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Calcium Lactate Treatment after Salting of Chinese Cabbage Improves Firmness and Shelf-life of Kimchi

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Abstract

In order to enhance the firmness and shelf-life of kimchi, as well as to increase the content of well-absorbed digestible calcium, the effect of calcium lactate (CaL) treatment of salted Chinese cabbage on pH, titratable acidity, total microbes, lactic acid bacteria, alcohol insoluble substance (AIS) content, firmness, mineral content and tissue structure were investigated. Treatment with the Cal solution increased pH and decreased titratable acidity, which was more pronounced at higher concentrations. The edible period evaluated by pH was 7~8 days for non-treated kimchi, 10 days for 1% treated kimchi, 15 days for 2% treated kimchi and 20 days for 3% treated kimchi. Total microbes were reduced, but lactic acid bacteria counts were higher in the treated group. CaL treated kimchi showed higher AIS content and firmer texture, which was more conspicuous in the 2 and 3% CaL treated groups. Calcium content in kimchi fermented for 15 days was 40.75~41.53 mg%, which is 42~45% higher than that in the control group. The sodium content was 23~54% less in the treated groups. The epidermis and vascular bundle tissue of kimchi fermented for 15 day was damaged more severely in the control group than in the treated group. CaL treated kimchi has a crispier taste and the development of sour taste was delayed. Therefore, addition of CaL can produces a kimchi with high calcium as well as superior texture and shelf-life, when adjusting the concentration according to the fermentation periods.

Key words: kimchi, calcium lactate, shelf-life, firmness, black snail

INTRODUCTION

Kimchi is a Korean traditional fermented food made with salted vegetables with red pepper powder, garlic and salted fish. It is highly hygienic because of the preservation effects of salt and the anti-microbial characteristics of lactic acid bacteria. Reported health benefits of kimchi include: strengthening intestinal activities, anti-cancer, anti-oxidant, lowering cholesterol, preventing anemia, anti-diabetes and anti-obesity properties (1-4). However, it is necessary to store kimchi at low temperatures because it will otherwise become softened and putrefactive during fermentation resulting from excessive acid production and weakened lactic acid bacteria (5), propagation of yeast (6), actions of cell wall related enzymes (7). Therefore, diverse production methods have been studied such as low temperature storage (8), adding preservatives (9), improved packaging of kimchi (10) and adding natural substances (11) in order to increase preservation and enhance quality. The softening phenomenon of kimchi tissue occurs when salt penetrates into the tissue of the vegetables during the salting process. Calcium combined with cell wall pectin becomes separated or erupted, which can reportedly delay the softening (7). Lee et al. (12) reported that they could lengthen the edible period for 5 days when they add Sepiaeos, which is high in calcium, to kimchi. Lee and Lee (13) reported that when adding organic salts, which contain calcium, were added to kimchi, the content of alcohol insoluble substance and the solidity of kimchi are highly maintained. Park et al. (3) reported that calcium powder materials coerced the creation of acid in kimchi. Additionally, Kim et al. (14) reported the effects of adding crab shell to kimchi were a result of the calcium contained in the shell. On the other hand, calcium lactate is a non-toxic water-soluble calcium that provides health benefits such as prevention of osteoporosis, cancer and plagues and is widely used as a calcium supplement (15). Although it is well known that CaL delays acidification and strengthen the tissue of vegetables (14), there are few studies on the different effects according to different treatment. In this study, the effects of calcium lactate made from the shell of black snail (16), which has been a history as a popular ingredient because the blue pigment from the boiled black snail has been reported to be an effective intervention for liver infection, hepatocirrjosis and liver cancer. To produce kimchi with an

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extended shelf-life and high calcium content using the calcium lactate, we treated salted cabbage with different concentrations of calcium lactate solutions and investigated the effects of the solutions on organoleptic properties and shelf-life of kimchi.

MATERIALS AND METHODS

Materials

For preparation of kimchi, Chinese cabbage (*Brassica campestris* var. pekinensis Galacsin No. 1) harvested in the fall (3 kg) was used. Black snail (*Swnisulcopira bensoni*), averaging about 35 mm in length and 14 mm in diameter, were originated from North Korea, Gyeongju Awa National Agricultural Cooperative Federation was used. Red pepper powder, garlic, ginger, fermented anchovy juice were used as subsidiary materials.

