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Progress In The Classification, Bacterial Flora And Main Flavor Substances Of Fermented Bean Curd

Shuangying Jia[a], Feng Ji[a], Xinyu Shi[b], Mengying Li[a], Yingjie Mei[c],

Yueqin Xue[d], Xinhe Zhao[a,E*]

[A] *Shandong (Zibo) Prefabricated Food Research Center, College Of Agricultural Engineering And Food Science, Shandong University Of Technology, Shandong, China*
[B] *Zibo Product Quality Testing Research Institute, Shandong, China*
[C] *Zibo Institute For Food And Drug Control, Shandong Province, China*
[D] *Traditional Chinese Medicine Pharmacy，Linyi Central Hospital, Linyi, China, 546700*
[E] *Shanli Health Food Technology Co., Ltd, Shandong, China*

Abstract
Fermented bean curd is a traditional Chinese fermented food, usually used as an appetizer, side dish, or condiment. During fermentation, compounds such as soybean proteins and fats are hydrolyzed by microbial action to form flavor substances. At the same time, microorganisms are also essential for the food safety of fermented bean curd. In order to comprehensively understand the characteristics and research progress of fermented bean curd, this paper combines the current research status at home and abroad, gives a detailed overview of the classification of curd, outlines the main fermentation strains of curd and their roles, and elaborates on the main volatile flavor substances and influencing factors of curd. It also describes the main volatile flavor compounds and factors affecting them to guide the process optimization and quality improvement of fermented bean curd.

Keywords: *fermented bean curd; classification; fermentation strains; flavor substances*

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I. Introduction

With the prevalence of health and nutritional concepts in recent years, there has been an increased interest in plant-derived proteins [1], with soybeans being a significant contributor. It is rich in vegetable proteins, fats, carbohydrates, and some other nutrients, such as vitamins and minerals [2], which have a good effect on reducing the incidence of cardiovascular diseases, increasing the metabolic rate, and enhancing the strength of human bones [3]. However, soybean odor and anti-nutritional factors limit its consumption [2], so soybean shortcomings are often improved by fermentation to make various types of soybean-based fermented products, including soy sauce, dairy products, and fermented bean curd.

Fermented bean curd, also known as bean curd milk, shaggy bean curd, and moldy bean curd, has a long production history, originating in ancient China. Its production history is over a thousand years, and it is one of China's four traditional fermented soybean products [4]. Its ease of digestion, variety of flavors, delicate texture,

and nutrient richness have made it popular in China and other Asian countries [5-7]. Fermented bean curd is made from soybeans with water, white wine, salt, and spices as auxiliaries by processing, grinding, dehydration, coagulation, fermentation, inoculation, and flavoring [8]. During fermentation of fermented bean curd, proteins, fats, and other compounds in soybeans are hydrolyzed by microorganisms such as Mucor and bacteria to produce small molecules such as polypeptides, amino acids, and fatty acids, as well as to form aroma components [9-10]. The study of changes in the main microflora and primary flavor substances in fermented bean curd fermentation is essential for improving fermented bean curd quality and quality assurance.

This paper presents an overview of the classification and production process of fermented bean curd to give a preliminary understanding of the types and processing of fermented bean curd. The major microflora of fermented bean curd fermentation were also analyzed to promote improving fermented bean curd's safe and controllable process. Finally, the main volatile flavor substances and influencing factors of fermented bean curd were reviewed in detail, and recent research has been conducted to guide further improvements in the quality of fermented bean curd and promote the safe production of the fermented bean curd industry.

II. Classification Of Fermented Bean Curd

Classification According To The Surface Color Of Fermented Bean Curd

Based on the surface color of fermented bean curd, fermented bean curd can be classified into red fermented bean curd, white fermented bean curd, and gray fermented bean curd. Red fermented bean curd, also known as bean curd milk, is made from a mixture of yellow sorghum, red yeast rice, and other ingredients. The surface of fermented bean curd has a natural red color, and the cut surface is yellowish-white, with a mellow texture and unique flavor. It is commonly used as a culinary condiment in addition to accompanying meals [3]. White fermented bean curd is made from soybeans and other raw materials by making a tofu embryo and then using Trichoderma inoculation on the tofu embryo by fermentation [11]. Produced tofu milk has a slight, smooth, and fluffy texture, a transparent surface of orange and yellow, delicious flavor and aroma, and rich nutrients. It is also suitable for enjoying the cooking of condiments. Fermented bean curd belongs to the "green side"; gray fermented bean curd refers to stinky fermented bean curd, which is added to the bitter syrup water and salt water during the pickling process so that it is soybean green in color [12]. The stinky fermented bean curd fermentation process is more thorough and richer in amino acids than other varieties. Mainly, it contains more alanine and esters; when eating stinky tofu, milk feels a unique sweet and ester flavor [3].

