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Microbiological Study of Tofu

KOORANEE TUITEMWONG and DANIEL Y. C. FUNG*

Department of Animal Sciences and Industry, Kansas State University, Manhattan, Kansas 66506

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ABSTRACT

The microbiological qualities of tofu juice and cake were studied. Seven brands of tofu from four grocery stores were tested, at day 1 and after 30 d of storage in a refrigerator. The microbial load at day 1 was different from brand to brand, but cell counts in juice and cake of the same brand were correlated. The number of cells observed at day 30 was different from brand to brand but was not related to the initial cell count. The pH had a great effect on the type of contaminating microorganisms present. All brands spoiled after 30 d of storage at 7°C; 112 isolates from both the fresh juice and cake at day 1 and at day 30 were obtained. The most common gram-positive organisms isolated were *Streptococcus* sp., *Pediococcus* sp., and *Lactobacillus* sp., and the most common gram-negative bacteria were *Pseudomonas putida*, *P. aeruginosa*, *Enterobacter agglomerans*, and *E. cloacae*.

Tofu is an important nonfermented soybean product (curd). It has been reported that tofu was first invented 2000 years ago by a Chinese king of the Hsi-Han Dynasty. Now, tofu is a popular food in Oriental countries and in the United States (*6*).

According to data from the Soy Foods Center of America, tofu has been commercially made in the United States by Asian immigrants since 1904. After that time, non-Oriental people began to produce tofu, and the number of producers increased during the period 1975 to 1981. More than 11,000 tons of soybeans are used annually to make tofu. In addition, the Chinese proverb, "Tofu is meat without bones" is well known in the United States (*12,13*).

Tofu is made from soybean milk, which is a liquid extracted from soybeans. Soybeans are soaked in water, blended, and the liquid and solid portions are separated. The soymilk is heated to the boiling point and filtered. Then it is precipitated with coagulants such as calcium or magnesium sulfate or calcium or magnesium chloride. A cheese-like curd is formed and is pressed into cakes of tofu (*7,13*).

There are two commercially popular methods of making tofu: the traditional method, as described above, and the hot grind method. Tofu made from the traditional method has a beany flavor. In contrast, the hot grind method, in which the lipoxygenase enzyme in soybean curd is inactivated, yields a bland taste with a less beany flavor (*13,15*).

There are three different types of tofu: soft, hard, and dried. Soft tofu has a high water content (84-89%), and the curd is smooth and fragile. Hard tofu, on the other hand, has a firmer texture and contains less water (79-87%), so it can be cut into different sizes for frying. Dried tofu is the firmest variety and has a moisture content of 60-82%. Tofu colors range from white or pale yellow to dark brown. Furthermore, a chewy or meaty texture varies with the type of tofu (*14*).

Tofu compositions vary depending on the soybean varieties and the methods of production. The approximate composition of nutrients in 100 grams of regular Japanese tofu solidified with calcium sulfate is 7.8% protein, 4.3% fat, 2.3% sugars, 0.7% ash, and 84.9% water (*13*). Because of the rich nutrients in tofu, it is perishable by contamination with microorganisms. Microbiological study of tofu is important because its shelf life, without preservatives, is very short. Normally, tofu will spoil within 1 to 3 d without refrigeration or any method of preservation (*8*). The shelf life can be extended with refrigeration, depending on the types and brands of tofu. In the United States, tofu may stay fresh for many days, if it is stored at a cool temperature, and some manufacturers predate their tofu for a 28-d shelf life (*2*). Researchers have attempted to increase tofu shelf life through study of its microflora and attempts to find harmless preservatives (*5,16*).

Tofu can be contaminated by microorganisms during curd pressing to form cake and during cake handling before packaging (*11*). Normally, the microorganisms that spoil tofu are lactic acid bacteria (*4*). Fujii et al. (*5*) reported that a high level of *Escherichia coli* was found in soybean curd during transportation from the producers to the retail stores. The storage temperature during transportation was very important in extending tofu's shelf life. In addition, the microbiological quality of commercial tofu varied within the cake. The microorganism counts were higher at the top than in the center or the bottom portion of the tofu cake (*11*). Moreover, aerobic organisms, psychrotrophic bacteria, and coliform bacteria were found to vary among lots of the same brand.

