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ARTICLE

Changes in the Contents of Sugars and Organic Acids during the Ripening and Storage of Sufu, a Traditional Oriental Fermented Product of Soybean Cubes

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In the present study, sufu, an oriental fermented product of soybeans, was prepared by ripening the tofu cubes in *Aspergillus oryzae* fermented rice–soybean koji mash for 16 days at 37 °C. The sufu product was further kept at room temperature for another 30 days. Examining the changes in the sugar content revealed that stachyose, raffinose, and sucrose contents of tofu and rice–soybean koji granules decreased while levels of glucose and fructose increased during the ripening period and after storage. Glucose was the most abundant sugar detected. Four organic acids, including oxalic, lactic, acetic, and citric acid, were detected in the sufu product and koji granules after ripening. Generally, the contents of these organic acid increased as the ripening period was extended. Among them, acetic acid was the most highly detected.

KEYWORDS: Sufu; *Aspergillus oryzae*-fermented koji; ripening; sugar; organic acid

INTRODUCTION

Sufu, which has existed in China for more than 1000 years, is a highly flavored, soft, cheese-like fermented product of soybean curd (tofu) (*1*). This fermented product possesses a characteristic flavor and a relatively high protein content. With an estimated annual production of over 300,000 tons, sufu is widely consumed by Chinese people as a relish in their daily diet (*2*).

On the basis of production method, sufu is categorized into various types (*2*), with mold-fermented and enzyme-ripened sufus as the primary types of sufu available in the market. The former is manufactured by first growing a fungus such as *Actonomucor*, *Rhizopus*, or *Mucor* sp. on the surface of tofu cubes to prepare pehtze. Then, the prepared pehtze is aged in a saline solution for a period of 4–6 months (*2, 3*). On the other hand, during the manufacturing of enzyme-ripened sufu, salted tofu cubes and koji mash are first prepared simultaneously. Koji mash is obtained by growing *Aspergillus oryzae* on a mixture of steamed rice and soybean before being soaked in syrup solution (65% sucrose). Then, the salted tofu cubes are ripened in the *Asp. oryzae*–koji mash at 37 °C for approximately 2–3 weeks. In addition to a shorter required ripening period, the enzyme-ripened sufu is generally sweeter and less salty than the mold-fermented sufu. For these reasons, enzyme-ripened sufu is becoming more popular in the market.

Due to the lack of scientific documentation, and in an attempt to provide information to improve the quality of enzyme-ripened sufu, biochemical and physical properties of enzyme-ripened sufu were investigated (*4*). It was noted that hydrolytic enzymes such as protease, lipase, α-amylase, and β-amylase leached out from the koji granules into syrup solution and penetrated into the tofu cubes during the sufu ripening period. At the same time, the total nitrogen content, pH, and hardness of sufu decreased. However, the titratable acidity, protein dissolution ratio, fatty acid content, and free amino acid content increased. Furthermore, it was found that the sufu product possessed higher amounts of aglycone, the bioactive form of isoflavones, and also greater antioxidant activity when compared with the unfermented tofu cube (*5*). This paper further explores the changes of sugar and organic acid contents in the sufu (tofu cube) and koji granules during the ripening and storage of sufu.

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MATERIALS AND METHODS

Preparation and Ripening of Sufu. In this study, the preparation and ripening of sufu essentially followed the procedure described by Li et al. (*4*). Briefly, tofu cubes approximately 2.5 cm × 2.5 cm × 2 cm in size were first mixed with salt and water at a ratio of 4:1:3 (w/w/w) and kept at room temperature for 24 h. They were then air-dried for 24 h to obtain salted tofu cubes. *Asp. oryzae* was grown in a mixture of steamed rice and soybean at 35 °C in relative humidity of 70% for 40 h to pepare the rice–soybean koji (koji). To obtain the rice–soybean koji mash, the prepared koji was mixed with syrup (65% sucrose) at a ratio of 1:5 (w/w) and stored at room temperature for 48 h. The sufu was then ripened by mixing 195 g of salted tofu cubes (approximately 14–15 cubes) with 175 g of koji mash in a 400 mL glass jar. The ratio of koji granules to syrup was 1:4 (w/w). This mixture was then held at 25, 37, or 45 °C for a period of 16 days. Additionally, the sufu-containing jar was placed at room temperature (approximately 25 °C) for another 30 days. This is the practice employed in some factories before sufu products are distributed for sale in the market.

