EFFECTS OF USING CHEMICAL PRESERVATIVES TO EXTEND THE SHELF LIFE OF SOYBEAN CURD

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INTRODUCTION

Soybeans are one of the most important cash crop in the United States, and they contribute more protein and fat to our food economy than any other single source (Liener, 1978). Soybean protein is unique among plant proteins because of its relative high biological value (Schroder et al., 1973). The amino acid distribution of soybean protein is close to that recommended by the Food and Agricultural Organization of the United Nations. In general, proteins of animal origin rank highest and plant protein rank lowest when their total essential amino acid content is expressed as a fraction of total nitrogen. However, soybean protein assumes an intermediate position (Liener, 1978).

One of the most popular Oriental soybean foods is soybean curd or tofu (Wang, 1984). According to industry statistics gathered by Shurtleff and Aoyagi (1983) of the Soyfoods Center in California, tofu has been made commercially by Asian immigrants in the United States since 1904. A Chinese proverb, "Tofu is meat without bones," is growing rapidly in popularity in the West.

Currently there are two popular methods for preparing tofu: the traditional method (Shurtleff and Aoyagi, 1983) and hot-grind method (Wilkens et al., 1967). The traditional

method of making tofu yields a product with a typical beany flavor. However, the hot-grind method inactivates the enzyme, lipoxygenase, and produces tofu with a bland taste that Westerners prefer (Johnson and Wilson, 1984).

The shelf life of tofu is normally one to three days without refrigeration or any other method of preservation. Some studies have been conducted to investigate the microbiological quality of tofu (Anon., 1983b; Dotson et al., 1977; Fujii et al., 1978.; Rehberger et al., 1984). Researchers also have attempted to increase the shelf life of tofu (Fujii et al., 1978; Pontecorvo and Bourne, 1978; Wu and Salunkhe, 1977). However, little information was found in the literature on the use of chemical preservatives to extend the shelf life of soybean curd.

The purpose of this project was to study the effects of chemical preservatives on the shelf life of tofu with microbiological analyses, physical measurements, and sensory evaluation.

REVIEW OF LITERATURE

Soybean curd, or tofu, is an important nonfermented soybean product that is used widely in a variety of dishes by Oriental people. It has been reported that tofu was invented by Liu-An, a Chinese King of the Hsi-Han Dynasty, who ruled

over the eastern region of China about 2000 years ago (approximately 160 B.C.). Tofu was first introduced into Japan in 1183 and its usage spread to other countries. Therefore, tofu has been a popular food in Eastern Asia for a long time (Hsu, 1978).

According to data from the Soyfoods Center of California (Shurtleff, 1982), the number of non-Oriental tofu producers in North America rose from none in 1975 to 167 in 1981. More than 11,000 tons of soybeans are used annually in making tofu in the United States (Shurtleff, 1982). The United States is the world's leading soybean producer, harvesting some 135 billion pounds annually (Leviton, 1982). Although the bulk of the soybean crop still is used for animal feeds and oil, the use of whole soybeans for human consumption is increasing steadily (Leviton , 1982). The Tofu Market Survey showed that 33 % of Americans had heard of tofu, 18 % had tasted it, and 10 % had purchased it. Each American, on a per capita basis consumes 2.13 pounds of lightly processed soyfoods per year, and spends approximately $1.77 for it. Per capita tofu consumption approximates the annual consumption of yogurt at 2.7 pounds (Leviton, 1982). Soyfood industry observers have spoken of tofu becoming the "yogurt of the 1980s" (Leviton, 1982).

Types of soybean curds

Three types of soybean curd are produced in the Orient: soft tofu, hard tofu, and dried tofu. In addition, many different products related to tofu, such as tou-chi (bean-chicken), savory toukan, chien-chang, yuba, fried tofu and fermented tofu also are made (Tsai et al., 1981).

Soft tofu, with water content as high as 87-90 % and a smooth, fragile curd, is especially popular in Japan (Anon., 1954; Tsai et al., 1981). It generally is topped with soy sauce and eaten with a spoon. Hard tofu has a firmer texture than soft tofu, contains less water because more is expressed in preparation, and can be cut into various sizes for direct use in tofu dishes as well as for frying. Dried tofu (toukan) is the firmest variety of Chinese tofu and has a moisture content of approximately 8 %. Dried tofu is usually boiled in a mixture of soy sauce and seasonings to make savory toukan. This tofu ranges in color from light to dark brown and has a chewy, meaty texture like that of the textured soy protein. Savory toukan can be consumed without further preparation or cooked with meat and vegetables in the home (Tsai et al., 1981).

Tofu in United States markets contains 75-80 % water. According to United States tofu producers, western consumers prefer tofu with a firm, chewy texture (Wang et al., 1983).

Biological value

The approximate composition of soybean curd is 6 % protein, 3.5 % fat, 1.9 % carbohydrates, 0.6 % ash, and 88 % water (Shurtleff and Aoyagi, 1983). Muto et al. (1963) tested tofu as a source of protein in the solid diet of weanling infants; its performance was evaluated with respect to acceptability, weight gain, nitrogen balance and serum levels. Tofu was nutritionally equivalent to the protein derived from a mixture of eggs, fish, and liver. On the basis of essential amino acid composition, the sulfur-containing amino acids limit the nutritional value of soybean protein (Liener, 1972). However, soybean protein is higher in lysine than other plant proteins (Zimmerman et. al., 1967). Composition of essential amino acids with Minimum Daily Requirements (MDR) in a 100 g portion of Japanese tofu containing 7.8 % protein is shown in Table 1.

Tofu is unique among high protein foods in being low in calories and saturated fats and entirely free of cholesterol. A typical 8-ounce serving contains only 147 calories. Tofu contains only 4.3 % fat; 80 % of the fatty acids are in the polyunsaturated form and 15 % are saturated fatty acids. Tofu is high in linoleic acid, one of the most important unsaturated fatty acids. By comparison, beef fat is high in

Table 1. Amounts of essential amino acids and their percentages of Minimum Daily Requirements in 100-gram portion of tofu

Amino Acids[a]	MDR (g)	Tofu (g)	MDR (%)
(Methionine-cysteine)	1.10	0.20	17
Tryptophan	0.25	0.12	47
Methionine	0.20	0.10	52
Leucine	1.10	0.59	52
Valine	0.80	0.43	53
Isoleucine	0.70	0.41	59
(Phenylalanine-tyrosine)	1.10	0.75	67
Lysine	0.80	0.57	71
Threonine	0.50	0.37	72
Phenylalanine	0.30	0.48	160
Protein, usable	43.10	5.06	12

[a] Amino acids in shortest supply are listed first. Those in parentheses are important combinations of essential and non-essential amino acids with common properties. (Shurtleff and Aoyagi, 1983).

saturated fats (48 %), low in unsaturated fats (47 %) and contains only 9 % linoleic acid (Shurtleff and Aoyagi, 1983). Because of its low carbohydrate content (approximately 5 grams in 8 ounces of tofu), it can be used for starch-restricted dieters (Shurtleff and Aoyagi, 1983). It also is used by individuals who are intolerant of lactose in cow's milk (PAG, 1972).

Composition of soybean seeds

Proximate composition. Soybeans contain about 600 mg phosphorus, 70-80 % of which exists as phytic acid and is extractable in soybean milk and co-precipitates with the proteins in tofu during coagulation (Saio, 1979). Soybeans contain proteins, lipids, carbohydrates, and minerals. On a moisture-free basis, the average composition of whole soybean seeds is 40.4 % protein, 22.3 % fat, 31.9 % nitrogen-free extract plus fiber, and 4.9 % ash (Horan, 1974). The proximate composition of soybeans and seed parts is shown in Table 2.

Soybean proteins. Soybean proteins are heterogeneous, but most of them are globulins. According to sediment coefficients, water extractable soybean proteins of defatted

Table 2. Proximate composition of soybeans and seed parts

Fraction	Protein (N x 6.25) (%)	Fat (%)	Carbohydrate (%)	Ash (%)
Whole bean (100%)	40	21	34	5
Seed coat (8%)	9	1	86	4
Cotyledon (90%)	43	23	29	5
Hypocotyl (2%)	41	11	44	4

(Wolf, 1977)

meal may be classified into four fractions: 2, 7, 11, and 15 S (Wolf, 1972). Relative amounts and molecular weights for the components that make up these four fractions are given in Table 3. The 2 S fraction contains trypsin inhibitors, cytochrome c, allantoinase, and two globulins with no known biological activity. The 7 S fraction represents slightly over one-third of the total soluble proteins and contains at least four different proteins. The 7 S globulin comprises about one-half of the 7 S fraction. It is a glycoprotein consisting of 12 glucosamines and 31 mannose residues per mole. The 11 S globulin, the major protein of soybeans, accounts for the bulk of the 11 S fraction which makes up about one-third of the total soluble soybean proteins. The 15 S fraction has not been isolated and characterized, and may be a polymer of other proteins (Orthoefer, 1978; Wolf, 1972).

The principal protein components of soybean storage proteins are 7 S and 11 S. The concentration of these two components depends on soybean varieties, and the 7 S/11 S ratio in soybean milk seriously affects the textural properties of tofu (Saio and Watanabe, 1973).

Soybean curd processing

For centuries the process of making tofu has been

Table 3. Ultracentrifuge fractions of soybean proteins

Protein fraction	Percentage of total	Components	Molecular weight
2 S	22	Trypsin inhibitors	8,000 21,500
		Cytochrome C	12,000
		2.3 S globulin	18,200
		2.8 S globulin	32,000
		Allantoinase	50,000
7 S	37	Beta-amylase	61,700
		Hemagglutinins	110,000
		Lipoxygenases	108,000
		7 S globulin	186,000-210,000
11 S	31	11 S globulin	350,000
15 S	11	-	600,000

(Wolf, 1972)

controlled by tradition and long experience; without the benefit of scientific knowledge, tofu "craftsmen" have skillfully carried on the process (Wang et al., 1983). In recent years, studies have been made on gel formation of proteins isolated from defatted soybean meal as well as from water extracts of whole soybeans. Processing conditions (such as type and concentration of coagulants, temperature, mode of mixing and pressure applied) that affect the quality and quantity of gel formation in tofu have been investigated (Lu et al., 1980; Saio, 1979; Tsai et al., 1981; Watanabe et al., 1960; Wang and Hesseltine, 1982).

In addition to processing conditions, soybean variety has been reported to affect the yield and quality of tofu. Watanabe et al. (1960) found that Japanese varieties were more desirable for making tofu than the United States varieties. But Smith et al. (1960) reported that the most important differences between Japanese and United States soybeans, as viewed from Japanese custom, were in texture and color of tofu produced from them. Although yield and composition of tofu varied with soybean variety, the average yield from U.S. soybeans was the same as that from Japanese beans. More recently, Skurray et al. (1980) used 15 soybean varieties grown under the same agricultural conditions for

making tofu and found that the amount of calcium salt used had a greater effect on the quality of tofu than did the variety of soybeans. Nevertheless, a problem persists in selecting the most suitable variety of soybeans for making tofu.

Preparation of soybean milk. Traditionally soybean milk is processed from whole beans. Beans are soaked in cold water until hydrated, then finely ground with added water in a stone mill to form a slurry. Water is added to the slurry to the desired concentration (usually bean to water in the ratio of 1 to 8 by weight); the slurry is filtered to remove the insoluble residue, and the milk is boiled for 20-30 minutes to improve flavor. Heat treatment also is necessary to destroy microorganisms and anti-nutritional factors which occur in soybeans causing a decrease in nutritional value (Del Valle, 1981; Klose et al., 1948).

Characteristic flavor of soybean milk is considered an undesirable flavor to non-Oriental populations. Numerous modifications of the traditional process have been reported to improve the flavor. These modifications include hot water extraction (Wilkens et al., 1967), acid grinding (Kon et al., 1970), and alkaline soaking (Badenhop and Hackler, 1970;

Khaleque et al., 1970). Nelson et al. (1976) blanched beans before grinding and homogenized whole soybean slurries without filtration to reduce the beany flavor and increase total solids.

As soaking time for soybeans is increased, larger quantities of water-soluble solids are leached into the soaking water. For a minimum loss the soaking time should be only long enough to permit the soybeans to almost double their initial dry weight. This facilitates grinding of the beans (Lo et al., 1968a). Soaking at higher temperatures drastically reduces the yield of soybean milk (Wilkens and Hackler, 1969). When the temperature of extraction is above 85 °C there are substantial reductions in the solids extracted in soybean milk and difficulties are encountered in filtering the soybean milk (Lo et al., 1968b). Johnson and Snyder (1978) found that heating reduced the solids yield of soybean milk and heating before grinding of the beans decreased the yield more than heating while grinding the beans. Hackler et al. (1965) reported that the protein efficiency ratio of heat-processed soybean milk is dependent upon both time and temperature treatment. Maximum nutritional value of the protein of soybean milk is

attained within 5-10 min when the soybean milk is treated at 121 °C or in 60 min at 93 °C. These conditions are required to inactivate about 90 % of the trypsin inhibitor activity.

Coagulant type and concentration. It has been recognized that one of the most important steps in the tofu-making process is the addition of a coagulant to the soymilk to precipitate the soy protein and thus form curds. It has been shown that the amount of the coagulant required is related to the per cent solids of the soymilk (Watanabe et al., 1964). Lu et al. (1980) showed that the amount of chemicals added to precipitate soy protein varied depending upon the type of compound used, but in all cases, soy protein was precipitated when the pH of soymilk was about 6.0. Calcium acetate and calcium chloride were good precipitants for soybean curd preparation when compared to other calcium salts. The quality and sensory scores of the soybean curd prepared from these salts required less skill than when calcium sulfate was used. The amount of calcium acetate or calcium chloride needed to precipitate soy protein was less than one-half that of calcium sulfate (Lu et al., 1980).

Wang (1984) reported that the coagulation step was significant in terms of yield and texture of tofu. She reported that both ionic concentration and type of coagulant

affected the gross weight and moisture content of the final product, as well as total solids and nitrogen recoveries. When calcium sulfate was used, gross weight and moisture content of the final product, and total solids recovery decreased as the salt concentration increased from 0.01 to 0.02 M, remained about the same between 0.02 and 0.04 M, and then steadily increased at higher concentrations. When the concentration was higher than 0.1 M and lower than 0.008 M, no curds appeared. However, the percentage of nitrogen recovery increased as the concentration of salt increased, remained the same at 0.02-0.04 M, and then decreased at higher concentrations. Appurao and Rao (1975) reported that at higher concentrations of calcium ions, precipitation decreased and protein became soluble again. Saio (1979) reported that for tofu preparation, calcium chloride reacted with soybean protein faster than calcium sulfate and the concentration range of calcium chloride that gave optimum texture for tofu was narrower than that of calcium sulfate. He noted that although the optimum concentration of calcium salt was about 0.02 N, the hardness of tofu increased with concentrations up to 0.04-0.05 N and decreased at higher concentrations. He reported that when glucono-delta-lactone (GDL) was used, it caused soybean milk to coagulate only after reheating. This characteristic would be adaptable to

modern mechanical procedures for the manufacture of tofu. Wang (1984) noted that (calcium chloride, magnesium sulfate, or magnesium chloride) as the concentration of coagulant increased from 0.01 to 0.02 M, there was an increase in hardness, brittleness, cohesiveness, and elasticity in tofu. There were no significant effects observed at concentrations between 0.02 to 0.04 M, but above that range, curd yield decreased steadily. Wang said that these data indicated that the use of a salt at a level between 0.02 to 0.04 M was more likely to yield reproducible firm products. Tsai et al. (1981) studied yield and characteristics of tofu using various coagulants at concentrations ranging from 0.01 to 0.08 M. They found that based on coagulability and texture, coagulant concentrations between 0.025 to 0.03 M were suitable for making Chinese-style tofu. Wang (1984) reported that calcium chloride resulted in curds with much greater hardness and brittleness than did calcium sulfate and magnesium sulfate, indicating that anions have greater effect than cations on these two parameters. Aoki (1965) studied the effect of salts on the gelation of soybean protein and found that anions have a stronger effect on water-holding capacity than cations. Wang and Hesseltine (1982) reported that the temperature of soybean milk and the mode of mixing greatly affected the yield and texture of the resulting tofu. When

temperature was increased, the gross weight and moisture content of the curd decreased, but its hardness increased. Increased mixing also decreased tofu volume and increased hardness (Saio, 1979; Wang and Hesseltine, 1982).

Wang (1984) reported that the chemical composition of soybeans also affected tofu texture. Saio et al. (1969) found that gels made from 11 S proteins isolated from defatted meal were much harder than those made from 7 S proteins. As increasing amounts of phytic acid were added to soybean milk, the hardness of tofu also increased. Soybean variety could also have an effect on tofu texture (Saio et al. 1969). Skurray et al. (1980) compared 15 varieties and found no significant correlation between the ratio of 7 S to 11 S proteins or phosphorus content and quality of tofu. However, they, too found that the quality of tofu was affected by the amount of calcium ion added.

