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Comparative Studies on Some Quality Attributes of Firm Tofu Sterilized with Traditional and Autoclaving Methods

TZOU-CHI HUANG,*,† HUI-YIN FU,‡ AND CHI-TANG HO§

Department of Food Science, National Pingtung University of Science and Technology, 912 Pingtung, Taiwan; Department of Food Sanitation, Tajen Institute of Technology, 912 Pingtung, Taiwan; and Department of Food Science, Rutgers University, 65 Dudley Road, New Brunswick, New Jersey 08901-8520

An innovative retort cooking method was developed for firm tofu sterilization to replace the traditional hydrogen peroxide treatment. The caramelized firm tofu was sterilized by autoclaving at 105 °C for 20 min, which led to a 3 log cycle reduction of total plate count. Shelf life of the processed firm tofu was extended at least 3 months. As compared with chemical treatment, thermal treatment degraded total and individual isoflavones to a lesser extent, and only minor changes were observed. The percent DPPH free radical scavenging capacity of autoclaved firm tofu was significantly higher than that of the hydrogen peroxide treated samples. Even after prolonged heat treatment, the three-dimensional network structure of the autoclaved firm tofu did not change significantly. Texture profile analysis and sensory evaluation confirmed autoclaved firm tofu to be acceptable.

KEYWORDS: Soy isoflavones; tofu; quality attributes; isoflavone stability

INTRODUCTION

Diets rich in soy foods containing a high concentration of isoflavones are associated with a decrease in the incidence of several chronic inflammatory diseases (*1*). Soy isoflavones appear to prevent the progression of arteriosclerosis through multiple interactions, including lowering of plasma lipids and lipoproteins, increased vasodilatation, and decreased activation of blood platelets and vascular smooth muscle cells (*2*).

Four basic types of tofu are manufactured in Taiwan, namely, soft, regular, firm, and dry (tou-kan or tau kwa), depending on the amount and type of coagulant used and the force that is applied to press the curds into blocks of tofu (*3*). The manufacturing procedures for both soft and firm tofu have been described (*4*). However, nothing has been published concerning the detailed steps for dry tofu processing (*5*). Traditionally, firm tofu is prepared following a procedure similar to that of commercial tofu. Most dry tofu makers in Taiwan are equipped with a grinder, a kettle, a conical filter, and some coagulating tanks and wooden molds, similar to that described by Tsai et al. (*6*) for tofu processing. Commercial dry tofu was pressed further, to lower water content, followed by an alkaline treatment, and then colored by caramelization. After soaking in a hydrogen peroxide solution, the firm tofu was dried in an oven.

Many researchers have studied the chemical modification of soybean isoflavone during processing (*7*−*10*). They concluded that the variety of soybean, method of processing, and addition of other components affect the retention and distribution of isoflavones in soy foods. Naturally occurring soybean isoflavones exist mainly in conjugated forms, for example, glucoside, malonyl glucoside, or acetyl glucoside (*11*). Matsuura et al. (*12*) found that daidzein and genistein increased through the action of β-glucosidase in soybean during the soaking processing of soy milk manufacturing. The percent of the isoflavone aglucones to the total isoflavones increased from approximately 3.3 to 12.4% after soaking at 20 °C for 16 h. There are differences between the profiles of soy isoflavones in nonfermented and fermented foods. Nonfermented foods have greater levels of glucosides; on the other hand, greater levels of aglycons have been found in fermented foods, indicating that enzymatic hydrolysis occurred during fermentation. Processed soy products such as tofu, soy milk, soy sauce, soy milk film, and dry spiced tofu are currently sold in Asian countries (*11*). Asian fermented soy foods contain predominantly isoflavone aglycons, whereas in nonfermented soy foods of both American and Asian origin, isoflavones are present mainly as β-glycoside conjugates.

A packed, firm tofu, which has a longer shelf life compared to conventional ones, is now commercially available in Taiwanese markets. These are mainly manufactured following a procedure similar to the traditional method except for using retort cooking instead of H_2O_2 soaking. The effect of hydrogen peroxide treatment on soybean isoflavone stability has not been reported. The objective of this study is to compare the stability of isoflavones, DPPH scavenging capability, shelf life, and textural properties in both traditional and innovative firm tofu processing. The results may help to replace hydrogen peroxide treatment with the autoclaving process in the firm tofu industry throughout Taiwan.

