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Physiochemical Characteristics of Functional Tofu Fortified with Garlic (*Allium sativum*) and Calcium Lactate as a Coagulant.

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ABSTRACT

The objective of the fourth study was to investigate the effects of calcium lactate as a coagulant and garlic (Allium sativum) on the qualities of tofu including color, textural properties and shelf-life. Statistical analysis of data was carried out using the SPSS 12.0 program. In this experiment, the yield of garlic tofu was high in order of: 0.75% addition of garlic juice > 1.00% addition of garlic juice > no addition of garlic juice > 0.50% addition of garlic juice > 0.25% addition of garlic juice. Therefore, we decided to add garlic juice 0.75% to tofu coagulated by calcium lactate. The lightness (L value) decreased significantly with the increase of the concentration of garlic juice. The hardness and cohesiveness decreased significantly with the increase of the concentration of garlic juice addition. The tofu with the addition of garlic juice (0.75%) showed significantly more fractural texture than the other samples. The hardness of tofu with calcium lactate as a coagulant was significantly lower than the other samples using different coagulants. All tofu had initial bacterial concentration of 9.6×103 CFU/g at 0 day of storage. Tofu prepared with 0.75% and 1.0% addition of garlic juice did not show any change in viable microbial counts by the second day and the fourth day of storage, respectively. However, viable microbial counts of tofu prepared with 0.0%, 0.25% and 0.50% addition of garlic juice increased more rapidly than those of tofu prepared with 0.75% and 1.0% addition of garlic juice during longer storage periods. These results indicate that the garlic may be used to improve the quality of tofu and extend shelf-life period by inhibition of microbial growth during storage.

Keywords: Garlic (*Allium sativum*), Calcium lactate, Functional tofu, Shelf-life

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INTRODUCTION

Soybeans have been garlic processed into various forms, among which tofu is the most widely accepted soy food [1]. Tofu (soybean curd) as a kind of traditional oriental food has attracted interests worldwide due to its good nutritional and healthful benefits. Soybean products including tofu are good sources of proteins and carbohydrates, are low in fat and rich in various mineral contents, and are part of the principal meals in eastern countries [2]. Soybean seeds have a protein content of 35~40% on dry weight basis, which makes them a relatively inexpensive source of protein for human consumption [3]. Especially, it is consumed in large quantities in Asian countries because of its inexpensive high quality protein [4]. Also the incorporation of tofu into western diet can be an important means of preventing and treating chronic diseases such as cancer and cardiovascular diseases as supported by several previous studies [5-7].

In general, tofu is made by coagulation of heated soy milk with a coagulant, followed by moulding and pressing the curd to draw the whey. And it is produced by exposing filtered soybean mash to calcium or magnesium salts, which precipitate the soy solid into a curd. Thus, tofu has a high content of calcium [7, 8]. Also the yield, quality, texture and shelf life of tofu are influenced by several factors such as the variety of soybeans, the soybean phytate content, the soaking time and temperature of soybeans, storage conditions, the concentration of coagulant, the rate of stirring and coagulation temperature, pressing time and weight [2, 8-11]. Especially, the coagulation of soymilk is the most important step in the tofu-making process. Calcium sulfate, calcium chloride, magnesium sulfate, magnesium chloride and glucono-δ-lactone are some of different types of coagulants used on an industrial scale for the preparation of tofu. Coagulation occurs due to the cross-linking of protein molecules in soymilk with divalent cations [1].

In Asia, about 90% of soybean protein is consumed in the form of tofu. However, because of its high moisture content and rich nutrients, tofu is prone to spoilage. Some tofu purchased in the fresh form at an Asian market or health food store may have a shelf life of only 3~4 days. Therefore, to extend the shelf life of tofu, microwave treatment, coagulation with organic acid and pH adjustment of immersion solutions have been tried [12]. Also the phytic acid of soymilk decreases the utilization of calcium due to the formation of insoluble calcium salts according to binding with calcium [13]. On the other hand, calcium lactate is known as non-toxic, soluble calcium and it is used as a food additive for bread, orange juice and yogurt. Also garlic contains mainly vinyldithiins (2-vinyl-4H-1,3-dithiin and 3-vinyl-4H-1,2-dithiin), ajoenes [(E,Z)-4,5,9-trithiadodeca-1,6,11-triene-9-oxide)], and a small amount of sulfides. Particularly, ajoene is well known as an antimicrobial compound [14].

