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The effect of oyster shell powder on the extension of the shelf life of tofu

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Abstract

The effects of oyster shell powder addition (0%, 0.05%, 0.1% and 0.2% of soy milk) on quality and shelf life of tofu (soybean curd) were investigated. Yield and moisture of tofu prepared with 0.1% of shell powder were higher than that coagulated with a single use of $MgCl_2$. Syneresis was low when shell powder was added to tofu, but higher when $MgCl_2$ was added. Shell powder addition (0.05% and 0.2% of soymilk) showed a high level in hardness and gumminess comparing with tofu prepared with a single use of $MgCl_2$. However, there was no significant difference ($p > 0.05$) between 0.05% and 0.2% addition of shell powder. When 0.05% of shell powder was added, overall acceptability showed a high score because tofu had a good mouthfeel, moderate firmness and lower beany-flavour. However, there was not a significant difference ($p > 0.05$) between no addition and 0.05% and 0.1% addition of shell powder. Tofu prepared with shell powder (0.05% and 0.1% addition) had a shelf life of above 2 days longer than that prepared with a single use of $MgCl_2$. The addition of shell powder (0.05% and 0.1%) for tofu manufacturing resulted in a good sensory evaluation and the extension of shelf life.

Keywords: Shell powder; Tofu; Soybean curd; Shelf life; Sensory evaluation

1. Introduction

Soybean seeds have a protein content of 35–40% on a dry weight basis, which makes them a relatively inexpensive source of protein for human consumption (Derbyshire, Wright, & Boulter, 1976). Soybeans have been transformed into various forms of soy foods, tofu being the one most widely accepted throughout the world.

In Asia, for example, 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. In order to obtain a longer shelf-life for tofu, considerable attention has been given to the development of methods for extending the shelf life of tofu. Some researchers have studied quality improvement and shelf-life extension of tofu. To extend the shelf-life of tofu, microwave treatment, coagulation with organic acid and pH adjustment of immersion solutions have been tried (Champagene, Aurouze, & Goulet, 1991; Pontecorvo & Bourne, 1978; Wu & Salunkhe, 1977). Chitosan was used as an additive to tofu for the purpose of shelf-life extension because it has antimicrobial activity (No & Meyers, 2004). However, none of these methods have been employed by commercial tofu manufacturers. Thus, there is need for a more practical and efficient method.

At present, in oyster shell-harvesting districts, large amounts of shells are piled up near the seaside, which creates several serious problems such as the emission of

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offensive odors and soil pollution from heavy metals contained in the viscera. The main component of shell is $CaCO_3$, and by heat treatment, $CaCO_3$ in the shell is converted to CaO, which exhibits antibacterial activity. In fact, there were reports that shell powder heated to over 700 °C exhibited a bactericidal activity (Sawai, Shiga, & Kojima, 2001; Shiga, Sawai, & Kojima, 1999). Oyster shell powder was applied to prepare noodles, fried chicken, sardine ball (Suhara, 1995) and *kimchi* (Choi, Whang, Kim, & Suh, 2006) for quality improvement or extension of shelf life. Therefore, the use of this material in food processing is expected not only to prolong the shelf life of foodstuffs, but also to be a source of minerals.

The objective of this study was to investigate the effect of oyster shell powder on the quality of tofu in order to extend the shelf life of tofu.

2. Materials and methods

2.1. Materials

To prepare tofu, soybean was purchased from a local supplier. The seed density was 40.29 g/100 beans. Food grade $MgCl_2 \cdot 6H_2O$ and oyster shell powder was purchased from Samwoo TD. (Seoul, Korea). Shell powder was composed of 93% crude ash containing 65.4% of calcium. And, shell powder contained other minerals as follows; magnesium 476 mg/100 g, iron 53 mg/100 g, phosphorus 21 mg/100 g, potassium 7 mg/100 g, and sodium 2 mg/100 g.