Innovations in tofu processing. With recent commercial popularity of soybean curd, industrial interest in tofu production has led to innovative research concerning the processing and sensory qualities of tofu (Johnson and Wilson, 1984; Tsai et al., 1981; Wang et al., 1983).

Bagged tofu is made by placing soybean milk and calcium salt into a plastic bag. The closed bag is dropped into a

tank of hot water for one hour to coagulate and sterilize the packaged tofu (Smith and Circle, 1972). A Japanese patent introduced packaged tofu which could be stored for at least a month without deterioration by using soymilk processed in a series of heating and cooling steps (MMIC,1981). Asahimatsu Koridofu Company (1981) improved shelf life of soybean curd by coagulating soymilk with acid or salt which is dispersed in water with egg whites and then heated under pressure. A strong, stable soybean curd with improved shelf life stability can be prepared without substantial modifications of the flavor and textural properties of the soybean curd by blending or mixing dehydrated food substances with the soybean curd so that the water activity is lowered to below 0.90-0.95, preferably from about 0.65 to about 0.80. By this means, refrigerated shelf life has been extended to several weeks (Anon., 1981). A British patent (MMIC, 1979) introduced tofu with an extended shelf life developed by heat sterilizing soybean milk followed by coagulating, dehydrating, and then hermetically sealing it in a container. Yagi (1972) patented a packaging technique of soaking dried soybean curd in a bag with a soy sauce mixture containing preservatives, spices, and binders. Bags were sealed after removing air and steam heated.

In the United States soybean curd sold in retail stores

usually is water-packed. Recently introduced vacuum packaging can prevent surface drying and protect against air-borne bacteria. It reduces distribution costs by eliminating water, thus decreasing the shipping weight (Anon., 1983a). Pasteurization of tofu in the package also has been introduced. Studies have suggested that pasteurization (60 °C for 8 min) or chlorination could be used to safeguard against Yersinia enterocolitica contamination (Anon., 1983b). Surface sterilizing of tofu with boiling water in the package prior to sealing and aseptically packaging also is practiced.

Quality of tofu

Shelf life and microbiological studies. Good soybean curd has a bland taste and is white or pale yellow in color. However, it is perishable even when commercial refrigeration is used in retail stores. The shelf life of soybean curd varies from one to three days (depending on temperature) without any preservation.

Presently there are no comprehensive standards regarding the bacteriological safety of soybean curds (Rehberger et al., 1984). Processing of tofu, which includes boiling the soymilk, can be effective in eliminating much of the initial vegetative microflora. However, the post boiling pressing of the curds to form cakes and handling of cakes before

packaging allows for possible contamination (Rehberger et al., 1984). Tofu often is displayed in the produce section of grocery stores where temperature fluctuations are possible, making tofu a potential public health hazard (Anon., 1983b; Rehberger et al., 1984). Recently the Food and Drug Administration reported a Class 2 recall of tofu because of adulteration with Yersinia enterocolitica (Aulisio et al., 1983). Bacteria generally responsible for the spoilage of soybean curds are lactic acid bacteria (Dotson et al., 1977). Fujii et al. (1978) conducted a study to determine microbiological quality of soybean curd obtained from tofu makers and retail stores. They found large amounts of E. coli contamination in soybean curd attributed to transportation from tofu makers to retail stores. These researchers pointed to the importance of storage temperature during the transportation from production to retail stores.

Rehberger et al. (1984) also investigated the microbiological quality of commercial soybean curd available in local retail outlets. They found variations in microbial counts among samples of tofu taken from different locations within tofu cakes as well as from different lots of the same brand of commercial soybean curd. Counts were higher in the top corner than in the center portion or bottom corner.They accounted for the variation of total aerobic counts within a

piece of tofu as the result of apparent nonhomogeneous distribution of the soybean curd pieces before packaging. Large variations in counts of aerobic organisms, psychrotrophs, and coliforms also were found between the lots and within a brand of tofu. To improve the microbiological quality of tofu, processors need to reduce the initial bacterial load by improving sanitation and processing techniques, and retailers should provide more consistent and cooler refrigeration. Pigott (Anon., 1983b) conducted shelf life tests for fresh packaged soybean curd held at various temperatures ranging from 38°F (the recommended holding temperature) to 53°F (the internal temperature of some soybean curd found in the produce cases). He found that soybean curd held at 53°F showed over 100 million bacteria per gram and were completely spoiled. Pigott's warning was effective enough to switch soybean curd displays from the warm produce section to the much cooler dairy section.

Some researchers have attempted to increase the shelf life of soybean curd. Pontecorvo and Bourne (1978) used three simple techniques of preservation: immersion in aqueous solutions of sodium chloride, smoking the soybean curd for a period of 12, 24, 36, and 48 hours, and a combination of smoking and immersion in sodium chloride solutions. Their studies showed that shelf life of soybean curd could be

extended to 10-15 days without refrigeration by smoking and storing in salt brine acidified with lemon juice.

Wu and Salunkhe (1977) introduced in-package microwave treatment on fresh soybean curd to extend its shelf life. Soybean curds pretreated with microwave heating to 65°C, 80°C, and 95°C had shelf lives of 16, 21, and 27 days, respectively, compared to 7 days for the control. They also found that a decrease in pH, increase in titratable acidity, or increase in viable cell count in the soaking water was accompanied by a decrease in quality of soybean curd. This was in agreement with the findings of Dotson et al.(1977). Although microwave heating treatments for extending shelf life of soybean curds are apparently economically feasible, application to industrial scale requires consideration of such limiting factors as capacity of the microwave apparatus and the amount of soybean curds to be treated.

pH. Soybean curd, decomposed by the action of microorganisms, shows changes in pH values. Pontecorvo and Bourne (1978) reported that immersion solutions prepared for tofu storage would best act as antimicrobial agents when their pH was below 4.5, since higher pH values caused potential problems with growth of *Clostridium botulininum*. The researchers used immersion solutions of sodium chloride, lemon juice, and both agents in different proportions

to preserve soybean curd. They found noticeable changes in odor and flavor when the immersion solutions had reached a pH of 2.8 after 10 days of storage.

Dotson et al. (1977) found that pH values of the liquid surrounding the packaged tofu were generally about 0.2-0.25 units lower than that of the tofu in the cake. They also found that pH changes correlated with flavor deterioration during storage of the tofu cake immersed in water. This also was in agreement with the findings of Wu and Salunkhe (1977) when they attempted to extend the shelf life of soybean curd using in-package microwave treatment.

Fujii et al. (1978) studied the changes in pH in soybean curd during 10 days of storage under various conditions: cold temperature (8 °C), running tap water, and room temperatures of 27 °C and 37 °C. They found that pH in soybean curd decreased rapidly in one to two days of storage at 27 °C and 37 °C. However, pH increased rapidly from the fifth day of storage at 37 °C. Fujii et al. (1978) suggested that 37 °C was suitable for growth of microorganisms, which caused rapid decomposition of protein, resulting in pH changes. Putrefaction occurred initially, then acidic compounds were produced; basic compounds were estimated to form at the middle and end of the process of decomposition (Fujii et al., 1978). The researchers found that pH of

soybean curd preserved in running water decreased slowly, possibly due to washing away of microorganisms from the surface. At 8 °C, pH decreased slightly on the fourth day of storage of the curd during the ten day observation period.

Titratable acidity. Soybean curd is a suitable medium for microorganisms because it is a rich source of protein and moisture (Fujii et al., 1978). Acid production in soymilk is dependent on bacterial load, growth rate, and ability to utilize the carbohydrate available in the medium (Angeles and Marth, 1971; Mital and Steinkraus, 1974; Patel and Gupta, 1982). Soymilk provides a satisfactory growth media for most lactic acid bacteria. When soymilk was supplemented with glucose, sucrose, and lactose or partially degraded protein, there was an increased yield of lactic acid production (Angeles and Marth, 1971).

Acid development in the immersion solutions of soybean curd during the storage period was titrated by Wu and Salunkhe (1977). They found that a notable increase in titratable acidity of the immersion liquid occurred at the time of a decrease in quality of soybean curd. Pontecorvo and Bourne (1978) reported the titratable acidity equivalence of citrus fruit juices prepared for use in immersion solutions

for preservation of soybean curd. They suggested that vinegar be substituted for citrus fruit juices because of flavor problems when fruit juices were used.

Fujii et al. (1978) investigated the daily changes in the amount of true protein in soybean curd and the free amino acids in soybean curd stored at 8°C and 37°C. At 37°C, the amount of true protein decreased rapidly from the fifth day of storage as the amount of free amino acids by titration increased. At 8°C, the amount of true protein decreased slowly and the amount of free amino acids increased slowly. Fujii et al.(1978) noted in this study that at 37°C microorganisms were growing rapidly and protein was decomposing quickly in soybean curd during the storage period.

Sensory analysis. Fresh tofu has a characteristic odor, flavor, and mouthfeel. Spoilage of soybean curd is characterized by a sour taste and sometimes an acidic odor. When soybean curds are placed in immersion solutions for preservation, they may acquire the characteristic flavors of the solutions. Pontecorvo and Bourne (1978) conducted a sensory study to compare immersion solutions used in preservation of soybean curds. The panelists evaluated flavor, aroma, mouthfeel, texture, color, and general appearance of the samples on the basis of their own

individual preferences, indicating at each session which sample was preferred. Samples were presented randomly to equalize the sample sequence effect on food preferences as recommended by Eindhoven et al. (1964). Panelists chose immersion of tofu in a solution of 4 % sodium chloride and 10 % lemon juice as the best method in the study. Dotson et al . (1977) evaluated the flavor of soybean curd by comparing flavor of the test samples during the storage period with that of freshly made soybean curd. Results from Dotson's trained sensory panel showed that a decrease in soybean flavor corresponded to microbiological count, pH, and optical density changes observed in the liquids that surrounded the soybean curds.

Texture and structural composition. The microstructure of tofu as well as physical and chemical factors that regulate its textural development have been studied extensively. Curd strength is related to the interactions between protein molecules, such as hydrogen bonding, ionic bonding, disulfide bonding and hydrophobic association. Interactions between or within protein molecules determine the microstructure of the protein network. The microstructure integrates into the structural network which is interpreted as mechanical properties during processing and textural properties upon consumption (Lee and Rha, 1978).

Saio (1979) studied the structure of soybean curd using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). His studies showed that the density of the network of tofu correlated with hardness of tofu. Density of tofu varies with amount and type of coagulant, phytic acid content, and loss of whey during coagulation. Saio also showed that large protein aggregates resulted in harder tofu. Because sulfhydryl-disulfide interchange reactions occur predominately in heat aggregation of 11 S, the larger aggregates of 11 S result in a network with a large protein aggregate. Precipitability of 7 S and 11 S with calcium salt differed: 11 S began to precipitate faster than 7 S in lower calcium concentrations and formed a larger aggregate. Formation of the soluble aggregate of 11 S was accelerated in the presence of calcium salt (Saio and Watanabe, 1973).

Lee et al. (1983) showed that tofu's deformability mode is regulated by both the solid matrix properties and internal hydrostatic pressure. Lee and Rha (1978) studied the relation of protein interactions and the microstructure of soybean protein aggregates to the textural properties of the curd. They found that isoelectric point precipitation and calcium coagulation did not change the globular structure of the native soybean protein. However, heating induced the destruction of the native protein body as protein

denaturation was necessary in formation of the structural network of the aggregates. When protein aggregates were frozen, their structures became better defined and enlarged. From heated soybean protein, the three-dimensional network structure of the aggregate showed low sedimentation rate, high curd yield, high water-holding capacity, low value of hardness, and high springiness compared to unheated precipitates of globular structure (Lee and Rha, 1978).

Instrumental assessment of texture. The textural properties of various types of tofu were considered major quality attributes in the product. Textural properties of tofu can be evaluated instrumentally using Texturometer or Instron texture profiling analysis (Lee and Rha, 1978; Saio, 1979).

Skurray et al. (1980) evaluated the textural properties of tofu using an Instron Universal Testing Machine (Model 1140) to measure hardness and cohesiveness. Their studies showed that a slight variation in the texture of tofu was due to the 7 S and 11 S proteins in the soybean, but an important factor affecting the texture of tofu was found in the amount of calcium ions added. A linear relationship was found between the protein content of the beans and the calcium sulfate concentration required for good quality tofu.

Wu and Peng (1983) studied the textural properties of

soy-cheese whey curd with an Instron Universal Testing Machine. Five parameters were determined: stiffness, bioyield point, firmness, relaxation, and plasticity. When curds were prepared at different soymilk concentrations, stiffness and firmness were affected but other textural parameters were not altered. The researchers believed, therefore, that stiffness and firmness best defined the textural parameters for their soy-cheese whey curd.

Lu et al. (1980) used an Instron Universal Model 1132 with a compression fixture attached to determine hardness of soybean curd. Tsai et al. (1981) studied the textural properties (jelly strength, softness, and chewiness) of tofu with a Rheometer (NRM 2002J, Fu do Kabu shiki Kaishia, Japan).

Lee et al.(1983) investigated the rheological behavior of tofu by considering its compressive behavior and using three methods: stress vs true strain, stress relaxation, and percentage recoverable work at different strains. The researchers noted that textural studies are dependent on the test geometry (e.g. the specimen or penetrating plunger dimensions) and the test conditions (especially the arbitrarily selected deformatory level). For this reason, they cautioned that results published by different authors cannot be compared meaningfully or expresesed in terms of fundamental mechanical parameters such as module or strength.

Peleg (1976, 1977) cited innate theoretical complications and Culioli and Sherman (1976) noted imperfect or inappropriate testing conditions as contributors to inaccurate determination of textural properties.

Food grade chemicals for preservation of soybean curd

Little information was found in the literature on the use of chemical preservatives to extend the shelf life of soybean curd. Pontecorvo and Bourne (1978) prepared immersion solutions of methyl and propyl parabens to preserve the soybean curd. Parabens were not acceptable because of their medicinal odor and flavor, although they could extend the shelf life of the soybean curd. Ogawa (1970) patented a preservation technique using hydrogen peroxide and high molecular weight phosphates to extend the shelf life of soybean curd. Kamiya and Murai (1975) introduced the use of synthetic bactericides, such as AF-2, to prevent susceptibility of soybean curd to putrefaction. Fujii et al. (1978) studied the effects of magnesium chloride, delta-gluconolactone and sodium hexametaphosphate on growth of microorganisms in soybean curd. They found that delta-gluconolactone was effective in controlling microbial growth during production of soybean curd and for 36 hours after addition of the chemical.

Addition of chemical preservatives to food is a useful approach toward inhibition of growth of food spoilage microorganisms and the extension of shelf life of food. However, use of chemical preservatives in foods is governed by legislative requirements in most countries of the world. Since most chemical preservatives are pH dependent and some are effective in the undissociated form at low pH, a moderate degree of acidification of foodstuffs often is attempted (Jarvis and Paulus, 1982).

Phenolic antioxidants for preservation. Phenolic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary-butyl-hydroquinone (TBHQ) commonly are used to prevent rancidity in lipids and lipid containing products. These antioxidants also have antimicrobial activity against bacteria, molds, viruses, and protozoa (Ahmad, 1979; Fung et al., 1984; Kim et al., 1978; Snipes et al., 1975; Surak et al., 1976; Vardaman et al., 1978; Wanda et al., 1976; Yousof and Marth, 1984). The general consensus is that Gram positive bacteria were more affected by antioxidants than Gram negative bacteria, although exceptions exist. Differences also were found among strains of the same bacteria species (Fung et al., 1984).

Ward and Ward (1967) were the first to report the antimicrobial activity of antioxidants. They found that a concentration of 1.0 % BHT was inhibitory to S. senftenberg on commercial fish meal, although inhibitory effects were slight. Inhibition beyond 24 hours would require the impractical high concentration of at least 1.0 % BHT. Hence, the control of Salmonella in fish meal by BHT alone had little practical value. Inhibition of general food spoilage microflora has also been investigated. Trelease and Tompkin (1976) found 500 ppm of BHA to be totally inhibitory to microorganisms' preventing spoilage of frankfurters. In studying antimicrobial effect of BHA and potassium sorbate against S.typhimurium in cooked turkey, Morad et al. (1982) showed that there was a reduction in counts of S.typhimurium during 8 days of refrigerated storage in all treatments. Sorbate was more effective than BHA in reducing the test organisms and the combination of BHA and sorbate had additive effects. Their report was the first to indicate the effectiveness of BHA to reduce bacteria in meat systems although the concentrations of BHA exceeded the legal limits of usage.