Therefore, the objective of this study was to investigate the effects of calcium lactate as a coagulant and garlic on the quality of tofu in order to extend the shelf life of tofu.

MATERIALS AND METHODS

Preparation of garlic juice

Raw garlic bulbs were harvested from the Danyang area in Korea. The garlic juice was prepared by homogenizing the required amount of garlic cloves in an appropriate volume of water to give a concentration of 12.5 mg/ml [15]. The homogenate was centrifuged at 3,000 rpm for 10 min to remove garlic sediment and the supernatant fraction was used for the preparation of tofu.

Preparation of tofu

Figure 1 shows the flow chart of the garlic tofu process manufactured with Danyang garlic juice. To prepare tofu, soybean was purchased from a local supplier. Food grade $CaSO_4$, $CaCl_2$, and $MgCl_2$ were purchased from CS Trading Co. (Seoul, Korea). Washed soybean (≈100 g) was soaked in 500 ml tap water in a 1 liter beaker at room temperature (25°C) for 12 hr. After the stipulated soaking time, the bean was drained and ground with 1000 ml tap water in a Waring blender for 3 min at high speed. The mash was strained through a muslin cloth and pressed to obtain soya milk. The soya milk was heated to boiling, and then mixed with 1.5% calcium lactate, $CaSO_4$, $CaCl_2$, and $MgCl_2$ alone or together with 0.25%, 0.50%, 0.75%, and 1.00% garlic juice at 85°C, followed by being held for 10 min to coagulate. The other coagulants including $CaSO_4$, $CaCl_2$, and $MgCl_2$ were added same amount with calcium lactate. The curd was gently transferred to a perforated stainless steel container (9.0 × 5.0 × 7.0cm depth) lined with a single layer of cheesecloth and pressed for 30 min using bricks weighing 3.0 kg.