* Author to whom correspondence should be addressed (fax 886-87740213; e-mail tchuang@mail.npust.edu.tw).
† National Pingtung University of Science and Technology.
‡ Tajen Institute of Technology.
§ Rutgers University.

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Table 1. Effect of Processing on Isoflavone Concentration in Firm Tofu

	isoflavones[a] (μg/g of dry wt)									
step	malonyl-daizein	daizin	daizein	malonyl-genistein	genistin	genistein	malonyl-glycitin	glycitein	glycitin	total
raw bean	436a	215a	44a	914a	230a	31a	75a	34a	96a	2075a
pressed	106b	115b	82b	308b	132b	98b	15b	32a	60b	948b
alkali	85c	106c	92b	96c	222c	92b	nd	nd	56c	749c
autoclaved	91c	102c	95b	85c	219c	86b	nd	nd	52c	730c
H_2O_2	77d	98c	89b	81d	208d	91b	nd	nd	47d	691d

[a] Means of triplicate trials. Within the same row, means with no letter in common are significantly different ($p < 0.05$). nd, not detectable.

MATERIALS AND METHODS

Materials. Soybeans (Proto cultivar) were obtained from a local seed farm. Antifoaming agent was obtained from Koah Co. (Wakayama, Japan), and coagulant, food grade, was from Taiwan Salt Workers (Tainan, Taiwan). Authentic standards for genistein, daidzein, daidzin, and genistin were obtained from the Indofine Chemical Co. (Somerville, NJ). All chemicals were of reagent grade from Sigma Aldrich (St. Louis, MO) unless noted otherwise. HPLC grade water was used.

Preparation of Firm Tofu. Soybeans were processed into firm tofu using an automated system (model PT-60, Ta Ti Hsing Machinery Co., Taoyuan, Taiwan) following the method of Cai et al. (*13*). The system was composed of an autosoaking tank, a direct steam injection cooker, a cooling coagulant mixing tank equipped with a stirrer, four tofu trays (40 L × 40 W × 4.5 H cm/tray), an air cylinder press, and an NaOH soaking tank. Washed soybeans (8000 g) were soaked in water at 25 °C for 6 h. The soaked soybeans were milled using a grinder with tap water at a flow rate of 2.5 L/min and then cooked at 80° C for 10 min. The cooked milk was mixed at 420 rpm for 20 s with 1000 mL of a 10% coagulant suspension containing 200 g of $CaSO_4$. The mixture was then poured onto eight tofu trays (each covered with a cheesecloth) and allowed to stand to coagulate for 10 min. The bean curd was then pressed sequentially with air press gauge readings of 1 kg/cm^2 for 10 min, 2 kg/cm^2 for 15 min, and 3 kg/cm^2 for 20 min. The pressed tofu was cooked in an NaOH solution (pH 10) at 95 °C for 4 min to remove the hardened skin, for coloring with caramel, and then packed in a plastic bag.

H_2O_2 Treatment of Firm Tofu Samples. The alkaline-treated firm tofu prepared in the above method was soaked in a 0.25% H_2O_2 solution for 10 min for sterilization and then packed in a plastic bag as described previously. All of the packaged firm tofu samples were stored in a refrigerator at 10 °C, similar to that in a supermarket.

Retort Cooking. Retort cooking (105 °C for 10, 20, 30, and 40 min) was performed using a retort cooker (Hisaka Seisakusyo, Osaka, Japan).

Determination of Textural Properties of Firm Tofu. Texture profile analysis (TPA) for firm tofu was performed using an Instron Universal testing machine (model 1011; Instron Corp., Canton, MA) to determine objective measurements of fracturability (FR), hardness (HA), springiness (SP), cohesiveness (CO), and gumminess (GU) as described by Bourne (*14*). Cylindrical samples (5 cm diameter × 1.0 cm height) were cut from the central portion of the firm tofu cakes with a stainless steel cylindrical cutter. A cylindrical plunger with a 5 cm diameter and a weight beam of 5 kg was used. Three samples taken from the center of one curd of firm tofu from each batch were measured by pressing twice to 25% of the original height of each cake (*15*).

