a lightly fermented tea, is one of China's six major tea categories with the highest total flavonoids compared to other teas (Quan *et al.*, 2021). The other 5 categories are green (non-fermented), yellow (another lightly fermented), Oolong (semi-fermented), black (fully fermented) and dark (post-fermented) teas (Zheng *et al.*, 2015). White tea has a relatively more straightforward production process, consisting of tea leaves/bud tips picking, withering and low-heat drying (Li *et al.*, 2021) among the 6 categories. Based on the quality, white tea can be divided into 4 grades: *Shou Mei* (SMT), *Gong Mei* (GMT), *Bai Mu Dan* (White Peony, WPT) and *Bai Hao Yin Zhen* (Silver Needle, SNT) (Ning *et al.*, 2016; Tan *et al.*, 2017). Among these 4 grades, SNT (first grade) is the most expensive white tea as only unopened buds of the tea (*Camellia sinensis*) plant are used. The infused tea from SNT is light yellow with a sweet, vegetal and delicate flavour (Pan *et al.*, 2018).

The difference between WPT (second grade) and GMT (third grade) is in the buds-to-leaves ratio, whereby a proportion of either 1:1 or 1:2 is used to prepare WPT, while a ratio of either 1:2 or 1:3 is used for preparing GMT. The lowest (fourth) grade is SMT, made of mature tea leaves (Ning *et al.*, 2016).

The primary forms of flavonoids in tea leaves are catechins, theaflavins, anthocyanins, leucoanthocyanidins, flavonols and traces of theasinensins, apigenin and patuletin (He *et al.*, 2021). Numerous studies have proven the effects of flavonoids on lowering blood pressure, neuroprotection, anti-tumour, anti-ageing, decreasing blood sugar levels, treating hepatic injury and reducing the morbidity of cardiovascular diseases (Sanlier *et al.*, 2018; Sha *et al.*, 2020; Hinojosa-Nogueira *et al.*, 2021; Li *et al.*, 2021; Li *et al.*, 2022). Various conventional flavonoid extraction techniques were investigated, including maceration percolation, hydro-distillation, boiling, reflux, soaking

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and Soxhlet (Benito-Román *et al.*, 2021; Vo *et al.*, 2021; Zhang, Wang, He *et al.*, 2022). Due to the limitations of conventional techniques (e.g., the use of a large volume of solvent, lower yield, long extraction time, low selectivity, more expensive and adverse impacts on the bioactive compounds and environment), advanced techniques are being developed. These advanced techniques include ultrasound-assisted extraction (He *et al.*, 2018; Zhou *et al.*, 2021; Ji *et al.*, 2022), microwave-assisted extraction (Rehder *et al.*, 2021; Yeong *et al.*, 2021) and solid-phase extraction (Chen *et al.*, 2022). Among these advanced techniques, ultrasound-assisted extraction allows the extraction of flavonoids using less solvent, shorter extraction time, is cheaper, able to preserve the integrity of the flavonoids and can be used for the extraction of types of bioactive compounds, such as polysaccharides, pigments and phenolic compounds (Carreira-Casais *et al.*, 2021).

This study aimed to investigate the ultrasound-assisted extraction technique for extracting flavonoids from different grades of Zhenghe white tea. The effects of various extraction parameters, i.e., ethanol concentration (EC), liquid-solid ratio (LSR), extraction time (ET), extraction temperature and ultrasonic power (UP), on the flavonoid yield were examined before optimising these extraction parameters using response surface methodology.

2. Materials and methods

2.1 Chemicals

All chemicals used were of analytical grade and were purchased from Sigma-Aldrich Co., St. Louis, USA.

2.2 Preparation of white tea powder

Four different grades of Zhenghe white tea (arranged from lowest to highest), i.e., *Shou Mei* (SMT), *Gong Mei* (GMT), White Peony (WPT) and Silver Needle (SNT), were purchased from the Institute of Zhenghe Yungen White Tea Co. Ltd. All the tea samples were from the exact origin and grown in the Zhenghe region of the Fujian province in China. The tea samples were milled into powder using a high-speed pulveriser (Tianjin Tester Instrument, PW80, Tianjin, China). The powders were sieved through a 50-mesh screen and stored in an airtight container.

