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Sufu Production Through Enzymes

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Abstract: Sufu is a kind of Chinese traditional fermented soybean food. This article analyzes the problems existing in the process of sufu production, such as semi-manual operations, high salinity, long producing cycle and so on in process control. It is suggested that the application of biotechnology in producing paste sufu with enzymes could overcome the problems and help to realize the industrialization of sufu production.

Key words: sufu; enzyme; paste

What is sufu: Sufu, also called tosufu, is a kind of traditional fermented soybean food developed from soybean curd in China. Sufu production has a long history. It started in Bei Wei Dynasty (more than one thousand years ago) and was popularized in Ming and Qing Dynasty (several hundreds years ago).

Sufu contains rich nutritional ingredients, which are shown in Table 1 and 2. The protein content in sufu reaches more than 12%. Besides free amino acid and free fatty acid, there are considerable lactoflavin and vitamin B12 in it due to the activities of microorganisms. The content of lactoflavin is 6 to 7 times higher than that in soybean curd. Sufu also contains abundant calcium, phosphorus and iron, and is characteristic of cholesterollessness, delicious taste and tenderness. Therefore, it has been one of the most favorite appetizers in China and some other countries for centuries and dominating in soybean foods. Due to the similarity to soft creamy cheese, it is called Chinese cheese in Europe and America (Li, 1998)

Analysis by HPLC (High Pressure Liquid Chromatogram) shows that there are many kinds of peptides in sufu. The molecular weight distribution of these peptides is almost continuous, which implies that some anti-oxidation peptides and blood pressure reducing peptides may be available and sufu may be an important resource of functional peptides. After fermentation, the composition of amino acids in sufu is changed. On the one hand, the ratio of essential amino acids to total amino acids changes from 31.0% to 36.1%. On the other hand, water-aloof amino acids content in soybean protein rises from 36.7% to 45.6%. The high proportion of water-aloof amino acids means that sufu may become cholesterol-reducing food (Chen, 1997). All of these facts have been stimulating the research of sufu in decades.

Current sufu producing process: Sufu is produced in many places in China, especially in Beijing, Suzhou and Wuxi in Jiangsu province, Shaoxing in Zhejiang province, Guangdong, Sichuan and Hunan provinces, and Guangxi Zhuang Autonomous Region. Due to different weather and climate conditions, different dietary habits and tastes, sufu producing techniques have their own characteristics in different places. However all of the techniques use soybean as raw material, and make sufu through a series of physical and chemical changes. There are two major types of sufu according to different production processes: bloat-type and mould-type. The latter includes natural fermentation and pure cultural fermentation which use mucor or bacteria as the main productive fungus. Today, mucor pure cultural fermentation is used most popularly. Four steps are normally involved in making this type of sufu: (1) Preparing soybean curd, (2) Preparing pehtze (Pizi)by fungal fermentation of soybean curd, (3) Salting of pehtze, and (4) Aging in dressing mixture.

The physical-chemical processes in sufu production mainly occur in soybean curd preparation, including soaking, milling, sieving, heating, curdling, squeezing and dividing. The biochemical reactions mainly occur in preparation of pehtze (pre-fermentation or the first fermentation), salting and aging (post-fermentation or the second fermentation). During pre-fermentation, the spore suspensions of mucor are inoculated, incubate and excrete enzymes in soybean curd. After pre-fermentation, the mycelia enwraps the pehtzes and part of soybean proteins is hydrolyzed into soluble proteins. Post-fermentation is the process in which huge molecule substances such as the left proteins and lipids are degraded, and consequently the degraded matters are esterified by the co-operation of enzymes produced by microorganisms and other chemical substances in sufu dressing (Zhao, 1994). After fermentation, sufu becomes a kind of nutritional food with special color, flavor, taste and shape.

Sufu production through enzymes

The basis for sufu production through enzymes: It is evident that fermentation is most important in sofu production and enzymes are crucial to sufu fermentation. That is to say, sufu can not be produced without cooperation of enzymes or sufu fermentation is an enzymatic process.

The chemical ingredients in soybean curd for making sufu are shown in Table 3. The proteins are almost soybean globins of 11S (Glycinin). After inoculating, the spores incubate and secrete hide enzymes with the protease system as the major part. With the secretion of enzymes, the macro-molecular ingredients such as protein begin to be hydrolyzed. The hydrolyzed protein is water-soluble protein and its content is raised from 3.61% to 55.54% through pre-fermentation. The protease system has three members: acid protease, neutral protease and alkaline protease. Each of them has its particular action pH value (pH 1~6.3, pH 4~10 and pH 5~10 respectively). The pH value of sufu is usually maintained between 5 and 6. Therefore, it is the cooperation of whole protease system that results in the degradation of soybean protein (Li, 1998).

Tofu pizies become sufu pehtzes through pre-fermentation. After brining, bottling, adding dressing mixtures, they begin to post-ferment, which is the major process for the degradation of macro-molecule matters and the formation of flavors. The hydrolyzed extent of protein during ripening is considered as the most important factor for sufu flavor and quality. The SDS-PAGE (Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis) analysis of soybean protein in red sufu (Han, 2002) shows that there is no intact soybean protein whether in the dressing mixtures or on the surface and in the internal of sufu due to the action of protease system secreted by microorganisms. This indicates that the structure of soybean proteins is destroyed and the proteins are hydrolyzed to peptides, amino acids and so on. This is the main reason that sufu is tender, delicious and easy for digestion. After ripening, the proportion of nitrogen content in small peptides to total reaches 86.4% to 88.9 %, which is closely related to sufu flavor.

