Glycative Stress Research

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Original Article

Effect of tea (*Camellia sinensis*) and herbs on advanced glycation endproduct formation and polyphenol metabolism by post-fermentation

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Abstract

***Objective*:** Fermentation, a procedure for producing tea leaves, includes oxidative fermentation and post-fermentation; the former acted by enzymes within the tea leaves and the latter is induced by microorganisms, such as molds and bacteria, *i.e.*, lactobacillus. The purpose of the present study is to examine the influence of fermentation on polyphenol components and anti-glycation activity, using test samples of catechin-rich tea derived from *Camellia sinensis* (CS) and other herb teas.

***Methods*:** Test samples were 13 kinds of CS-derived tea and 18 kinds of herb tea, from each 1g of which extracts were prepared by 1 hour incubation in 40 mL hot water at 80°C. Then, 7 kinds of catechin contents were measured by reverse-phase high-performance liquid chromatography (HPLC). The results of anti-glycation effect (half maximum concentration; IC_{50}) were quoted from our tentative report of advanced glycation endproduct (AGE) formation inhibition tests using fluorescent AGEs as a marker in an *in vitro* reaction model between glucose and albumin.

***Results*:** Total amount of 7 catechins was 0.461 mg/mL as the catechin equivalent in CS tea and 0.063 mg/mL in herb tea. Post-fermentation reduced the catechin content in CS tea to 0.009 ~ 0.427 mg/mL, a level which was 1/10 ~ 1/4 of the level in non-treated green tea. While, oxidative fermentation reduced the catechin content in CS tea to 0.398 ~ 0.575 mg/mL, or 2/5 ~ 3/5 the level in green tea. The catechin reduction was larger in post-fermentation. In CS tea (green tea, Oolong tea and black tea), a significant correlation was noted between the anti-glycation activity and catechin contents; r = 0.930 in theaflavin and r = 0.926 in (-)-epigallocatechin (EGC), while no correlation was noted in herb tea. In herb tea of rooibos (*Aspalathus linearis*), hama-cha (*Chamaecrista nomame*), tien-cha (*Rubus suavissimus*) persimmon (*Diospyros kaki*) and dokudami (*Houttuynia cordata*) in which post-fermentation enhanced the anti-glycation activity, no relationship was found between the catechin component change and the activity.

***Conclusion*:** Oxidative fermentation on CS tea increased theaflavin content and decreased EGC content that may be related to the reduction of anti-glycation activity. The polyphenol contents tended to increase in the tea extracts in which post-fermentation enhanced anti-glycation activity. The post-fermentation procedure in herb tea may act on polyphenols other than catechins and it may be useful for the prevention of AGE formation.

***KEY WORDS*:** advanced glycation endproducts (AGEs), tea (*Camellia sinensis*), fermentation, polyphenol, catechin

Introduction

Glycative stress is the total concept whereby reducing sugars, glucose and aldehydes, once combined with protein and lipids in the body, cause a variety of reactions and finally form advanced glycation end products (AGEs), followed by additional *in vivo* reactions, tissue damage and inflammation. Glycative stress is one of risk factors for age-related diseases and deterioration, for example, which play a role in the pathogenesis of atherosclerosis or osteoporosis [1,2)]. We have reported the effect of tea (*Camellia sinensis*; CS) leaf extracts, containing abundant catechins [3)], which show AGE formation inhibitory activity (anti-glycation effect) [4)]. Also, 31 samples of CS-derived tea and herb tea extracts were examined using an *in vitro* albumin/glucose reaction model and as a result, all 13 CS tea samples and 4 of 18 herb tea samples [rooibos (*Aspalathus linearis*), tien-cha (*Rubus suavissimus*), guava (*Psidium guajava*) and dokudami (*Houttuynia cordata*)], showed anti-glycation activity the same as or higher than

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aminoguanidine (AG). Further, in the tea leaves of which the glycation activity was augmented by the post-fermentation procedure, the polyphenol content tended to increase [5].

Fermentation is an important procedure in the tea leaf production which can give the tea additional value. Fermentation tea is divided into two groups: one is oxidative fermentation tea which occurs by polyphenol oxidase contained in tea leaves, the other is post-fermentation tea due to microorganism *i.e.*, lactobacillus. Green tea makes Oolong tea by half-way oxidative fermentation and black tea by further fermentation. Catechins, rich in tea leaves, are mainly classified into (+)-catechin (C), (-)-epicatechin (EC), (-)-epicatechin gallate (ECg), (-)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCg), and (-)-theaflavin. Oxidative fermentation causes marked reduction of EGCg and EGC and the formation of (-)-theaflavin by catechin polymerization [6]. Post-fermentation tea includes molding-induced aerobic fermentation tea (batabata-cha, pu'er tea), bacteria (*i.e.*, lactobacillus)-induced anaerobic fermentation tea (Awa-ban-cha), and aerobic and anaerobic-combined fermentation tea (goishi-cha, Ishizuchi-kuro-cha). The advantage of tea leaf post-fermentation is the augmentation of mild taste and better preservation quality [7]. However, the influence of post-fermentation on the biochemical changes of tea components or anti-glycation activity remains unclear.

In the present study, to examine the relation between fermentation and anti-glycation activity, and especially focus the post-fermentation procedure, we measured catechin components using same samples of CS teas and herb teas [5] in comparison with the anti-glycation activity.

Methods

Test samples

Test samples used, the same as in the previous report, [5] were extracts of 13 CS-derived tea leaves and 13 herb tea leaves and 21 kinds of polyphenols. As reagents, C, EC, ECg, and EGC were purchased from Kurita Water Industries Ltd. (Nakano-ku, Tokyo, Japan), EGCg, GC and theaflavin were from Nagara Science Co., Ltd. (Gifu, Japan), and caffeine was from Wako Pure Chemical Co., Ltd. (Chuo-ku, Osaka, Japan).

