Kimchi fermentation and characteristics of the related lactic acid bacteria

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I. Introduction

Kimchi is a Korean fermented vegetable food that are salted, blended with various ingredients and fermented for a certain period of time at ambient temperature. The characteristics of kimchi differ depending on the varieties of kimchi. The varieties are due to the raw materials used, processing methods, seasons, localities and functional properties of kimchi. More than 200 kinds of kimchi are available in Korea, but kimchi can be classified as two major groups, ordinary and mul-kimchi. Ordinary kimchi without added water includes baechu kimchi (diced Chinese cabbage), tongbaechu kimchi (whole Chinese cabbage), yeolmoo kimchi (young oriental radish) and kakdugi (cubed radish kimchi). Mul-kimchi includes baik kimchi (baechu kimchi with water), dongchimi (whole radish kimchi with water) and nabak kimchi (cut radish and Chinese cabbage). 1)

The raw materials used for kimchi preparation are divided into three groups, major, sub-ingredients (spices) and optional ingredients. A recipe for the simplest kimchi may include cabbage 100 g, garlic 2 g, red pepper powder 2 g, green onion 2 g, ginger 0.5 g, with optimum salt content of 2-3%. 2)

The optimum pH for the best taste of kimchi is 4.2-4.5 and the optimum acidity is 0.6-0.8% as lactic acid. The best taste is attained after 2-3 days of fermentation at 20°C with 2-3% salt. Kimchi has a unique sour, sweet, carbonated taste and usually is served cold. Also, kimchi contains a lot of live lactic acid bacteria (LAB). In this respect, kimchi differs from western sauerkraut and Japanese asatsuke, and the former is only acidic in taste and served warm, and latter is not a fermented product and has few live LAB.

Total amount of kimchi production is estimated to 1,500,000 M/T in 2000 and one fourth of total consumption of kimchi was commercially produced in the same year. According to a national survey, an adult consumes 50-100 g/day of kimchi in summer and 150-200 g/day in winter. 2)

During the last 50 years, many genus and species of bacteria, yeasts, and fungi has been isolated and reported from kimchi samples, but it was confirmed that major microorganisms responsible for kimchi fermentation are LAB and yeasts are known to be role for softening of kimchi texture and off-flavor. The major genus and species of LAB isolated and identified from kimchis are Leuconostoc mesenteroides, Leuconostoc dextranicum, Leuconostoc citreum, Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus plantarum, Pediococcus
The sugars in raw materials are converted to lactic acid, acetic acid, carbon dioxide and ethanol by hetero fermentative LAB during kimchi ripening, and these acids and carbon dioxide are responsible for the fresh and carbonated taste of kimchi. However, after a certain period of time, excessive lactic acids are formed and off flavors are developed due to the growth of homo fermentative LAB and yeasts.

The total number of kimchi microorganisms reaches its maximum level (1x10^8-9 cells/ml) at optimum-ripening time and after then number of microorganisms decrease slowly and again increase and maintains 2nd maximum level (1x10^6-7 cells/ml) as kimchi fermentation carried. In the kimchi fermentation system, it seems clear that hetero fermentative LAB producing organic acids and carbon dioxide from sugars are major species in the early stage of fermentation, and homo fermentative LAB producing excessive lactic acid are major species in the late stage of fermentation.

It was also confirmed that low salt concentration and low temperature (eg, 2% and 10°C) favor growth of hetero fermentative LAB, while high salt concentration and high temperature (eg, 3.5% and 30°C) favor growth of homo fermentative LAB. Therefore, salt concentration and temperature are the most important key factors for controlling kimchi fermentation.

Besides the two key factors for controlling kimchi fermentation, many other factors such as raw materials used, processing methods, addition of natural preservatives and starters affecting kimchi fermentation and preservation has been reported in previous reviews and books.

In this article, the factors affecting kimchi fermentation, microbial and chemical changes during kimchi fermentation, and some characteristics of LAB from kimchi will be reviewed.

II. Factors affecting kimchi fermentation

Kimchi fermentation occurs mainly by the microorganisms naturally present in the raw materials that contain numerous microflora including LAB. Various LAB may initiate fermentation, but hetero fermentative type LAB increased rapidly with organic acids accumulation and homo fermentative type LAB increased thereafter. Numerous chemical, physical, and biological factors may contribute directly to the growth of microorganisms and the extent of fermentation.

The important factors that affect kimchi fermentation are microorganisms, temperature, salt concentration, fermentable carbohydrates, other available nutrients, or any inhibitory compounds in raw materials used, as well as oxygen and pH. In this chapter, the salts, temperature, raw materials, natural preservatives and selected starter cultures related to the kimchi fermentation
will be discussed in detail.

1. Salts

Salt is one of the key factors for controlling the kimchi fermentation and preservation of good quality of kimchi at various temperatures. There are more than 200 kinds of kimchi available in Korea. However, salt concentrations of those kimchi are all different depending on the makers. A flow-chart for processing baechu kimchi is shown in Fig. 1.

Prior to kimchi preparation the major raw materials such as Chinese cabbages and radishes may be salted with either salt solution or dry salt and washed with clean water. This treatment is the most important step for fermentation and maintenance of kimchi quality. It was reported that the optimum salt concentration of kimchi is about 2.0-3.0%, while acceptable level is individually determined by housewife’s experiences. Therefore, it is necessary to optimize the salting condition. Of two types of salting methods, direct addition method is widely used conventionally at household level, but this method has the disadvantage of difficulty in control of the final salt content of kimchi. Brine method is more preferable for the commercial production of kimchi. Satisfactory quality of kimchi could be obtained when cabbages are salted for 3-6 hours using 15% salt solution.

Salting is carried out over a wide range of time from 3 to 15 hr depending on the salt concentration, temperature, varieties, and cutting way and size of cabbages. For baechu kimchi, the final salt concentration is adjusted to 2.0 - 3.0% of the overall ingredients, and this concentration is the best for optimum fermentation. This concentration is maintained during fermentation and preservation. If the salt concentration is below the optimum concentration, fermentation proceeds too fast and frequently causes quick acidification and softening. On the other hand, color and flavors are not acceptable when the salt concentration is over 6%. Depending on the salting time, free sugars and amino acids were reduced in raw cabbages and texture, chemical and physical properties, and total microflora have been changed during salting.

Generally, salting reduces the moisture content (10-12%), relative volume, and weight, as well as the internal void space of the cabbage. These changes affect the physical properties of the vegetable, especially the flexibility and firmness of the tissue, which give a typical textural property to the final product. As a result of brining, the total microorganisms, such as aerobic counts in salted cabbage are reduced (11-87%) and LAB are increased (3-4 times), and reducing sugars also decreased (7-17%).

Washing conditions in salted cabbage on the quality of kimchi is also important for the retention of quality of characteristics. Chinese cabbage treated with 1000 ppm solution of grapefruit seed extracts or citric acid at 10°C
showed a retarded increase in titratable acidity and decrease in pH and reducing sugar content, and kimchi made from Chinese cabbage soaked in a heated 10% salt solution at 40°C has improved quality and shelf-life of kimchi.

Fig. 2 shows the effect of salt concentration and temperature on acid production during kimchi fermentation. Total acid was more at lower salt content (2.25%) than high concentration of salt at any temperature tested. 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% was reached in 5 days and maintained at 1.6% thereafter, but at 5.0 and 7.0% salt content, acidity reached 1.4 and 1.1% after 5 and 6 days, respectively.

Recently, to examine the quality of mul-kimchi, the temperature (4, 15, and 25°C for 10 days) and salt concentration (0, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0%) in water was conducted, and found that the pH was lowest and acidity was highest in the mul-kimchi containing 1.0% salt.