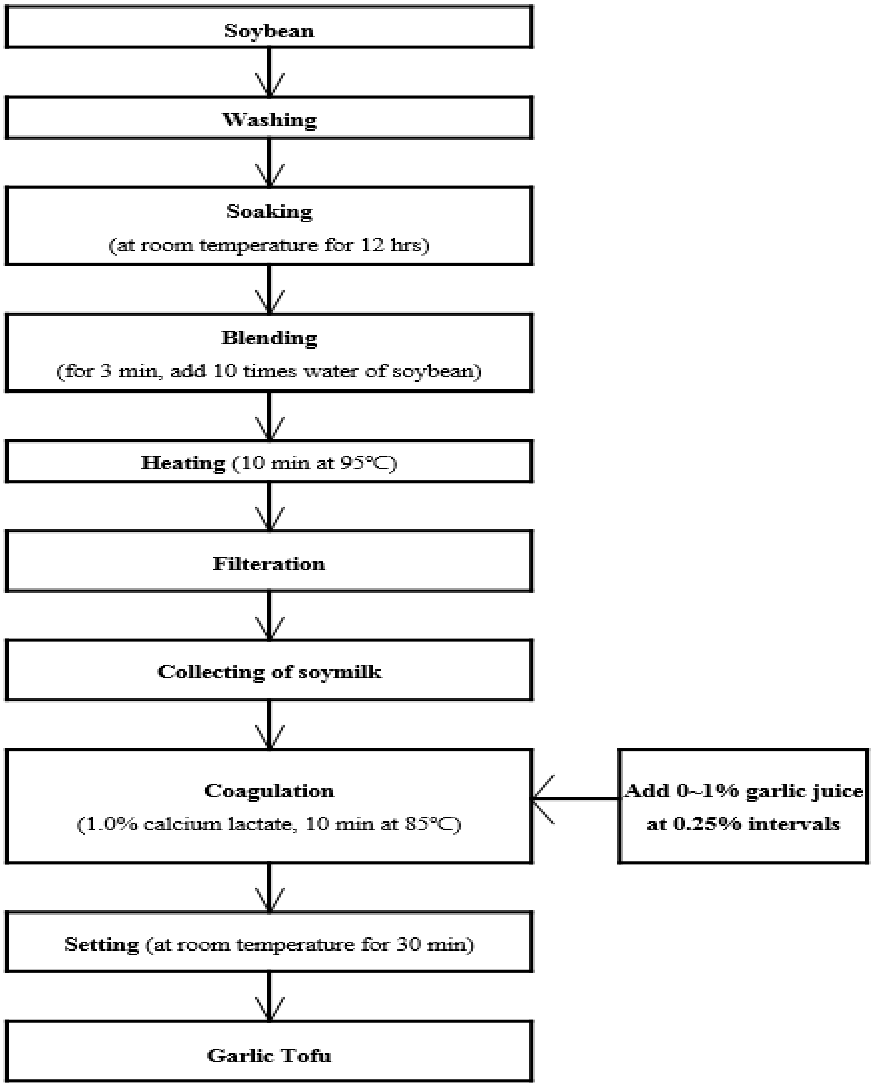

Figure 1: The flow chart of the garlic tofu process manufactured with Danyang garlic juice.

Yield and color of tofu

The yield of tofu was expressed as the fresh weight of tofu (9.0 × 5.0 × 7.0 cm) obtained from 1000 ml of soymilk. Color evaluation was performed on fresh tofu samples using a spectrocolorimeter (Hunterlab model CQⅡ/UNI-120) equipped with a D25 circumferential optical sensor. A standard white tile with reflectance values of X=83.24, Y=85.23 and Z=100.92 was used as a reference. A representative sample was placed into a 6 cm Petri dish and covered to avoid stray light. Hunter L (lightness), +a (red) to -a (green), and +b (yellow) to -b (blue) were then determined for each sample. Each value represented a mean value of five replicate determinations. The coefficients of variations for all measurements were less than $P = 0.05$.

Texture measurement of tofu

The textural properties of tofu were evaluated with TA XT2 Texture Analyser (Stable Micro Systems, Goldaming, Surrey, UK). Cube samples of tofu (10 × 10 × 10 mm) were axially compressed to 50% deformation of their original height in a two-cycle compression test by a compression plunger (D: 25 mm). Force time deformation curves were obtained using a 5 kN load cell applied at a cross speed of 60 mm/min. The textural parameters of tofu such as hardness, cohesiveness, springiness, gumminess and adhesiveness were evaluated. Eight samples were analyzed for each treatment.

Storage test of tofu

The tofu (9.0 × 5.0 × 7.0 cm) obtained above was placed in a polypropylene container (15.0 × 8.0 × 10.0 cm) containing 100 ml of sterilized distilled water as an immersion solution. The container with a plastic cover was stored at 25°C for 15 days. All experiments were triplicated. In order to determine the viable bacterial counts, the tofu and immersion solution were homogenized together by using Polytron homogenizer (RT-1200C, Switzerland), and centrifuged at 3,000 rpm for 20 min. The supernatant was diluted with 0.1% peptone water. Plate count agar (Difco, St. Louis, USA) was used for the determination of the total viable counts. All plates were triplicated, incubated at 30°C for 48 hr, and the viable cell numbers were determined as colony forming units (CFU) per ml.

Statistical analysis

Statistical analysis of data was carried out using the SPSS 12.0 program. All results were expressed as mean ± standard deviation (SD) for each of the groups, and the statistical significance of differences among the groups were assessed by using analysis of variance (ANOVA) and Duncan's multiple range test. The level of significance used was $P < 0.05$.

RESULTS AND DISCUSSION

The yield of garlic tofu and the quantity of whey according to the garlic juice concentration

Figure 2 shows the yield of garlic tofu and the quantity of whey according to the concentration of garlic juice. In the present study, the yield of garlic tofu was high in order of: 0.75% addition of garlic juice > 1.00% addition of garlic juice > 0.00% addition of garlic juice > 0.50% addition of garlic juice > 0.25% addition of garlic juice. However, there was not significant difference between 0.75% and 1.00% addition of garlic juice. In a previous study, it was reported that the addition of carrageenan to soymilk prior to coagulation resulted in a significant increase in the yield of tofu [8], which is similar to the effect of adding garlic juice in this study. The increase in the yield of tofu adding 0.75% and 1.00% garlic juice was reflected by the higher moisture content and corresponding decrease in the yield of whey. On the other hand, the quantity of whey in tofu by adding 1.00% garlic juice was significantly lower than the others ($P < 0.05$). Therefore, we decided to add 0.75% garlic juice to the tofu coagulated by calcium lactate. In general, it has been known that the moisture content of tofu samples varied with the use of different coagulants. Also the high moisture content accounted for a higher tofu yield since the tofu yield and moisture content are highly correlated with each other [16].

