Effect of ultrasonic-assisted and microwave-assisted technology on bioactive compounds and aroma quality of instant green tea

Xiaozhou Zhou[1,*], Xiuhua Fu[2], Zhusheng Fu[2], Xingqian Ye[1]

[1] School of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China; psu@zju.edu.cn

[2] Zhejiang Minghuang Natural Products Research Institute, Zhejiang, Hangzhou 310058, China; fxh@minghuang-cn.com(X.F.); mhgs@minghuang-cn.com(Z.F.)

[*] Correspondence: 642926385@qq.com

Abstract: The production of instant green tea requires hot-water extraction, which is time consuming and contributes to losses in aromatic compounds. In this study, an ultrasonic-assisted technology was used to improve the extraction efficiency of green tea, thereby shortening extraction time from 45 to 15 min. In pure water, the dissolution of caffeine and theanine did not change significantly, but total catechin dissolution increased by 0.23 mg/mL and total tea polyphenol dissolution decreased by 3.2 mg/mL. In 76.2% ethanol, the dissolution of caffeine and theanine did not change significantly, but total catechin dissolution increased by 1.57mg/mL and total tea polyphenol dissolution decreased by 1.5 mg/mL. Additionally, we used microwave-assisted technology to further improve the extraction efficiency of green tea, which shortened the extraction time to 2 min. However, the extraction rate remained unchanged. In pure water, the dissolution of caffeine and theanine did not change significantly, but the dissolution of total catechins increased by 0.41 mg/mL and the dissolution of tea polyphenols decreased by 2.9 mg/mL. Ultrasonic treatment increased the proportion of 3-hydroxybutan-2-one, (5S)-5-(hydroxymethyl)oxolan-2-one and 2-phenylethanol, which were the main aroma compounds in tea. Microwave treatment changed the aroma compounds in tea, causing losses in aroma compounds with low boiling point and maintaining (5S)-5-(hydroxymethyl)oxolan-2-one. The taste and aroma of instant green tea improved based on sensory evaluation results.

Keywords: ultrasonic wave; microwave; instant green tea; extraction rate; active components; aroma

1. Introduction

The traditional preparation of instant tea involves a hot-water extraction of tea leaves, followed by concentration, sterilization, and drying [1-3]. Soaking tea leaves in hot water for a prolonged period of time contributes to losses in aroma compounds, which requires the addition of artificial flavors to the final product. The flavor of instant tea differs greatly from that of the original leaves [4-5].

Vilkhu et al. [6] reported that the cavitation effects of ultrasonic waves promote the dissolution of tea components. Xia et al. [7] observed that the extraction rate of tea polyphenols, caffeine, theanine, and aroma compounds was higher by ultrasonic-assisted extraction than by traditional extraction. In addition, Horžić et al. [8] concluded that the antioxidant activity of tea increased when ethanol was used in ultrasonic-assisted extraction.

According to Pan et al. [9], the selective heating characteristics of microwaves contribute to a greater dissolution effect of polyphenols and caffeine than traditional extraction. Microwave-assisted extraction can significantly improve the dissolution of tea polyphenols, especially of EGCG [10-11]. In addition, Giorgia et al. [12] reported that microwave-assisted extraction of tea requires a short time, which protects tea polyphenols.

Other technologies have been used in tea extraction. The ultra-high pressure technology accelerates cell breakage and promotes the dissolution of tea components [13-15]. The enzymatic auxiliary technology, suitable when equipment modification is challenging or when raw materials require mild treatment, hydrolyzes cellulose and glycosides, promotes the dissolution of tea components, and improves tea clarity [16-20].

Compared with other extraction methods, ultrasonic and microwave-assisted extraction methods are more energy-efficient and do not introduce impurities. According to preliminary studies, 76.2% ethanol is the optimal extraction solution for tea; however, water is used in industrial extraction methods. In this study, we compared ultrasonic and microwave-assisted extraction of instant green tea.

2. Materials and methods

2.1. Materials

Xinwei Yunnan green tea was obtained on April 9, 2019 from Yunnan Province, China.

2.2. Reagents

Ethanol was of analytical grade, and methanol, acetonitrile, and acetic acid were of chromatographic grade. Besides the above reagents, ferrous sulfate, sodium tartrate, disodium hydrogen phosphate, dipotassium hydrogen phosphate, trichloroacetic acid, EDTA, ascorbic acid, caffeine, gallic acid, catechin standard, and theanine standard were also obtained from Aladdin Biochemical Technology Co., Ltd, Shanghai, China.

2.3. Instruments

The instruments used in the study were a high performance liquid chromatography analyzer (Agilent 1260, Agilent Inc. USA), equipped with a 50/30 m dvb/car/pdms solid-phase microextraction head (US Supelco Company), a 7890B gas chromatograph, and a 5977A DMS mass spectrometer (Agilent Technologies Co., Ltd., USA); a UV9000 ultraviolet spectrophotometer (Shanghai Yidian Analytical Instrument Co., Ltd); a FC5706 centrifuge

(Ohouse International Trading co., Ltd); a water bath (Guohua Electric Appliance Co., Ltd); a laboratory scale (AEL200, Shimadzu Company, Japan); an ultrasonic cleaning machine (KQ5200DB, Kunshan Sumei Ultrasonic Instrument Co., Ltd); and a microwave oven (M1-211A, Guangdong Mei Mei Electric Appliance Co., Ltd).

2.4. Ultrasonic-assisted extraction

Tea leaves (5 g) were placed in a 50-mL centrifuge tube. Extraction solvent was added at a tea-to-water ratio of 1:7 for the first extraction and 1:5 for the second extraction (hereinafter, the ratio of tea to water represents the ratio of tea to 76.2% ethanol solution). The ultrasonic extraction conditions consisted of 70℃, 120 w, and 40 kHz. The first extraction time ranged from 5 to 30 min with a 5-min interval. Following the first extraction, the tea leaves were filtered and transferred to a reagent bottle, and mixed with extraction solvent (tea-to-water ratio of 1:5) for a secondary extraction (extraction temperature did not change). The secondary extraction time was set to 0, 1, 3, 5, 7, and 9 min, where 0 was the filtrate obtained by rinsing the tea residue with solvent after the first extraction. We detected the compounds in the filtrate and calculated the extraction rates and set three replicates for each sample. After determining the optimal extraction time, we optimized the tea-to-water ratios (1:7, 1:8, 1:9, 1:10, 1:11, and 1:12) and extraction temperatures (30℃, 40℃, 50℃, 60℃, and 70℃).

2.5. Microwave-assisted extraction

Tea leaves (5 g) were placed in a 50-mL centrifuge tube, and the extraction solvent, which was mixed with the tea leaves, was added at a tea-to-water ratio of 1:7 for the first extraction and 1:5 for the second extraction. The microwave extraction conditions were 600 w (low power). The first extraction time ranged from 0.5 to 3 min with a 0.5-min interval. Following the first extraction, the tea leaves were filtered and transferred to a reagent bottle, and mixed with extraction solvent (tea-to-water ratio of 1:5) for a secondary extraction. The secondary extraction time ranged from 0 to 1 min with an interval of 0.25 min, where 0 was the filtrate obtained by rinsing the tea residue with solvent after the first extraction. We detected the compounds in the filtrate and calculated the extraction rates and set three replicates for each sample. After determining the optimal extraction time, we optimized the microwave power (750 w, medium power and 900 w, high power) and the tea-to-water ratios (1:7, 1:8, 1:9, 1:10, 1:11, and 1:12).

2.6. Extraction rate

Water extraction was performed based on method GB/T 8305-2013. A clean empty aluminum pan with a lid was weighed (m_1). Leaching filtrate was added and evaporated to dryness on a boiling water bath pan. The aluminum pan was placed in an oven at 120℃ for 1 h, placed in a dryer for 1 h, and weighed (m_2). Subsequently, the aluminum pan was placed in an oven at 120℃ for another 1 h, placed in a dryer for 30 min, and weighed (m_3). When $m_2 - m_3$ was < 0.001 g, the pan was dried to constant weight. Otherwise, the steps were repeated until the aluminum pan had a constant weight of m_n. Leaching rate was calculated using the following equation,

$$\text{Leaching rate} = \frac{(m_n - m_1)}{5} \times 100\%$$

2.7. Determination of Tea Polyphenols

Tea polyphenols were determined based on method GB/T 21733-2008. To 5-mL filtrate, we added 4 mL of water and 5 mL of ferrous tartrate and adjusted the volume to 25 mL using phosphoric acid buffer (pH 7.5). Ferrous

tartrate and phosphoric acid buffer were prepared according to method GB/T 21733-2008. Absorbance was measured at 540 nm. The same volume of the sample without ferrous tartrate was used as a blank. Tea polyphenol concentration was calculated based on the following equation,

$$\text{Tea polyphenol concentration} = \frac{(A_1 - A_2) \times 1.957 \times 2 \times K}{5} \times 10^6 \quad \text{(mg/mL)}$$

where A_1 is the absorbance of the test solution after color development, A_2 is the absorbance of the base color of the test solution, 1.957 is the factor when absorbance is 0.5 in a 10-mm cuvette (the content of tea polyphenols in 1-mL tea is equivalent to 1.957 mg), and K is the dilution factor.

