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Microbial Spoilage of Tofu (Soybean Curd)

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ABSTRACT

Plate counts and pH measurements were performed on tofu from different aged lots. The pH declined from to 5.8 to 5.2 with age. Aerobic plate counts of 1- and 30-d-old samples were approximately 10^6 CFU/g. Older samples had counts of about 10^8 CFU/g. Anaerobic counts rose from 10^6 CFU/g in the 1-d-old lot to a high of 10^9 CFU/g in the 30-d-old lot. The major species from the different age lots were lactic acid bacteria, enteric bacteria, and *Pseudomonas* species. Representatives from each of these groups were inoculated into autoclaved tofu and incubated at 5°C for 23 d. CFU/ml and pH of both water and cake were measured. Turbidity, mg protein per ml, and mg NH_4^+ per ml of the water were measured. All species tested increased in numbers in the tofu and caused changes in at least some of the characteristics measured. Samples were taken during the manufacture of tofu, and all organisms found in each sample were characterized. All organisms that were shown capable of causing spoilage in tofu were present in large numbers early in manufacture but were no longer found in samples subsequent to pressure cooking. All of the spoilage organisms reappeared later during manufacture. These data indicate that tofu is spoiled by enteric bacteria and *Pseudomonas* species, as well as by lactic acid bacteria. The spoilage organisms are probably introduced by equipment and personnel with which the product comes in contact after it is pressure cooked. In addition, aerobic plate counts can underestimate the bacterial load in tofu, and therefore, anaerobic plate counts should also be performed.

Tofu (soybean curd) is a versatile protein-rich food prepared from soybeans. In the West, tofu is commonly found in 12 oz. (ca. 340 g) to 21 oz. (ca. 595 g) cakes packaged in plastic cartons covered with plastic film (*14,19*). The tofu used for this project is sold in 1-lb (ca. 456 g) cakes sealed in airtight plastic bags packaged in boxes.

Soybeans have traditionally been used as a source of protein or meat substitute in Eastern diets (*14*). It has been suggested that tofu might be an aid in solving world food problems because soybeans have both high quality protein and a high yield of protein per acre (*10,13,14*). The high nutritional value and low cost of soybean products make them a suitable solution for malnutrition problems for poor people living on grain centered diets (*14*). The versatility of tofu and other soybean products makes them easily adaptable to traditional diets even in countries where they are not usually served (*13,14*).

Tofu is relatively easy to make (*14,19*). Soybeans are first soaked in water, coarsely ground, and then heated. The cooked, ground beans, called *go* in Japanese, are pressed to extrude the soymilk, leaving bean solids, called *okara*, as a byproduct. A solidifier is added to the soymilk, such as concentrated sea salts, called *nigari*, calcium sulfate (either in refined form, or as naturally occurring gypsum), or magnesium sulfate. The mixture is gently stirred so that the resulting curds have the desired size and consistency. The exact method of stirring varies from manufacturer to manufacturer and is considered to be part of the "art" of tofu making. The resulting curds and whey are formed into pressed cakes. Tofu is usually cut into smaller blocks in the cooling water.

Traditionally in the East, tofu is eaten on the same day it is made, or shortly afterward, so spoilage and microbiological quality have not been important concerns. In the West, tofu is produced in factories, then shipped, often to another city, where it may remain for days or weeks in the retail outlet before purchase. The consumer may store the tofu in the refrigerator for days before consumption. Microbiological safety of tofu has become a concern due to these recent changes in tofu consumption patterns (*7*). Even so, no microbiological standards currently are set for tofu (*1,12*).

Hankin and Hanna (*5*) evaluated 17 soy products, including 11 tofu products, according to standards set for dairy products. Most of the products failed to meet these standards. Their findings have been criticized on a number of counts (*1*). The application of dairy standards to soy products may not be appropriate because vegetable foods can be safe with a bacterial load which might be considered too high in a food of animal origin, due to the difference in the types of bacteria one is likely to encounter in these two sources.

Dotson et al. (*4*) developed criteria for measuring spoilage of tofu and suggested that spoilage is caused by lactic acid bacteria. Tuitemwong and Fung (*17*) tested seven brands of tofu for microbial flora present, cell counts, and pH after 1 and 30 d of storage. They found that spoilage varied according to the bacteria initially present and the physical properties of the tofu itself. Other studies of the microbiological quality of tofu have been reported (*12,16,18*).

Two food poisoning outbreaks related to tofu have been reported. *Yersinia enterocolitica* was implicated in one outbreak (*3*) and *Shigella sonnei* in the other (*8*). The ability of foodborne pathogens to grow and produce toxins in tofu has also been evaluated (*7*).