2.2. Preparation of tofu

Tofu was prepared by a modification of the methods proposed by Park, Nam, Jeon, Oh, and In (2003). Washed soybeans (≈110 g) were soaked in 500 mL tap water in 1litre beaker at room temperature (28 °C) for 16 h. After the stipulated soaking time, the beans were drained and ground with 1100 mL tap water in a Waring blender for 2 min at high speed. The mash was strained through a muslin cloth and pressed to obtain soymilk. Soymilk (1000 mL) was heated to boiling, and, then, mixed with 1.8% $MgCl_2 \cdot 6H_2O$ alone or together with 0.05%, 0.1%, and 0.2% shell powder at 80 °C, followed by being held for 15 min to coagulate. The curd was gently transferred to a perforated stainless steel container (9.5 × 5.0 × 7.0 cm depth) lined with a single layer of cheesecloth and pressed for 15 min using bricks weighing 3.5 kg. The tofu was immersed in the running tap water for 30 min, drained for 30 min to remove excess moisture, and subjected to the analysis and sensory evaluation.

2.3. Yield, moisture content and colour

Yield of tofu was expressed as fresh weight of tofu obtained from 1000 mL of soymilk. Moisture content was determined by drying 5 g of fresh tofu at 105 °C in air oven to constant weight (Tsai, Lan, Kao, & Chen, 1981).

Colour evaluation was performed on fresh tofu samples using a Hunterlab Model D25 Tristimulus Colorimeter, 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. Coefficients of variations for all measurements were less than 3%.

2.4. Syneresis

Syneresis was measured by a modified method of Amstrong, Hill, Schrooyen, and Mitchell (1994). Three pieces of tofu samples of 1.5 cm diameter were weighed and filled into Visking tubing (2.5 cm diameter). The tube was wrapped with plastic wraps and tied to a wire frame placed over a 2 litre beaker in a hanging position for 24 h at 4 °C. Percentage syneresis was calculated as the weight of water released from the tofu in 24 h divided by the weight of sample and multiplied by 100.

2.5. Texture measurement of tofu

Texture profile analysis was applied to evaluate the textural properties of tofu samples using TA-XT2 Texture Analyzer (Stable Micro Systems, Goldaming, UK). Cube samples of tofu (10 mm × 10 mm × 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. Textural parameters of the tofu, hardness, cohesiveness, springiness, gumminess, chewiness and adhesiveness, were evaluated for each treatment according to the definitions given by Bourne (1982). Ten samples were analyzed for each treatment.

2.6. Storage test of tofu

The tofu (9.5 × 5 × 7 cm) obtained above was placed in a polypropylene container (12 × 8 × 12 cm) containing 100 mL of sterilized distilled water as an immersion solution. The container with plastic cover was stored at 10 °C for 11 days. All experiments were triplicated.

In order to determine viable bacterial counts, the tofu and immersion solution were homogenized together by using Polytron homogenizer (RT-1200C, Switzerland), and centrifuged at 3000*g* for 20 min. The above supernatant was diluted with 0.1% peptone water. Plate count agar (Difco, St. Louis, USA) was used for the determination of total viable counts. All plates were triplicated, incubated at

30 °C for 48 h, and viable cell numbers were determined as colony forming units (CFU) per mL.

2.7. Sensory evaluation

Twenty panelists who were trained to recognize and score the sensory attributes participated in the sensory evaluation. The list of attributes selected by the authors as appropriate for defining the quality of the tofu are mouth-feel, firmness, beany-flavour and overall acceptability, and the results were expressed on a 5-point scale (1 point = very poor (weak) to 5 point = very good (strong)) for each attribute. Samples were steamed before being served in the form of a cube (1.5 cm). All samples were coded and always presented in a randomized arrangement.

Data were analyzed using an ANOVA procedure of the SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL). When significance was indicated, means were separated using Fisher's Least Square Difference Test. Statistical tests were conducted at the 5% probability level.

3. Results and discussion

3.1. Yield and moisture

Yield of tofu in this experiment was of the order: 0.1% addition of shell powder (198.4 g) > 0.05% addition of shell powder (186.4 g) > 0.2% addition of shell powder (176.1 g) > a single use of $MgCl_2$ (171.0 g) (Table 1). However, there was not a significant difference ($p > 0.05$) between 0.05% and 0.1% addition of shell powder. And there were not significant differences ($p > 0.05$) among 0.05% and 0.2% addition of shell powder, and a single use of $MgCl_2$.