Preparation of calcium lactate

100 mL of 10% lactic acid solution was placed in a 500 mL Erlenmeyer flask with a cooling device-attached and was heated at 70°C while adding 18 g of ash of black snail and constantly stirring with a magnetic stirrer/hot plate (Misung, MS-300, Korea) and finally neutralized at pH 7.0. The neutralized lactate solution was then passed through a glass filter to eliminate the residue and was then dehydrated at 120°C to obtain anhydrous calcium lactate (17).

Calcium lactate treatment of Chinese cabbage after salting

The Chinese cabbage with the outer leaves removed was divided into four parts, salted and soaked in 10% NaCl solution for 24 hours at room temperature. The ratio of 10% NaCl solution to the fresh weight of the Chinese cabbage was 2.5 : 1 (v/w). After salting, kimchi was treated with the calcium solutions (1, 2 and 3%) at a 1.5 : 1 (v/w) ratio to salted cabbage for 5 hours. The soaked cabbage was rinsed under running tap water three times and drained for 3 hours at 4°C.

Preparation of kimchi

Four different kimchis were prepared, the control kimchi which was not treated with calcium lactate, and kimchis prepared with the Chinese cabbage dipped in 1~3% calcium lactate solution for 3 hours after salting. To each 100 g of salted cabbage (final salt concentration: 2.5%), garlic 2.0 g, chopped ginger 0.8 g, red pepper powder 2.0 g, fermented anchovy sauce 4.8 g were added and mixed well. Prepared kimchi was packed in 350 mL of plastic vessels without headspace and fermented for 20 days at 10°C.

Measurement of pH and titratable acidity

The cabbage tissue and kimchi juice were homogenized together by using Polytron homogenizer (PT-1200C, Swizerland), and filtered through three layers of cheese cloths. The pH was measured with a pH meter (Methrom 632, Switzerland), and titratable acidity, calculated as lactic acid %, was determined by the pH-metric method.

Measurement of total microbe and lactic acid bacteria

The cabbage tissue and kimchi juice were homogenized together with Polytron homogenizer, and diluted a gradation with 0.1% peptone water. Total viable counts and lactic acid bacteria were determined on Plate Count Agar (Difco), MRS agar with 0.002% of bromophenol blue. All plates were triplicated and incubated at 37°C for 48 hours and viable cell numbers were counted a colony forming units (cfu) per mL (6).

Measurement of AIS and firmness

Alcohol insoluble substance (AIS) was determined according to the methods of Kim et al. (18). Ethanol was added to 50 g of kimchi to make alcohol concentration of 80%. The sample was then homogenized, and boiled at 80°C for 20 min. Whatman No. 5 filter papers were used to filter the homogenate, and the residue was washed three times with 80% ethanol and vacuum dried at 40°C. Firmness of the kimchi was measured using a Rheometer (RE-3305, Yamaden, Japan) on a cube cut (2×2 cm) from the mid rib area of the kimchi.

Measurement of crude ash and mineral content

Crude ash and mineral content was determined according to the methods of Kim et al. (19). Crude ash content was measured, seasonings on the surface of kimchi tissue were removed with cheese cloth, 10 g of sample dried at 80°C for 24 hours and ashed at 600°C in a muffle furnace (HY-4500, Hwashin Co., Korea). Mineral content was determined in the ash after it was solublized with 25 mL 6 N HCl and filtered through Whatman No. 5 filter papers, using an ICP-AES (JY 38 Plus, France). Measurement conditions were: frequency 40.66 MHz, plasma gas flow 12 L/min, sheath gas flow 0.2 L/min, auxiliary gas flow 0.1 L/min, sample flow rate 1 L/min.

Microscopic observation

Kimchi tissue (1×1 cm) from around the midrib (epidermis, vascular bundle) was cut and freeze dried for 48 hours. The dried kimchi was then coated with gold by using Carbon Coater (108-CA, Jeol, Japan). Microscopic observation of epidermis and vascular bundle tissue was performed by using 15 kV, 10 μÅ Scanning Electron Microscope (JSM-6335F, Jeol, Japan).