The object of the present project was to determine some of the organisms that cause spoilage of tofu. It was also to ascertain at what point these organisms are introduced during the manufacturing process. In addition, the microbial quality of the commercial tofu product used was evaluated.

MATERIALS AND METHODS

Tofu

Tofu samples used in all experiments were obtained from a nearby soyfood company. Their method of tofu production is as follows: 6.8 kg dry weight of yellow soybeans is placed in a large plastic barrel. To wash the beans, the barrel is filled with water, stirred, and drained. The beans are soaked in water from between 8 h in the summer to 12 h in the winter, which increases the volume of the beans 2 1/4 to 3 times. Soaked beans are put in to a hopper to drain the water and then are rinsed in running water. The beans are ground into meal in a grinder. The ground beans are cooked in a steam pressure cooker which starts at 54.4-60°C and finishes at 102-103°C and 0.68 atm after about 15 min. The slurry is drained out through a valve into two filter bags, one coarse, one fine. A hydraulic press extrudes milk into a large plastic barrel. This yields 121-127 L of milk and 13.6-18 kg *okara*. The milk temperature is about 93°C and is reduced to 76.7-82.2°C. In summer, a fan blowing across the surface of the milk is used to speed cooling. The tofu samples from different aged lots were prepared during the winter months. The sampling during tofu manufacture was performed during the summer. Approximately 250 g calcium sulfate is added. The mixture is stirred, then left to set about 10 min. It is periodically "lifted" with a paddle for 15-20 min to cause the curds to drop. The yield is about half curds, or tofu, and half whey, or leftover liquid from the soymilk. Whey is removed by siphoning and dipping. Curds are put into a pressing form lined with cheesecloth and then are covered with cheesecloth. A pressing board is placed on top and weighted with large jugs of water for 15-30 min. The warm tofu is sliced into blocks. The tofu blocks are placed in ice water and cooled to 15.6°C. They are then weighed, packaged, and stored at 2-3°C.

Tofu of different ages

Tofu samples were held at 2-3°C by the manufacture until they were brought to the laboratory. Samples were 1, 30, 37, 62, and 101 d old. Tofu cakes from each sample were homogenized in an equal volume of phosphate buffered saline (PBS) (*15*), diluted in PBS, and 0.1 ml of 10^{-3}, 10^{-5}, and 10^{-7} dilutions were plated on trypticase soy agar (TSA). Some of the liquid from each sample was used as an inoculum for streak plates. Plates were incubated both aerobically and anaerobically at room temperature for 4 d. BBL GasPak^R anaerobic systems were used for anaerobic incubations. After counting, well-isolated colonies of the most common colony types were picked from the 10^{-5} or 10^{-7} plates. These were incubated at 30°C, except for a few strains which failed to grow at 30°C and were incubated at 20°C. All strains were purified by streak plating. Strains incubated in anaerobic jars during isolation were incubated in anaerobic jars during purification. All strains found to be facultative anaerobes upon purification were subsequently maintained aerobically.

Total direct cell counts

A package of tofu was purchased from a retail outlet 18 d after manufacture. A sample from the tofu was homogenized, appropriate dilutions were made in PBS, and aerobic and anaerobic counts performed, as described above. A sample was prepared for a total direct cell count by adding 0.04 ml glutaraldehyde (50% weight to weight, Fisher), 0.001 ml 4'6-diamidino-2-phenylindole (DAPI) (1 mg/ml), and 0.001 ml Triton X-100 to 0.96 ml of tofu homogenate diluted 1:1 in PBS. A total direct cell count using a Petroff-Hauser cell counter was performed using epifluorescence microscopy with optics as described by Porter and Feig (*11*).

Taxonomy

For the experiment concerning tofu of differing ages all gram-negative, facultatively anaerobic bacteria were classified using the Enterotube II^R or Oxiferm^R tube identification systems. Other strains were characterized using established methods, as described in Holt (*6*). All tests were performed as described by Smiebert and Krieg (*15*), except the DNAase test, described by Difco (*2*).

Strains obtained during manufacture of tofu were classified as described above, except that rather than using prepared testing systems, the tests were prepared by us and then were keyed according to the material provided with the prepared tests. A test for the presence of *Escherichia coli* was performed on all samples according to established methods by Mehlman and Andrews (*9*).

Sampling during tofu manufacture

Samples were taken at different stages during the production of a single batch of tofu. All samples were placed in sterile Whirlpak^R bags (NASCO) which were stored until use at ≤7°C. A collection control was made by opening one of the bags in the factory, leaving it open long enough to have put a sample into it, and then closing it and storing it with the other samples. A bag of tapwater served as a temperature control. All samples were plated within 36 h.

