Purification of theaflavins and its pH stability

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Summary

Theaflavin (TF_1), theaflavin-3-gallate (TF_2A), theaflavin-3′-gallate (TF_2B) and theaflavin 3,3′-digallate (TF_3) are four main individual theaflavins from black tea, and are the most important components in black tea contributing to its quality and bioactivity. Theaflavins of 80% purity (named TF80) were isolated from a crude theaflavins solution on AB-8 macroporous resin using step-gradients of aqueous ethanol. Under appropriate conditions, the yield reached 50%.And pH stability of TF80 at 37□ was studied. Theaflavins were much more stable in a pH range of 2.5 to 5.5 relative to 6.5 to 7.5. The relative stability of the individual theaflavins was determined to be $TF_3 \geq TF_2A \approx TF_2B > TF_1$.

Introduction

Theaflavins are a group of polyphenol pigments formed during the fermentation process of making black tea. Each of the theaflavins, - theaflavin (TF_1), theaflavin-3-gallate (TF_2A), theaflavin-3′-gallate (TF_2B) and theaflavin 3,3′-digallate (TF_3), has a benzotropolone skeleton in common. TF_1 contains no gallate ester, TF_2A and TF_2B each have one gallate, and TF_3 has two gallates.

Materials and methods

The crude theaflavins, also named TF40, containing catechins (mainly 7.0% EC, 8.8% EGCG and 11.0% ECG), TF_1, TF_2A, TF_2B and TF_3 (total theaflavins account for about 50%), was prepared according to our patented method[1].

Further purification was optimized through a column chromatograph equipped with different macroporous resins (AB-8, NKA-9, SP-207). The absorption capacities and desorption capacities of the three resins were evaluated by static tests. Dynamic adsorption experiments evaluated elute flow rate and gradient elution. The bed volume (BV) of the resin was 100 mL. The eluate was collected in 25ml fractions, followed by HPLC analysis. The solutions in which the concentration of theaflavins was over 80% were pooled, and dried under vacuum to obtain purified theaflavins (TF80). The yield of purified theaflavins was calculated.

The pH stability of TFs was assessed as follows: prepare 1mg/ml TF80 in sodium phosphate-citric acid buffer at different pH values, varying from 2.5-7.5, incubating at 37°C for 24 h. The levels of theaflavins remaining were determined by HPLC analysis every 3 h. The reversion of TF80 was appraised as following: after incubating in sodium phosphate-citric acid buffer at pH 6.5 and 7.5 at 37°C for 12h, adjust pH values back to 5.5, and then theaflavins were determined every 1.5h to observe the change of content.

Results and discussion

Three macroporous resins (AB-8, NKA-9, SP-207) were tested through static adsorption tests for preliminary selection, The adsorption capacity/desorption capac ity of AB-8, NKA-9, and SP-207 were determined to be 76.8%/73.6%, 44.6%/68.1%, and 47.1%/94.6%, respectively. Thus the recovery ratio of total theaflavins of AB-8, NKA-9, SP-207 were 56.5%, 30.4%, 44.5%, respectively. Therefore, AB-8 was significantly better than the others according to the statistical analysis ($P<0.05$). Comparison

of yields by different elution conditions was shown in table 1. The total theaflavins content of TF80 reached 83.8%, including 10.2% TF_1, 15.1% TF_2A, 12.5% TF_2B and 46.0% TF_3 according to the HPLC-UV analysis. Most catechins were removed under the appropriate purification conditions, and purified (-)-epicatechin gallate (ECG), the most abundant catechin in TF40, was additionally obtained during the elution (Figure 2).