Effect of salt concentration on dongchimi (ponytail Chinese radish kimchi) fermentation was also studied. Diced Chinese radishes were fermented at 4°C with salt concentration of 1.5-6.3%. The pH level reduced during the fermentation, while the total acid content of dongchimi increased and the salt concentration of dongchimi liquid decreased. Equilibrium for salt concentration between dongchimi liquid and solid radish was achieved after fermentation of 15-22 days.

2. Temperature

The most important factor affecting kimchi fermentation is temperature, since the kimchi fermentation occurs mainly by the microorganisms naturally present in raw materials. Kimchi is now available in all year round but the quality of kimchi differs depending on localities and seasons. Ambient temperature is applied for making kimchi at household level. Kimchi fermentation and over-acidification occurs simultaneously at ambient temperature.

Fig. 3 shows the changes of pH and total acids during kimchi fermentation at various temperatures. Ripening time of kimchi 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 with 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.

The optimum-ripening time and the edible period of kimchi varied
depending upon the fermentation temperature and salt content as shown in Table 1. At 30°C, the optimum-ripening period was 1 day and the edible period was also 1-2 days. But at lower temperature, the optimum ripening time and the edible period were longer than those of 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 even after 180 days fermentation.

Chemical changes, LAB and yeast counts in kimchi prepared by commercial manufacturer in large scale were monitored at different fermentation temperature. It has been confirmed that the optimum pH of kimchi was around 4.2 and reached within 2 days at 25°C, 3 days at 15°C, and 23 days at 5°C fermentation, respectively.

In order to investigate the fermentation characteristics of kimchi which was made at 12°C and fermented at 17°C and 4°C, the pH, total acid, total microorganisms, LAB, dissolved carbon dioxide content, reducing sugars and temperature at the center of a kimchi jar were studied. The pH and the total acid content of kimchi that was fermented at 17°C for 4 days were almost the same as those of kimchi fermented at 4°C for 48 days. The total cell counts of microorganisms and LAB in kimchis which were fermented at 17°C for 2 days and 4°C for 9 days were $1.5 \times 10^9$ and $6.3 \times 10^8$ cells/ml, and $2.0 \times 10^8$ and $8.7 \times 10^7$ cell/ml, respectively. The results showed that it took 23 and 35 hr, respectively, to reach the temperature of 17°C and 4°C at the center of a jar during bulk fermentation of kimchi from the fermentation of initial temperature of 12°C.

The effects of fermentation temperature (0-15°C) and salt concentration (1.5, 2.75, 4.0%) on fermentation parameters of kimchi were also analyzed by response surface methodology. pH decreased and acidity increased with increasing fermentation time, reduction and incremental rates were increased as temperature increased and salt concentration decreased. The optimum pH of 4.2 was achieved within 14-24 days at 5-15°C, but at 5°C, pH was still >4.2 after 24 days. Maximum edible acidity (0.75%) was reached within 8 days at 15°C, but at 0°C, acidity was only 0.35-0.43% after 24 days of fermentation. Edible period for kimchi, based on the acidity range 0.4-0.75%, were 4, 10, and 18 days for the fermentation at 15, 10, and 5°C, respectively, with 2.75% NaCl.

Differences in quality characteristics such as pH, acidity, reducing sugar content, microbial counts and sensory properties between whole Chinese cabbage kimchi (pogi kimchi) and sliced Chinese cabbage kimchi (mat kimchi) were examined during the fermentation at 20°C for 10 days and 5°C for 50 days. Pogikimchi showed delayed fermentation of approximately 2 days at 20°C and 10 days at 5°C than mat kimchi. Order, color and flavor scores of kimchi were higher in the samples fermented at 20°C than those fermented at 5°C, also there were no great differences between sensory properties of pogi and mat.
kimchi. Gas composition of the packages containing the kimchi fermented at 20 °C showed increased CO₂ concentration and decreased O₂ concentration after 3 days for both kimchi varieties. ²⁰)

The effect of fermentation temperature on the sensory, physicochemical and microbiological properties of *kakdugi* (cubed radish kimchi) and on free sugar, organic acid and volatile compound levels in *kakdugi* during fermentation was also investigated. ²¹-²²) After the initial fermentation for 12, 24 and 36 hr at 20 °C, *kakdugi* was fermented for 57 days at either 4 °C, 10 °C or 20 °C, respectively.

The pH was decreased to the range of 4.1~4.3 from the initial pH 5.8, and total acidity was increased 2~4 times than that of in the initial period (0.24%). The number of LAB was remarkably increased in palatable period and was gradually decreased thereafter. ²¹)

Free sugar levels decreased at each temperature during fermentation (with the exception of mannitol, levels of which increased) although decreases were less marked in samples fermented at 4 °C. Of the organic acids tested, lactic acid production was the most pronounced, increasing substantially with time and temperature. In contrast malic acid, which was the most abundant organic acid initially, decreased in concentration during fermentation, and this decrease was most pronounced at higher temperature.

Levels of the volatile compound, methyl allyl sulfide, were initially very low, but increased dramatically up to approximately 45 days after which levels decreased. The increase corresponded to the increased aroma in sensory evaluations. These results suggest that fermentation at 4 °C, following an initial fermentation at 20 °C for 36 hr, is suitable for the production of good quality *kakdugi* with high free sugar and organic acid contents. ²²)

Recently, the characteristics of natural lactic acid fermentation of radish juice were investigated at different salt concentration (0-2%) and temperature (10-30 °C) with a view of low salt kimchi (a kind of water kimchi). It was found that LAB isolated from radish juice fermented at 2% salt concentration was mainly *Leu. mesenteroides*, *Lac. plantarum* and *Lac. brevis*. Growth rate of LAB increased with increasing temperature at 1% salt concentration and LAB were still active at 10 °C. The time to reach pH 4.0 during the fermentation of juice containing 1% salt was 11~13 days at 10 °C and 2~3 days at 30 °C. ²³)

3. Raw materials

Kimchi, a fermented vegetable food with various sub-ingredients (spices), has been the most popular side dish that is served with cooked rice for hundreds of years in Korea. In total, there are about 200 kinds of kimchi, whose raw materials are mainly Chinese cabbages and radishes. Dried red pepper powder, garlic, green onion and ginger are the widely used seasonings. The types and amounts of seasonings used in kimchi vary greatly between
manufacturers and processors. Therefore, the quality and species of major ingredients may significantly affect fermentation and the product characteristics of kimchi.

The important raw materials of kimchi are divided into three groups such as the major raw materials, sub-ingredients (spices) or fermented fishery products, and optional (minor) ingredients.

As seen in Table 2, *baechu* (Chinese cabbages) are the most common major raw materials for preparing kimchi and radishes are other raw materials for *kakdugi* (cubed radish kimchi) and *dongchimi* (whole radish kimchi with water) preparation. Cabbages, whole radish, cucumber, mustard leaves, green onion, and leeks that are available in various seasons and localities are the raw materials for making special kimchi in Korea.

The sub-ingredients include garlic, hot pepper powder, green onion, ginger and fermented fishery products such as shrimp and anchovy. The optional (minor) raw materials include leek, parsley, glue plant, mustard leaves, pear, chestnut, oyster, frozen pollack, starches, monosodium glutamate (MSG), and sweetener. One of the important criteria for making good taste of kimchi is the selection of good quality of raw materials, and the next is the good formulation of kimchi ingredients and seasonings.

