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Advances in Research of Preparation and Application of Pure Functional Yaoqu by Adding Traditional Chinese Medicine

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Abstract This paper introduces the origin and research status of traditional Yaoqu (Chinese medicinal koji) in China, and advances in the research of preparation and development of pure functional Yaoqu. The study results showed that the quality of the products was significantly improved when pure Yaoqu was used as saccharifying agent in the brewing of sake, soy sauce and other products. Based on the high saccharification performance and special functional components, Yaoqu can be applied to the development of fermented food.

Key words Yaoqu (Chinese medicinal koji), Chinese medicinal herbs, Health care, Strain

0 Introduction

Traditional Chinese medicine (TCM), as an important part of traditional Chinese medicine, has a long history of thousands of years. Yaoqu (Chinese medicinal koji), as an important part of it, not only has a long history and a wide variety, but also shows diversity in efficacy. The concept of homology of medicine and food has been deeply rooted in the hearts of the people, which reflects the close relationship between traditional Chinese medicine and daily diet. However, due to the diversity of Chinese herbal medicine resources and the complexity of compatibility, it is difficult to study modern Chinese medicine deeply in pharmacology and human metabolic mechanism. In addition, the existence of "secret recipes" in TCM industry has led to the fact that the production of traditional Chinese medicine is still largely dependent on traditional handicraft workshops, and the scale and modernization level of the whole industry is relatively low. The production process of traditional Yaoqu is similar to that of Daqu and Xiaoqu (both are (fermentation starters), which depends on the natural fermentation of microorganisms. Through this process, the physical, chemical and biochemical characteristics and efficacy of raw materials are transformed, so as to produce traditional Chinese medicine or health food raw materials for both medicine and food. The microorganisms in this process mainly come from natural inoculation or yeast powder transmission. In the brewing industry, the practice of adding Chinese herbal medicines to food also has a long history. In 2019, TCM was included in the Global World Health Program, which marked the further recognition of the status of TCM in the world. With the rapid growth of demand for traditional and natural medicines in the international pharmaceutical market, the development of innovative drugs or health products based on these has become a new trend in global drug research and development. With the increasing emphasis on Yaoqu and the improvement in material living standards, the basic research on Yaoqu has been gradually deepened, the application development has been gradually strengthened, and the productivity has been growing. Therefore, Yaoqu is gradually becoming an important branch of TCM and health food.

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1 Origin and research status of Chinese traditional Yaoqu

1.1 Origin of Chinese traditional Yaoqu Food brewing, as one of China's important traditional handicraft industries, has a history of thousands of years. As early as the Shang Dynasty, China has started to make and use koji skillfully, among which the koji for brewing was the most popular. During the Western Han Dynasty, koji-making technology had a greater development, and there were more kinds of koji, barley, wheat and other grain raw materials could be used to make koji, and there was a difference between mildew and non-mildew on the surface of koji. The original koji was loose koji, and later there was cake-like koji. In the Jin Dynasty, herbal medicines were added to distiller's yeast, which was also the first Chinese herbal medicine used in food processing. Because many herbs contain factors beneficial to the growth of microorganisms, the addition of them is more conducive to the preparation of distiller's yeast and the improvement of its saccharification performance, and the brewed wine also has a unique flavor[1]. With the gradual application of Chinese herbal medicine in koji-making, the dosage and types of Chinese herbal medicine gradually increased, and the application of koji gradually developed from brewing, fermented food to pharmaceutical field, and the practice of preparing Yaoqu by fermentation has been handed down to this day.