Measurement of catechins and polyphenols

High performance liquid chromatography (HPLC) was used for the measurement of catechins and polyphenols. For catechin measurement, each sample was diluted 2 fold with pH 2.6 phosphate buffered saline (PBS), 20 μL of which was put into the HPLC (Tosoh, Minato-ku, Tokyo, Japan) and measured with flowing conditions; column, Cadenza CD-C18 (75 x 4.6 mm); (Imtakt, Kyoto, Japan) eluent A, 10.0 mM PBS (pH 2.6); eluent B, acetonitrile; flow rate, 1.0 mL/min; column temperature, 40°C; detection wave length, UV 270 nm. For measurement of the catechin component, reverse-phase HPLC was used and each polyphenol was identified based on the retention time (RT) of standardized polyphenols. The results were expressed as a catechin equivalent (Eq).

For polyphenol measurement, each polyphenol sample was dissolved in dimethyl sulfoxide (DMSO) and diluted 2 fold with PBS (pH 2.6), then 20 μL of solution was put into the HPLC in the same way as described above.

Results

Catechin component of tea extracts

The catechin and caffeine contents were measured by reverse-phase HPLC based on RT, which was 2.4 ~ 2.5 in GC, 4.8 ~ 5.0 in ECG, 6.2 ~ 6.4 in C, 7.1 ~ 7.3 in caffeine, 11.5 ~ 11.7 in EC, 12.2 ~ 12.4 in EGCg, 17.7 ~ 18.0 in ECg, and 25.7 ~ 25.8 in theaflavin. The results of each concentration (mg catechin Eq/mL) were calculated using these data and are presented in ***Table 1***.

The total amount of 7 catechins averaged 0.063 mg catechin Eq/mL in 18 herb tea samples and 0.461 mg catechin Eq/mL in 13 CS tea samples. Catechin contents were 0 mg/mL in several herb teas and averaged lower in herb teas than in CS teas.

Catechin contents in post-fermentation tea were 0.009 ~ 0.427 mg catechin Eq/mL, which was 1/10 ~ 1/4 of green tea (leaf). Catechin contents in oxidative fermentation tea were 0.009 ~ 0.427 mg catechin Eq/mL, which was 2/5 ~ 3/5 of green tea (leaf). The values tended to be lower by post-fermentation than by oxidative fermentation.

In the correlation analysis between catechin contents and glycation activity, there was no significant correlation in herb tea, maybe because the catechin contents were very low. While in CS tea, a significant correlation was noted; r = 0.930 (y = 2.2937x -0.0171) between IC_{50} and theaflavin in green tea (leaf and tea bag), Oolong tea (leaf and tea bag), black tea and Darjeeling tea (***Fig. 1***). Also, the logarithmic correlation was noted between IC_{50} elevation and EGC content elevation (r = 0.926, y = -0.026ln(x) - 0.0296, ***Fig. 2***).

The correlation analysis was conducted between anti-glycation activity and EGC or theaflavin contents in post-fermentation tea was conducted. There was a moderate correlation between EGC contents and IC_{50} (r = 0.878), on the condition that values of dan-cha and batabata-cha were excluded, since the EGC amount was 0.

Comparison of chromatograph

Figures 3-12 show the difference in chromatograph between before and after the post-fermentation procedure in the tea leaf samples which have a complete set of two data. In CS-derived post-fermentation tea, except for pu'er tea (***Figs. 13-17***), chromatographs were compared with that of green tea (leaf). There were some changes noted in the peak area by post-fermentation. Post-fermentation partially augmented the peak area, however this was reduced or disappeared more frequently. The peak area reduction was marked especially at RT 15 ~ 25 minutes in herb tea and at RT 10 ~ 20 minutes in CS tea.

For an example of oxidative fermentation, the chromatograph of black tea was compared with that of green tea (leaf) (***Fig. 18***). Peak area change was not marked by oxidative fermentation compared to post-fermentation.

Table 1. Catechin contents in tea extracts

Name	C	EC	ECg	GC	EGC	EGCg	Theaflavin	Catechin total	Caffeine	Character
Herb tea	(mg/mL)									
Rooibos	0.000	0.000	0.036	0.033	0.024	0.000	0.005	0.097	0.000	OF
+PF	0.004	0.000	0.000	0.018	0.016	0.000	0.008	0.047	0.000	OF & PF
Green rooibos	0.006	0.000	0.044	0.021	0.010	0.030	0.009	0.121	0.000	
+PF	0.005	0.120	0.010	0.017	0.000	0.000	0.014	0.166	0.000	PF
Hama-cha	0.008	0.009	0.003	0.000	0.025	0.003	0.018	0.065	0.000	
+PF	0.008	0.000	0.000	0.000	0.015	0.000	0.029	0.051	0.004	PF
Tien-cha	0.037	0.000	0.036	0.000	0.048	0.028	0.047	0.197	0.003	
+PF	0.000	0.000	0.024	0.000	0.000	0.014	0.009	0.047	0.000	PF
Guava	0.005	0.014	0.000	0.000	0.029	0.000	0.000	0.048	0.000	
+PF	0.004	0.015	0.009	0.000	0.000	0.005	0.000	0.032	0.001	PF
Persimon leaf	0.002	0.001	0.000	0.000	0.013	0.000	0.000	0.016	0.000	
+PF	0.000	0.000	0.002	0.018	0.020	0.000	0.019	0.060	0.003	PF
Houttuynia (Dokudami)	0.004	0.000	0.000	0.028	0.020	0.000	0.003	0.054	0.000	
+PF	0.000	0.000	0.000	0.022	0.015	0.000	0.011	0.048	0.002	PF
German chamomile	0.007	0.004	0.000	0.000	0.040	0.000	0.000	0.052	0.000	
+PF	0.000	0.000	0.002	0.000	0.015	0.000	0.000	0.017	0.023	PF
Perilla (Shiso-cha)	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	
+PF	0.001	0.000	0.002	0.000	0.012	0.000	0.000	0.015	0.013	PF
Tea										
Green tea (leaf)	0.034	0.173	0.068	0.062	0.295	0.317	0.009	0.958	0.111	
(tea bag)	0.035	0.081	0.075	0.037	0.154	0.142	0.017	0.542	0.058	
Oolong tea (leaf)	0.019	0.050	0.042	0.043	0.085	0.138	0.021	0.398	0.111	OF
(tea bag)	0.026	0.050	0.053	0.066	0.060	0.153	0.032	0.044	0.011	OF
Black tea	0.052	0.070	0.012	0.047	0.021	0.185	0.038	0.425	0.111	OF
Black tea (Darjeeling)	0.045	0.088	0.109	0.065	0.039	0.200	0.028	0.575	0.119	OF
Pu'er tea	0.066	0.386	0.249	0.078	0.187	0.421	0.013	1.401	0.185	
+PF	0.028	0.037	0.054	0.000	0.021	0.017	0.035	0.193	0.015	PF
Goishi-cha	0.020	0.027	0.018	0.065	0.022	0.025	0.007	0.184	0.105	PF
Ishizuchi-kuro-cha	0.061	0.073	0.016	0.104	0.094	0.018	0.014	0.380	0.080	PF
Batabata-cha	0.000	0.000	0.005	0.000	0.000	0.004	0.000	0.009	0.078	PF
Awa-ban-cha	0.021	0.058	0.034	0.049	0.163	0.093	0.010	0.427	0.073	PF
Dan-cha	0.000	0.000	0.023	0.000	0.000	0.000	0.034	0.058	0.198	PF

