Variations of Enzymatic Activity and Main Quality Components During the Fermentation Process of Acanthopanax senticosus Tea

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Abstract. Acanthopanax senticosus fresh leaf was used as the experimental material to produce tea, Eurotium cristatum was used to ferment the Acanthopanax senticosus tea. The variations of main quality components and enzymatic activity were examined during the fermentation process. The results showed that tea polyphenols, amino acid, and soluble sugar were decreased from 16.36%, 4.33%, 1.96% to 5.42%, 1.34%, 0.88%. The theabrownin was increased from 14.0% to 16.61%, but theaflavins and thearubigins were increased and then decreased during the fermentation process of Acanthopanax senticosus tea. In addition, cellulase, pectinase and protease had been there as the endogenous enzyme before generating microorganisms, while polyphenol oxidase were exogenous enzymes. The main quality components and enzymatic activity were changed obviously as the time was prolonged. The quality of Acanthopanax senticosus tea was improved effectively, which provided theoretical basis for the production and application of fermented-tea of Acanthopanax senticosus.

1. Introduction
Acanthopanax senticosus, the unique Chinese herbal medicine in northern China, contains a variety of active quality components[1-2]. The function of Acanthopanax senticosus includes anti-oxidant, anti-fatigue, anti-radiation, anti-cancer. Acanthopanax senticosus attracted great attentions as the special value of both medicine and food, and it also had been used widely in food industry. Eurotium cristatum was a kind of natural probiotics with many functions such as improvement of tea quality, reduction of blood pressure, anti-oxidant and loss-weight[3-4].

The fresh leaves of Acanthopanax senticosus were made into fermented-tea by solid state fermentation with Eurotium cristatum, the variations of main enzymatic activity and active components in Acanthopanax senticosus during fermentation were examined, in order to provide scientific basis for developing fermented tea of Acanthopanax senticosus.

2. Materials and methods

2.1. Materials
The freshly leaves of *Acanthopanax sentieosus* was collected from Benxi city, Liaoning province. *Eurotium cristatum* which extracted from fu-brick tea was purified, identified and frozen in the refrigerator at -20°C for use.

### 2.2. Preparation of Eurotium cristatum-fermented tea

The preselected and preserved *Eurotium cristatum* was directly cultured in plates with PDA medium using the streaking method. The *Eurotium cristatum* was incubated in 28°C for 7 days. The autoclaved sterilized water (5 mL) was added to the plate, and a sterilized glass rod was used to scrape the mycelia from the plate into sterilized water, then transfer to a 50mL sterilized centrifuge tube. The tubes were shaken for 10 min, and then centrifuged for 5 min at 3000 r/min. The supernatant was filtered with absorbent cotton. The spores were cultured in PDA liquid medium for 7 days (28°C, 180 r/min) and adjusted the spore concentration to 1.0×10^7 cfu/mL.

The plucked fresh tea leaves were handled with fixation, rolling and baking. The baked leaves were placed into flasks (30 g/flask), and then the flasks were sterilized at 121°C for 20 min. 7 mL of the *Eurotium cristatum* spore solution was inoculated into four flasks and an unfermentation flask when the flasks were cooled. The fermented teas were placed at 28°C to ferment for 2 d, 4 d, 6 d and 8 d. Then the fermented teas were dried at 80°C for 2 h.

### 2.3. Preparation Determination of enzymatic activity and main components

Determination of tea polyphenols: GB/T 8313-2018, Determination of amino acids: GB/T 8314-2013, Determination of tea pigments: system analysis,[5] Determination of soluble sugar: anthrone colorimetric method,[6] Determination of cellulase and pectinase: DNS method,[7] Determination of protease: Folin method,[8] Determination of polyphenol oxidase: spectrophotometry.[9]

### 2.4. Statistical analysis

The experimental data were expressed as mean ± standard error with three parallel experiments. The drawings were handled by Origin Pro 8.5 and Excel 2010.

### 3. Results and discussion

#### 3.1. Variations of polyphenols oxidase and tea polyphenols during the different fermentation times

As shown in figure. 1, the enzymatic activity of polyphenol oxidase were increased and then decreased during the fermentation process. The enzymatic activity of polyphenol oxidase in unfermentation tea was 0.028 U/(g·min), it reached the maximum of 0.283 U/(g·min) on the 4th day, then the enzymatic activity were decreased from 4 d to 8 d. The polyphenol oxidase was mostly derived from exogenous enzymes produced by *Eurotium cristatum*.

