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Research Article

Research on the Release of Theanine in the Exist of Metal Ions in Natural Water

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Abstract: Theanine is a main component in tea leaf, it is the key factor to influence the nutrition value and flavor when the tea leaf is brewing. Natural water is the most-used extractant to dissolve out the theanine. It is recorded in ancient books that dissolution rate is quite different in different kinds of natural water. Recent study shows its correlation with the complexing abilities of metal ions. The thesis is trying to explain the law between the release of theanine and metal ions through designed model test. Response surface experiments showed that in the mixed solution of 1.74 mg/L Ca^{2+}, 21.63 mg/L Na^{+}, 5.55 mg/L Mg^{2+} and 4.86 mg/L Al^{3+}., the release of theanine reaches the peak value 0.88 mg/mL. It is also found that in the exist of Ca^{2+}, free theanine is increasing with total metal ions while free theanine is decreasing with the increasement of total metal ions in the absence of Ca^{2+}.

Keywords: Complexation, metal ion, natural water, tea, theanine

INTRODUCTION

Tea is a popular natural healthy drink worldwide after water and is generally seen as a relaxing drink whereas coffee is seen as more of an energizing drink. In this unique drink, free amino acids is a main kind of nutritional components, L-Theanine accounts for approximately 50% of the total free amino acids in tea. L-Theanine is a non-proteinogenic amino acid found in tea and a derivative of glutamic acid (Danrong *et al*., 2009). In ancient Tang, there are many books and articles announced that different kinds of natural water can promote the release of the nutrition components of tea and improve the flavored substance. There are detailed records about water in The famous book named 《Tea Scripture》 by Lu-Yu, Tang Dynasty, (Lu *et al*., 2011) it says: 'Among the natural water, mountain water is the best, river water is the medium, well water is the poorest quality of 3 for tea brewing.' (Cabrera *et al*., 2006) Natural water can be divided in several groups by its origin named as mountain spring, stream, river, lake, well, rain, snow and so on.' The Top-quality water for tea brewing can be appraised by 5 important indexes as 'Clear, Light, Sweet, Chill, Active' (Cesarettin *et al*., 2012).

The components in the tea solution can be reacted with metal ions easily thus to make change on the flavor of tea. Under the research on completing abilities of several metal ions with components of green tea, 22 metal ions are observed complexing action, when Fe^{2+}, Fe^{3+}, Al^{3+}, Ca^{2+}, Mg^{2+}, Pb^{2+}, Mn^{2+}, Cr^{2+}, Ni^{2+}, Ag^{+}, Zn^{2+} are excessive in water, (Jun-Feng *et al*., 2009) it will generate bad effect on the flavor of tea because of complexation supersaturation, tea solution tasted thin and astringent. While the amount of Zn^{2+}, Ca^{2+}, Cu^{2+} is appropriate, they can stabilize the color of green tea drink and enhance the stability with the increase of their concentration. A comparison between natural water before and after brewing shows that the content of Ca^{2+} in natural water after brewing is much higher than before, the complexation is the main reason of the phenomenon. So, the content of free amino acids is the key factor of tea flavor. Because theanine is the largest amount of free amino acids, we are trying to explain the law of theanine release in the solution of several metal ions (Keenan *et al*., 2011). The thesis is trying to explain the law between the release of theanine and metal ions through designed model test.

MATERIALS AND METHODS

Instruments and reagents: Theanine was purchased from Adamas Reagent Co., Ltd. (Swiss), purity≥99%, NaCl, KCl, $MgCl_2$, $CaCl_2$, $AlCl_3$, $FeCl_3$, SnCl, K_2HPO_4, KH_2PO_4, Ninhydrin hydrate were purchased From Sinopharm Chemical Reagent Co., Ltd. (China).