The samples taken and the method used to prepare them for dilutions and plate counts were as follows: 5 g of raw beans was washed in 5 ml of PBS by manually rubbing the beans in the sampling bag, and this wash was used. Then, 10 ml of PBS were added to 15 ml of the two washed bean samples. These were agitated by hand and the wash was used. The *go* and *okara* samples were diluted 1:1 in PBS, and then the liquid portions were diluted and plated. The soymilk, whey, and cooling water were diluted and plated without prior treatment. The calcium sulfate powder was suspended in PBS in a 1:1 weight/volume ratio and the suspension was used undiluted. The sample taken just after the coagulant was added to the soymilk and stirred was homogenized. The trim from the freshly pressed cakes and the sample from the final packaged product were diluted 1:1 with PBS and homogenized. The sample control bag and a sample of the packaging were rinsed with 5 and 9 ml, respectively, of PBS, and this wash was plated undiluted.

All samples were plated on TSA and incubated both aerobically and anaerobically at 25°C. Plate counts were performed after 4 d. Plates were examined and samples of each and every colony type observed in each sample were counted, transferred, purified, and identified.

Introduction of spoilage organisms into sterile tofu

Samples of tofu obtained within 24 h of manufacture were cut into chunks of 40 ± 4 g .and then autoclaved in side-arm flasks containing 250 ml water. Bacterial strains isolated from the tofu of differing ages were chosen arbitrarily and then grown in Trypticase soy broth to density of 15 to 20 Klett units [red (#66) filter, Klett-Summerson photoelectric colorimeter], which is about 10^8 CFU/ml. In order to put about 10^7 CFU/g into each block of tofu, 0.1 ml of this cell suspension per g of tofu was injected into

the middle of each block of tofu using a glass pipet. The flasks were aseptically sealed with sterile rubber stoppers, covered with aluminum foil, and then incubated at 5°C until the end of the period indicated by the manufacturer's pull date (23 d). The organisms present at the end of the spoilage experiment were examined microscopically, macroscopically, and biochemically for purity and for identity with the starting organisms. Samples were diluted in PBS and plated as before. Due to the change in texture of the tofu caused by autoclaving, it was not possible to homogenize the tofu cakes completely. Tofu cakes were ground by placing them in a sterile Waring Blender cup with sufficient PBS to make a 10^{-1} dilution, and then blending them three times for a few seconds each time. This produced a suspension of finer, sand-sized chunks which were diluted and plated. Both the suspension of the cakes and liquid surrounding the tofu were examined microscopically. Plates were incubated at room temperature for 3 d prior to counting.

The amount of protein in the liquid surrounding the tofu was determined by biuret test. Readings were made on a Beckman spectrophotometer, Model 2400, at 530 nm. The pH readings of the tofu cakes were taken at a distance of about 2 cm from the site where the bacteria were originally injected into the cake.

To determine the amount of ammonia released into liquid surrounding tofu, measurements were performed in a Klett-Summerson photoelectric colorimeter using a green filter (No. 54). Samples were prepared by putting 2 ml of the liquid surrounding the tofu cake into a screw-capped tube, then adding 8 ml distilled water and 1 ml Nessler's reagent (*15*). The sample was mixed by inversion and then inserted into the colorimeter to be read. The blank was prepared with 10 ml distilled water and 1 ml reagent. A standard curve was prepared in the same manner by using solutions containing a known amount of ammonia, added in the form of ammonium chloride (NH_4Cl), in place of the liquid from the tofu.

RESULTS

In tofu from lots of different ages, the pH declined with increasing tofu age (Fig. 1). The 1- and 30-d aerobic counts were approximately 10^6 CFU/g. The older samples had counts of about 10^8 CFU/g. The anaerobic count was highest at 30 d at 1.25 x 10^9 CFU/g, then declined to 9 x 10^7 CFU/g at 62 d (Fig. 2). The major flora differed in tofu of different ages (Table 1). *Serratia liquefaciens* is the only major organism which was found in all age lots. *Lactobacillus* spp. were a major species in all age lots except the 30-d lot. Lactic *Streptococcus* spp. were major species in all lots except the 101-d lot. *Enterococcus* spp. were major species in all lots except the 1-d-old lot. Although no *Pseudomonas* spp. appeared as a major species in all lots,

