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Research Article

Separation of Caffeine and Tea Poly-phenols from Instant (Soluble) Tea Waste Liquor by Macro-porous Resins

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Abstract: Instant tea is presently manufactured by spraying and freeze drying the concentrated brew of processed tea leaves and dust. The purpose of this study was to develop a technique using macro-porous resins for separating Caffeine (Caf) and Tea Poly-phenols (TP) from the waste liquor generated by manufacturing instant tea. Optimum adsorption conditions were obtained using an initial concentration of Caf solution of 80 mg/L and a flow rate of 1.5 mL/min at 60°C; The optimal desorption conditions were determined using a flow rate of 1.0 mL/min, 80% aqueous ethanol of, eluent volume of 4 times of Bed Volume (BV). After column separation, the purity of Caf was enhanced from 6 to 23%, TP from 38 to 61% and with the final yield of 16.9% following the separation by D101 resin. This study demonstrated macro-porous resin can effectively separated Caf and TP from instant tea waste liquor.

Keywords: Caffeine (Caf), instant tea waste liquor, macro-porous resins, separation, Tea Poly-phenol (TP)

INTRODUCTION

Tea is consumed by two-third of the world's population and it is more popular than any other beverage. Increasing interest in the functional properties of poly-phenols has led to the inclusion of green tea in the group of beverages with the potential to positively impact human health (Luczaj and Skrzydlewska, 2005; Cabrera *et al*., 2006; Judith *et al*., 2007; Bancirova, 2007). Instant (soluble) tea is a processed tea product of, usually in powder form that is generally prepared by the aqueous extraction of tea followed by concentration and drying (Wells *et al*., 1998). Due to its convenience, instant tea is becoming more and more popular and it is one of the most widely consumed beverages worldwide. The instant tea industry has developed quickly to meet the demands of an expanding market (Sinija *et al*., 2007). The global annual output of instant tea is more than 70,000 tons currently and the instant tea industry in China occupies an important place and plays a critical role in the national economy (Yue and Wang, 2004). Instant tea is manufactured in several countries, but its production and consumption in the United States is greater than in the other parts of the world (Kang *et al*., 2006).

Instant tea waste liquid is a byproduct of the industrial production of instant tea. Instant tea liquor contains some useful ingredients, such as proteins, poly-phenols, caffeine and theanine, which have not been recycled effectively, i.e., there are at least 200 tons of dried instant tea waste liquid in a instant tea powder factory with an annual production capacity of 3000 tons. Most of the time, the waste liquid was simply discarded. Instant tea liquid waste recycling can improve the economic benefits of the enterprise and be socially beneficial.

Several industrial methods are used to separate the major effective components from tea, such as resin adsorption, solvent extraction, membrane separation and supercritical fluid extraction (Chen, 2005; Chen *et al*., 2005; Zhu *et al*., 2007; Wu *et al*., 2009; Fang *et al*., 2012). Resin adsorption methods have been widely used due to their simplicity, low energy consumption, safety and the feasibility of mass production (Li *et al*., 2009). Chen *et al*. (2005) purified theanine and tea poly-phenols from the extract of green tea with ZJL macro-porouscation resin column followed by ZJX macro-porous adsorption resin column chromatography, at the same time caffeine was removed effectively. Additionally, resin materials have several advantages, including a large capacity of absorption, fast adsorption speed, accurate selectivity, easy regeneration and low cost. Industrial resins have been used in many kinds of natural products extraction methods (Cai *et al*., 2008).

At present, there have been reports concerning the utilization of waste liquid from the production of TP, although approaches for recycling instant tea waste has rarely been reported (Pi *et al*., 2008; Shan *et al*., 2010).

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Zhu *et al*. (2007) studied the technology of isolation and purification of theanine extracted from the spent tea water after the tea poly-phenols had been removed. After being pretreated by ultra-filtration and de-colorization, theanine was adsorpted and separated by ion exchange. The purpose of our study was to develop a macro-porous resin separation method for Caf and TP from instant tea waste liquor. In order to achieve instant tea waste liquid resource recycling system, the macro-porous resins was firstly used to separate Caf and TP from the instant tea waste liquor. Six kinds of macro-porous resin were selected to separate Caf and TP from instant tea waste liquor. Dynamic absorption and desorption experiments were used to identify optimum operating conditions. The aim of this study was to select the most suitable resin and determine the optimal adsorption separation conditions. This study will provide theoretical and technical basis for instant tea waste recycling.

