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Effect of Temperature and Salt Concentration on *Kimchi* Fermentation

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김치발효에 미치는 온도 및 식염농도의 영향

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Abstract

Chemical and microbial changes during *Kimchi* (a group of Korean seasoned pickles) fermentation were carried out at various temperatures and salt concentrations. The time reaching optimum ripening of *Kimchi* varied depending upon fermentation temperature and salt concentration. At high temperature and low salt content *Kimchi* fermentation was faster than at low temperature and high salt content. The ratio of volatile to non-volatile acids reached its maximum at the optimum ripening time of *Kimchi* and decreased thereafter. *Leu. mesenteroids, Lac. brevis, Lac. plantarum. Ped. cerevisiae, Str. faecalis* and low acid producing *Lactobacilli* were isolated from *Kimchi* samples. However, the main microorganism responsible for *Kimchi* fermentation was *Leu. mesenteroides* and *Lac. plantarum* was the main acidifying organism. Total viable count increased rapidly in the begining of fermentation and reached its maximum number at optimum ripening time and then decreased slowly as the acidity of *Kimchi* increased. While the total aerobic bacteria and fungi decreased during *Kimchi* fermentation, the yeast increased significantly at lower temperature.

Introduction

Kimchi is the name given to a group of delicate, fermented vegetable foods of long tradition in Korea and is a popular side dish served at every meal along with cooked rice and other dishes. According to a national nutritional survey, and adult consumes 50-100 g/day in summer and 150-200 g/day in winter[1] which constitutes 12.5% of total daily food intake.[2] Many kinds of *Kimchi* are available depending on the raw materials, processing methods, seasons and localities. Although the proper combination of minor ingredients has been said to be a key for delicious and palatable *Kimchi*, the more important factors seem to be the salt concentration and fermentation temperature.

Kimchi is characteristic in its palatibility giving sour, sweet and carbonated taste and greatly differs in this respect to sauerkraut which is popular in the west. Much research has been done in the past on the microflora of *Kimchi* and organisms isolated from *Kimchi* included anaerobic bacteria such as *Lactobacillus plantarum, Lactobacillus brevis, Streptococcus faecalis, Leuconostoc mesenteroides,* and *Pediococcus cerevisiae*,[3,4] and aerobic bacteria such as *Achromobacter, Flavobacterium, and Pseudomonas species*.[5] It was also reported that after the ripening of *Kimchi*, in certain cases yeast and moulds destroy the product and make it unfit for consumption by softening the texture and imparting undesirable odour.[6]

In the present studies the effect of temperature and salt content on *Kimchi* fermentation have been thoroughly investigated in order to identify different microorganisms desirable for good *Kimchi* fermentation.

Materials and Methods

Preparation and Fermentation of *Kimchi*

The Chinese cabbages cut into small pieces (4-5cm) were salted with 15% brine for 3 hrs. and washed twice with 2.0% brine. After draining, the other minor ingredients were added and mixed. The minor ingredients were blended to paste before addition. *Kimchi* contains Chinese cabbage 100g, garlic 2g, green onion 2g, red pepper powder 2g, and ginger 0.5g. The content of water and soluble sugar of the prepared *Kimchi* were 90% and 3.0% respectively.

Final salt concentrations of 2.25, 3.5, 5.0 and 7.0% were adjusted by adding required amount of salt. Each samples of *Kimchi* prepared consisted of 200g of material filled in nylon film bags of 12x20cm size and was sealed. Fermentation of *Kimchi* were carried out at 30, 20, 14, and 5 °C for a period of 10, 20, 40, and 180 days respectively.

Analysis

The samples from each bag were blended and filtered by sterilized gauze, and the analysis for miarobial and chemical changes of *Kimchi* during fermentation were carried out.

pH was measured using Heath pH meter. Acidity was determined by titrating with 0.1 N NaOH solution using phenolphthalein as indicator and calculated on the basis of lactic acid.

After steam distillation of sample, 150ml of the distilate was titrated with 0.1 N NaOH solution and calculated for volatile acid on the basis of acetic acid and the ramaining distilate was also titrated with 0.1 N NaOH for non-volatile acids.