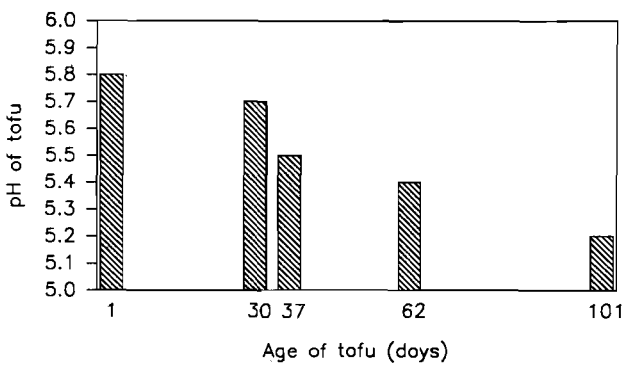

Figure 1. *Change in pH of tofu with age.*

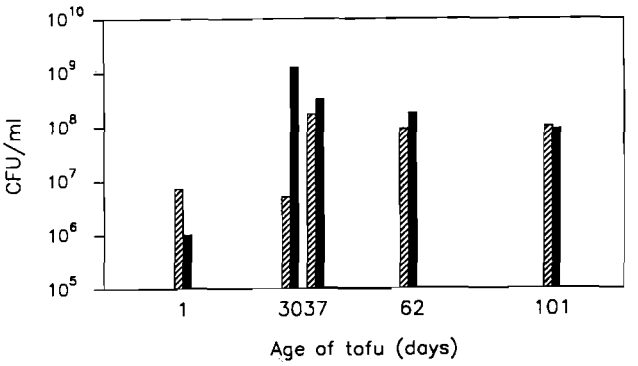

Figure 2. *Bacterial counts of tofu of differing ages. Hatched bars represent aerobic plate counts. Solid bars represent anaerobic plate counts.*

TABLE 1. *Major organisms isolated from tofu of differing ages.*

Organism	Tofu age (days)				
	1	30	37	62	101
Lactic acid bacteria					
Lactobacillus	x		x	x	x
Lactic *Streptococcus*	x	x	x	x	
Pseudomonas					
Pseudomonas cepacia	x	x		x	
Pseudomonas fluorescens	x				
Pseudomonas putida				x	x
Pseudomonas stutzeri				x	
Pseudomonas vesicularis			x		
Enteric bacteria					
Citrobacter freundii					x
Enterobacter agglomerans		x			x
Klebsiella pneumoniae			x		
Serratia liquefaciens	x	x	x	x	x
Streptococci					
Enterococcus		x	x	x	x
Others					
Staphylococcus epidermidis		x			
Rods, gram +, catalase +, Non-sporeforming		x	x		

the group as a whole was represented by at least one major species in each lot. The other types of bacteria were major species in only some of the lots. No attempt was made to determine the exact number of the major species found in each age lot, but each was present in amounts of at least 10^5 CFU/g.

A total direct cell count performed on a batch of tofu showed 1.6 x 10^8 CFU/g of tofu. Aerobic and anaerobic counts performed on the same batch of tofu were 1.26 x 10^8 CFU/g and 1.07 x 10^8 CFU/g, respectively. Of the total, 79% were detected by the aerobic plate count and 67% by the anaerobic plate count.

Total aerobic and anaerobic counts of organisms found in samples taken during the manufacture of tofu are shown in Fig. 3. Organisms originally present on the beans multiplied during the time the beans were soaked. Grinding and cooking the beans decreased counts of organisms by one log unit. The lowest numbers were found in the samples

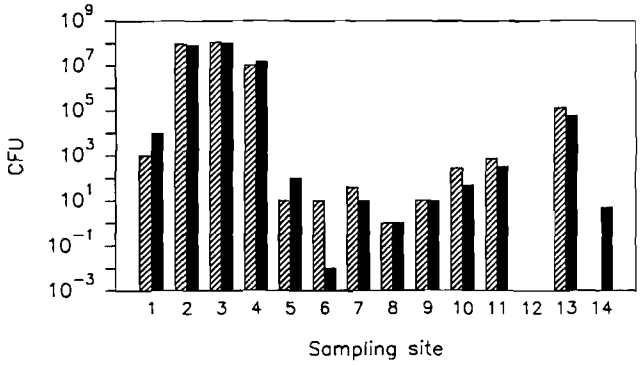

Figure 3. *Total aerobic and anaerobic counts of samples collected during tofu manufacture (per gram). Hatched bars represent aerobic plate counts. Solid bars represent anaerobic plate counts. Sampling sites are numbered as follows: 1 = raw beans, 2 = washed beans, 3 = beans washed twice, 4 = cooked, ground beans (go), 5 =* okara, *6 = freshly extruded soymilk, 7 = calcium sulfate, 8 = stirred, coagulated soymilk, 9 = whey, 10 = pressed tofu cake, 11 = cooling water, 12 = empty package, 13 = packaged tofu cake, 14 = sampling control.*