2.3 Single-factor experimental design for extraction of total flavonoids

Tea powders (1 g) were weighed into Erlenmeyer flasks, whereby ethanol (range: 10 to 50 mL) was added to achieve liquid-solid ratios of 20:1 to 50:1 mL/g (at 5

different proportions). The EC ranged from 40 to 80% (at 5 different concentrations). The extractions were carried out at 55 to 75°C (at 5 different temperatures) for 20 to 100 mins (at 5 different times) using an ultrasonic machine (Kunshan Ultrasonic Instrument, KQ5200DE, Kunshan, China) at 80 to 160 W (at 5 different ultrasonic powers) and 40 kHz. After the extraction, the mixtures were separated using a centrifuge (Heal Force, Neofuge23R, Shanghai, China) at 8,700×g for 10 mins at -5°C. The supernatants were kept at -20°C until analysis.

2.4 Optimisation for extraction of total flavonoids

Based on the single-factor experimental designs, the flavonoid extraction from Zhenghe white tea was optimised using the Box-Behnken design method. Four factors, EC (X_1), LSR (X_2), UP (X_3) and ET (X_4), with 3 levels for each factor (Table 1), were selected due to their significant effects on the extraction yield. The extraction temperature for SMT was 70°C, while 75°C was used for GMT, WPT and SNT. After the extraction, the mixtures were treated similarly, as described in Section 2.3.

2.5 Extraction yield

The total flavonoids content (TFC) of the supernatants obtained from the ultrasound-assisted extraction process was determined using aluminium nitrate colourimetry using rutin as standard (Zhang, Wang, He *et al.*, 2022). Briefly, samples (500 µL) were mixed with 150 µL of sodium nitrite (5%, w/v). After 6 mins, 150 µL of aluminium nitrite (10%, w/v) was added to the mixtures. After another 6 mins, 2 mL of sodium hydroxide (1 M) was added to the mixtures before topping up to 5 mL using deionised water. The diluted remedies were mixed thoroughly and left at room temperature to react for 20 mins. Absorbance at 510 nm was measured using a UV-Vis spectrophotometer (Shanghai Meipuda, V-1100D, Shanghai, China). TFC expressed as µg rutin equivalent per mL, can be obtained by comparing it with a rutin standard curve (0–70 µg/mL). The extraction yield (%) was calculated using Eq. 1.:

$$\text{Extraction yield (\%)} = [(C \times V \times DF) / (W \times 10^4)] \quad (1)$$

Where C is the concentration of the total flavonoids (µg/mL), V is the volume of the supernatant (mL), DF is the dilution factor and W is the weight of tea powder used (g).

2.5 Statistical analysis

Analysis of variance (ANOVA) was used for analysing the data. IBM SPSS Statistics version 20 (IBM Corporation, New York, USA) was used for the statistical analysis. All optimisation of the flavonoid

Table 1. Experimental design and value for the four different grades of Zhenghe white teas: *Shou Mei* (SMT), *Gong Mei* (GMT), White Peony (WPT) and Silver Needle (SNT).