Sodium chloride was determined by the method of Mohr.[7]

Microbial Changes

Tryptone-glucose-yeast extract (TGY) agar mentioned by Pederson and Albury[8] was used for the counting of total viable organisms in *Kimchi*. For counting the yeast, aureomycin-rose bengal agar[9] was used and sodium azide-sucrose agar[10] was utilized for the counting of lactic acid producing bacteria. Following the

Ⅰ. Morphology
Cocci ———— Rod (Gram+)
Ⅱ. Physiology
Fermentable hoxose
Heterofermentative Type —┴— Homofermentative Type

Heterofermentative Type	Homofermentative Type
End product:	End product:
Lactic acid, Acetic acid	Lactic acid
Ethanol, CO, etc	
Rod: *Lac. brevis*	Rod: *Lac. plantarum*
Cocci: *Leu. mesenteroides*	Cocci: *Ped. cerevisiae*
Dextran	*St. faecalis*

Fig. 1. Identification of lactic acid bacteria

method of Stamer,[11] and Pederson and Albury,[8] individual lactic acid bacteria were counted. 30 colonies growth on TGY agar were isolated and tested for their morphological and physiological properties according to Fig. 1. Each lactic acid bacteria isolated was cultivated for 10 days at 30 °C using TGY broth containing 1.5% glucose. Total acid produced was determined by methods described above.

Heterofermentative cocci producing total acid of about 0.6-0.8% and dextran forming from sucrose agar were counted as *Leuconostoc mesenteroides*, heterofermentative rods producing 0.8-1.0% of total acid were *Lactobacillus brevis*, homofermentative rods producing 1.0-1.5% of total acid were *Lactobacillus plantarum*, homofermentative tetrad cocci producing about 1.0% of total acid were *Pediococcus cerevisiae* and total acid below 0.4% were regarded as *Streptococcus faecalis*.

Homofermentative rods producing 0.6-1.0% of total acid were tentatively identified as low acid producing *Lactobacilli* in this study.

Results and Discussion

Chemical Changes During *Kimchi* Fermentation

Temperature is one of the important factors to control *Kimchi* fermentation. Hence, the changes of pH, total acid, volatile and non-volatile acid, and salt concentration were examined under various temperature conditions with *Kimchi* containing 3.0% salt. Also optimum ripening period and eatable period of *Kimchi* were investigated through panel test.

As seen in Fig. 2, ripening time of *Kimchi* varied

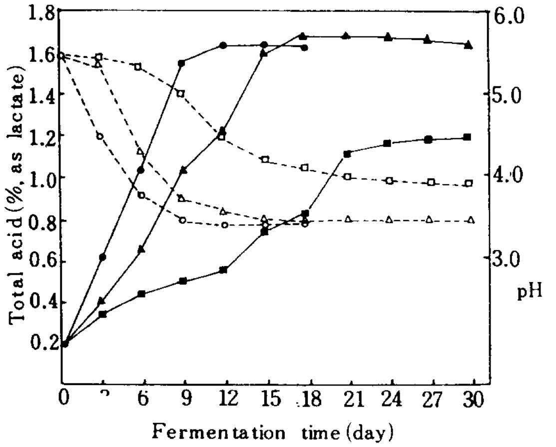

Fig. 2. Changes of total acid and pH during *Kimchi* fermentation at various temperatures (3.0% salt)

Total acid pH
—●— 20℃ ···○···
—▲— 15℃ ···△···
—■— 10℃ ···□···

depending on fermentation temperature, accordingly the changes in pH and acidity showed notable differences. At 20 °C, pH dropped sharply with increasing acidity, but pH and acidity at 10 °C changed more slowly compare to high temperature tested. Maximum total acid produced in *Kimchi* at 20 °C and 15 °C is 1.6% but it never exceeds total acidity of 1.2% at 10 °C. As a result of panel test, it was evaluated that the pH and acidity of optimum ripening period of *Kimchi* were 4.2 and 0.6% (as lactic acid) respectively.

Fig. 3 shows the effect of salt content and temperature on acid production during *Kimchi* fermentation. As seen in Fig. 3, total acid was more at lower salt content (2.25%) than high concentration of salt at any temperature tested in this study. At the lower salt content maximum acidity was reached in a shorter period. At 30 °C and 2.25-3.5% salt content, acidity of *Kimchi* was maintained in the same pattern throughout. The acidity of 1.55% is reached in 5 days and is maintained at 1.6% thereafter, but at 5.0 and 7.0% salt content acidity reached 1.4 and 1.05% after 5 and 6 days, respectively.