Compared to protease enzymatic activities of microorganisms in other soybean fermentation foods, the protease enzymatic activities of mucor are lower, usually about 1/10 of other molds. The highest neutral protease activity is less than 65.52 U/g (dry weight) (Li, 1998). The varieties of whole enzyme system are limit. It is not enough to produce sufu with good flavor. For decades, it is always the goal for people to select strains with rich enzymes and highly active. People also study on fermentation with multiple-microbes in production, aiming to speed up the degradation of macro-molecular matters and the formation of flavor, and to shorten the cycle of production by the cooperation of enzyme system (Wang, 1998).

In traditional production, people used the way of adding dressing mixture in post-fermentation to compensate the shortage of enzyme varieties and raise enzymatic activities. According to the study (Li 1998), it is the added materials that offer rich enzymes for the fermentation, which is the basis for the sufu production by enzymes and bloating. During post-fermentation, the protease from sufu pehtze accounts only about 1/3, while 2/3 of total proteases come from the added materials. Traditional added materials are not only red rice and alcohol but also starch, sugar, lipids and other ingredients contributing to flavor. The optimal flavor of sufu can be formed just through complex chemical changes of all components and delicate operations.

Problems in traditional sufu production: After decades of development and adjustment, the process has been improved a lot. However, there are still many problems, especially related to strains, which limit sufu development.

The popular strains used in current production are mucor strains, including AS-3.25 M wutungliao, Mucor sufu and As -3.27 Acinomucor elegans. Compared to other molds, these strains are more difficult to produce spores and be incubated. Moreover, they can not bear high temperature and may die even under 35?, which often makes manufacturing impossible in summer and causes much loss in finance. It is a major goal to choose strains with high-temperature-tolerance, fast growth and high enzymatic activity in a long term.

Traditionally, the shape of sufu is cubic. In order to keep the perfect shape, there are strict requirement to the growth of mycelia and people always perform much manual work such as bottling. These make the production complex, not easy to control and low in productivity. The package of cubic products is comparatively coarse. After ripening, the products must be covered by dressing mixture, which makes sufu inconvenient to eat and a lot of wastefulness of dressing mixture. People also have a deep guard towards the unevenness of sufu. Consequently, the development is very limited.

There is no cubic concept from the literal meaning of sufu. The current cubic shape should not be the only shape for sufu and the production should not be limited by it (Ling, 2000). If discarding the cubic shape in sufu production, the role of pre-fermentation is only to produce enzymes. Since the enzymes can be massivelyproduced by modern bio-engineering method, the process becomes more simple.

Sufu is a kind of high salinity food. Salt takes important role in the production. Firstly, the pehtze absorbs the salt and penetrates out redundant water, which is helpful to the taste and the cubic shape. Secondly, salt can inhibit the growth of harmful bacterial and benefit to the normal fermentation. Thirdly, salt can slower the hydrolyzing of protein to format more flavors.

It is well known that sufu is a highly nutritious value soybean food. With increasing of knowing its nutritious value, more and more people may pay attention to it. Nowadays, the Chinese sufu has already sold in Japan, America and many other countries. However, scientists believe the adult hypertension and many other diseases are closely related to dietary sodium intake. Internationally, it becomes a trend to keep food low salinity in recent years. Biologically, high salinity is unhelpful to the degradation of soybean protein and lipid, which is the major reason for the long cycle of sufu production and consequently taking up huge

Table 1: The nutritional ingredients in sufu (Han, 2001)

Ingredients	Water	Protein	Fat	Fibre	Starch	Ash
Content %	58-70	12-17	8-12	0.2-1.5	6-12	4-9
ingredients	Calcium mg/100g	Phosphorus mg/100g	Iron mg/100g	VB_1 mg/100g	VB_2 mg/100g	B_{12}
Content	100-230	150-300	7-16	0.04-0.09	0.13-0.36	1.7-22

Table 2: The amount of amino acids recommend by FAO and contained in Sufu (Li, 1998)

Amino acids	Ile	Leu	Lys	Met+Tyr	Phe+Tyr	Thr	Val	Trp
Amount needed for adult everyday (Mg/50kg)	500	980	600	650	700	350	500	175
Amount contained is Sufu (mg/100g)	510	700	610	700	970	510	640	90

Table 3: Routine chemical ingredients in tofu pehtze (Yuan 1982)

Terms	Water	Fat	Protein	Sugar	Cellulose	Ash
Content (%)	75.80	7.20	16.00	0.10	---	0.99

space. All of these make sufu development not really optimistic.

Sufu production through enzymes: Sufu and soybean sauce are all Chinese traditional fermented condiments. In 1970s?Japanese experts purified the protease system in sauce production and did further study on the enzymes attributes. Based on these investigation, they can produce high quality sauce by the enzymatic method only in several hours. Studies show that flavors can be made by enzymes (Xing, 1994). The essence of sufu fermentation is biochemical transformation of ingredients in tofu pizi and dressing mixture by enzymes. Therefore, sufu can be produced by the enzymatic method.

The process of sufu production with paste shape by enzymes can be realized by two major steps: enzyme preparation and sufu fermentation. The former includes four steps: strains selection, cultivation, enzyme extractiion and enzyme preparation. The latter includes the following steps: preparing soybean curd, mixing soybean curd with additional materials, homogenizing, sterilizing, hydrolyzing, ending the hydrolyzing and packaging. Due to the increasing development of modern bio-engineering technology, the enzyme preparation can be massively produced in enzyme plant and the whole process of hydrolyzing can be realized in bio-reactor. This can not only make the process simpler and shorten the production cycle, but also be helpful to manage the product quality and online process monitor. Meanwhile, the paste shape is helpful to promote low salty, functional, convenient sufu and its market.

In short, sufu production by enzymes is a critical reform to current process, which is an effective way to realize stable and automatic production without the limitation of seasons.

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