Table 1 Comparison of yields by different elution conditions

Elution conditions	Yield/%
40% ethanol aqueous solution, 1 BV/h	26.45±0.01 [c]
40% ethanol aqueous solution, 2 BV/h	39.29±0.01 [b]
40% ethanol aqueous solution, 3 BV/h	40.34±0.02 [b]
20%-40%-60% ethanol aqueous solution, 2 BV/h	51.88±0.02 [a]
20%-30%-40%-50%-60% aqueous solution, 2 BV/h	54.76±0.02 [a]

[c,b,a] represent significant difference between the yields ($p < 0.05$), and [a] is better than [b], [b] is better than [c]

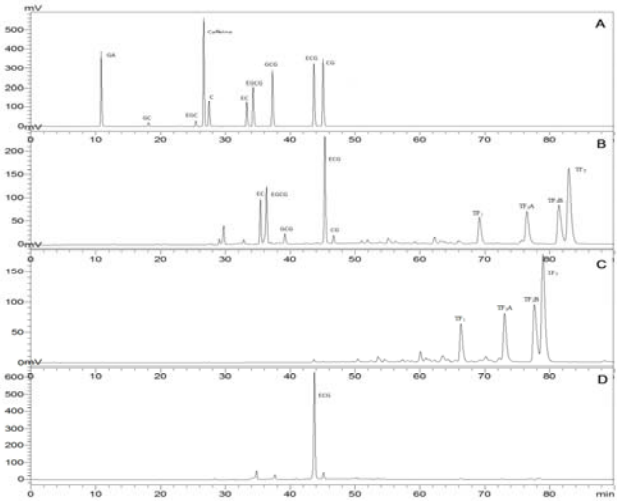

Figure 1. HPLC chromatogram of catechins, TF40, TF80 and ECG.

As illustrated in Figure 2, TFs demonstrated pH-dependent stability; the lower the pH values of the sodium phosphate-citric acid buffer, the greater the stability. This supports previous results. Simply, theaflavins should be stable in the acidic environment of the stomach, but easily destroyed when in the more alkaline environment of the small intestine. The pH-dependent stability should be taken into consideration when theaflavins are used for health-food or medicine. In addition, we observed a red substance after incubation in pH 7.5 buffer for 25 h. This might be evidence of thearubigins or other oxidation products of theaflavins. According to the HPLC analysis, catechins did not appear to be degradation products of theaflavins, but unidentified peaks did appear between catechins and theaflavins. That means that alkaline conditions might induce theaflavins to form intermediates, whose structures and bioactivity await further study.

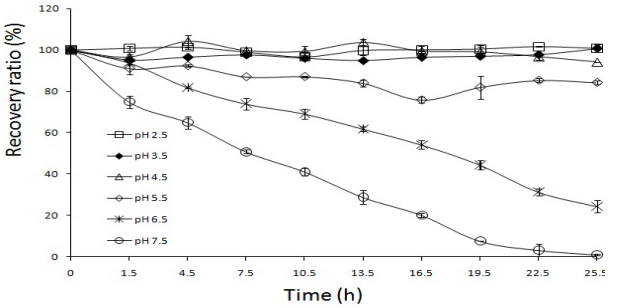

Figure 2. Recovery ratio of total theaflavins at pH value ranging from 2.5 to 7.5 at 37°C. Data are expressed as means±S.D. of n=3 samples.

Individual theaflavins demonstrated differences in stability. In all, the stability is $TF_3 \geq TF_2A \approx TF_2B > TF_1$ (Figure 3). This might be due to the more stability of galloyl group than hydroxyl group. When in alkaline solution, more hydroxyl group had reactions with galloyl group. TF_3 has one more galloyl group than TF_2 and two more than TF_1. The biochemical reaction of TFs in high pH value was irreversible.

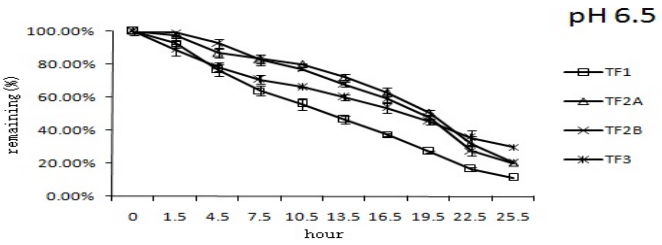

Figure 3. pH-dependent stabilty of individual theaflavins at pH value 6.5 at 37°C. Data are expressed as means±S.D. of n=3 samples.

References

Tu, Y.Y., Xu, X.Q., Xia, H.L., Watanabe, N. (2005) Optimization of theaflavin biosynthesis from tea polyphenols using an immobilized enzyme system and response surface methodology. Biotechnol Lett. 27(4): 269-274.