Moisture contents of tofu added with shell powder were higher than that with a single use of $MgCl_2$, which is reflected in the lower yield of tofu (Table 1). Both tofu prepared with a single use of $MgCl_2$ and with shell powder gave clear whey, indicating that the level of coagulant added was sufficient for complete coagulation of the soy proteins. The increase in yield was reflected by the higher moisture content of tofu prepared with shell powder.

The higher moisture in tofu prepared with shell powder, compared to tofu prepared with a single use of $MgCl_2$, is probably due to the differences in the gel network effected by the ionic strengths of the coagulants and/or the effect of the different anions on the water-holding capacity of the soy protein gels (Wang & Hesseltine, 1982).

3.2. Syneresis and colour

When stored at 4 °C for 24 h, syneresis of tofu prepared with shell powder was significantly ($p < 0.05$) lower than that of tofu prepared without shell powder (Fig. 1). Especially, 0.05% addition of shell powder showed more reduction of syneresis than 0.1% and 0.2% addition. It is generally accepted that syneresis in the protein gel during storage is caused by an increased cross-linking among protein molecules through various interactions, making the protein gel matrix denser (Sun & Breene, 1991). In addition of shell powder, the decrease in syneresis could result from enhanced water retention in the gel microstructure.

Tofu of good quality is generally white or light yellow in colour. All the tofu samples prepared in this study had a light yellow colour. Tofu prepared without shell powder showed a slightly higher L and a values, but lower b value than that prepared with shell powder (Table 2). However, a and b values of tofu prepared without shell powder were not significantly ($p > 0.05$) different from those of tofu prepared with 0.05% addition of shell powder. The yellowness (b value) increased with increasing amount of shell powder. No significant difference ($p > 0.05$) in a and b values were also observed between no addition and 0.05% addition of shell powder. With increasing the amount of shell powder addition, there were trends of decrease for L value, but

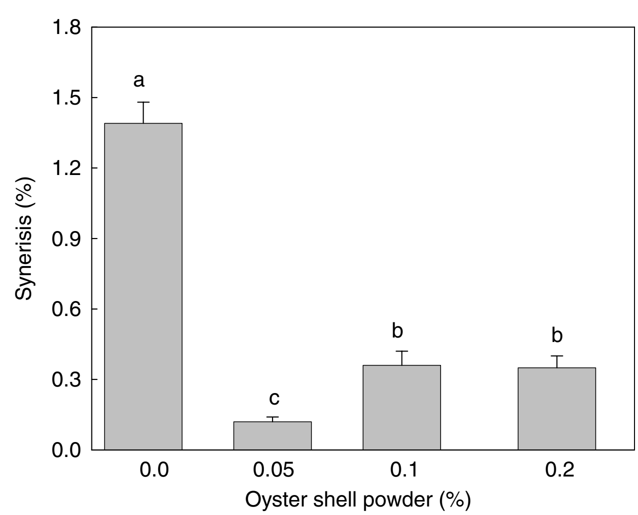

Fig. 1. The effect of shell powder on syneresis of tofu. Percentage syneresis was calculated as the weight of water released from the tofu in 24 h at 4 °C.

Table 1
Yield and moisture content of tofu[A]

Characteristics	Shell powder (%)			
	0.0	0.05	0.10	0.20
Yield (g)	171.0 ± 1.65[b]	186.4 ± 1.55[ab]	198.4 ± 6.55[a]	176.1 ± 4.28[b]
Moisture (%)	83.0 ± 0.09[b]	84.0 ± 0.09[ab]	84.7 ± 0.39[a]	83.0 ± 0.69[b]

[A] Means ± SD of quadruplicates. For each type of tofu, the same superscripts in the same shell are not significantly different ($p > 0.05$).

Table 2
Effect of shell powder on the colour of tofu[A]

Shell powder (%)	*L*	*a*	*b*
0.0	90.6 ± 0.2^{a}	0.48 ± 0.05^{a}	12.17 ± 0.12^{c}
0.05	88.9 ± 0.3^{b}	-0.07 ± 0.13^{ab}	13.37 ± 0.02^{c}
0.1	88.0 ± 1.0^{b}	-0.79 ± 0.11^{c}	15.94 ± 0.31^{b}
0.2	87.9 ± 2.1^{b}	-0.21 ± 0.52^{b}	19.34 ± 1.32^{a}

[A] Means ± SD of 10 replicates. For each type of tofu, the same superscripts in the same column are not significantly different ($p > 0.05$).

no significant differences ($p > 0.05$) in L value were observed among the addition of shell powder.