C,(+)-catechin; EC, (-)-epicatechin; ECg, (-)-epicatechin gallate; GC, (-)-gallocatechin; EGC, (-)-epigallocatechin; EGCg, (-)-epigallocatechin gallate; PF, post-fermentation; OF, oxidative fermentation.

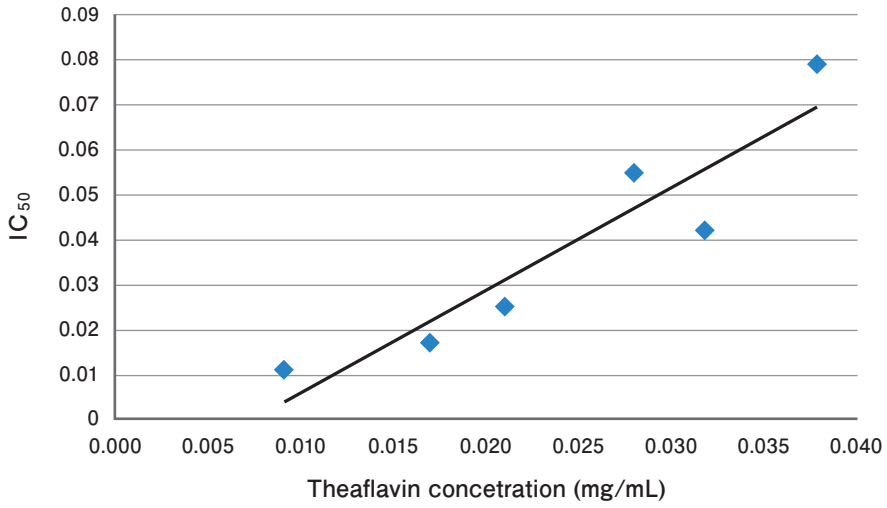

Fig 1. **Correlation between theaflavin and antiglycation effect in the oxidative fermentation tea.**

y = 2.2937x - 0.0171, r = 0.930, r < 0.05. IC_{50} data are quoted from Ref.5. IC_{50}, half maximum concentration; EGC, (-)-epigallocatechin.

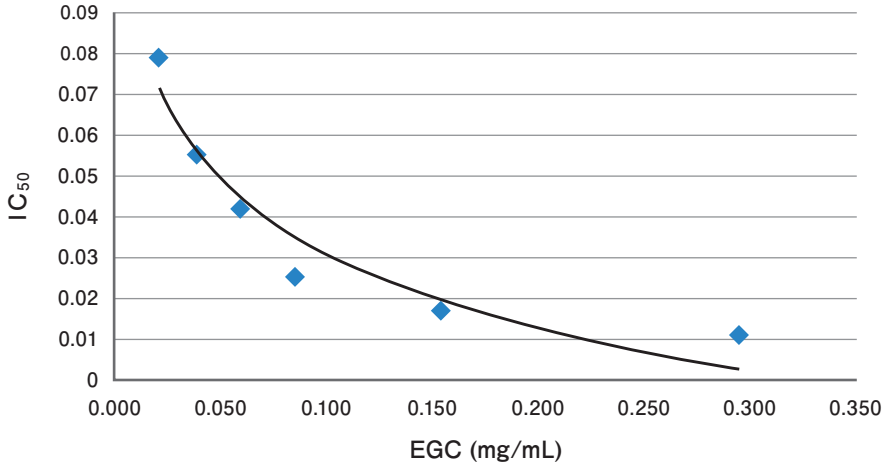

Fig 2. **Correlation between EGC and anti-glycation activity in the oxidative fermentation tea.**

y = -0.026ln(x) - 0.0296, r = 0.926, p < 0.05. IC_{50} data are quoted from Ref. 5. IC_{50}, half maximum concentration; EGC, (-)-epigallocatechin.

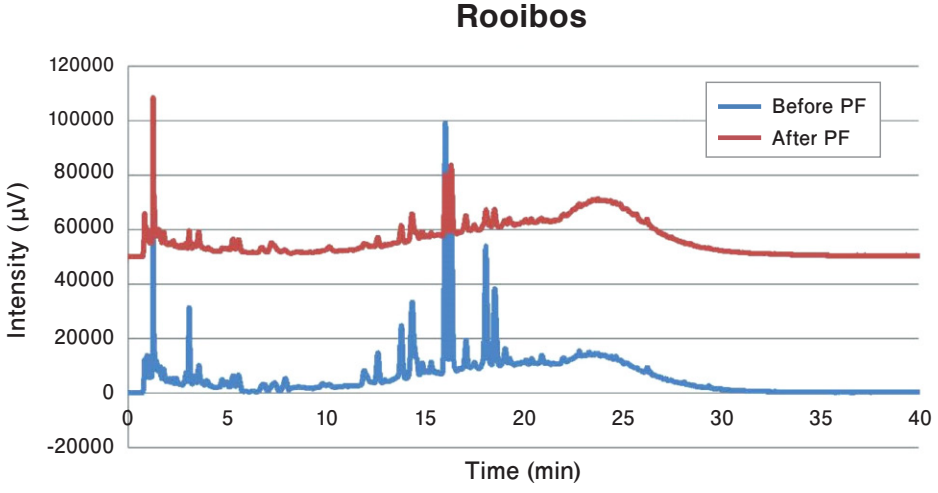

Fig 3. **Reverse-phase HPLC chromatograph before and after PF of rooibos.**

PF, post-fermentation.