Measurement of color

Seasonings on the surface of white leaves of kimchi tissue were removed with cheese cloth. The color of L* (lightness), a* (redness), b* (yellowness) were measured using a Chromameter (CR-200, Minolta, Japan).

Sensory evaluation

Sensory tests on the sour taste, crispy taste, overall taste were evaluated by twenty five panelists using a five-point scale test (20). Crispy taste and sour tastes were evaluated as very strong (5 points), strong (4 points), medium (3 points), low (2 points), very low (1 point). Overall tastes were evaluated as very good (5 points), good (4 points), medium (3 points), poor (2 points), very poor (1 point).

Statistical analysis

All experiments except sensory evaluation were triplicately determined and represented as mean or mean ± standard deviation (SD). The results of the sensory evaluation was calculated from the mean value and SD of the twenty five panelists. Significance was verified by performing Duncan's multiple range test using SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA) software package.

RESULTS AND DISCUSSION

pH and titratable acidity

Fig. 1, 2 shows the pH and titratable acidity of kimchi made from salted chinese cabbage soaked in 0, 1, 2 and 3% dehydrated calcium lactate solution manufactured from black snail for five hours. There were no significant differences between the control group and the experimental groups in pH on the soaking day, but during the fermentation periods, the experimental group maintained

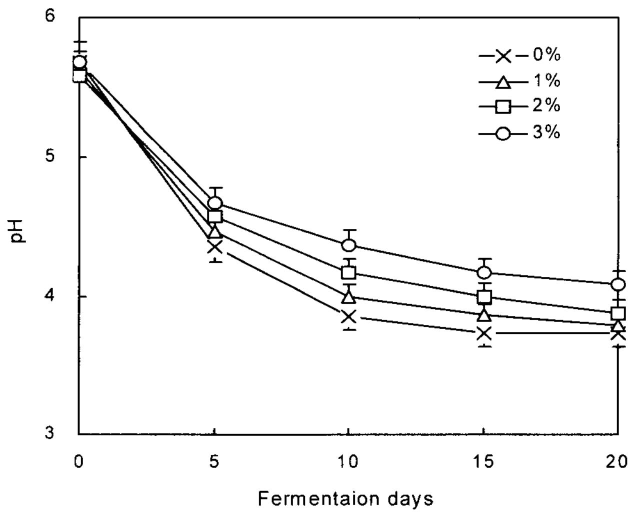

Fig. 1. Changes in pH of kimchi prepared with Chinese cabbage treated with different concentration of calcium lactate (0~3%) after salting during fermentation at 10°C.
Values are mean ± SD of triplicate determinations.

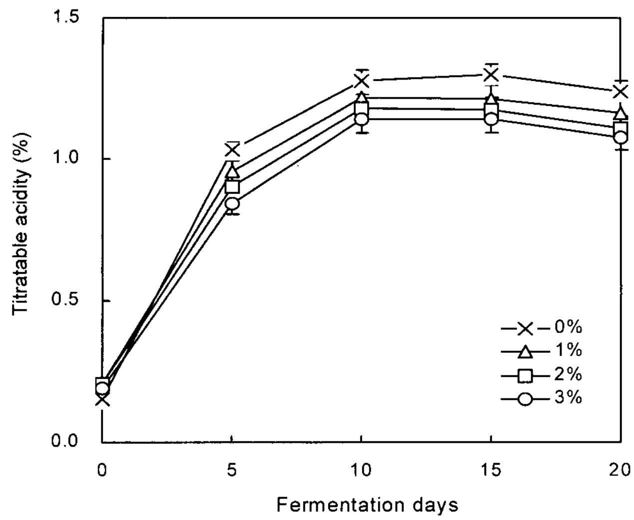

Fig. 2. Changes in the titratable acidity of kimchi prepared with Chinese cabbage treated with different concentration of calcium lactate (0~3%) after salting during fermentation at 10°C.

a higher pH than the control group. The higher the calcium lactate (CaL) concentration, the higher the pH was maintained. In summary, the pHs of the control group at the 10th and 20th day of fermentation were 3.85 and 3.73, respectively, but the treated groups using 3% CaL solution showed 4.37 and 4.08, respectively. Titratable acidity was 1.03 in the control group on the 5th day, while it was 0.84~0.96 in the treated group. On the 10th and 20th day of fermentation, they were 1.28 and 1.24, respectively, in the control group, but they were 1.14~1.22 and 1.08~1.16 in the treated group. These results are consistent with the results of Park et al. (3) and Kim et al. (15) that adding calcium powder or calcium lactate to kimchi increased the pH. Kim et al. (15) proposed that adding calcium lactate to kimchi would increase acidity because of the buffering actions between calcium salt and lactic acid from kimchi, which may account for the lower difference in acidity than in pH in this study.