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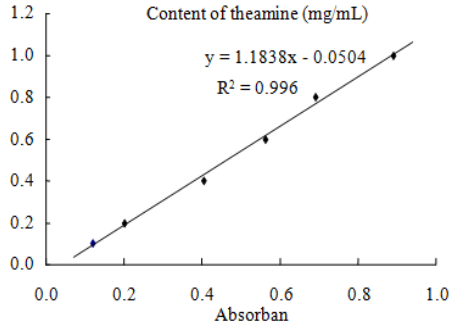

Fig. 1: Working curve of theanine

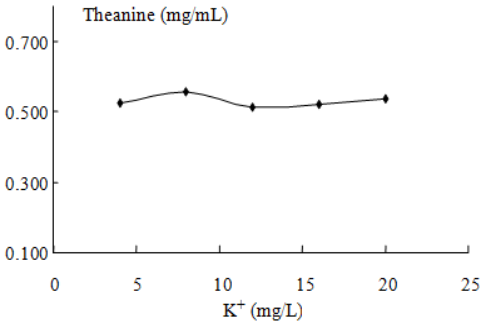

Fig. 2: Relation curve between free theanine with K^+

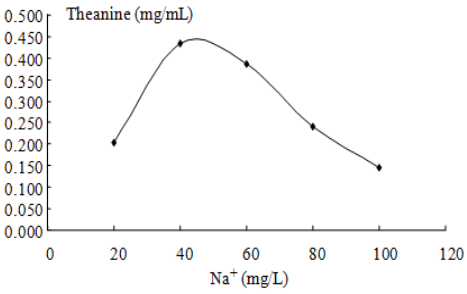

Fig. 3: Relation curve between free theanine with Na^+

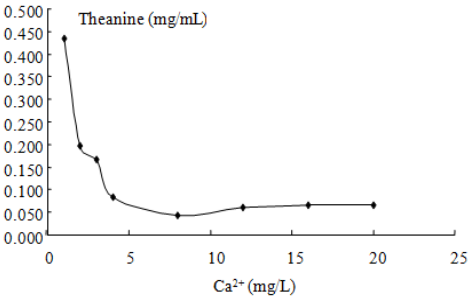

Fig. 4: Relation curve between free theanine with Ca^{2+}

The main instruments used in the experiments are digital display thermostatic water bath pot, Mettler PL403 type electronic analytical balance (0.001 g), Hitachi UV-3310 ultraviolet visible spectrophotometer (Alcaa *et al*., 2007).

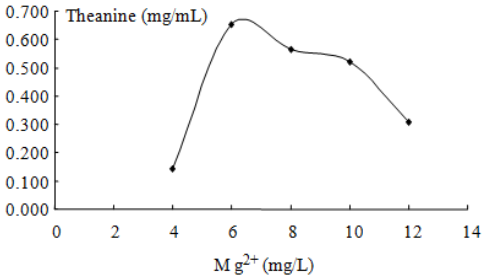

Fig. 5: Relation curve between free theanine with Mg^{2+}

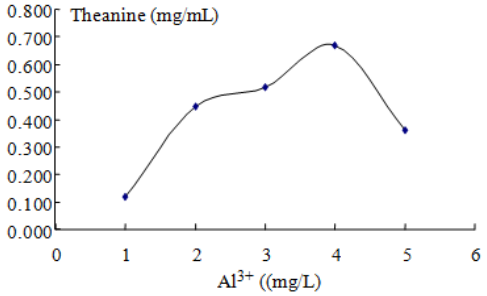

Fig. 6: Relation curve between free theanine with Al^{3+}

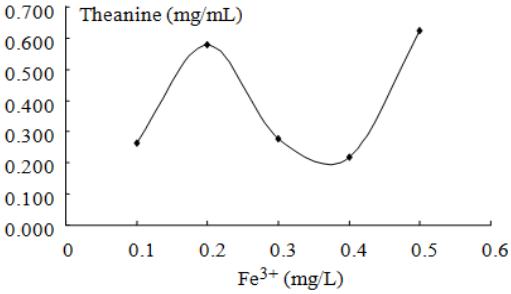

Fig. 7: Relation curve between free theanine with Fe^{3+}

Methods and conditions: One hundred milligram theanine (purity ≥99%) was dissolved in 100 mL distilled water to get theanine standard solution; To prepare 2% Ninhydrin solution, 2 g Ninhydrin hydrate (purity ≥99%) and 80 mg $SnC1\cdot 2H_2O$ were dissolved in 50 mL distilled water, stir well and leave in the dark for 24 h, then filter and set the mixture to 100 mL with distilled water.

Draw of working curve of theanine: One milliliter theanine standard solution, taking concentration gradient as 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL, respectively was adding with 0.5 mL phosphate buffer solution (pH 8.0), 0.5 mL 2% Ninhydrin solution, boil and keep it for 15 min, then cool and set to 25 mL with distilled water, measure its absorbance at 570 nm, the working curve of theanine was drawn as Fig. 1, fitted equation is y = 1.1838x - 0.0504, R^2 = 0.996, the correlation is significant (Syu *et al*., 2008).