3.3. Textural properties

The addition of shell powder (0.05 and 0.2%) significantly ($p < 0.05$) hardened the texture of tofu and subsequently increased the gumminess and chewiness while did not significantly ($p > 0.05$) influence the cohesiveness and adhesiveness of the tofu samples (Table 3). However, no significant ($p > 0.05$) further increase in textural properties such as hardness, gumminess and chewiness were observed when the amount of added shell powder increased from 0.05% to 0.20%. The result indicated that the presence of calcium ion in the mixed coagulant system increased the hardness of tofu sample, and a similar effect of calcium salt has previously reported by Kim, Lee, and Hong (1997). They reported the increase of tofu hardness with the increase of calcium salt fraction in the coagulant mixture of calcium salt (calcium chloride or calcium sulfate) and glucono-δ-lactone.

The hardening of tofu by the addition of calcium ion can be affected by the way protein interacts with calcium and other constituents, e.g. phytic acid, in soy milk and anions to form the microstructure into gel (Lim, de Man, de Man, & Buzzel, 1990; Wang & Hesseltine, 1982). Gelation of food protein involves heat denaturation followed by aggregation. If aggregation is relatively slower than denaturation an ordered structure will be promoted, by allowing the denatured molecules to orient themselves in a systematic fashion prior to aggregation (Hermansson, 1978). Conditions that retard the intermolecular interaction will result in a more homogeneous and regular network and consequently a stronger tofu structure (Bernal, Smadja, Smith, & Stanley, 1987).

When magnesium sulfate was used as a coagulant the corresponding tofu had textural properties much different from other coagulants. The hardness and chewiness of magnesium sulfate coagulated tofu was less than the hardness and chewiness of every other tofu prepared in calcium ions. According to Wang and Hesseltine (1982), cross-linking between protein molecules along with the presence of calcium ions is required for soy protein coagulation. But magnesium ions can also be used instead of calcium ions, since this divalent cation can form cross-linking between protein molecules. However, the sites of cross-linking in the protein molecules may be different for both calcium and magnesium causing the latter to form a loose network.

3.4. Sensory characteristics

Tofu samples were evaluated by a panel for mouthfeel, firmness, beany-flavour and overall acceptability on a 5-point scale. Tofu prepared with $MgCl_2$ alone and together with shell powder were evaluated, and the results are shown in Table 4. Mouth-feel decreased with increasing amount of shell powder addition, but there was not a significant difference ($p > 0.05$) between 0.1 % and 0.2% addition of shell powder. Firmness increased with increasing amount of shell powder addition, but no significant differences ($p > 0.05$) in firmness were observed in tofu prepared with shell powder. With increasing the amount of shell powder addition, there were trends of decrease for mouth-feel, but increase for firmness. Hardness measured by texture analyzer did not coincide with the firmness of sensory evaluation. Among them, tofu prepared with 0.05% addition of shell powder showed the highest score of overall acceptability. However, there was not a significant difference ($p > 0.05$) between no addition and 0.05% and 0.1% addition of shell powder. According to the sensory evaluation, the addition of shell powder with 0.05% and 0.1% resulted in the manufacturing the most palatable tofu.

3.5. Storage test of tofu

Fig. 2 shows the changes of the viable microbial counts of tofu prepared with shell powder and a control tofu prepared with single use of $MgCl_2$ during storage at 10 °C for 11 days. All tofu had initial bacterial concentrations of 208 CFU/g at 0 day of storage. These values are similar to ini-

Table 3
Texture profile analysis of tofu[A]