Table 3 shows the basic ingredients for making *baechu* kimchi, *kakdugi*, *dongchimi*, and other mul-kimchi (water-kimchi, but the simplest kimchi recipe includes salted cabbages 100 g, hot pepper powder 2 g, garlic 1.5 g, green onion 2 g, ginger 0.5 g and final salt concentration of 2-3%.16)

Generally softer texture and higher sugar content of vegetables are more desirable for making good quality and taste of kimchi than harder texture and lower sugar content. However, harden texture of vegetables are more favorable for long-term preservation without softening.2)

Among the many kind of kimchi, *baechu* kimchi has been consumed for a long time as a traditional fermented vegetable food in Korea. The ingredients involved in *baechu* kimchi preparation, such as red pepper, garlic, ginger, green onion, leek, fermented shrimp or anchovy, sugar, etc., affect the kimchi fermentation rate differing with kinds and amounts of ingredients. Effects of raw materials and ingredients on kimchi fermentation have been extensively studied.25,29-32)

Besides the temperature and salt concentration, types of kimchi are also the factors for controlling kimchi fermentation. Therefore, three main types of Chinese cabbage kimchi, *baechu* kimchi and *tongbaechu* kimchi (semi-solid types of the cabbage kimchi prepared with a large amount of hot pepper powder and a small amount of water) and *baek* kimchi (a liquid type of kimchi prepared with a small amount of hot pepper powder and a large amount of water), were selected as models to compare fermentation characteristics of kimchi. In this result, the amount of hot pepper powder and water are believed to be the
primary (or external) and the secondary (or internal) factors, respectively, those affect and control kimchi fermentation.\(^{33}\)

The difference of qualitative characteristics between whole Chinese cabbage kimchi \((pogi\) kimchi\) and sliced Chinese cabbage kimchi \((mat\) kimchi\) was examined during the fermentation at 20°C and 5°C. \(Pogi\) kimchi showed a delayed fermentation about 2 days at 20°C and 10 days at 5°C.\(^{20}\)

The growth of microorganisms could be controlled by the appropriate addition of raw materials. Among the sub-ingredients, radishes,\(^{34}\) hot pepper powder,\(^{29-32}\) fermented anchovy and shrimp,\(^{35-38}\) starches,\(^{35,39}\) reducing sugar,\(^{31,40}\) and protein sources\(^{42}\) have been probed their accelerated effect on kimchi fermentation, but garlic and leek has showed delayed effect,\(^{29-31,43,44}\) and green onion and ginger had controversial effect on kimchi fermentation.\(^{30-32}\) On the other hand, mustard or leaf mustard,\(^{45}\) and extracts of leek\(^{44}\) were known to delay the fermentation period of kimchi. Addition of dried methanol extracts of radish stimulated the growth of LAB, especially \(Lac.\) \(fermentum, Lac.\) \(leichmannii, Lac.\) \(sake,\) and \(Lac.\) \(brevis.\)^\(^{34}\)

The composition of pigments in hot pepper consists of 40 different carotenoids with capsanthin and capsorubin as a major carotenoid and carotenes as provitamin A. The average amount of hot pepper added to kimchi was found to be 2.24% with frequency of use of 97.3%. In spite of the fact that hot pepper was reported to promote the fermentation of kimchi, it did not show any significant increase in sour taste and did not affect sweet, salty, palatable and crispy tastes.\(^{31}\)

Garlic is used most frequently as a sub-ingredient (100%) in kimchi. Since ground garlic is used in kimchi-making, alliin is changed to allicin with an intensive taste. This allicin takes part in the desirable fermentation of kimchi by inhibiting the growth of various unnecessary microorganisms derived from sub-ingredients in the initial fermentation period and to delay the fermentation of kimchi. Garlic improves storage capacity by prolonging fermentation period by LAB and is beneficial to less acidification.\(^{29,30,32,43,46}\) With fermentation of kimchi, the intensive hot taste of garlic was slowly changed to the harmonized taste and flavor.\(^{31}\)

Green onion and leek contain various allyl sulfides, carotenes and vitamin C. The frequency of use in the general cabbage kimchi was 72.8% for green onion and 32.4% for leek, which both are much lower compared with that of 97.3% for hot pepper and 100% for garlic. The amounts of green onion and leek used for kimchi making are still high, ie, 0.6-0.9% for green onion and 2.0-6.0% for leek. Recently, it has been reported that leek could retard the fermentation of kimchi because of its antimicrobial activity.\(^ {44}\)

Ginger contains unique components like citral and linalol, and hot taste components such as gingerone and shogaol. Ginger caused a delay in fermentation and there were no significant differences in the sour, sweet, salty,
hot, palatable, unpleasant and overall tastes, acidic odor and color between the kimchi fermented with and without ginger. \cite{30,31}

Fermented anchovy and shrimp contain a lot of proteins and amino acids, and have their own unique taste and flavor. Therefore, those sub-ingredients would affect not only the nutritional balance but also the improvement of the sensory quality of kimchi. These fermented fish products and other protein sources such as skim milk, soy protein isolate, beef extract, and fish protein produced high lactic acid and promoted the growth of LAB. \cite{37,38,42}

The frequency of addition of starch and sugar in kimchi making was 27% with a concentration of 0.4-3.0%, and sugar was used for sweet and harmonized tastes. However, since starch and sugar are utilized as a carbon source by various microorganisms present in kimchi, they are expected to affect the kimchi fermentation and sensory quality. In the results, starch and sugar promote the fermentation of kimchi and contribute greatly to harmony of tastes by reducing the hot and overall tastes, and acidic and garlic odors. \cite{31,39}

Yulmoo, a young radish, is a vegetable that has been a major raw material of kimchi just as much as Chinese cabbage. Compared with Chinese cabbage, \textit{yulmoo} is chiefly cultivated in summer and thus is cheaper during this season. \textit{Yulmoo} kimchi is also different from \textit{baechu} kimchi in that it requires less seasoning. Owing to the use of various seasonings like red pepper powder and fermented fish products, Chinese cabbage kimchi is more expensive and at the same time more prone to turn sour than \textit{yulmoo} kimchi in summer. Therefore, \textit{yulmoo} kimchi would not only taste better but also be economical during summer. To evaluate the effect of starch on \textit{yulmoo} kimchi fermentation wheat flour was used as starch source and compared with glutinous rice. It was found that both wheat flour and glutinous rice flour hastened the fermentation of \textit{yulmoo} kimchi. \cite{39}

\section*{4. Natural preservatives}

The groups of LAB involved in kimchi fermentation continuously produce organic acids after the optimum ripening, and cause changes in the composition of kimchi. These changes are called the over-ripening or acid-deterioration of kimchi, which is often observed in summer kimchi and in winter kimchi stored for an extended period. The over ripening of kimchi is the most serious problem in the storage of kimchi. Since the over-ripening of kimchi is mainly due to activities of lactic acid-forming LAB, the best way to overcome it is to control the growth of LAB without destroying the quality of kimchi.