immediately subsequent to the cooking process. This is probably because organisms were not exposed to higher temperatures long enough for substantial killing to occur during cooking. Since the batch size was large enough to prevent rapid cooling, organisms were exposed to elevated temperatures long enough for most cells to be killed during cooling of the batch, so few would be left by the time coagulant was added. The tofu cake sampled immediately after pressing had higher counts of organisms than soymilk with coagulant added. Still higher counts were found in the final product. Approximate counts for each individual organism and the samples in which each organism was found are shown in Fig. 4.

All organisms used in the spoilage experiment altered tofu in at least some of the characteristics measured (Table 2). It is interesting to note that all organisms tested lowered pH of tofu, although lactic acid organisms lowered the pH more than did other organisms. Turbidity correlated well with pH (Table 3). All organisms tested released more protein per ml from the tofu than was released from uninoculated tofu. The enteric species, *S. liquefaciens*, caused release of more protein per ml from the tofu than did the others, and *Enterobacter agglomerans* also caused release of high amounts. However, the lactic and *Pseudomonas* groups had organisms that released both high and low amounts of protein from the tofu. The only organisms that produced significant amounts of ammonia were two of the lactic acid bacteria. The measurements of mg protein per ml liquid correlated poorly with other measures of spoilage we applied, with r values ranging from 0.30 to -0.28. Mg NH_4^+ per ml correlated slightly with pH of the liquid (r = -0.65) and quite loosely with pH of the cake (r = -0.48) and with turbidity (r = 0.39), but did not correlate with any other measurement.

The second *Lactobacillus* species and the *S. liquefaciens* did not alter the smell or texture of the tofu. The lactic *Streptococcus* and the first *Lactobacillus* sp. caused a beany odor in the tofu cake, while the two *Pseudomonas* spp. and *E. agglomerans* caused a putrid odor. All except the lactic *Streptococcus* and *Pseudomonas vesicularis* caused a slimy texture in the cake. Odor of the tofu correlated well with CFU/ml in the liquid surrounding the tofu (r = 0.88), but otherwise subjective criteria did not correlate with objective measurements.

DISCUSSION

Counts in older samples for organisms which were obtained anaerobically were higher than for those which were obtained aerobically. This is probably due to the fact that the packaging is impermeable to air, so that with increasing time, the environment becomes more suitable for organisms that can grow anaerobically.

In the present study, lactic acid bacteria in pure culture had a strong effect on pH of liquid surrounding tofu but a measurable effect was also exerted by the *S. liquefaciens* and the *E. agglomerans* and by the *Pseudomonas cepacia* and the *P. vesicularis*. Since lactic acid bacteria, enteric bacteria, and *Pseudomonas* spp. are among the major types of bacteria found in aged tofu samples, and since they do exert a measurable effect on pH, it is likely that they are important in spoilage of tofu.

Evidence from the pH measurements, measurements of protein and NH_4^+ in the liquid surrounding the tofu, and the CFU in the tofu and surrounding liquid supports the idea that spoilage of tofu is more complicated than has been assumed, and that organisms in addition to lactic acid bacteria play an important role. Non-sporeforming, gram-positive rods other than lactic acid bacteria were among the major types of organisms observed in the aged tofu samples. Their presence in large numbers in aged tofu suggests that it could be prudent to perform catalase tests on rod-shaped, gram-positive organisms observed in spoiled tofu before assuming that they are lactic acid organisms.

We used two spoilage criteria which were not used by previous researchers, i.e., measures of mg protein per ml and mg NH_4^+ per ml of the liquid surrounding the tofu. The release of soluble protein had no consistent relationship to any of the other measurements of spoilage, so it alone is not a good indicator of spoilage. Possibly bacteria which are efficient at releasing protein into the liquid have less effect on flavor deterioration of tofu than do organisms releasing less protein. That is, some products of bacterial degradation of tofu might be more palatable than others. This may not be true of organisms which were not tested in this study, however.

Some organisms released significant amounts of NH_4^+ into the liquid, whereas others either had no effect on the amount of NH_4^+ present, or appeared to decrease it. Ammonia tended to increase with decreasing pH. The decrease in pH is probably due to other compounds, such as lactic acid, which were not monitored, and not to the higher amounts of NH_4^+. Since mg NH_4^+ per ml did not correlate well with CFU/ml and turbidity of the liquid, considered by Dotson et al. (*4*) to be good indirect measurements of spoilage, mg NH_4^+ per ml cannot be considered to be a reliable indicator of tofu spoilage.