Sampling. During the sufu fermentation and storage period, bottled sufu samples were withdrawn at specific intervals. Sufu samples (tofu cubes) were first collected and soaked in deionized water for 2 min. Then, the remaining koji mash was filtered through a screen to separate the koji granules. The collected sufu samples and koji granules were subjected to freeze-drying and analysis for various sugars and organic acids.

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Table 1. Changes in Various Sugar Contents of Tofu during Sufu Fermentation and after Storage

sugar	nonfermented tofu (mg/g of dried matter)	ripened at 37 °C for 2 h[a] (mg/g of dried matter)	8 days (mg/g of dried matter)	16 days (mg/g of dried matter)	held at room temperature for 30 days (mg/g of dried matter)
stachyose	8.63 ± 0.73a[b]	8.84 ± 0.76a	8.81 ± 1.74a	6.85 ± 1.67ab	5.93 ± 0.52b
raffinose	13.82 ± 1.16b	21.57 ± 1.45a	13.09 ± 0.56b	9.77 ± 1.39c	7.55 ± 0.67d
sucrose	1.39 ± 0.18b	28.50 ± 4.47a	4.96 ± 1.09b	2.93 ± 0.39b	2.68 ± 0.19b
maltose	ND[c]	ND	0.85 ± 0.07c	1.37 ± 0.12b	1.72 ± 0.12a
glucose	0.27 ± 0.08d	98.49 ± 12.27c	432.64 ± 15.42a	358.35 ± 12.69b	376.78 ± 20.62b
fructose	65.05 ± 18.32b	256.23 ± 30.36a	258.22 ± 5.53a	257.56 ± 21.91a	261.75 ± 23.74a
galactose	ND	ND	5.99 ± 1.46b	8.60 ± 1.23a	9.12 ± 0.27a

[a] Tofu cubes were collected 2 h after mixing with koji mash. [b] Values are presented as means ± SD ($n = 3$). Means in the same row with different letters are significantly different by Duncan's multiple-range test ($P < 0.05$). [c] None detected.

Preparation of Samples for the Determination of Sugars and Organic Acids. The extraction procedures described by Lefebvre et al. (*6*) were followed when the samples were prepared for the determination of sugar and organic acid levels. Essentially, 10 g of the sample was homogenized with 90 mL of distilled water in a grinder (model H F-365, Shivn Feng Enterprise Co. Ltd., Taipei, Taiwan) for 30 s. Five milliliters of $HClO_4$ solution (1 M) was added to 10 mL of the homogenate. The mixture was centrifuged for 15 min at 4000*g* and 15 °C. Then, the supernatant was neutralized (pH 7.0 ± 0.1) with KOH solution (2 M), and the volume was adjusted to 25 mL with distilled water. After 30 min of precipitation on ice, the solution was filtered through a 0.45 μm Millipore PVDF filter (Schleicher & Schuell, Dassel, Germany).

Measurements of Sugar and Organic Acid Contents. Sugar and organic acid levels in the samples were determined according to the HPLC analysis procedure described by Lefebvre et al. (*6*), with minor modifications. The HPLC equipment used was a chromatograph (model 7200, Jasco Co., Tokyo, Japan). For sugar determination, it was equipped with a Phenomenex RNM Carbohydrate column (300 × 7.8 mm, Phenomenex Co., Torrance, CA), an RI detector (830-RI, Jasco Co.), and a data processor (EC 2000 data system 1.0, Analab Cooperation, Taipei, Taiwan). When samples were analyzed, the mobile phase was HPLC grade water. The flow rate and column temperature were 0.35 mL/min and 75 °C, respectively. Sugar standards used included stachyose (ABCR, Karlsruhe, Germany), fructose and raffinose (Chem Service, Inc., West Chester, PA), sucrose and maltose (Supelco Analytical, Bellefonte, PA), and glucose and galactose (Sigma-Aldrich Co., St. Louis, MO).