Screening of natural preservatives such as edible plants, herbs and spices, antimicrobial agents and related compounds to inhibit kimchi fermentation were studied extensively. \cite{47} Among 42 oriental medicinal plants tested, Baicall skullcap (\textit{Scutellaria baicalensis}), and Assam indigo (\textit{Cimicifuga foetida}) were very
effective for preserving kimchi. Among 32 herbs and spices tested, peppermint (\textit{Mentha piperita} L.), cinnamon (\textit{Cinnamomum verum} Presl), lemon balm (\textit{Melissa officinalis} L.), clove (\textit{Eugenia caryophyllate} Thunb.), hop (\textit{Humulus lupulus} L.), rosemary (\textit{Rosmarinus officinalis} L.), sage (\textit{Salvia officinalis} L.), horse radish (\textit{Moringa oleifera} Lam.) and thyme (\textit{Thymus vulgaris} L.) showed high antimicrobial activity against microorganisms in kimchi. Clove was the most effective microbial inhibitor, when added to fresh kimchi. However, sensory test was not good for evaluation of the effect of herbs and spices, since their highly specific flavors affected the taste of kimchi. Among 28 fruits, vegetables and related plants tested, leaves of pine tree (\textit{Pinus rigida}), persimmon (\textit{Diospyros kaki}) and oak leaves (\textit{Quercus glauca}) also demonstrated a significant bactericidal effect. In addition, of 21 natural preservatives added individually to fresh kimchi, only nisin and caffeic acid were able to inhibit fermentation.\(^{47}\)

The changes of pH and acidity of \textit{baechu} kimchi and \textit{mul} kimchi were remarkably inhibited by adding the tea catechins at the level of 2 mg/g fresh \textit{baechu}, results suggesting that the tea catechins can be successfully used for the extension of shelf-life of kimchi.\(^{48}\)

Studies were carried out to investigate the effects of \textit{Lithospermum erythrorhizon}, \textit{Glycyrrhiza uralensis} (LG) with and without dipping of salted Chinese cabbage in 1% chitosan solution (LGDC) on fermentation of kimchi at 10\(^{\circ}\)C during 25 days. The sour taste of LG and LGDC added kimchi was changed more slowly than that of control during fermentation of kimchi. The shelf-life of LGDC added kimchi was extended over 10 days compared with control.\(^{49}\)

By the addition of 1% mixed extracts of \textit{Lithospermum erythrorhizon} and \textit{Scutellaria baicalensis} and 1% crab shell treated with ozone to kimchi, color, flavor, and sourness were slightly inferior, while texture and overall acceptability were found to be the same or slightly superior compared with untreated kimchis.\(^{50}\)

It was also found that the kimchi containing 2% of ozone treated crab shell powders showed both strong neutralization action for 0-25 days and buffer action after 25 days during fermentation at 10\(^{\circ}\)C.\(^{51}\)

Addition of 500 ppm grape fruit seed extract showed the highest pH during fermentation at 20\(^{\circ}\)C and lowest titratable acidity compared with control kimchi. The total microbial counts were higher than LAB counts right after the preparation of kimchi, but they were similar after three days. However, sensory evaluation of 3 day old kimchi samples showed a significant difference (P<0.05) between the control and the treatment in odor, color and taste, except for the one with 50 ppm.\(^{52}\)

Recently, fermentation characteristics of mustard leaf kimchi added with green tea and pumpkin powder has been studied and found that the sensory scores of flavor, aroma and overall acceptability were highest in the kimchi with
0.3% pumpkin powder and 0.2% green tea powder.\textsuperscript{53)}

The effect of chitosan (0.5\%) on properties of kimchi was studied during the fermentation at 20 °C for 8 days. It was confirmed that chitosan reduced the total number of microorganisms and levels of \textit{Leuconostoc sp. and Lac. plantarum} in kimchi, and the lower mol. wt. chitosan fraction had the greatest effect on levels of \textit{Leuconostoc sp.} Also, chitosan reduced the intensity of sour and stale flavor notes in kimchi, and the content of reducing sugar in control kimchi samples was lower than in those containing chitosan for the first 6 days of fermentation at 20°C. Malic acid content was lower and lactic and acetic acid contents were higher for control kimchi than in those with chitosan for the first 4 days of fermentation. Control kimchi contained more succinic acid than those with chitosan for the first 2 days of fermentation.\textsuperscript{54-55)} \textit{Leuconostoc sp. and Lac. plantarum} were higher in the control than in the kimchis containing chitosan dissolved in 0.3% acetic acid and 0.05% sodium benzoate.\textsuperscript{55)} It is also found that addition of chitosan to kimchis influences pectic substance levels and improves their textural properties.\textsuperscript{56)}

Two % ginseng in kimchi had the best overall preservative and quality, and promoted the growth of \textit{Lac. plantarum} and \textit{Lac. fermentum} and inhibited the growth of \textit{Leu. mesenteroides} and \textit{Ped. cerevisiae}.\textsuperscript{57)}

One or two % of omija (\textit{Schizandra chinensis}, a fruit of maximoviczia typica) extracts had strong growth inhibitory properties against isolated LAB were shown by fumaric and itaconic acid among various organic acids of \textit{Schizandra chinensis}.\textsuperscript{58)} The growth of \textit{Sac. cerevisiae} and \textit{Lac. casei} in the culture media containing 1.0 and 1.5% of methanol extracts of kukija (\textit{Lycii fructus}, a fruit of the Chinese matrimony vine) were increased, whereas that of \textit{E. coli} was somewhat decreased.\textsuperscript{59)}

Addition of sap from pine needles (\textit{Pinus densiflora}) delayed kimchi fermentation by slowing down the decrease in pH and inhibiting the growth of \textit{Lactobacillus sp.}.\textsuperscript{60)}

Water extract of pine needles had more inhibitory effects against \textit{Lac. plantarum} than against \textit{Leu. mesenteroides}, and the vascularity of kimchi tissue of control kimchi was degraded more than that of water extracts of pine needles added kimchi.\textsuperscript{61,62)}

It was found that mustard oil (200 ppm) had antimicrobial effect on the major LAB of kimchi such as \textit{Lac. plantarum}, \textit{Lac. brevis}, \textit{Leu. mesenteroides} and \textit{Ped. cerevisiae}, and mustard and mustard oil could control the shelf life after 15 days storage at 15°C.\textsuperscript{63)}

The extract of bamboo leaves had a wide range of antimicrobial activity against \textit{Brettanomyces custersii}, \textit{Klebsiella oxytoca}, \textit{Pichia membranaefaciens} which cause kimchi softening.\textsuperscript{64)} Recently, the effects of \textit{Sepiae os}, cuttlefish born,\textsuperscript{65)} enoki mushroom (\textit{Flammulina velutipes}),\textsuperscript{66)} \textit{Monascus koji},\textsuperscript{67)} and boiled-dried fusiforme, a kind of seaweed\textsuperscript{68)} on the fermentation and quality of
kimchi have been studied.

5. Starters

Qualities of kimchi can be controlled by desirable microorganisms, and various fermentation conditions such as temperature, salt concentration, and nutrients in raw materials.

The major preparation procedure for kimchi includes lactic acid fermentation by the addition of various kinds of vegetables, spices, edible salt to Chinese cabbage and radishes. Kimchi is generally acknowledged to be more nutritious than other vegetable foods such as sauerkraut, pickles, and Japanese tsukemono or asatsuke. In addition, it is also known that the fermentation processes of kimchi involve more complex biochemical and microbiological processes compared with other fermented vegetable foods.\(^{2,16,69}\)

During the entire stage of natural fermentation of kimchi, lactic acid-producing bacteria are major population, and after prolonged fermentation, various and excess organic acids are produced by the LAB species. Those excess organic acid productions in kimchi fermentation are called the acidification of kimchi. After that stage, other microorganisms including yeasts grow on the surface of kimchi, and that growth causes the softening of the texture of the ingredients.\(^{70}\) Therefore, generally, it can be defined that the edible good taste of kimchi is obtained before the texture softening and the acidification.