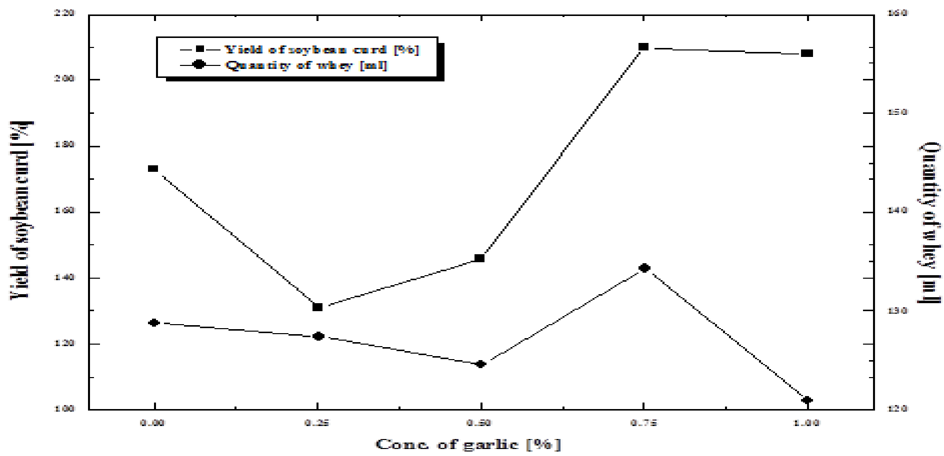

Figure 2: The yield of garlic tofu and the quantity of whey according to the concentration of garlic juice.

Changes in pH of soaking solution containing garlic tofu during storage

Figure 3 shows the changes in pH of soaking solution containing garlic tofu during storage. The changes in pH of soaking solution showed a gradual decline for up to 4 days and then showed a rapid increase in all the groups. Immediately after the preparation of tofu with garlic juice added, the pH range of soaking solution was from 6.4 to 6.6, which was not significantly different among the groups. In a previous study, it was reported that the pH of dough with calcium lactate was higher than that of the control [17]. These results showed that the solution of calcium lactate is alkaline.

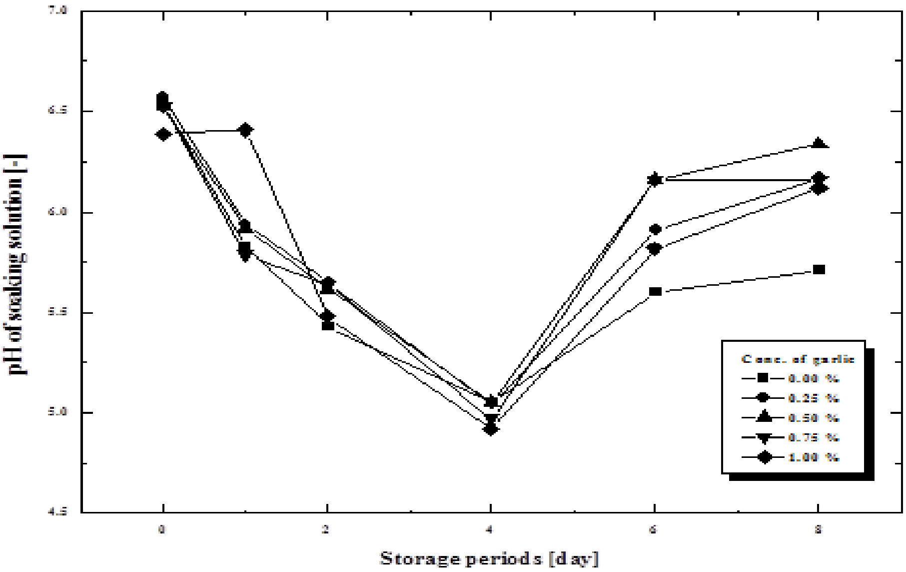

Figure 3: Changes in pH of soaking solution containing garlic tofu during storage at 25°C.