Isoflavone Extraction and HPLC Analysis. Isoflavones were extracted following the method of Murphy et al. (*16*) with slight modification. A freeze-dried sample (1 g) was mixed with 10 mL of 80% methanol and 1.5 mg of internal standard (fluorescein sodium salt, Sigma Chemical Co., St. Louis, MO), stirred at room temperature for 1 h, and then centrifuged at 4000*g* for 10 min. All analyses were performed using a Hitachi HPLC model 6000A equipped with a photodiode array model L-4500 detector (220−400 nm) with a 25 × 0.46 cm Shimpak (C-18) column under isocratic conditions at ambient temperature. The solvent was 0.033 M potassium phosphate, pH 2.2, at a flow rate of 1.0 mL/min, which yielded resolution of all components.

Concentration of isoflavones on a dry sample basis was calculated by comparison of the internal standard. Identification of the individual isoflavone was conducted by comparison of retention time and UV absorption patterns with authentic isoflavone standards or with values given in the literature (*11*).

Measurement of Total Antioxidant Activity. The method for measuring total antioxidant activity estimates the relative ability of the antioxidant substance to scavenge the radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol, as compared to standard amounts of daidzein (*17*).

Microbiological Analysis. An amount of 10 g (from the surface through the center of each triplicate sample) of each sample was homogenized in a Stomacher with 90 mL of sterile tryptone water (0.1% w/v) for 1 min following the method of Prestamo et al. (*18*). Decimal dilutions were prepared by mixing 1 mL aliquots with 9 mL of sterile tryptone water (0.1% w/v), and 0.1 mL of the dilutions was plated on agar. Total plate counts were determined on aerobic count plates (Petrifilm Microbiology Products 3M Health Care, St. Paul, MN) and enumerated after an incubation period of 72 h at 30 °C following the method of Prestamo et al. (*18*).

Scanning Electron Microscopic (SEM) Technique. An SEM technique was used for the investigation of the three-dimensional structure of the retort cooked firm tofu, as compared to the hydrogen peroxide treated firm tofu at room temperature according to the method of Fuchigami and Teramoto (*19*).

Sensory Evaluation. Nine students, who were familiar with firm tofu products, were selected and trained to subjectively evaluate the change in mouthfeel (*20*). Firm tofu, produced by the conventional method, was purchased from a local supermarket and used as a reference for training. Each of the attributes was scored on a 9-point hedonic scale, where 9 = extremely liked and 1 = extremely disliked, according to the method of Obatolu (*21*).

Statistical Analysis. All sample analyses were run in triplicate. Statistical analysis was done using the SAS package (version 6.03, 1998) developed by SAS Institute Inc. (Cary, NC). Analyses of variance using the ANOVA were conducted. Differences between the sample means were analyzed by Fisher's least significant difference (LSD) test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The concentration and distribution of isoflavones in firm tofu were evaluated by high-performance liquid chromatography (HPLC) equipped with photodiode array detection. The levels and compositions of the isoflavones in the raw beans, pressed curd, alkali-treated curd, and H_2O_2-treated bean curds are shown in **Table 1**. Nine of the 12 known isoflavones in soybean were detected and quantified. No detectable amounts of acetyl glucoside of three isoflavones were determined. Dry heat increased the percentage of acetyl derivatives at the expense of malonyl derivatives due to heat-induced decarboxylation (*22*). In addition, Wang and Murphy (*23*) reported that acetyldaizin and acetylgenistin were generated only during heat processing. The traditional method for firm tofu production involves soaking and cold grinding of beans, yielding soybean milk with a beany flavor, which Asian people favor (*24*). The samples analyzed in this experiment follow a procedure similar to that of

traditional tofu processing, which is under relatively low temperatures, not high enough to evoke a decarboxylation. This can explain the extremely low concentration of acetyl glucoside in the current firm tofu.

The combination of soaking, grinding, coagulation, and pressing steps causes significant loss of isoflavones as follows: daidzin derivative (56%), genistin derivative (54%), and glycitein derivative (57%) in firm tofu as compared with that in raw soybean. The losses of malonylated derivatives, 6″-*O*-malonyldaidzin, 6″-*O*-malonylgenistin, and 6″-*O*-malonylglycitin, were 75.7, 66.3, and 57.3%, respectively. Their corresponding isoflavone glycosides dominated in the pressed firm tofu. Processing might cause de-esterification of 6″-*O*-malonyl glucosides and 6″-*O*-acetyl glucosides, thereby leading to the underivatized β-glucosides (daidzin and genistin) as the predominant forms. Some malonyl isoflavones might be changed into glucosides and aglycons because of hydrolysis. As a consequence of the more hydrophilic nature (and hence aqueous solubility) of glucosides and aglycons compared to malonate and acetate ester derivatives, the major isoflavone losses in traditional firm tofu processing could be attributed to the leaching to whey. Different from that of regular tofu processing, for firm tofu, the bean curd is broken down vigorously to remove more water during pressing (*6*). This step further increases the leaching of isoflavones. In addition to the chemical reaction, microbial degradation of isoflavones in firm tofu should be taken into consideration. For commercial mass production, it takes a full day to finish the entire process. Endogenous microbial growth may also account for the remarkable loss of daidzein, daidzin, genistein, and genistin during the coagulation step.

