Study on the changes of chemical constituents of Pueraria lobata during fermentation

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Abstract: At present, Pueraria lobata has become an important raw material in the field of health food and medicine, and research on its processing has gradually increased. As a result, Pueraria lobata fermentation technology has also been further researched and expanded. Based on this, this article discusses the fermentation of Pueraria lobata from a chemical point of view, and discusses in detail the changes of the main constituents as well as the change mechanism, in order to provide references for similar work in the future to improve the quality of the fermentation products of Pueraria lobata.

1. Introduction

Pueraria lobata is the dried root of the leguminous plant Pueraria lobata, which is a kind of medicinal and edible plant. It is rich in nutrients and contains a variety of essential amino acids and vitamins. Especially the abundant flavonoids are very effective in lowering blood pressure and enhancing immunity. At present, most of the domestic processing of Pueraria lobata is still mainly to obtain Pueraria lobata starch, which easily destroys the beneficial ingredients. Therefore, it is necessary to further develop Pueraria lobata through fermentation to ensure that the Pueraria lobata industry develops in the direction of high added value.

2. Basic experimental flow of Pueraria lobata fermentation

2.1. Materials and reagents

The main raw material of the experiment is Pueraria lobata, and the experimental reagents include puerarin standard substance, aminobutyric acid standard substance, ethanol, phthalaldehyde, etc. At the same time, the experiment is carried out by using lactic acid bacteria and yeast strains that are independently separated and screened.

2.2. Laboratory equipment

The main equipment used in this experiment includes acidity meter, induction cooker, biochemical incubator, spectrophotometer and gas chromatograph.
2.3. Experiment methods

2.3.1. Preparation of Pueraria liquor. Firstly, it needs to select the qualified Pueraria lobata block, pulverize it with a pulverizer, pass a 100-mesh sieve to obtain powder, then add an appropriate amount of pure water to mix, beat and steam. After cooling, it should use six layers of gauze to filter to obtain Pueraria liquor for reservation.

2.3.2. Activation of yeast and lactic acid bacteria. A certain amount of yeast and lactic acid bacteria are added to a certain concentration of sterile sugar water, and the activation is performed on a shaker at 37°C. When a large number of bubbles appear in the sugar water, the activation is proved to be completed.

2.3.3. Fermentation of Pueraria lobata liquor. Since the pH of untreated Pueraria lobata liquor is not suitable for the growth of bacteria, it is easy to inhibit the growth of bacteria [1]. In this regard, it can use a certain concentration of sodium bicarbonate solution to adjust the pH value of pueraria lobata liquor, adjust it to about 5.5-5.6, and then add a certain amount of sodium glutamate and brown sugar. Finally, the activated lactic acid bacteria and yeast strains are inoculated into the Pueraria lobata liquor, and ultrasonicated. After the ultrasonic, the fermentation is carried out at 30°C. When the residual sugar is less than 1.2g/L, the fermentation is finished. At the same time, in order to minimize the possible errors of a single experiment, repeated experiments are used to conduct multiple optimization experiments, and the experimental results are comprehensively evaluated using fuzzy mathematics to obtain more accurate results [2].

3. Analysis of main chemical constituents changes during the fermentation

The original chemical constituents of Pueraria lobata are shown in table 1. After the fermentation, various main constituents will change to different degrees.

| Constituents   | Starch | Protein | Fattiness | Cellulose | Total Flavonoid | Ash content |
|---------------|--------|---------|-----------|-----------|-----------------|-------------|
| Mass fraction | 59.51  | 6.46    | 0.20      | 2.35      | 0.51            | 3.19        |

On the whole, there are many physical and chemical indicators that change during the fermentation of Pueraria lobata, mainly including changes in alcohol, acid, ester, total flavonoid, free amino acid and other indicators. These different changes also correspond to different biochemical reaction mechanisms, so they need to be studied and analyzed one by one.

3.1. Determination and change analysis of total flavonoid

In the determination experiment of total flavonoid, standard solutions of different concentrations should be used for comparison, and distilled water is used as the blank control group. The absorbance value is measured at a wavelength of 250nm, and the value of the blank control group is subtracted from the measurement result, which is the actual value. Then it can establish the standard curve equation of puerarin mass concentration and absorbance value by the least square method, and then calculate the total flavonoid in the puerarin sample [3]. According to the experimental results, it can be seen that during the fermentation, the total flavonoid in Pueraria lobata liquor increases steadily at the initial stage, and it is in equilibrium at the later stage of fermentation indicating that the total flavonoid in Pueraria lobata rises after fermentation. The reason may be that various enzymes produced by lactic acid bacteria and yeast during the fermentation destroy the structure of the cell wall of Pueraria lobata, which cause a large amount of flavonoid to be dissolved. It is also possible that a series of biochemical reactions during the fermentation produced flavonoid [4-5].