MATERIALS AND METHODS

Raw material: Instant tea waste liquor was generously provided by Nanjing Apogee Food Technology Co. Ltd. Waste liquor was dried into powder whose mass fractions of TP and Caf were 6.00 and 38.03%, respectively. Five kinds of macro-porous resins, D101, NKA-II, D4020, AB-8, NKA-9, were obtained from the chemical factory of Nankai University (Tianjin, China) while another macro-porous resin, HZ-806, was from Anhui Sanxing Resin Factory (Anhui Hefei, China). The rest of the reagents and solvents were of analytical grade.

Experiment equipment: The resin dynamic adsorption and desorption experimental device is shown in Fig. 1.

Liquid sample processing: The dried powder of instant tea waste liquor was dissolved with deionized water at a concentration of 20 g/L. The liquid samples were centrifuged at 3500 r/min for 10 min to remove solid debris.

The static adsorption experiment: Six different kinds of processed macro-porous resin samples (5 g) were individually placed in 250 mL conical flasks. Then, 100 mL of sample liquor (20 g/L) was added to each flask. The samples were agitated on an orbital shaker at 120 r/min for 24 h at 25°C. Then the concentration was measured as C_1. Then the saturated adsorption resin was removed and added 100 mL 80% ethyl alcohol, then vibrated at 120 r/min for 24 h, 25°C. Then the concentration was measured as C_2. The resin adsorption and desorption performance were tested according to the adsorption rate and the desorption rate. The calculation formula used to calculate the resin adsorption capacity and adsorption rate are as follows:

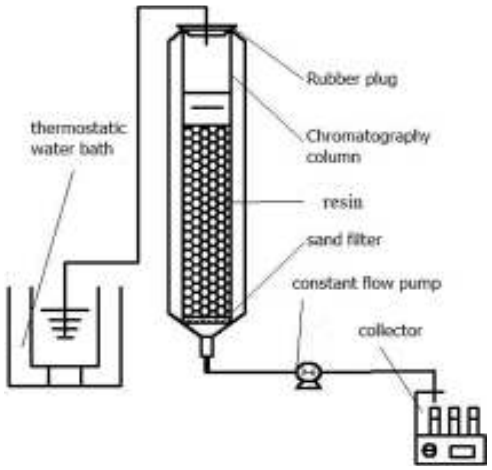


Fig. 1: Schematic diagram of experimental setup

$$\text{Adsorption rate (\%)} = \frac{C_0 - C_1}{C_0} \times 100$$

$$\text{Desorption rate (\%)} = \frac{C_2}{C_0 - C_1} \times 100$$

$$\text{Desorption quantity (mg/g)} = \frac{C_2 \times V}{G(1-a)}$$

where,

C_0 = The original concentration of sample liquor
C_1 = The concentration of sample liquor after absorption to the resin
C_2 = The concentration of eluent (mg/mL)
V = The volume of sample liquor
G = The wet weight of resin
a = The moisture content of resin

So, the yield was determined using the formula followed:

$$\text{Yield} = \frac{\text{Recovery sample quality}}{\text{Experimental sample quality}} \times 100\%$$

Dynamic adsorption experiment:

Single factor design: Five kinds of loading buffer velocity, 0.5, 1.0, 1.5, 2.0, 2.5 mL/min, respectively, were tested at 60°C, while Caf concentration in the sample liquor was 80 mg/L. Five temperatures, 40, 50, 60, 70 and 80°C, respectively were also tested using 80 mg/L Caf concentration and 1.5 mL/min loading buffer velocity. In addition, five Caf concentrations, 20, 50, 80, 110, 140 mg/L, respectively, were tested at 60°C, while the loading buffer velocity was 1.5 mL/min. These three single factor designs aimed to determine the resin adsorption rate so as to identify the optimal conditions for separating Caf and TP.

Orthogonal experimental design: After performing the single factor experiment, an orthogonal experiment of three factors and three levels was adopted to

Table 1: Factors and levels of the orthogonal experiment

	Factor		
Level	Liquor velocity A/mL/min	Temperature B/°C	Concentration C/mg/L
1	1.0	40	50
2	1.5	50	80
3	2.0	60	110

Table 2: Factors and levels of the orthogonal experiment

	Factor		
Level	Parsing velocity A/mL/min	Eluent concentration B/ %, v/v	Dosage of eluent C/BV
1	0.5	60	3
2	1.0	70	4
3	1.5	80	5

optimize the resin adsorption technology of Caf and TP (Table 1).