A hetero fermentative type of LAB, *Leuconostoc mesenteroides*, is a major bacterial population of kimchi from the initial to the middle stage of fermentation. During those stages, hetero fermentative type LAB produce various metabolites such as lactic acid, acetic acid, ethanol, carbon dioxide, mannitol, and dextran which are associated with the taste of kimchi, and the number of LAB reaches the highest during the optimum-ripening period. However, the total number of *Leu. mesenteroides* decreases sharply when the pH of kimchi is decreased to 4.0. On the other hand, a homo fermentative type LAB, *Lac. plantarum*, which has a strong pH tolerance under high organic acid concentrations, has been continuously increased in their number during kimchi fermentation to the last stage. It has been reported that the acidification of kimchi is mainly caused by *Lac. plantarum*.\(^{16}\)

As already mentioned, kimchi fermentation and ripening are carried out by the microorganisms present in raw materials. Sugars in raw materials are converted to lactic and acetic acid, carbon dioxide, ethanol and mannitol by the LAB growing at 1-3% salt concentration.

As seen in Table 4, many LAB were isolated from kimchi, and among them, *Lac. plantarum, Lac. brevis, Lac. acidophilus, Lac. bavaricus, Lac. homohiochii, Ped. cerevisiae, Leu. mesenteroides, Leu. dextranicum, and Leu. paramesenteoides* have
been tried as kimchi starters to improve the quality and shelf-life extension of kimchi.  

The combination of various strains such as *Leu. mesenteroides*, *Lac. brevis*, *Lac. plantarum* and *Ped. cerevisiae* which were isolated from kimchi could be used as starters for kimchi fermentation. These starters increase the fermentation rate, and mixed strains are more effective than a single strain to produce better organoleptic quality of kimchi.  

Generally, kimchi fermentation has been carried out relatively at low temperature, and psychrotrophic LAB were isolated and characterized from kimchi fermented at 5°C. Therefore, psychrotrophic LAB isolated from kimchi fermented at low temperature were studied as starters for their effect on kimchi fermentation. The results indicated that the fermentation period could be shortened by using the LAB starters which isolated from low temperature kimchi. Among the LAB used as kimchi starters, it was confirmed that *Leuconostoc* species were more effective than any other *Lactobacillus* species tested for kimchi fermentation.  

Because the acid production from the hetero fermentative type LAB is lower than that of the homo fermentative type LAB, the addition of an acid-tolerant mutant strain of *Leu. mesenteroides* as starter of a kimchi fermentation may inhibit the rapid pH decrease and lactic acid production during kimchi fermentation. Therefore, a mutant strain, *Leu. mesenteroides* M-10 which could grow in low pH (3.0) at 10°C and produce more CO₂ than wild type was mutagenized to be improved as a kimchi starter. With respect to total acceptability, the kimchis prepared using the mutant strain M-10 were better than the other strains, and use of the mutant strain extended the optimum-ripening period of kimchi by two fold compare with that of control. This report suggested that a mutant strain of *Leu. mesenteroides* which has more stable growth ability in acidic conditions was able to extend the edible period of kimchi.  

Psychrotrophic yeast, *Saccharomyces fermentati* YK-19 that showed better growth at 10°C than at 25°C in the medium containing 0.3% lactic acid and 0.6% acetic acid, was isolated from kimchi and used as a kimchi starter in order to prevent over-acidification of kimchi. The addition of *Sac. fermentati* YK-19 prolonged the period of optimum fermentation (at pH 4.2 and 0.6-0.8 acidity) by >63%. The lactic acid content increased rapidly in control samples, followed by the kimchi with *Saccharomyces* sp. Yk-17 and *Sac. fermentati* YK-19 as starters. The growth of *Lactobacillus* species was inhibited by the addition of yeast starter, particularly by *Sac. fermentati* YK-19. Furthermore, sensory scores such as acidic and moldy flavor were reduced by starter addition, while flavor scores for freshness were increased.  

*Leu. paramesenteroides* P-100, a psychrotrophic mutant which grow well at pH 4.0, and 10°C, and in organic mixture (lactic acid:acetic acid;1:2) was improved
from wild type *Leu. paramesenteroides* Pw. A wild strain Pw and a mutant strain P-100 were inoculated as starters and the total acceptability of the kimchi were evaluated.\(^{80,81}\) Kimchi added with mutant strain had better tastethan that of control kimchi by sensory test and the optimal pH range of kimchi extended up to about 2.2-2.5 times. In the kimchi added with *Leu. paramesenteroides* P-100, the succinic acid content was higher than that of others, and the total number of *Lac. plantarum* was reduced about 2.5 fold with respect to control kimchi.\(^{80}\)

The effects of the addition of *Leu. mesenteroides* M-100, an acid-tolerant mutant improved from wild type strain *Leu. mesenteroides* Mw,\(^{82}\) and *Sac. fermentati* YK-19, an acid-utilizing and aromatic flavor-producing yeast, were tested for the retardation of acidification and the prolongation of the edible period of kimchi. The addition of *Leu. mesenteroides*M-100 to kimchi preparation may induce the prolonged acidification of kimchi because of its low production of lactic acid and increased growth in comparison with *Lac. plantarum*. Also *Sac. fermentati* YK-19 may lengthen the edible period of kimchi by reducing the content of lactic acid and acetic acid in the later period of kimchi fermentation, and the good flavor in the starter-added kimchi group may be due to the various compounds, carbon dioxide, and succinic acid produced by *Leu. mesenteroides* M-100 and *Sac. fermentati* YK-19.\(^{83}\)

Recently, the effects of the addition of mutant strains of *Leu. mesenteroides* and *Leu. paramesenteroides* which have increased adipic acid resistance compared with wild type species on shelf life of kimchi were evaluated.\(^{84}\) The addition of a combination of both mutants was more effective than that of single strain in extending shelf-life of kimchi. The optimum inoculation was 0.005% of a 1:10 mixture of *Leu. mesenteroides*:*Leu. paramesenteroides* according to the results from acidification tests and sensory analysis.\(^{84}\)

To extend the storage period and inhibit contamination of *E. coli*, conditions for kimchi brining in lactic acid solution and effect of the halophilic *Lactobacillus* HL-48 starter were investigated. The results showed that the pH value of the starter inoculated kimchi was 4.2 after 18 hr fermentation at 25\(^{\circ}\)C, while the pH of the starter non-inoculated kimchi was 3.3 at the same condition.\(^{85}\)

### III. Microbial and chemical changes during kimchi fermentation

#### 1. Microbial changes

The number and species of major microorganisms in kimchi fermentation vary widely, and are influenced by raw materials and other ingredients. However, the growth, activity, and role of the microorganisms participating in the fermentation are influenced more by environmental conditions, especially by temperature and salt concentration.
Generally, total LAB was highly distributed throughout the whole fermentation period. However, they were slightly declined as the acidity increased. The growth pattern of the major LAB during fermentation at 20 to 30 °C is that the number of *Leuconostoc* species increase initially, and then at pH 4.0 to 4.5 they decrease rapidly with an increase in the number of *Lactobacillus* species. However, at 5 to 15 °C, the times for the increase and decrease of LAB are delayed.\(^{16,69}\)

The total counts at the optimum-ripening time were 1x10\(^{8-9}\) cells/ml and *Leuconostoc* spp. appeared maximum levels at this time, and after then total counts decreased and increased again by little. *Lactobacillus* and *Pediococcus* appeared maximum level at this period (1x10\(^{6-7}\) cells/ml).\(^{16,69}\) The number of *Leu. mesenteroides* reached its maximum value at the optimum-ripening stage of kimchi and decreased when kimchi became acidic. At the same fermentation temperature, the total number of *Leu. mesenteroides* was higher at lower salt content than at higher one. 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 the number of *Leu. mesenteroides* reached maximum with the corresponding increase in the total viable count. *Lac. breviss* grew better at higher temperature and lower salt content at over-ripening period of kimchi. *Ped. cerevisiae* was dominant at higher salt content (5-7%) and also appeared generally at over-ripening stage of kimchi. *Lac. plantarum* appeared at the time when the *Leu. mesenteroides* decreased in number and after the appearance of *Lac. breviss* and *Ped. cerevisiae*. The population of *Lac. plantarum* was dominant at the over-ripening and acid-deteriorated stage of kimchi. *Lac. breviss*, *Ped. cerevisiae*, and *Lac. plantarum* were not detected at any levels of salt contents at 5°C. *Str. faeculis* appeared rarely at all temperature and salt contents but its number was negligible.\(^{16}\)