Classification According To The Production Process Of Fermented Bean Curd

Pickled Fermented Bean Curd

Pickled fermented bean curd is a tofu blank that goes directly to post-fermentation without the pre-fermentation stage of microbial growth [13]. Dehydration of fermented bean curd occurs in the presence of brine due to a change in osmotic pressure, which inhibits the growth and multiplication of microorganisms while imparting a salty flavor to the fermented bean curd [4]. Reducing the water content of fermented bean curd is also beneficial in keeping the block intact. However, due to the shortcomings of its long fermentation period, single-product flavor, and insufficiently delicate quality, it is seldom used in the food industry. The production process is shown in Figure 1.

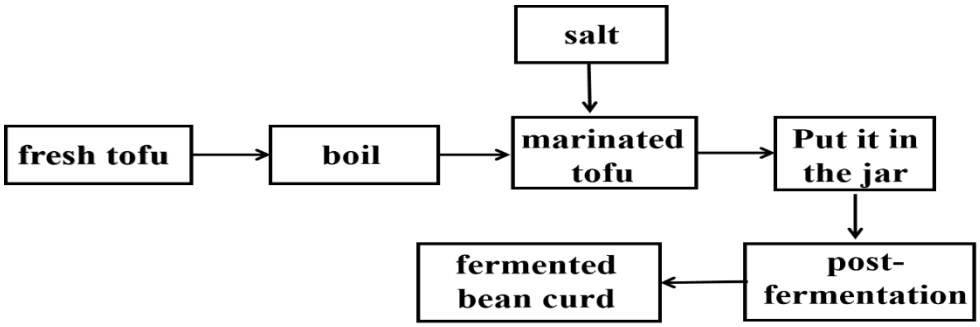

Fig. 1 Production Process Of Pickled Fermented Bean Curd

Mouldy Type Fermented Bean Curd

Moldy fermented bean curd is a pre-fermentation stage of microbial growth of tofu blanks using natural or artificial pure inoculation followed by a natural or holding post-fermentation stage with the addition of ingredients [14]. A complex microbial community is formed during fermentation of fermented bean curd, and the microorganisms act synergistically to produce a rich enzyme system that breaks down proteins, fatty acids, and other substances into smaller molecules and leads to the lineage of aroma substances [13]. Bacterial strains used for pre-fermentation of fermented bean curd do not produce toxins, have a fast growth rate, are resistant to heterotrophic bacteria, and secrete protease and lipase enzymes, which give fermented bean curd a more delicate texture and a richer aroma [15]. A diagram of the production process is shown in Figure 2.

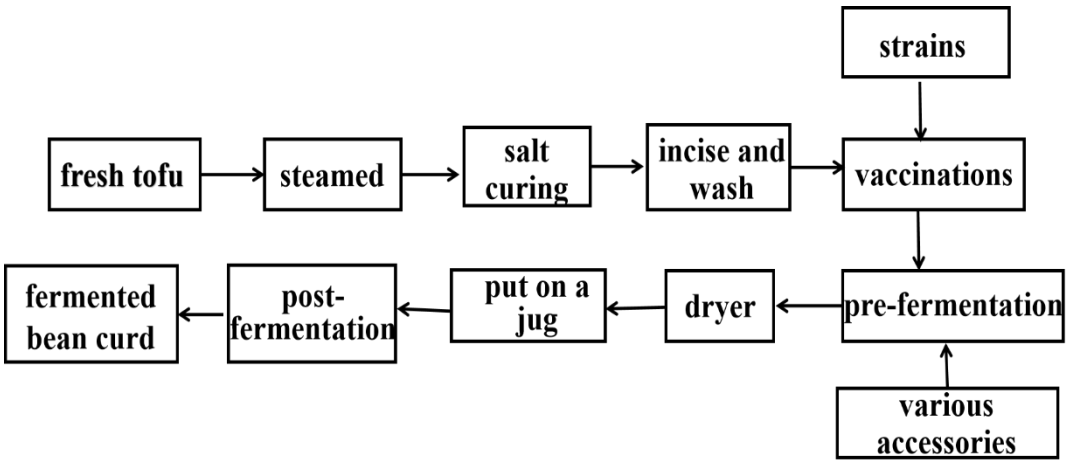

Fig. 2 Mold-Based Fermented Bean Curd Production Process

III. Main Strains Of Fermentation Of Fermented Bean Curd

Microorganisms play an essential role in fermentation. Symbiotic or alternate relationships exist between different microorganisms. Functional microorganisms can enhance fermented bean curd flavor, color, and texture and promote the production of healthy active compounds [16-17]. The main strains currently used for the fermentation of fermented bean curd are Trichoderma, Rhizopus, and Bacteria. Table 1 shows the classification of the three commonly used strains.