Little work has been done on elucidating the mechanism of microbial inhibition by phenolic antioxidants. They possibly act on the cytoplasmic membrane of the microorganisms in a

manner similar to the action of other phenolic compounds (Branen et al., 1980). Eletr et al. (1974) and Singer and Wan (1977) used ^{22}Na efflux and electron spin resonance studies to show that BHT disrupts liposomes. Davidson et al. (1979) found that BHA caused leakage of intracellular protein from Pseudomonas fluorescens, apparently as a result of reaction with the cell membrane. Surak et al. (1976) and Surak (1977) found that the principal effect of BHA and TBHQ on Tetrahymena appeared to be inhibition of DNA, RNA, and protein synthesis. TBHQ also inhibited metabolism of ^{14}C-acetate. Ahmad (1979) found that BHA was more effective in preventing mold growth in low-fat products than in high-fat products. In low-fat products such as applesauce or agar, 200 ppm BHA totally inhibited mold growth, while 400 ppm BHA were required to totally inhibit mold growth in high-fat products such as processed cheese. There are two principal theories explaining that the presence of lipid decreases the antimicrobial activity of BHA. Since BHA has a nonpolar character, it could migrate and solubilize in any lipid present in a medium, making it unavailable to act on microorganisms. Secondly, the antimicrobial property of BHA may be related to its antioxidant properties; therefore, if BHA is no longer effective in prevention of autoxidation, the

antimicrobial activity also may be lost (Branen et al., 1980).

Most studies have shown that the antioxidants are not as effective in food systems as they are in broth or agar systems. Robach et al. (1977) found that in broth 50 ppm BHA totally inhibited the growth of Vibrio parahaemolyticus but that 400 ppm was required in crab meat. Shelef and Liang (1982) evaluated the antibacterial effects of BHA against Bacillus species in nutrient broth and in cooked rice and strained chicken. They found that food components counteracted the effectiveness of BHA against B. subtilis species. Growth inhibition of BHA on non-enterotoxigenic strain of B. cereus, two toxigenic strains of B. cerus, B. subtilis, and B. megaterium was found to be 75 ppm in nutrient broth. However, in cooked rice, the level increased to 1000 ppm, and in strained chicken they were not effective until at 500 ppm.

In studying the effects of BHA and TBHQ, Poerschke (1981) found that in laboratory liquid media, 0.2 % potassium sorbate plus 25 ppm TBHQ caused total inhibition of growth of S. aureus S-6. When similar combinations of BHA and TBHQ with sorbate, optimal in synergistic inhibition of S. aureus S-6 in laboratoy media, were tested in fresh ground beef, their effects were diminished. This diminished effect was attributed to autoxidation of ground beef lipids.

Raccach and Henningsen (1982) studied the effect of TBHQ on Pediococcus pentosaceus from the standpoint of fermentation activity of this organisms . They reported the minimum inhibitory concentration of TBHQ for Log 3-5 cells/ml was 15 ppm and for Log 6 cells/ml was 20 ppm. Synergistic inhibitory effects were observed when heat stress and sodium chloride were used along with TBHQ. TBHQ inhibited the fermentation of sucrose at 5 ppm, L-arabinose, D-galactose, and maltose at 10 ppm, and glucose at 15 ppm. The production of both L-and D-lactic acid was inhibited by TBHQ. Raccach et al. (1984) also showed that fermentation activity of lactic acid bacteria in meat was inhibited by TBHQ.

Erickson and Tompkin (1977) applied TBHQ as an antimicrobial agent in various food products. They were able to delay spoliage of a 30 % gelatin dispersion by use of 0.01 % TBHQ and to prevent the growth of normal spoilage bacteria in pasteurized fluid whole milk at 36 ^{o}C with 0.005 % TBHQ.

It is unlikely that BHA or BHT could be used alone to control the growth of microorganisms in foods, since the required amounts appear to far exceed the legally allowable amounts (0.02 % based on the weight) (Branen et al., 1980). Antioxidants could be useful in combination with other antimicrobial agents, such as monoglycerides which show synergistic action with antioxidants.

Davidson et al. (1981) found that S. aureus was 95 % inhibited by 150 ppm of BHA, and completely inhibited by TBHQ at 25 ppm. Sorbate/BHA and sorbate/TBHQ combinations were effective in synergistically delaying growth of S. aureus. B. aureus was reported to be inhibited by TBHQ in a report by Erickson and Tompkin (1977).

Ayaz et al. (1980) studied the effects of BHA and BHT on three enterotoxigenic strains of S. aureus in brain heart infusion. Complete inhibition of S. aureus growth occurred with 200 ppm BHA or 150 ppm BHT, while 150 ppm BHA or 100 ppm BHT inhibited enterotoxin formation in the same organism. The combination of BHA and BHT provided greater inhibition than when the either antioxidant was used alone. Inhibition of S. aureus growth by BHA or BHT was substantial at pH 7 and 2 % NaCl.

One precaution in the use of antioxidants is their highly selective inhibitory effect. For example, 150 ppm BHA could inhibit growth of lactic acid bacteria in meat but not growth of Pseudomonas. Recent work by Davidson et al. (1979) indicated that P. fluorescens may become tolerant to BHA. Growth of this organism at a non-toxic level of BHA appears to increase its subsequent resistance to higher levels of BHA.

Antimicrobial properties of lipids. Lipids and lipid derivatives occurring naturally in foods play a role in

preventing the spoilage of foods. Although most lipids are considered Generally Recognized As Safe (GRAS) and are used as emulsifying agents in foods, little use has been made of them as food additives to control microbial growth. The various short-chain fatty acids, although quite active as antimicrobials, can produce undesirable off-flavors in many food products as a result of their high volatility (Branen et al., 1980).

Acetic acid, has been known as a preservative since ancient times. It is more effective against yeasts and bacteria than against molds (Ingram et al., 1956). Acetic acid and its salts have higher antimicrobial activity as pH is lowered and the undissociated form of the acid is increased.

Sorbic acid , is a 2,4 trans ,trans, hexadienoic fatty acid. In the pH range of 5.0-6.0 where the acidity is not inhibitory, this fatty acid has a marked inhibitory effect; thus, toxicity to the microorganism is due partly to pH and partly to the anion (Beuchat, 1980). The increased upper pH limit for sorbate effectiveness makes it the preservative of choice for most mildly acidic, easily preserved food (Beuchat, 1980).

The salts of sorbic acid, especially the potassium salt, are important in food applications due to their high

solubility in water. Sorbate can be used as a direct additive, as a spray or dip, and as a component coating in or on packaging materials. Sorbic acid can be added directly in the dry form to cakes and salads or it can be mixed easily with shortening or dressing for distribution throughout a product. Stock solutions of 10 to 20 % of potassium sorbate are used for direct addition of sorbate to beverages and pickle products (Chichester and Tanner, 1972). Levels of incorporation into the product vary from 0.01 to 0.3 % sorbic acid. The major commercial use of sorbate is as a fungistatic agent (Sofos and Busta, 1981).

Phillips and Mundt (1950) and Jones and Harper (1952) reported that 0.1 % sorbic acid prevented growth of surface yeasts in cucumber fermentations. Borg et al. (1955) observed that 0.1 % sorbic acid not only inhibited growth of fermentative yeast in cucumber fermentations, but it also retarded growth and acid prodcution by the acid-forming bacteria. Additional research by Costilow et al. (1957) demonstrated that the inhibitory effect of sorbic acid on lactic acid-producing bacteria in cucumber fermentations depended upon brine strength. The usefulness of sorbic acid as a mold inhibitor in cheeses has been demonstrated by several workers (Deuel et al., 1954; Melnick and Luckman, 1954; Smith and Rollin, 1954).

Vaughn and Emard (1951) and Emard and Vaughn (1952) reported that sorbate inhibited several species of bacteria in laboratory media. Results presented by Doell (1962) indicated that sorbate concentrations as low as 0.075 % were effective against S. typhimurium and Escherichia coli. S. typhimurium also was inactivated by sorbate in laboratory media, milk and cheese (Park and Marth, 1972; Park et al., 1970).

Tompkin et al. (1974) undertook a detailed study of sorbate as a sausage preservative. It was shown that sorbate retarded growth and toxin production formation by C. botulinium. Davidson et al. (1979) reported a synergistic antimicrobial effect against S. typhimurium by BHA with potassium sorbate at levels of 0.05 % sorbate/50 ppm BHA, 0.05 % sorbate/100 ppm BHA, and 0.1 % sorbate/50 ppm BHA. Robach and Statler (1980) studied the influence of potassium sorbate alone and in combination with sodium chloride, TBHQ, BHA, or ethylenediamine tetraacetic acid (EDTA) on growth of two strains of Staphylococcus aureus (S-6 and 12600). They found that a combination of sorbate and BHA were synergistic against both strains.

Lahellec et al. (1981) found that potassium sorbate at 1 %, 3 %, and 5 % levels in combination with BHA, BHT, or propyl gallate (PG) (50 and 100 ppm) exerted greater

bactericidal and bacteriostatic effects on S. aureus strains at pH 5 than at pH 7; at pH 6 the effects were more pronounced with 3 % and 5 % than with 1 % sorbate. They also found that higher concentrations of the antioxidants exerted greater bactericidal and bacteriostatic effects on the test organisms.

Lipids, like phenolic antioxidants, prevent microbial growth by altering the bacterial cell membrane. Kodicek and Worden (1945) theorized that fatty acids formed a monolayer around the bacterial cells which resulted in inhibition of growth either by blocking the transport of nutrients into the cell or by increasing leakage of essential metabolites from the cell. Many workers have shown that blockage of transporting nutrients seemed to be the most logical explanation of the mechanisms of antimicrobial activity of lipids (Galbraith and Miller, 1973; Kondo and Kanai, 1976).

MATERIALS AND METHODS

Preliminary study on commercial soybean curd

A study was done to investigate the microbiological quality of commercial soybean curd obtained from six local retail stores in Manhattan, Kansas, to determine the feasibility of purchasing tofu for the study versus preparing it in the laboratory. Two types of tofu were available: water-packed and pasteurized water-packed. Duplicate samples of commercial tofu were purchased within 24 hours after delivery to the retail stores. Viable cell count and pH measurements were obtained from the soaking solutions of the tofu samples on the same day that they were obtained. Samples were stored at refrigeration temperature (4 -7 °C) and viable cell counts and pH measurements were made after storage periods of 1, 3, 5, 7, and 9 days.

Preparation of soybean curd

Based on preliminary work, a decision was made to prepare tofu in the laboratory where control conditions could be optimized.

Soybean curd was made according to the procedure of Shurtleff and Aoyagi (1983) with modifications. Six hundred grams of whole dry soybeans were washed, soaked in 2.8 L tap water at ambient temperature (22 -25 °C) for 10-12

hours. After soaking, the beans were rinsed with water and drained. Hydrated beans were weighed into seven portions. Each portion was blended until smooth with 300 ml of hot water in a Waring blendor (Waring Products Corp., Model 700B). The puree was heated in an aluminum cooking pot containing 2.8 L hot water, stirred, and transferred to a cloth filter bag (Tofu Kit, Soyfoods Center). The filter bag was pressed repeatedly against a colander to express as much soymilk as possible. Boiling water (700 ml) was poured over the contents in the filter bag and pressure was applied again. The resulting soymilk was heated to 100 °C and then simmered for an additional 7 min before removal from the heat source. Food grade calcium sulfate dihydrate (Charles B. Chrystal Corp., Inc.), was used as the coagulating agent. A dispersion was made with 14.3 g calcium sulfate in 500 ml water. One-third of the calcium sulfate dispersion was poured into the hot soymilk (80 °C) while the mixture was stirred rapidly with a wooden spoon. The soymilk was stirred 5-6 times after the addition of the first portion of coagulant. When movement of the liquid had ceased, another third of the calcium sulfate dispersion was sprinkled over the liquid areas with slow stirring of the upper 1 cm layer of the curdling soymilk.

After the final addition of coagulant, the mixture was covered and set aside for 6 min. Then the surface layer was

stirred for 20-30 sec, and a wooden spoon was pushed down the inside of the pot in several places to free soymilk that was trapped below the curds. Curds were ladled by layers into a wooden forming container, dimensions of 19.7 x 10.6 x 10 cm (Tofu Kit, Soyfoods Center), that was lined with a double layer of cheese cloth (The Bandage Corp.). Excess cheesecloth was folded over the top of the container holding the curds and an 8-kg weight was placed on the cover for 15 minutes ($0.038\ kg/cm^2$). The tofu was inverted from the forming box and immersed in cold water for about 5 minutes until firm.

Treatments

The freshly made soybean curd was cut into three blocks (6.85 x 10.6 x 10 cm) weighing approximately 330 g and each was placed into a white plastic food tray (GFW-26, Gage Industries, Inc.) with an immersion solution. The following immersion solutions were used:

1) sterilized, distilled water (no additive)
2) 0.5 % acetic acid (AA); J.T. Baker Chemical Co.
3) 0.1 % citric acid (CA); Mallinckrodt, Inc.
4) 0.005 % tertiary-butyl-hydroquinone (TBHQ); Eastman Kodak Co.
5) 0.15 % potassium sorbate (PS); Pfaltz and Bauer, Inc.
6) 0.15 % PS + 0.5 % AA
7) 0.15 % PS + 0.1 % CA
8) 0.15 % PS + 0.005 % TBHQ

Samples were covered with aluminum foil, stored at 10 -15 °C, and removed as necessary.

Microbiological analyses

Samples of soybean curd in the immersion solutions were stored at refrigeration temperature (10 -15 °C). Viable cell counts were made for microbiological study by plating 1 ml serial dilutions of soybean curd soaking solutions in Trypticase Soy Agar (Difco) according to specifications and standard methods of USP, APHA and AOAC, as stated in Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures (1977). Colonies were counted after incubation at 32 °C for 48 hours and at subsequent testing periods. Selective agar, Rogosa SL. Agar (Difco), was used similarly to determine the lactic acid bacteria count.

Chemical analyses

pH was determined with a Horizon Model 599-10 pH meter (Ecology Co.) with a glass electrode immersed in 10 ml soaking solution. Titratable acidity was measured (Ruck, 1969) by titrating the immersion solution with 0.025 N NaOH. A 5-g sample of soaking water was added to 100 ml distilled water and boiled for 2 minutes to release carbon dioxide. The liquid was titrated with NaOH to pH 8.1. Titratable acidity was calculated as percentage lactic acid using the formula

(Ruck, 1969):

$$\% \text{ Lactic Acid} = \frac{\text{TITER} \times N \times \text{EQ.WT.} \times 100}{1000 \times \text{ml. OF SAMPLE}}$$

where VOLUME OF SAMPLE = 5 ml. soaking solution

TITER = ml. NaOH (adjusted) in titration

EQ.WT. = lactic acid (90.08)

The titer was adjusted by subtracting the titration value obtained for each testing storage period from the initial titration obtained for each immersion solution on day 0 when the tofu was freshly made.

Viable cell counts, lactic acid bacteria counts, pH and titratable acidity were measured on the immersion solutions during the storage period of 1, 4, 7, 10, 13, 16, 19, and 23 days

Sensory analysis

Panelists. Six trained sensory panelists were employed for evaluation of soybean curd. In the orientation sessions, panelists became familiar with the product, score card, and terminology used in this study. Procedures for testing samples were developed also. Sessions were scheduled with panelists on four consecutive weeks to analyze the tofu held for 1, 9, 16, and 23 days of storage.

Sample preparation. Stored tofu was drained of immersion

solution and placed in a miter box. A serrated knife was used to remove 2.54 cm from the outer edges on four sides of the tofu block. The remaining interior block of tofu was cut into samples with dimensions of 2.54 x 1.27 x 1.27 cm. Six samples were obtained for sensory analysis and two were used for instrumental measurement of texture.

Sample evaluation. Each panelist was presented with a sample of tofu placed in a 30-ml plastic cup coded with a randomized three-digit number, and covered with a watch glass. Three test samples were presented in a series with a control identified as freshly prepared tofu immersed in sterilized distilled water. Samples were evaluated at room temperature (25 °C). Panelists were instructed to drain moisture from the tofu and transfer the sample onto the concave surface of the watch glass. Panelists then cut the tofu into 0.62-cm cubes with plastic knives, and covered them with the inverted plastic cup on the watch glass. Panelists were required to rinse the mouth with distilled water before tasting. One cut piece of tofu was placed as a slice on the tongue with a toothpick. The tongue with the tofu was worked against the roof of the mouth. Panelists were instructed to keep the mouth closed while chewing and swallowing the slice. They evaluated the control for total flavor intensity, tofu flavor intensity, sourness, and texture and then the stored

samples. The score card which was used to record observations for the multiple comparison evaluation is included in the Appendix (Figure A-1). Comparisons to the control were converted to a numerical score for intensity where 1 = much less than control, 3 = same as control, and 5 = more than control.

Instrumental assessment of texture

A Warner/Bratzler shearing blade attached to the Instron Universal Testing Machine (Model 1122) was used to determine the hardness of the stored tofu. A crosshead speed of 200 mm/min and a chart speed of 200 mm/min was used with a 2-kg load. Duplicate samples (2.54 x 1.27 x 1.27 cm) were tested at room temperature (25 ^{o}C). Hardness was determined as the maximum peak force on the force-deformation curve and reported in kilograms. Measurements were taken on the freshly prepared sample and stored samples after 1, 9, 16, and 23 days of storage.