TSA is commonly used for growth of organisms found in tofu (*4,17*). However, it does not duplicate the conditions

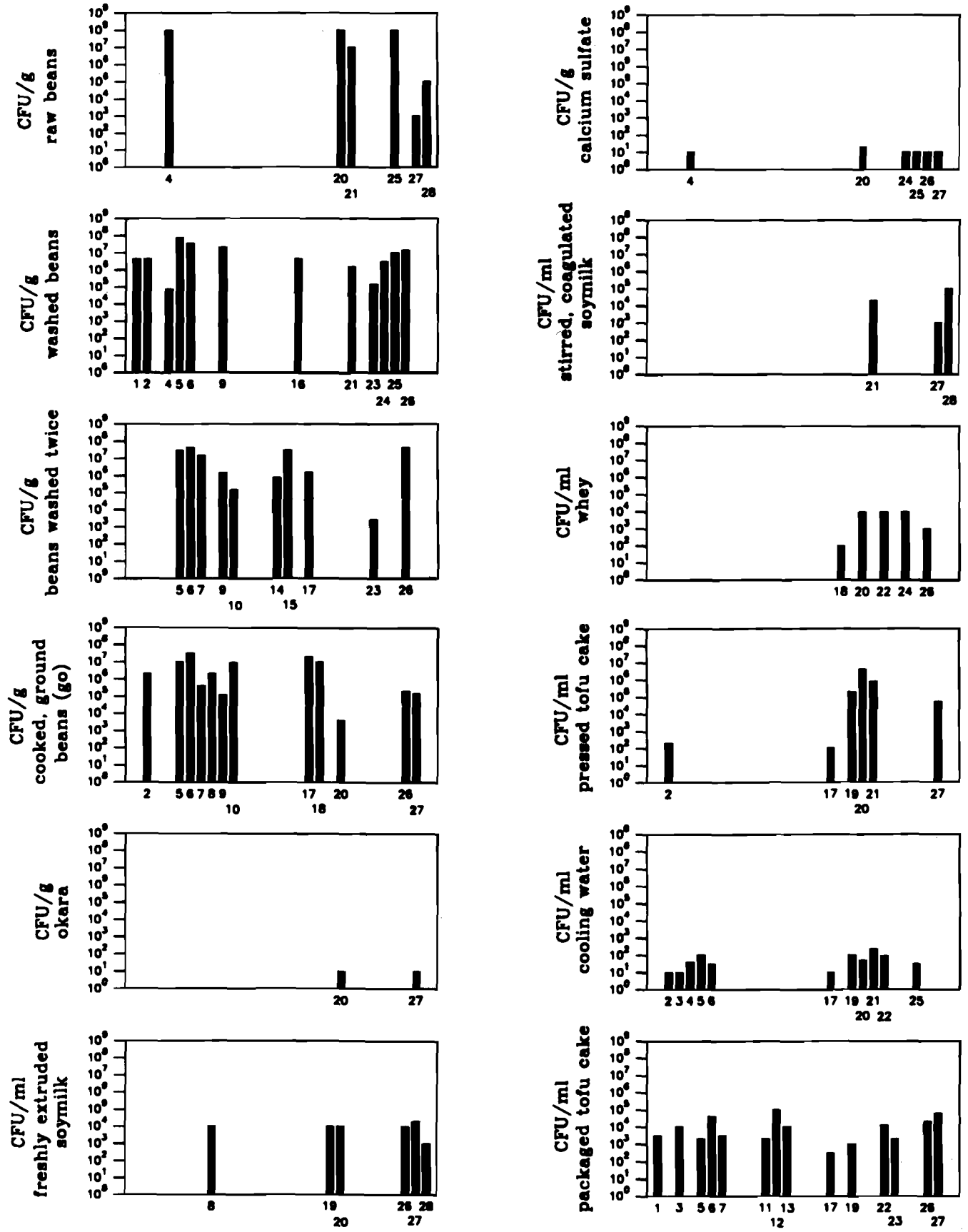

Figure 4. *Counts of organisms found during the manufacture of tofu. Bars are numbered as follows: 1* = Lactobacillus, *2* = *lactic* Streptococcus, *3* = Aeromonas hydrophila, *4* = Flavobacterium *sp.*, *5* = Pseudomonas cepacia, *6* = Pseudomonas putida, *7* = Pseudomonas vesicularis, *8* = Xanthamonas *sp.*, *9* = Citrobacter freundii, *10* = Citrobacter freundii *or* Enterobacter agglomerans, *11* = Enterobacter sakazakii, *12* = Escherichia coli, *13* = Klebsiella ozaenae, *14* = Serratia liquefaciens, *15* = Serratia rubidea, *16* = Shigella boydii, *17* = Enterococcus *sp.*, *18* = *oral* Streptococcus, *19* = Staphylococcus *sp.*, *20* = *aerobic coccus, 21* = *faculatative coccus, 22* = Actinomyces *sp.*, *23* = Bacillus *group 1, 24* = Bacillus *sp., 25 rods, gram-, oxidase-, 26* = *rods, gram-, oxidase+, 27* = *rods, gram+, catalase+, non-sporeforming, 28* = *fungi. The sampling control (not shown) had 50 CFU/ml each of* Bacillus *sp. and fungi.*

TABLE 2. *Measurements of tofu spoilage.*[a]

Organism tested	pH Liquid	pH Cake	O.D.	CFU/ml cake x 10^8	CFU/ml liquid x 10^8	Protein mg/ml	NH_4 mg/ml
Control	5.66	5.70	0	0	0	4.05	1.25
Lactic *Streptococcus*	4.80	5.11	47.5	1.6	26	5.6	2.97
Lactobacillus sp. #1	5.30	5.37	24	2	16	5.8	0.72
Lactobacillus sp. #2	5.31	5.49	17.8	0.5	5	6.36	3.27
Pseudomonas cepacia	5.54	5.67	20.5	7	7	8.9	0.65
Pseudomonas vesicularis	5.59	5.68	16.2	9	7	5.4	1.82
Serratia liquefaciens	5.49	5.53	15.2	3	10	11.28	1.39
Enterobacter agglomerans	5.62	5.52	22	7	44	8.28	1.07

[a] For all tests, n = 1.

TABLE 3. *Correlations of all data from spoilage of autoclaved tofu.*

	pH Water	pH Cake	Protein mg/ml	NH_4 mg/ml	CFU cake	CFU water	O.D.	Odor	Texture
pH Water	1.00								
pH Cake	0.94	1.00							
Protein mg/ml	0.19	0.10	1.00						
NH^4 mg/ml	-0.65	-0.48	-0.28	1.00					
CFU cake	-0.22	-0.49	0.20	-0.06	1.00				
CFU water	0.43	0.40	0.30	-0.38	0.27	1.00			
O.D.	-0.87	-0.88	0.03	0.39	0.56	0.02	1.00		
Odor	0.16	0.12	0.06	-0.37	0.46	0.88	0.30	1.00	
Texture	0.22	0.03	-0.02	-0.62	0.65	0.48	0.15	0.75	1.00

actually present in a cake of tofu. For instance, the lactic *Streptococcus* used in the spoilage experiment did not grow well on TSA or Trypticase soy broth. However, it grew well in the tofu itself; it was one of the major species found in the tofu samples from different age lots. It also grew well in autoclaved tofu. Some species, such as *Clostridium perfringens*, that can grow in tofu cannot grow on TSA (L. S. McClung, personal communication), although it is not known if this organism is actually found in tofu.