3.2. Determination and variation analysis of alcohol content

The measurement of alcohol content is actually the measurement of ethanol content. The experiment is
mainly carried out by gas chromatography by using a hydrogen ion flame detector. After the determination is completed, the least square method is used to establish a linear regression equation based on the mass concentration of ethanol and the peak area. Used to calculate the ethanol content, and then it can simply calculate the value of the alcohol content. According to relevant calculation results, during the fermentation process of Pueraria lobata, the ethanol content begins to increase in the early stage. After reaching the maximum in the middle stage, it begins to slowly decrease [6]. The main reason is that in the initial stage of fermentation, the sugar and oxygen content in the entire reaction system is relatively sufficient. Therefore, the yeasts multiply and consume the oxygen in the reaction system. When the oxygen concentration drops to a critical value, the yeast begins anaerobic fermentation to produce a large amount of ethanol. However, with the decrease of sugar and the increase of ethanol concentration, the anaerobic fermentation of yeast is inhibited. In addition, ethanol will undergo a certain degree of esterification reaction with the organic acid in the reaction system, so the ethanol content in the entire reaction system will slightly decrease in the later stage.

3.3. Changes and analysis of ester content
Since esters play an important role in the sensory quality of Pueraria lobata fermentation products, they are also important indicators in the fermentation of Pueraria lobata. The esters in the fermentation products are mainly obtained from the esterification reaction of ethanol and other organic acids produced during fermentation. With the continuous advancement of the fermentation, the acidity in the reaction system gradually increases, and esters are not easily hydrolyzed. Therefore, it gradually begins to accumulate and the content gradually increases. However, due to the inhibition of the fermentation of related bacteria in the late fermentation stage, the rate of increase of esters gradually slows down and eventually remains stable [7].

3.4. Determination, change and analysis of organic acid content
According to theoretical analysis, the organic acids contain in Pueraria lobata liquor after fermentation mainly include oxalic acid, tartaric acid, ascorbic acid (vitamin C), lactic acid, acetic acid, citric acid, malic acid and succinic acid. For the determination of these acids, high-performance liquid chromatography is usually used, and the peak area and the mass concentration of organic acids are used as variables to simulate the regression equation. Generally, the organic acid regression equation is shown in table 2. The correlation coefficients of these equations all exceed 0.999, and the fitting effect is good.

| Organic acid species | Regression equation |
|---------------------|---------------------|
| Oxalic acid         | y=1871590x+1357920  |
| Tartaric acid       | y=588319x+684075    |
| Ascorbic acid       | y=2126380x-743678   |
| Lactic acid         | y=171533x+16601     |
| Acetic acid         | y=161086x-3630      |
| Citric acid         | y=322000x-12895     |
| Malic acid          | y=194312x+8713      |
| Succinic acid       | y=176589x-1860      |

Specifically, there are two main sources of acidic substances from Pueraria lobata after fermentation. One is the organic acids contained in Pueraria lobata liquor itself, and the other is the metabolites of yeast and lactic acid bacteria in the fermentation. In fact, in the fermentation of Pueraria lobata, not only the types of acid substances are not obvious, but the content of them also does not change much. The main newly generated organic acids are lactic acid and a small amount of acetic acid. The lactic acid mainly comes from the fermentation process of lactic acid bacteria. The source of acetic acid is relatively complicated, and it may come from the metabolism of other organic acids or the fermentation of different types of lactic acid. In addition, from the test results in table 3, it is not difficult to find that the content of citric acid, malic acid and ascorbic acid is relatively low, especially the citric acid content decreased in the late fermentation stage. After preliminary inference, it may be due to the fact that citric
acid participates in the metabolic reaction of the fungi during the fermentation process, and part of it is converted into ketones.