Variables ^a	Coded Level (x)											
	SMT			GMT			WPT			SNT		
	-1	0	+1	-1	0	+1	-1	0	+1	-1	0	+1
X_1	50	60	70	60	70	80	40	50	60	50	60	70
X_2	30	40	50	30	40	50	20	30	40	20	30	40
X_3	100	120	140	80	100	120	100	120	140	80	100	120
X_4	20	40	60	20	40	60	40	60	80	40	60	80
Run ^b	Coded Level for Input Factors ^c				Observed Extraction Yield (%)							
	X_1	X_2	X_3	X_4	SMT	GMT	WPT	SNT	SMT	GMT	WPT	SNT
1	-1	-1	0	0	11.31	11.18	13.86	15.06				
2	+1	-1	0	0	8.54	10.08	13.54	14.93				
3	-1	+1	0	0	11.55	11.50	14.99	15.07				
4	+1	+1	0	0	11.44	8.28	11.21	16.02				
5	0	0	-1	-1	9.11	8.64	14.36	15.39				
6	0	0	+1	-1	11.73	10.14	13.30	15.52				
7	0	0	-1	+1	12.33	11.13	13.49	14.91				
8	0	0	+1	+1	12.54	10.74	12.98	15.94				
9	-1	0	0	-1	11.47	10.40	15.10	15.07				
10	+1	0	0	-1	8.68	7.78	14.49	16.00				
11	-1	0	0	+1	12.07	10.27	14.36	16.07				
12	+1	0	0	+1	12.03	9.97	11.98	16.00				
13	0	-1	-1	0	9.83	10.73	14.38	14.40				
14	0	+1	-1	0	11.98	9.30	14.64	15.63				
15	0	-1	0	0	12.17	11.02	14.18	15.83				
16	0	+1	0	0	12.46	11.61	11.26	15.55				
17	-1	0	-1	0	11.30	10.23	14.71	14.52				
18	+1	0	-1	0	10.27	9.20	14.94	15.39				
19	-1	0	+1	0	11.64	11.04	14.46	15.90				
20	+1	0	+1	0	12.03	10.79	11.98	15.36				
21	0	-1	0	-1	9.89	9.66	14.70	14.89				
22	0	+1	0	-1	12.09	8.34	14.26	15.76				
23	0	-1	0	+1	12.40	10.79	15.04	15.04				
24	0	+1	0	+1	12.57	10.91	11.99	16.66				
25	0	0	0	0	13.32	12.16	15.10	16.84				
26	0	0	0	0	12.72	11.86	15.29	17.03				
27	0	0	0	0	13.14	11.99	15.79	16.52				
28	0	0	0	0	12.93	12.50	15.23	16.61				
29	0	0	0	0	13.02	12.24	15.04	16.81				

^aIndependent variables. X_1 : ethanol concentration (%); X_2 : liquid-solid ratio (mL/g); X_3 : ultrasonic power (W); X_4 : extraction time (min)

^bThe order of the runs was randomised during the experiment to minimise selection error.

^cThe extraction temperature for SMT was 70°C, while 75°C was used for GMT, WPT and SNT.

extraction parameters was performed using Design Expert version 13 (Stat-Easa Inc, Minneapolis, USA).

3. Results and discussion

3.1 Effects of extraction parameters on flavonoids extraction

When the EC increased from 40 to 80%, the

extraction yield of the total flavonoids of the four grades of Zhenghe white tea showed an initial increasing trend followed by a decreasing trend (Figure 1A). The change in the extraction yield may be because when the EC is too high, the polarity of the solvent will be reduced. The lipophilic and viscous substances in the tea will be dissolved in large quantities, hindering the extraction of total flavonoids (Ferreira and Pinho, 2012). Based on the