Optimum acidity (0.6%) of *Kimchi* was reached within 1 day at 30 °C and at 2.25-3.5% salt content, and the same level of acidity was reached in 2-4 days at 5.0 and 7.0% salt content. At 20 °C, maximum acidity (1.6%) was reached after 16 days at 2.25 and 3.5% salt content, and the maximum of 1.4 and 1.0% acidity was reached after 20 days at 5.0 and 7.0% salt content, respectively.

At 14 °C and 2.25% salt content, total acid increased more slowly than at higher temperature (30-20 °C) and

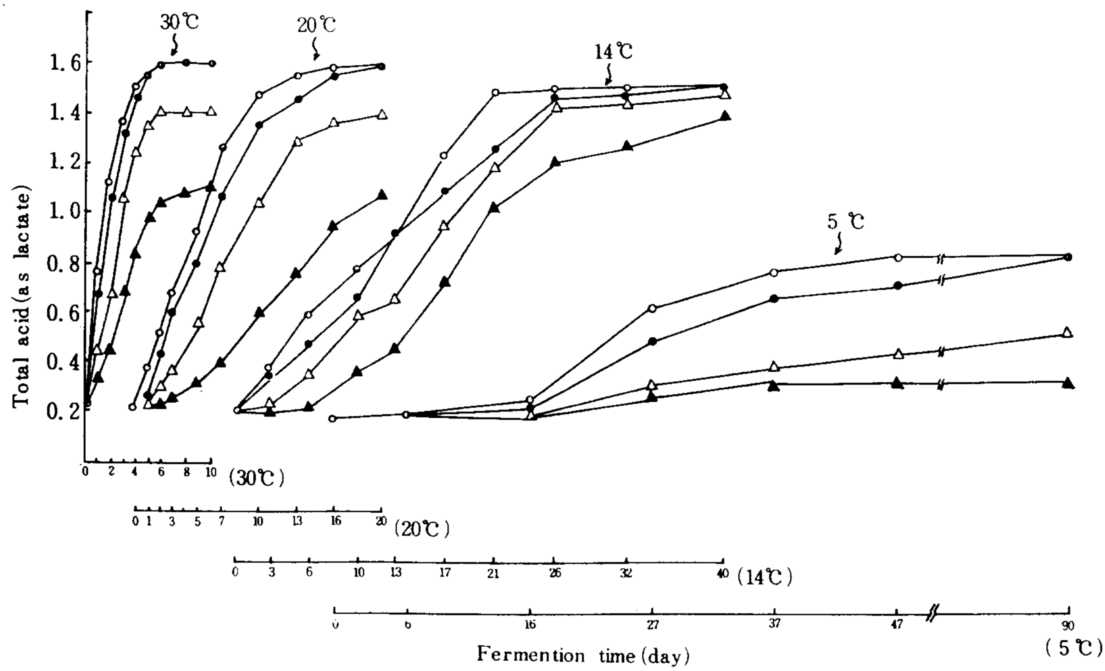

Fig. 3. Changes of total acid during *Kimchi* fermentation at different temperatures and salt concentrations
NaCl —○— 2.25%, —●— 3.5%, —△— 5.0%, —▲— 7.0%.

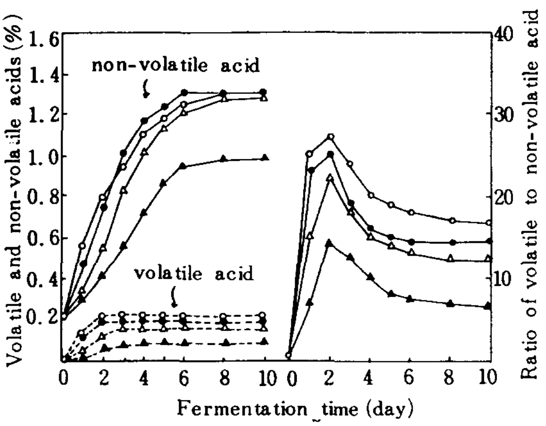

Fig. 4. Volatile and non-volatile acid and its ratio during *Kimchi* fermentation at different salt concentrations at 30℃

Non-valatile acid	Volatile acid	
—○—	···○···	2.25% NaCl
—●—	···●···	3.5 % NaCl
—△—	···△···	5.0 % NaCl
—▲—	···▲···	7.0 % NaCl

reached upto 1.5% in 21 days and the same level of acidity was maintained thereafter (40 days). But at 3.5 and 5.0 and 7.0% salt content, total acid increased much more slowly than at 2.25% salt content and reached 1.48 and 1.44 and 1.20% acids respectively in 26 days. After 40 days, the level of acidity was maintained 1.5% at 3.5 and 5.0% salt content.