Soaking in alkali was used in the processing of traditional firm tofu. The alkali solution was applied to dissolve the gelated skin structure on the surface of firm tofu, thus enhancing the coloring with caramel. A total of 4 min of cooking in an alkali solution lowered the levels of the isoflavones slightly. The total isoflavone content of soy protein isolates was only about half of that in whole soybeans or soy flour (*11*). Soy protein isolate was prepared by diluted alkali extraction of soluble protein from defatted soy flakes, precipitation at the isoelectric point, neutralization, and drying of the protein fraction. Similarly, firm tofu was cooked in an alkali solution (pH 10) for 4 min to remove the gelated skin. Alkali treatment not only increased the leaching of water-soluble isoflavone but also degraded at least part of the malonyl ester and glycosides, leading to a significant increase ($p < 0.05$) in the aglycons. Significant loss of malonyldaidzin (81.8%) and malonylgenistin (89.5%) as compared with that of raw soybean was observed during alkaline treatment.

A further degradation of soybean isoflavones was observed after the H_2O_2 sterilization process. To extend the shelf life of firm tofu, soaking in a 0.25% H_2O_2 solution for 10 min has been used for sterilization. The loss of daidzin derivative, genistin derivative, and glycitein derivative in H_2O_2-sterilized firm tofu was determined to be 62, 68, and 77%, respectively. Hydrogen peroxide has long been used for the decontamination of *Escherichia coli* in fruits and vegetables. Therefore, washing with hydrogen peroxide solution achieved a population reduction as great as 3−4 log 10 colony-forming units (CFU)/g. However, the 5-log reduction proposed by the U.S. Food and Drug Administration for fruit and vegetable juices could not be attained by washing (*25*). The oxidant activity of hydrogen peroxide may destroy some of the isoflavones in firm tofu.

The effect of autoclaving on isoflavone stability was compared with that of H_2O_2 sterilization. A similar degradation pattern for isoflavones was observed in the aseptic style firm tofu, which was processed by a procedure similar to the conventional one, except for retort cooking. In processing of aseptic style firm tofu, the caramelized firm tofu was packed in a plastic bag and then retort cooked. Although extra heat treatment (105 °C, 20 min) was undertaken to inactivate the microbial population in packed firm tofu, the loss of daidzin derivative, genistin derivative, and glycitein derivative in aseptic style firm tofu as compared with that of raw soybean was found to be 59, 67, and 75%, respectively, less than that of H_2O_2-sterilized firm tofu.

Table 2. Changes of Antioxidative Activity of Isoflavones Extracted from Firm Tofu

step	DPPH free radical scavenging capacity[a] (%)
pressed bean curd	54.2 (3.9)a
alkali treated	51.6 (3.1)b
autoclaved	51.1 (3.4)b
H_2O_2 treated	48.5 (2.7)c

[a] Each value is expressed as the mean of three replications with standard deviation in parentheses. Means with different letters differed significantly ($p < 0.05$). DPPH free radical scavenging capacity of raw soybean is 100%.

Comparison of Antioxidative Activity of Firm Tofu Prepared by Different Methods. Soybean isoflavones were found to possess antioxidative capacity (*26*). It has been noted that the extent of the antioxidative capacity of these isoflavones is positively correlated with the number of hydroxyl groups in the isoflavone. Glycosidation of isoflavones depressed their antioxidative activity considerably. The free radical scavenging activities of methanolic extract (1 g of powder in 10 mL of methanol) from lyophilized raw soybean, pressed, alkali-autoclaved (105 °C, 20 min), and H_2O_2-treated bean curd were evaluated by the decrease in absorption of the stable DPPH radical at 517 nm. The antioxidant activity of the methanol extract, as tested using the DPPH radical scavenging method, showed a decrease as processing progressed. The remaining percent DPPH free radical scavenging capacity of pressed soybean curd was 54.29% in the methanol extract as compared to 100% for the lyophilized raw soybean powder. Alkali treatment decreased percent radical scavenging capacity further to 51.69%.