Table 1 List of common Mucor, Rhizopus and Bacterial species used for fermented bean curd fermentation

Strain name	Strain classification	Growing temperature
	Actinomucor elegans	25-28℃
	Mucor of Wutong Bridge	15-25℃
Mucor	Mucor racemosa	30-35℃
	Tall corrycosm	28-32℃
	Mucor sufu	20-40℃
	Rhizopus oryzae	30-35℃
Rhizopus	Rhizopus chinensis	15-45℃
	No Root Mold	30-35℃
	Lactic acid bacteria	20-45℃
Bacteria	Bacillus subtilis	10-40℃
	Micrococcaceae	25-30℃

Mucor

Mucor is the most widely used strain in fermented bean curd production. The modern fermented bean curd of Mucor type production process involves inoculation of pure species of Mucor to fresh tofu blanks in a sterilized environment and incubation at a suitable temperature for 3-5 days, when mycelium grows on the surface of the tofu block and forms a skin film, Mucor makes an essential contribution to the formation of the unique texture and aroma of fermented bean curd [3]. Fermented bean curd with Mucor as a fermentation strain can reduce the content of biogenic amines. Mucor has high protease activity compared to other different fermentation types of fermented bean curd, which can decompose the proteins in the tofu to produce peptides, amino acids, organic acids, and esters, so that fermented bean curd is enriched with a variety of nutrients, and improve the texture and quality of the fermented bean curd [18].

Rhizopus

Rhizopus has a high affinity with Mucor so that it can ferment fermented bean curd. Rhizopus has the characteristics of high-temperature tolerance, a wide range of growth temperatures, and a slight influence by season. Rhizopus secretes a more active amylase enzyme than Mucor, which saccharifies starch to produce alcohol. In addition, during post-fermentation, small amounts of ethanol, glucose, and organic acids can be produced by saccharification of starch by root molds to enrich the flavor of fermented bean curd [19]. However, the mold filaments of Rhizopus will be thinner and lighter in color, and the proteases and peptidases present in it are less active, which is not as good as the hairy mold type of fermented bean curd in terms of taste and color in the production of fermented bean curd. Therefore, industrial production of fermented bean curd is often mixed with Mucor and Rhizopus in a particular proportion, co-inoculated with tofu billet on the mixed bacteria fermentation of fermented bean curd, which can effectively improve the quality and flavor of fermented bean curd, shorten the fermentation cycle, and enrich the nutritional value [3].

Bacteria

The role played by bacteria in the fermentation of fermented bean curd is similar to that of Mucor and Rhizopus, but the fermentation period is extended. In daily life, the principal strains commonly used to ferment fermented bean curd are Micrococcus and Bacillus subtilis [3].

Micrococcus produces enzymes such as lipase and glutaminase. Glutaminase has good salt tolerance, which facilitates fermentation of fermented bean curd under high salt conditions, and glutaminase converts L-glutamic acid, which provides fresh flavor for fermentation of fermented bean curd. Bacillus subtilis has the characteristics of non-toxicity, heat resistance, and a solid ability to secrete proteins, which can provide a good material basis for the fermentation of fermented bean curd and make fermented bean curd more thorough.

IV. Study On The Flavor Components Of Fermented Bean Curd

As a folk cuisine inherited from China for thousands of years, it is popular in China and Southeast Asia [20]. Flavor is an essential sensory attribute that determines the overall quality and consumer acceptance of fermented bean curd products [21]. In recent years, there has been an increasing number of studies on the composition of flavor substances in different varieties of fermented bean curd. The volatile flavor substances of fermented bean curd were determined and analyzed during the study of volatile flavor substances of fermented bean curd by gas chromatography-sniffing coupling technique, headspace solid-phase microextraction coupled with mass spectrometry, distillation extraction, dynamic headspace method, and dynamic headspace method. Since, in modern times, fermented bean curd is widely classified based on color, we investigated the volatile flavor substances of fermented bean curd of different colors in the light of domestic and international studies.