However, there was no significant increase in acidity up to 16 days at 5 °C and then increase slowly depending on the salt content. At 2.25 and 3.5% salt content total acidity reached 0.63 and 0.67% in 27 and 37 days, respectively, and 0.84% of acidity was maintained after 90 days. At 5.0 and 7.0% salt content, maximum acidity was not more than 0.55-0.3% during the completed period of fermentation. These acidities refer no optimum ripening of *Kimchi* by panel test.

Fig. 4 shows the patterns of volatile and non-volatile acids, and their ratio during *Kimchi* fermentation at different salt concentration at 30 °C.

Volatile acid was produced more at low salt content (2.25%) reaching only 0.22% to the maximum. Non-volatile acid at 2.25-3.5% salt content showed the variation at the beginning stage but finally it reached the same level. At any level of salt concentration used, ratio of volatile to non-volatile acids was higher only after 2 days of fermentation. This was found to be the optimum time of fermentation to produce the best quality *Kimchi* as proved by the panel tests.

At lower temperature, the ratio of volatile to non-volatile acids was higher than when tested at higher temperature as shown in Fig. 5 The optimum ripening time and estable period of *Kimchi* varied depending upon the fermentation temperature and salt content as shown in Table 1. At 30 °C, optimum ripening period was 1 day and estable period was also 1-2 days. But at lower temperature, the optimum ripening time and eatable period were longer that at higher temperature At 5 °C and above 5.0% salt content, *Kimchi* was ripened very slowly and at 7.0% salt content it was not ripened

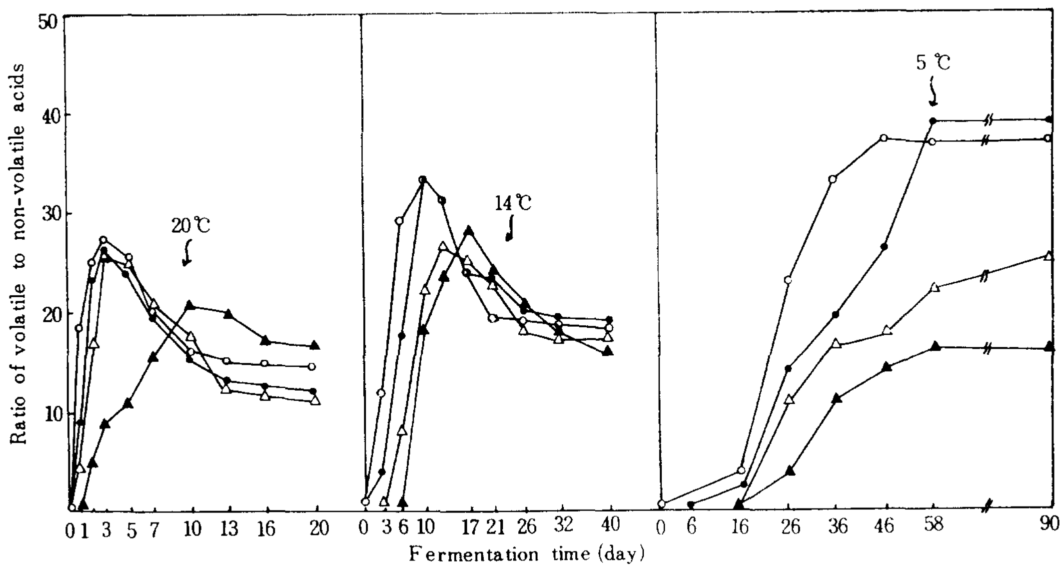

Fig. 5. Ratio of volatile to non-volatile acids during *Kimchi* fermentation at 20, 14, and 5℃
NaCl —○— 2.25%, —●— 3.5%, —△— 5.0%, —▲— 7.0%.