In the retort cooked firm tofu, thermal treatment did not lower the antioxidative activity significantly ($p < 0.05$) as shown in **Table 2**. Although recent studies conducted by Kudou et al. (*27*) showed that 6″-*O*-malonyl glucosides are the principal conjugated forms of the isoflavones in soybean, these malonate ester derivatives are labile to heat and acidic conditions. Fukutake et al. (*28*) reported that the genistein and genistin levels in tofu were lower than those in soy nuts and attributed this loss to filtration steps during tofu processing but not thermal treatment. A recent investigation on the distribution of isoflavones in soybeans and soybean products from Australia and Indonesia showed similar trends (*29*). They noted that processing and preparation did not affect the level of the isoflavones in soy foods at retail level. Similarly, the isoflavones in firm tofu were not destroyed by the heating steps but were fractionated into okara and whey as well. In retort sterilized firm tofu, the whey remained in the plastic bag. The total free radical scavenging capacity of firm tofu under different processing steps may be attributed to the retention of total isoflavones. Contrary to that, in the retort cooked firm tofu, the strong oxidant, H_2O_2, may destroy at least part of the isoflavone in bean curds. Only 48.5% of radical scavenging activity remained after H_2O_2 treatment ($p < 0.05$).

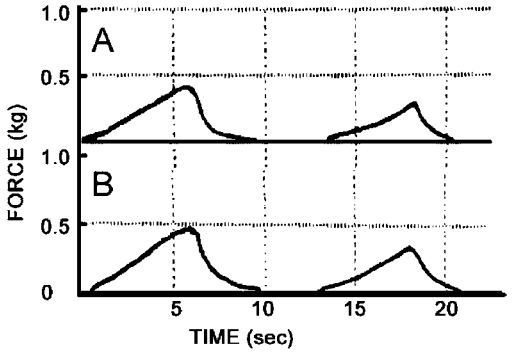

Figure 1. Force penetration curve for (A) hydrogen peroxide treated and (B) retort cooked (105 °C, 20 min) firm tofu.

Table 3. Comparison of Textural Qualities of Firm Tofu Sterilized by Different Methods

	autoclaved[a]					H_2O_2
	0 min	10 min	20 min	30 min	40 min	treated
hardness (kg)	0.47a	0.47a	0.48a	0.49a	0.52b	0.42c
chewiness (kg·cm)	0.026a	0.026a	0.026a	0.027a	0.031a	0.025a
cohesiveness (kg·cm)	2.01a	2.01a	2.02a	2.02a	2.04b	1.98c
gumminess (kg)	0.023a	0.024a	0.023a	0.025a	0.042b	0.038b
springness (mm)	1.13a	1.12b	1.11b	1.11b	1.10b	1.19c
mouthfeel	7.4a	7.1a	6.8a	5.1b	4.1c	7.1a

[a] Means of triplicate trials. Within the same row, means with no letter in common are significantly different ($p < 0.05$).

Comparison of Textural Quality of Processed Firm Tofu. Firm tofu texture is an important quality attribute that affects product acceptability. Firm tofu with greater hardness means harder and firmer, with greater cohesiveness. It requires more work to break down the internal bonding, and with higher springiness it possesses higher elasticity and greater chewiness, becoming stiffer and more difficult to eat. To study the effect of sterilization on the firm tofu matrix, texture profile analysis was conducted. Typical TPA profiles for both autoclaved and H_2O_2-sterilized firm tofu are shown in **Figure 1**. There is a significant increase in hardness value after 40 min of heat treatment. Heating of the isolated soy protein pastes at temperatures between 60 and 110 °C induced "hard" gels (*30*). It was observed that the hardness of tofu increased with the increase in applied pressure from 0.186 to 0.744 P (*3*).

There is no significant difference in chewiness for the various treatments. Cohesiveness and gumminess increased slightly after 40 min of autoclaving as shown in **Table 3**. In studying the effect of pressure on texture profile parameters of soybean curd (tofu), Gandhi and Bourne (*3*) reported that the chewiness and gumminess increased with increasing pressure, whereas springiness, cohesiveness, and adhesiveness declined slightly with increasing pressure. The breaking and pressing steps in firm tofu processing increased the thickness of the cell wall of the pressed firm tofu. Physical force, including thermal and pressure during autoclaving, applied on the firm tofu, increased the thickness of the cell wall further after 40 min of autoclaving. Springiness, which indicates the rate at which a deformed material goes back to its undeformed condition after the deforming force is removed, decreased with the duration of heat treatment, as shown in **Table 3**. Generally speaking, retort cooking did not alter the textural properties of firm tofu significantly, except after 40 min of autoclaving.