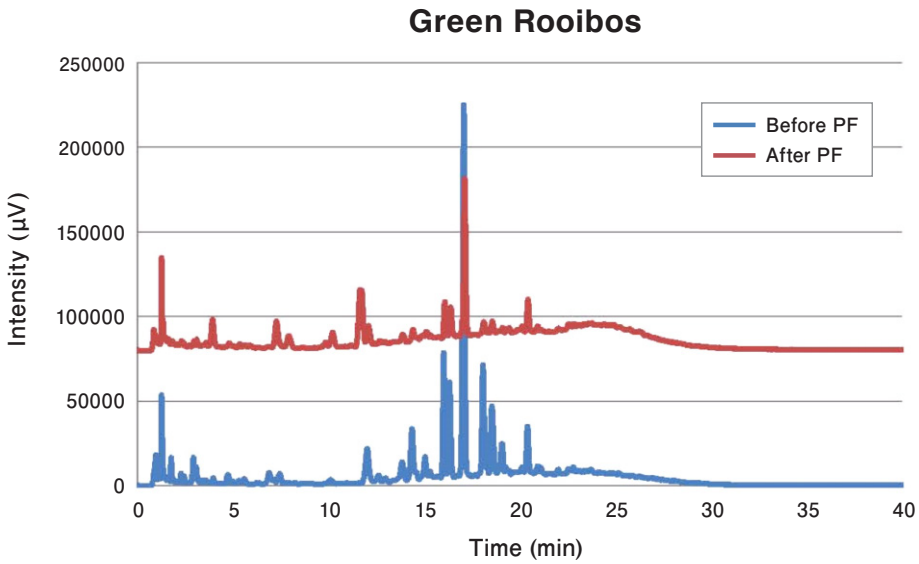

Fig 4. **Reverse-phase HPLC chromatograph before and after PF of green rooibos.**
PF, post-fermentation.

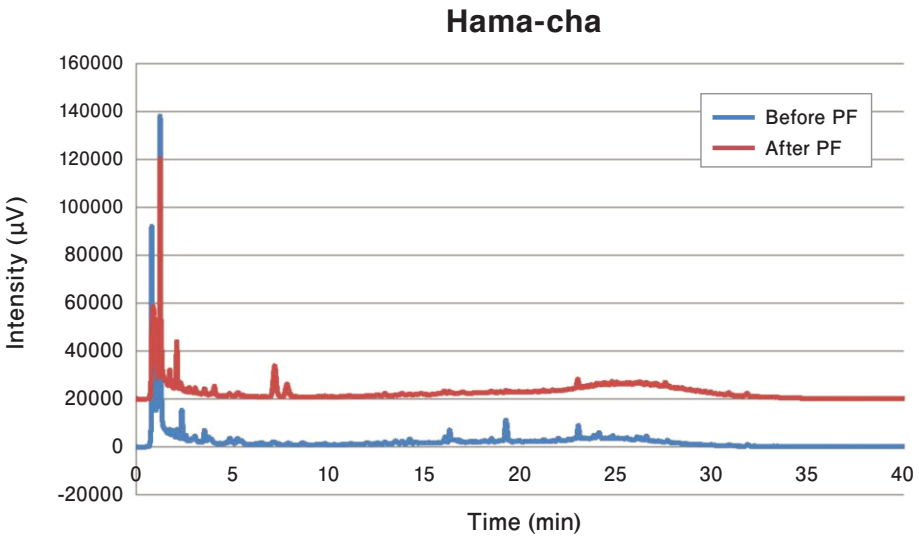

Fig 5. **Reverse-phase HPLC chromatograph before and after PF of Hama-cha.**
PF, post-fermentation.

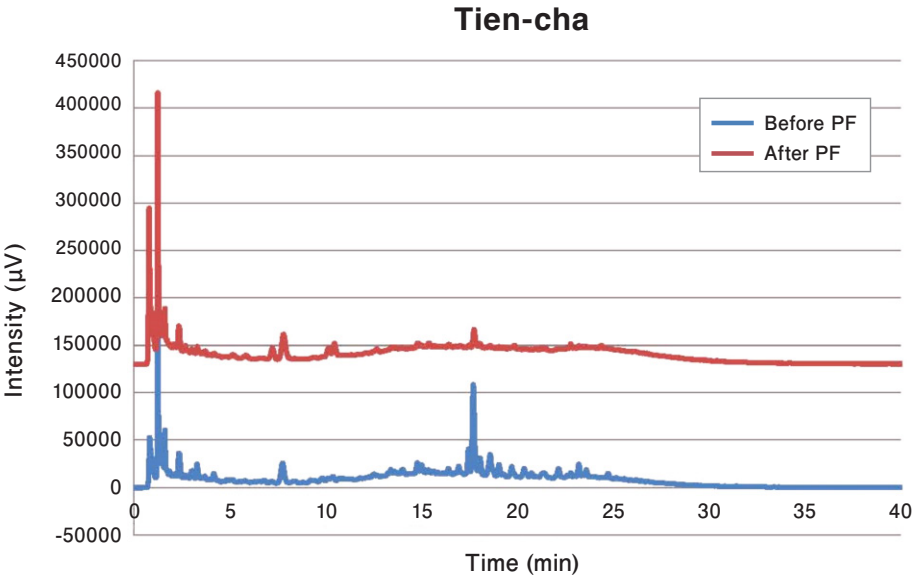

Fig 6. **Reverse-phase HPLC chromatograph before and after PF of Tien-cha.**
PF, post-fermentation.