2.8. Determination of Caffeine

Caffeine was determined according to method GB/T 5009.139-2014. To 1 mL filtrate, we added 9 mL of trichloroacetic acid, prepared according to method GB/T 5009.139-2014. The solution was filtered. We injected a standard series of working solutions of caffeine into the liquid chromatograph and measured the corresponding peak areas. We generated a standard curve by plotting the concentration of the working solutions against the peak areas. Caffeine concentration in the samples was calculated according to the following formula,

$$\text{Caffeine concentration} = \frac{c \times V}{v} \times 10^3 \quad \text{(mg/mL)}$$

where c is the mass concentration of caffeine in the sample solution (μg/mL), V is the total volume of the tested sample (mL), and v is the volume of the sample (1 mL).

2.9. Determination of Catechin

Catechin was determined based on method GB/T 8313-2018. An aliquot of filtrate (2 mL) obtained using a 0.45-μm membrane was added to a 10-mL volumetric flask after filtration followed by 8 mL of stabilizer. The solution was passed through a 0.45-μm membrane after shaking into chromatographic column for testing. Stabilizers and catechin working solution were prepared based on method GB/T 8313-2018. Catechin concentration was determined using the following formula,

$$\text{Catechin concentration} = \frac{(A - A_0) \times f_{std} \times v \times d}{V \times 10^3} \quad \text{(mg/mL)}$$

where A is the peak area of the tested component in the sample, A_0 is the peak area of the corresponding tested component in the blank, f_{std} is the correction factor of the measured component (concentration/peak area, concentration unit is μg/mL), v is the volume of sample extract (mL), V is the total volume of the sample to be tested (mL), and d is the dilution factor (5). The total amount of catechins was the sum of five catechins.

2.10. Determination of Theanine

Theanine was determined based on method GB/T 23193-2017. Filtrate (1 mL) was centrifuged at 13,000 rpm for 10 min and analyzed by liquid chromatography. The chromatographic conditions and preparation of theanine were based on method GB/T 23193-2017. Theanine concentration was determined using the following formula,

$$\text{Theanine concentration} = \frac{c \times V \times 1000}{m \times w \times 1000}\ (\text{mg/mL})$$

where c is the concentration of the measured sample (mg/mL), V is the volume of the sample after final volume determination (mL), m is the mass of the sample (g), and w is the dry matter content of sample (%).

2.11. Aroma Analysis

Extract (30 g) obtained by different treatment methods was transferred to a 60-mL bottle and placed in a 50°C water bath for 10 min. Adsorption and desorption time were 30 and 5 min, respectively.

Gas chromatographic conditions: chromatography was performed using a DB-WAX elastic Shi Ying capillary column (30 m×0.25 mm×0.25m) with a carrier gas flow rate of 1.0 mL/min. The program was set to 50°C for 5 min, increased to 160°C at 5°C per min, held at 160°C for 5 min, increased to 250°C at 10°C per min, and held at 250°C for 5 min. The inlet temperature was 250°C.

Mass spectrometry conditions: the ionization energy was 70 eV with an interface temperature of 280°C, an ion source temperature of 300°C, a temperature of the four-stage pole at 150°C, and a scanning quality range of 30–500 u.

2.12. Sensory Evaluation

Samples were concentrated and dried under similar conditions. Sensory analysis was performed using a double-blind test design, and the scoring standard referred to the sensory evaluation method for green tea recorded in GB/T 23776-2018. A total of 10 participants (five were knowledgeable on tea) from the research and development and sales personnel of Hangzhou Branch of Zhejiang Minghuang Natural Food Development Co., Ltd. participated in the analysis.

2.13. Statistical analysis

All measured or calculated parallel data were analyzed using SPSS19.0 for single-factor significance difference.

3. Results and Analysis

3.1. Effect of Ultrasonic Time on Extraction Rate

In the industry, the optimal extraction time for traditional one-time extraction is 30 min. Our findings showed that the extraction rate during a 30-min period was lower with water than with 76.2% ethanol. The extraction rate with water followed the equation $f(t) = -0.0019t^2+0.0847t+0.0295$, $R^2=0.9903$ (Fig 1). At $t_1=15$ min, the extraction rate was 16.8% (maximum value without auxiliary extraction), and at $t_1 = 22.3$(min), the extraction rate was 19.4% (maximum value with ultrasonic treatment). Similarly, the extraction rate with 76.2% ethanol had the following equation, $f(t)= -0.0025t^2+0.1179t+0.0536$, $R^2=0.9919$. At $t_1=15$ min, the extraction rate was 25.6% (maximum value without auxiliary extraction), and at $t_1=23.6$(min), the extraction rate was 28.8% (maximum value with ultrasonic treatment). The extraction rates of the water extraction and 76.2% ethanol extraction groups were significantly different at the two time points. However, considering that the secondary extraction could be carried out and the

extraction time could be reduced to improve product quality, $t_1=15$(min) was selected, followed by a secondary extraction to assess whether the total extraction rate reached a maximum value.
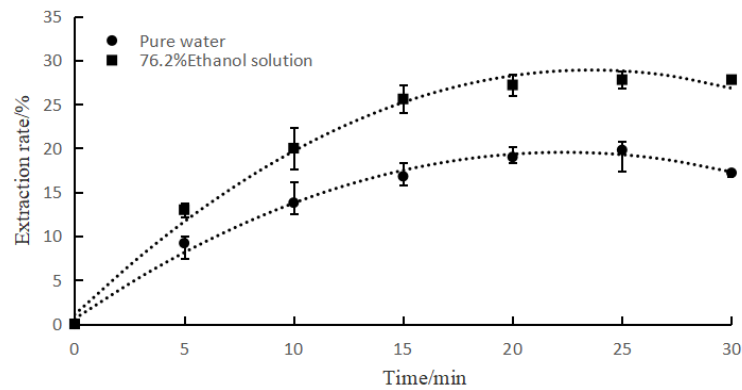

Figure 1. Effect of ultrasonic time on the extraction rate of Xinwei

Based on the conventional industrial operation conditions, the optimal time for the traditional secondary extraction is 15 min. The extraction rate over a 9-min period was measured. Extraction rate was calculated by pooling the filtrates of the two extractions. Extraction rate was lower in the water extraction group than in the 76.2% ethanol extraction group. As shown in Figure 2, the total extraction rate of the water extraction and 76.2% ethanol extraction groups did not show significant changes with time. At $t_2 = 0$(min), the extraction rates were 29.2% in the water extraction group and 39.8% in the 76.2% ethanol extraction group (maximum values without assistance). Moreover, total extraction rate did not increase significantly with time. Therefore, the extraction rate and time of the two extractions were comprehensively evaluated. The optimal extraction time under ultrasonic treatment was 15 min + 0 min, i.e., the first extraction was 15 min, while the secondary extraction was 0, with maximum extraction rates of 29.2% and 39.8%, respectively.
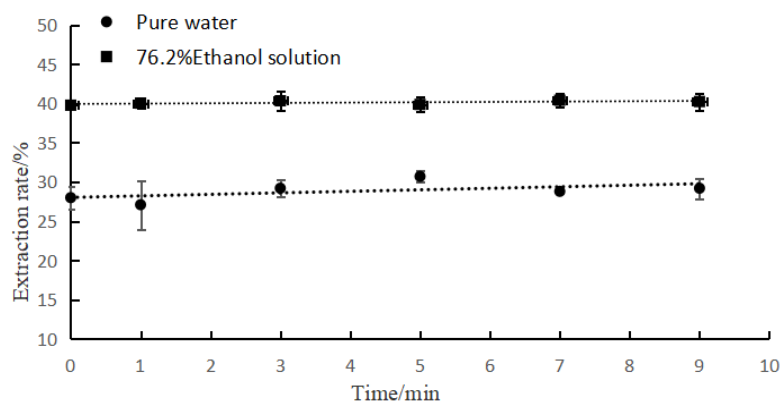
Figure 2. Effect of ultrasonic time on the extraction rate of Xinwei