Kim et al. (15) reported that the edible period of kimchi would be 10 to 15 days. However, considering the optimal pH of kimchi was over 4.0 (21), the edible period of non-treated kimchi was only 7 to 8 days. This may be because the composition of materials makes considerable effects on fermentation. Additionally, the edible period of kimchi to which calcium lactate was added extended to 10 days for 1% treatment, 15 days for 2% treatment and 20 days for 3% treatment. These results demonstrate that the edible period can be extended by treatment with calcium lactate after the salting process.

Total microbe and lactic acid bacteria

Fig. 3 and 4 shows the number of total microbes and lactic acid bacteria during the fermentation of kimchi made with salted Chinese cabbage treated in 1~3% of

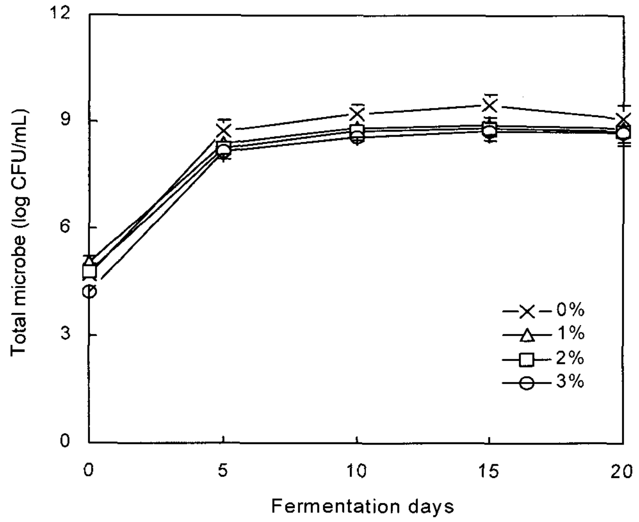

Fig. 3. Changes in the number of total microbes in kimchi prepared with Chinese cabbage treated with different concentration of calcium lactate (0~3%) after salting during fermentation at 10°C.

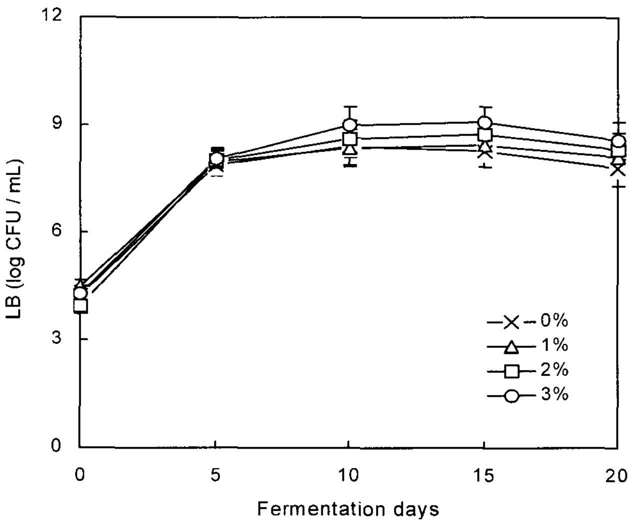

Fig. 4. Changes in the number of lactic acid bacteria (LB) of kimchi prepared with Chinese cabbage treated with different concentration of calcium lactate (0~3%) after salting during fermentation at 10°C.