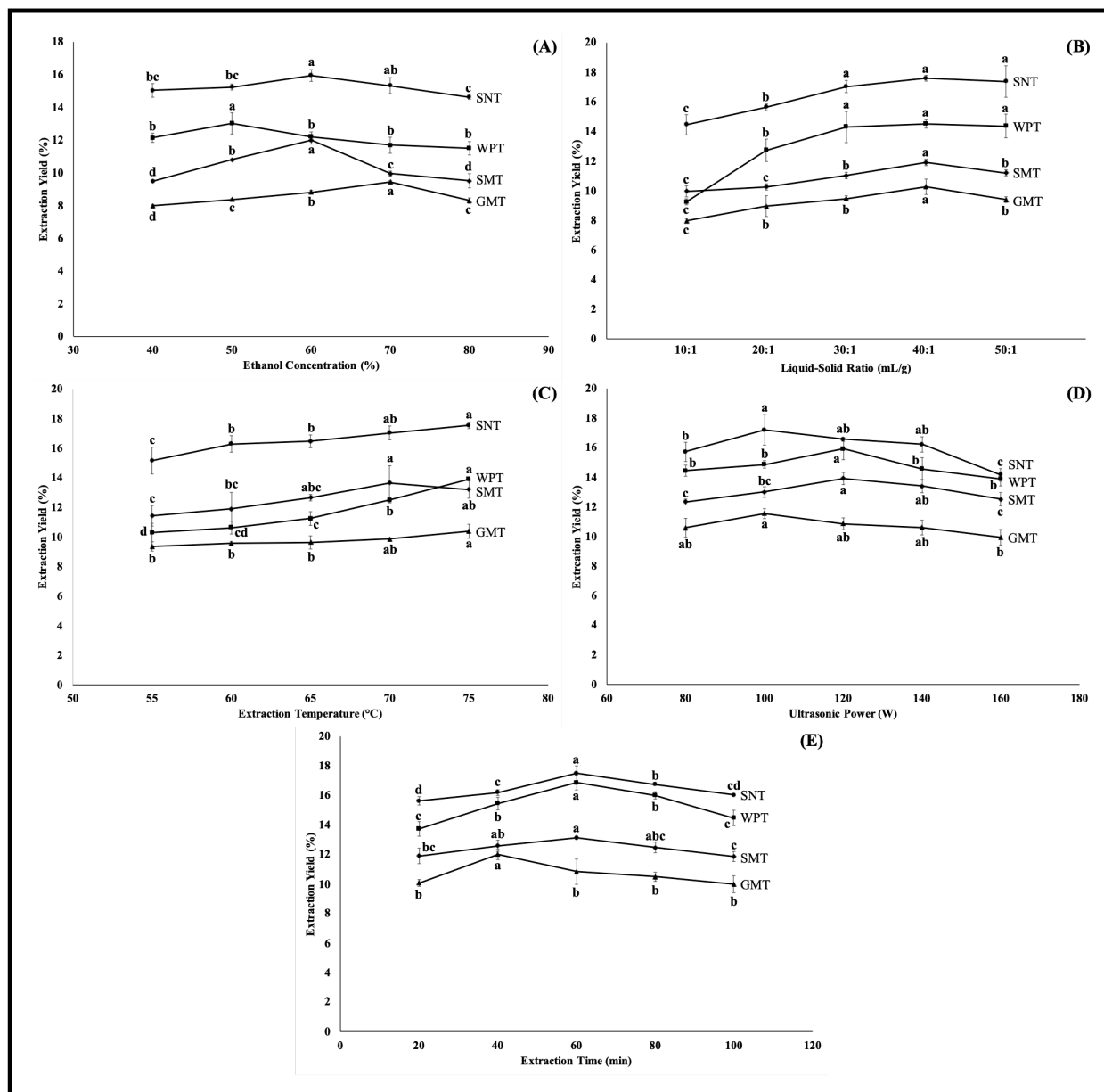


Fig-1. The effects of (A) ethanol concentration, (B) liquid-to-solid ratio, (C) extraction temperature, (D) ultrasonic power and (E) extraction time on the extraction yield of total flavonoids from four different grades of Zhenghe white teas; *Shou Mei* (SMT, ◆), *Gong Mei* (GMT, ▲), *White Peony* (WPT, ■) and *Silver Needle* (SNT, ●). Markers with different letters are significantly different. The highest extraction yield obtained in this single-factor experiment, the optimal EC should be 60% for SMT, 70% for GMT, 50% for WPT and 60% for SNT. In the single-factor experiment, the optimal LSR for all 4 grades should be set at 40:1 mL/g.

When the LSR is in the range of 10:1–50:1 mL/g, the extraction yield of all four grades of Zhenghe white tea showed an initial increasing trend followed by a decreasing trend (Figure 1B). The change in the extraction yield may be due to the increase in the LSR, whereby the contact between tea granules and ethanol solution also increases (Makanjuola, 2017). Hence, this promoted the extraction of flavonoid compounds. However, when the LSR is too high, it could also lead to the excessive dissolution of some alcohol-soluble substances and other impurities (Rosero *et al.*, 2022), thus affecting the dissolution of total flavonoids and reducing the extraction yield. Based on this single-factor