TPA data revealed that a higher hardness level was determined for the retort cooked tofu than for the H_2O_2-sterilized firm tofu. The average moisture contents were 76.1% for autoclaved and 79.3% for H_2O_2-sterilized firm tofu. The softer texture of the H_2O_2-sterilized samples may be attributed to the higher moisture content due to the absorption of water during the soaking process. Chewiness, cohesiveness, and gumminess exhibited similar trends. On the contrary, the springiness of retort cooked firm tofu is lower than that of a H_2O_2-treated firm tofu. The scores of mouthfeel were found to be 6.8 and 7.1 for the autoclaved (105 °C for 20 min) and hydrogen peroxide treated firm tofus, respectively. The mouthfeel scores for the samples autoclaved longer than 30 min were significantly lower than that of the hydrogen peroxide treated firm tofu. The sensory evaluation results revealed that the mouthfeel of the autoclaved firm tofu (105 °C for 20 min) was in the range of as good as the control samples and was acceptable to the panelists.

Comparison of Structural Quality of Processed Firm Tofu. The microstructures of firm tofu after retort cooking and with hydrogen peroxide treatment in the scanning electron micrographs were compared and are shown in **Figure 2**. The result revealed that both firm tofu samples possess a fine network structure. Thermal treatment is usually undertaken to achieve the desired shape and texture in tofu processing. Gel formation in firm tofu, similar to that in regular tofu, consists of two steps: protein denaturation and coagulation (*31*). The hydrophobic regions of the soy protein molecules in the native state are exposed to the outside by heat denaturation (*32*). The denatured soybean protein was coagulated either by calcium ions from $CaSO_4$ or by thermal denaturation. Stabilization of the three-dimensional network gel structures may involve hydrogen bonding, hydrophobic associations, ionic interaction, or disulfide linkage. SEM images of the caramelized firm tofu revealed a characteristic porous structure having membranous walls of thin compact film (**Figure 2A**). Retort cooking tends to develop a thicker membranous wall structure, as shown in **Figure 2C**,**D**. Prolonged thermal treatment leads to the development of a network structure similar to that of transglutaminase-induced retort resistant properties in tofu. Retort-resistant tofu has been prepared by adding transglutaminase to increase the cross-linkage between soy protein molecules. Transglutaminase treatment with an enzyme concentration >5.0 units/g of solid was found to suppress the retort-induced changes of tofu breaking strength and strain (*33*). As compared with that of an autoclaved firm tofu, the H_2O_2-treated firm tofu showed a structure similar to the characteristic three-dimensional network in the caramelized one.

Effect of Sterilization on Firm Tofu Microbial Population. A number of studies have found tofu to harbor *Streptococcus* sp., *Enterobacter* sp., and *Pseudomonas* sp. (*34*, *35*). Rehberger et al. (*36*) reported that of the tofu sampled immediately after purchase, 83% of the lots tested had total counts $>10^6$ CFU/g and psychrotrophic counts $>10^4$ CFU/g. Very low levels (<10 CFU/g) of all other microbial groups tested for (sporeformers, yeasts and molds, and coagulase-positive staphylococci) were found in the majority of lots. However, conventional processing of tofu is sufficient to completely destroy vegetative bacterial cells. Total bacterial counts in beans that had been soaked overnight diminished from 2×10^6 to $<10^2$ organism/g after first heating. The unacceptably high bacterial count seen in final products could be due only to unsanitary postheating manipulation (*37*).