For the analysis of various organic acids, a Phenomenex Capcell Pak C18 UG-120A column (4.6 mm × 250 mm, 5 μm) was used. Operational conditions were as follows: mobile phase, 5 mM sulfuric acid (prepared using distilled deionized water); flow rate, 0.5 mL/min; column temperature, 30 °C; detector wavelength, 220 nm (UV−vis detector, model UV-970, Jasco Co.). Reference standards of organic acid included lactic acid, acetic acid, and citric acid (Supelco Analytical) and oxalic acid and succinic acid (Chem Service).

The contents of the organic acids and sugars, expressed as milligrams per gram of dried sample, were calculated from the standard curves of the area responses for authentic sugar or organic acid standards.

Measurement of Dry Weight. Dry weights of samples were measured by drying to a constant weight at 105 °C as described by AOAC (*7*).

Statistical Analysis. The mean values and standard deviation were calculated using the data obtained from three separate experiments. Means were compared using Duncan's multiple-range test method in SAS, version 8 (SAS Institute, Cary, NC). The level of significance was chosen at $P < 0.05$, and the results are presented as mean ± standard error.

RESULTS AND DISCUSSION

Changes in the Sugar Contents of Tofu during the Ripening and Storage of Sufu. During the preparation of the enzyme-ripened sufu, it is reasonable to expect that the hydrolytic enzymes which leached from the koji to the syrup and those that penetrated into the tofu cubes (*4*) may catalyze the hydrolysis of sugar components in the tofu substrate. **Table 1** shows the changes in the content of various sugars during the 16 days of ripening at 37 °C and further storage at room temperature for 1 month. The total content of the sugars examined was found to be 89.16 mg/g of dried matter in the nonfermented tofu, whereas the content increased to 645.43 mg/g of dried matter after 16 days of ripening. The main sugars noted in the nonfermented tofu, the raw material used as the substrate for sufu fermentation, were stachyose, raffinose, sucrose, glucose, and fructose. Among these sugars, fructose was the most abundant. Stachyose and raffinose, the main oligosaccharides in soy products, are the factors causing flatulence in humans after eating soybean foods (*8*).

In comparison with the nonfermented tofu, the sufu sample, taken after 2 h of soaking of the tofu cubes in the koji mash, exhibited markedly high contents of raffinose, sucrose, fructose, and glucose. Apparently, the increased amounts of these sugars in the 2 h sufu came essentially from the koji mash.

As the ripening proceeded further, contents of stachyose, raffinose, and sucrose declined. For example, the contents of stachyose and raffinose noted in the nonfermented tofu reduced significantly ($P < 0.05$) from 8.63 and 13.82 mg/g, respectively, to 6.85 and 9.77 mg/g of dried matter after 16 days of ripened.

Further reduction in the amounts of stachyose and raffinose were noted in the sufu product after storage at room temperature for another month. It was reported that *Asp. oryzae* was capable of producing α-galactosidase (*9*) in addition to β-glucosidase, protease, amylase, and lipase (*4*, *10*, *11*). α-Galactosidase may catalyze the hydrolysis of α-1,6-linked α-galactoside residue (*12*). The catalytic action of α-galactosidase may thus lead to the reduced contents of stachyose and raffinose in tofu substrate during the fermentation period. These results, in accordance with reports of Chumchuere and Robinson (*13*) and Wang et al. (*10*), demonstrate that fermentation may reduce the levels of stachyose and raffinose, both flatulence factors, in sufu products.