The extraction yield for SMT increased within the range of 55–75°C, reaching a maximum at 70°C, and then began to decline (Figure 1C). Meanwhile, the extraction yield of total flavonoids from GMT, WPT and SNT gradually increased with the increase in temperature. The extraction yield is the highest at 75°C. According to the thermal motion of the molecule, an increase in temperature leads to a faster movement of the flavonoid compounds, which promotes the migration of these compounds in the solvent (He *et al.*, 2021). Thus, increasing the extraction yield. However, if the temperature is too high, ethanol will evaporate, reducing the EC and decreasing the extraction yield (Zhang, Su, Chu *et al.*, 2022). Furthermore, when exposed to high

temperatures, flavonoids can be degraded (Mello *et al.*, 2010). Based on these findings, the extraction temperature range is very narrow. Hence, extraction temperature is not included in the response surface methodology study and was treated as a fixed variable. Therefore, the extraction temperature for SMT is 70°C, while 75°C was used for GMT, WPT and SNT.

When the UP is in the 80–160 W range, the extraction yield of all four grades of Zhenghe white tea shows a familiar inverted-u shape trend (Figure 1D). The change in the direction may be because cavitation and mechanical effects are enhanced when the UP is too high (Al-Dalalmeh *et al.*, 2022; Zhou *et al.*, 2022), and then the molecular structure of flavonoids is destroyed (Ji *et al.*, 2022). These cause a decrease in the extraction yield. Based on the highest extraction yield obtained in this single-factor experiment, the optimal UP should be 120 W for SMT and WPT, while 100 W for GMT and SNT.

A similar inverted-u shape trend was observed when the ET ranged from 20 to 100 mins (Figure 1E) in all 4 grades of Zhenghe white tea. When more ET is provided, this increases the extraction yield. However, when extended ET was provided, more impurities would also end up in the solvent (Vo *et al.*, 2021), affecting the total flavonoids in the extract (Azahar *et al.*, 2017). Based on the highest extraction yield obtained in this single-factor experiment, the best ET should be 40 mins for SMT, while 60 mins for GMT, WPT and SNT

3.2 Response surface analyses on flavonoids extraction for different grades of Zhenghe white tea

The extraction yield ranged from 7.78–17.03% (Table 1). The highest yield for different grades of Zhenghe white tea was obtained under different extraction parameters. The order of the 4 grades affecting the extraction yield is SNT (17.03%) > WPT (15.79%) > SMT (13.32%) > GMT (12.50%). This trend could be linked to the material and ratio of these grades. The flavonoid concentration in tea leaves tends to diminish as they mature (Li *et al.*, 2015; Lv *et al.*, 2022). Since the ratio of buds to leaves is highest in SNT compared to the 4 grades of Zhenghe white tea (Ning *et al.*, 2016), it is no surprise that it has the highest yield.

The correlation coefficients obtained were more than 90%, indicating that these models can explain at least 90% of the data changes. In addition, all the $P_{\text{lack-of-fit}} > 0.05$ suggests that the lack of fit is insignificant. The intensity of each extraction parameter on the extraction yield varies and depends on the grade of Zhenghe white tea; SMT (UP > LSR > EC > ET), GMT (ET > EC > UP > LSR), WPT (EC > UP > LSR > ET) and SNT (LSR > UP > EC > ET). The results obtained in this study show

that the response surface models are effective and reliable for the data of extraction yield for the 4 grades of Zhenghe white tea. The significant interactions ($P < 0.05$) between ultrasound-assisted extraction parameters on the extraction yield for the 4 different grades of Zhenghe white teas are shown in Figure 2. The data were fitted to second-order polynomial models (Eq. 2–5) for SMT, GMT, WPT and SNT, respectively.