The pH values of the tofu cake and juice change during storage because of decomposition by microorganisms. The juice or immersion solution around the cake is bacteriostatic when its pH is below 4.5. At a higher pH value, *Clostridium botulinum* was shown to grow in this product (*10*). Dotson et al. (*4*) found that the pH of the juice surrounding the tofu cake between the first and the second

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days of storage was normally about 0.2-0.25 units lower than the pH of the cake. They also found that pH change during storage affected the flavor and texture of the tofu cake. Fujii et al. (5) reported that growth of microorganisms causes rapid decomposition of protein and a change in the pH. The tofu cake is a suitable medium for microorganisms because of its high content of protein and moisture. Acid production in soybean milk depends on the numbers and types of bacteria, the growth rate, and the ability to utilize carbohydrates in the medium (*1,9*). In addition, Angeles and Marth (*1*) reported that soy milk promotes the growth of lactic acid bacteria.

The purposes of this study were to investigate the microbiological quality of different brands of tofu, including both the juice surrounding the curd in the package and the tofu cake; to compare the number of gram-positive and gram-negative bacteria in each brand at day 1 and day 30; to observe the relationship between the pH of tofu and the types of bacteria; and to investigate genera of contaminating bacteria in different tofu brands.

MATERIALS AND METHODS

Seven different brands of commercial soft tofu were purchased from four local stores in Manhattan, KS. All brands were immediately analyzed for microbiological quality. The date of analysis was counted to be day 1 of the experiment. The tofu juice and tofu cake were aseptically separated into separate sterile containers. Samples of tofu were stored at a temperature of 7°C for 30 d, then the microbiological quality at day 30 was analyzed.

Microbiological analysis

Viable cell count of tofu juice and homogenized tofu cake was made using the Spiral System (Spiral System Instruments Inc., Bethesda, MD 20814). The juice was directly pipetted, and serial dilutions were made. Ten g of tofu cake was aseptically transferred to a stomacher bag. Ninety ml of sterile phosphate buffer (pH 7.2) was added to the bag. The sample was blended by the Stomacher for 2 min. Serial dilutions were made, and the liquid portion was used to evaluate cell numbers. Tryptic soy agar (TSA) was used as the medium for enumeration. All samples were done in duplicate. Petri dishes were incubated at 32°C for 48 h. A cell count was obtained by the Laser Bacterial Colony Counter 500A and the Colony Viewer MV of the Spiral Plate System. Viable cell numbers were counted at day 1 and day 30. At the same time, the pH was also measured.

TABLE 1. *pH and viable cell count of juice and cake of tofu at day 1 and day 30.*

Brands		Day 1		Day 30	
		pH	*VCC (CFU/ml or gm)	pH	VCC (CFU/ml or gm)
A	juice	6.10	1.6×10^{2}	6.44	7.8×10^{7}
	cake	6.20	2.3×10^{3}	6.10	3.3×10^{9}
B	juice	4.31	1.2×10^{4}	5.50	6.1×10^{8}
	cake	4.49	4.1×10^{4}	5.17	2.0×10^{8}
C	juice	4.16	5.0×10^{6}	4.17	3.5×10^{6}
	cake	4.79	1.5×10^{6}	6.18	2.8×10^{8}
D	juice	6.10	2.3×10^{3}	5.60	3.4×10^{6}
	cake	5.42	6.2×10^{4}	5.70	2.9×10^{8}
E	juice	5.96	1.8×10^{2}	4.31	3.4×10^{6}
	cake	6.06	3.6×10^{4}	6.34	9.4×10^{7}
F	juice	6.48	1.9×10^{2}	5.20	2.3×10^{7}
	cake	6.18	7.5×10^{3}	5.89	5.1×10^{9}
G	juice	4.50	5.3×10^{5}	4.70	1.1×10^{7}
	cake	4.51	1.1×10^{6}	5.38	2.2×10^{8}

*VCC = viable cell count.

Microbial identification

Colonies from each countable dilution plate of each tofu brand were randomly selected. Eight colonies from each tofu cake and each soaking solution (juice) were isolated. Therefore, 112 colonies were selected from seven brands both at day 1 and at day 30. All cultures were Gram stained and checked for catalase reactions.