From the perspective of changes in the total content of organic acids, the overall trend is that the rate of increase is relatively fast 3 days before fermentation, and the rate of increase begins to slow down on the fourth day. This is mainly due to the high activity of yeast and lactic acid bacteria in the early stage of fermentation, and their large numbers of reproduction and frequent metabolism produce large amounts of acids. The accumulation of acids leads to a rapid increase in the total content of organic acids. In the middle and late stages of the fermentation process, the nutrients in the reaction system are already very scarce, and the growth of yeast and lactic acid bacteria is inhibited. Some acids have undergone carboxylation reactions under special circumstances, which significantly reduces the increase in organic acids.

| Organic acid species | Before fermentation | After fermentation |
|---------------------|---------------------|--------------------|
| Oxalic acid         | 1.136               | 1.568              |
| Tartaric acid       | 1.323               | 1.746              |
| Ascorbic acid       | 0.315               | 0.420              |
| Lactic acid         | 0.642               | 2.442              |
| Acetic acid         | 1.227               | 2.129              |
| Citric acid         | 0.826               | 0.535              |
| Malic acid          | 0.183               | 0.860              |
| Succinic acid       | 0.533               | 1.211              |

(All organic acid content units are mg/mL in the table)

3.5. Analysis on the change of free amino acid content
Although the protein content in Pueraria lobata is relatively low, it contains many kinds of essential amino acids, reaching 5 kinds, and there are 10 kinds of non-essential amino acids. For the specific determination of the content of these amino acids, the S433D amino acid analyzer is used for direct determination. The measurement results showed that during the fermentation of Pueraria lobata, the content of aspartic acid, alanine, cysteine, histidine and arginine decrease, while the content of other amino acids increase. This indicates that during the fermentation of Pueraria lobata, the fungus will consume some amino acids and produce new amino acids. Specific analysis shows that these amino acids come from the decomposition of the protein itself. In the initial stage of fermentation, the protein in the raw materials is used as a nitrogen source by yeast and lactic acid bacteria, which significantly reduces the content of some amino acids. With the deepening of the fermentation process, the fermentation of yeast and lactic acid bacteria is inhibited and begins to decay. After decay, the bacterial cells will precipitate some amino acids due to autolysis. In the later stage of fermentation, the activity of the fungi was weak overall, and the rate of utilization of amino acids is also significantly reduced, so that the content of some amino acids increase compared with before.

3.6. Changes of soluble solids content in Pueraria lobata during fermentation
According to the relevant theoretical analysis, it can be known that in the initial stage of the fermentation, the carbohydrates contained in Pueraria lobata liquor will be rapidly consumed with the proliferation of yeast and lactic acid bacteria. Therefore, at this stage, the content of soluble solids will drop rapidly, usually by more than 50%. In the later stage of the fermentation, since the nutrients available for yeast and lactic acid bacteria have been greatly reduced, the bacteria generally appear to be aging, and the change of soluble solids tends to be slow. On this basis, the researchers conduct actual experiments, and the results show that the analysis results are correct.

4. Conclusion
In the fermentation process of Pueraria lobata, a large number of complex chemical changes are often
involved. To ensure the high quality of Pueraria lobata fermentation products, these chemical changes must be studied to realize the understanding and control of the direction and degree of chemical changes, and then to realize the knowledge of Pueraria lobata effective control of various chemical components in fermentation products. The processing of Pueraria lobata products is not limited to the main fermentation stage. Because the products at this stage are still not stable enough, further research is needed to ensure that the various components reach a balanced and stable state.

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References
[1] Dong Qi, Zhang Hucai, Liu Feixiang. Research on the technology of thick mash fermentation of Pueraria lobata wine[J]. Liquor-making Technology, 2020, (9): 87-92.
[2] Zhu Deyan. Process optimization of pueraria lobata fermented by yeast[J]. Food Industry Science and Technology, 2020, 41(12): 82-87.
[3] Hu Meiyiing, Huang Ping. Research progress of Pueraria lobata fermentation products[J]. Science and Technology Vision, 2019, (26): 88, 84.
[4] Wang Zhenbin, Wang Qing, Liu Jiayou. Study on the changes of nutrition and flavor components of Pueraria lobata liquor before and after fermentation[J]. China Brewing, 2016, 35(7): 93-98.
[5] Cai Fengjiao, Jiang Yanming, Song Qingying, Wang Jiangbo. Optimization of fermentation conditions for Pueraria lobata fermented milk[J]. China Brewing, 2020, 39(01): 209-214.
[6] Zhong Zhenyu, Yang Xiaosheng. Research on total flavonoid and antioxidant activity of Pueraria lobata fermentation broth[J]. Journal of Guizhou Medical University, 2017, 42(12): 1379-1383.
[7] Wei Jinsong. Research on the fermentation technology and quality analysis of Pueraria lobata wine[D]. Xihua University, 2019.