$$Y = 13.02 - 0.5289X_1^\# + 0.6638X_2^\# + 0.6468X_3^\# + 0.9140X_4^\# + 0.6647X_1X_2^\# + 0.3538X_1X_3 + 0.6861X_1X_4^\# - 0.4664X_2X_3^* - 0.5066X_2X_4^* - 0.6004X_3X_4^\# - 1.2800X_1^{2\#} - 0.7932X_2^{2\#} - 0.6471X_3^{2\#} - 0.7074X_4^{2\#} \quad (2)$$

$$Y = 12.15 - 0.7092X_1^\# - 0.2933X_2^* + 0.5095X_3^* + 0.7377X_4^\# - 0.5299X_1X_2^* + 0.1941X_1X_3 + 0.5789X_1X_4^* + 0.5039X_2X_3^* + 0.3606X_2X_4 - 0.4712X_3X_4^* - 1.1400X_1^{2\#} - 0.8068X_2^{2\#} - 0.6594X_3^{2\#} - 1.380X_4^{2\#} \quad (3)$$

$$Y = 15.29 - 0.7782X_1^\# - 0.6129X_2^\# - 0.6978X_3^\# - 0.5307X_4^\# - 0.8630X_1X_2^\# - 0.6754X_1X_3^* - 0.0422X_1X_4 - 0.7987X_2X_3^\# - 0.6540X_2X_4^* + 0.1367X_3X_4 - 0.6986X_1^{2\#} - 0.8956X_2^{2\#} - 0.8165X_3^{2\#} - 0.6463X_4^{2\#} \quad (4)$$

$$Y = 16.76 + 0.1671X_1^* + 0.3770X_2^\# + 0.3216X_3^\# + 0.1653X_4^* + 0.2680X_1X_2 - 0.3538X_1X_3^* - 0.2493X_1X_4 - 0.38067X_2X_3^* + 0.1876X_2X_4 + 0.2251X_3X_4 - 0.6601X_1^{2\#} - 0.7284X_2^{2\#} - 0.7927X_3^{2\#} - 0.4296X_4^{2\#} \quad (5)$$

Where, Y is the extraction yield (%), X_1 is the EC (%), X_2 is the LSR (mL/g), X_3 is the UP (W) and X_4 is the ET (min). Asterisks (*) and hashes (#) indicate significant effects ($P < 0.05$ and $P < 0.01$, respectively).

The optimal ultrasonic-assisted flavonoid extraction conditions for the highest extraction yield for the 4 grades of Zhenghe white tea were obtained using the Box-Behnken design method (Table 2). The optimal processes were modified to suit the actual extraction process conditions. Verification was carried out under the adjusted conditions, and the extraction yields obtained were near the predicted values (using Eq. 2–5). The variation observed could be due to the modifications made to the extraction parameters.

4. Conclusion

Under the optimal flavonoid extraction process parameters, the extraction yield in ascending order is GMT < SMT < WPT < SNT. Overall, the models generated from this study can be utilised to optimise the flavonoid extraction for these four grades of white tea. However, it should be remembered that these regression equations are sound within the limits of experimental factors and grades of white tea used. Hence, any attempt

Table 2. Verification of optimum flavonoid extraction^a process parameters^b for the four different grades of Zhenghe white teas: *Shou Mei* (SMT), *Gong Mei* (GMT), White Peony (WPT) and Silver Needle (SNT).

Grades	Input Factors ^c				Extraction Yield (%)	
	X_1	X_2	X_3	X_4	Predicted	Observed ^d
SMT	60	42	130	45	13.3	12.9±1.7
GMT	67	40	100	43	12.4	12.2±1.5
WPT	47	31	110	52	15.6	15.0±3.7
SNT	61	33	100	65	16.9	16.5±2.1

^aOptimised values were adjusted to suitable values that are practical for verification.

^bThe extraction temperature for SMT was 70°C, while 75°C was used for GMT, WPT and SNT.

^c X_1 : ethanol concentration (%); X_2 : liquid-solid ratio (mL/g); X_3 : ultrasonic power (W); X_4 : extraction time (min).

^dMean value of three determinations ($n = 3$).