Table 1. Optimum ripening time and eatable period of *Kimchi*

Temperature (℃)	Salt content (%)			
	2.25	3.5	5.0	7.0
30	1-2*	1-2	2	2
20	2-3	2-3	3-5	10-16
14	5-10	5-12	10-18	13-22
5	35-180	55-180	90-180	

* Days
\- Not ripened

even after 180 days fermentation.

Song *et al.*[12] found that initial pH of *Kimchi* starts from 5.5-5.8, reducing to 4.5-4.2 at optimum ripening period and dropped to 4.0 upon over-ripening. They also reported that salt concentration is not changed during *Kimchi* fermentation. Lee and Yang[13] have reported that acidity at the optimum ripening period of *Kimchi* is 0.4-0.75 percent (as lactic acid), reaching 1.0 percent upon over-ripening and reaches 1.5-2.0 percen' at the stage of spoilage.

It has been reported that *Kimchi* fermented at lower temperature (6-7 °C) contains more organic acids than that fermented at higher temperatures (22-23 °C)[14] and that *Kimchi* fermented at higher salt concentration, the acetic acid production was low.[15] In this study, we found that optimum pH, acidity and salt content of *Kimchi* were 4.2, 0.6-0.8% (as lactic acid) and 3.0%, and *Kimchi* fermented low temaperature (5-14 °C) is more tasty than that of fermented at higher temperature (20-30 °C).

Microbial Changes during *Kimchi* Fermentation

The salt content of *Kimchi* is slightly higher than sauerkraut and the optimum salt content also varies depending on the localities and users. Therefore, the effect of various salt concentration and temperature on *Kimchi* fermentation was carried out to identify the major microorganisms responsible in *Kimchi* fermentation.

Changes of total viable counts: As seen in Fig. 6A-6D, total number of microorganisms and the time for the maximum phase varied depending on the temperature and salt concentration. However, general tendency of fermentation pattern is that total count increases rapidly in the beginning and then decreased slowly as the acidity increases.

At 30 °C and 2.25% salt content, total viable count reached miximum number (1.0×10^9 calls/*ml*) in 1 day, and the same level was maintained upto 3 days and then decreased slowly and finally reached $2,9\times10^7$ cells per *ml* in 10 days.

At 20 °C, maximum number reached after 3 days at 2.25, 3.5 and 5.0% salt content and decreased to $4.0\times10^7-1.0\times10^8$ cells per *ml* after 20 days. However, at 7.0% salt content maximum number reached after 3 days and the number remained the same until 20 days.

At 14 °C, the maximum number ($7.0\text{-}8.0\times10^8$ cells/*ml*) reached after 6 days at 2.25% salt content, then slowly decreased to 1.0×10^8 cells per *ml* after 40 days. But at 3.5% salt content maximum number (2.9×10^8 cells/*ml*) reached after 6 days and decreased to 5.0×10^7 cells per *ml* after 40 days. At 5.0% salt content maximum number was reached (2.5×10^8 cells/*ml*) after 6 days and decreased to 3.0×10^7 cells per *ml* after 40 days. However, at 7.0% salt content maximum number (2.0×10^8 cells/*ml*) reached after 10 days, then slowly decreased to 2.0×10^7 cells per *ml* in 40 days.

When *Kimchi* was fermented at 5 °C, the maximum number reached after 27 days at 2.25 and 3.5% salt centent, but at 5.0% salt content maximum number reached only after 37 days. However, at 7.0% salt content the total viable count was not significantly increased and remain constant thereafter (90 days).

Changes of fungi and yeast: As seen in Fig. 6A-6D, the fungal flora during *Kimchi* fermentation decreased in number as the acidity of *Kimchi* increases. However, yeast population during *Kimchi* fermentation showed typical changes depending upon the temperature and salt concentration. At the temperature range of 20-30 °C (Fig. 6A and 6B) total number of yeast was somewhat changed during *Kimchi* fermentation, but at 14 °C (Fig. 6C) the population of yeast increased significantly and slowly decreased later. The changes of total yeast population have close relationship with salt concentration. More the salt content, more was the growth. At all levels of salt content, the maximum number reached after 26 days. However, when *Kimchi* was fermented at lower temperature the number of yeast was somewhat increased and decreased slowly. At this temperature maximum number reached after 37-57 days (Fig. 6D).