Selection of metal ion and handling: Set concentration gradient of metal ions as Table 1

Table 1: Concentration of metal ions selected (mg/L)

	1	2	3	4	5	6
K^+	4	8	12	16	20	-
Na^+	20	40	60	80	100	-
Ca^{2+}	1	2	3	4	8	12
Mg^{2+}	4	6	8	10	12	-
Al^{3+}	1	2	3	4	5	-
Fe^{3+}	0.1	0.2	0.3	0.4	0.5	-

Table 2: Design of response surface experiment

	Level		
Factor	-1	0	1
Na^+	20	40	60
Ca^{2+}	0	1	2
Mg^{2+}	4	7	10
Al^{3+}	3	4	5

Table 3: Results of response surface experiment

	Ca^{2+} (mg/L)	Na^+ (mg/L)	Mg^{2+} (mg/L)	Al^{3+} (mg/L)	Metal ions (mmol/L)	Theanine (mg/L)
1	2	20	7	4	2.00	0.846
2	1	60	10	4	3.94	0.372
3	1	40	7	4	2.82	0.505
4	2	60	7	4	3.74	0.234
5	0	40	7	5	2.88	0.354
6	2	40	4	4	2.62	0.557
7	1	60	7	3	3.58	0.378
8	0	20	7	4	1.90	0.261
9	1	40	7	4	2.82	0.516
10	1	60	4	4	3.44	0.485
11	1	20	4	5	1.81	0.864
12	1	40	4	5	2.68	0.518
13	2	40	7	3	2.76	0.344
14	2	40	7	5	2.98	0.427
15	1	60	7	5	3.80	0.516
16	1	20	7	3	1.84	0.772
17	0	40	7	3	2.66	0.659
18	0	40	4	4	2.52	0.396
19	1	20	10	4	2.20	0.661
20	1	40	4	3	2.46	0.626
21	1	40	10	5	3.18	0.505
22	1	40	7	4	2.82	0.516
23	2	40	10	4	3.12	0.316
24	1	20	7	5	2.06	0.716
25	1	40	7	4	2.82	0.516
26	0	60	7	4	3.64	0.868
27	1	40	10	3	2.96	0.558
28	0	40	10	4	3.02	0.754
29	1	40	7	4	2.82	0.515

according to the content in natural water. Add certain amount of NaCl, KCl, $MgCl_2$, $CaCl_2$, $AlC1_3$, $FeCl_3$ in distilled water to prepare different concentration gradient solution, keep pH at 4.5. Take such solution 1 mL, react with 1 mL theanine standard solution in boiling water for 5 min, then left for future determination. Mix 0.5 mL phosphate buffer solution (pH 8.0), 0.5 mL 2% Ninhydrin solution with the mixture to determine, keep 15 min in boiling water, then cooled to measure its absorbance at 570 nm, check the content of theanine according to the working curve. Mossinon *et al*. (2008) draw the relation curve between metal ions with free amino acids as Fig. 2 to 7, use distilled water for blank control (Iyer and Singhal, 2008).

From Fig. 2, there is slight variance in the content of free theanine when it is added into the K^+ solution of different concentration. It indicates that K^+ has no significant impact on the release of theanine. In the solution of Na^+, Ca^{2+}, Mg^{2+}, Al^{3+}, Fe^{3+}, the content of free theanine presents significant peak value, (Yong-Quan *et al*., 2011) it is clear that theanine has combined with metal ions in certain degree. In the solution of Fe^{3+}, there are 2 peak values because Fe^{3+} can form 2 kinds of complexing compounds with 4 or 6 ligands (Yu and Yi-Ming, 2009). When the complexing reaction is converted, theanine is released, the peak is occurring. Due to the complicate reaction between Fe^{3+} and theanine, we just leave Fe^{3+} for future research, just select Na^+, Ca^{2+}, Mg^{2+}, Al^{3+} to get regular pattern of the theanine release in the optimal ration of the metal ions above.

Experiment design of response surface: According to the requirements of statistics, experiments are arranged as Table 2, the results are listed in Table 3.