Film-forming yeasts appear on the surfaces of the kimchi preserved for long term period, and they produce polygalacturonase and contribute to softening the texture of kimchi by degrading of pectic substances.\(^{70}\)

The number of fungal flora during kimchi fermentation decreased in number at higher temperature (30°C), but the total number of fungi decreased and remained constant at temperature below 20°C. However, yeast population during kimchi fermentation showed typical changes depending upon the temperature and salt concentration. Total number was not changed at 20-30°C, but it showed gentle curve of initial increase to slow decrease in the later phase at lower temperature (14-5°C).\(^{16}\)

The typical changes of major LAB during kimchi fermentation at 20°C are shown in Fig. 4. The patterns of microbial changes in each lactic acid bacterial group, *Leuconostocs*, *Lactobacilli*, and *Streptococci*, were similar at different fermentation temperature, and the microbial changes accelerated by increasing the temperature. Among them, *Leuconostocs* showed higher number at high
temperature than the others. The numbers of Leuconostocs and Streptococci increased the beginning but they rapidly decreased after the optimum-ripening period. Pediococci increased their number after Streptococci, but they rapidly disappeared later.69)

The strains of Leuconotocs, Streptococci, Pediococci and Lactobacilli were identified as Leu. mensenteroides subsp. mesenteroides, Str. faecalis, Str. faecium, Ped. pentosaceus, Lac. plantarum, Lac. sake and Lac. brevis. Among the LAB isolated, Lac. brevis and Lac. plantarum appeared mainly at the beginning and around the over-ripening period of fermentation, respectively.69)

Kimchi fermentation is dominated by Lactobacillus spp. at 25 °C and Leuconostoc spp. at 5 °C.86) The levels of Streptococcus and Pediococcus species are lower than those of Leuconostoc and Lactobacillus species and decrease considerably at lower temperature. Like Leuconostoc species, Streptococcus and Pediococcus species increase initially, and then decrease rapidly after the optimum-ripening period during the fermentation. Lac. sake and Lac. brevis also are included among the Lctobacillus species. The Lac. sake especially is isolated from an early stage and is not able to survive under pH 4.0 at the later stages. Lac. brevis are also isolated at early stages, but smaller numbers are only found in the later stages. Lac. plantarumis isolated from all the stages during the fermentation period, but especially at the later stages after the optimum-ripening period.69,86)

Lactobacillus sp. and Leuconostoc sp. were the main bacteria isolated from optimum-ripened kakdugi (53 and 43%, respectively) as well as from over-ripened kakdugi. (63 and 37%, respectively). Leuconostoc mesenteroides subsp. paramesenteroides was the dominant strain among the LAB from kakdugi. The isolates from optimum-ripened kakdugi required more amino acids for growth compared to those from over-ripened samples, while no difference was observed with respect to vitamins. The physiological characteristics of the isolates, such as amino acid and vitamin requirements, were different from those of 9 type strains of various Leuconostoc spp.26)

Recently, 88 strains of LAB were isolated from dongchimi, and the predominant strains were identified as Leu. mesenteroides subsp. mesenteroides at the initial stage of fermentation (on the 5th day), Lc. lactis subsp. lactis at the middle stage (30th day), and Lac. curvatus and Lc. lactis subsp. lactis at the final stage (50th day). The 88 strains of isolates were classified into 15 groups according to the fermentation properties, of which, 6 groups of Leu. mesenteroides, 3 groups of Lac. curvatus, 2 groups of Lac. cellobiosus, and each of 1 group of Leu. citreum, Lc. lactis. subsp. lactis Lac. acidophilus and Lac. delbrueckii subsp. delbrueckii.87)

In the early 1960’s, LAB such as Lac. brevis, Lac. plantarum, Leu. mesenteroides, Ped. cerevisiae, and Str. faecalis were isolated from kimchi and it was reported
that the main microorganisms responsible for kimchi fermentation are *Leu. mesenteroides*, *Lac. brevis*, *Ped. cerevisiae*, and *Lac. plantarum* which was just the same as in sauerkraut fermentation.\(^8^8,8^9\) However, kimchi is less acidic than sauerkraut and the optimum acidity and pH of kimchi are 0.6-0.8% and 4.5-4.2, respectively, while those of sauerkraut are 1.6-2.0% and 3.7-3.5. Also, sauerkraut fermentation is completed after 20 days at 23°C with 2.25% salt content, whereas kimchi fermentation is completed only within 3 days under similar fermentation conditions. Therefore, it has been suggested that the main microorganism in kimchi fermentation is *Leu. mesenteroides* rather than *Lac. plantarum* and *Lac. plantarum* is a main acid-deteriorating organism in kimchi fermentation unlike in sauerkraut where it constitutes main responsible microorganism.\(^1^6\)

Recently, many new LAB were isolated and identified from various kimchis using molecular techniques. However, the role of new LAB has not yet been confirmed by testing as kimchi starters. New LAB from kimchi will be discussed more in chapter IV-1.

### 2. Chemical changes

The chemical compositions of kimchi may be different basically depending on the varieties of cabbage, kinds and amounts of minor ingredients such as garlic, green onion, leek, hot pepper powder, ginger, and fermented anchovy or shrimp. However, kimchi fermentation was carried out continuously by LAB existing in the raw materials. The sugars in kimchi raw materials were converted to organic acids, carbon dioxide, ethanol, and other flavoring compounds by lactic acid fermentation.

Generally, the best quality of kimchi can be obtained at pH 4.2-4.5 and at the time being maximum levels of carbon dioxide production. Acidity at the optimum-ripening period is 0.6-0.8% as lactic acid, and reaches 1.5% upon over-ripening and becomes 1.5-2.0% at the stage of acid-deterioration of kimchi. The optimum-ripening time and shelf-life of edible period were changed depending on the salt content, fermentation temperature and time as seen in previous Table 3.

In most cases, the kimchi fermentation process has several stages based on the changes of pH, acidity and reducing sugar content. The first stage has a rapid decrease of pH, an increase of acidity and a decrease of reducing sugar. The second stage shows a gradual decrease in pH, an increase in acidity, but a rapid decrease of reducing sugar. The last stage has no or only gradual changes in pH, acidity, and reducing sugars.\(^1^6\)

Generally, during the kimchi fermentation, pH and reducing sugars decrease while total acids increase. The pH starts from 5.5-6.0, reaches 4.5-4.2 at optimum
ripening period, and drops further down upon over-ripening period. High temperature and low salt content showed faster fermentation than low temperature and high salt content.

Lactic, acetic, citric, malic, fumaric, succinic, oxalic, tartaric, malonic, maleic, and glycolic acid were identified from kimchi samples. Among the organic acids identified, lactic acid and acetic acid are the major acid that increased by fermentation. However, the kimchi fermented at lower temperature (6-7°C) contains more lactic and succinic acid, and less oxalic, malic, tartaric, malonic, maleic and glycollic acid than the kimchithat fermented at higher temperature (22-23°C), while no difference is noted in citric acid level. Higher salt concentration brings about a lower acetic acid, and large amounts of acetic acid and carbon dioxide make kimchi tasteful.