To conserve water, it is necessary to increase the tea-to-water ratio in the extraction conditions. However, due to the imbibition effect of tea leaves, decreasing the water volume during each extraction would contribute to a reduction in yield. Therefore, to assess the feasibility of increasing the ratio of tea to water, we combined the two extractions. The water consumption for one extraction was increased, and the total water consumption was lower than the total water consumption for the original two extractions. Under the assumption that the optimal extraction time remained at 15 min, the effect of the tea-to-water ratio on extraction rate was investigated. As shown in Figure 3, by decreasing the tea-to-water ratio, the extraction rate gradually increased. At 1:12, the extraction rates with water and ethanol increased to 22.4% and 30.4%, respectively. The total volume of water and ethanol reached the total volume of the two extractions without assistance, but the extraction rates were lower than 29.2% and 39.8% of the two extractions. Therefore, the ratios of tea to water were 1:7 and 1:5 for the two extractions.
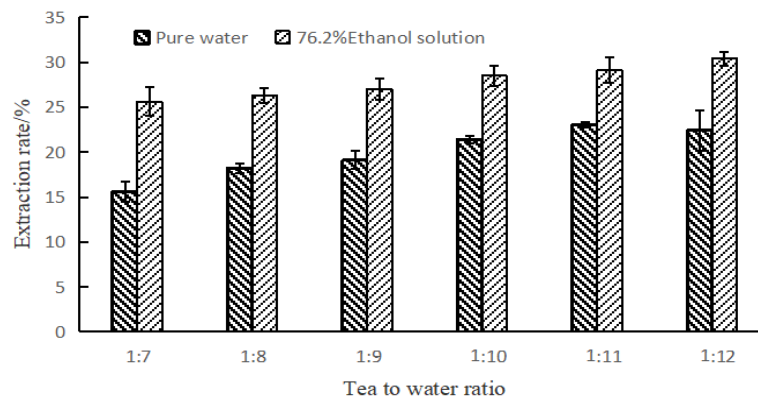

Figure 3. Effect of tea-to-water ratio of two solvents on extraction rate

Considering the average temperature in the workshop of factory throughout the year, the normal leaching temperature was set at 30℃. For energy consumption, the maximum temperature did not exceed 70℃ for leaching without auxiliary conditions. The change of leaching rate at this temperature range was investigated. As shown in Figure 4, the extraction rate of the water extraction and 76.2% ethanol extraction groups increased with increasing temperature. The trend of the two groups was linear, with $R^2=0.9934$ in the water extraction group and $R^2=0.9977$ in the 76.2% ethanol extraction group. The effect of temperature on extraction rate had no maximum value in the selected temperature range; therefore, 70℃ was set as the optimal extraction temperature.
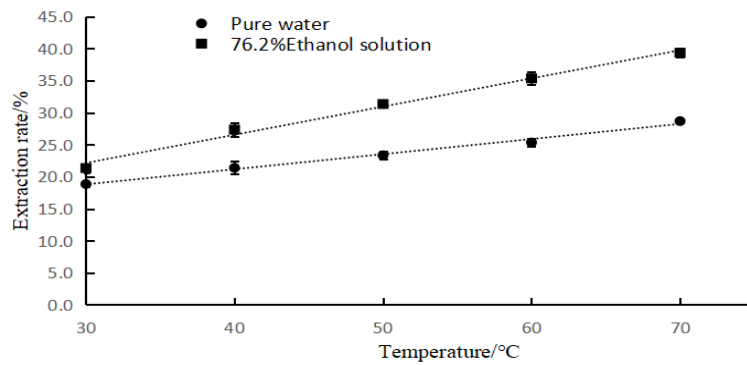
Figure 4. Effect of ultrasonic temperature on extraction rate

3.2. Effect of Microwave Power and Time on Extraction Rate

We investigated the effect of microwave treatment at 600 W during a 3-min period (Figure 5). Extraction rate increased with time. The change of extraction rate in the water extraction group was linear with $R^2 = 0.9958$. The extraction rate was 17.3%, and the energy consumption was 108 kJ at 3 min. The extraction rate of the 76.2% ethanol extraction group increased rapidly before 2 min and reached a constant maximum after 2 min. At 2 min, the extraction rate was 19.3%, and the energy consumption was 72kJ.
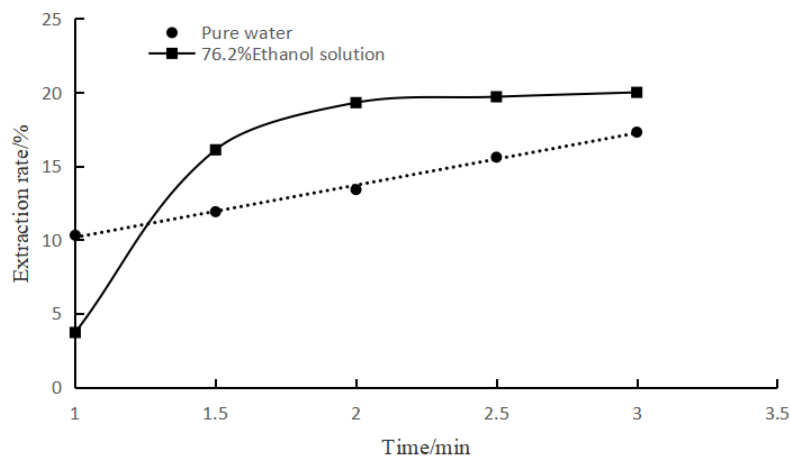

Figure 5. Effect of low power microwave time on extraction rate

We investigated the effect of microwave treatment at 750 W between 0.5 and 3 min (Figure 6). In the water group, extraction rate increased before 1.5 min, decreased from 1.5 min to 2 min, and remained relatively constant after 2 min. The extraction rate at 1.5 min was 19.5%, which was 16.8% higher than the optimal extraction rate at 15 min of ultrasonic treatment, and the energy consumption was 67.5 kJ. In the ethanol group, extraction rate gradually increased before 2 min. After 2 min, the solution began to boil. The extraction rate could not be accurately measured. The extraction rate at 2 min was 23.2%, which did not reach the maximum extraction rate at one time during ultrasonic treatment, and the energy consumption was 90 kJ.
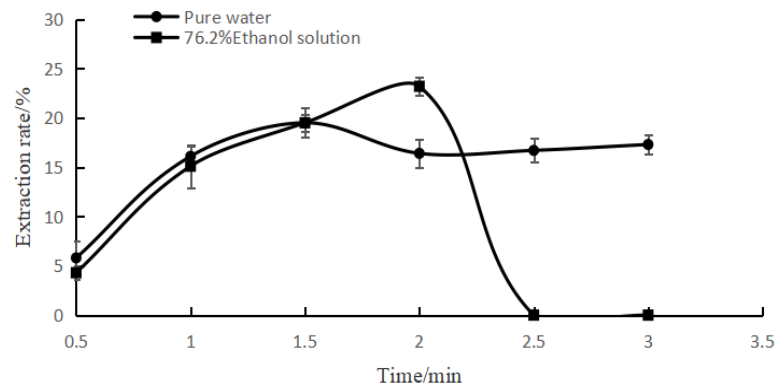
Figure 6. Effect of medium power microwave time on extraction rate

The maximum extraction rate of the microwave first extraction was reached at 1.5 min at medium power. The secondary extraction time ranged from 0 to 1 min. At time 0, the tea residue was rinsed with 1:5 solvent to obtain the filtrate. The filtrates of the two extractions were pooled, and the extraction rate was determined. Figure 7 shows that at 2 min, the extraction rate reached a maximum value of 29.1%. With increasing secondary extraction time, the total extraction rate stabilized. When total extraction time was 1.5 min, the extraction rate was 26.3%. The energy consumption was 90 kJ and 67.5 kJ in the first and second extractions, respectively, and the energy consumed per unit extraction rate was 3.1 kJ and 2.5 kJ, respectively. Therefore, the 1.5-min total extraction time resulted in the lowest energy consumption, which may improve production efficiency, but the extraction is not sufficient, while the extraction rate at 2 min was higher, which is consistent with that of ultrasonic extraction for 15 min.
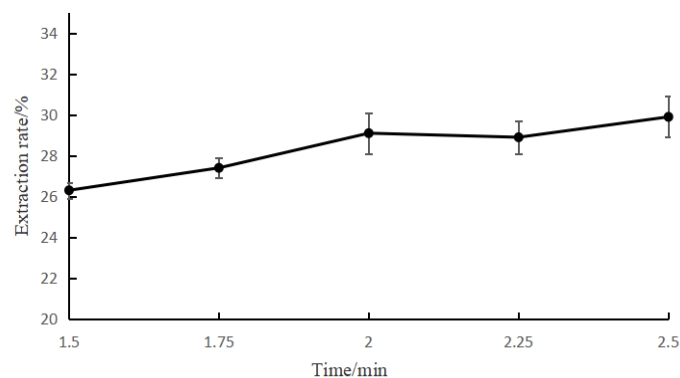

Figure 7. Effect of medium power microwave time on extraction rate.