CaL solution. Total microbes increased rapidly up to the 5th day of fermentation both in the treated group and the control group, with counts of log 8.74 cfu/mL in the control group and 8.17~8.40 cfu/mL in the treated group. Up to the 10th day of fermentation, there was no significant difference between the control group and the treated group. However, after the 10th day, the increase in total microbes was lower in the treated group. After the 15th day of fermentation, bacteria counts decreased in both groups. The numbers of total microbes in the control group on the 15th day and 20th day were 9.46 and 9.10 cfu/mL, respectively. In the treated group, they were 8.75~8.93 and 8.70~8.82 cfu/mL, respectively. The lower number of total microbes in the treated group may be partially due to the anti-microbial functions of CaL (22). But it may also be due to differences in the concentration of the salt solution when soaking the salted Chinese cabbage in the CaL solution. Although the number of total microbes becomes low when salt concentration is high, Hahn et al. (23) suggested that kimchi fermented in 1.25% salt concentration adding alcohol and adipic acid showed a lower number of total microbes than the kimchi fermented in 2.5% salt concentration. The number of lactic acid bacteria increased upto the 15th day of fermentation and then began to decrease in both groups. In contrast to the total microbes, lactic acid bacteria were 8.26 and 7.78 cfu/mL at the 15th and 20th day of fermentation in the control group and they were 8.46~9.10 and 8.08~8.57 cfu/mL in the treated group, therefore lactic acid bacteria continued to increase in the CaL treated kimchi. Among the treated groups, the one treated with 3% CaL solution had the highest number of lactic acid bacteria. The CaL treatment effect was most noticeable after the 15th day. Cho and Rhee (24) reported that increases in lactic acid bacteria would be consistent with the decrease in pH, which is in agreement with our results. The ratio of lactic acid bacteria to total microbes during fermentation was higher in the treated group than in the control group. The higher the ratio is, the higher the quality fermentation products in kimchi, and also indicates a more hygienic kimchi. Lee et al. (25) suggested that the ratio of lactic acid bacteria to total microbes would be as follows; in the beginning of the fermentation, total microbes would increase because of the growth of aerobic and non-aerobic bacteria. As fermentation proceeds, organic acid would be created and acid-durable non-aerobic germs would increase. Thereby, the number of lactic acid bacteria would increase and consequently the ratio of lactic acid bacteria would be increased during latter stages of kimchi fermentation, which is consistent with the result of this experiment.

Content of AIS and firmness

The AIS content (alcohol insoluble substance) in the CaL treated kimchi (Fig. 5) decreased upto the 15th day of fermentation, in both in the control group and the treated group and increased gradually after that. On the 20th day of fermentation, the AIS concentration was 2.14 g/100 g in the control group and 2.17~2.52 g/100 g in the treated group. The firmness of kimchi tissue on the 15th day of fermentation (Fig. 6) was 21.80×10^6 dyne/cm^2 in the control group and $24.20\sim29.80\times10^6$ dyne/cm^2 in the treated group, with the higher values realized with higher CaL concentrations. The AIS in the Chinese cabbage tissue is mostly cell wall pectin. It

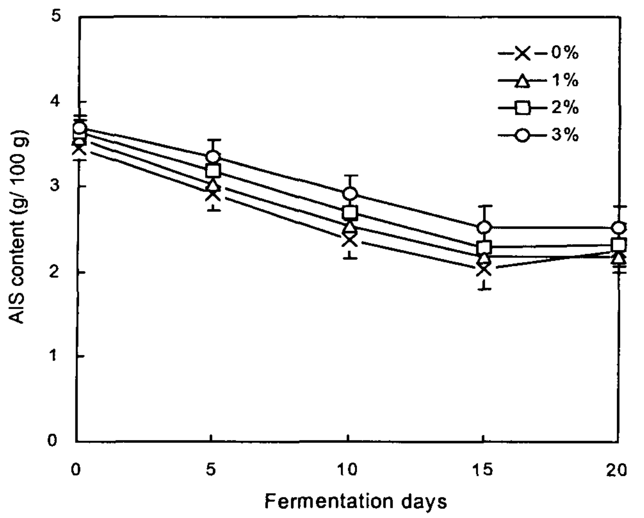

Fig. 5. Changes in the AIS (alcohol insoluble substance) content of kimchi prepared with Chinese cabbage treated with different concentration of calcium lactate (0~3%) after salting during fermentation at 10°C.