Shell powder (%)	Hardness (N)	Cohesiveness	Gumminess (N)	Chewiness (N mm)	Springiness (mm)	Adhesiveness
0.0	3.71 ± 0.57^{c}	0.54 ± 0.024^{a}	1.99 ± 0.31^{c}	1.71 ± 0.26^{c}	8.64 ± 0.13^{ab}	-0.12 ± 0.04^{a}
0.05	8.17 ± 0.69^{a}	0.54 ± 0.019^{a}	4.42 ± 0.48^{a}	3.78 ± 0.39^{a}	8.53 ± 0.17^{ab}	-0.13 ± 0.07^{a}
0.1	5.91 ± 1.02^{b}	0.57 ± 0.027^{a}	3.33 ± 0.50^{b}	2.95 ± 0.46^{b}	8.93 ± 0.22^{a}	-0.12 ± 0.03^{a}
0.2	7.31 ± 0.49^{a}	0.56 ± 0.027^{a}	4.08 ± 0.37^{a}	3.46 ± 0.43^{ab}	8.52 ± 0.30^{b}	-0.12 ± 0.02^{a}

[A] Means ± SD of 10 replicates. For each type of tofu, the same superscripts in the same column are not significantly different ($p > 0.05$).

Table 4
Effect of shell powder on the sensory quality of tofu[A]

Shell powder (%)	Mouth-feel	Firmness	Beany-flavour	Overall acceptability
0.0	3.57 ± 0.98^{ab}	2.14 ± 0.90^{b}	2.14 ± 1.07^{ab}	3.43 ± 1.27^{ab}
0.05	3.71 ± 1.11^{a}	3.00 ± 0.82^{ab}	1.14 ± 0.38^{b}	4.00 ± 1.00^{a}
0.1	2.71 ± 0.76^{ab}	3.33 ± 0.76^{a}	2.00 ± 1.00^{ab}	3.00 ± 1.00^{ab}
0.2	2.57 ± 0.53^{b}	3.43 ± 0.98^{a}	2.57 ± 1.62^{a}	2.43 ± 0.53^{b}

[A] Means ± SD of twenty sensory evaluations. For each type of tofu, the same superscripts in the same column are not significantly different ($p > 0.05$). Score scale of 1–5 with 1 = very poor (weak) and 5 = very good (strong).

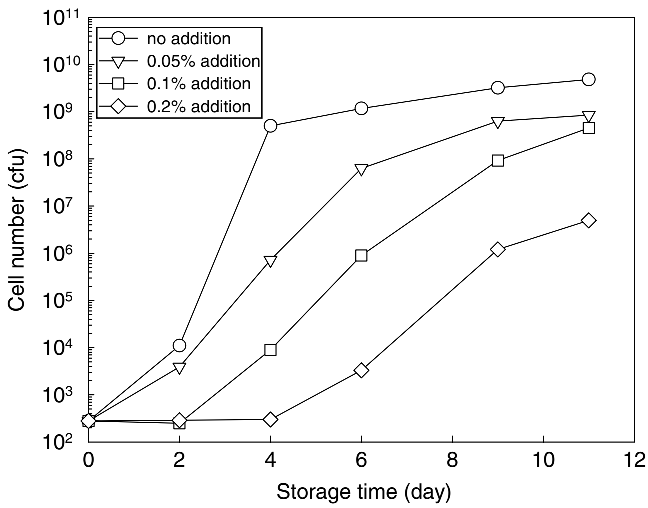

Fig. 2. The effect of shell powder on total microbes of tofu.

tial concentrations (102 CFU/mL) found in the solution which immerses tofu (Miskovsky & Stone, 1987). Tofu prepared with 0.1% and 0.2% addition of shell powder did not show any changes of viable counts by the second day and the fourth day of storage, respectively. However, 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 longer storage periods.

Kim and Lee (1992) postulated that tofu spoilage would start when viable counts were above 10^7 CFU/mL. Based on this, the addition of shell powder in the range of tested concentrations can extend the shelf life of tofu. Tofu prepared with shell powder (0.05% addition) had a shelf life which was 2 days longer than the one prepared with a single use of $MgCl_2$. This was probably due to the antimicrobial activities of CaO in shell powder (Sawai et al., 2001; Shiga et al., 1999). Choi et al. (2006) also reported that the addition of 0.5% shell powder enhanced the shelf life and the quality of *Kimchi* for preservation and consumption.

The effect of the addition of shell powder on tofu preparation was investigated. The tofu prepared with 0.05% and 0.1% shell powder showed a similar sensory quality of the one prepared with $MgCl_2$ alone. As for the storage test, tofu prepared with 0.05% and 0.1% shell powder had a longer shelf life, above 2 days, than tofu prepared with $MgCl_2$. Therefore, this study concluded the use of 0.05% and 0.1% of shell powder for tofu preparation resulted in a good sensory evaluation and the extension of shelf life.