Experimental design and analysis

Fourteen large blocks of tofu were prepared and sectioned into three small blocks (approximately 330 g each) in sequential order with randomized numbers assigned to each block. Treatments in preservation solutions and controls were

randomized and placed in refrigeration (10 -15 °C) pH, titratable acidity, viable cell counts, and lactic acid bacteria counts were measured on the following day and at three-day storage intervals at 3-day storage intervals (days 1, 4, 7, 10, 13, 16, 19, and 23).

On Day 1, fourteen large blocks of tofu were prepared for storage periods of 16 and 23 days. The first seven large blocks were sectioned into three small blocks (approximately 330 g each) in sequential order according to preparation. Treatments were assigned to these twenty-one blocks of tofu obtained from the large blocks according to a balanced incomplete block design for the sixteenth day of storage. The small blocks were immersed in the chemical preservative solutions and stored at refrigeration temperature (5 -15 °C). Similarly another seven large blocks were sectioned and treated for the twenty-three day of storage.

For the four taste panel sessions, twenty-one treatments were presented to the six panelists in randomized order and the panelists tasted the samples according to the order of presentation. Three treatment samples were given to the panelists so that they could compare them to a freshly made samples of tofu. Seven series with three treated samples and the control presented in each taste panel session were conducted to evaluate the treated samples stored for 1, 9, 16, and 23 days.

Instrumental assessment of texture was measured on the same days as sensory analysis.

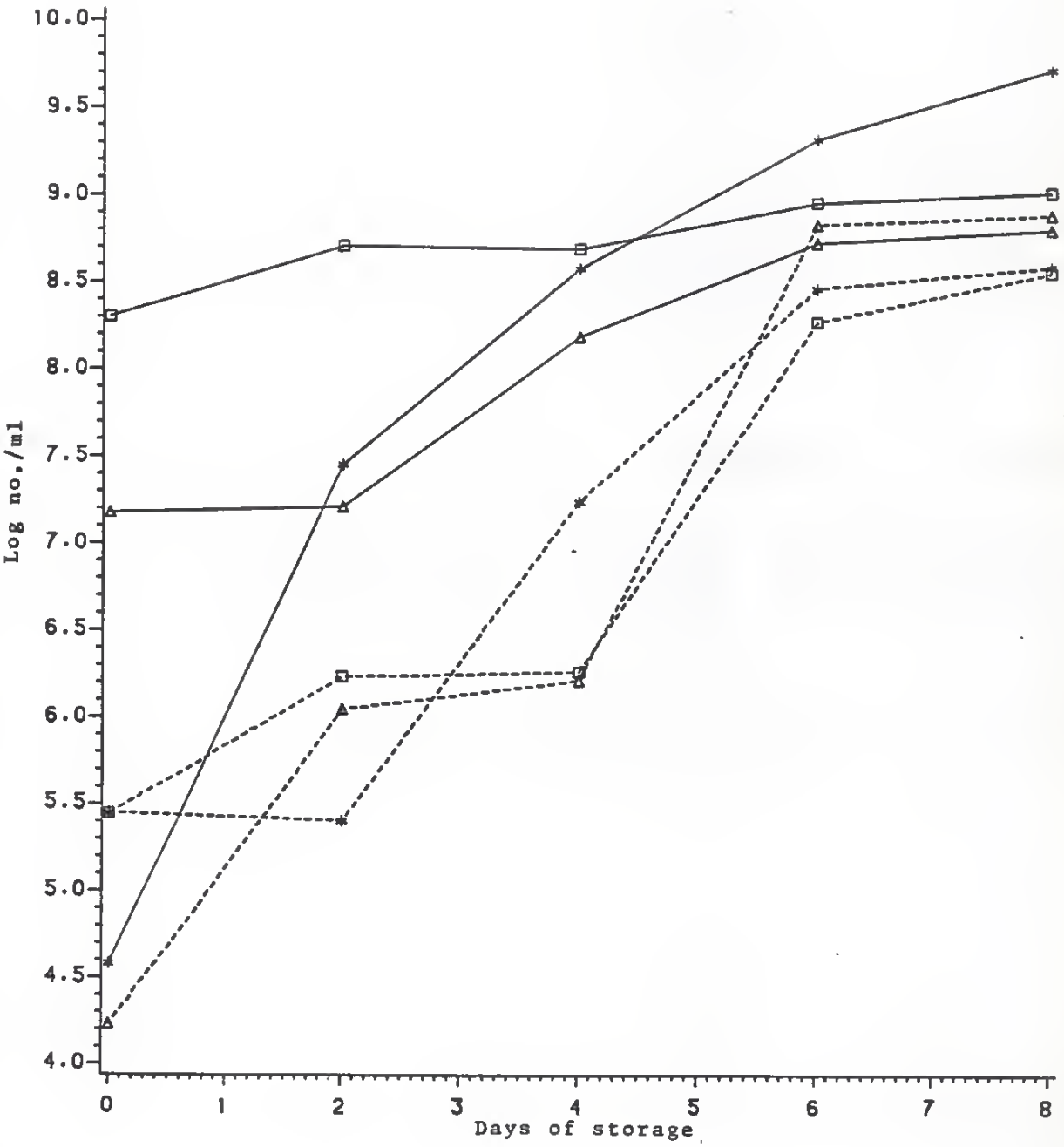
This experimental design allowed for five replications for the microbiological studies, and three replications of the sensory analysis and instrumental assessment of texture. Data were analyzed by analysis of variance, and when significant differences were shown, least squares means were determined. Correlation coefficients also were determined on physical measurements and sensory data for tofu stored in various immersion solutions.

RESULTS AND DISCUSSION

Analysis of commercial tofu

The immersion water in the commercially prepared tofu (Figure 1) had initial bacterial loads ranging from 10^4 to 10^8 colony forming units (CFU)/ml, although they were purchased within 24 hours after they were received by the stores. Similar findings were reported by Rehberger et al. (1984) who found variations in bacterial loads within commercial tofu samples purchased from four retail stores in Ames, IA. Assessment of microbiological quality of commercial tofu is difficult, and presently there are no comprehensive standards regarding the bacteriological safety of tofu. Three of the six tofu samples purchased in Manhattan, KS, had immersion water with greater than 10^8 CFU/ml within four days of storage, and all samples had 10^8 CFU/ml within six days of storage (Appendix, Table A-1). No differences were apparent between water-packed and pasteurized water-packed tofu during the study. Initially high bacterial loads in immersion water caused rapid growth of microorganisms, since tofu is a good medium for microbial growth. Because of variability in

Figure 1. Effects of storage on least squares means for viable counts in immersion solutions of commercial tofu


10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
Log no./ml
0
1
2
3
4
5
6
7
8
Days of storage

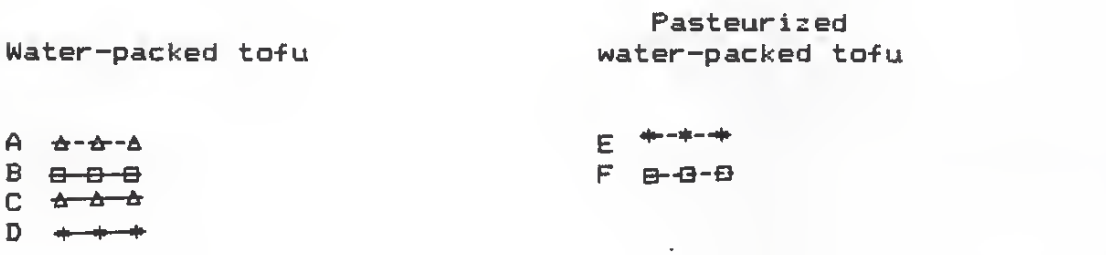
growth. Because of variability in handling, storage, and initial bacterial loads of the commercial samples tested, it was decided to prepare tofu for the remainder of the study in the laboratory where controlled conditions could be optimized. To improve the microbiological quality of commercial tofu, processors need to reduce initial loads by improving sanitation and processing techniques, and retailers should provide more consistent and colder refrigerated storage (Rehberger et al., 1984).

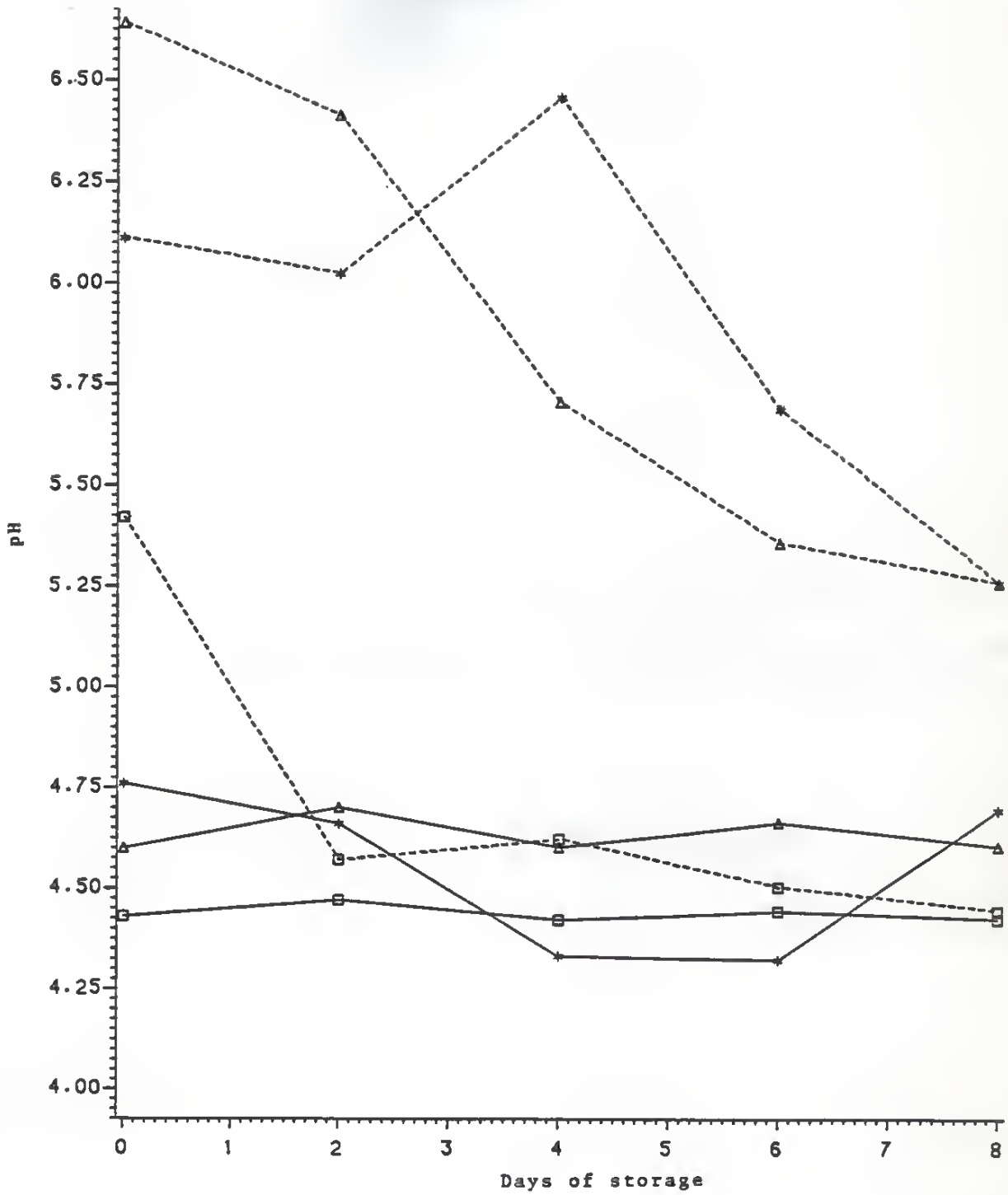
Variations existed also in initial pH of soaking water from tofu purchased in retail stores (Figure 2). Generally, lower pH values of soaking solutions corresponded to higher bacterial loads in commercial tofu when tested immediately after purchase. Higher initial pH values of soaking solutions declined rapidly as storage time increased. However, soaking solutions with lower initial pH values showed slow pH declines with storage time (Appendix, Table A-2). Dotson et al. (1977) also found that a decrease in pH corresponded with an increase in bacterial load in immersion water of commercially prepared tofu.

Microbiological analyses of treated immersion solutions

Least square means for logs of viable counts per ml

Figure 2. Effect of storage on least square means for pH in immersion solutions of commercial tofu



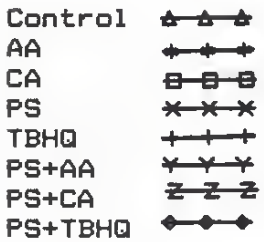

6.50
6.25
6.00
5.75
5.50
5.25
5.00
4.75
4.50
4.25
4.00
pH
0
1
2
3
4
5
6
7
8
Days of storage

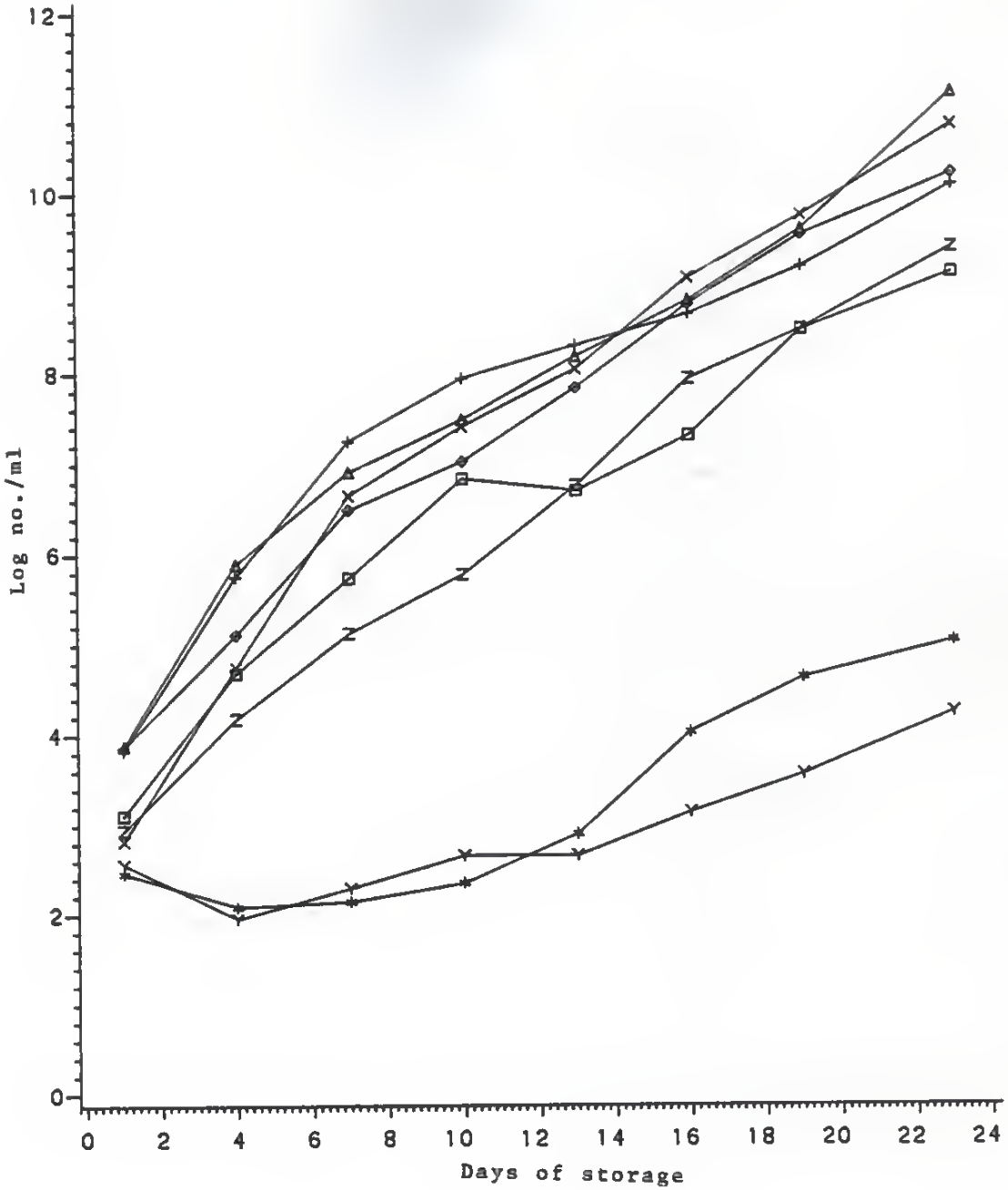
shown in Figure 3, and viable counts expressed as bacteria CFU/ml are found in the Appendix (Table A-3). All experimental immersion solutions had an initial bacterial load of 10^2 - 10^3 CFU/ml on the first day of storage which is substantially lower than the bacterial load reported for commercial tofu. The initial bacterial load may come from bacterial contamination during tofu processing as well as packaging before storage (Fujii et al., 1978; Rehberger et al., 1984).