Color of garlic tofu

Table 1 shows the color of garlic tofu according to the concentration of garlic juice. Tofu of good quality is generally white or light yellow in color. All the tofu samples in this study had a light yellow color. The tofu prepared without garlic juice showed a significantly higher L value, but lower b value than that prepared with garlic juice. The lightness (L value) decreased significantly with the increase of the concentration of garlic juice addition ($P < 0.05$). However, the yellowness (b value) increased significantly with the increase of the concentration of garlic juice addition ($P < 0.05$). With the increase of the concentration of garlic juice addition, there was the trend of increase in a value ($P < 0.05$). In a previous study of tofu with carrageenan added, the greenness increased with the increase of the concentration of carrageenan in tofu prepared by calcium sulfate as a coagulant [8]. In other previous studies about the physical properties of prepared firm tofu according to different coagulants, it was reported that, with regard to color, either white or creamy white color is the desirable tofu characteristic. The L value for the tofu ranged from 85.93 to 86.41, and the white a and b values ranged from 0.003 to 0.657 and 22.36 to 24.95, respectively. Also it was reported that different coagulating agents had no significant effect on the color of tofu [1].

Table 1: Hunter's color value of tofu manufactured with garlic juice

Hunter's color value			
Concentration of garlic juice	L value	a value	b value
0.00 %	94.71 ± 0.52^{d}	-3.93 ± 0.06^{d}	16.09 ± 0.44^{a}
0.25 %	92.60 ± 0.30^{c}	-3.06 ± 0.10^{bc}	16.60 ± 0.45^{a}
0.50 %	91.77 ± 2.08^{bc}	-3.28 ± 0.14^{c}	17.04 ± 0.51^{b}
0.75 %	90.01 ± 1.17^{b}	-2.94 ± 0.05^{b}	17.23 ± 0.49^{b}
1.00 %	88.84 ± 1.26^{a}	-2.68 ± 0.06^{a}	19.57 ± 0.56^{c}

L value ; Lightness (white +100 ↔ 0 black).
a value ; Redness (Red +100 ← 0 → -80 Green).
b value ; Yellowness (Yellow +70 ← 0 → -80 Blue).
$^{a, b, c, d}$; Values with the same letter are not significantly different ($P < 0.05$).

Textural properties of garlic tofu

Table 2 shows the textural properties of garlic tofu according to the concentration of garlic juice. Hardness and cohesiveness decreased significantly with the increase of the concentration of garlic juice addition ($P < 0.05$). It is considered that the moisture content was increased with the increase of the concentration of garlic juice addition in preparing the tofu. The tofu with the addition of garlic juice (0.75%) showed significantly more fractural texture than the other samples ($P < 0.05$). On the other hand, springiness was not significantly different among the samples. These results indicated that the presence of up to 1.0% garlic juice does not have positive influences in preparing garlic tofu. Also contrary to expectation, interaction between soy protein and garlic resulted in the reduction of the hardness of tofu, which is a desirable sensory attribute. Baek et al. [18] reported that, contrary to elasticity, cohesiveness, gumminess and brittleness, the hardness value decreased in 1% sea mustard added tofu compared to that in tofu prepared from whole soybean milk. Karim et al. [8] reported that hardness, cohesiveness, elasticitym gumminess and chewiness decreased with the increase of the concentration of carrageenan for tofu prepared by calcium acetate and calcium sulfate. However, the effect of carrageenan on the other two parameters adhesiveness and fracturability was not consistent. On the other hand, it was reported that the gel strength of tofu containing chitosan decreased with the increase of the degree of deacetylation of chitosan [19].