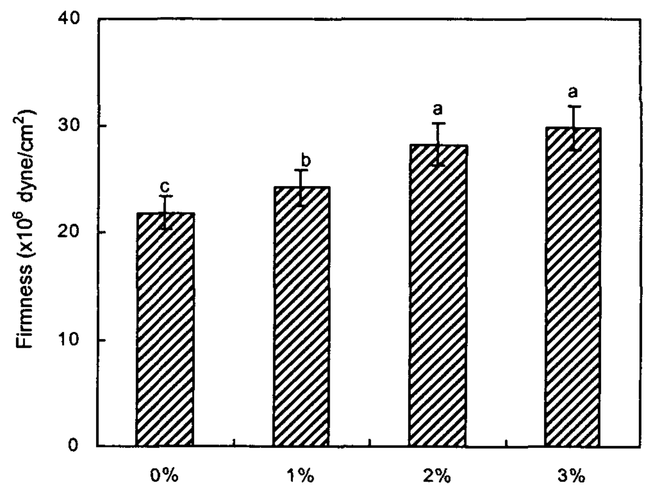

Fig. 6. Firmness of kimchi fermented for 15 days at 10°C prepared with Chinese cabbage treated with different concentration of calcium lactate (0~3%) after salting.
Values are mean ± SD of triplicate determinations, different superscripts on the bar indicate significant differences at $p<0.05$.

becomes available during the fermentation through the action of pectinesterase and polygalacturonase (26) or solubilized into the acid solution produced by fermentation (7). Oh and Kim (27) reported that the penetration of Na^{+} into the tissue of Chinese cabbage would elute Ca^{++}, which would stimulate enzymes that decompose cell wall polysaccharides. Therefore, CaL treatment can prevent the actions of enzymes by replenishing Ca^{++} eluted during the salting and delay fermentation by preventing the elution of saccharides, the nutrient sources of lactic acid bacteria. On the other hand, the increased AIS in the control group during the later periods of fermentation may be due to the increased dry materials resulting from water elution during fermentation. Kim and Kim (18) investigated changes in AIS content during the fermentation and reported that AIS content had increased because of the increased water elution from the tissue. Kaneko et al. (28) and Kim and Lee (29) reported the same results.

Content of crude ash and minerals

Table 1 shows the content of crude ash and minerals in kimchi fermented for 15 days. The content of crude ash was 1.90 mg% in the control group, 1.43 mg% in the 1% treated group, 1.73 mg% in the 2% treated group, 2.06 mg% in the 3% treated group. In 3% treated group, the crude ash content was higher than in the control group. The calcium content of the treated groups were 40.75~41.53 mg%, which was 42~45% higher than in the control group (28.60 mg%). However, the content of Na was 172.33 mg% in the control group and 35.49~92.26 mg% in the treated group, which is 23~54% of that in the control group. Additionally, Fe content in the treated group was 66~83% of the control group. In other words, the sodium content was increased by the salt treatment and calcium removed, however, subsequent soaking in the CaL solution removed much of the sodium while simultaneously restoring calcium concentration to higher levels than in the original cabbage. This process produces a higher calcium and lower sodium kimchi than obtained by conventional methods. Kim et al. (14,15) suggested that the calcium content in the tissue of the Chinese cabbage would be closely related with the firmness of kimchi, and that softening would occur during

Table 1. Content of crude ash and minerals of kimchi fermented for 15 days at 10°C prepared with Chinese cabbage treated with different concentration of calcium lactate after salting

CL (%)[1)]	Crude ash (%)	Ca (mg%)	Fe (mg%)	Na (mg%)
0	1.90 (100)[a2)]	28.60 (100)[b]	1.49 (100)[a]	172.33 (100)[a]
1	1.43 (75)[c]	40.75 (142)[a]	1.08 (72)[bc]	39.60 (23)[c]
2	1.73 (91)[b]	41.57 (145)[a]	1.23 (83)[b]	35.49 (21)[c]
3	2.06 (108)[a]	41.53 (145)[a]	0.98 (66)[c]	92.26 (54)[b]

[1)]CL: calcium lactate prepared with black snail.
[2)]Values are means of triplicate determinations, different superscripts within a column indicate significant differences at $p<0.05$. Parenthesis denote percent against zero % of calcium lactate.