References

Amstrong, H. J., Hill, S. E., Schrooyen, P., & Mitchell, J. R. (1994). A comparison of the viscoelastic properties of conventional and Maillard protein gels. *Journal of Texture Studies, 25*, 285–298.

Bernal, V. M., Smadja, C. H., Smith, J. L., & Stanley, D. W. (1987). Interactions in protein/polysaccharide/calcium gels. *Journal of Food Science, 52*, 1121–1125, 1136.

Bourne, M. M. (1982). Instrumental texture profile analysis. In M. M. Bourne (Ed.), *Food texture and viscosity: Concept and measurement* (pp. 114–117). New York: Academic Press, Inc.

Champagene, C. P., Aurouze, B., & Goulet, G. (1991). Inhibition of undesirable gas production in tofu. *Journal of Food Science, 56*, 1600–1603.

Choi, Y. M., Whang, J. Y., Kim, J. M., & Suh, H. J. (2006). The effect of shell powder on the extension of the shelf life of Kimchi. *Food Control, 17*, 695–699.

Derbyshire, E., Wright, D. J., & Boulter, D. (1976). Legumin and vicilin storage protein of legume seeds. *Phytochemistry, 15*, 3–24.

Hermansson, A. M. (1978). Physicochemical aspect of soyprotein structure and gel formation. *Journal of Texture Studies, 9*, 33–58.

Kim, D. H., & Lee, K. S. (1992). Effects of coagulants on storage of packed tofu. *Korean Journal of Food Science and Technology, 24*, 92–96.

Kim, J. W., Lee, J. K., & Hong, J. H. (1997). Effect of mixed coagulant on the rheological properties of soybean curd. *Journal of Korean Society of Food Science and Nutrition, 26*, 1096–1101.

Lim, B. T., de Man, J. M., de Man, L., & Buzzel, R. I. (1990). Yield and quality of tofu as affected by soybeans and soymilk characteristics. Calcium sulphate coagulant. *Journal of Food Science, 55*, 1088–1092, 1111.

Miskovsky, A., & Stone, M. B. (1987). Effects of chemical preservatives on storage and nutrient composition of soybean curd. *Journal of Food Science, 52*, 1535–1537.

No, H. K., & Meyers, S. P. (2004). Preparation of tofu using chitosan as a coagulant for improved shelf-life. *International Journal of Food Science and Technology, 39*, 133–141.

Park, Y. J., Nam, Y. L., Jeon, B. R., Oh, N. S., & In, M. J. (2003). Effects of garlic addition on quality and storage characteristics of soybean curd (tofu). *Journal of Korean Society of Agricultural Chemistry and Biotechnology, 46*, 329–332.

Pontecorvo, A. J., & Bourne, M. C. (1978). Simple methods for extending the shelf life of soy curd (tofu) in tropical areas. *Journal of Food Science, 43*, 969–972.

Sawai, J., Shiga, H., & Kojima, H. (2001). Kinetic analysis of the bacterial action of heated scallop-shell powder. *International Journal of Food Microbiology, 71*, 211–218.

Shiga, H., Sawai, J., & Kojima, H. (1999). Utilization of heated shell powder in biocontrol. *Transactions Materials Research Society of Japan, 24*, 557–560.

Suhara, H. (1995). Application of antimicrobial calcium agent in food products. *The Food Industry, 38*, 32–44 (in Japanese).

Sun, N., & Breene, W. M. (1991). Calcium sulphate concentration influence on yield and quality of tofu from five soybean varieties. *Journal of Food Science, 56*, 1604–1607.

Tsai, S. J., Lan, C. Y., Kao, C. S., & Chen, S. C. (1981). Studies on the yield and quality characteristics of tofu. *Journal of Food Science, 46*, 1734–1740.

Wang, H. L., & Hesseltine, C. W. (1982). Coagulation conditions in tofu processing. *Process Biochemistry*(Jan/Feb), 7–12.

Wu, M. T., & Salunkhe, D. K. (1977). Extending shelf life of fresh soybean curds by in-package microwave treatments. *Journal of Food Science, 42*, 1448–1450.