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Use of Nisin as an Aid in Reduction of Thermal Process of Bottled *Sikhae* (Rice Beverage)

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Conventional commercial thermal process for preparing *Sikhae* (Rice beverage) in a hermetically sealed container was evaluated to solve the nutritional deterioration and organoleptic inferiority problem caused by severe heat treatment. A milder thermal process with an aid of Nisin, a GRAS-grade , selectively germicidal compound, was introduced to destroy the putrefactive microorganisms. In this experiment, hot-filling method with Nisin, and thermal processing (at 110°C for 15 minutes with Nisin, at 121°C for 25 minutes without Nisin) were compared. The quality of *Sikhae* could be enhanced and over 90% of the thermal process could be conserved by this process in terms of sterilizing value without quality deterioration when processing the bottled *Sikhae* at 110° for 15 minutes $\{(F^{10}_{121})_{process}=1.54\}$.

Sikhae is a famous traditional rice beverage with a strong malt flavor liberated by amylolytic digestion of starch from rice by enzymes in malt. It is also known as either *Dansool* or *Gamjoo* in Korea (1). This was prepared by houswives at home, however, local packers have already commercialized the *sikhae* in tin cans or retort pouches. They process the *Sikhae* at 121°C for 25 minutes and the approximate $(F^{10}_{121})_{process}$ value of the process is 19 (2). The *Sikhae* processed under these conditions is inferior in nutritional quality and color. However, it is hard to find any publication in regard of the thermal process of *Sikhae*. Most of the papers are simply oriented to studing the saccharification process (1, 12). Major factors of spoilage of *Sikhae* filled in aseptic bottle after boiling were gram-positive bacilli and yeasts in the preliminary experiment. Therefore, we postulated that a milder processing condition could be introduced to destroy the heat-sensitive microorganisms if the germination and growth of heat-resistant sporeformers could be retarded by a unique GRAS grade compound (3). In this study, Nisin was added to control the gram-positive sporeforming bacteria with a mild heat treatment to control the heat-sensitive yeast and non-sporeforming gram negative bacteria.

MATERIALS AND METHODS

Preparation of Bottled *Sikhae*

Sikhae was prepared as outlined in Fig. 1. Malt (5 Kg, commercial grade, spring product) was soaked and extracted with distilled water (3.5 liter). The extract was allowed to stand overnight in a cold room (4°C) and the supernatant was decanted. The steamed (30 minutes over boiling water) rice (5 Kg before steaming) was saccharified with the clear malt extract at 65°C for about 2.5 hours. The juice separated from rice solution was heated up until boiling to stop further enzymatic reaction and sweetness was adjusted to 15° Brix by adding sugar. The boiled juice (180 m*l*) mixed with or without 400 IU/m*l* Nisin was added into a 200 m*l* bottle (53.5×138 cm) previously filled with 4 g of saccharified rice. They were thermally processed (15 minutes at 110°C, 25 minutes at 121°C) and cooled in water both controlled by an electric heating system (Agar sterilizer, Agarmatic AS-8, NBS, U.S.A.). Samples were also prepared by hot-filling method without any thermal processing. The boiling juice with Nisin was filled into a bottle and capped. The bottled *Sikhae* was allowed to stand upside down for 30 minutes and cooled.

Heat Penetration Characteristics

A heat penetration curve was obtained by installing a copper-constantan temperature sensor on the geometric center of the bottle (4 g saccharified rice and 180 m*l* juice, bottle size: 53.5×138 cm) and the temperature was recorded by Temperature Microprocessor (CMC 821-AK, Denmark). The heating parameters such as f_h, j_{ch} and come-up time were calculated by plotting the data on the inversed semi-logarithmic paper (4). $(F^{10}_{121} = F_o)_{process}$ value was calculated by converting the product temperature to lethal rate and summing up the

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lethal rate.

Analysis

Total sugar was determined after HCl hydrolysis and reducing sugar was analyzed by Somogyi method. Total nitrogen was determined by Kjeldahl method and pH was determined by Orion Model 520 pH meter (5). β-Amylase activity was assayed by determining the maltose liberated from soluble starch and α-amylase activity was assayed by determining the amount of liberated sugars as determined by dinitrosalicylic acid (6). Free sugar was analyzed by HPLC (model 510, Waters, U.S.A.) equipped with a carbohydrate analysis column and RI detector (model 410, Waters, U.S.A.). The mobile phase was acetonitrile and water (80/20 : v/v). Minerals were analyzed by using an Inductively Coupled Plasma Atomic Emission Spectrometer (ISA Inst. S.A. Co. Model JY 38 Plus, France). Color was determined by a color difference meter (Yasuda Seiki Sesakusho Ltd., UC 600-IV).