Immersion solutions of AA, CA, or in combination with PS were effective in controlling bacterial growth in the immersion water for 13 days of storage. Tofu stored in these solutions retained less than 10^6 CFU/ml in their soaking solutions. CA and PS+CA were not as effective in maintaining low bacterial growth after the thirteenth day, and the soaking solution increased to a bacterial load of 10^7 CFU/ml by the sixteenth day of storage. The remaining chemical solutions (PS, TBHQ, PS+TBHQ) were similar in bacterial load to the control during the storage period. These chemical solutions had bacterial loads ranging from 10^6 to 10^7 CFU/ml after seven days of storage.

Generally AA, CA, or combined with PS produced increased antimicrobial effects for the first 13 days by lowering the

Figure 3. Effect of storage on least squares means of viable counts for immersion solutions of tofu




Log no./ml
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6
8
10
12
0
2
4
6
8
10
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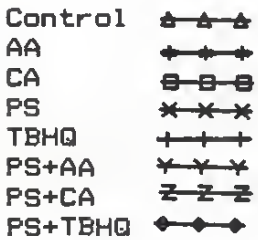
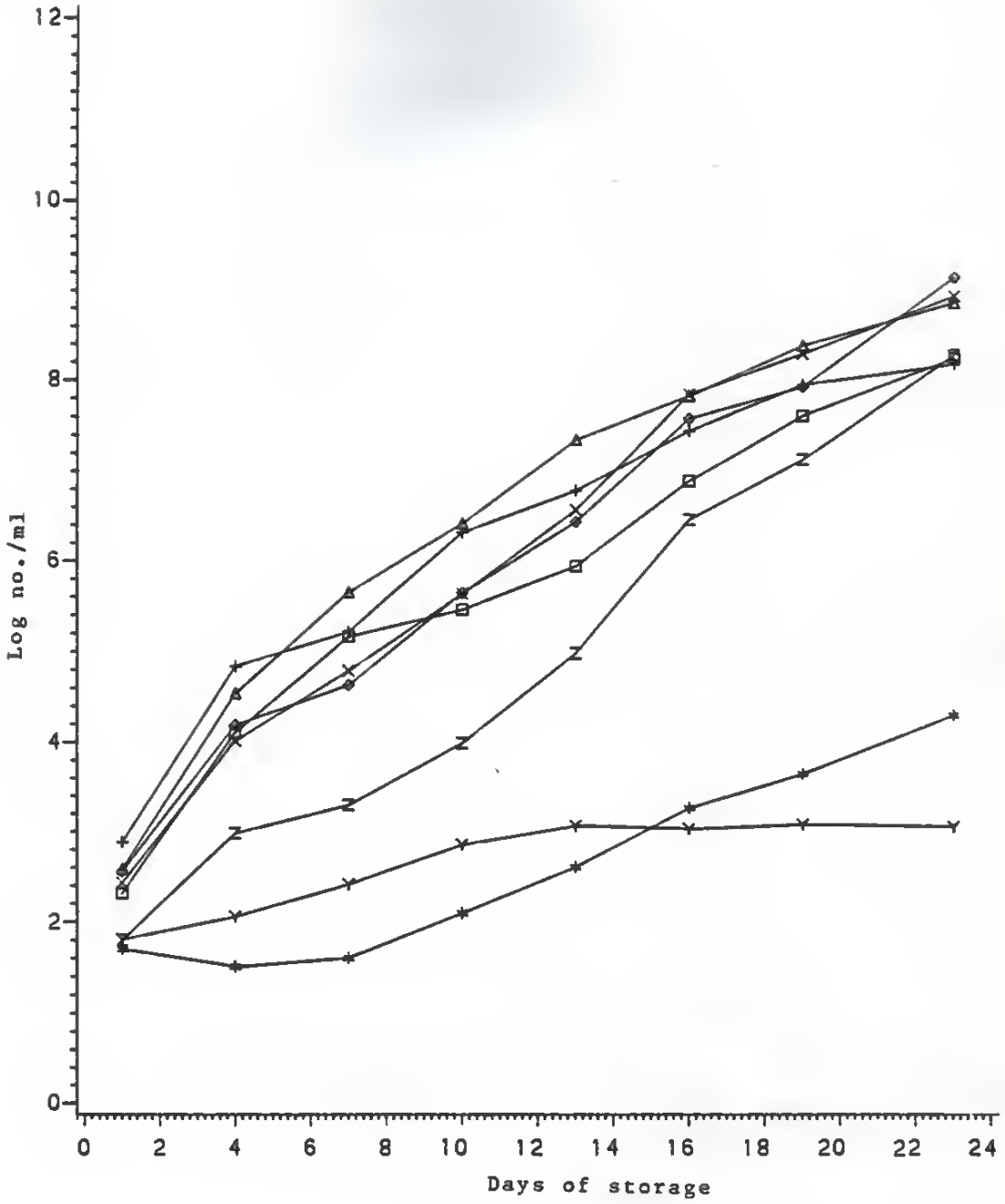
14
16
18
20
22
24
Days of storage

pH of the immersion solutions to levels closer to the dissociation constant of potassium sorbate (pKa=4.75). At this pH value, 50 % of the acids are in the undissociated form causing them to function more effectively as antimicrobial agents (Sofos and Busta, 1981). Tofu immersion solutions of AA, CA, or in combinations with PS showed lower bacterial loads than the other chemical preservatives used during each storage period tested for viable counts and for lactic acid bacterial counts (Figure 4; Appendix, Table A-4). These results paralleled findings by Emard and Vaughn (1952), Nomoto et al. (1955) and Sofos and Busta (1981) who found that sorbate was an effective antimicrobial agent in low pH foods. Other reports also indicated synergistic interactions between sorbate and pH level (Cowles, 1941; Hoffman et al., 1939; Rahn and Conn, 1944).

Synergistic sorbate-acetic interactions have been reported. Sheneman and Costilow (1955) found that acetic acid used with sorbate in sweet cucumber pickles prevented growth of lactic acid bacteria. However, PS was not as effective an antimicrobial agent as AA or PS+AA when used alone in storage solutions for tofu as indicated by high total bacterial loads and lactic acid bacteria counts. Chemical solutions containing PS, TBHQ, or PS+TBHQ, as well as the control showed increased viable and lactic acid bacteria counts as storage time increased.

Figure 4. Effect of storage on least squares means of lactic acid bacteria for immersion solutions of tofu




Log no./ml
0
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8
10
12
14
16
18
20
22
24
Days of storage

Several reports have suggested that sorbates exert a selective inhibition against different microorganisms, generally catalase-negative lactic acid bacteria and Clostridia (Emard and Vaughn, 1952; Phillips and Mundt, 1950; Vaughn and Emard, 1951). Some researchers reported that variations in tolerance to sorbic concentrations among species and strains of microorganisms tested were contributing factors in the effectiveness of sorbates as antimicrobial agents (Emard and Vaughn, 1952; Phillips and Mundt, 1950; Vaughn and Emard, 1951). However, there were major indications from the conclusions of other researchers that pH of the media affected the selective power of sorbates (Bell et al., 1959; Cowles, 1941; Hoffman et al., 1939; Nomoto et al., 1955; Sofos and Busta, 1981).

There were no significant differences in viable cell counts or lactic acid bacteria counts of soaking solutions when TBHQ was used alone or in combination with PS in comparison to the control. This indicated that TBHQ was not functional as an antimicrobial agent in a tofu food system. This was in agreement with the findings of Ahmad and Branen (1981), Lin and Fung (1983), and Gailani and Fung (1984) who concluded from their studies that an antioxidant's antimicrobial activities were reduced greatly in food and food components compared to test cultures.

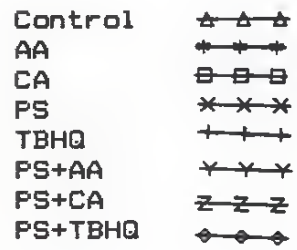
TBHQ showed no synergistic effect when used in combination with PS and compared to the control. Hence, sorbates may have selective inhibition against certain strains of microorganisms and pH influences their effectiveness as antimicrobial agents. Similar findings also were reported by Sofos and Busta (1981).

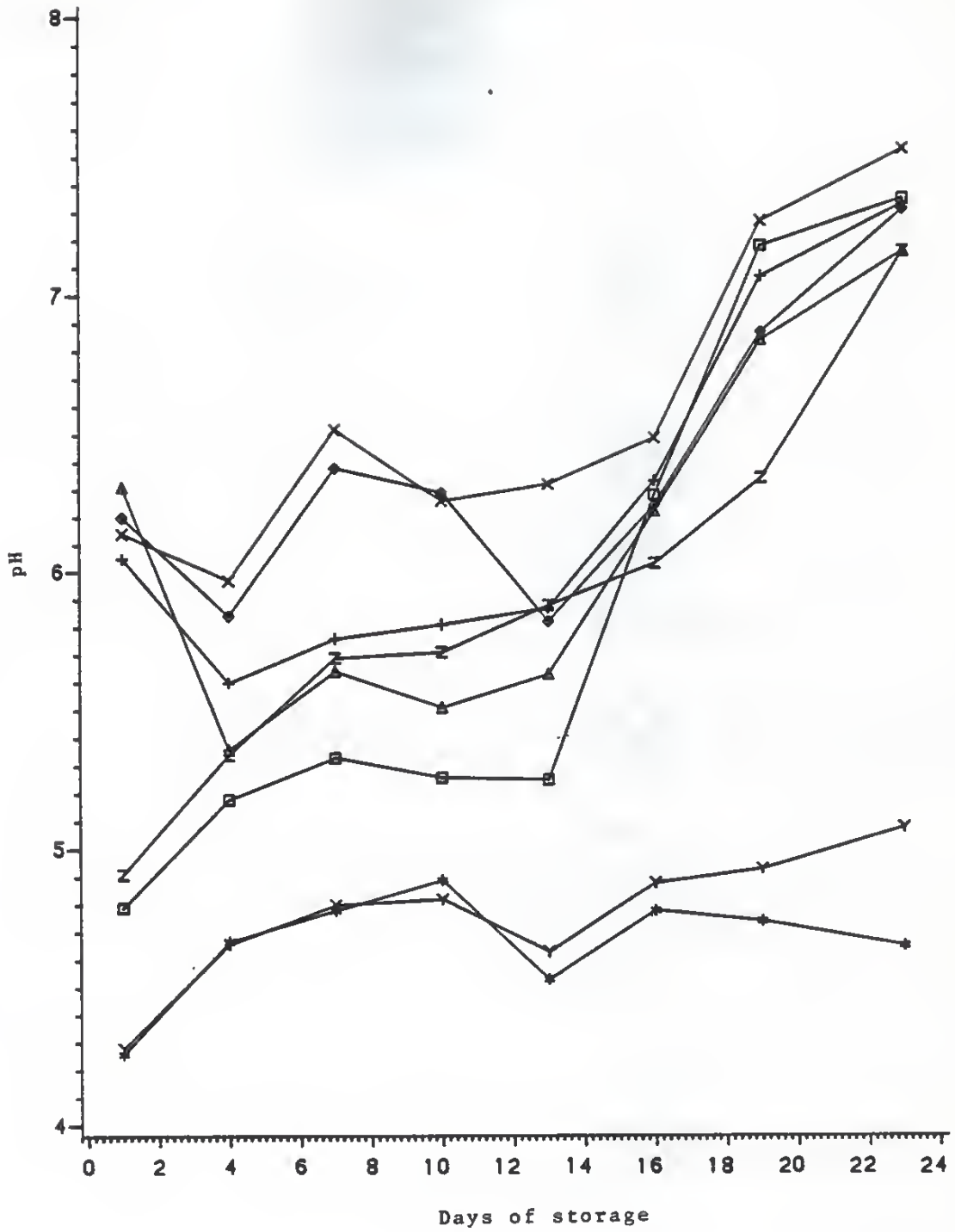
As expected, the bacterial load for lactic acid bacteria was lower (Figure 4) than the viable counts (Figure 3) of the soaking solutions during the initial storage. Rehberger et al. (1984) identified microorganisms other than lactic acid bacteria (higher numbers of psychrotrophs, total aerobic counts, coliforms, and some yeasts and molds) in commercially produced tofu.

The lactic acid bacteria increased markedly as storage days increased in all samples except PS+AA. Dotson et al. (1977) identified lactic acid bacteria as responsible for tofu spoilage during storage. The increase in lactic acid bacteria counts also indicated that tofu is a good medium for the growth of this microorganism.

pH of the soaking solutions fluctuated as the storage period increased (Figure 5; Appendix, Table A-5). The increase in lactic acid bacteria may have contributed to decomposition of soybean protein in the tofu. Fujii et al. (1978) speculated that protein decomposition in soybean curd

Figure 5. Effect of least squares means for pH for immersion solutions of tofu




8
7
6
5
4
pH
0
2
4
6
8
10
12
14
16
18
20
22
24
Days of storage

affected pH. They hypothesized that at the first stages of microbial growth primarily acidic compounds are produced, and basic compounds form as proteins are putrefied. This could account for pH fluctuations observed in the present study.

Chemical analysis

pH. After only one day of storage, immersion solutions of AA, CA, or in combination with PS were lower in pH ($p < 0.05$) than were the other three chemical treatments (Figure 5). Immersion solutions of PS, PS+TBHQ, and TBHQ were higher in pH and approached that found in the control (pH=6.3). The control showed the largest pH decrease in the study (0.97 units), and it occurred between the first and fourth days of storage. Immersion solutions of AA, CA or in combination with PS showed a small increase in pH after four days of storage (approximately 0.4-0.43 units) but these solutions were lower in pH than all other treatments ($p < 0.05$). The remaining solutions showed a small decrease in pH from the first to fourth day of storage, but were higher than the control in pH value. Between the fourth and seven days of storage, pH of all immersion solutions containing chemical preservatives showed increases in pH values (0.15-0.55) with the exception of CA which remained the same. No distinct trends were evident in pH between storage days seven and ten. However,

all immersion solutions showed an increase in pH on the sixteenth day of storage and throughout the remainder of the 23-day study. Storage solutions of AA and PS+AA were lower in pH than the other storage solutions throughout the entire study ($p < 0.05$).

In general, pH findings were in agreement with Fujii et al. (1978) who found that pH in soybean curd initially decreased and then increased because of putrefaction. Acidic compounds were produced initially from decomposition of soybean protein and then basic compounds were produced during the middle and end of the storage period.

Soaking solutions of AA and PS+AA retained acidic pH values (pH=4-5) during the 23-day storage period. However, the remaining chemical additives used to prepare immersion solutions for tofu storage showed a trend of increasing pH from initial values of 4.8-6.2 to final values of 7.2-7.5. There was no significant pH differences between the remaining immersion solutions and the control on the sixteenth, nineteenth, and twenty-third days of storage, when pH of the control was 6.2, 6.8, and 7.2, respectively.

Titratable acidity. Generally, the lactic acid production increased slowly with storage time (Figure 6; Appendix, Table A-6). Chemical additives used in soaking solutions influenced lactic acid production by the lactic acid bacteria.

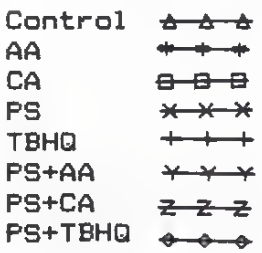
TBHQ, PS+TBHQ, and distilled water (control) showed the highest amounts of lactic acid production after the fourth day of storage and through the testing period when compared to the remaining immersion solutions. PS had an inhibitory effect on lactic acid production when used alone or in combination with AA or CA. These results also paralleled the findings of Borg et al. (1955) who reported that sorbic acid retarded the growth and acid production by the acid-forming bacteria in cucumber pickling.