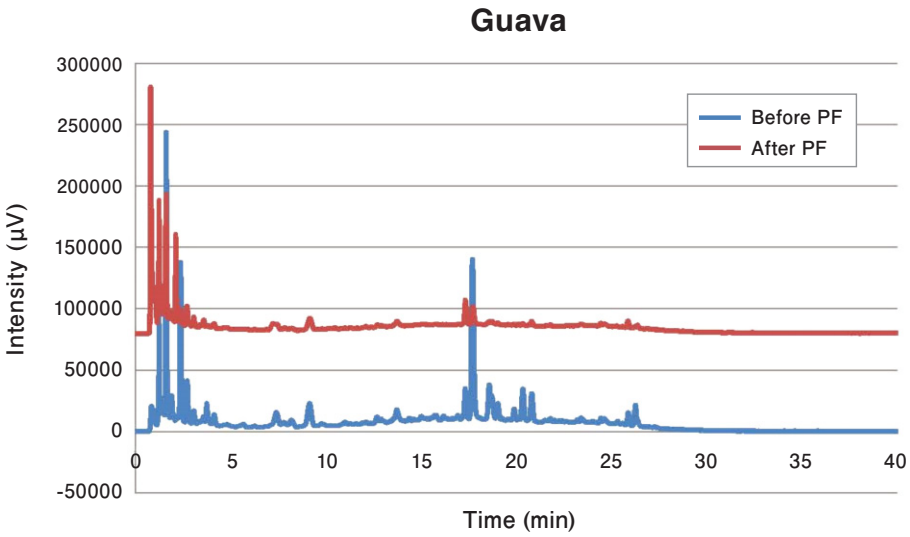

Fig 7. **Reverse-phase HPLC chromatograph before and after PF of guava.**
PF, post-fermentation.

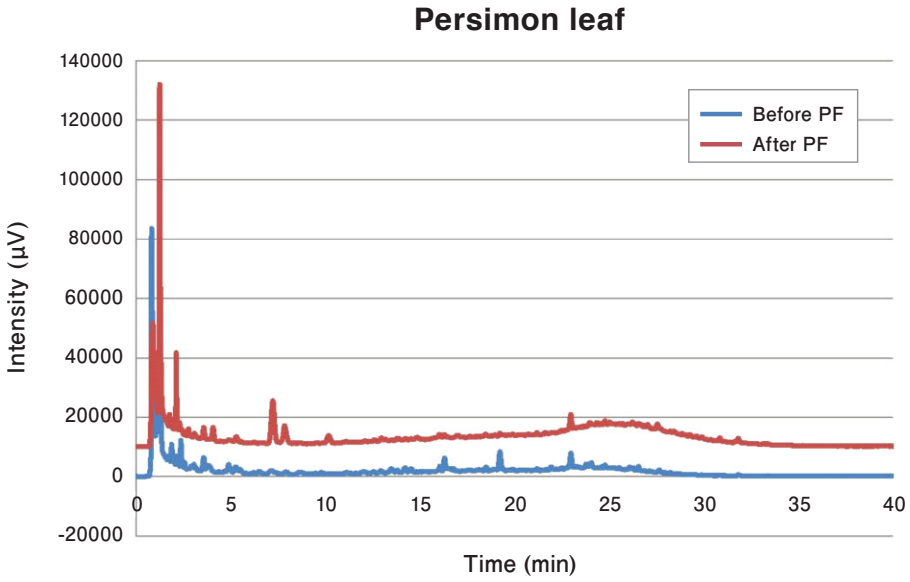

Fig 8. **Reverse-phase HPLC chromatograph before and after PF of persimon leaf.**
PF, post-fermentation.

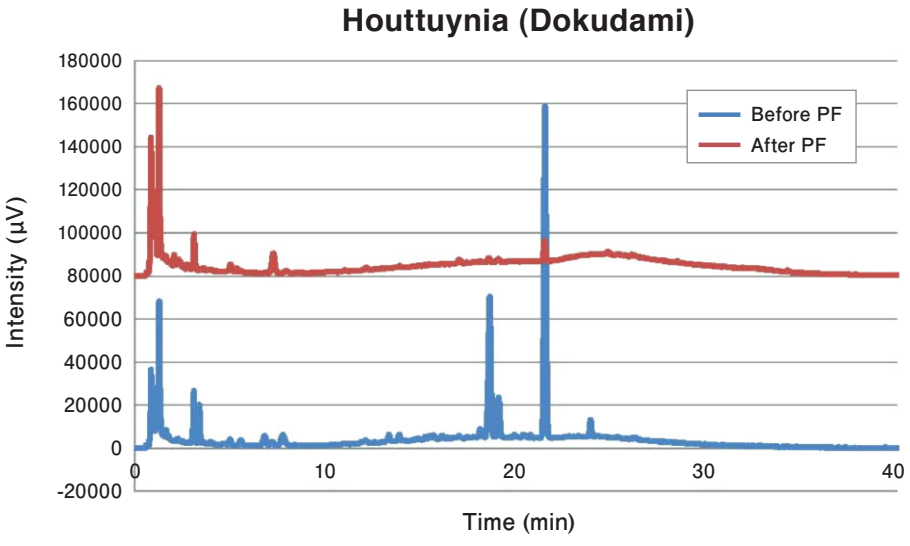

Fig 9. **Reverse-phase HPLC chromatograph before and after PF of houttuynia (dokudami).**
PF, post-fermentation.

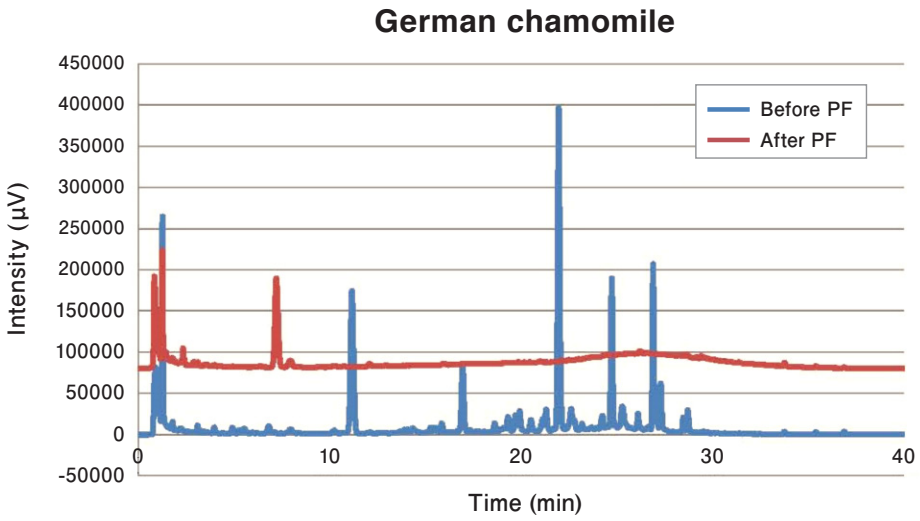

Fig 10. **Reverse-phase HPLC chromatograph before and after PF of German chamomile.**
PF, post-fermentation.