Gram-positive bacteria were restreaked on MRS agar for purification. Morphological and biochemical tests of each isolate were performed (*3*). Colonies were identified by genus.

Gram-negative bacteria were restreaked on TSA and EMB agar. Single colonies that could grow on TSA and EMB agar were selected. An oxidase test was done with all selected colonies. Oxidase-negative isolates were identified by Micro ID test kits (Organon Teknika Co., Durham, NC 27704), the oxidase-positive isolates were identified by Oxi/Ferm tube test kits (Hoffman-La Roche Inc., Nutley, NJ 07110). The identify organisms were obtained by comparing the results with the Micro ID manual and Oxi/Ferm manual, respectively.

RESULTS AND DISCUSSION

Figure 1 shows the log cell number of microorganisms from tofu cake and juice. At day 1, the juice had initial bacterial loads ranging from 10^{2} to 10^{6} CFU/ml, and the cake had initial bacterial loads ranging from 10^{3} to 10^{6} CFU/g tofu. Brand A had the smallest number of microorganisms in all samples tested at day 1. In contrast, brand C had the highest initial cell number in both juice and cake at day 1. The data for pH and microbial load at day 1 and day 30 are shown in Table 1. The pH of tofu was not related to the initial cell count. Lim (*8*) and Rehberger et al. (*11*) found that initial cell count of different brands of tofu ranged from 10^{4} to 10^{8} CFU/ml or greater in some cases. However, this study found lower initial cell count of both tofu juice and cake of the same brand.

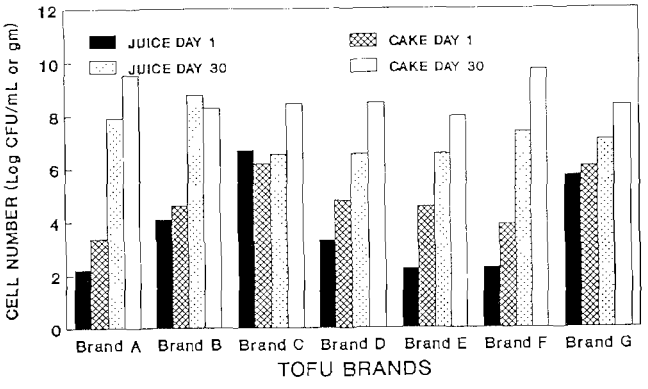

Figure 1. *Log cell number of tofu cake and juice at day 1 and day 30.*

All tofu brands were white in color and the juice was clear at day 1. Thirty days later, all tofu samples were spoiled. All samples had a bad odor and a creamy loose texture. The color changed according to the color of bacteria present. The juice was cloudy. The viable cell count increased to the range of 10^6 to 10^9 CFU/ml or g. The cakes of all brands had a higher cell count than the juices. However, brand E had the lowest cell count in both juice and cake at day 30 (Table 1, Fig.1).

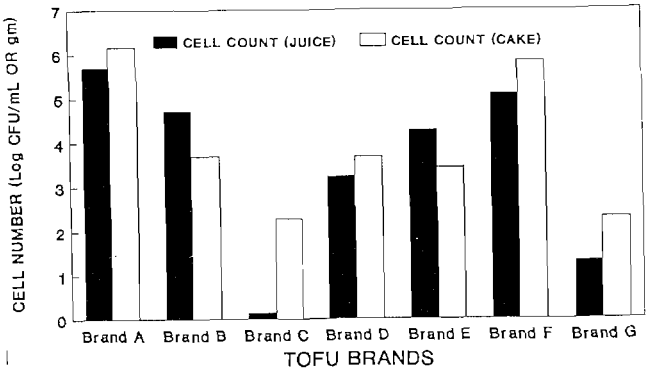

Figure 2. *Difference between cell counts of tofu cake and juice between day 1 and day 30.*