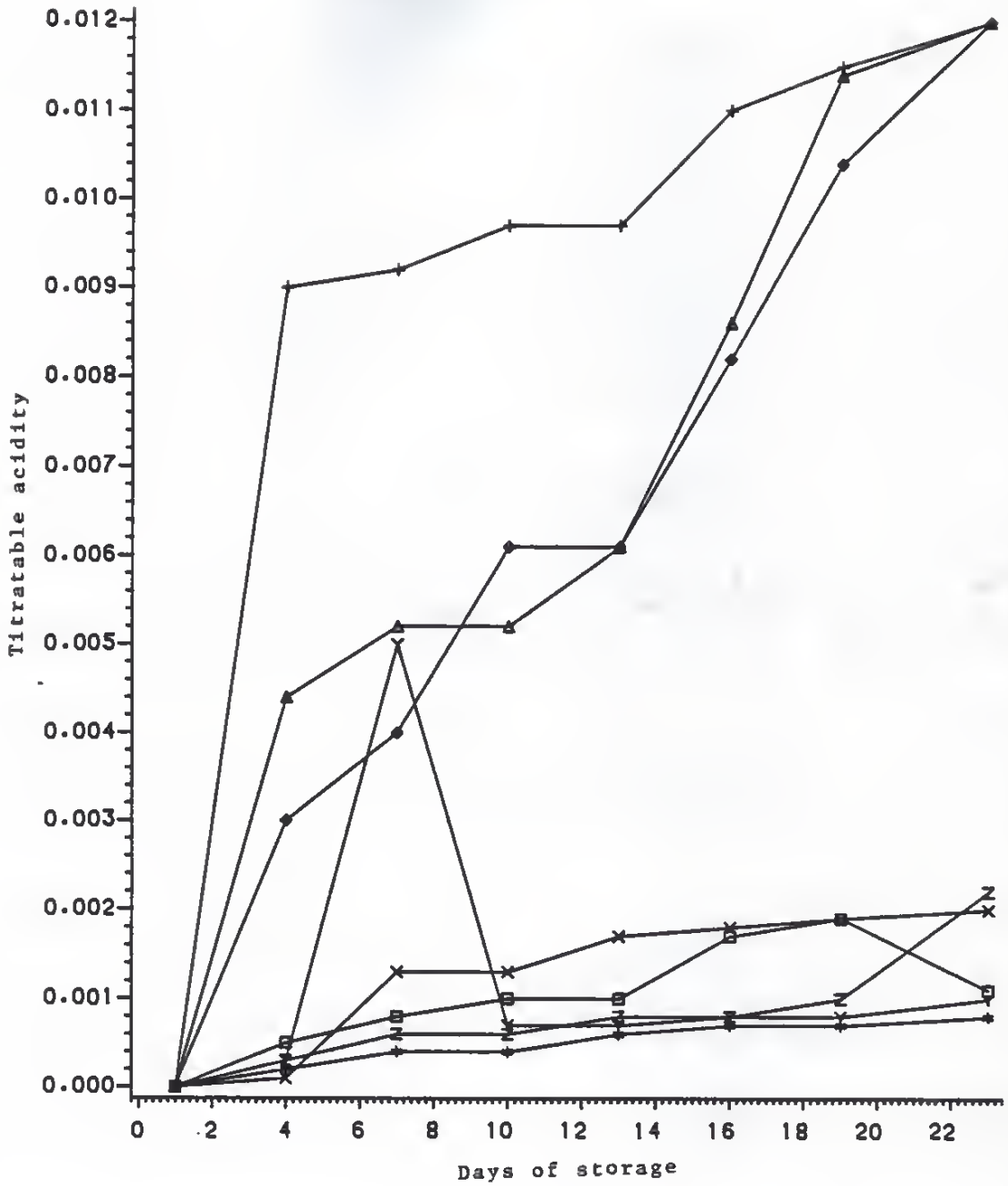
Correlations among measurements. According to Falkner's (1962) analysis, the correlation between pH and titratable acidity would be classified as moderate. Falkner (1962) considered the relationship between variates low when the correlation coefficient, regardless of sign, falls within the range of 0.00 to 0.39, moderate for 0.40 to 0.79, and high for 0.80 and above. The correlation coefficient of pH vs titratable acidity was 0.59 ($p < 0.01$). Correlation coefficients between pH and viable cell counts ($r=0.77$) and pH and lactic acid bacteria counts ($r = 0.71$) were moderate ($p < 0.001$).

Sensory analysis

Analysis of variance for sensory characteristics of tofu stored in immersion solutions is shown in Table 4. Treatment,

Figure 6. Effect of storage on least squares means for percentage titratable acidity as lactic acid in immersion solutions of tofu




0.012
0.011
0.010
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
Titratable acidity
0
2
4
6
8
10
12
14
16
18
20
22
Days of storage

i.e., presence of chemical preservatives in immersion solutions, was found to significantly influence total flavor, tofu flavor, and sourness scores after one day of storage. Total flavor and sourness were the attributes affected significantly following nine days of storage. Treatment was found to significantly influence sourness and texture (firmness) after sixteen days storage and at the end of twenty-three days. Least square means attributed to each treatment for sensory characteristics of tofu during twenty-three days of storage are shown in Table 5.

Total flavor. Tofu stored in the immersion solutions containing AA and PS+AA had the greatest total flavor intensity (more than the freshly prepared sample) after one and nine days of storage ($p < 0.05$). There were no significant differences in total flavor found in tofu stored in the other immersion solutions after one day of storage, and all were scored about the same as the control in total flavor intensity. The lowest intensities of total flavor for Day 16 were noted in tofu immersed in PS, PS+CA, and TBHQ solutions ($p < 0.05$), although they were scored near the total intensity of the control. After 16 and 23 days of storage, there were no significant differences observed in total flavor intensity of tofu immersed in any of the seven

Table 4. Mean squares and F-values[a] for sensory characteristics of tofu stored in immersion solutions

Source of Variation	df	Total flavor	Tofu flavor	Sourness	Texture
			One day storage		
Block	6	0.12	0.21	0.01	0.30
Treatment	6	0.63 * (5.44)	0.58 * (5.02)	0.86 ** (7.22)	0.21 (0.91)
Error	8	0.11	0.11	0.95	0.23
			Nine days storage		
Block	6	0.35	0.20	0.05	0.22
Treatment	6	0.86 *** (24.61)	0.29 (2.80)	1.38 *** (33.52)	0.43 (1.12)
Error	8	0.04	0.10	0.04	3.19
			Sixteen days storage		
Block	6	0.07	0.06	0.05	0.25
Treatment	6	0.24 (2.01)	0.06 (1.24)	0.05 ** (11.34)	0.25 ** (7.09)
Error	8	0.12	0.10	0.04	0.18
			Twenty-three days storage		
Block	6	0.08	0.04	0.08	0.69
Treatment	6	0.12 (1.87)	0.07 (1.84)	0.70 ** (6.70)	3.15 ** (6.69)
Error	8	0.06	0.04	0.10	0.47

[a] F-values in parentheses

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Table 5. Least squares means for sensory characteristics of tofu stored in immersion solutions[a]

Treatments	Storage days 1	9	16	23
		Total flavor		
AA	3.59 bc	3.29 de	3.87	3.62
CA	3.25 a	3.23 c	3.80	3.50
PS	3.16 a	2.61 ab	3.66	3.60
TBHQ	3.05 a	2.99 bc	3.59	3.86
PS+AA	4.25 c	4.11 a	3.80	4.00
PS+CA	2.62 a	2.54 a	3.25	3.55
PS+TBHQ	3.02 a	2.87 abc	4.30	4.05
		Tofu flavor		
AA	1.90 bcdef	1.28	1.25	1.30
CA	2.70 f	1.87	1.17	1.68
PS	2.55 ef	2.21	1.60	1.30
TBHQ	1.98 bcdef	1.44	1.46	1.37
PS+AA	1.24 a	1.21	1.48	1.28
PS+CA	2.27 cdef	1.68	1.60	1.49
PS+TBHQ	2.44 def	1.59	0.98	1.18
		Sourness		
AA	3.99 bc	4.72 c	4.63 f	4.43 bc
CA	3.13 a	3.39 a	3.96 cd	3.50 a
PS	2.85 a	3.15 a	3.84 bcd	3.29 a
TBHQ	2.65 a	3.10 a	3.75 abcd	3.40 a
PS+AA	4.22 c	4.65 bc	4.44 ef	4.60 c
PS+CA	2.93 a	3.08 a	3.39 a	3.29 a
PS+TBHQ	2.96 a	2.96 a	4.06 de	3.67 a
		Texture		
AA	4.12	3.79	4.56 c	4.85 bc
CA	3.67	3.10	2.71 a	3.33 a
PS	3.70	2.60	3.18 a	2.73 a
TBHQ	3.92	2.82	3.09 a	2.21 a
PS+AA	3.78	3.48	4.18 bc	4.87 c
PS+CA	3.50	2.70	3.11 a	3.09 a
PS+TBHQ	3.18	2.89	2.61 a	2.37 a

[a] Each value is a mean for 18 determinations. Means in a column bearing different letters differ significantly ($p < 0.05$)

treatment solutions. The chemicals used in preparing the immersion solutions could affect the overall perceptions of taste and aroma in the stored tofu. Panelists described the tofu stored in PS+TBHQ as having an "herby" aromatic characteristic when swallowed. Some panelists described the tofu stored in TBHQ as having a chalky mouthfeel. Tofu stored in AA and PS+AA were described by some panelists as having an acidic odor and sour taste. As stated earlier in this thesis, acetic acid is a short chain fatty acid which is very volatile and has an acidic off-flavor when used in foods (Branen et al., 1980). A future recommendation for the use of acetic acid in the immersion solution would be to lower the concentration of acetic acid to less than 0.5 % in order to prevent acidic off-flavors in the stored tofu.

Tofu flavor. Regardless of storage time or type of chemical additives used in soaking solutions, all tofu samples were scored as having less tofu flavor than the freshly prepared sample. Tofu flavor was most intense in the sample immersed in the CA solution after one day, and least intense in those immersed in AA or PS+AA ($p < 0.05$). No significant differences were found in tofu flavor intensity among the seven treatment solutions on the ninth day of storage and thereafter. Generally, tofu flavor intensity decreased as storage days increased. Dotson et al. (1977)

also found a decrease in tofu flavor with increased storage time; however, in their study, tofu was stored in water alone.

Sourness. After one day of storage tofu immersed in AA and PS + AA solutions were more sour ($p < 0.01$) than tofu stored in other immersion solutions or in the freshly made product. Treatment of tofu in TBHQ produced lowest sourness intensity scores when compared with other immersion solutions. However, there were no significant differences in sourness among the five remaining treatment solutions.

On Day nine, there were no significant differences in sourness among tofu samples except that tofu immersed in AA and PS+AA were judged more sour, and also more sour than the freshly prepared sample ($p < 0.001$). These treatment solutions consistently produced lower pH values than did the other chemical preservatives (Figure 5).

By the sixteenth day of storage tofu immersed in PS+CA was scored lower in sour intensity than the other samples ($p < 0.01$). Tofu immersed in AA or PS+AA were most sour, although scores were slightly lower in sourness than they had been on the ninth day. Generally, pH of immersion solutions increased also after the thirteenth day of storage (Figure 5).

On the final day of sensory testing sensory scores for sourness intensity in tofu decreased except for PS+AA. This paralleled findings for pH of immersion solutions which were highest at the end of the storage period (Figure 5). Tofu stored in PS+AA had the highest sensory score for sourness ($p < 0.01$) followed by tofu immersed in AA. All other treatment solutions produced tofu with significantly lower sourness scores that approached the intensity of freshly prepared tofu.

Texture. After one day of storage, as expected, there were no significant differences in sensory scores for firmness in any treated samples of tofu.

On storage day nine, sensory scores for firmness tended to decrease for all tofu treatments. However, no significant differences existed among samples of tofu in the different immersion solutions.

On the sixteenth day and again on the twenty-third day of storage tofu immersed in AA and PS+AA solutions produced the firmest samples ($p < 0.01$). Tofu stored in PS+TBHQ solution was the softest sample compared to other treatments on Day 16, while TBHQ solutions produced the softest sample on Day 23. However, all samples were scored as softer than AA and PS+AA samples ($p < 0.01$) and Days 16 and 23.

Correlations among sensory characteristics. The nature of the experimental design allowed for comparisons of sensory characteristics with different treatment solutions for a storage period of either 1, 9, 16, or 23 days. In order to determine if correlations existed among the different sensory characteristics, the sensory scores were combined for the seven treatments and four storage periods when sensory evaluation was conducted to obtain twenty-eight determinations. Values are presented graphically in the Appendix (Figures A-2--A-5) and correlation coefficients are given in Table 6.

When total flavor was correlated with tofu flavor intensity, there was a correlation coefficient of 0.7 ($p < 0.001$). This would indicate that total flavor intensity could be used as an indicator to determine tofu flavor of tofu during storage. However, several factors should be considered prior to extrapolation from these data. Tofu has a bland taste and storage in immersion solutions consistently produced low scores for tofu flavor in multiple comparison tests with freshly prepared samples. Total flavor was judged consistently as more than the control. When using differrent types of chemicals in immersion solutions for storage, tofu could absorb flavors of the chemicals. Depending on their intensity they could diminish tofu flavor or mask changes

Table 6. Correlation coefficients for physical measurements and sensory characteristics for soybean curd

Total flavor score vs	
Tofu flavor score	0.70 ***
Sourness score	0.41 *
Tofu flavor score vs	
Sourness score	0.34 ns
pH vs	
Viable cell count	0.77 ***
Lactic acid bacteria count	0.71 ***
Titratable acidity	0.59 **
Physical hardness value vs	
Texture (firmness) score	0.62 **

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

in tofu flavor. Furthermore, according to Falkner's analysis (1962) the correlation between total flavor intensity and tofu flavor intensiy would be moderate.

Correlation coefficients calculated for other sensory characteristics for tofu in different immersion solutions indicated that there was a moderate significant relationship ($p < 0.05$) between sourness and total flavor intensity. Sour flavors in tofu usually are associated with decreasing quality. Data from this study might be related to decreased quality, but also would be attributed to acidic compounds used in some immersion solutions.

A correlation coefficient of 0.34 resulted between sourness and tofu flavor intensity. Tofu flavor intensity was dependent on the type of preservatives used in immersion solutions which affected the overall perception of taste. With low correlation coefficient for tofu flavor and sourness which was not significant, tofu flavor could not be used as an accurate determinant of sourness in tofu with increasing time of storage or vice versa.

Instrumental assessment of texture. Means squares and F-values for Instron measurements for hardness (kg) of tofu stored in immersion solutions for 1, 9, 16, and 23 days are reported in Table 7. No significant differences in hardness

were observed among the tofu samples immersed in different chemical preservative solutions on day one or day nine when tested with an Instron Universal Testing Machine. However, there were significant differences in hardness ($p < 0.01$) when tofu was stored for 16 days (Table 7). Tofu stored in PS was the softest treated sample, and comparable in hardness to the tofu stored in water, when tested with an Instron Universal Testing Machine (Table 8). Tofu stored in PS+AA had the highest degree of hardness. No significant differences in hardness were observed among tofu samples stored in immersion solutions of AA, PS+AA, and PS+CA solutions.

On Day 23 there were significant differences ($p < 0.001$) in textural hardness attributable to treatment (Table 7). Tofu stored in AA and PS+AA were firmest when compared with tofu stored in TBHQ which was softest (Table 8). However, there was no significant differences in hardness of tofu immersed in PS or TBHQ after 23 days of storage. CA, PS+CA, and PS+TBHQ produced tofu intermediate in hardness among the previously mentioned samples. No significant differences in hardness was found in the tofu stored in their solutions.

Regardless of storage time or type of chemical additives used in soaking solutions, all tofu samples were scored by panel as about the same as fresh tofu in firmness for the

Table 7. Mean squares and F-values for Instron measurements for hardness of tofu stored in immersion solutions

Days of storage	Source of variation	df	Mean square	F-value
1	Block	6	0.003	
	Treatment	6	0.004	1.54
	Error	8	0.002	
9	Block	6	0.003	
	Treatment	6	0.01	1.11
	Error	8	0.03	
16	Block	6	0.25	**
	Treatment	6	1.28	7.09
	Error	8	0.18	
23	Block	6	0.002	***
	Treatment	6	0.12	30.40
	Error	8	0.004	

** $p < 0.01$
*** $p < 0.001$

Table 8. Least squares means[a] for hardness of tofu using an Instron Universal Testing Machine

	Storage days			
Treatments	1	9	16	23
	Hardness (kg)			
Control	0.27	0.34	0.27 a	0.29 a
AA	0.34	0.38	0.50 de	0.87 e
CA	0.26	0.34	0.47 bcd	0.53 c
PS	0.27	0.24	0.32 a	0.33 ab
TBHQ	0.29	0.29	0.40 abcd	0.30 a
PS+AA	0.35	0.32	0.61 e	0.77 de
PS+CA	0.34	0.33	0.48 cde	0.42 bc
PS+TBHQ	0.28	0.35	0.42 abcd	0.42 bc

[a] Each value is a mean for 6 determinations. Means in a column bearing different letters differ significantly ($p < 0.05$).

first and ninth days of storage (Table 5). Instrumental assessment of hardness showed no significant differences for both one and nine days of storage.

After sixteen days of storage, tofu immersed in AA and PS+AA solutions were firmest when compared with the freshly made tofu ($p < 0.01$). Panel scores did not show significant differences in firmness among the other tofu treatments and the freshly made tofu. However, hardness when measured with an Instron showed significant differences among the tofu treatments and the control (freshly made tofu). Tofu stored in PS, TBHQ, and PS+TBHQ remained about the same as the control in hardness. However, AA, PS+AA, CA, and PS+CA solutions increased in hardness after 16 days ($p < 0.01$).

On the twenty-third day of storage, tofu immersed in AA and PS+AA solutions were scored firmer than tofu stored in other immersion solutions or in the freshly made tofu ($p < 0.01$). There were no significant differences in panel assessment of firmness among tofu stored in other immersion solutions and the fresh sample. Tofu measured with an Instron produced some significant differences among samples in the immersion solutions. Treatment of tofu in PS and TBHQ resulted in about the same degree of hardness as the control. Tofu stored in AA and PS+AA had the hardest texture after 23 days of storage.