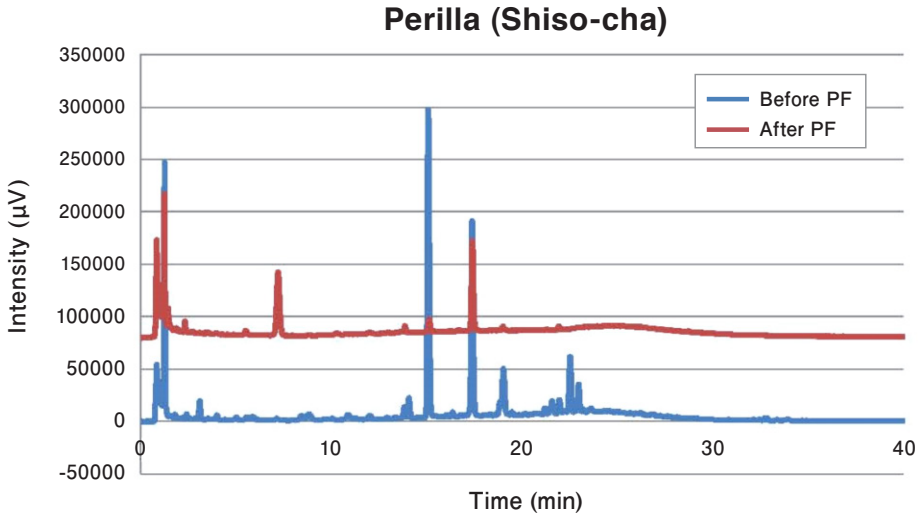

Fig 11. **Reverse-phase HPLC chromatograph before and after PF of perilla (shiso-cha).**
PF, post-fermentation.

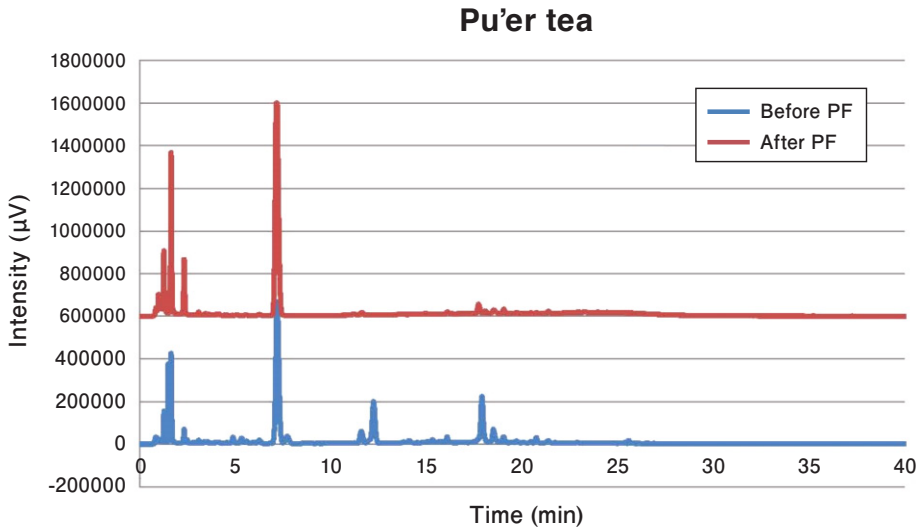

Fig 12. **Reverse-phase HPLC chromatograph before and after PF of Pu'er tea.**
PF, post-fermentation.

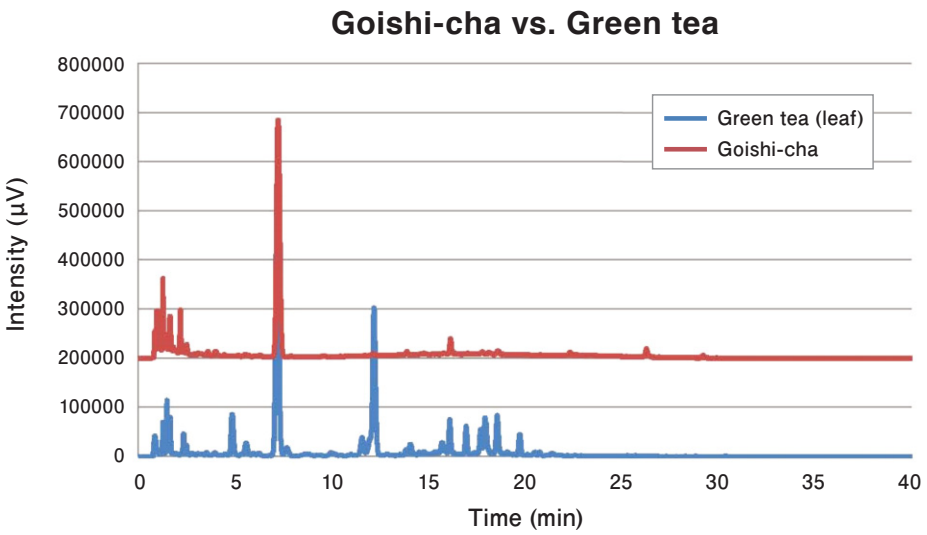

Fig 13. **Reverse-phase HPLC chromatograph of Goishi-cha (designated PF) and green tea (leaf).**
PF, post-fermentation.