We investigated the effect of microwave treatment at 900 W from 0.25 to 1.25 min. Figure 8 shows that extraction rate of the water extraction group increased with increasing time before 1 min reaching a maximum value at 1 min (15.2%). Energy consumption was 54 kJ. The extraction rate of the 76.2% ethanol extraction group gradually increased before 1 min. After 1 min, the solution began to boil. The value of the extraction rate could not be

accurately measured. The extraction rate at 1 min was 21.6%, which did not reach the maximum extraction rate of ultrasonic treatment, and the energy consumption was 54 kJ.
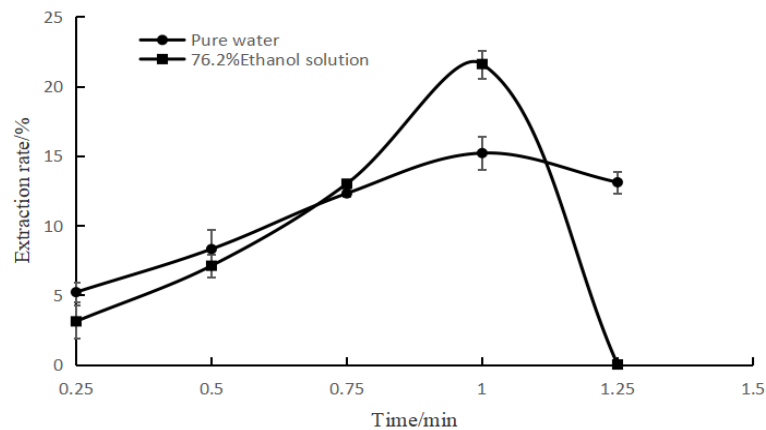

Figure 8. Effect of high-power microwave time on extraction rate

The 1.5-min and 2-min total extraction times had advantages and disadvantages. Therefore, the tea-to-water ratio was evaluated at both times. As shown in Figure 9, the change in extraction rate in the water extraction 1.5-min group and the water extraction 2-min group increased gradually with decreasing tea-to-water ratio, i.e., increasing water consumption. The change in the water extraction 1.5-min group was less significant. When the tea-to-water ratio was 1:12, the extraction rate was 22.3% in the 1.5-min group and 27.6% in the 2-min group. The extraction rate of single extraction was lower than that of extraction twice. Therefore, the optimal ratio of tea to water remained at 1:7 for the first extraction and 1:5 for the second extraction.
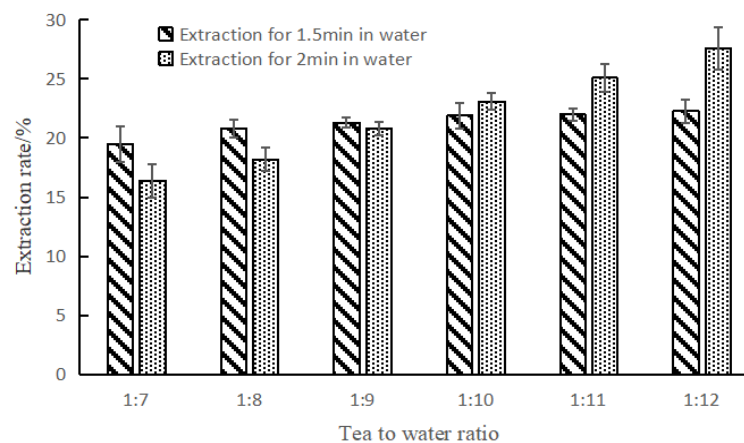

Figure 9. Effect of tea-to-water ratio at different microwave treatment times on extraction ratio

3.3. Effect of Ultrasound and Microwave on Extraction of Tea Polyphenols

As shown in Figure 10, the ultrasonic treatment had a significant effect on the extraction of tea polyphenols in the water extraction and 76.2% ethanol extraction groups. Due to the boiling phenomenon of organic solvents during microwave treatment, we only detected the samples extracted with water. The tea polyphenol content in the water

extraction group was 7.9±0.2 mg/mL, which was not significantly different from the tea polyphenol content in the 76.2% ethanol extraction group (8.5±0.1 mg/mL). However, the tea polyphenol content in the water extraction group was reduced to 4.7±0.2 mg/mL following ultrasonic treatment. In the 76.2% ethanol group, the tea polyphenol content was reduced to 7.0±0.2 mg/mL after ultrasonic treatment. The water-extracted tea polyphenols were reduced to 5.0±0.1 mg/mL by microwave treatment. Ultrasonic and microwave treatment conditions in the following section were the same as those in this section. Different letters in Fig. 10 and 11 represent differences among groups (P < 0.01).
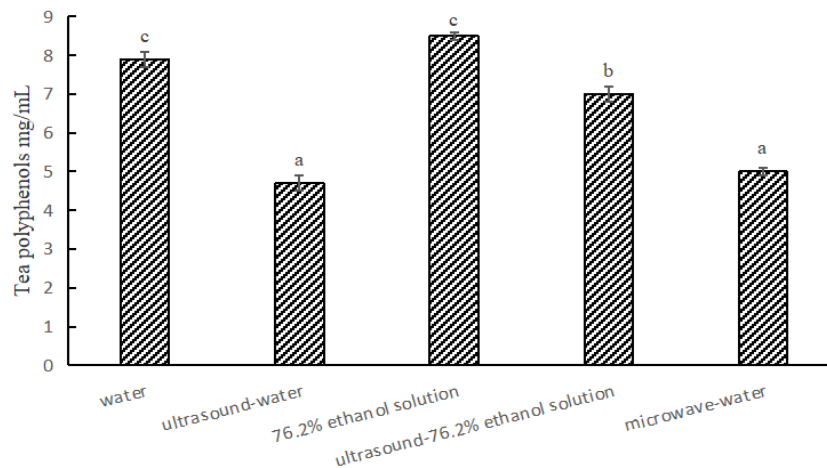

Figure 10. Effect of ultrasonic and microwave treatments on the extraction of tea polyphenols

3.4. Effect of Ultrasound and Microwave on Extraction of Caffeine

Figure 11 shows that the caffeine content was 1.1±0.08 mg/mL in the water group, 1.0±0.06 mg/mL in the ultrasonic group, and 1.1±0.04 mg/mL in the microwave group. The effect of ultrasound and microwave treatments on the water extraction of caffeine was not significant. Caffeine content was 1.5±0.06 mg/mL in the 76.2% ethanol extraction group and 1.5±0.06 mg/mL in the ultrasonic treatment group. The ultrasonic treatment had no effects on the extraction of caffeine with 76.2% ethanol.
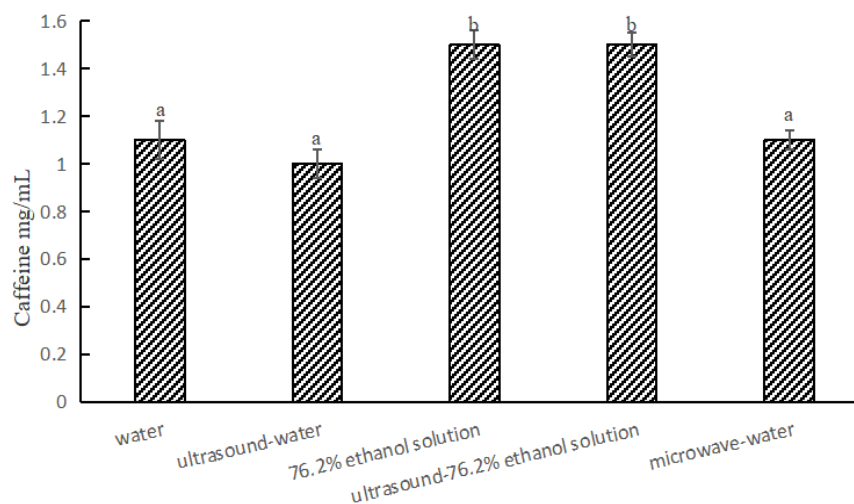
Figure 11. Effect of ultrasonic and microwave treatments on the extraction of caffeine

3.5. Effect of Ultrasound and Microwave on Extraction of Catechins

As shown in Figure 12, 76.2% ethanol promoted a higher degree of catechin dissolution than pure water. The distribution of catechins in the samples was similar. EGC and ECG contents changed following ultrasonic and microwave treatments, and EGC content was slightly higher in the ultrasonic-water extraction group and microwave-water extraction groups than in the pure water extraction samples. The ultrasonic-76.2% ethanol extraction group had higher EGC content than the 76.2% ethanol extraction group. Opposite results were obtained for ECG content. Ultrasonic and microwave treatments had a promoting effect on the dissolution of EGCG and EGC in water-extracted samples, with little effect on other catechins. Therefore, the total catechin content was higher in the ultrasonic-water extraction group and microwave-water extraction group than in the water extraction group, and the promoting effect of microwave treatment was more obvious. The total amount of catechins in the ultrasonic-76.2% ethanol extraction group reached 5.06±0.06 mg/mL, which was much higher than 3.49±0.04 mg/mL in the 76.2% ethanol extraction group, mainly because the dissolution of EGCG and EGC increased following ultrasonic treatment.