RESULTS AND DISCUSSION

Results of response surface: Analysis by Design Expert 8.0.5, fitted model is expressed by R^2, its significance in statistics is tested by F value, the recommended model is 2F1, the results of analysis are shown as Table 4 and Fig. 8. The analysis by Design Expert 8.0.5 notes that free theanine will get the

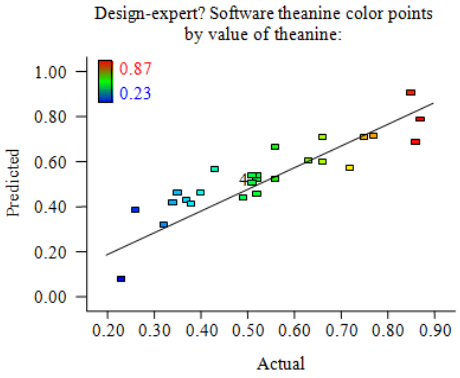

Fig. 8: Analysis on predicted vs. actual number of theanine

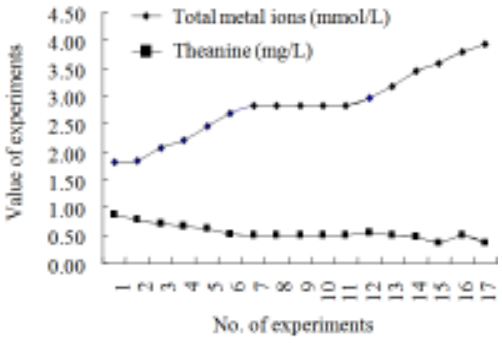

Fig. 9: Relation on metal ions with content of FAAs

Table 4: Significant indexes of model in response surface experiment

Source	S.S.	df	M.S.	F-value	p-value	Notes
Model	0.69	10	0.069	7.01	0.0002	Significant
Lack of fit	0.18	14	0.013	632.04	<0.0001	Significant

S.S.: Sum of square; M.S.: Mean square

Table 5: Relation on variation of metal ions with content of FAAs

Ca^{2+} (mg/L)	Metal ions* (mmol/L)	FAA (mg/L)
0	1.90	0.26
0	2.52	0.40
0	2.66	0.66
0	2.88	0.56
0	3.02	0.75
0	3.64	0.87
2	2.00	0.85
2	2.62	0.56
2	2.76	0.34
2	2.98	0.43
2	3.12	0.32
2	3.74	0.23

: Metal ions = 2 $[Ca^{2+}]$ /40+ $[Na^{+}]$ /23+2* $[Mg^{2+}]$ /24+3* $[Al^{3+}]$ /27

maximum value as 0.88 mg/mL in the mixed solution of 1.74 mg/L Ca^{2+}, 21.63 mg/L Na^{+}, 5.55 mg/L Mg^{2+} and 4.86 mg/L Al^{3+}.

Key influence of Ca^{2+}: From the results above, 4 kinds of metal ions can be complexed by theanine, the complexing ability is varied by the ionic radius. In the same solution, 4 kinds ions are competitive to complex with theanine, theanine will come to the peak value when the mixed metal ions get balance (Khan and Mukhtar, 2007). It is found that the release of theanine is highly related with the total amount of metal ions. When it is absent from Ca^{2+}, the release of theanine is increasing with the increase of metal ions. It is maybe caused by the mutual interference of metal ions, the complexing ability get lower (Ekborg-Ott *et al*., 1997) When the Ca^{2+} reaches 2 mg/L, free theaninie is decreasing with the increase of the total metal ions, it indicates the enhancement of complexing ability, the data are showed in Table 5; When Ca^{2+} is 1 mg/L, free theanine is also decreasing with increase of metal ions, but the trend is flat, shown in Fig. 9.

CONCLUSION

Ca^{2+} is a key metal ions in natural water, the hardness of water is usually divided by its intensity. But from our research, Ca^{2+} is not the unique factor to decide the brewing of tea, especially for the theanine, the important appraisal index. Several metal ions are affecting the flavor and nutrition value of tea, there are really rules to follow though it is complicated. If get tea polyphenol and terpene series substance as analog to react with metal ions, there are most probably to get a optimal brewing method with confirmed proof. In this way, tea will be strengthened by its healthy function and will attract more and more people to have a drink (Singh *et al*., 2013).

ACKNOWLEDGMENT

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