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Optimization of ultrasonic-assisted extraction from chicory root via orthogonal

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Introduction

- ***Cichorium intybus* L.** **Chicory (*Cichorium intybus* L.) is a perennial plant of the Asteraceae family and is native to the Mediterranean region, Central Asia and Northern Africa. It was first cultivated as a medicinal plant and a vegetable crop in ancient Rome and Greece (Plmuier, 1972). Today, it is popularly cultivated in Europe and North America and has many commercial uses.**
- **Chinese Medicine: diuretic, digestive, lightly laxative and cholagogic properties**
- **efficacy of anti-allergy**

- Ultrasonic-assisted extraction: Ultrasound-assisted extraction (UAE) has been widely utilized to isolate bioactive substances from different parts of plants
- Orthogonal array designs: Orthogonal array designs have been used very efficiently to discover how different parameters interact and how they affect product recovery (Stenlund et al., 2009). In this study, a new approach was developed that determined the overall best parameters for the UAE setting

Materials and methods

- **1. Plant materials preparing: Five-year-old Puna chicory roots, freshly harvested in September 2009 after growing in an experimental field of the Grassland Science Department, Northwest A&F University, Shaanxi Province, China, were washed several times with water, cut into pieces 3 to 5 mm in thickness and dried in an oven at 50℃ until a constant weight was obtained (Chen et al., 2009). The dried material was then ground into powder**

- **2. Sample preparation** :

Oven dried *Cichorium intybus* L. roots

- **3. Experimental design : Table 1**
- **4. Preparation of crude extracts: 16 different treatments will to be done in the experiment. 500g chicory root powder was taken every time, put in to the jars and added corresponding ethanol solution stirring with a glass rod. By vacuum pump and Buchner funnel, the mixtures was filtered through 2 layers of filter parers after sonicated, collected the filtrate and was rotary-evaporated under vacuum pump at about 40℃[49] till to concrete yield of chicory**

Table 1 Assignment of controlled factors and levels of the experimental design using mixed orthogonal matrix L_{16} ($4^3 \times 2^6$)

Factors[a]	A(%)	B(v:v)	C(℃)	D(h)	E	F(min)	G(w)	H	I
Level I	0	8	20	24	1	30	200	1	
Level II	50	16	35	48	2	120	400	2	
Level III	75	24	50						
Level IV	100	32	65						

[a]Column A, B, C, D, E, F, G, H and I stand for ethanol concentration, solvent-to-solid ratio, ultrasound temperature, impregnation time, impregnation times, ultrasound time, ultrasound power, ultrasound times and vacancy for statistical error, respectively.

Results

- **1 visual analyses:**

The results of the L16 (43×26) orthogonal experimental design are shown in Fig. 1 and Table 2. The highest extraction percentage was observed in treatment 1 (97.36%, bolded in Table 2), which occurred at visually optimal conditions, as was the case for the treatment A_1 B_1 C_4 D_1 E_2 F_1 G_2 H_2

Table 2 Assignment of factors and levels of experiment by using $L_{16}(4^3\times2^6)$ matrix along with response (extraction rate)

Factors No.treat	A (%)	B (v:w)	C (℃)	D (h)	E (time)	F (min)	G (W)	H (time)	I	extraction rate（%）
1	**1(0)**	1(8)	4(60)	1(24)	2(2)	1(30)	2(400)	2(2)	1	**97.36**
2	2(50)	1	1(20)	2(48)	1(1)	1	1(200)	2	2	25.20
3	3(75)	1	3(50)	2	2	2(120)	1	1(1)	1	25.89
4	4(100)	1	2(35)	1	1	2	2	1	2	2.04
5	**1**	2(16)	3	2	1	2	2	2	2	**72.96**
6	2	2	2	1	2	2	1	2	1	28.32
7	3	2	4	1	1	1	1	1	2	21.50
8	4	2	1	2	2	1	2	1	1	4.29
9	**1**	3(24)	1	1	2	2	1	1	2	**71.64**
10	2	3	4	2	1	2	2	1	1	24.60
11	3	3	2	2	2	1	2	2	2	33.94
12	4	3	3	1	1	1	1	2	1	4.39
13	**1**	4(32)	2	2	1	1	1	1	1	**54.60**
14	2	4	3	1	2	1	2	1	2	33.80
15	3	4	1	1	1	2	2	2	1	32.09
16	4	4	4	2	2	2	1	2	2	10.43