Figure 12. Effect of ultrasonic and microwave treatments on the extraction of catechins

3.6. Effect of Ultrasonic and Microwave Treatment on Extraction of Theanine

As shown in Figure 13, 76.2% ethanol was more unfavorable to theanine dissolution than pure water. The highest and lowest theanine contents were obtained in the ultrasonic-water extraction group (0.39±0.02 mg/mL) and 76.2% ethanol extraction group (0.29±0.03 mg/mL), respectively. In addition, ultrasonic and microwave treatments had no significant effect on theanine dissolution.
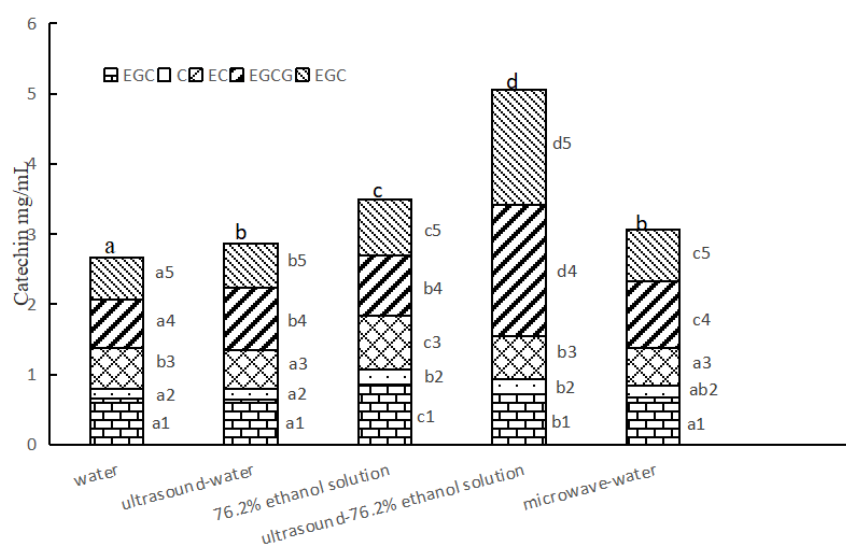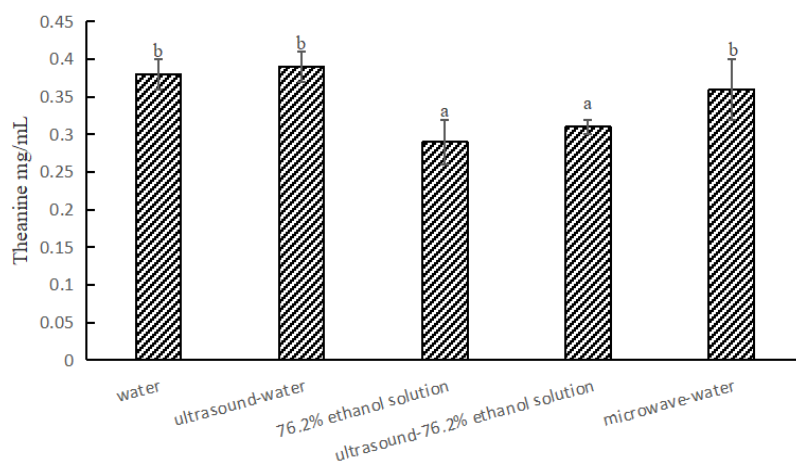
Figure 13. Effect of ultrasonic and microwave treatments on the extraction of theanine

3.7. Effect of Ultrasonic and Microwave Treatment on Extraction of Aroma Compounds

As shown in Figure 14, the distribution of aroma compounds was relatively average in the water extraction and ultrasonic-water extraction groups. The aroma compounds were higher in the ultrasonic-water extraction group than in the water extraction group, and the aroma compounds were lower in the microwave-water extraction group than in the water extraction group. The aroma compounds of the 76.2% ethanol solution group were relatively concentrated in the highly volatile part, and the content of aroma compounds extracted with 76.2% ethanol improved by ultrasonic treatment.

ultrasound-water

microwave-water

76.2% ethanol solution

ultrasound-76.2% ethanol solution
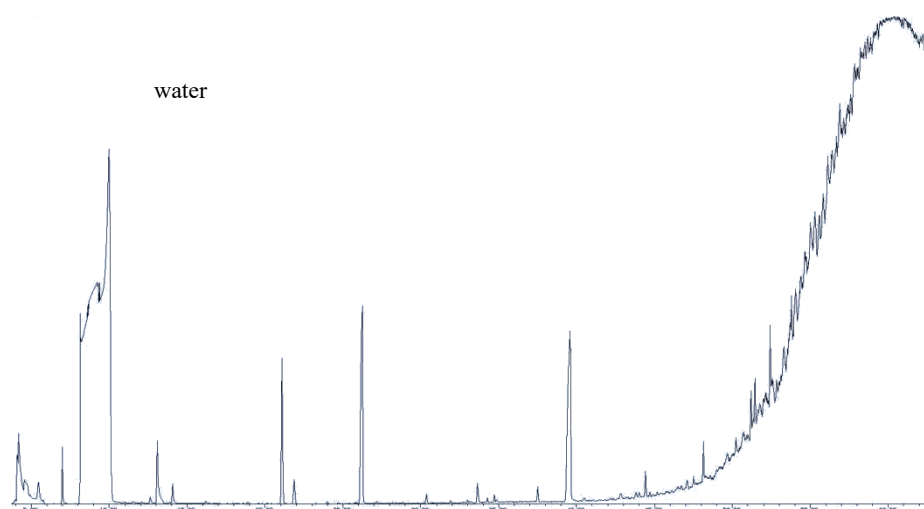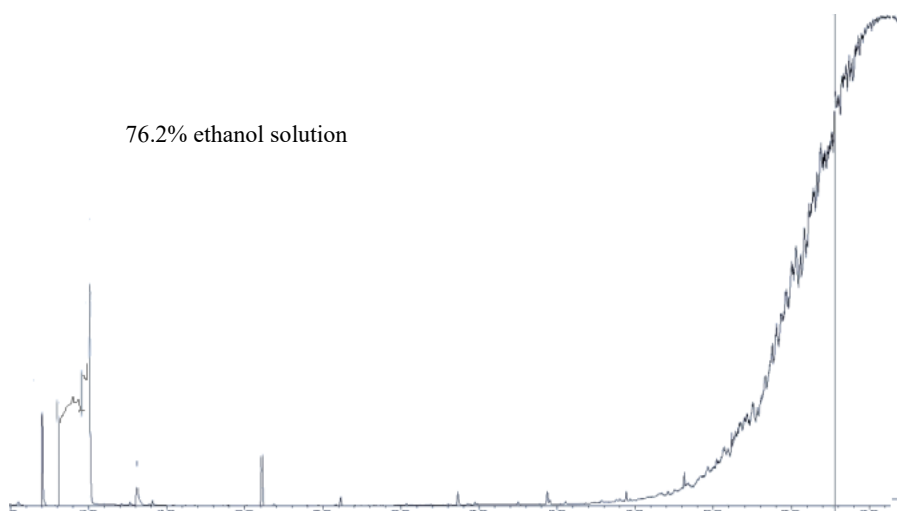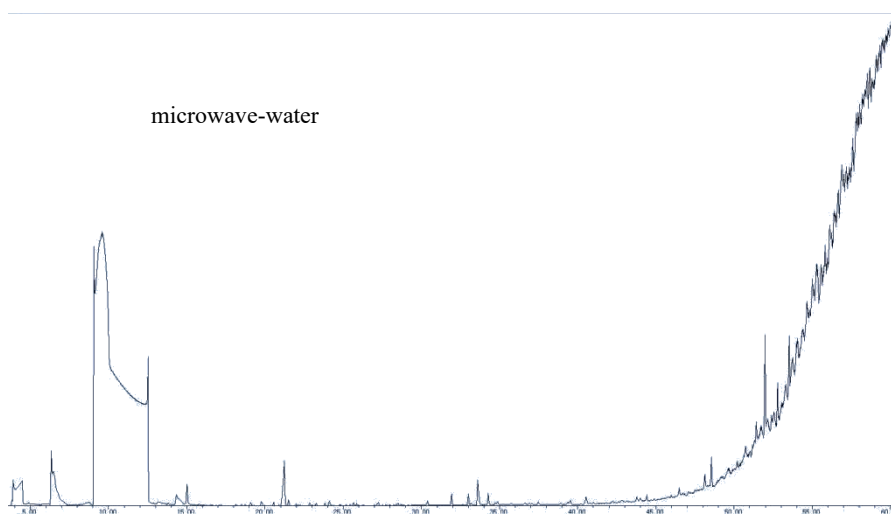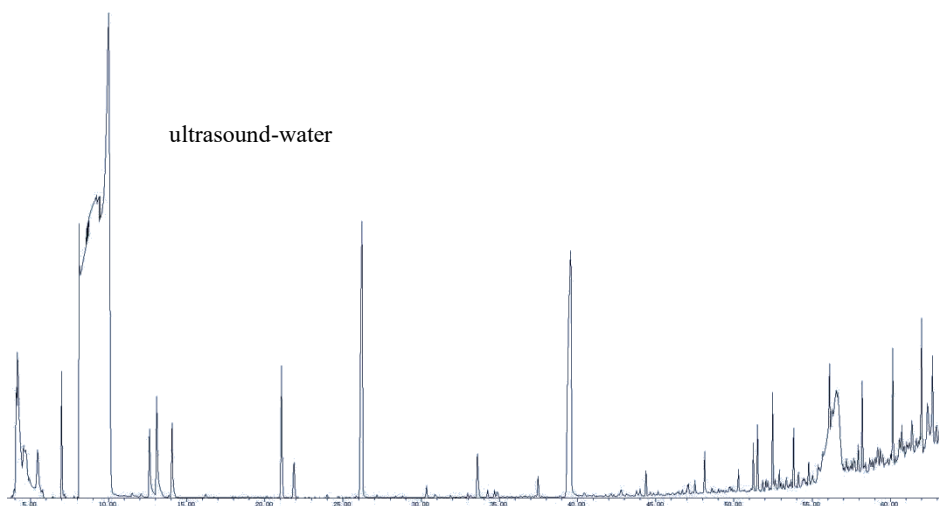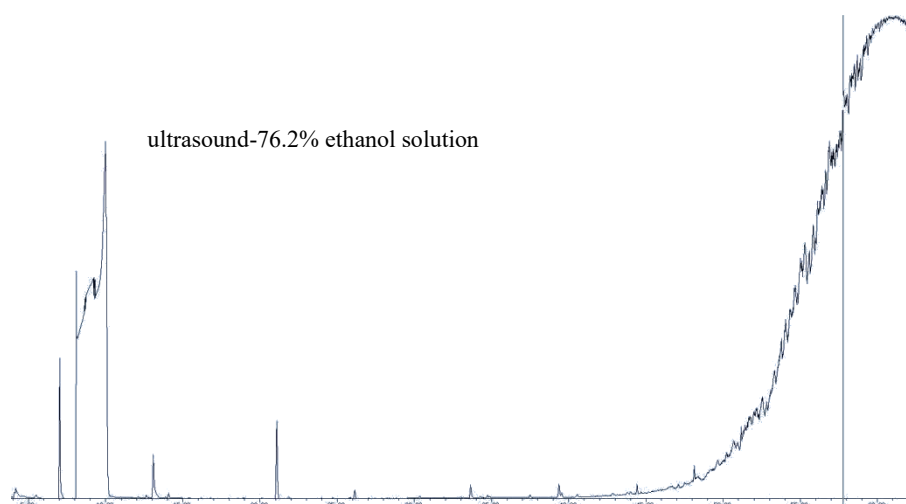
Figure 14. Aroma analysis