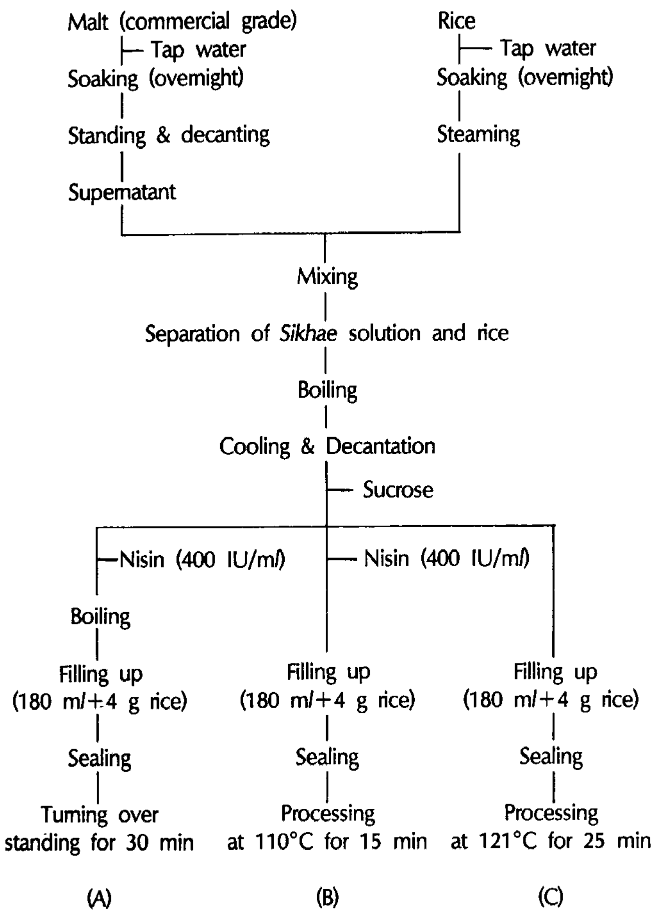

Fig. 1. Preparation of bottled *Sikhae*.
A; Hot filled *Sikhae* with Nisin, B; *Sikhae* mixed with Nisin and processed at 110°C for 15 min. C; *Sikhae* processed at 121°C for 25 min.

Viable Cell Count (7, 8)

Viable cell counts during storage were done by pouring method after serial dilution of the sample. The medium was plate count agar (Difco, U.S.A., 37°C) for aerobic plate count, Slanetz-Bartley medium (37°C, Oxoid, England) for Streptococci count, Potato Dextrose agar (37°C, Difco, U.S.A.) for yeast, and Violet Red Bile Agar for coliform count.

Sensory Evaluation (9)

Sensory scores of *Sikhae* underewent different thermal processes were obtained by the ranking method and preference test.

RESULTS AND DISCUSSION

Composition of Malt

The composition of malt used in this study was shown in Table 1. Malt contained 13.1% moisture, 2.8% reducing sugar (glucose and maltose were 0.6 and 1.9%, respectively), 47.9% total sugar. The level of β-amylase was 155 unit/g. Major minerals were 25.5 mg% of potassium, 21.2 mg% phosphorus, 5.3% calcium and 2.3 mg% sodium.

Changes During Saccharification

After mixing the aqueous malt extract and steamed rice, the mixture was left to stand at 65°C. Saccharification ended after 2.5 hours. The Brix increased from 1.8° initially to 9.8° in the end (Fig. 2). This is a very rapid process yielding a high level of reducing sugar in a very short period compared other reports. Kim *et al.* (1984) (10) reported that level of reducing sugar reached up to 8.9% of reducing sugar after 8 hours. HPLC analysis of free sugar showed that the glucose level changed from 0.1% to 1.1% during the saccharification process but the maltose level increased remarkably due to the high level of β-amylase activity in the malt used here. Final maltose concentration was 6.0%. The level of α-amylase activity was at constant level (11 Unit/ml), however, that of β-amylase activity was maximum (22 Unit/ml) after 1 hour when maltose liberation was very fast. Final concentration of reducing sugar was 10.5% and therefore sweetness was adjusted with sucrose (6.5

Table 1. The composition of malt

Moisture (%)	R.S[a] (%)	T.S[b] (%)	T.N[c] (%)	pH	β-A[d] unit/g	Free sugar (%)		Mineral (mg%)			
						Glu[e]	Mal[f]	Ca	Na	K	P
13.12	2.83	47.91	2.19	5.73	155.44	0.62	1.93	5.30	2.34	25.47	21.16

[a] R.S: Reducing sugar, [b] T.S: Total sugar, [c] T.N: Total nitrogen, [d] β-A unit/g: The amount of maltose (μmol) produced by β-amylase at 37°C for 1 min. [e] Glu: Glucose, [f] Mal: Maltose.