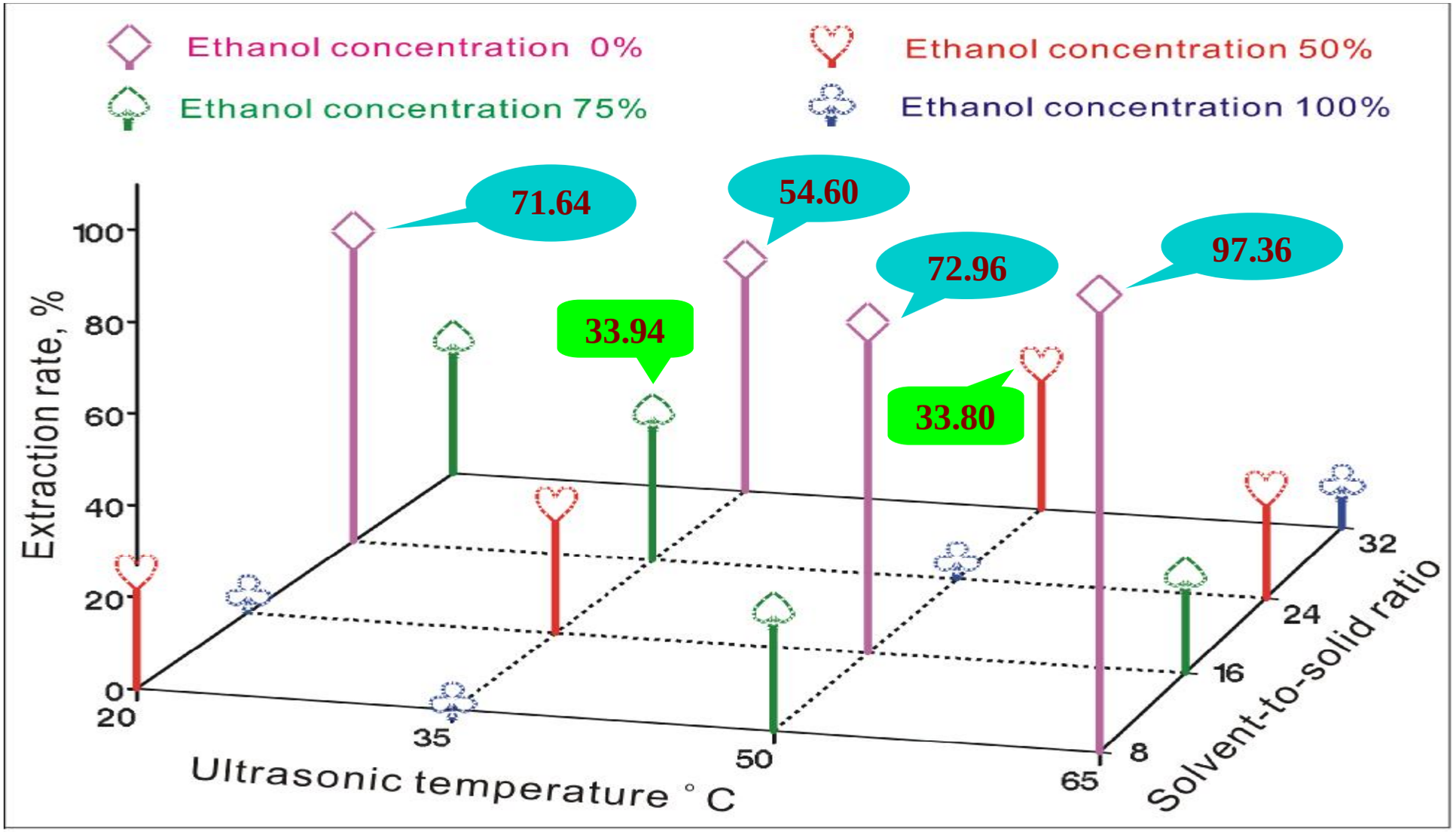

Fig. 1. The interaction of ultrasonic temperature and solvent-to-solid ratio for extraction rate

2. Range and variance analyses

- The ranges of the average and sum of the factors are shown in Table 3 and Table 4, respectively. The ranges in column I, which had statistical errors of 0 and 0.03 in Table 3 and Table 4, respectively, indicated that the experiment was statistically accurate given its designed intention (Hedayat et al., 1999). Furthermore, according to the orthogonal method, as the range increases, the factor becomes more important (Bagheri et al., 2000; Chi and Bloebaum, 1996). As such, the ranges for the averages in decreasing order were A, C, E, H, G, B, D and F (indicated in the last row in Table 3), and the ranges for the sums in decreasing order were A, E, H, G, D, C, B and F (indicated in the last row in Table 4). This ordering of factors (Table 3 and Table 4) thus conveyed their relative importance. Additionally, the results showed that the eight factors were significantly different according to variance analysis (Table 5)