The dynamic desorption experiment:

Single factor design: Five kinds of elution velocity, 0.5, 1.0, 1.5, 2.0, 2.5 mL/min, respectively, were tested, while the eluent volume fraction was 80% and the dosage of eluent is 4 BV. Seven kinds of eluent volume fraction, 10, 20, 50, 60, 70, 80 and 90%, respectively were test at the condition of 1.5 mL/min elution velocity and 4BV eluent dosage. Five kinds of eluent dosage, 2, 3, 4, 5 and 6 BV, respectively were also tested at the condition of 1.5 mL/min elution velocity and 80% eluent volume fraction. These three single factor tests aimed to inspect the resin desorption rate so as to determine the optimal conditions for separating Caf and TP.

Orthogonal experimental design: On the basis of single factor dynamic desorption experiment, the orthogonal experiment (3 factors and 3 levels) (Table 2), was designed to inspect the amount of parsing Caf and TP, so as to determine the best desorption technology.

Determination methods: The Caf content and TP content were determined according to the procedures described in the Chinese National Standard GB/T 8312-2002 (SAC, 2002) and GB/T 21733-2008 (SAC, 2008), respectively.

RESULTS AND DISCUSSION

The screening of proper resin type: The adsorption rate and desorption rate of 6 kinds of resin were tested. The results are shown in Table 3. The Caf adsorption rate and Caf desorption rate of D101 resin are superior to the other 4 resins. As for the TP, the adsorption rate and desorption rate of D101 resin was higher than 4 kinds of resin, NKA-II, AB-8, D4020 and HZ-806. Based on the above results, D101 resin was selected to be the most suitable materials for separating Caf and TP from instant tea waste liquor.

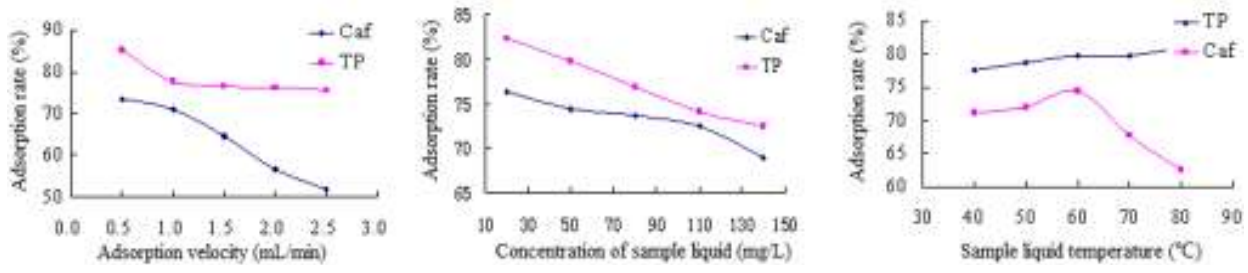
Adsorption parameters influence on the adsorption rate:

The single factor design result on the adsorption rate: The single factor design experiment demonstrated the effects of different adsorption conditions on the adsorption rate (Fig. 2).

Figure 2a to c showed the variation adsorption tendency of TP and Caf at different conditions including different adsorption velocity, different concentration of sample liquid, different sample liquid temperature. Both the adsorption rate of TP and Caf are decreased by an increase of flow velocity and concentration (Fig. 2a and b). There was a relatively

Table 3: Adsorption and desorption characteristics of macro-porous resins for Caf and TP seprating from instant tea waste liquor

	Caf		TP	
Resin type	Adsorption rate (%)	Desorption rate (%)	Adsorption rate (%)	Desorption rate (%)
D101	70.27	66.98	61.48	58.55
NKA-9	37.23	33.02	72.38	61.43
NKA-II	18.08	15.75	21.48	18.76
AB-8	49.89	38.53	25.37	20.82
D4020	45.40	42.47	55.10	45.93
HZ-806	64.19	60.44	60.74	56.10



(a) (b) (c)

Fig. 2: Effect of velocity and concentration and temperature on adsorption rate of TP and Caf