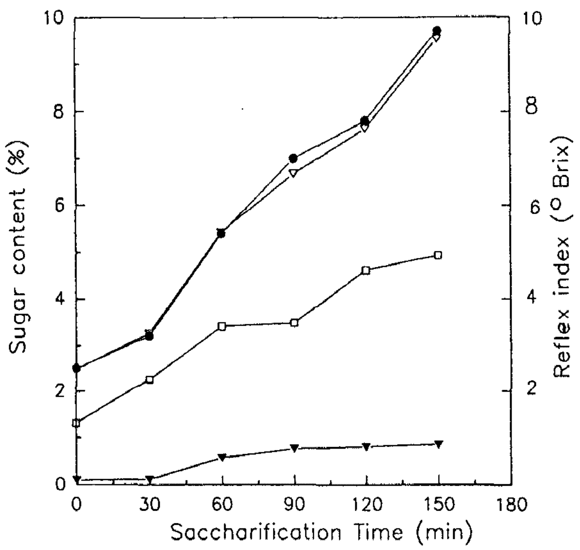

Fig. 2. Changes of sugar components in *Sikhae* solution during saccharification process at 65°C.
●; °Brix, ▽; Reducing sugar, □; Maltose, ▼; Glucose.

Table 2. The composition of *Sikhae*

Moisture (%)	R.S[a] (%)	°Brix	T.A[b] (%)	T.N[c] (%)	pH	Free Sugar (%) Glu[d]	Mal[e]	Suc[f]
80.25	10.47	15.0	0.13	0.18	5.70	0.85	5.93	6.55

[a] R.S: Reducing sugar (as maltose), [b] T.A: Total acidity (as lactic acid), [c] T.N: Total nitrogen, [d] Glu: Glucose, [e] Mal: Maltose, [f] Suc: Sucrose.

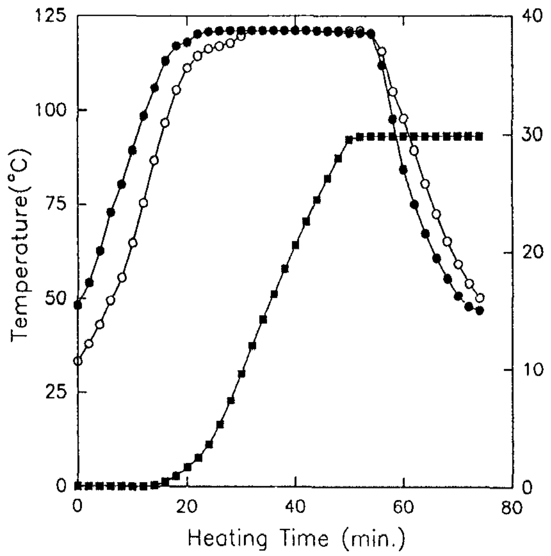

Fig. 3. Heat penetration and $F_{process}$ accumulation profile of bottled *Sikhae* during thermal processing at 121°C for 25 minutes.
○; Bottled *Sikhae*, ●; Retort, ■; Fo value.

%) to 15° brix (Table 2).

Heat Penetration Characteristics

Typical heat penetration curves of bottled *Sikhae* at 121°C for 25 minutes and 115°C for 15 minutes are as in Fig. 3 and 4. The initial temperature (IT) of bottled *Sikhae* was set to 33°C. Fig. 3 showed heating and cooling curve when processing at 121°C for 25 minutes. The product temperature at the slowest heating point reached the processing temperature after 32 minutes. $(F^{10}_{121})_{process}$ after heating and cooling was 29.84. The product temperature was 116°C when the retort temperature reached to the processing temperature after