1.2 Research status of Chinese traditional Yaoqu Traditional Yaoqu, such as Jianqu, Liushenqu, Banxiaqu, Dannanxing, light fermented soya bean and black soybean product, are still used clinically. Their processes are all traditional solid fermentation, and their raw materials include Chinese medicinal materials, grain and grain by-products, and health food raw materials with the same source of medicine and food. For example, in the preparation of Jianqu, 21 kinds of traditional Chinese medicines such as *Polygonum flaccidum* are crushed into fine powder, mixed with wheat bran, sieved, and then the flour is made into a proper amount of thin paste, which is kneaded evenly with the above medicinal powder while hot, kneaded into a ball by hand, thrown to disperse, made into cubes, placed in a fermentation box, with gaps between the cubes, covered with sacks or straw, fermented in a closed room until the medicinal blocks are covered with white mildew, taking out when the medicinal blocks have wine fragrance, and dried to use. It is a yellowish brown rectangular lump with fragrant smell and slightly bitter taste. It is used for headache due to cold and heat, food stagnation, vomiting and fullness[2]. The process for preparing Liushenqu is characterized in that Armeniacae Semen Amarum and *Vigna umbellata* are crushed into coarse powder and mixed with flour and wheat bran; *Polygonum flaccidum*, *Artemisia carvifolia*, and *Xanthium sibiricum* are decocted in water for 1 h, filtered, and the filtrate is concentrated into a clear paste, which is then mixed with the above medicinal powder while hot, kept at a proper temperature and humidity, and naturally fermented until the surface is covered with yellow-white or gray mildew. Taking out, crushing and drying to obtain Liushenqu. It is irregular, fine or coarse particles, stale and slightly bitter in taste, and is used to invigorate the spleen and stomach, promote digestion and regulate the middle energizer[3]. Another example is the soybean yellow made of black soybeans, whose fermentation process is "a bucket of black soybeans, fried to yellow, covered with Artemisia on the mat, such as the sauce method, when yellow, take out and dry in the sun", which is sweet and warm in nature, can dispel dampness and relieve itching, strengthen the spleen and benefit qi[4]. Unfermented black soybeans are bitter in nature and have the functions of promoting blood circulation, promoting diuresis and detoxifying[5]. The active components of Chinese herbal medicines often exist in the cell wall, and their content is low and their structure is complex. At present, remarkable achievements have been made in modifying the structure of active components of medicinal materials and using microorganisms to produce new active substances of Chinese medicinal materials[6-7]. Professor Zhuang Yi from Nanjing University of Traditional Chinese Medicine[8-9] proposed the concept of mycoplasm, which uses modern technology to form various fermentation combinations of different fermentation substrates composed of effective fungi and Chinese herbal medicines, and ferments them under certain conditions to produce various mycoplasms with different properties. Zhuang Yi proposed that traditional Chinese medicine could be used as an integral part of the culture medium to construct medicinal mycoplasm, compare the changes of related components of traditional Chinese medicine before and after fermentation, find a breakthrough point for the combination of traditional Chinese medicine and medicinal fungi, and develop medicinal koji with good efficacy.

Studies have shown that koji fermentation of traditional Chinese medicine materials or compatible traditional Chinese medicine materials can change the original properties of traditional Chinese medicine, produce new pharmacological effects or enhance the original efficacy, and expand the variety of drugs[10]. With the development of modern biotechnology, Yaoqu preparation has liquid fermentation besides solid fermentation. The liquid fermentation of Yaoqu is based on the production process of antibiotics, which adds microbial cells or mycelium to the culture medium, mixes them with medicinal materials and ferments them at an appropriate temperature. Liquid fermentation has high material transfer efficiency and is easy to realize automatic control of fermentation process[11].

2 Development of Yaoqu in new functions and the analysis of its application

Hao Pingping *et al.*[12] took the lead in introducing Japan's excellent koji-making strains and the purebred koji-making technology of high temperature culture followed by low temperature culture. The novel microbial starter-making fermentation technology is formed by effectively combining the Japanese modern brewing technology of pure strain starter-making culture, pure strain liquid high-activity fermentation, biological extraction and analysis technology of functional ingredients and the like with the Chinese traditional brewing technology of solid-state natural inoculation starter-making technology, Chinese herbal medicine compatibility multi-strain coordinated culture technology, solid-liquid-gas three-phase interface microbial fermentation technology and the like. The fermentation product becomes a novel and efficient food-grade saccharifying agent, a fermenting agent, a flavoring agent, a health care functional preparation and the like.