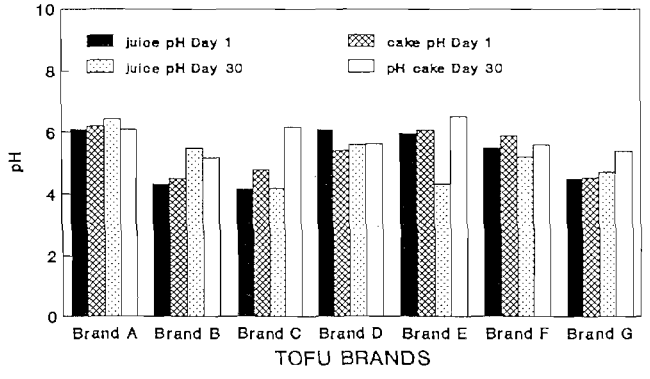

Figure 3. *The pH of tofu cake and juice at day 1 and day 30.*

The differences in cell count between day 1 and day 30 are shown in Fig. 2. Different brands exhibited different levels of cell count increase. Brand A had the greatest growth of cells after 30 d of incubation. In contrast, brand C had the smallest increase in cell count. However, the increase in cell count did not correspond to the initial cell number. This indicates that the pH value, type of microorganisms, and growth rate of the microorganisms should be considered in studying spoilage pattern of tofu.

The pH was an important indicator of the types of contaminating microorganisms in tofu. Fig. 3 shows the pH values for all brands at day 1 and day 30. At day 1, brands A, D, E, and F had pH values between 5 and 6, whereas brands B, C, and G had pH's between 4 and 5. At day 30, all brands of tofu were spoiled. The pH of the samples increased or decreased depending on the type of contaminating microorganisms.

Both gram-negative and gram-positive microorganisms were identified. Fig. 3 and 4 show the percentage of gram-positive bacteria isolated from the tofu cake and juice and

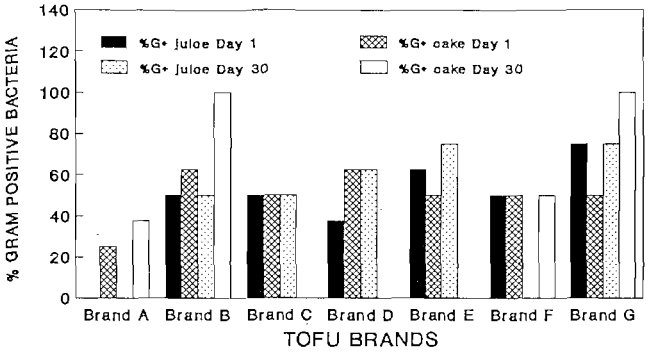

Figure 4. *Percentage of gram-positive bacteria in tofu cake and juice at day 1 and day 30.*

the relationship between the pH and percentage of gram-positive bacteria. Approximately 45% of the bacteria were gram-positive when the pH of tofu ranged between 4 and 6 at day 1. One month later, more than 63% of the bacteria were gram-positive in the pH range of 4 to 6. The observed numbers of gram-positive bacteria were only 0 to 50% at a pH higher than 5.5 at day 30. Thus, lower pH levels were conducive to the growth of gram-positive organisms in these products; 100% of the bacteria in brands B and G were gram-positive at day 30, and these brands had lower pH values (5.1-5.4) than the others. On the other hand, no gram-positive bacteria were found in brands C, D, and E at day 30, which had higher pH ranges of 5.7 to 6.4.

All gram-positive isolates were catalase negative; all gram-negative isolates were catalase positive (Tables 2 and 3). The morphological and biochemical characteristics of the gram-positives were studied and they exhibited rapid growth. Gram-positive colonies in the MRS agar were 1-2 mm larger than those in TSA. Glucose was fermented without gas being produced. They grew well at pH values of 4 to 6 and were of two different morphologies: cocci and bacilli. Two different cocci arrangements, short-chain and tetrad, were observed. The cocci were identified as *Streptococcus* sp. and *Pediococcus* sp., respectively. The bacilli found were *Lactobacillus* sp.

For the gram-negative bacteria isolated, both oxidase-negative and oxidase-positive reactions were observed. The oxidase-positive isolates were identified as *Pseudomonas putida* and *P. aeruginosa*. On the other hand, the oxidase-negative isolates were identified as *Enterobacter agglomerans* and *E. cloacae*.

Coliform bacteria were identified most often in fresh tofu. *Pseudomonas* sp. was identified most often in spoiled tofu and was the psychrotrophic bacteria responsible for spoilage at refrigeration temperature (*11*). Rehberger et al. (*11*) reported that the significant increase in psychrotrophic bacteria indicated poor quality tofu stored at low temperature, and the decrease of coliform bacteria was due to low storage temperature.