As shown in **Table 1**, a markedly higher amount of sucrose was noted in the 2 h sufu when compared with the nonfermented tofu. This may be attributed to the penetration of sucrose from the koji mash into tofu cubes because the koji mash was prepared with syrup containing 65% sucrose. However, similarly to the changes of stachyose and raffinose, and in accordance with that observed on the mold-fermented sufu, which was prepared with different starter organisms according to a different manufacture process (*2*), sucrose content noted on the 2 h sufu reduced significantly ($P < 0.05$) as the ripening period was further extended. This was probably due to the enzymatic degradation of disaccharides to monosaccharides such as glucose and fructose.

Although maltose and galactose were not detectable in the nonfermented tofu and 2 h sufu samples, the presence of these sugars was noted in sufu ripened for 8 days or longer. Along with the reduction in stachyose, raffinose, and sucrose contents, increases in maltose and monosaccharides such as glucose, fructose, and galactose were noted during the ripening of sufu. Catalytic hydrolysis of stachyose, raffinose, and sucrose by the

Table 2. Changes in Various Sugar Contents of Rice−Soybean Koji during Sufu Fermentation and after Storage

		ripened at 37 °C for			
sugar	koji (mg/g of dried matter	2 h[a] (mg/g of dried matter)	8 days (mg/g of dried matter)	16 days (mg/g of dried matter)	held at room temperature for 30 days (mg/g of dried matter)
stachyose	37.45 ± 10.16a[b]	3.34 ± 0.11b	2.41 ± 0.71b	1.17 ± 0.42b	ND[c]
raffinose	50.57 ± 6.55a	5.05 ± 0.92b	2.67 ± 0.33b	1.95 ± 0.25b	1.04 ± 0.21b
sucrose	0.44 ± 0.16c	23.07 ± 1.49a	2.19 ± 0.61b	1.61 ± 0.42bc	1.58 ± 0.19bc
maltose	0.07 ± 0.06e	0.52 ± 0.11d	0.98 ± 0.12c	1.15 ± 0.04b	1.33 ± 0.03a
glucose	59.07 ± 14.19c	399.70 ± 24.80a	358.07 ± 11.90b	348.26 ± 15.75b	335.42 ± 29.79b
fructose	0.98 ± 0.23d	145.34 ± 16.06c	276.75 ± 14.75a	261.27 ± 51.14ab	212.34 ± 36.43b
galactose	13.26 ± 0.94a	6.36 ± 0.94b	6.02 ± 1.23b	3.65 ± 0.37c	3.66 ± 0.46c

[a] Koji mash was prepared by mixing koji with syrup (65% sucrose) for 2 days before sufu fermentation was begun. 2 h koji refers to the koji collected 2 h after mixing koji mash with tofu. [b] Values are presented as means ± SD (n = 3). Means in the same row with different letters are significantly different by Duncan's multiple-range test ($P < 0.05$). [c] None detected.

Table 3. Changes in Various Organic Acid Contents of Tofu during Sufu Fermentation and after Storage

		ripened at 37 °C for			
organic acid	nonfermented tofu (mg/g of dried matter)	2 h[a] (mg/g of dried matter)	8 days (mg/g of dried matter)	16 days (mg/g of dried matter)	held at room temperature for 30 days (mg/g of dried matter)
oxalic	0.12 ± 0.01b[b]	0.17 ± 0.03b	0.51 ± 0.09a	0.58 ± 0.07a	0.47 ± 0.13a
lactic	0.14 ± 0.12b	ND[c]	1.79 ± 0.21a	2.35 ± 0.26a	2.12 ± 0.71a
acetic	ND	1.13 ± 0.22b	18.01 ± 1.14a	17.18 ± 1.09a	18.03 ± 1.14a
citric	ND	ND	0.15 ± 0.13c	0.91 ± 0.44b	2.83 ± 0.34a
succinic	ND	ND	ND	ND	ND

[a] Tofu cubes were collected 2 h after mixing with koji mash. [b] Values are presented as means ± SD (n = 3). Means in the same row with different letters are significantly different by Duncan's multiple-range test ($P < 0.05$). [c] None detected.