2.1 Development of new purebred Yaoqu and its products China has a vast territory, abundant natural resources and a long history of traditional Chinese medicine culture. The use of traditional Chinese medicine and the promotion of homology of medicine and food have a far-reaching impact on the development of traditional Yaoqu in China. Zhang Yu *et al.*[13] analyzed and discussed the selection of medicinal materials and formula rules of 55 kinds of brewing and medicinal koji, and found that among the 185 kinds of medicinal materials involved, 38 kinds of raw materials belong to both medicine and food.

Among them, the use of Cinnamomi Cortex, Armeniacae Semen Amarum, Zingiberis Rhizoma Recens, and Glycyrrhizae Radix Et Rhizoma has a high frequency; Armeniacae Semen Amarum. Cinnamomi Cortex. Atractylodis Macrocephalae Rhizoma, and Glycyrrhizae Radix Et Rhizoma are often used for compatibility with a variety of medicinal materials in koji-making formula;

most of the brewing koji and some of the medicinal koji formulas were warm and pungent in nature, and entered the liver, spleen and stomach meridians, which were consistent with the characteristics of liquor. The efficacy of each Yaoqu formula is generally manifested as relieving exterior syndrome, warming interior, clearing heat and regulating qi. Luo Fang *et al.* [14] systematically analyzed the feasibility of preparing purebred distiller's yeast from 21 kinds of Chinese medicinal materials and the influence of Chinese medicinal materials on the performance of purebred Yaoqu. Panacis Quinquefolii Radix, Astragali Radix and Rhodiolae Crenulatae Radix Et Rhizoma were selected as the best koji-making materials. We further determined and optimized the best adding mode and adding amount, 0.2% to 0.4% of Panacis Quinquefolii Radix powder, 0.1% to 0.4% of Astragali Radix powder and 0.1% to 0.4% of Rhodiolae Crenulatae Radix Et juice. Lei Xuejun *et al.* [15] further analyzed the correlation between Chinese medicinal materials and the performance indicators of distiller's yeast on the basis of the relevant research on the selection of Chinese medicinal materials based on the preparation of pure Yaoqu, and conducted a statistical stepwise regression analysis on the performance indicators of rice yeast and the types, medicinal properties and main chemical components of medicinal materials. The results showed that the addition of Codonopsis Radix, Rhodiolae Crenulatae Radix Et Rhizoma and Paeoniae Radix Alba could significantly improve the saccharification power and liquefaction power of rice koji.

Based on the statistical analysis of the main chemical components of the medicinal materials, we speculated that the triterpenes, saponins and polyphenols in the medicinal materials were the main reasons for the improvement of the saccharification power of rice koji. Li Li *et al.* [16] prepared red koji Yaoqu based on the research of pure rice koji Yaoqu, and used it to brew sake, and found that when red koji Yaoqu was used as the main saccharification starter, its physical and chemical indicators and *in vitro* oxidation resistance were superior to those of general rice koji sake. In the selection of raw materials for the preparation of pure koji, wheat, rice, corn and other cereal raw materials were compared and analyzed, and it was found that the saccharifying power, liquefying power and other performance indicators of pure koji prepared with rice as raw material were the best[17]. Based on the above research, pure Yaoqu mainly focuses on the preparation of pure Yaoqu with rice as raw material and Chinese herbal medicine. In the current research on the preparation of pure Yaoqu, some researchers have tried to use other raw materials instead of some rice to make koji. Su Yao *et al.* [18] studied the preparation process of *O. europaea* and Panacis Quinquefolii Radix Yaoqu, and used *O. europaea* waste residue to replace part of rice and added Chinese medicinal materials to prepare *O. europaea* Yaoqu, and it was found that Panacis Quinquefolii Radix was the most suitable medicinal material for preparing *O. europaea* Yaoqu. The optimal process parameters were Panacis Quinquefolii Radix extract 2% (*v*/*w*), the saccharification performance of the *O. europaea* Yaoqu was better, the liquefaction power could reach 13.33 U/g, and the saccharification power could reach 1 350 U/g. This study opens up a new way for the preparation of pure Yaoqu, and effectively utilizes the waste liquid of *O. europaea*, which is of great significance for environmental protection and the comprehensive utilization of by-product resources.