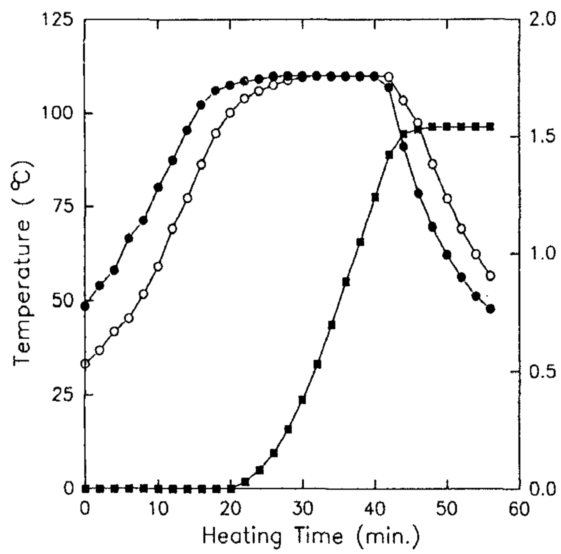

Fig. 4. Heat penetration and $F_{process}$ accumulation profile of bottled *Sikhae* during thermal processing at 115°C for 15 minutes.
○; Bottled *Sikhae*, ●; Retort, ■; Fo value.

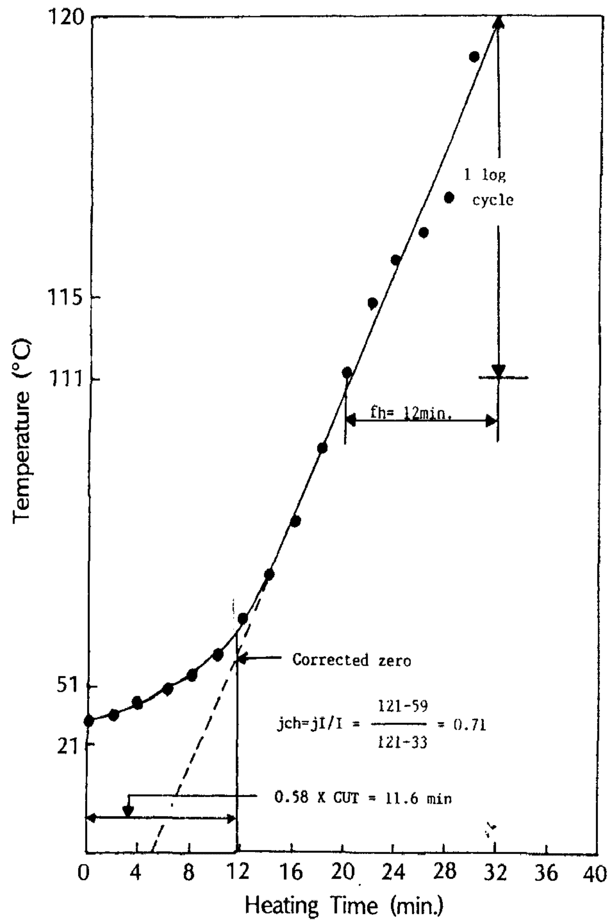

Fig. 5. Heat penetration curve of bottled *Sikhae*

20 minutes of come-up time. The heating curve was plotted on the inversed semilogarithmic paper to get heat penetration parameters (Fig. 5). Heating lag factor (j_{ch}) and f_h value were 0.71 and 12 minutes (13). The heat penetration seemed to be by convection (13). When processing at 110°C for 15 minutes (Fig. 4), the product temperature reached at 105°C after come-up time and $(F^{10}_{121})_{process}$ was 1.54.

Sensory Evaluation

Thermally processed *Sikhae* was subjected to sensory evaluation to evaluate the quality. Fresh *Sikhae* without thermal processing was provided as a control (Table 3). *Sikhae* processed at 121°C for 25 minutes was significantly inferior in color to fresh, hot-filled *Sikhae* and that processed at 110 °C for 15 minutes. This color difference was verified by using a color difference meter (Table 4). Samples became darker as the *Sikhae* was processed under the severe heating condition. Panel members preferred the color and flavor of *Sikhae* processed at 110°C for 15 minutes. There was no significant difference in flavor and taste among fresh, hot-filled *Sikhae* and that processed at 110°C for 15 minutes. This result implies the possibility that *Sikhae* of good quality could be prepared at the lower processing temperature for shorter heating time. The effect of the addition of Nisin on the sensory scores was evaluated (Table 5). It was not possible to observe any difference between the two samples. Therefore, it could be concluded that there is no adverse effect of Nisin on the quality at the added level.