hydrolytic enzymes such as α-galactosidase and β-fructosidase/invertase produced by *Asp. oryzae* extracellularly during the fermentation might lead to this result (*14*, *15*). After being kept at room temperature for another month, the sufu was found to contain 1.72, 376.78, 261.75, and 9.12 mg/g of dried matter of maltose, glucose, fructose, and galactose, respectively. Contents of these sugars were all significantly higher ($P < 0.05$) than those found with the nonfermented tofu. On the other hand, glucose content was found to reach a maximum after 8 days of ripening before decreasing as time went on. It was observed that the color of tofu cubes changed from pale yellow to yellowish brown after 16 days of ripening due to enzymatic and nonenzymatic browning reactions at the expense of amino acid and glucose (*4*). This might lead to the observed reduction of glucose content.

Changes in the Sugar Contents of Rice−Soybean Koji Granules during the Ripening and Storage of Sufu. Unlike the mold-fermented sufu product, the koji enzyme-ripening sufu is sold packed in jars with the koji mash, which contains granules of rice and soybean koji. The koji granules, along with sufu, are also consumed as condiments. Changes in sugar content in the koji granules during the ripening and storage of sufu product were also investigated. Results obtained are shown in **Table 2**. Similar to that observed on the 2 h sufu (**Table 1**), a significantly higher ($P < 0.05$) sucrose content was also noted with the koji granules collected after 2 h of ripening when compared with koji before it was mixed with sucrose syrup for the preparation of koji mash. Penetration of sucrose from the syrup to the koji granules may lead to the increased sucrose content observed. Unlike the tofu cubes, maltose and galactose was found in the koji granules before mixing with syrup for sufu fermentation. This was probably due to the action of hydrolytic enzymes such as amylase and α-galactosidase produced by *Asp. oryzae* during the preparation of koji. Compared with koji granules before ripening, koji granules collected during ripening and after 1 month of storage at room temperature showed reduced contents of stachyose, raffinose, and galactose, but increased contents of maltose, glucose, and fructose (**Table 2**). Besides, the occurrence of browning reaction (*4*) might also lead to the reduction of fructose content of koji granules as the ripening period is extended to more than 8 days or after storage. The trend in the changes of these sugars' content in koji granules was generally similar to that observed with the tofu substrate (**Table 1**).

Changes in the Organic Acid Contents of Tofu during the Ripening and Storage of Sufu. Acids often impart flavors besides a sour taste. Whereas the dominant flavor of organic acids is sourness, they also contribute to bitterness and astringency, and the proportions of these sensations vary with different acids (*16*). Additionally, antimicrobial activity of organic acids may also improve the keeping quality of a food product (*17*). It was noted that the tofu cubes showed an initial acidity and pH of 0.16% and 6.04, respectively, before ripening; the acidity and pH changed to 1.50% and 5.55, respectively, after 16 days of ripening (*4*). As shown in **Table 3**, only small amounts of oxalic and lactic acids were detectable in the nonfermented tofu samples. However, four organic acids, including oxalic, lactic, acetic, and citric acid, were detected in the tofu samples that had ripened for 8 days or longer. It was generally found that the content of these organic acids, except citric acid in the tofu cube, increased to the highest point after 8 days of fermentation. Further extending the ripening time did not change the contents of these organic acids significantly ($P > 0.05$). On the other hand, the citric acid content of tofu was found to continually increase with extension of ripening time.