The production of carbon dioxide during kimchi fermentation was stimulated by higher temperatures and was affected by seasonal factors. Kimchi made from winter baechu (Chinese cabbage) produced much more fermentative gas than that made from summer baechu. It was suggested that the changes in carbon dioxide concentration could be used as a characteristic index for indicating the fermentation course of packaged kimchi products.

The changes of the content of organic acids, carbon dioxide, alcohols and carbonyl compounds of the various kimchi which were made from cabbage with green onion, garlic, ginger, and red pepper fermented at 12°C-16°C were investigated. The identified nonvolatile organic acids were acetic, formic, propionic, butyric, valeric, n-caproic and malic acid. The identified carbonyl compounds were acetaldehyde and acetone. The content of lactic acid was increased with fermentation, and higher in kimchis containing red pepper, garlic and green onion. The content of acetic acid wasincreased with fermentation, especially in the kimchi containing garlic. The content of carbon dioxide was higher in the kimchi containing garlic. Alcohols identified in all kimchis were only ethanol. Carbonyl compounds had no direct effect on off-flavor of kimchi.

The production of free non-volatile and volatile organic acids in kimchi during fermentations at 30, 20, and 5°C, were determined by gas chromatography. The order in the amount of non volatile organic acid, soon after preparation, was malic,citric, tartaric, pyroglutamic, oxalic, lactic, succinic, and L-ketoglutaric acids. The major non volatile acids at the optimum ripening time were malic, tartaric, citric and lactic acids, and as the temperature was lowered, the amount of lactic, succinic, oxalic, pyroglutamic and fumaric acid increased, while that of malic, and tartaric acids decreased. The order in the amount of volatile acids at the beginning was acetic, butyric, propionic and formic acids. Among these acids, acetic acid was significantly increased in its amount during fermentation. The kimchi fermented at low temperature produced more acetic acid than that fermented at high temperature.
Of 17 volatile flavor components tentatively identified from kimchi, ethanol was the most abundant, and increased in fermented kimchi while the others decreased with the time of fermentation.\(^{96}\) It was also found that the ratio of volatile to non volatile acids found to be highest at the optimum-ripening time of kimchi.\(^{16}\)

Vitamin B1, B2, B12, and niacin reached the highest levels -twice the original content- at maturation. It was also found that kimchi had the most palatable taste at this period and subsequently there was a sharp decrease in B vitamins with a sudden development of acids. On the other hand, although both vitamin C and carotene decrease during fermentation, their residual contents were still significant.\(^{97}\)

Many kinds of free amino acids were identified from mustard leaf kimchi samples, and it was found that major free amino acids were proline, glutamic acid, alanine and histidine. It was also found that nucleic-acid related compounds were hypoxanthine, inosine monophosphate (IMP), and guanine monophosphate (GMP).\(^{98}\)

Major flavor components were identified as dimethyl disulfide, dimethyl trisulfide, dipropyl disulfide, 1-butane-1-isothiocyanate, and diallyl disulfide from kimchi fermented at 5°C by GC and GC-MS. The contents of organic acids such as lactic and citric acid increased during fermentation. Free amino acids were important in kimchi flavor. Total concentration of free amino acids increased from 316 to 600 mg/100g of kimchi on fermentation, and glutamic acid, alanine, valine, leucine, lysine and arginine levels were high. Sensory evaluation showed that kimchi flavor was closely related to the contents of non volatile organic acids and free amino acids and pH.\(^{99}\)

In \textit{woong} kimchi, a specialty kimchi prepared form burdock (\textit{Arctium lappa} L), fermented at 15°C, it was found that the major volatile components were identified as ethanol, hexanol, 2-hexanol, disulfidedi-2-prophenyl, zingiberene, and beta-sesquiphellandrene. The relative amounts of hexanal, 1-hexanal and ethanol decreased, while the relative amounts of acetic acid ethyl ester, 3-hydroxy-2-butanone and acetic acid increased gradually during fermentation.\(^{100}\)

Aroma-active compounds in kimchi prepared with or without fish sauce were analyzed. The most intense aroma compounds in kimchi included dimethyl trisulfide, diallyl disulfide isomers, diallyl trisulfide, and methylallyl disulfide. However, addition of fish sauce did not noticeably affect the aroma profile of kimchi.\(^{101}\)

Recently, flavor compounds of \textit{dongchimi} soup fermented at different temperature and salt concentration were analyzed. In this study four organic acids including acetic, lactic, malic, and succinic acid were identified. The flavor compounds of \textit{dongchimi} soup were composed of mainly dimethyl disulfide and ethanol followed by 3-(methylthio)-1-propene. Acetic acid and dimethyl...
trisulfide were also detected significantly. Dongchimi soup fermented at 4 °C after prefermentation at 12 °C for 12 hr contained the largest amount of flavor compounds.28)

The taste and flavor components have been identified from various kimchi samples. However, the exact amounts of those compounds during kimchi fermentation were not clearly analyzed. Further researches are needed to improve the tastes and quality of kimchis.

Ⅳ. Characteristics of LAB from kimchis

1. Identification of kimchi microorganisms

Kimchi fermentation and ripening were carried out by the microorganisms present in the raw materials. Sugars in the raw materials are converted by the LAB surviving at 2~3% salt concentration to lactic, acetic acid, carbon dioxide, ethanol and mannitol.

The quality of kimchi depends on the composition of the LAB involved in the fermentation process. Therefore, in order to study the ecology of kimchi microorganisms, it is important to understand the components of the microbial community and identification of the physiologically active microorganisms for kimchi fermentation.

Isolation and identification of microorganisms have long been attempted in kimchi, conventionally prepared and fermented with the mixture of salted cabbage and spices such as sliced radish, red peppers, garlic, ginger, green onions, and fermented fish sauces. Kimchi is a fermented food with the variety of vegetables. Therefore, it is expected that various LAB propagate in its habitat.

Since 1960, many aerobic bacteria, anaerobic bacteria including LAB, yeast, and fungi were isolated and identified from kimchi and other vegetable fermented products and were reported in Korea as seen in Table 5 and 6.

Seven genera of LAB include Lactobacillus, Leuconostoc, Weissella, Pediococcus, Streptococcus, Lactococcus, and Enterococcus were isolated and identified from kimchis as seen in Table 5. Among them, Lac. brevis, Lac. plantarum, Leu.citreum, Leu. lactis, Leu. mesenteroides subsp. mesenteroides, Leu. mesenteroides subsp. dextranicum, Weis. pramesenteroides, Str. faecium, and Ped. pentosaceus have been commonly isolated.

Six genera of aerobic bacteria from kimchi were Achromobacter, Flavobacterium, Pseudomonas, Aerococcus, Aeromonas, and Bacillus, and ten genera of yeast from kimchi were Bretanomyces, Candida, Cryptococcus, Citeromyces, Debaryomyces, Kluyveromyces, Pichia, Rhodotorula, Saccharomyces and Torulopsis as seen in table 6. However, there is no clear evidence how those aerobic bacteria and yeasts except Saccharomyces species have been involved.
and affected kimchi fermentation.