In conclusion, tofu is rich in nutrients that support the growth of bacteria. However, the chemical properties of tofu, for example pH, have a great influence on the number, type, and growth of contaminating bacteria. Tofu spoilage was indicated by a high number of bacteria and changes in pH, texture, and odor. The major bacteria found were *Streptococcus* sp., *Enterobacter* sp., and *Pseudomonas* sp.

TABLE 2. *Catalase and Gram stain reaction (+/-) of bacteria isolated from juice and cake of tofu at day 1.*

Brands		Test	Selected colony c1*	c2	c3	c4	c5	c6	c7	c8
A	juice	Catalase	+	+	+	+	+	+	+	+
		Gram	-	-	-	-	-	-	-	-
	cake	Catalase	+	-	+	+	-	+	+	+
		Gram	-	+	-	-	+	-	-	-
B	juice	Catalase	+	+	-	+	-	-	+	-
		Gram	-	-	+	-	+	+	-	+
	cake	Catalase	+	-	-	-	+	+	-	-
		Gram	-	+	+	+	-	-	+	+
C	juice	Catalase	+	-	+	+	-	+	-	-
		Gram	-	+	-	-	+	-	+	+
	cake	Catalase	+	-	-	+	+	+	-	-
		Gram	-	+	+	-	-	-	+	+
D	juice	Catalase	+	-	+	+	-	-	+	+
		Gram	-	+	-	-	+	+	-	-
	cake	Catalase	-	+	-	-	-	-	+	+
		Gram	+	-	+	+	+	+	-	-
E	juice	Catalase	-	-	+	-	+	-	-	+
		Gram	+	+	-	+	-	+	+	-
	cake	Catalase	-	-	-	+	+	-	+	+
		Gram	+	+	+	-	-	+	-	-
F	juice	Catalase	+	+	-	-	-	+	+	-
		Gram	-	-	+	+	+	-	-	+
	cake	Catalase	-	-	-	+	-	+	+	+
		Gram	+	+	+	-	+	-	-	-
G	juice	Catalase	-	-	-	-	-	-	+	+
		Gram	+	+	+	+	+	+	-	-
	cake	Catalase	-	+	-	+	+	+	-	-
		Gram	+	-	+	-	-	-	+	+

*c1 to c8 are inidividual isolates from each brand of tofu juice and cake.

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TABLE 3. *Catalase and Gram stain reaction (+/-) of bacteria isolated from juice and cake of tofu at day 30.*

Brands		Test	Selected colony							
			c1*	c2	c3	c4	c5	c6	c7	c8
A	juice	Catalase	+	+	+	+	+	+	+	+
		Gram	-	-	-	-	-	-	-	-
	cake	Catalase	+	-	+	+	-	+	-	+
		Gram	-	+	-	-	+	-	+	-
B	juice	Catalase	+	+	+	-	+	-	-	-
		Gram	-	-	-	+	-	+	+	+
	cake	Catalase	-	-	-	-	-	-	-	-
		Gram	+	+	+	+	+	+	+	+
C	juice	Catalase	-	-	-	+	-	+	+	+
		Gram	+	+	+	-	+	-	-	-
	cake	Catalase	+	+	+	+	+	+	+	+
		Gram	-	-	-	-	-	-	-	-
D	juice	Catalase	-	-	+	+	-	-	+	-
		Gram	+	+	-	-	+	+	-	+
	cake	Catalase	+	+	+	+	+	+	+	+
		Gram	-	-	-	-	-	-	-	-
E	juice	Catalase	-	-	-	-	-	-	+	+
		Gram	+	+	+	+	+	+	-	-
	cake	Catalase	+	+	+	+	+	+	+	+
		Gram	-	-	-	-	-	-	-	-
F	juice	Catalase	+	+	+	+	+	+	+	+
		Gram	-	-	-	-	-	-	-	-
	cake	Catalase	-	-	+	+	-	+	-	+
		Gram	+	+	-	-	+	-	+	-
G	juice	Catalase	-	-	-	+	+	-	-	-
		Gram	+	+	+	-	-	+	+	+
	cake	Catalase	-	-	-	-	-	-	-	-
		Gram	+	+	+	+	+	+	+	+

*c1 to c8 are inidividual isolates from each brand of tofu juice and cake.