A variety of extracellular enzymes were produced during the fermentation process owing to the metabolism of *Eurotium cristatum* was vigorous, so the content of tea polyphenols were decreased from 16.36% to 5.42%. The tea polyphenols were oxidized into quinones under the oxidation of polyphenol oxidase,[10] so the content of tea polyphenols were decreased and the bitterness and astringent of fermented tea were reduced.
Figure. 1 Variations of polyphenol oxidase and tea polyphenols in *Acanthopanax Senticosus* tea during the different fermentation times

3.2. Variations of tea pigments contents during the different fermentation times

As shown in figure. 2, the content of theaebrownin was increased from 14.001% to 16.61%, but theaflavins and thearubigins were increased and then decreased during the fermentation process. The content of theaflavins and thearubigins in unfermentation tea were 0.19% and 6.18%, and theaflavins reached the maximum of 0.28% on the 4th day of fermentation and thearubigins reached the maximum of 7.48% on the 6th day.

Due to the analysis of the change of polyphenols oxidase and polyphenols during the different fermentation times, the catechins in tea polyphenols were oxidized to quinones and subsequently react to form theaflavins and theaflavins under the oxidation of polyphenol oxidase. Therefore, the content of theaflavins and thearubigins increased during the fermentation prophase. Theaflavins and thearubigins were oxidized to produce theaebrownin and the content of theaflavins were increased gradually, while the content of theaflavins and thearubigins were decreased as the prolonged time\[^{[11]}\].

Figure. 2 Variations of tea pigments contents in *Acanthopanax Senticosus* tea during the different fermentation times
3.3. Variations of protease and amino acid during the different fermentation times
As shown in figure. 3, the protease activity of unfermentation tea were 63.96 U/(g· min), because the protease were existed in tea as endogenous enzymes before the growth of Eurotium cristatum. The change of protease and amino acid was similar during the whole fermentation process. The activity of protease decreased to 60.00 U/(g· min) after fermentation, and the content of amino acids in the original tea were decreased significantly from 4.27% to 1.34%.

Protease can catalyze the hydrolysis peptide bonds to produce polypeptides and amino acids. Amino acids can be further reacted to form aromatic substances in fermented tea and improve the quality\(^{[12]}\). The result showed that the reduction of amino acid content in fermented tea effectively increased the aroma and improved the sensory quality of tea.

![Variations of amino acid and protease in Acanthopanax Senticosus tea during the different fermentation times](image)

3.4. Variations of polysaccharide hydrolases and soluble sugar during the different fermentation times
As shown in figure. 4, the cellulase and pectinase activities of unfermentation tea were 167.49 U/(g· min) and 317.95 U/(g· min), because the cellulase and pectinase were existed in tea as endogenous enzymes before the growth of Eurotium cristatum. The change of cellulase and pectinase was similar during the whole fermentation process. The activity of cellulase reached the maximum of 181.42 U/(g· min) on the 2nd day of fermentation, and pectinase reached the maximum of 358.87 U/(g· min) on the 4th day. Subsequently, the enzymatic activity began to decrease. The content of soluble sugar in the original tea was decreased from 1.96% to 0.88%.

Cellulose is the main polysaccharide in plant cell wall, which can be degraded to produce soluble sugar by cellulase. Pectinase can catalyze pectin in raw materials to produce small molecule sugar which can dissolve in water\(^{[13,14]}\). The change of enzyme activity can cause the change of soluble sugar, because soluble sugar needs to provide carbon source during the growth of Eurotium cristatum, so the content of soluble sugar reduced.
Figure 4 Variations of polysaccharide hydrolases and soluble sugar in Acanthopanax Senticosus tea during the different fermentation times

4. Conclusion
In this study, Acanthopanax senticosus fresh leaf was used as the experimental material to produce tea, Eurotium cristatum was used to ferment the Acanthopanax senticosus tea. The variations of main quality components and enzymatic activity were examined during the fermentation process. The results showed that cellulase, pectinase, protease and polyphenol oxidase were changed during the different fermentation times. The tea polyphenols, amino acid and soluble sugar were decreased while theabrownin was increased, but theaflavins, thearubigins were increased and then decreased. The fermentation of Eurotium cristatum has significantly changed the main quality components in fermented tea and effectively improved the taste of Acanthopanax senticosus.

Acknowledgments
This study was supported by the Program of Key R&D Program of Liaoning province (No. 2018205001).

5. References
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