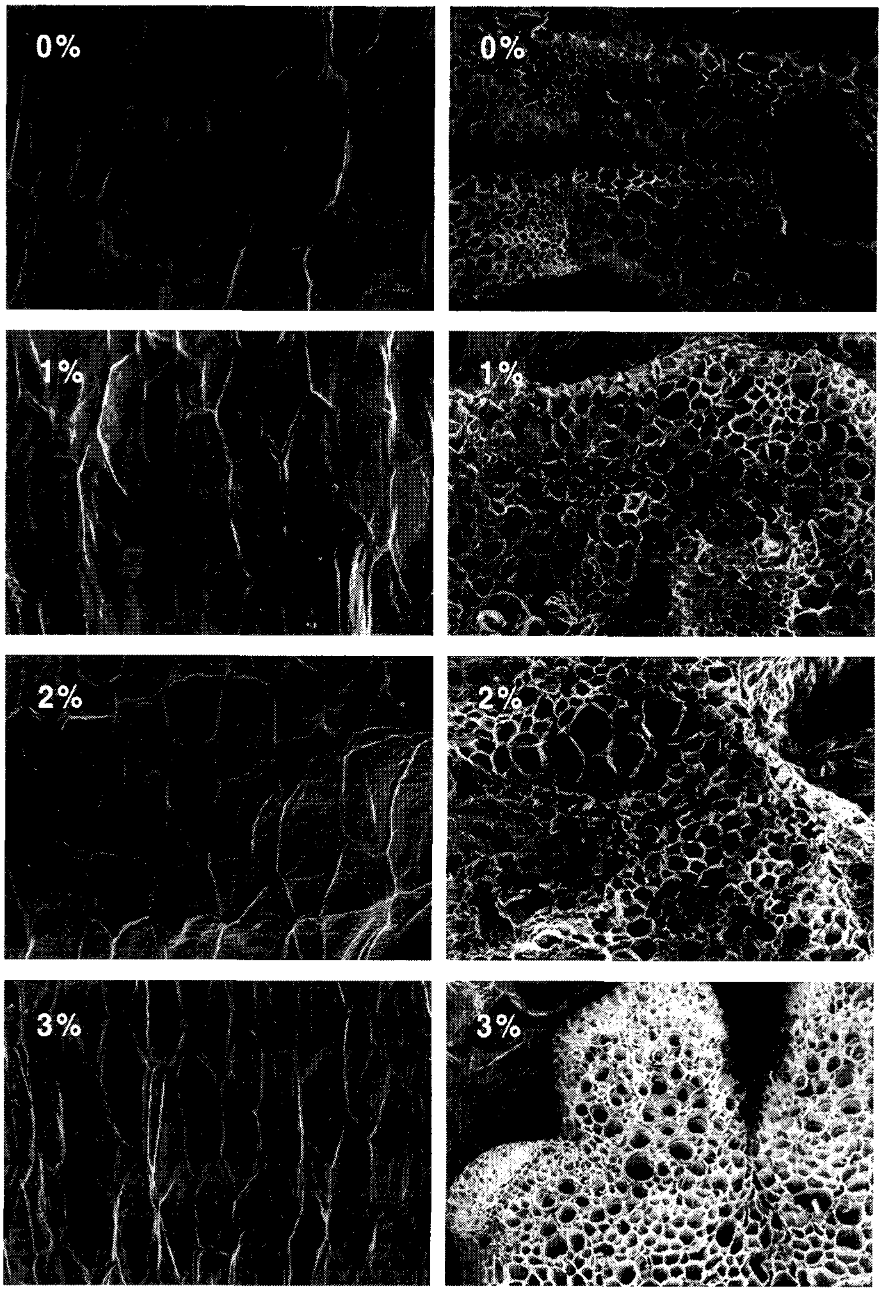

Fig. 7. Scanning electron micrograph photograph of epidermis (left: ×200) and vascular bundle tissue (right: ×100) of white leaf of the Chinese cabbage kimchi prepared from the cabbage treated with different concentration of calcium lactate (0~3%) after salting.

the fermentation because calcium in the tissue is eluted and pectin destroyed. Accordingly, CaL treatment after the salting process can extend the edible shelf-life of kimchi by maintaining firmness of tissue with the supplying providing calcium. Furthermore, the high calcium content contributes significant nutritional value to the kimchi.