Changes of lactic acid bacteria: The number of *Leu. mesenteroides* reached its maximum at the optimum ripening period of *Kimchi* and decreased when *Kimchi*

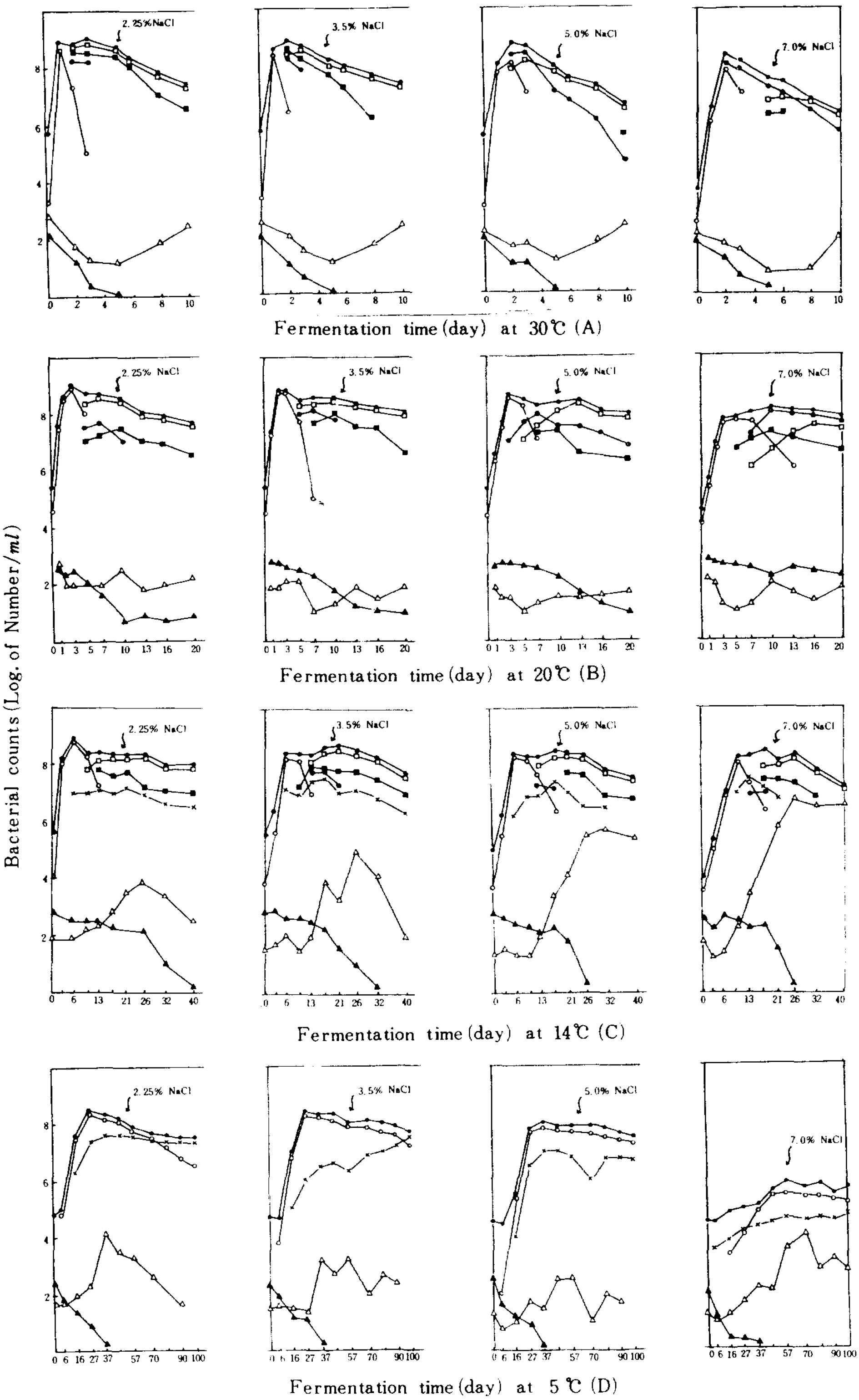

Fig. 6. Microbial changes during *Kimchi* fermentation at various temperatures and salt concentrations

—●— Total viable count
—○— *Leu. mesenteroides*
--◐-- *Ped. cerevisiae*
--■— *Lac. brevis*
--×-- Low acid producing *Lactobacilli*
△ Yeast

becomes acidic. At the same fermentation temperature, the total number of *Leu. mesenteroides* were more at lower salt content than at higher salt content. The maximum number reached after 1 day at 30 °C, 3 days at 20 °C, 6 days at 14 °C, and 27 days at 5 °C. It is very interesting to note that at the optimum ripening period of *Kimchi*, the number of *Leu. mesenteroides* reached maximum with the corresponding increase in the total viable count.