Some strict anaerobes which could conceivably grow in anaerobic microenvironments in tofu may not have been detected by the anaerobic techniques used here. Any anaerobes detected using this system would have to be aerotolerant in order to survive handling and the lag time before anaerobiosis occurring in the anaerobic jar. *C. perfringens* and *Clostridium sporogenes* strains were capable of growth under these conditions on appropriate media (data not shown), but more research is needed in order to determine whether other anaerobes which might be present in tofu would be detected under these conditions.

The comparison between direct cell count of a sample and counts obtained by plating the sample showed that the majority of organisms present in tofu are detectable by plating them on TSA, aerobically and in anaerobic jars. Since aerobic plate counts were one-fifth lower, and anaerobic plate counts one-third lower than direct cell counts, it is probable that some types of organisms were missed entirely, and that other methods would be needed to detect them. Some of the difference between the total direct cell count and plate counts was probably due to killing of bacteria during processing of the samples prior to plating, and to presence of nonviable cells in the sample.

All of the organisms tested in the present work produced changes in tofu which are consistent with normal spoilage. That is, they altered the smell or texture of tofu, or lowered its pH, or both. This evidence lends support to the idea that organisms found in the older age lots of tofu are indeed responsible for spoilage. Not all organisms responsible for spoilage were among the major species present in the 1-d-old tofu sample. One possible explanation for this is that, in the selective environment of packaged tofu, some organisms initially present in low numbers grow to become abundant. An alternative explanation is that the initial flora differed among different lots and, therefore, these organisms were simply not present in the 1-d-old lot. Since none of the species present only in small numbers were characterized, it is not possible to decide between these two alternatives. Another possibility is that part of the initial flora was missed due to sampling error.