Table 1 shows that 3-hydroxy-2-butanone, (S)-5- hydroxymethyldihydrofuran-2-one, and 2-phenylethanol improved after ultrasonic-assisted extraction compared to water extraction, while the main aroma compounds extracted by microwave were significantly changed. The loss of low-boiling point aroma compounds was considerable, and (S)-5-hydroxymethyldihydrofuran-2-one and acetic acid were the main aroma compounds. The 76.2% ethanol extraction sample contained neophytadiene, limonene, and other olefinic compounds, chlorophyllin, and methyl salicylate. The aroma composition was significantly different in the water-extracted sample.

Table 1. Aroma compounds

Aroma compounds	Retention time/min	Relative percentage content/%				
		Water	Ultrasound -water	Microwave -water	76.2% ethanol solution	Ultrasound-76.2% ethanol solution
3- pentene -2- one	12.08	0.29	0.34	1.36	/	/
1- pentene -3- ol	19.56	0.07	0.07	/	/	/
3- pentene -2- ol	20.12	/	0.15	0.66	/	/
Isoamyl alcohol	21.88	1.92	1.29	1.32	/	/
1- pentanol	23.99	0.31	0.31	/	0.09	0.48
Styrene	24.70	0.16	0.22	0.85	/	/
3- hydroxy -2- butanone	26.23	41.32	53.17	10.32	/	/

2- heptanol	27.17	0.30	0.32	/	0.53	0.62
2,5- dimethylpyrazine	27.85	0.05	/	/	/	/
Hexyl alcohol	28.81	0.11	/	/	/	/
Butyl alcohol	30.36	/	/	/	/	0.07
Myrcene	19.78	/	/	/	0.36	0.75
Terpinolene	20.56	/	/	/	0.71	0.77
Limonene	21.53	/	/	/	1.26	1.59
3- isopropyl -6- methylene -1- cyclohexene	22.00	/	/	/	/	0.46
Ocimene	23.28	/	/	/	0.68	0.71
Gamma terpinene	23.84	/	/	/	0.36	0.37
Leaf alcohol	30.36	1.06	1.05	/	0.75	0.76
Nonanal	30.87	0.31	/	/	0.25	/
Linalool oxide (I)	32.97	0.45	0.51	1.87	0.77	0.81
Acetic acid	33.58	5.95	4.79	23.83	10.81	9.60
Linalool oxide (II)	34.25	0.69	0.62	/	3.06	3.04
2-ethyl hexanol	34.84	0.26	0.17	/	0.41	0.30
Linalool	37.47	1.93	2.21	2.91	0.61	0.59
(2S,3S)-(+)-2,3-butanediol	39.37	/	/	16.95	/	/
Linalool dihydrogen	41.00	/	/	/	/	0.17
Acetophenone	43.78	0.31	0.64	1.91	/	0.75
Linalool oxide	47.52	1.10	0.96	/	/	/
Methyl salicylate	48.55	0.37	/	/	13.61	7.95
Benzyl alcohol	51.45	6.31	7.09	/	/	/
N- ethylsuccinimide	51.84	1.03	/	/	/	/
Neophytadiene	52.04	0.61	/	/	18.28	23.86
Chlorophyll alcohol	52.78	/	/	/	8.86	8.11

2- phenylethanol	52.42	9.10	12.50	/	/	/
2,6- dimethyl -3,7- octadiene -2,6- diol	52.89	1.39	/	/	/	/
Heptylic acid	53.17	0.29	/	/	/	/
Trans -3- hexanoene	53.34	0.73	/	/	/	/
Trans -2- hexanoene	53.65	0.56	/	/	/	/
Methyl maltol	54.11	0.69	/	/	/	/
2- acetylpyrrole	54.13	0.67	/	/	/	/
Pelargonic acid	57.95	1.57	/	/	/	/
2,4- di-tert- butylphenol	60.73	2.15	/	/	/	/
2,3- dihydrobenzofuran	62.70	8.48	/	/	7.21	5.04
(S)-5- hydroxymethyldihydrofuran -2- one	65.12	9.48	13.61	38.01	16.96	21.43
Diisobutyl phthalate	65.71	/	/	/	2.22	3.15
Vegetable alcohol	66.68	/	/	/	12.21	8.63

3.8. Sensory analysis

The high pigment content in the sample extracted with 76.2% ethanol was easily identified, affecting the sensory analysis results; therefore, the sample extracted with 76.2% ethanol was not included in the sensory analysis. The scores obtained in Table 2 represent average scores. The taste scores were higher than other sensory scores in the water extraction group. The taste scores generally revealed a level of astringency. Sensory analysis scores were higher in the ultrasonic-water extraction group than in the other two groups. The flavor score was lower in the microwave-water extraction group than in the other two groups, but the scores of other indicators were higher IN WHICH GROUP? than in the water extraction group.

Table 2. Sensory evaluation of instant tea

Indicators	Scoring standard (1-10)	Score		
		Water	Ultrasound-	Microwave-

			water	water
Color	1. Orange yellow and bright, 10 points.	6.2	8.6	7.3
	2. Those with turbidity, sedimentation, peculiar smell and suspended solids get 5-9 points.			
	3. 1~4 points for malignant sediment or suspended matter.			
Texture	1. Mellow and rich, 10 points.	7.1	7.7	7.5
	2. The taste of the tea after the entrance is not thick and mellow, and the taste is weak, scoring 5-9 points.			
	3. The taste is not strong, and the liquid is viscous, with a score of 1-4.			
Flavor	1. Pure aroma without peculiar smell, 10 points.	6.5	7.3	3.1
	2. The fragrance is insufficient, and the aroma is not straight, with different fragrance, etc., 5-9 points.			
	3. The aroma is not harmonious, and the miscellaneous smell is heavy, 1-4 points.			
Taste	1. Sweet without astringency, 10 points.	5.9	7.9	7.2
	2. Bitter taste, spicy feeling, other miscellaneous flavor, etc., 5~9 points.			
	3. Taste uncoordinated, mixed flavor, or too sweet, 1~4 points.			
Overall taste	Good, 10 points. Ordinary, 5 points. Bad, 1 point.	7.0	8.0	6.5
	Total score (5-50)	32.7	39.5	31.6

4. Conclusion

4.1. Ultrasonic-assisted extraction

At 120 w and 40 kHz ultrasonic treatment, pure water and 76.2% ethanol reduced extraction time. The most ideal extraction time was 15 min with a water-to-tea ratio of 1:7 for the first extraction, and 0 min for the second extraction with a water-to-tea ratio of 1:5 at 70℃. At 15-min extraction time, the total extraction rate was the highest. The maximum extraction rates were 29.2% in the pure water and 39.8% in the 76.2% ethanol, respectively, which were the same with those of treatments without auxiliary.