Table 2: Changes in the textural characteristics of tofu manufactures with garlic juice

Textural characteristics				
Conc. of garlic	Hardness	Fracturability	Springiness	Cohesiveness
0.00 %	996.57 ± 61.37^{e}	10.31 ± 1.91^{cd}	$0.96\pm0.09^{N.S.}$	0.53 ± 0.02^{d}
0.25 %	590.91 ± 67.52^{c}	8.44 ± 1.12^{a}	0.91 ± 0.03	0.54 ± 0.02^{d}
0.50 %	900.83 ± 62.15^{d}	8.93 ± 1.42^{abc}	0.93 ± 0.04	0.50 ± 0.04^{c}
0.75 %	419.32 ± 60.92^{b}	10.86 ± 1.33^{d}	0.91 ± 0.06	0.44 ± 0.01^{b}
1.00 %	297.67 ± 57.23^{a}	9.10 ± 2.01^{bc}	0.90 ± 0.05	0.41 ± 0.01^{a}

$^{a\sim e}$; value with the same letter are not significantly different ($P < 0.05$).

Textural properties of garlic tofu prepared with different coagulants

Table 3 shows the textural properties of garlic tofu (0.75% garlic juice addition) prepared with different coagulants. The lowest concentration of coagulant applied by Lu et al [20] to obtain coagulation was 0.1% for calcium chloride, 0.2% for calcium lactate and 0.3% for calcium sulfate. Calcium lactate is rarely used as coagulant for tofu manufacturing. However, in this study, we checked its possibility as suitable coagulant in preparing tofu. Coagulants used in this study were $MgCl_2$ (1.0%), $CaSO_4$ (1.0%), $CaCl_2$ (1.0%), and calcium lactate (1.0%) after due consideration with adding garlic juice. Oboh [21] observed that since the amount and the type of coagulated isoflavones vary with the type of coagulant this will inevitably alter their biological activity. The hardness of tofu with calcium lactate was significantly lower than other samples using different coagulants ($P < 0.05$). Also the springiness and cohesiveness of tofu using calcium lactate were significantly lower than those using $CaSO_4$ as coagulant ($P < 0.05$). However, Lee and Hwang [22] reported that the hardness, cohesiveness, springiness and gumminess of tofu prepared by $CaCl_2$ and $MgCl_2$ were significant higher than those prepared by $CaSO_4$ and glucono-δ-lactone. Prabhakaran et al. [1] reported that calcium sulfate was found to be the most suitable coagulant for tofu making in terms of its high yield, retention of the maximum amount of isoflavones and in obtaining a firm, but not hard texture of tofu. Also the decrease in the yield of tofu with the increase of the concentration of calcium sulfate can be caused by the increase of syneresis and the loss of whey from the curd as more binding occurs and thus makes the protein matrix denser and compact [23]. Kim et al [24] reported that the addition of shell powder (0.05 and 0.2%) makes the texture of tofu significantly harder and subsequently increases the gumminess and chewiness while not having a significant influence on the cohesiveness and adhesiveness of the tofu samples. In a previous study, calcium lactate was found to be a better coagulant than calcium acetate in terms of the yield and retention of isoflavone in tofu [1]. Lee and Kim [13] reported that lower hardness showed softer and smaller particles. The particle of tofu prepared by calcium lactate was small and uniform but the size of tofu prepared by black snail powder was coarse. In this study, fracturability was not significantly different among the samples. The hardening of tof u by the addition of calcium ion can be affected by the way that protein interacts with calcium and other constituents including phytic acid in soy milk and anions to form the microstructure into gel [25].

These results indicated that calcium lactate may be usable as coagulant for tofu manufacturing because of its high absorbability in human body.

Table 3: Changes in the textural characteristics of garlic tofu prepared with different coagulants

	Textural characteristics			
Coagulants	Hardness	Fracturability	Springiness	Cohesiveness
$MgCl_2$	799.52±41.42^{b}	9.79±2.41$^{N.S.}$	0.98±0.06^{ab}	0.50±0.07^{ab}
$CaSO_4$	972.77±37.56^{c}	9.97±2.06	1.00±0.09^{b}	0.55±0.02^{b}
$CaCl_2$	1051.40±24.70^{c}	9.58±3.04	0.96±0.09^{ab}	0.54±0.02^{b}
calcium lactate	697.67±57.23^{a}	9.10±2.01	0.90±0.05^{a}	0.49±0.01^{a}

$^{a, b, c}$; Values with the same letter are not significantly different (P < 0.05).