Table 3. The sensory evaluation scores of *Sikhae* prepared by different processes

Sample	Color[2]	Flavor	Taste
A[1]	2.8^b	$2.3^{a,b}$	2.2^a
B	$2.1^{a,b}$	$2.5^{a,b}$	1.7^a
C	1.6^a	1.7^a	2.3^a
D	3.6^c	3.4^b	3.9^b

[1] A: Fresh *Sikhae* without thermal processing, B: Hot filled *Sikhae* with Nisin, C: *Sikhae* mixed with Nisin and processed at 110°C for 15 min, D: *Sikhae* processed at 121°C for 25 min.
[2] Mean of rank-order evaluated by 16 persons. 1; First order, 2; Second order 3; Third order 4; Fourth order. ^{a,b,c} n=16, $F_{(0.01,3,60)}=4.13$.

Table 4. Comparison with color between *Sikhae* prepared different thermal processes

Sample	L	a	b	ΔE
A	88.4	−1.63	13.7	18.0
B	82.9	−1.33	19.3	25.8
C	85.5	−1.13	27.5	31.0

A: Hot filled *Sikhae* with Nisin
B: *Sikhae* mixed with Nisin and processed at 110°C fro 15 min.
C: *Sikhae* processed at 121°C for 25 min.

Table 5. The sensory evaluation scores[2] of *Sikhae* with or without Nisin

Sample	Color	Flavor	Taste
A[1]	9	8	8
B	7	8	8

[1] A: *Sikhae* processed at 110°C for 15 min without Nisin
B: *Sikhae* mixed with Nisin and processed at 110°C for 15 min.
[2] Sum of comparison preference evaluated by 16 persons n=16, $t_{(0.01,15)}=2.98$.

Changes During Storage

Chemical, physical and microbiological changes of the products during storage at 30°C were determined

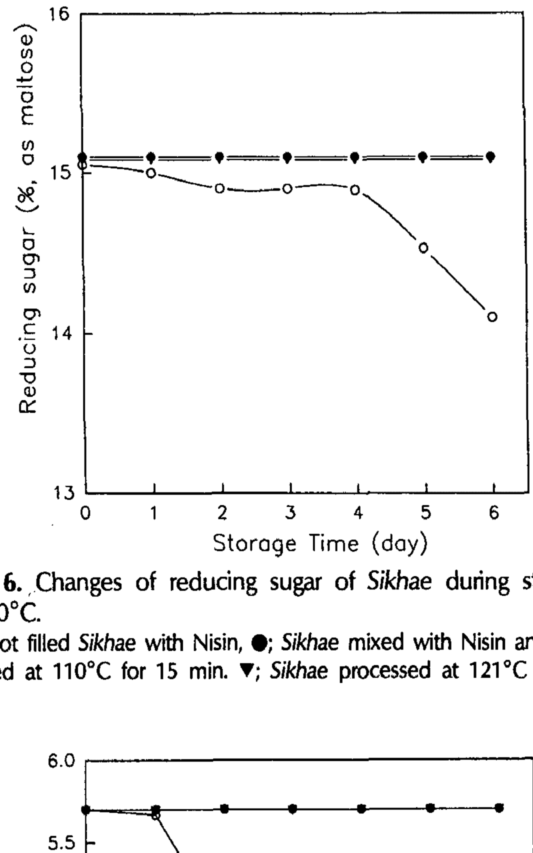

Fig. 6. Changes of reducing sugar of *Sikhae* during storage at 30°C.
O; Hot filled *Sikhae* with Nisin, ●; *Sikhae* mixed with Nisin and processed at 110°C for 15 min. ▼; *Sikhae* processed at 121°C for 25 min.

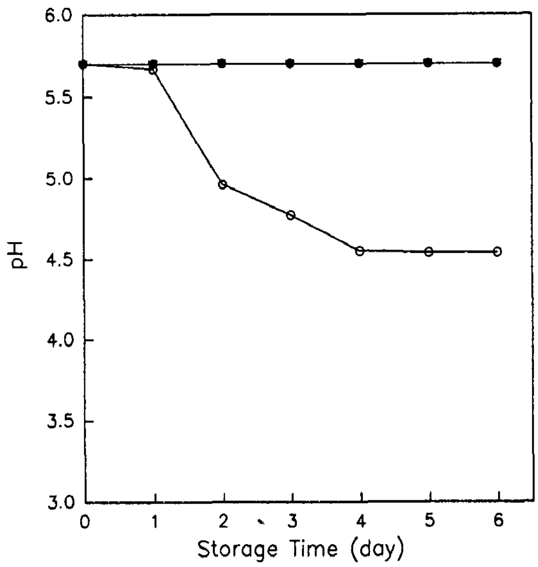

Fig. 7. Changes of pH of *Sikhae* during the storage at 30°C.
O; Hot filled *Sikhae* with Nisin, ●; *Sikhae* mixed with Nisin and processed at 110°C for 15 min. ▼; *Sikhae* processed at 121°C for 25 min.