Table 4: Result of the orthogonal experiment

	Test	Factor			Adsorption rate (%)	
	No.	A	B	C	Caf	TP
	1	1	1	1	81.71	79.56
	2	1	2	2	85.06	79.72
	3	1	3	3	86.81	72.43
	4	2	1	3	86.80	75.35
	5	2	2	1	85.26	76.64
	6	2	3	2	87.79	74.19
	7	3	1	2	84.51	70.15
	8	3	2	3	87.89	73.95
	9	3	3	1	85.91	76.12
Caf	k_1	84.53	84.340	85.80		
	k_2	86.62	86.070	85.92		
	k_3	86.10	86.840	85.53		
	R	2.09	2.249	0.40		
TP	k_1	77.90	75.020	76.90		
	k_2	76.39	76.770	78.40		
	k_3	74.74	77.250	73.74		
	R	3.16	2.230	4.66		

Table 5: Result of the orthogonal experiment

	Test	Factor			Desorption quantity (mg/g)	
	No.	A	B	C	Caf	TP
	1	1	1	1	0.64	28.32
	2	1	2	2	0.81	29.78
	3	1	3	3	1.02	30.36
	4	2	1	3	0.67	28.17
	5	2	2	1	0.75	32.00
	6	2	3	2	1.19	33.00
	7	3	1	2	0.61	25.43
	8	3	2	3	0.82	27.54
	9	3	3	1	1.17	31.39
Caf	k_1	0.82	0.64	0.88		
	k_2	0.87	0.80	0.88		
	k_3	0.87	1.13	0.79		
	R	0.05	0.49	0.09		
TP	k_1	29.49	27.31	29.62		
	k_2	31.06	29.77	31.58		
	k_3	28.12	31.58	29.26		
	R	2.94	4.28	0.52		

higher adsorption rate when the flow velocity and sample liquor concentration were low (Tang *et al*., 2011; Gong *et al*., 2005; Zhang *et al*., 2009). However, this will lengthen the production cycle and reduce production efficiency. Considering the adsorption rate and production efficiency, we selected the adsorption velocity of 1.0 mL/min, sample liquor concentration of 50 mg/L. TP and Caf adsorption rate increased as the temperature's increased from 40 to 60°C ((Fig. 2c). The Caf adsorption rate reached a maximum when the temperature was 60°C and then decreased at increasing temperature, while the TP adsorption rate increased slowly when the temperature was greater than 60°C (Tao *et al*., 2013). Considering both the adsorption efficiency of Caf and TP, we selected 60°C as the optimum temperature for loading sample.

Determination of optimal adsorption condition: The orthogonal experimental design was conducted on the basis of single factor design results of different adsorption conditions. Results are shown in Table 4. We know that factors affected the Caf absorption rate, with $B>A>C$. The optimal level combination is $A_2B_3C_2$ (velocity, 1.5 mL/min, temperature, 60°C, concentration, 80 mg/L). The factors affecting TP absorption rate line were *C*, *A*, *B* and the optimal level combination was $A_1B_3C_2$ (velocity, 1.0 mL/min, temperature, 60°C, concentration, 80 mg/L). Analyzed from the above result, $A_2B_3C_2$ (velocity, 1.5 mL/min, temperature, 60°C, concentration, 80 mg/L) was chosen to be the optimal combination of factors for separating Caf and TP.

Elution parameters also influenced the desorption rate:

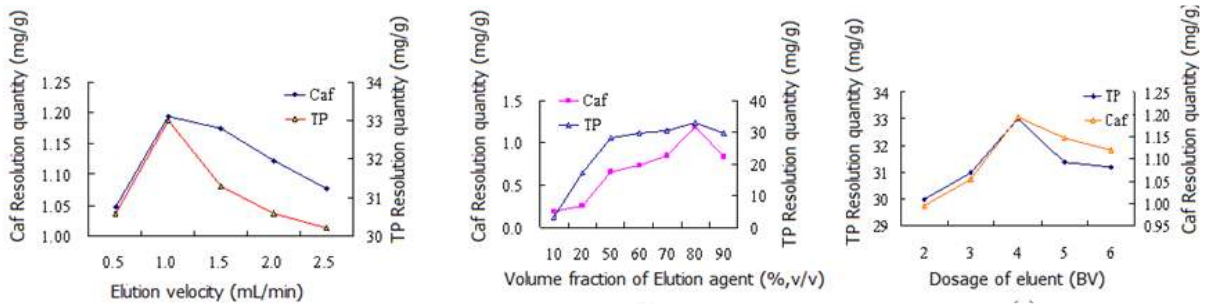
Single factor test result of elution parameters influence on the desorption rate: The single factor test result of influence of different elution parameters on the desorption rate were showed in Fig. 3.