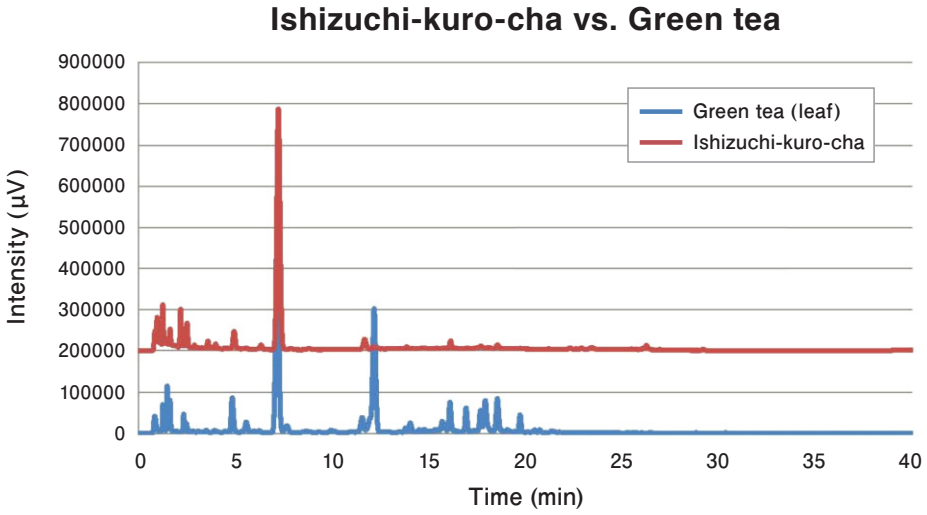

Fig 14. **Reverse-phase HPLC chromatograph of Ishizuchi-kuro-cha (designated PF) and green tea (leaf).**
PF, post-fermentation.

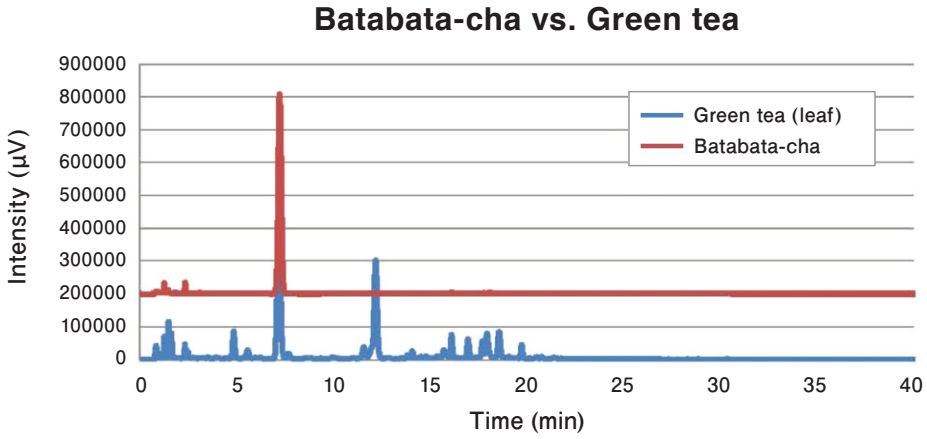

Fig 15. **Reverse-phase HPLC chromatograph of Batabata-cha (designated PF) and green tea (leaf).**
PF, post-fermentation.

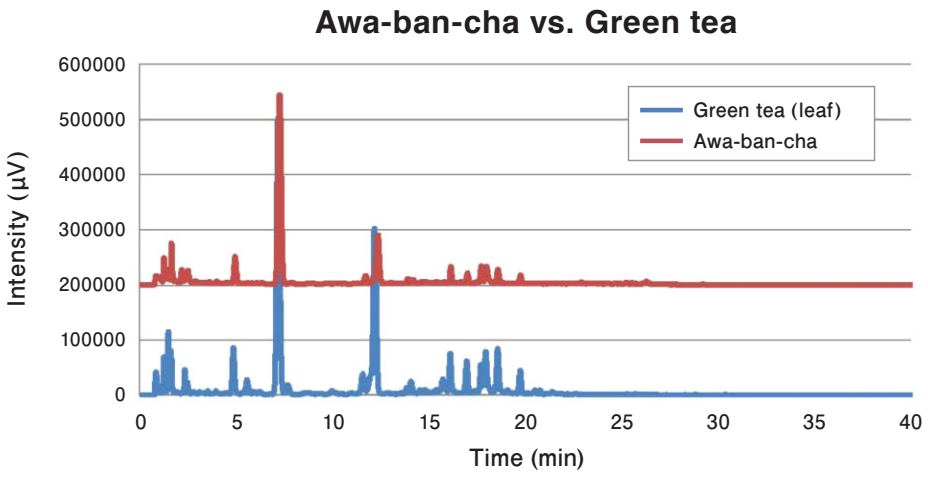

Fig 16. **Reverse-phase HPLC chromatograph of Awa-ban-cha (designated PF) and green tea (leaf).**
PF, post-fermentation.

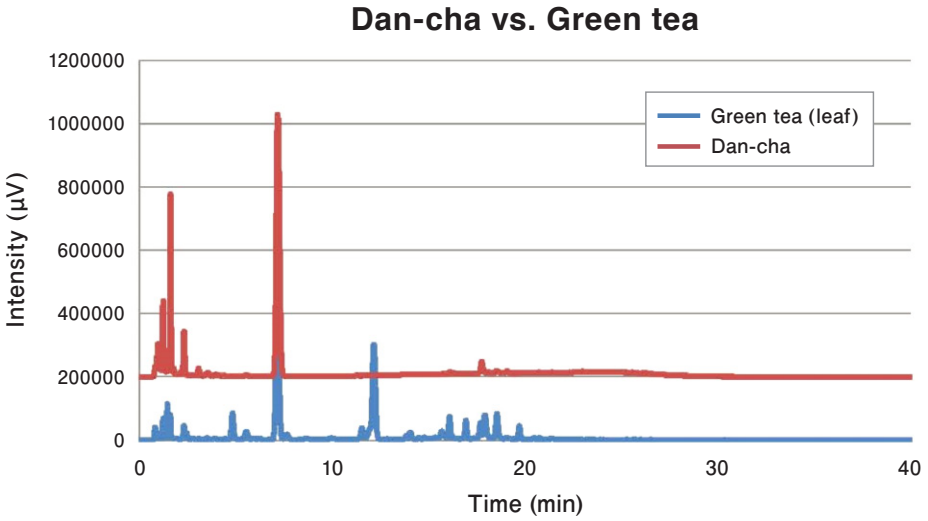

Fig 17. **Reverse-phase HPLC chromatograph of Dan-cha (designated PF) and green tea (leaf).**
PF, post-fermentation.