2.2 Analysis of application research status of purebred Yaoqu Chinese medicine Yaoqu with specific health care effects can be used as a product itself, and can also be used as saccharifying and fermenting agents for fermented foods such as wine, soy sauce, vinegar and the like, thereby forming a new functional food chain. Many researchers have studied the application of purebred Yaoqu and the development of functional foods. Luo Song *et al.* [19] brewed sake by semi-solid and semi-liquid fermentation with pure Yaoqu prepared by adding Panacis Quinquefolii Radix as saccharifying agent. Sake with good quality was obtained by optimizing the fermentation process in the study, which reached the first-class standard of refreshing semi-sweet rice wine in standard *Rice Wine* (GB/T 13662-2018). Yi Xin *et al.* [20] identified 43 and 31 kinds of aroma components in Yaoqu purple sweet potato wine added with Rhodiolae Crenulatae Radix Et Rhizoma and pure rice-koji purple sweet potato wine separately using pure rice-koji without Rhodiola as control. Their contents accounted for 97.83% and 92.95% of the total aroma, respectively. The results showed that the aroma components of Yaoqu purple sweet potato wine were more abundant than those of pure rice koji purple sweet potato wine, which was consistent with the results of sensory evaluation, indicating that the addition of Rhodiolae Crenulatae Radix Et Rhizoma in Yaoqu purple sweet potato wine could not only play a certain role in health care, but also improve the sensory flavor of purple sweet potato wine. Yang Jun *et al.* [21] used Astragali Radix purebred Yaoqu as saccharifying agent, combined with the traditional solid-state fermentation method to brew soy sauce, and analyzed and evaluated the quality of soy sauce. The results showed that the soy sauce fermented by Astragali Radix Yaoqu reached the national standard, and its basic physical and chemical indicators and some antioxidant indicators were also better than control group. Therefore, the study showed that the addition of Astragali Radix in Yaoqu fermented soy sauce could improve the quality of soy sauce.

3 Conclusions

With the gradual mechanization and intelligent development of brewing technology, the traditional Yaoqu is bound to encounter severe challenges. Therefore, it is particularly important to gradually adopt controlled modern biotechnology fermentation as a new technical means. At present, the production process of Yaoqu in the new purebred mostly adopts quantitative control, which makes industrial production easy to achieve. In addition, based on the stable and reliable quality of a series of health foods developed by Chinese medicine Yaoqu, we believe that these products will have broad market prospects in the future.

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3 Conclusions

Through the experiment of grafting of virus-free *C. reticulata* Hongmeiren onto different rootstocks, we can draw the following conclusions. In terms of survival rate and preserving rate, *C. trifoliata* L. rootstock performed the best; in terms of growth potential, *S. mandarin* cv. Miyagawa wase had the greatest tree height, while *C. unshiu* Marc. cv. Owari had the best performance in scion stem diameter, shoot length, shoot thickness and leaf area, showing the best growth potential; as for fruit quality, *C. unshiu* Marc. cv. Owari had the greatest single fruit weight, the highest soluble solid content, the best flavor and excellent overall fruit quality.

Considering the growth, development and fruit quality after grafting of *C. reticulata* Hongmeiren, among the three kinds of rootstocks selected in this experiment, *C. unshiu* Marc. cv. Owari showed the best comprehensive performance on virus-free *C. reticulata* Hongmeiren, followed by *S. mandarin* cv. Miyagawa wase.

When planting *C. reticulata* Hongmeiren in the south bank plain of Hangzhou Bay, *C. unshiu* Marc. cv. Owari was used as the rootstock for virus-free *C. reticulata* Hongmeiren. Because of its vigorous growth potential, excellent fruit quality and high yield, it got the highest comprehensive score and showed the strongest local adaptability. Therefore, when building a new *C. reticulata* Hongmeiren orangery in the south bank plain of Hangzhou Bay, it is recommended to choose *C. unshiu* Marc. cv. Owari as high-quality seedling for rootstock.

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