When sensory hardness was compared with an instrumental assessment of hardness (Table 6), the correlation coefficient was 0.62 ($p < 0.01$). Texture analysis of hardness using an Instron correlated moderately with the sensory attribute described by panelists as firmness.

CONCLUSIONS

Based on the conditions of this study, one can conclude:

1) Immersion solutions of acetic acid alone and potassium sorbate + acetic acid were lower in viable cell counts and lactic acid bacteria counts than other immersion solutions during the 23-day study.

2) Immersion solutions of citric acid alone and potassium sorbate + citric acid had low viable cell counts and lactic acid bacteria counts through 13 days and then increased markedly.

3) Tertiary-butyl-hydroquinone alone and potassium sorbate + tertiary-butyl-hydroquinone were not effective as antimicrobial agents in immersion solutions for tofu, and resulted in high pH values and high titratable acidity when compared to other chemical preservatives in solution.

4) Potassium sorbate was more effective as an antimicrobial agent when used in acidic media than when used alone.

5) pH of immersion solutions generally decreased through the thirteenth day of storage and then increased through the remainder of the study.

6) pH was lowest in immersion solutions of acetic acid alone and potassium sorbate + acetic acid throughout the storage period.

7) Lactic acid production increased slowly with storage time in immersion solutions containing chemical preservatives other than tertiary-butyl-hydroquinone alone and potassium sorbate + tertiary-butyl-hydroquinone.

8) pH of immersion solutions correlated positively with viable cell count, lactic acid bacteria count, and titratable acidity of the solutions.

9) Intensity of tofu flavor in treated samples was less then the control throughout the 23 day study.

10) Intensity of sourness in all treated samples of tofu was greater than the control on the sixteenth and twenty third days of storage.

11) No differences were attributable to type of preservatives used for firmness of tofu when analyzed by sensory panel or instrumentally through the first nine days of refrigerated storage. After the sixteenth day of storage, tofu was measured by panel or instrument as firmest when immersed in acetic acid alone or potassium sorbate + acetic acid and least firm when immersed in tertiary-butyl-hydroquinone alone or potassium sorbate + tertiary-butly-hydroquinone.

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APPENDIX

Table A-1. Viable cell counts for eight days' storage for water-packed and pasteurized water-packed commercial tofu purchased in Manhattan, Kansas[a]

Days of storage	Viable count (Colony forming units/ml)					
	Retail store					
	A	B	C	D	E	F
	Water-packed tofu				Pasteurized water-packed tofu	
0	3.8×10^{4}	2.0×10^{8}	1.5×10^{7}	1.7×10^{4}	2.8×10^{5}	2.8×10^{5}
2	2.8×10^{7}	5.0×10^{8}	1.6×10^{7}	1.1×10^{6}	2.5×10^{5}	1.7×10^{6}
4	3.7×10^{8}	4.8×10^{8}	1.5×10^{8}	1.6×10^{6}	1.7×10^{7}	1.8×10^{6}
6	2.0×10^{9}	8.7×10^{8}	5.1×10^{8}	6.5×10^{8}	2.8×10^{8}	1.3×10^{8}
8	4.9×10^{9}	9.8×10^{8}	6.0×10^{8}	7.3×10^{8}	3.7×10^{8}	3.4×10^{8}

[a] Each value is a mean of four determinations.

Table A-2. pH[a] of immersion solutions for eight days' storage for water-packed and pasteurized water-packed commercial tofu purchased in Manhattan, Kansas

Days of storage	Retail store A	B	C	D	E	F
	Water-packed tofu				Pasteurized water-packed tofu	
0	4.76	4.43	4.60	6.64	6.11	5.42
2	4.66	4.47	4.70	6.41	6.02	4.57
4	4.33	4.42	4.60	5.70	6.45	4.62
6	4.32	4.44	4.66	5.35	5.68	4.50
8	4.69	4.42	4.60	5.25	5.25	4.44

[a] Each value is a mean for four determinations.

Table A-3. Least square means[a] for viable counts in immersion solutions of tofu during storage

Treatments	Viable count (colony forming units/ml) — Storage days							
	1	4	7	10	13	16	19	23
Control	7.6×10^{3}	7.9×10^{5}	8.3×10^{6}	3.1×10^{7}	1.5×10^{8}	6.5×10^{8}	4.0×10^{9}	1.3×10^{11}
AA	2.5×10^{2}	1.2×10^{2}	1.4×10^{2}	2.3×10^{2}	8.1×10^{2}	1.1×10^{4}	4.4×10^{4}	1.1×10^{5}
CA	1.3×10^{3}	4.9×10^{4}	5.5×10^{5}	6.9×10^{6}	5.1×10^{6}	2.1×10^{7}	3.0×10^{8}	1.3×10^{9}
PS	6.8×10^{2}	5.8×10^{4}	4.6×10^{6}	2.6×10^{7}	1.1×10^{8}	1.1×10^{9}	5.8×10^{9}	5.8×10^{10}
TBHQ	6.8×10^{3}	5.8×10^{5}	1.8×10^{7}	8.9×10^{7}	2.0×10^{8}	4.6×10^{8}	1.5×10^{9}	1.1×10^{10}
PS+AA	3.7×10^{2}	9.1×10^{1}	2.0×10^{2}	4.7×10^{2}	4.7×10^{2}	1.4×10^{3}	3.7×10^{3}	1.8×10^{4}
PS+CA	8.9×10^{2}	1.5×10^{4}	1.3×10^{5}	6.0×10^{5}	6.9×10^{6}	8.7×10^{7}	3.1×10^{8}	2.5×10^{9}
PS+TBHQ	7.4×10^{3}	1.3×10^{6}	3.2×10^{6}	1.1×10^{7}	6.9×10^{7}	5.9×10^{8}	3.5×10^{9}	1.7×10^{10}

[a] Each value is a mean for 10 determinations.

Table A-4. Least square means[a] for lactic acid bacteria counts in immersion solutions of tofu during storage

Treatments	(Lactic acid bacteria no./ml) Storage days							
	1	4	7	10	13	16	19	23
Control	3.8×10^{2}	3.5×10^{4}	4.5×10^{5}	2.6×10^{6}	2.2×10^{7}	6.8×10^{7}	2.5×10^{8}	7.4×10^{8}
AA	5.0×10^{1}	3.2×10^{1}	4.0×10^{1}	1.3×10^{1}	4.1×10^{2}	1.9×10^{3}	4.5×10^{3}	2.0×10^{4}
CA	2.1×10^{2}	1.3×10^{4}	1.5×10^{5}	2.9×10^{5}	8.7×10^{5}	7.8×10^{6}	4.2×10^{7}	1.8×10^{8}
PS	2.7×10^{2}	1.0×10^{4}	6.2×10^{4}	4.3×10^{5}	3.7×10^{6}	7.1×10^{7}	2.0×10^{8}	8.7×10^{8}
TBHQ	7.6×10^{2}	6.9×10^{4}	1.7×10^{5}	2.1×10^{6}	6.0×10^{6}	2.8×10^{7}	9.1×10^{7}	1.5×10^{8}
PS+AA	6.4×10^{1}	1.1×10^{2}	2.6×10^{2}	7.2×10^{2}	1.2×10^{3}	1.1×10^{3}	1.2×10^{3}	1.1×10^{3}
PS+CA	6.3×10^{1}	9.5×10^{2}	2.0×10^{3}	9.8×10^{3}	9.5×10^{4}	2.9×10^{6}	1.1×10^{7}	1.3×10^{7}
PS+TBHQ	3.6×10^{2}	1.5×10^{4}	4.3×10^{4}	4.4×10^{5}	2.7×10^{6}	3.8×10^{7}	8.5×10^{7}	1.4×10^{9}

[a] Each value is a mean for 10 determinations.

Table A-5. Least square means[a] for pH of immersion solutions of tofu during storage

Treatments	Storage days							
	1	4	7	10	13	16	19	23
Control	6.31 cde	5.36 de	5.64 bcd	5.51 cde	5.63 cdef	6.22 cdefg	6.84 cdefg	7.16 cdefg
AA	4.26 a	4.67 a	4.78 a	4.89 ab	4.53 a	4.78 a	4.74 a	4.65 a
CA	4.78 a	5.34 cd	5.33 abcd	5.26 bcd	5.25 bc	6.28 efg	7.17 fg	7.35 fg
PS	6.14 de	5.97 g	6.52 f	6.26 fg	6.32 g	6.49 g	7.27 g	7.53 g
TBHQ	6.05 bcde	5.60 ef	5.76 de	5.81 ef	5.87 efg	6.33 fg	7.07 efg	7.33 defg
PS+AA	4.28 a	4.66 a	4.80 a	4.82 a	4.63 ab	4.88 a	4.93 a	5.08 a
PS+CA	4.91 a	5.18 bcde	5.69 cde	5.71 def	5.88 fg	6.03 bcdefg	6.34 bcd	7.16 bcdefg
PS+TBHQ	6.20 e	5.84 fg	6.38 e	6.29 g	5.82 defg	6.24 defg	6.87 defg	7.33 efg

[a] Each value is a mean for 10 determinations. Means in a column bearing different letters differ significantly ($p < 0.05$).

Table A-6. Least square means[a] for percentage titratable acidity as lactic acid of immersion solutions of tofu during storage

Treatments	Storage days							
	1	4	7	10	13	16	19	23
Control	0.0000	0.0044 d	0.0052 f	0.0052 fg	0.0061 de	0.0086 e	0.0114 de	0.0120 d
AA	0.0000	0.0002 ab	0.0004 a	0.0004 a	0.0006 a	0.0007 a	0.0007 a	0.0011 a
CA	0.0000	0.0003 ab	0.0008 cd	0.0006 bc	0.0008 ab	0.0008 a	0.0010 a	0.0022 c
PS	0.0000	0.0001 a	0.0013 d	0.0013 e	0.0017 c	0.0018 c	0.0019 b	0.0020 bc
TBHQ	0.0000	0.0090 e	0.0092 g	0.0097 h	0.0097 f	0.0110 f	0.0115 e	0.0120 d
PS+AA	0.0000	0.0003 ab	0.0007 bc	0.0007 cd	0.0007 a	0.0008 a	0.0008 a	0.0010 a
PS+CA	0.0000	0.0005 b	0.0008 cd	0.0010 de	0.0010 bc	0.0017 bc	0.0019 b	0.0022 c
PS+TBHQ	0.0000	0.0030 c	0.0040 e	0.0061 g	0.0061 e	0.0082 d	0.0104 c	0.0120 d

[a] Each value is a mean for 10 determinations. Means in a column bearing different letters differ significantly ($p < 0.05$).

Figure A-1. Sample score card used for sensory analysis of soybean curd

DATE:_____ COMPARISONS TO CONTROL JUDGE NO._____

	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#
Total Flavor																					
Much More																					
More																					
About The Same																					
Less																					
Much Less																					
Comments:																					

	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#
TofU Flavor																					
Much More																					
More																					
About The Same																					
Less																					
Much Less																					
Comments:																					

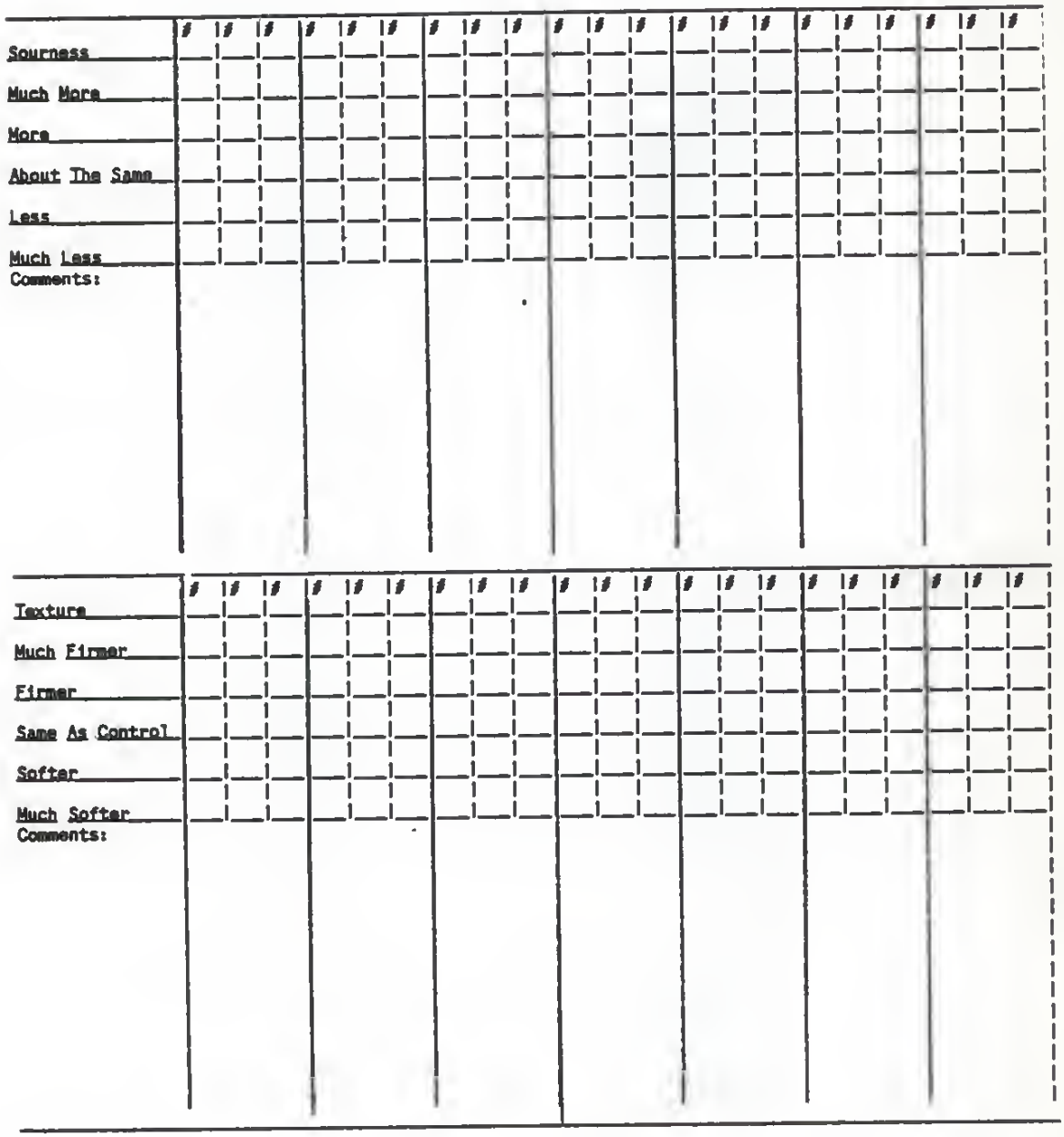
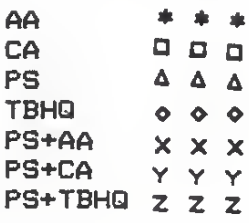

Sourness
Much More
More
About The Same
Less
Much Less
Comments:
Texture
Much Firmer
Firmer
Same As Control
Softer
Much Softer
Comments:

Figure A-2. Relationship of sensory total flavor vs sensory tofu flavor in immersion solutions of tofu