In the presence of pure water, ultrasonic treatment had no significant effects on the dissolution of caffeine and theanine, but it had a promoting effect on the dissolution of total catechins (increasing by 0.23 mg/mL) and an inhibitory effect on the total amount of tea polyphenols (reducing by 3.2 mg/mL). In the presence of 76.2% ethanol, ultrasonic treatment had no significant effects on the dissolution of caffeine and theanine, but it had a considerable promoting effect on the dissolution of total catechins (increasing by 1.57 mg/mL) and a significant inhibitory effect on the total amount of tea polyphenols (decreasing by 1.5 mg/mL).

Ultrasonic treatment promoted the release of the main aroma compounds in tea. The main aroma compounds in the water extraction group were 3-hydroxy-2-butanone, (S)-5-hydroxymethyldihydrofuran-2-one, and 2-phenylethanol, which impart, respectively, pleasant milk fragrance, fruit fragrance, and flower fragrance characteristics. The main aroma compounds in the 76.2% ethanol extraction group were neophytadiene, (S)-5-hydroxymethyldihydrofuran-2-one, and acetic acid, which impart a fresh sour aroma.

4.2. Microwave-assisted extraction

The optimal microwave treatment was achieved at 750 w. The extraction rate of pure water in one extraction reached 19.5% in 1.5 min, which was higher than the optimal extraction rate of 16.8% in 15 min of ultrasonic treatment. The second extraction time was 0.5 min, and the other conditions were the same as in the first extraction. The tea-to-water ratios of the two extractions were 1:7 and 1:5, and the total extraction rate was 29.1%. The maximum extraction rate of 76.2% ethanol under low microwave treatment was 19.3%, which was lower than 25.6% with ultrasonic treatment for 15 min. Under medium and high power, the boiling phenomenon occurred after 2 min and 1 min, respectively, while the maximum extraction rates before boiling were 23.2% and 21.6%, respectively, which were lower than 25.6% after ultrasonic treatment for 15 min.

In the presence of pure water, microwave treatment had no significant effects on the dissolution of caffeine and theanine, but had a significant promoting effect on the dissolution of total catechins (increasing by 0.41 mg/mL) and a significant inhibitory effect on the total amount of tea polyphenols (reducing by 2.9 mg/mL).

Microwave treatment denatured the aroma compounds with low boiling point, reducing 3-hydroxy-2-butanone levels, generating (2S,3S)-(+)-2,3-butanediol, and increasing the proportion of acetic acid and (S)-5-hydroxymethyldihydrofuran-2-one, thereby affecting overall freshness and introducing a ripe fruit flavor.

4.3. Sensory evaluation

Ultrasonic and microwave treatments improved color and taste of tea. Ultrasonic treatment promoted the release of aroma compounds and enhanced the aroma flavor of the sample, while microwave treatment negatively impacted the flavor, especially aroma compounds of low boiling point. A comprehensive evaluation showed that the total sensory score was the highest and lowest in ultrasonic and microwave treatments, respectively.

5. Discussion

5.1. Cost of Ultrasonic- and Microwave-Assisted Extraction

The maximum extraction rate with ultrasonic and microwave treatments was basically the same as that without these auxiliary treatments. Under optimum conditions, energy consumption was 108 kJ for ultrasonic treatment and

90 kJ for microwave treatment. The total extraction time was 15 min for ultrasonic extraction and 2 min for microwave extraction. However, the microwave extraction rate of the 76.2% ethanol extraction group was lower than the ultrasonic extraction rate, and was prone to boiling; therefore, it was difficult to apply microwave-assisted extraction with ethanol solution in production. Ultrasound and microwave treatments require additional energy consumption [21-22]; therefore, it is important to assess whether the value of the increased production capacity due to the improved production efficiency through auxiliary means is higher than the increased energy consumption cost. Alternative, the increased energy consumption could be accounted for by reasonably pricing the tea products.

5.2. Ultrasonic-Assisted Extraction Effect

Compared with the conventional industrial operation conditions, under optimized conditions, the ultrasonic extraction efficiency of tea leaves was greatly improved, from the original total extraction time of 45 min for two times to 15 min. However, total extraction rate did not change significantly: 28.7% for non-assisted water extraction, 29.2% for ultrasonic-water extraction, 40.0% for non-assisted-76.2% ethanol extraction, and 39.8% for ultrasonic-76.2% ethanol extraction. Due to the lack of ultrasonic power selection, there might be a shorter extraction time and higher ultrasonic extraction efficiency, which require further evaluation.

According to the evidence, the effect of ultrasonic treatment on the extraction of green tea components varies [23-25]. In this study, the effect of ultrasonic treatment on specific components was mainly concentrated on tea polyphenols. In the water extraction group and the 76.2% ethanol extraction group, the content of tea polyphenols was significantly reduced due to the effect of ultrasonic waves, which significantly reduced astringency and improved tea quality.

The aroma of tea treated by ultrasonic waves increased in proportion to the main components. The flavor of the water-extracted sample was closer to that of the original tea, and the flavor of the alcohol-extracted sample was more suitable for preparing fruit-flavored tea.

5.3. Microwave-Assisted Extraction Effect

Compared with the conventional industrial operation conditions, under optimized conditions, the microwave extraction efficiency greatly improved, from the original total extraction time of 45 min for two times to 2 min. Extraction rate did not change significantly, with 28.7% without auxiliary water extraction and 29.1% with microwave-water extraction. Microwave technology can be easily used in small-scale application. For mass production, large-scale microwave equipment are required.

The effect of microwave on the extraction components of green tea is variable [26-28]. In this study, the effect of microwave on specific components was mainly concentrated on tea polyphenols. The content of tea polyphenols in the water extraction group was significantly reduced due to ultrasonic treatment, which significantly reduced astringency. Microwave-assisted extraction might be suitable for making single-cup or large-pot tea.

The aroma of tea extracted by microwave treatment had obvious changes in the main components, and the natural and fresh aroma compounds with low boiling point were lost. The tea retained aroma compounds with high boiling point and had a heavy aftertaste.

5.4. Sensory Evaluation

Studies have reported that the color and aroma of tea improves following ultrasonic and microwave treatments [29-34]. However, in this study, the aroma of tea after ultrasonic and microwave treatments increased and decreased, respectively. The quality of tea obtained by water extraction following traditional methods was acceptable only in terms of taste. The scores of other sensory indicators were significantly lower in the traditional water extraction group than in the ultrasonic extraction group, while the sensory scores of aroma were lower in the microwave extraction group than in the traditional water extraction group. Other sensory indicators were higher in the microwave extraction group than in the traditional water extraction group. The ultrasonic-water extraction group had bright tea with soft taste, weak astringency, and great aroma. Compared with the traditional water extraction group, the microwave-water extraction group had superior flavor, but not aroma. The overall tea flavor of the instant tea sample was improved by ultrasonic-water extraction compared to traditional water extraction. Compared with traditional water extraction, microwave-water extraction results in a better tea flavor.