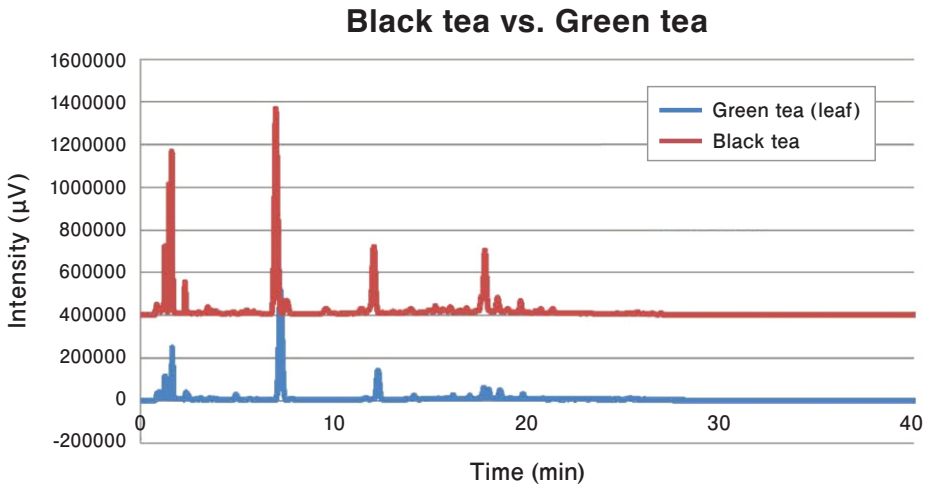

Fig 18. **Reverse-phase HPLC chromatograph of black tea (designated OD) and green tea (leaf).**
OD, oxidative fermentation.

Discussion

Influence of post-fermentation

In the tentative study, anti-glycation activity of CS tea or herb tea using an *in vitro* reaction model between glucose and human serum albumin (HSA) and all the CS tea showed anti-glycation activity equal to or greater than AG regardless of the presence or absence of post-fermentation [5]. Among herb teas, the anti-glycation activity was high in rooibos, tien-cha, guava and dokudami [5]. In the tea in which post-fermentation augmented anti-glycation activity, the total polyphenol contents tended to increase. Therefore, in this study, in order to more precisely examine the influence of post-fermentation, we measured the contents of catechins, which were especially focused among polyphenols in tea leaves.

The chromatography of extracts from the same kind of tea leaf was compered before and after post-fermentation and showed that the total catechin amount was more abundant in CS tea (0.461 mg catechin Eq/mL in average) than in herb tea (0.063 mg catechin Eq/mL in average). Regarding the influence of post-fermentation on the catechin contents it seemed that the procedure reduced it 1/10 ~ 1/4 from non-treated green tea (leaf) to the level of 0.009 ~ 0.427 mg catechinEq/mL in average CS tea.

In oxidative fermentation tea in which catechin contents were 0.398 ~ 0.575 mg catechin Eq/mL, the procedure reduced catechin 2/5 ~ 3/5 from non-treated green tea (leaf). Catechin content reduction was more marked by post-fermentation than by oxidative fermentation. Oxidative fermentation is reported to induce polymerization of EC and EGC and form theaflavin [8]. This report is compatible with our finding that theaflavin contents increased in all of the oxidative fermentation teas, *i.e.*, Oolong tea or black tea.

In CS tea, the influence of post-fermentation on theflavin contents was variable from undetectable (0 mg catechin Eq/mL) in batabata-cha to three-fold higher than non-treated green tea in pu'er tea or dan-cha. Catechin content reduction was more marked by post-fermentation than by oxidative fermentation. So, although post-fermentation degenerates catechins more abundantly than oxidative fermentation, theaflavin formation by catechin polymerization may depend on the kind of tea leaf.

As the next issue, it is interesting to examine the relation between catechin and anti-glycation activity. The correlation analysis between catechin contents and IC_{50} in CS tea showed a positive correlation between theaflavin content and the reduction of anti-glycation activity in green tea, Oolong tea and black tea ($r = 0.926$) and a negative logarithmic correlation between EGC contents and anti-glycation activity ($r = 0.926$). Therefore, oxidative fermentation seems to increase theaflavin and decrease EGC, thus reducing the anti-glycation activity.

A greater influence on the component was induced by post-fermentation than by oxidative fermentation, as shown by the comparison of the chromatograph between before and after the fermentation. Post-fermentation did affect ECG and theaflavin content, however, which were not correlated with anti-glycation activity.

Herb tea contains less catechin but it is rich in the other polyphenols. The correlation was not noted between catechin contents and anti-glycation activity in herb tea. Some herb teas showed enhanced in anti-glycation activity by post-fermentation, *i.e.*, rooibos tea, hama-cha, tien-cha, persimmon or dokudami, however, no relationship was noted between the catechin component change and the activity. In a tentative report [5], the total polyphenol content was confirmed to show an increase by fermentation, which may act on polyphenols other than catechins and then augment anti-glycation activity.

On the contrary, anti-glycation activity of guava was reduced by post-fermentation. Guava reportedly contains abundant polyphenols, of which more than 90% are flavan type and are mainly present as proanthocyanidin polymers [7]. Coupled with the finding that catechin degeneration was enhanced by post-fermentation, which may also degenerate proanthocyanidin polymers, possibly antiglycative, it can be speculated that reduction of proanthocyanidin may be responsible in the anti-glycation activity reduction of guava.

Conclusion

In the present study, catechin component was examined in CS tea and herb tea extracts and the relation to anti-glycation activity was investigated using an *in vitro* albumin/glucose model, and furthermore the influence of fermentation (oxidative fermentation and post-fermentation) was analyzed. In oxidative fermentation tea, the procedure increased theaflavin and decreased EGC, which was related to the reduction of anti-glycation activity. This may be a slight disadvantage from the viewpoint of glycative stress. Meanwhile, polyphenol contents tended to increase in the tea extracts in which post-fermentation enhanced anti-glycation activity. The reason that anti-glycation activity was not related to catechin degeneration may be due to the involvement of polyphenols other than catechins. It is possible to say that the post-fermentation procedure can be a useful technique not only for improving taste and fragrance but also for enhancement of AGE formation inhibition actions of tea.

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Conflicts of interest statement

The authors have no conflict of interest related to this study to declare.

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