A visit was made to a tofu manufacturer to attempt to determine where and how spoilage organisms enter tofu. A reasonable assumption is that they come from the soybeans. The beans did indeed have high bacterial counts, but none of the spoilage organisms were found on the raw beans. Beans that had been soaked overnight, then had the excess water drained off and were washed once again in tap water, yielded a wide variety of organisms, including all organisms which tested positive for their ability to spoil tofu. Many of these organisms were also seen in the *go*. In subsequent steps, total bacterial count decreased drastically,

and none of these organisms were detected again until they appeared in the pressed tofu cake.

Lactic acid bacteria first reappeared in the pressed tofu cake, *Pseudomonas* sp. in the cooling water, and enteric bacteria in the final packaged product. This suggests that these bacteria are reintroduced into the tofu from the equipment, cooling water, and personnel handling the tofu.

In addition, two bacterial species, *E. coli* and *Bacillus* group I (possibly *Bacillus cereus*), that could potentially pose a health hazard to consumers, were found in the finished product. *E. coli* is usually of human or animal origin, so it was probably introduced by persons who weighed and packaged the tofu. *Bacillus* group I (possibly *B. cereus*) may have come from the same source, or from surfaces the tofu contacted during packaging, or from the soybeans.

A standard test for *E. coli* in foods was performed on all samples obtained from the tofu factory, but results were negative. This could have been due to sampling error; that is, the sample used for the test had no *E. coli*, whereas the sample that was plated and characterized did. Alternatively, the strain of *E. coli* present might have been too few in numbers or might have an atypical reaction under the test conditions.

Recommendations for improving the shelf life of tofu

It is unlikely that the bacterial load present on the beans contributes significantly to spoilage organisms in the finished product. They are probably reintroduced late in processing. Therefore, efforts to reduce bacterial load should be concentrated on later stages of manufacture. Care should be taken to insure cleanliness of equipment between batches to prevent contamination from an earlier batch. The cooling water is a likely source of contamination, especially of *Pseudomonas* spp., and should be changed between batches.

Good sanitary practices are particularly important to those persons who handle the tofu press and pressed tofu, and who weigh and package tofu. Cleaning of all surfaces with which tofu comes in contact and handwashing before handling each individual batch would also decrease contamination from previous batches of tofu. This is important because bacteria could accumulate from batch to batch and multiply rapidly at room temperature in small pieces of tofu left on surfaces. Room temperature is often elevated in tofu manufacturing plants due to heat-producing machinery.

Recommendations for further study of microbial quality and spoilage of tofu

Many studies, including this one, have used TSA as the medium for microbiologically evaluating tofu. Although this medium is capable of detecting a wide variety of significant organisms, some important organisms, such as *C. perfringens*, do not grow on it. A better strategy would be to employ a variety of media, including one specific for this species.

Aerobic incubations are generally used for evaluation of tofu. These underestimate the number of organisms present in samples, especially those packed in airtight containers. For instance, our 30-d sample had an aerobic count of 5 x 10^6 CFU/ml and an anaerobic count of 1.25 x 10^9 CFU/ml. It is also important to perform total direct cell counts when attempting to estimate the total numbers of bacteria in a sample to insure that a large proportion of the organisms present is not being missed by the plating techniques employed.

General tests for "coliforms" should not be considered relevant for evaluation of tofu. Specific tests for known pathogens in this group should be performed instead. Many organisms that are harmless from a public health standpoint will appear in a coliform enrichment. If the focus of the research is spoilage rather than safety, general tests for *Enterobacteriaceae* might be appropriate, since we have shown that some members of this group can spoil tofu. However, it is not known if this is a general characteristic of the group or is peculiar to some species only. Further research is needed before a general test could be deemed appropriate.

One major problem with evaluations of microbial quality of tofu is lack of appropriate microbiological standards. If standards are set, they should take into account the microbial flora present in actual samples of tofu and the likelihood that these organisms would potentially threaten the health of consumers. Because the types and numbers of organisms that constitute a hazard in tofu and in other foods are different, it is not sufficient to "borrow" standards from another food and apply them to tofu. In studies requiring sterile tofu, it would usually be preferable to prepare tofu in a sterile manner, according to Kovats et al. (*7*) because autoclaving introduces additional variables by changing the characteristics of the tofu.

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