After further storage at room temperature for another 30 days, the sufu product showed a citric acid content of 2.83 mg/g of dried matter, which is significantly ($P < 0.05$) higher than the 0.15 and 0.91 mg/g of dried matter noted in the 8 and 16 day sufus, respectively. Acetic acid was found to be the predominant organic acid in the sufu examined. The nonfermented tofu contained no detectable acetic acid, whereas the 8 and 16 day sufus were found to contain acetic acid at levels of 18.01 and 17.18 mg/g of dried matter, respectively. The increased level of organic acids in the sufu samples observed in the present study is in agreement with

Table 4. Changes in Various Organic Acid Contents of Rice−Soybean Koji during Sufu Fermentation and after Storage

organic acid	koji (mg/g of dried matter)	ripened at 37 °C for 2 h[a] (mg/g of dried matter)	8 days (mg/g of dried matter)	16 days (mg/g of dried matter)	held at room temperature for 30 days (mg/g of dried matter)
oxalic	2.04 ± 0.36a[b]	0.66 ± 0.07b	0.18 ± 0.04c	0.25 ± 0.04c	0.36 ± 0.05bc
lactic	1.35 ± 0.39c	0.73 ± 0.13c	3.90 ± 0.42b	5.53 ± 0.84a	4.80 ± 0.09a
acetic	3.36 ± 0.72c	2.63 ± 0.45c	20.10 ± 0.53b	23.63 ± 0.86a	23.87 ± 0.99a
citric	ND[c]	ND	0.17 ± 0.06c	3.25 ± 0.45b	4.82 ± 0.29a
succinic	ND	ND	ND	ND	ND

[a] Koji mash was prepared by mixing koji with syrup (65% sucrose) for 2 days before sufu fermentation was begun. 2 h koji refers to the koji collected 2 h after mixing koji mash with tofu. [b] Values are presented as means ± SD ($n = 3$). Means in the same row with different letters are significantly different by Duncan's multiple-range test ($P < 0.05$). [c] None detected.

that observed in douchi, an *Aspergillus*-fermented product of fresh soybean (*18*). This increase may also account for the reduced pH level of sufu in comparison to the nonfermented tofu as reported by Li et al. (*4*). It is evident that that these organic acids were formed through the fermentation of sugar catalyzed by the enzyme produced by *Asp. oryzae* during the ripening periods. Some of these enzymes leached out from the koji into the infusion (syrup) and further penetrated into tofu cubes. These enzymes might catalyze the hydrolysis of complex sugars in the koji granules and tofu substrates, yielding the various hydrolytic products and organic acids detected in koji granules and tofu cubes.

Changes in the Organic Acid Contents of Rice−Soybean Koji Granules during the Ripening and Storage of Sufu. Table 4 shows the changes in organic acid contents in rice−soybean koji granules during ripening at 37 °C and after 30 days of storage at room temperature. The prepared koji granules before mixing with syrup contained oxalic, lactic, and acetic acid. Apparently they were formed through the catalytic action of enzymes produced by *Asp. oryzae* during the preparation of koji. With the extension of ripening and after storage, oxalic acid content in the koji granules decreased. Meanwhile, the lactic, acetic, and citric acid contents increased to 4.80, 23.87, and 4.82 mg/g of dried matter, respectively, after 30 days of further storage at room temperature. This phenomenon is similar to that observed for the tofu cube (**Table 3**).

In the mold-fermented sufu products manufactured by first growing *Actinomucor taiwanese* or *Act. elegans* on the surface of tofu cubes, oxalic acid was the main organic acid (*19*), whereas it was found to be a minor acid, quantitatively, in the enzyme-ripened sufu from the present study. Additionally, succinic acid was not detected in the sufu and koji samples examined, despite its presence in the mold-fermented sufu (*19*). This discrepancy may be attributed to the difference in the microorganisms involved and the fermentation process. However, the exact cause remains to be further investigated.

On the basis of the data obtained, it was concluded that as the ripening period continued, and after storage, generally, the stachyose, raffinose, and sucrose contents of tofu and koji granules decreased. At the same time, glucose and fructose contents increased. Glucose was the most abundant sugar detected in the sufu and koji granules after 30 days of storage. Oxalic, lactic, acetic, and citric acid were detected in the tofu and rice−soybean koji after ripening for 8 days or longer. Unlike what was observed for mold-fermented sufu (*19*), acetic acid was the most predominant organic acid detected in the enzyme-ripened sufu product in the present study.

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