Storage test of tofu

Figure 4 shows the changes of the viable microbial counts of tofu prepared with calcium lactate and garlic juice during storage at room temperature for 8 days. All tofu had an initial bacterial concentration of 9.6×10^3 CFU/g at day 0 of storage. Tofu prepared with 0.75% and 1.0% addition of garlic juice did not show any change of the viable counts by the second day and the fourth day of storage, respectively. However, the viable microbial counts of tofu prepared with 0.0%, 0.25% and 0.50% addition of garlic juice increased more rapidly than those of tofu prepared with 0.75% and 1.0% addition of garlic juice during longer storage periods. Several previous studies indicated the antioxidant and antimicrobial effects of garlic and garlic-derived organosulfur compounds [14, 26]. Kim and Lee [27] postulated that tofu spoilage would start when the viable counts were above 10^7 CFU/ml. Based on this, the addition of garlic juice in the range of tested concentration can extend the shelf life of tofu. Tofu prepared with 0.75% and 1.0% addition of garlic juice had a shelf life 2 days longer than the others samples. This was probably due to the antibacterial activities of CaO in calcium lactate and/or organosulfur compounds in garlic. Kim et al [28] reported that the minimum inhibitory concentration of fresh garlic extract was determined to be 0.1 mg/ml against gram-positive bacteria and 0.5 mg/ml against gram-negative bacteria. Also Chung et al [29] reported that an aqueous extract of garlic was bacteriocidal against gram-positive and gram-negative bacteria in all concentrations (0.1~2.5%, w/v). Moreover it was shown that garlic juice restrains the growth of pathogenic bacteria better than lactic acid bacteria. Shelef and Potluri [30] observed a pH reducing effect of calcium lactate in a meat medium. Luna-Guzmán and Barrett [31] observed that calcium lactate treatment is a potential alternative to calcium chloride for shelf life extension of fresh-cut cantaloupe since it provides similar or better tissue firming without providing undesirable bitterness. Kim et al [24] reported that all tofu had an initial bacterial concentration of 208 CFU/g at day 0 of storage. Tofu prepared with 0.1% and 0.2% addition of shell powder did not show any change of the viable counts by the second day and the fourth day of storage, respectively. However, the viable microbial counts of tofu prepared with a single use of $MgCl_2$ increased more rapidly than those of tofu prepared with shell powder during linger storage periods. Jung and Cho [32] reported that at the beginning of storage, the pH of tofu with green tea powder increased but decreased rapidly at the eighth day of storage.

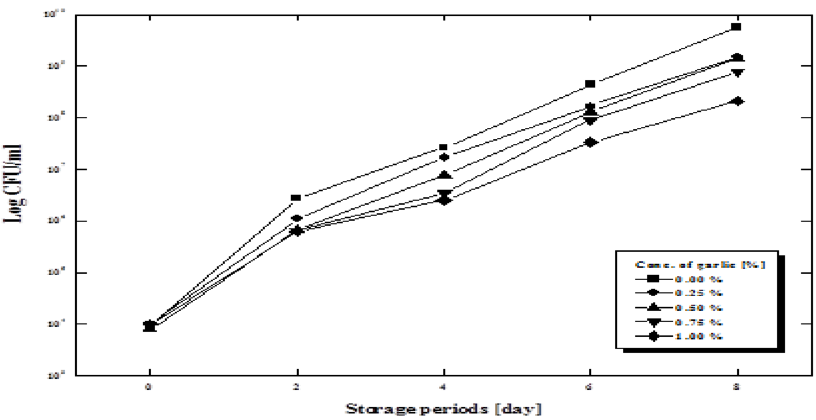

Figure 4: Changes in the total plate microbial count of tofu manufactured with garlic juice during storage at 25°C.

CONCLUSIONS

In conclusion, our results indicated that the garlic may be used to improve the quality of tofu and extend the shelf-life period by inhibition of microbial growth during storage. Our results can be useful in process optimization that becomes essential in retaining the maximum nutritive value in tofu fortified with garlic especially when the choice of coagulants including calcium lactate. Furthermore, it is expected that these functional foods with garlic and calcium lactate provide several bioavailabilities containing the healthful effects of garlic and high-quality protein as well as calcium for postmenopausal women.

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