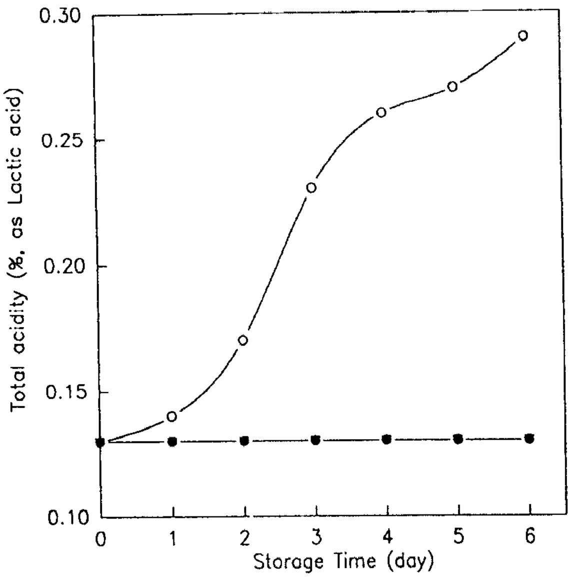

Fig. 8. Changes of total acidity of *Sikhae* during the storage at 30°C.

O; Hot filled *Sikhae* with Nisin, ●; *Sikhae* mixed with Nisin and processed at 110°C for 15 min. ▼; *Sikhae* processed at 121°C for 25 min.

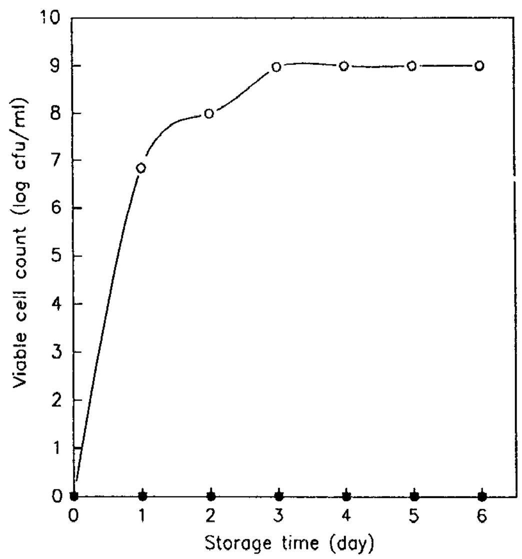

Fig. 9. Changes of viable cell count of *Sikhae* during the storage at 30°C.

O; Hot filled *Sikhae* with Nisin, ●; *Sikhae* mixed with Nisin and processed at 110°C for 15 min. ▼; *Sikhae* processed at 121°C for 25 min.

(Fig. 6). Sugar contents of hot-filled *Sikhae* with Nisin decreased after 4 days, however, *Sikhae* processed for 25 minutes at 121°C, and *Sikhae* processed for 15 minutes at 110°C with the aid of Nisin did not show observable change during 3 months of storage. The pH of hot-filled *Sikhae* with Nisin decreased to 4.5 and total acidity increased to 0.29% during storage (Fig. 7 and 8). This implies a possible growth of microorganisms that survived the thermal process and the increase of viable cell count was the evidence for this result (Fig. 9). Streptococci and coliforms were not detected. Microscopic examination of the spoiled *Sikhae* and alcoholic aroma from spoiled bottles revealed that yeast was the major cause of this spoilage (Data not shown). This is possible because Nisin does not inhibit the growth of eukaryotic cells (11). Gas evolution was also observed. *Sikhae* processed for 15 minutes at 110°C without Nisin showed turbid growth and microscopic examination revealed the growth of Gram-positive bacilli.

From this result, we concluded that bottled *Sikhae* of good quality could be prepared under the milder heating conditions with the aid of Nisin and that the current thermal process at plants could be reduced by over 90%. These kind of trials were reported in fruit and vegetable canning (14).

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