Lac. brevis appeared more at higher temperature and low salt content, at the stage of over-ripening period of *Kimchi*. It was also interesting to note that *Lac. brevis* did not appear at any levels of salt content tested at 5 °C.

Lac. plantarum appeared at 30, 20, 14 °C but could not be detected at lower temperature (5 °C). It appeared at the time when the *Leu. mesenteroides* decreased, and then its population became dominant. This will be the acidifying stage of *Kimchi* fermentation followed by spiolage stage of *Kimchi*.

St. faecalis appeared rarely at all temperature and salt contents but its number was negligible.

Homofermentative rod *Lactobacilli* producing 0.6-1.0% acid appeared after fermentation commended but the number was not more. These organisms appeared more at low temperature than at higher temperature.

Pederson and Albury[16] reported the effect of temperature and salt content on sauerkraut fermentation. They found that *Leu. meseneroides* and *Lac. brevis* grow at 1.0% salt content, and *Ped. cerevisiae* and *Lac. plantarum* grow at 3.5% salt content. At 23 °C and 2.25% salt content when acidity and pH were 0.77% and 4.0, respectively, they found only *Leu. mesenteroides* in sauerkraut. Even at 3.5%salt content, when acidity and pH were 0.64 and 4.03,respectively, *Leu, mesenteroides* was dominant and *Ped. cerevisiae* and *Lac. plantarum* rarely appeared. These results are very much similar to the present obervations.

In the microbial studies on *Kimchi Lac. plantarum, Lac. brevis, St. faecalis, Leu. mesenteroides, Ped. cerevisiae* have been already reported by previous workers.[4-5] Kim and Chun[4] concluded that *Leu. mesenteroides* appeared at the first stage of *Kimchi* fermentation followed by *Lac. plantarum* and *Lac. brevis* were the microorganism responsible for *Kimchi* fermentation. But *Kimchi* is less acidic product than sauerkraut and the optimum acidity and pH of *Kimchi* is 0.6-0.8% and 4.2 respectively while that of sauerkraut is 1.6% and 3.5 respectively.

Also, comparing sauerkraut to *Kimchi*, sauerkraut fermentation is completed after 60 days at 22 °C with 2.25% salt, whereas *Kimch* fermentation was completed in 3 days only. So, the main microorganisms of *Kimchi* fermentation seem to be those found at the initial stage of sauerkraut fermentation.

Therefore, it was claimed that main microorganisms in *Kimchi* fermentation is *Leu. mesenteroides* and *Lac. plantarum* is the main acidifying or deteriorating organisms in *Kimchi* fermentation, whereas this is the main microorganisms responsible in sauerkraut fermentation. However, further detailed studies on the role of *Lac. plantarum* on *Kimchi* fermentation will be needed.

요 약

온도와 식염농도를 달리하여 김치를 발효시키면서 생화학적 및 미생물의 변화를 검토하였다. 김치의 적숙시기는 발효온도와 식염농도에 따라 달랐다. 김치발효는 저온, 고식염농도에서 보다, 고온 저식염 농도에서 더 빨리 진행되었고 김치의 비휘발성 유기산에 대한 휘발성 유기산의 비는 김치 적숙시기에 최대로 되었다가 김치가 시어지면서 감소되었다. 식염농도와 발효온도를 달리하여 숙성시킨 김치에서 *Leu. mesenteroides*, *Lac. brevis*, *Lac. plantarum*, *Ped. cerevisiae*, *St. faecalis* 및 산생성이 낮은 *Lactobacilli*가 분리되었다. 그러나 김치발효에 관여하는 주 미생물은 *Leu. mesenteroides*이며, *Lac. plantarum*은 김치의 숙성보다는 산패와 더 관련이 있는 것으로 추정되었다. 총생균수는 발효초기에 급증하여 적숙기간에 최대로 되었다가, 그 이후 서서히 감소되었고, 호기성 세균과 사상균수는 발효가 진행되면서 계속 감소하였고, 효모는 10℃ 전후의 온도와 고 식염농도에서 그 수가 증가하였다가 다시 감소되었다.

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