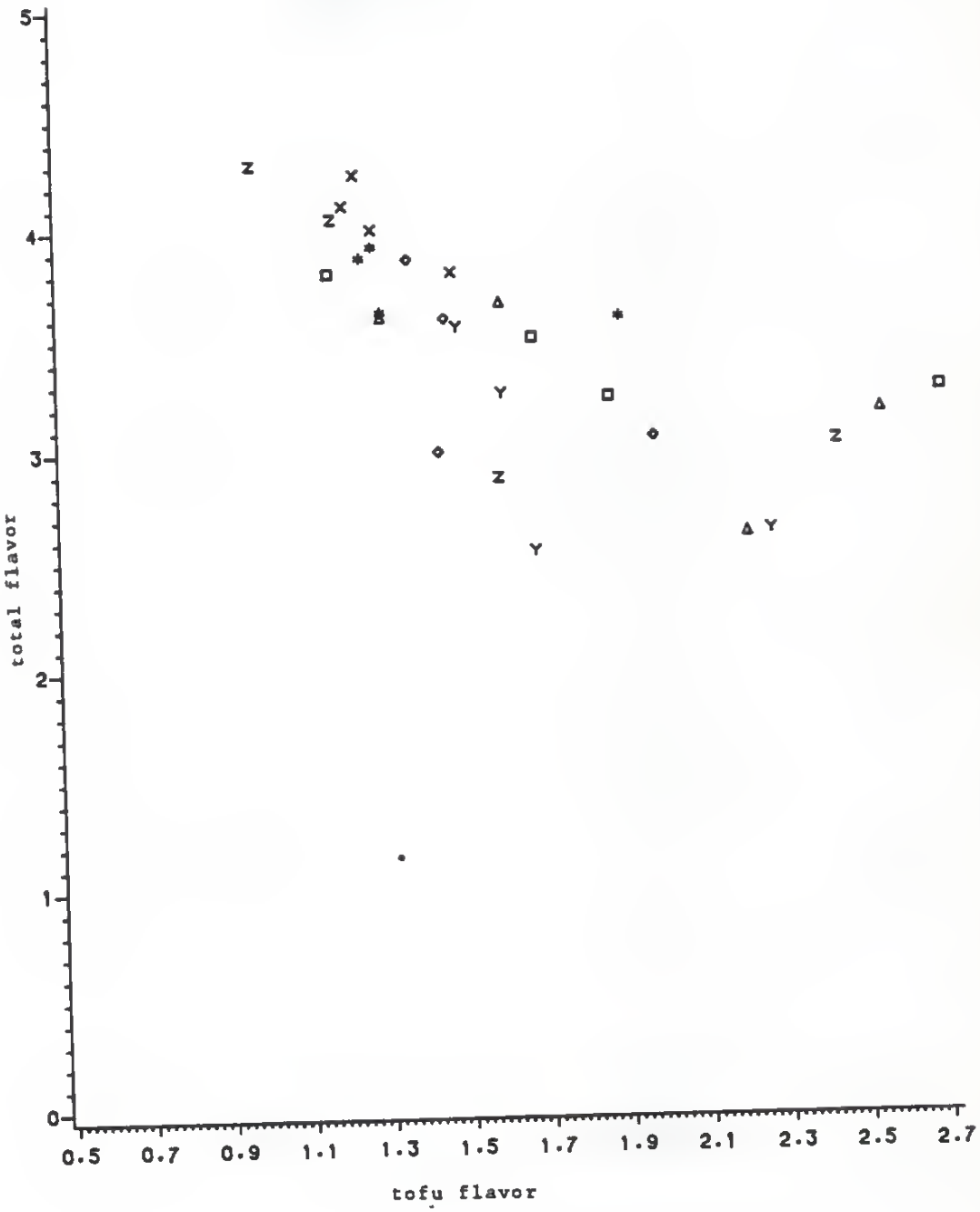
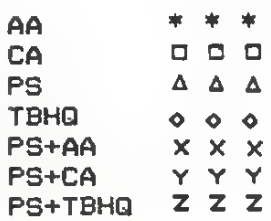

total flavor
tofu flavor
0
1
2
3
4
5
0.5
0.7
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
2.7

Figure A-3. Relationship of sensory tofu flavor vs sensory sourness in immersion solutions of tofu



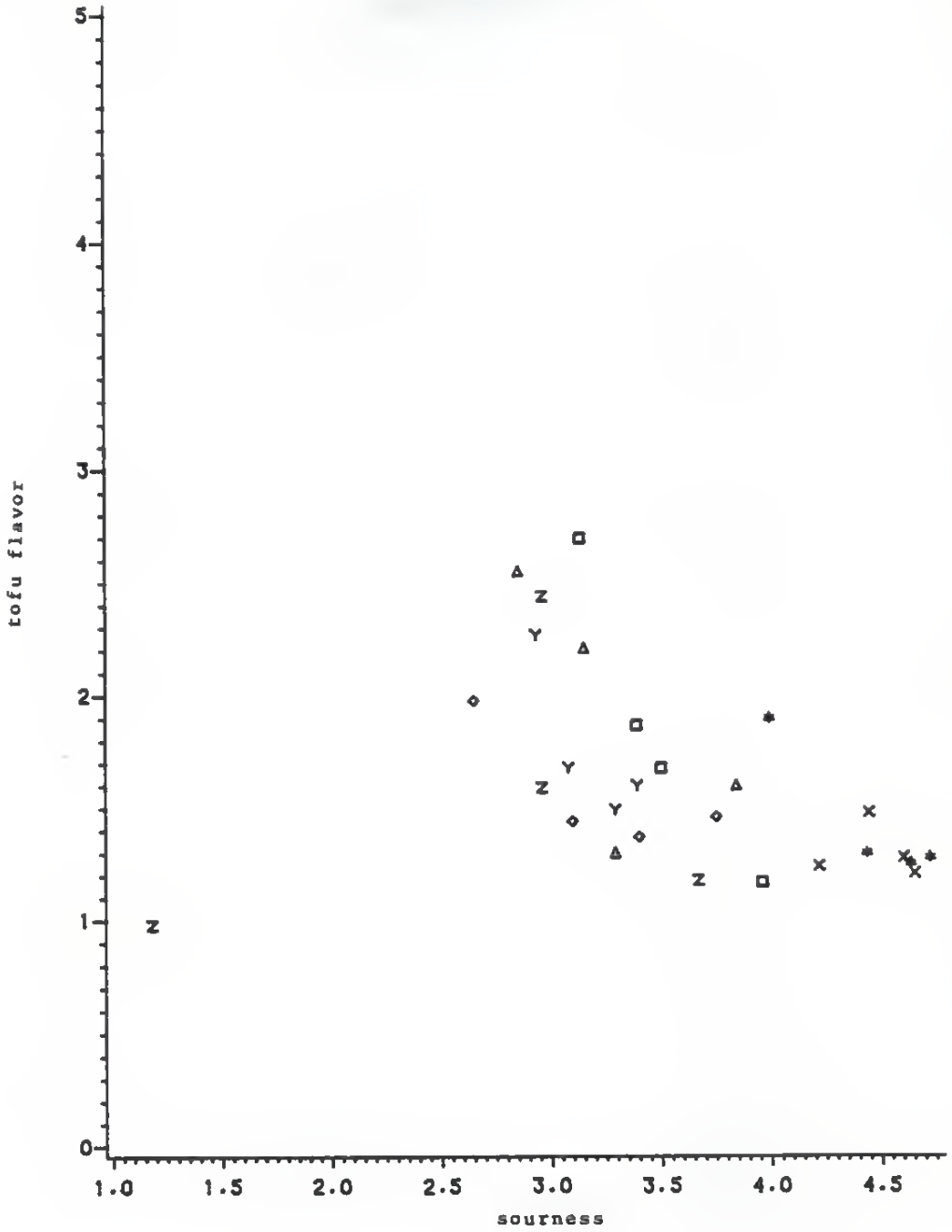
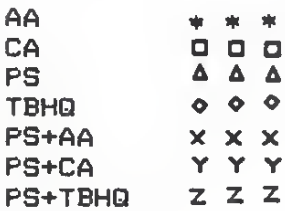

tofu flavor
5
4
3
2
1
0
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
sourness

Figure A-4. Relationship of sensory total flavor vs sensory sourness in immersion solutions of tofu



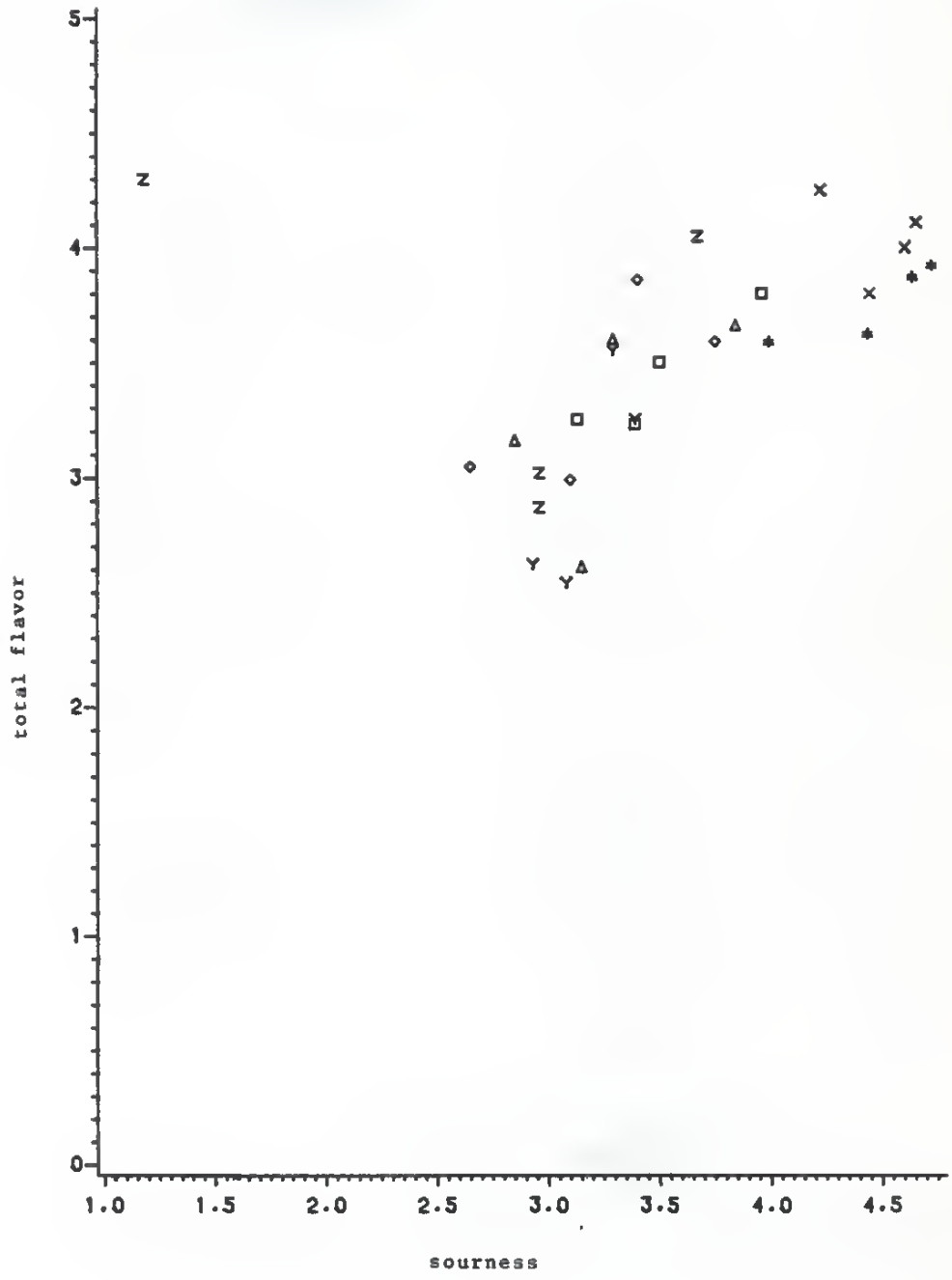
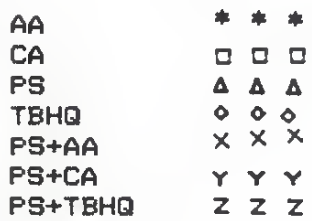
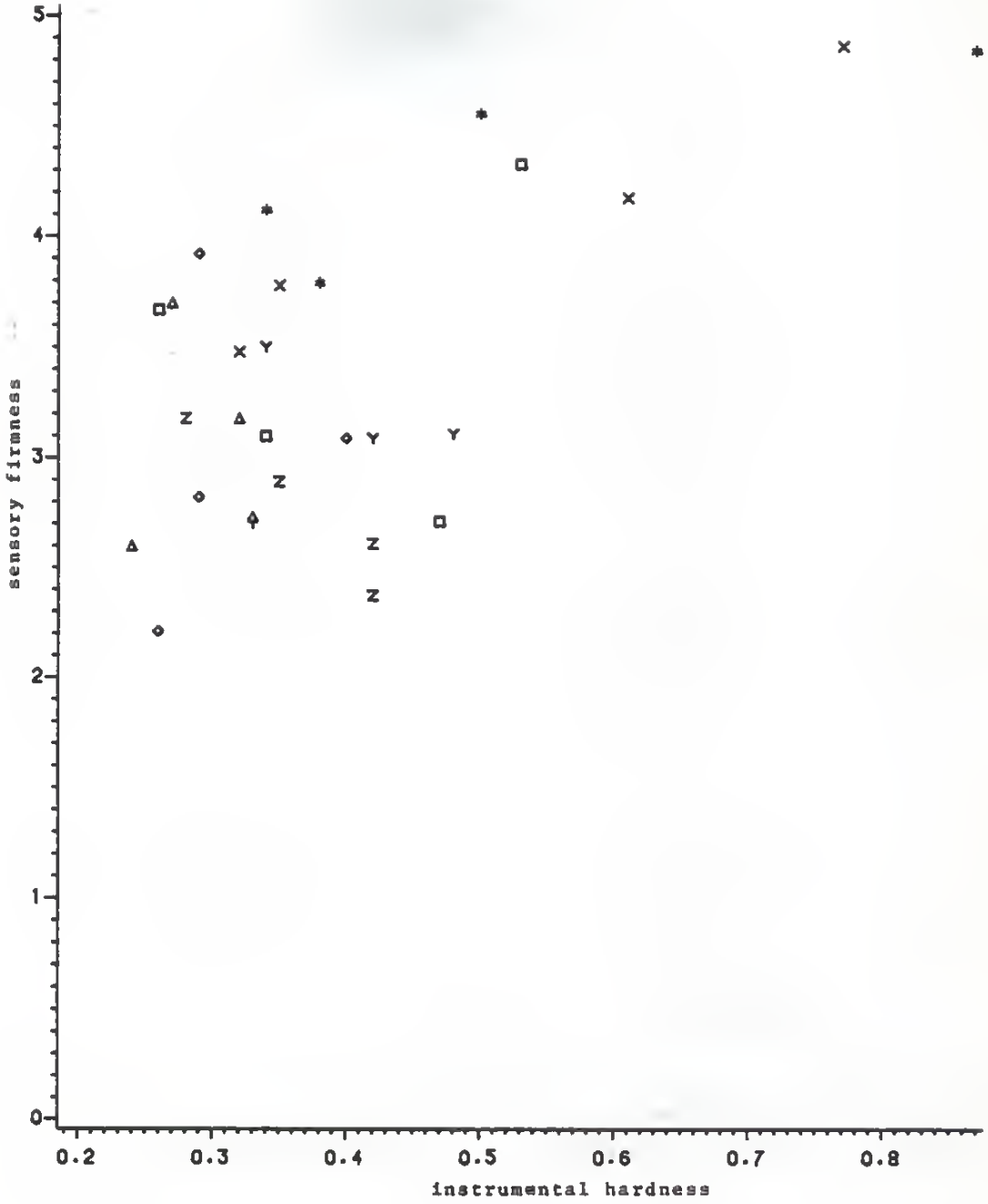

total flavor
sourness
0
1
2
3
4
5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5

Figure A-5. Relationship of sensory firmness vs instrumental hardness of tofu in immersion solutions




sensory firmness
instrumental hardness
0
1
2
3
4
5
0.2
0.3
0.4
0.5
0.6
0.7
0.8

EFFECTS OF USING CHEMICAL PRESERVATIVES TO EXTEND THE SHELF LIFE OF SOYBEAN CURD

by

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Manhattan, Kansas

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Effects of chemical preservatives on the shelf life of soybean curd (tofu) were investigated with microbiological analyses (viable cell counts and lactic acid bacteria counts), pH, titratable acidity, instrumental assessment of texture (hardness), and sensory analysis for total flavor, tofu flavor, sourness, and textural firmness. Tofu was made on a laboratory scale and immersed in solutions containing no additive; 0.5 % acetic acid (AA); 0.1 % citric acid (CA); 0.15 % potassium sorbate (PS); 0.005 % tertiary-butyl-hydroquinone (TBHQ); 0.15 % PS + 0.5 % AA; 0.15 % PS + 0.1 % CA; or 0.15 % PS + 0.005 % TBHQ. Samples were stored at 10-15 ^{o}C for a period of 23 days. Five replications were made for microbiological analyses, pH, and titratable acidity, and three replications were made for sensory analysis and instrumental assessment of texture.

Immersion solutions of AA and PS+AA were effective in controlling bacterial growth during the 23-day storage period. CA and PS+CA were effective as antimicrobial agents only during 13 days of storage. The remaining chemical preservatives were not effective in controlling bacterial growth. Lactic acid production in the immersion solutions increased slowly with storage time except for TBHQ and PS+TBHQ which increased markedly. Generally, pH of the immersion solutions decreased through the 13 days of storage

and then increased through the remainder of the study. pH of immersion solutions correlated positively with viable cell count, lactic acid bacteria count, and titratable acidity of the solutions. Tofu which had been stored in immersion solutions of AA and PS+AA had greatest total flavor after one and nine days of storage ($p < 0.05$). Panelists scored tofu in those solutions, however, as having an acidic odor and sour taste. Tofu from solutions of AA and PS+AA had the highest scores in sourness throughout the 23-days of storage ($p < 0.01$). All tofu samples were scored lower than freshly, prepared tofu in tofu flavor regardless of storage time or type of chemical additives used in soaking solutions. No significant differences were observed in firmness of tofu when analyzed by sensory panel or instrumentally for the first nine days of the storage study. After the sixteenth day of storage, tofu was assessed by panel and instrument as firmest after immersion in AA or PS+AA ($p < 0.01$) and least firm when immersed in TBHQ and PS+TBHQ ($p < 0.01$). Texture analysis of hardness using an Instron Univeral Testing Machine correlated moderately with the sensory scores for firmness ($r=0.62$).