Volatile Flavor Substances In Fermented Bean Curd Of Different Colors

Red Fermented Bean Curd

Red fermented bean curd is made by adding red yeast rice, yellow wine, sorghum wine, and other flavorings during the post-fermentation stage of fermented bean curd so that the surface of fermented, fermented bean curd is naturally red, with a mellow taste and attractive aroma [14]. Li et al. [22] used headspace solid-phase microextraction (HS-SPME) to analyze the flavor substances of red fermented bean curd, and a total of 53 flavor substances were detected, including 6 alcohols, four acids, 19 esters, 5 aldehydes, and ketones, two phenols, and 15 other miscellaneous compounds. The OVA method was used to determine the key components, and the assay revealed that 3-methyl-1-butanol, 2-pentyl furan, methyl salicylate, indole, and 3-methyl-1-butanol acetate were found to be the main flavor substances after red fermented bean curd. He et al. [23] used the HS-SPME-GC-MS method to study the flavor substances of commercially available red square fermented bean curd. The main flavor substances in commercially available red square fermented bean curd are ethyl valerate, phenylacetaldehyde, (E) and (2)-2phenyl-2-butenal, dihydro-5-pentyl-2(3H)-furanone, 5-methyl-2-pentyl-2-hexenal and ethyl oleate.

White Fermented Bean Curd

White fermented bean curd is white in color, tasty, soft, and fresh, with an orange-yellow and transparent surface. The post-fermentation stage does not require the addition of red fermented bean curd; it is fermented with soup made of spices, sorghum, and yellow wine. He et al. [24] used gas chromatography-mass spectrometry-olfactometry to analyze the flavor substances of Guilin peppercorn white fermented bean curd, and a total of 20 flavor substances were detected, including seven esters, five aldehydes, three alcohols, one phenol, one furan, one ether, one olefin, and one sulfur-containing compound. These aromatic active substances constitute the unique aroma of Guilin Huaqiao white fermented bean curd. There were 13 volatiles such as ethanol, ethyl 2-methyl butyrate, and hexanol with OAVS values exceeding one by OVA calculations, indicating that they were the main components that produced the aroma of white fermented bean curd of Guilin peppercorns. These essential flavor substances give fermented bean curd a rich fermented flavor and pronounced alcoholic, fatty, and sweet notes, accompanied by mild meaty and fruity flavors and a hint of floral notes [25].

Gray Fermented Bean Curd

Gray fermented bean curd, also known as Stinky Bean Curd, is brewed with low-salt brine and has a unique flavor that is fluffy and delicate. Tian et al. [26] used HS-SPME to analyze the critical flavor substances of gray fermented bean curd. They detected 272 volatile compounds, including 66 esters, 56 alcohols, 35 hydrocarbons, 32 ketones, 29 aldehydes, ten acids, ten furans, five phenols, and 29 other volatile compounds. The main volatile compounds in gray fermented bean curd are esters and alcohols, which give fermented bean curd a grassy, fruity,

and sweet flavor.

Composition Of Volatile Flavor Substances In Fermented Bean Curd

Volatile flavor substances of fermented bean curd are essential for the quality improvement of fermented bean curd. In this paper, we have summarized the volatile flavor substances of different colors of fermented bean curd, as shown in Annex 2, by combining the articles of recent years.

From Annex 2, it is clear that the wealthiest variety of fermented bean curd flavor substances are esters. The esters have multiple layers of present fruity flavor [33]. Esters are mainly derived from glucose and amino acids and are formed during microbial metabolism through enzymatic and non-enzymatic processes [26]. The content of ester compounds in fresh tofu blanks is low but increases gradually with fermentation time and finally stabilizes. The pre-fermentation stage produces more types of ester compounds due to the active growth and metabolism of microorganisms such as Trichoderma reesei [26]. In the post-fermentation stage, the type and content of ethyl ester compounds gradually increase due to the addition of ethanol [14], such as ethyl acetate and ethyl trans-oleate, which imparts a melon-fruity and alcoholic flavor to the fermented bean curd. At the same time, the degradation and generation of macromolecule products, such as proteins in the salts, and the synergistic effect of the chemicals in the ingredients continuously enrich the esters [26].