Figure 3a showed both TP and Caf desorption reached a maximum when the elution velocity was 1.0 mL/min. After that point, the desorption quantity decreased with increasing elution velocity (An *et al*., 2013). Figure 3b showed both TP and Caf desorption quantity increased along with the elution agent volume fraction. The data reached a maximum when the ethanol concentration was 80% and then decreased (Huang *et al*., 2007). Figure 3c showed that both TP and Caf desorption quantity increased along with the dosage of eluent at the beginning. The then data reached a maximum when the dosage of eluent was 4 BV and this decreased at higher concentrations. These experiments indicated that the optimal combination of elution parameters was an elution velocity of 1.0 mL/min, the elution agent volume fraction of 80% and the dosage of eluent was 4 BV.

Determination of optimal desorption conditions: The orthogonal experimental design was conducted on the basis of single factor test results of different desorption conditions. The results are shown in Table 5.

Table 5 showed the influence factors on the Caf and TP desorption quantity are $B>C>A$ and $B>A>C$, respectively. The optimal combination was $A_2B_3C_2$, the desorption velocity of 1.0 mL/min, the elution agent volume fraction of 80%, the eluent dosage of 4 BV.

Optimization test results and the analysis of product purity: Five times repeated trials were performed using optimum parameters, i.e., the adsorption velocity of 1.5 mL/min, temperature of 60°C. The Caf concentration was 80 mg/L. Then, the desorption experiments were conducted at the optimal conditions, the desorption agent concentration of 80% ethanol, desorption velocity



(a) (b) (c)

Fig. 3: Desorption curve of TP and Caf with various velocity and concentration and temperature

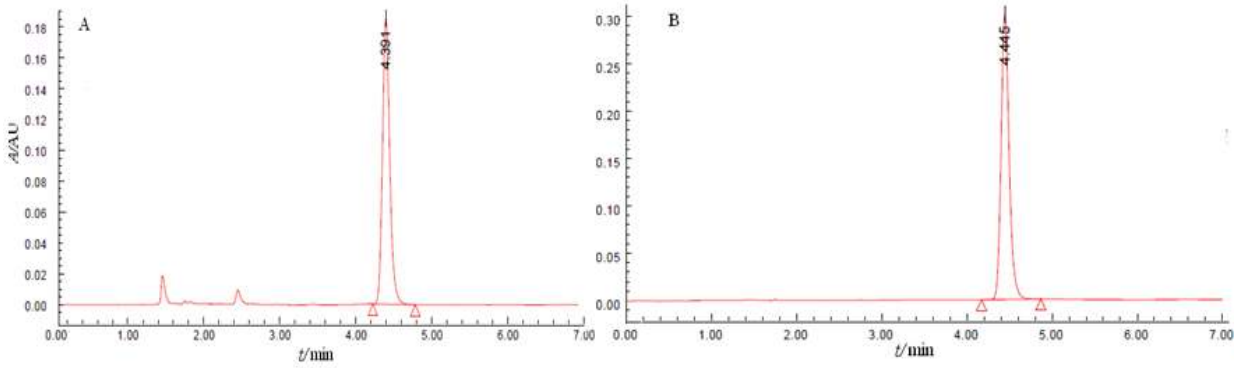


Fig. 4: Caf chromatograms of material (A) and product (B)

of 1.0 mL/min, the eluent dosage of 4 BV. The sample was collected after being enriched, frozen and dried. The average content of Caf and TP before and after the separation were detected respectively and the sample average yield was calculated.

The average mass fraction of Caf and TP seperated from instant tea waste liquor by resin were 23 and 61%. The average recovery was 16.9%. The content before and after the separation were shown in Fig. 4. The purity of Caf was improved after being separated by macro-porous resins.

CONCLUSION

The static adsorption and desorption experiments were adopted to select the proper adsorption carrier (D101). Dynamic absorption and desorption experiment were used to investigate the optimum operating conditions. The optimum parameters were as follows: the optimum adsorption conditions were with an initial concentration of the Caf solution of 80 mg/L, a flow rate of 1.5 mL/min and 60°C; The optimal desorption conditions were a flow rate of 1.0 mL/min, ethanol of 80% and ratio of eluent volume of 4 BV. After column separation, the purity of Caf could be enhanced from 6 to 23% and TP from 38 to 61% and with the final yield of 16.9% following the separation by D101 resin. This study demonstrates that macro-porous resin very effectively separated Caf and TP from instant tea waste liquor.

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