Microscopic observation

Fig. 7 shows the epidermis and bascular bundle tissue of kimchi tissue around mid rib in kimchi treated with 0, 1, 2 and 3% CaL solution and then fermented for 15 days at 10°C. The epidermis tissues in the control group showed serious damage and severe contraction. But in the treated group, the cells had a normal shape and sized and were normally distributed. More stable shapes were shown with the higher concentration of CaL. The thickness of cell walls was thicker in the treated group than in the control group, which is consistent with the study result of Kim et al. (15) who found that cell wall tissue would combine with calcium and consequently thicken the cell wall.

Table 2. Changes in sensory qualities of kimchi prepared with Chinese cabbage treated with different concentration of calcium lactate after salting during fermentation at 10°C

Attributes	CL (%)[1)]	Fermentation days				
		0	5	10	15	20
Crispy taste	0	$4.11^{aA2)}$	3.71^{bB}	3.40^{cB}	3.02^{dB}	2.02^{eC}
	1	4.19^{aA}	3.80^{bAB}	3.53^{cAB}	3.11^{dAB}	2.51^{eBC}
	2	4.20^{aA}	3.88^{bAB}	3.55^{cAB}	3.20^{dAB}	2.82^{eB}
	3	4.21^{aA}	4.03^{abA}	3.83^{bA}	3.50^{cA}	3.20^{cA}
Sour taste	0	1.10^{eA}	3.27^{dA}	3.41^{cA}	3.80^{bA}	4.95^{aA}
	1	1.12^{dA}	3.21^{cA}	3.32^{cA}	3.71^{bA}	4.50^{aB}
	2	1.10^{dA}	3.02^{cA}	3.14^{cAB}	3.55^{bAB}	4.37^{aB}
	3	1.12^{dA}	2.60^{cB}	3.01^{bcB}	3.37^{bB}	3.89^{aC}
Overall taste	0	2.75^{dA}	3.83^{bA}	4.20^{aB}	3.01^{cB}	2.10^{eC}
	1	2.62^{dA}	3.79^{bA}	4.47^{aA}	3.09^{cB}	2.78^{dB}
	2	2.57^{dA}	3.77^{bA}	4.50^{aA}	3.28^{cAB}	3.02^{cAB}
	3	2.63^{cA}	3.77^{bA}	4.35^{aA}	3.50^{bA}	3.27^{bcA}

[1)]CL: calcium lactate prepared with black snail.
[2)]Values are means of 25 panels, different superscripts within a row (a~e) and column (A~C) indicate significant differences at $p<0.05$.

Vascular bundle tissue were not significantly different between the control group and the treated group. However, we could see that the shape was maintained in the treated group better than in the control group. It is considered that epidermis tissues are contracted severely because of this. Rye et al. (30) suggested that during the salting process, middle lamella separation and cell wall destruction would arise because of salt. Accordingly, it is judged that this happens because CaL treatment after the salting process restores cell wall components and prevents the actions of enzymes and the elution of cell wall components by the acid.

Sensory evaluation

Table 2 shows the results of the sensory test of kimchi made with CaL treated cabbages. The crispy taste of kimchi was 2.02 on the 20th day of fermentation in the control group and 2.51 points in the 1% CaL, 2.82 points in the 2% CaL and 3.20 points in the 3% CaL, which are higher than in the control group. Additionally, although there was no significant difference between CaL 1% and CaL 2%, it was strikingly high in CaL 3%. Crispy taste is a kind of texture and it is a related function of the firmness of the tissue (31). The increased crispiness is consistent with the higher values for AIS and firmness in the CaL treated group (Fig. 5, 6).

Sour taste increased with fermentation time. From days 5 to 15 of fermentation, the average value was lower in the treated group, but not significantly ($p<0.05$). However, on the 20th day of fermentation, the sourness value was lower in the treated groups than in the control group. In particular, it was very low in the 3% treated group. The overall taste was better in the control group than in the treated group on the 5th day of fermentation, but there was no significant difference. As fermentation progressed, the treated group had increasing better values than the control group. On the 20th day of fermentation, the groups treated with higher CaL concentrations had higher values for overall taste.

In summary, making kimchi with CaL treated cabbages provides a crispy taste and delays the development of a sour taste. Therefore, the CaL concentration should be adjusted according to the fermentation period to enhance texture, to improve shelf-life, and to improved nutritive value of the kimchi.

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