Alcohols are also abundant in fermented bean curd in terms of type and content. They have good solubility, can synergize with other flavor compounds, and play an essential role in aroma enhancement [34]. During the post-fermentation stage, the type and content of alcohols increase by adding brine broth ingredients such as yellow wine and sorghum wine. However, as the fermentation time increases, many alcohols react with fatty acids to produce lipids, which decrease in type and content [35].

Aldehydes give fermented bean curd a light, fruity aroma. They are produced by the oxidative degradation of lipids [12]. The relative content of aldehydes was low at the time of white billeting but showed an increasing trend with fermentation time. Aldehydes are associated with the unpleasant soya flavor of tofu [34].

Ketones are produced during fermentation by fungal enzymatic action or a Meladic reaction [34]. Compared to other flavors, ketone content is relatively low and threshold high. Therefore, the compound has a low odor threshold and strong odor characteristics that give fermented bean curd a sweet, fruity taste.

Phenolics are produced by deleting lignin sugars and tyrosine [36]. Phenolics were higher in the pre- and post-fermentation stages of fermented bean curd. Its antioxidant properties help to protect against the harmful effects of free radicals and maintain cellular health [36], giving fermented bean curd a particular function.

Acids are mainly produced by fat oxidative degradation reactions and microbial fermentation, such as Trichoderma reesei [37]. In the pre-fermentation period of fermented bean curd, the relative content of acids is very small, but with the extension of fermentation time, their relative content and types increase [14].

The production of hydrocarbons is related to the degradation of fat [38]; alkane hydrocarbons do not contribute much to the overall flavor of fermented bean curd due to their large relative amounts and high threshold [39], and Shahidi et al. [40] concluded that alkane hydrocarbons play a lesser role in contributing to the formation of volatile flavor in food products. Therefore, they will not be analyzed here.

Factors Affecting The Volatile Flavor Substances In Fermented Bean Curd

Microorganisms

Microorganisms are an essential factor affecting the flavor of fermented bean curd. It mainly comes from the original microorganisms of the tofu blank, the air around the fermentation, and artificial inoculation. They secrete various catabolic enzymes in fresh tofu to form flavor substances [41]. Differences in microbial populations were mainly due to different sources and fermented bean curd production methods. During the pre-

fermentation period, the proteins in fermented bean curd are broken down into peptides and amino acids by proteases and peptidases secreted by microorganisms such as molds and bacteria, which provide sour, sweet, bitter, salty, and fresh flavors to the fermented bean curd [42]. Aldehydes, acids, alcohols, esters, and other flavor compounds with an aromatic flavor are produced by amino acid reactions catalyzed by enzymes secreted by fungi and bacteria. Huang [43] and others found that Lactococcus and Fusobacterium were the main contributors of esters and acids; Enterobacteriaceae and Enterococcus promoted the formation of flavor amino acids and sugars; and Lactobacillus and Moulds secreted and accumulated lipases during fermentation and catalyzed the release of fatty acids from fermented bean curd.

Raw Materials

Differences in the type and amount of raw materials are another critical factor affecting the flavor of fermented bean curd. Factors such as the amount of salt, type and amount of wine, fermentation temperature, and fermentation time all affect the flavor of fermented bean curd. The higher the salt content in fermented bean curd fermentation broth, the lower the amino acid content, affecting the production of fermented bean curd ketones and certain phenolic substances [44]. Yang et al. [45] studied the effect of salt concentration on fermented foods. They found that salt reduction during fermentation was beneficial in reducing osmotic stress on microorganisms, accumulating amino acid nitrogen and amino acids, and increasing volatile flavor compounds.

Production Process

The production process is also an essential factor in the flavor of fermented bean curd. The processing of fermented bean curd determines the quality of fermented bean curd. Cai et al. [46] found that the texture, microflora, and flavor substances of fermented bean curd varied considerably from one place of origin to another due to different production processes.

V. Summary

Fermented bean curd is a crucial fermented soya bean product, where raw materials and microbiota work together to form its variety and characteristics. Fermented bean curd contains a wide variety of flavor substances, including esters, alcohols, ketones, etc., which contribute to fermented bean curd's pleasing flavor. This paper presents an overview of the classification of curd to give a preliminary understanding of the types of fermented bean curd. It also analyses the main microflora of fermented bean curd fermentation to promote the improvement of the safe and controllable process. Finally, the main volatile flavor substances and influencing factors of fermented bean curd were reviewed to guide further improvement of curd quality. In the future, the preparation process for fermented bean curd should be further optimized to improve curd quality and safety continuously.

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