3. Optimized conditions

According to the orthogonal method, the level of the averages (or sum) with the highest yield corresponded to optimum conditions (Hedayat et al., 1999). Both in terms of average and sum, the highest extraction percentage of the levels (i.e., the optimal conditions) are highlighted in bold in Table 3 and Table 4 (including level one of A and B and level four of C, for factors A, B and C). Accordingly, the optimal conditions for calculation were A_1 B_1 C_4 D_1 E_2 F_1 G_2 H_2. Both visually (treatment 1 in Table 2) and by calculation (Table 3 and Table 4), the optimum conditions for the extraction were A_1 B_1 C_4 D_1 E_2 F_1 G_2 H_2. Without A_1 and A_4, the ranges in decreasing order were G, E, C, B, H, D and F, and the optimal conditions were B_4 C_3 D_1 E_2 F_1 G_2 H_2 (Table 6)

Table 3 Averages the levels with the factors and ranges

Factors	A	B	C	D	E	F	G	H	I
Level Ⅰ	74.14	37.62	33.31	36.39	29.67	34.39	30.25	29.80	33.94
Level Ⅱ	27.98	31.77	29.73	31.49	38.21	33.50	37.64	38.09	33.94
Level Ⅲ	28.36	33.64	34.26						
Level Ⅳ	5.29	32.73	38.47						
Range	68.85	5.86	8.75	4.90	8.54	0.89	7.39	8.29	0.00
Order	1	6	2	7	3	8	5	4	9

Table 4 Sum the levels of the factors and the ranges

Factors	A	B	C	D	E	F	G	H	I
Level Ⅰ	296.56	150.49	133.22	291.14	237.38	275.08	241.97	238.36	271.54
Level Ⅱ	111.92	127.07	118.90	251.91	305.67	267.97	301.08	304.69	271.51
Level Ⅲ	113.42	134.57	137.04						
Level Ⅳ	21.15	130.92	153.89						
Range	275.41	23.42	34.99	39.23	68.29	7.11	59.11	66.33	0.03
Order	1	7	6	5	2	8	4	3	9

Table 5 Variance analysis the experimental factors for significance

Variation source	SS	df	MS	F	$F_{0.01(m, n)}$
A	10014.88	3	3338.29	4768989.67**	5403
B	79.33	3	26.44	37777.43**	
C	155.26	3	51.75	73934.05**	
D	96.19	1	96.19	137410**	4052
E	291.47	1	291.47	416386**	
F	3.16	1	3.16	4513**	
G	218.37	1	218.37	311963**	
H	274.98	1	274.98	392827**	
I (error)	0.0007	1	0.0007		
Total variation	11133.64	15			

**: Statistically significant difference at 0.01.

Table 6 Average the levels of the factors and the ranges without factor A or I

Factors	B	C	D	E	F	G	H
Level Ⅰ	51.09	57.29	115.71	103.39	114.44	100.91	105.79
Level Ⅱ	49.82	59.69	109.63	121.95	110.9	124.43	119.55
Level Ⅲ	58.54	62.26					
Level Ⅳ	65.89	46.10					
Range	16.07	16.16	6.08	18.56	3.54	23.52	13.76
Order	4	3	6	2	7	1	5

Discussion

- The ranges of vacancy (I) were close to zero
- Sum up the three orders suggested the importance of them as A>E>G>C>H>B>D>F
- The ethanol concentration were variously reported

- A same optimum conditions of $D_1E_2F_1G_2H_2$
- Solvent-to-solid ratio, B at 8
- Sonicated temperature (C)
- The impregnating time (D) and times of impregnating (E)
- The sonicated time (F)
- Ultrasonic input power (G)

Conclusions

- (1) This study demonstrates that ultrasonic assisted was a great efficiency tool for the fast extraction from plant, and mixed orthogonal matrix was successfully screened the conditions of ultrasonic assisted extraction.
- (2) The importance of the eight factors in ultrasonic assisted extraction was orderly as ethanol concentration > impregnating times > ultrasonic input power > sonicated temperature > sonicated times > solvent-to-solid ratio > impregnating time > sonicated time.

(3) The optimal ultrasonic extraction conditions were impregnating time 24h, impregnating 2 times, sonicated time 30 min, ultrasonic input power 400W and sonicated 2 times, and they were independent of alcohol concentration, solvent-to-solid ratio or sonicated temperature.

(4) The alcohol concentration, solvent-to-solid ratio and sonicated temperature were optimized to two groups: 50~75%, 24~32 and 50~65℃, or 0%, 8 and 65℃, respectively.

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Thank you!