Because the possibility of recontamination from alkali and H_2O_2 treatment is very low, as compared with the cooling water that is used in tofu processing, total plate count was used to evaluate the effectiveness of H_2O_2 treatment and retort cooking in controlling the microbial count in firm tofu. The result showed

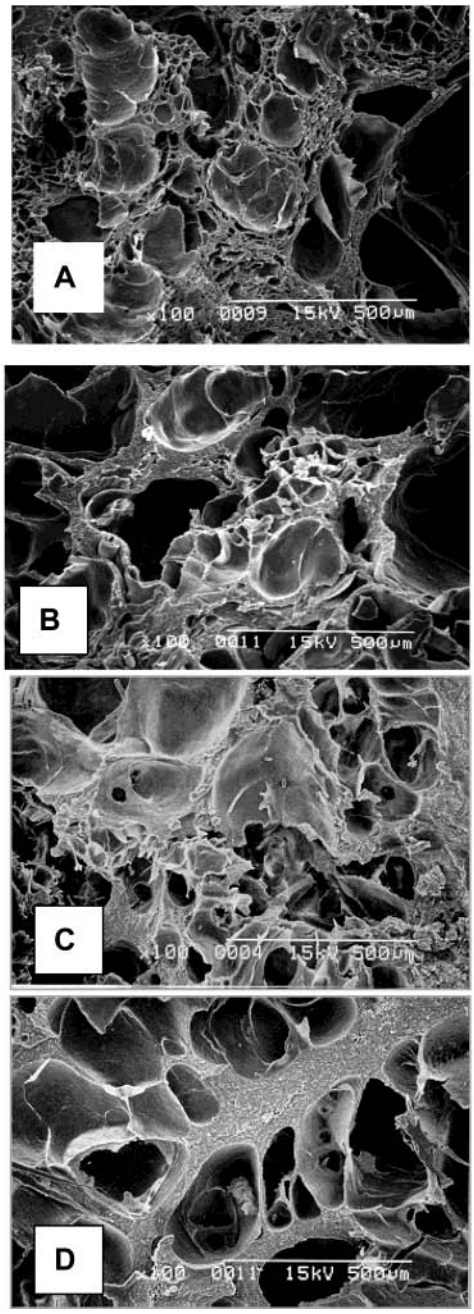

Figure 2. Scanning electron micrograms of inner structure of sterilized firm tofu: (A) caramelized; (B) hydrogen peroxide treated; (C) retort cooked (105 °C, 20 min); (D) retort cooked (105 °C, 40 min).

that the microbial population decreased after H_2O_2 soaking in traditional firm tofu. The total plate counts for pressed bean curd, caramelized, and H_2O_2-treated firm tofu were 5.4, 4.8, and 4.6 log CFU/g, respectively. The total plate counts increased to 3.1 and 7.4 log CFU/g after 7 and 14 days storage at room temperature, respectively, and spoiled after 90 days of storage, as shown in **Table 3**.

For the autoclaved firm tofu, the total microbial number was reduced to 0.9 log CFU/g, ~3 log units less as compared with that of the caramelized firm tofu. The total microbial population remained constant even after 90 days of storage in a plastic bag under conditions mimicking that of a supermarket. This is in good accordance with the finding of Ashraf et al. (*38*). They reported a significant difference ($p < 0.0008$) in plate counts between the packaged samples and the bulk tofu. Commercially available firm tofu, available in traditional Taiwanese markets, is usually soaked in 0.25% H_2O_2 for 10 min. The resultant products, with a shelf life of 2 days at room temperature, contained 95 ppm of H_2O_2, and the total count was found to be $<10^5$ CFU/g (*39*). These results indicate that both alkali and H_2O_2 treatments might reduce mainly the surface microbes. Retort cooking in innovative firm tofu manufacturing reduced the microbial population further. As shown in **Table 4**, after retort cooking, the population decreased by 3 log units and remained constant even after 14 days of storage.

Table 4. Results of Aerobic Population of Sterilized Firm Tofu during Storage

	total plate count[a] (log CFU/g of firm tofu)				
		retort cooked			
storage (days)	H_2O_2 sterilized	10 min	20 min	30 min	40 min
0	2.6 (0.2)c	2.5 (0.4)a	0.9 (0.1)a	nd[b]	nd
7	3.1 (0.1)c	2.9 (0.1)a	1.1 (0.1)a	nd	nd
14	7.4 (0.2)d	3.2 (0.3)a	1.1 (0.1)a	nd	nd
90	spoiled	4.4 (0.3)b	1.3 (0.2)a	1.8 (0.4)	1.3 (0.2)

[a] Each value is expressed as the mean of three replications with standard deviation in parentheses. Means with different letters in the same column differed significantly ($p < 0.05$). All of the samples were stored in plastic bags at 10 °C under conditions similar to that in a supermarket. [b] nd, not detectable.

It is concluded that the thermal treatment of firm tofu tends to destroy the endogenous microorganisms effectively, whereas it retained the residual isoflavone content, DPPH radical scavenging capacity, and characteristic texture.

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