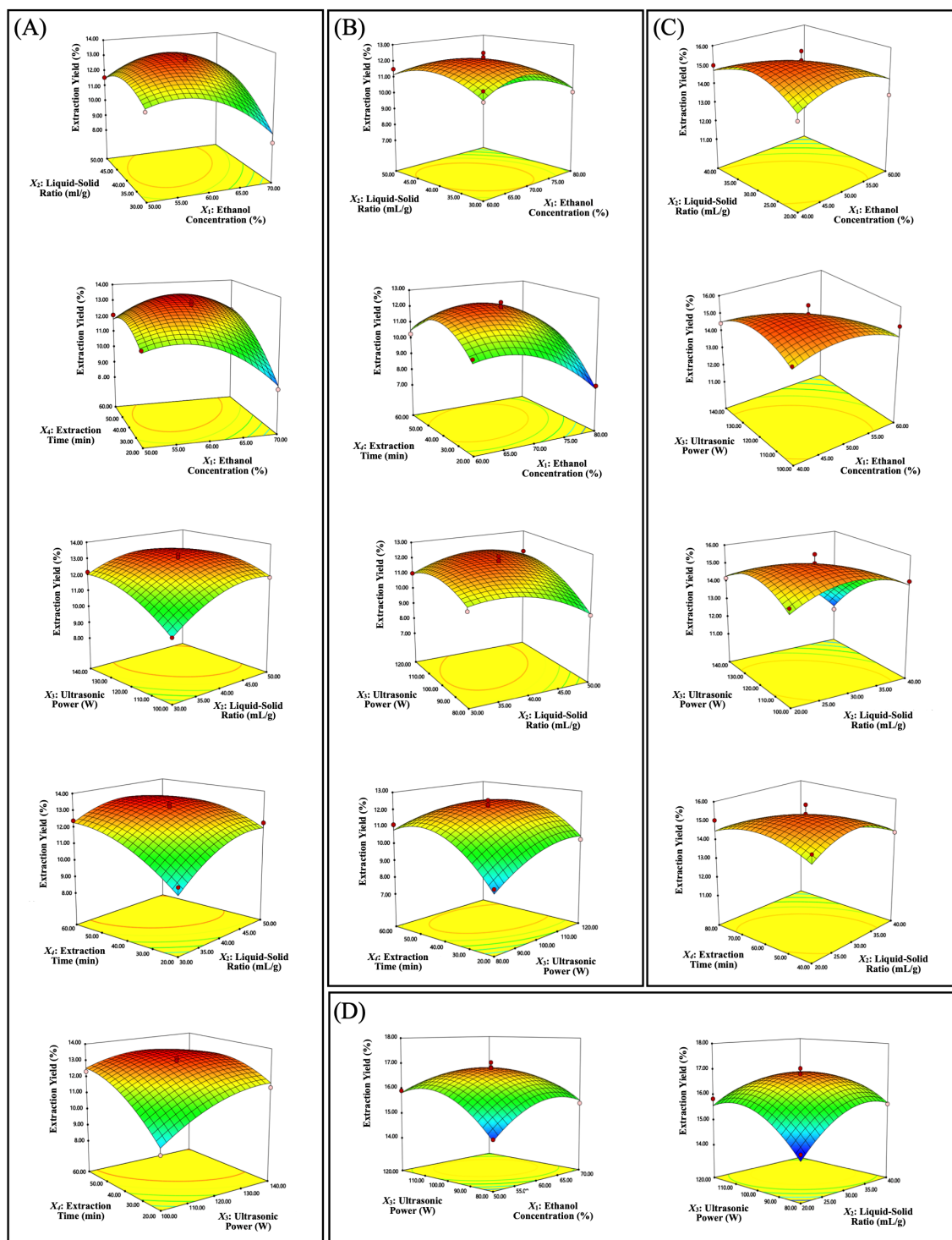


Figure 2. The effects of significant interaction between ultrasound-assisted extraction parameters on the extraction yield for four different grades of Zhenghe white teas; (A) *Shou Mei* (SMT), (B) *Gong Mei* (GMT), (C) White Peony (WPT) and (D) Silver Needle (SNT).

to extrapolate the extraction yield beyond the experimental range of the tested dependent variables used in this study may lead to inaccuracy in the outcome.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The research work was funded by the Fundamental Research Grant Scheme (FRGS/1/2019/STG01/USM/02/8) provided by the Malaysian Minister of Higher Education. This work was also supported by the University-Industry Cooperation Project (No. 2021N5015) of the Science and Technology Department in Fujian Province, China.

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