References

1. Someswararao, C.; Srivastav, P.P. A novel technology for production of instant tea powder from the existing black tea manufacturing process. *Innov. Food Sci. Emerg*. **2012**, *16*, 143-147. https://doi.org/10.1016/j.ifset.2012.05.005

2. Sinija, V.R.; Mishra, H.N.; Bal, S. Process technology for production of soluble tea powder. *J. Food Eng*. **2007**, *82*, 276-283. https://doi.org/10.1016/j.jfoodeng.2007.01.024

3. Ye, J.H.; Liang, Y.R.; Jin, J.; Liang, H.L.; Du, Y.Y.; Lu, J.L.; Ye, Q., Lin, C. Preparation of partially decaffeinated instant green tea. *J. Agr. Food Chem*. **2007**, *55*, 3498-3502. https://doi.org/10.1021/jf070046c

4. Chang, R.L.; Hsu, H.Y.; Li, C.F. Studies on instant tea. I. A comparison of the composition of instant teas with their original teas. *Int. J. Food Sci. Tech*. **1973**, *8*,217-220. https://doi.org/10.1111/j.1365-2621.1973.tb01709.x

5. Ahmed, T.; Sarwar, N. Evaluation of quality and antioxidant activity of developed instant black tea and commercial tea (Camellia sinensis) available in Bangladesh. *Ukr. J. Food Sci*. **2018**, *6*, 184-197. https://doi.org/10.24263/2310-1008-2018-6-2-4

6. Vilkhu K.; Mawson R.; Simons L.; Bates, D.. Applications and opportunities for ultrasound assisted extraction in the food industry-A review. *Innov. Food Sci. Emerg*. **2008**, *9*, 161-169. https://doi.org/10.1016/j.ifset.2007.04.014

7. Xia T.; Shi S. Q.; Wan X. C. Impact of ultrasonic-assisted extraction on the chemical and sensory quality of tea infusion. *J. Food Eng*. **2006**, *74*, 557-560. https://doi.org/10.1016/j.jfoodeng.2005.03.043

8. Horžić, D.; Jambrak, A.R.; Belš čak-Cvitanović, A.; Komes, D.; Lelas, V. Comparison of Conventional and Ultrasound Assisted Extraction Techniques of Yellow Tea and Bioactive Composition of Obtained Extracts. *Food Bioprocess Tech*. **2012**, *5*, 2858-2870. https://doi.org/10.1007/s11947-012-0791-z

9. Pan, X.J.; Niu, G.G.; Liu, H.Z. Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chem. Eng. Process*. **2003**, *42*, 129-133. https://doi.org/10.1016/S0255-2701(02)00037-510. Gulati, A.; Rawat, R.; Singh, B.; Ravindranath, S.D. Application of microwave energy in the manufacture of enhanced-quality green tea. *J. Agr. Food Chem*. **2003**, *51*, 4764-4768. https://doi.org/10.1021/jf026227q

11. Kubota, K.; Kumeuchi, T.; Kobayashi, A.; Osawa, Y.; Nakajima, T., Okamoto, Y. Effect of refining treatment with microwave heating dram on aroma and taste of green tea. *J. Jpn. Soc. Food Sci*. **1996**, *43*, 1197-1204. https://doi.org/10.3136/nskkk.43.1197

12. Spigno, G.; De Faveri, D.M. Microwave-assisted extraction of tea phenols: A phenomenological Study. *J. Food Eng*. **2009**, *93,* 210-217. https://doi.org/10.1016/j.jfoodeng.2009.01.006

13. Corrales, M.; Garc í a, A.F.; Butz, P.; Tauscher, B. Extraction of anthocyanins from grape skins assisted by high hydrostatic pressure. *J. Food Eng*. **2009**, *90*, 415-421. https://doi.org/10.1016/j.jfoodeng.2008.07.003

14. Xi, J.; Shen, D.J.; Li, Y.; Zhang, R. Ultrahigh pressure extraction as a tool to improve the antioxidant activities of green tea extracts. *Food Res. Int*. **2011**, *44*, 2783-2787. https://doi.org/10.1016/j.foodres.2011.06.001

15. Xi, J.; Zhao, S.; Lu, B.B.; Zhang, R.; Li, Y.; Shen, D.J.; Zhou, G.F. Separation of major catechins from green

tea by ultrahigh pressure extraction. *Int. J. Pharmaceut*. **2010**, *386*, 229-231. https://doi.org/10.1016/j.ijpharm.2009.10.035

16.   Kim, J.H.; Pan, J.H.; Heo, W.; Lee, H.; Kwon, E.G.; Lee, H.G.; Shin, D.H.; Liu, H.R.; Kim, Y.J. Effects of cellulase from aspergillus niger and solvent pretreatments on the extractability of organic green tea waste. *J. Agr. Food Chem*. **2010**, *58*, 10747-10751. https://doi.org/10.1021/jf102346p

17.   Kumar, C.S.; Subramanian, R.; Rao, L.J. Application of Enzymes in the Production of RTD Black Tea Beverages: A Review. *Crit. Rev. Food Sci*. **2013**, *53*, 180-197. https://doi.org/10.1080/10408398.2010.520098

18.   Lu, Y.; Jiang, J.G. Application of enzymatic method in the extraction and transformation of natural botanical active ingredients. *Appl. Biochem. Biotech*. **2013**, *169*, 923-940. https://doi.org/10.1007/s12010-012-0026-9

19.   Chandini, S.K.; Rao, L.J.; Gowthaman, M.K.; Haware, D.J.; Subramanian, R. Enzymatic treatment to improve the quality of black tea extracts. *Food Chem*. **2011**, *127*, 1039-1045. https://doi.org/10.1016/j.foodchem.2011.01.078

20.   Lu, M.J.; Chen, C. Enzymatic modification by tannase increases the antioxidant activity of green tea. *Food Res. Int*. **2008**, *41*, 130-137. https://doi.org/10.1016/j.foodres.2007.10.012

21.   Pradal, D.; Vauchel, P.; Decossin, S.; Dhulster, P.; Dimitrov, K. Kinetics of ultrasound-assisted extraction of antioxidant polyphenols from food by-products: Extraction and energy consumption optimization. *Ultrason. Sonochem*. **2016**, *32*, 137-146. https://doi.org/10.1016/j.ultsonch.2016.03.001

22.   Ait Amer Meziane, I.; Maizi, N.; Abatzoglou, N.; Benyoussef, E.H. Modelling and optimization of energy consumption in essential oil extraction processes. *Food Bioprod. Process*. **2020**, *119*, 373-389. https://doi.org/10.1016/j.fbp.2019.11.018

23.   Ferreira, A.F.; Dias, A.P.S.; Silva, C.M.; Costa, M. Effect of low frequency ultrasound on microalgae solvent extraction: Analysis of products, energy consumption and emissions. *Algal Res*. **2016**, *14*, 9-16. https://doi.org/10.1016/j.algal.2015.12.015

24.   Xia, T.; Shi, S.; Wan, X. Impact of ultrasonic-assisted extraction on the chemical and sensory quality of tea infusion. *J. Food Eng*. **2006**, *74*, 557-560. https://doi.org/10.1016/j.jfoodeng.2005.03.043

25.   Sonawane, S.S.; Patil, V.S. Effect of ultrasound on leaching of tannic acid from tea and its modeling. *Chem. Eng. Technol*. **2008**, *31*, 1304-1309. https://doi.org/10.1002/ceat.200800019

26.   Li, D.C.; Jiang, J.G. Optimization of the microwave-assisted extraction conditions of tea polyphenols from green tea. *Int. J. Food Sci. Nutr*. **2010**, *61*, 837-845. https://doi.org/10.3109/09637486.2010.489508

27.   Pan, X.; Niu, G.; Liu, H. Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chem. Eng. Process*. **2003**, *42*, 129-133.   https://doi.org/10.1016/S0255-2701(02)00037-5

28.   Spigno, G.; De Faveri, D.M. Microwave-assisted extraction of tea phenols: A phenomenological study. *J. Food Eng*. **2009**, *93*, 210-217. https://doi.org/10.1016/j.jfoodeng.2009.01.006

29.   Kraujalyte, V.; Pelvan, E.; Alasalvar, C. Volatile compounds and sensory characteristics of various instant teas produced from black tea. *Food Chem*. **2016**, *194*, 864-872. https://doi.org/10.1016/j.foodchem.2015.08.051

30.   Alasalvar, C.; Pelvan, E.; Özdemir, K.S.; Kocadağlı, T.; Mogol, B.A.; Paslı, A.A.; Özcan, N.; Özçelik, B.; Gökmen, V. Compositional, Nutritional, and Functional Characteristics of Instant Teas Produced from Low- and High-Quality Black Teas. *J. Agric. Food Chem*. **2013**, *61*, 7529-7536. https://doi.org/10.1021/jf4015137

31.   Perera, G.A.A.R.; Amarakoon, A.M.T.; Illeperuma, D.C.K.; Muthukumarana, P.K.P. Effects of raw material on the chemical composition, organoleptic properties, antioxidant activity, physical properties and the yield of instant black tea. *LWT-Food Sci. Technol*. **2015**, *63*, 745-750. https://doi.org/10.1016/j.lwt.2015.03.060

32.   Tao, M.; Liu, Z.Q. Influence of ultrasonic nebulization extraction, infusion temperatures, and matrices on aroma release and perception of green tea.   *LWT-Food Sci. Technol*. **2019**, *115*,108216. https://doi.org/10.1016/j.lwt.2019.05.114

33.   Sinija, V.R.; Mishra, H.N. Fuzzy Analysis of Sensory Data for Quality Evaluation and Ranking of Instant Green Tea Powder and Granules. *Food Bioprocess Tech*. **2011**, *4*, 408-416. https://doi.org/10.1007/s11947-008-0163-x

34.   Ye, D.; Zhang, L.; Sun, S.; Chen, J.; Fang, T. Production of high-aroma instant tea powder using various novel technologies. *J. Food Process Eng*. **2014**, *37*, 273-284. https://doi.org/10.1111/jfpe.12083