Recently, *Leu. mesenteroides*, *Lac. curvatus*, *Lac. cellobiosus*, *Leu. citreum*, *Lactococcus lactis* subsp. *lactis*, *Lac. acidophilus* and *Lac. delbrueckii* subsp. *delbrueckii* were isolated from *dongchimi* made by traditional method. Also, ethanol and low acid producing *Weis. paramesteroides* P30 were isolated from *kakduki* (cubed radish kimchi) and used as kimchi starter for reduced acid production. *Lac. plantarum* and *Lac. pentosus* from low salt (0.2-1.0%) *mul-kimchi* and *Lac. plantarum*, and *Lac. brevis* from medium salt (3.0%) *mul-kimchi* were isolated and identified. Other identified LAB were *Leu. citreum*, *Leu. mesenteroides* subsp. *mesenteroides* and *dextranica* and *Str. faecium* in *mul-kimchi* containing 0-3.0% salt, while *Pediococcus* sp. was not detected. As gram-negative organisms, *Aeromonas hydrophyla*, *Pseudomonas fluorescens*, *Pseudomonas aureofaciens* and yeast *Candida pelliculosa*, *Cryptococcus laurentii* were identified in the *mul-kimchi*.27)

Lactic acid bacteria are widely distributed in Korean traditional fermented food such as kimchi. Also many LAB have been isolated from kimchi, particularly, the genera *Lactobacillus*, *Leuconostoc*, *Weissella* and *Pediococcus* are known to play an important role in kimchi fermentation.69,89,111) Traditional identification methods based on physiological phenotypes are labor intensive and time consuming. Until recently, the identification of the LAB isolated from kimchi has mostly depended on traditional phenotypic analyses. However, this type of identification method using biochemical and morphological characteristics is limited in its discrimination and accuracy.108) Therefore, studies on their systematic taxonomy have been reported.104,106,118,119) Accordingly, the effective study of kimchi fermentation requires the development of rapid and accurate LAB identification methods, such as genotypic approach using modern molecular typing and identification tools.103,109,120)

Recently, LAB in kimchi were identified and differentiated rapidly by using the Biolog system. *Leuconostoc* and *Lactobacillus* are reported as the main genera associated with kimchi fermentation.118,106) The former genus was generally found at less than 15ºC and was comprised of isolated strains such as *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Leu. citreum*, *Leu. gelidum*, and *Leu. kimchi*.107,112) All species of the genus *Leuconostoc* isolated from kimchi have been found to produce slime from sucrose so far, which is a dextran, and dextran producing *Leuconostoc lactis* from sucrose media was also isolated from kimchi.108)

Until 90’s most taxonomic studies on isolates from kimchi have been based on phenotypic characteristics, but after 90’s a polyphasic approach including phenotypic, chemotaxonomic, and molecular methods is applied to determine the taxonomic position of kimchi microorganisms. Recently, novel strains such as *Leuconostoc kimchi*,112) *Lactobacillus kimchi*,113) *Weissella koreensis*,114) and *Weissella kimchi*117) from kimchi have been reported. However, further studies
are needed to understand the real role of those new strains on kimchi fermentation.

Aerobic yeasts and molds appear on the surface of the upper layer and on improperly covered vegetables at the later fermentation stages. Undesirable kimchi with off-flavors and softened texture is positively due to the excessive aerobic growth of moulds and film-forming yeasts. Particularly the film-forming yeasts appeared in long term stored kimchi at low temperature produce a polygalacturonase which degrade pectin to make kimchi’s texture soft.\(^7^0\) However, yeast appearing in low temperature (below 15\(^\circ\)C) fermented kimchi has a certain role in kimchi’s flavor and odor.\(^7^9\)

2. LAB producing bacteriocins from kimchi

Kimchi is prepared with various kinds of vegetables, spices and other ingredients and becomes palatable through proper fermentation. Kimchi fermentation initiated by various microorganisms present in the raw materials, but the fermentation is gradually dominated by LAB.\(^1^5\) The LAB play an important role in the taste of kimchi, as in dairy products such as cheese and fermented milk. The strains belonging to the genera *Leuconostoc*, *Lactobacillus*, *Streptococcus*, *Pediococcus* and *Lactococcus* in kimchi have been reported.\(^1^,1^6,2^4,1^0^4\) Accordingly, kimchi has been one of the important sources of LAB in Korea for a long time. Generally, the LAB are known to have the potential to inhibit the growth of microorganisms, especially pathogenic and spoilage bacteria. The antimicrobial activity of LAB is known to be due to the produced organic acids, hydrogen peroxide, diacetyl and bacteriocins. Many LAB have been isolated from kimchi in Korea and some of them have been shown to have antimicrobial activity and other useful properties.\(^1^2^1-1^2^3\)

The LAB have long been known to produce antimicrobial proteins called bacteriocins. Certain bacteriocins inhibit various food-born pathogens, including *Bacillus cereus*, *Clostridium perfringens*, *Listeria monocytogenes*, and *Staphylococcus aureus*. This suggests that bacteriocin-producing LAB may be useful in biocontrol of kimchi either by applying the culture directly or by adding the produced bacteriocin as a natural preservative. From this point of view, there are many reports on the isolation of bacteriocin-producing LAB from various kimchi samples.

As seen in Table 7, bacteriocins are produced by the genera of *Pediococcus*, *Lactobacillus*, *Leuconostoc*, and *Lactococcus* in kimchis.

The LAB isolated from kimchi have antimicrobial activity against *E. coli*, *Staphylococcus aureus*, *Bacillus cereus* and other microorganisms. Major LAB responsible for this activity are *Pediococcus pentosaceus*, *Leuconostoc* spp., *Lac. plantarum* and *Lactococcus lactis*. The properties of the inhibitory substances from these bacteria have been determined and purified. These bacteria could
produce adverse circumstances for the growth of other microorganisms.\textsuperscript{124,125)}

Due to the antibacterial activity of nisin against wide range of gram- positive bacteria and its stability under acidic conditions, nisin have been studied on its effectiveness in preventing over-acidification of kimchi. It has been reported that nisin added kimchi showed a slower rate of acid production.\textsuperscript{47)} However, controversial result was found that the use of nisin to prevent over-acidification of kimchi was not effective because of the nisin inactivation by vegetable components and the presence of nisin-resistant LAB on kimchi.\textsuperscript{125-127)}

V. Conclusion

Kimchi is characteristic in its palatability giving sour, sweet and carbonated taste with medium texture in tissue of kimchi. In this respect kimchi differs greatly from sauerkraut that is popular in the West. The optimum acidity, pH and salt content of kimchi are 0.6\% (as lactic acid), 4.5 and 2.0-2.5\%, respectively. Kimchi fermented at low temperature (7-14°C) was more tasty than that at higher temperature (20-30°C).

The total number of kimchi microorganisms reaches its maximum level (1x10^{6-9} cells/ml) at optimum-ripening time, and after then the number of LAB decreases slowly and maintains its 2\textsuperscript{nd} maximum level (1x10^{6-7} cells/ml).

Many aerobic and anaerobic bacteria, especially LAB were isolated and identified from kimchi. However, in kimchi fermentation system, it seems clear that hetero fermentative LAB producing organic acids, carbon dioxide, and dextrans from sugars are major species in the early stage and optimum fermentation period. Homo fermentative LAB producing excessive lactic acid is a major species in the final stage of fermentation. The total aerobic bacteria and fungi decreased and the yeasts somewhat increased and slowly decreased in number at lower temperatures (10-14°C).

Carbon dioxide and organic acids such as lactic, acetic, succinic and citric acids were detected during kimchi fermentation. Vitamin B1, B2, B12, and niacin increased two fold at optimum ripening period and decreased during over-ripening, while vitamin C and carotenoid decreased. It was also found that the ratio of volatile to nonvolatile acids and carbon dioxide reached maximum at optimum-ripening period of kimchi.

Kimchi fermentation is complex and is due mainly to certain LAB and yeasts naturally present in raw materials. Several physicochemical and biological factors such as salt and sugar concentration, temperature, raw materials, natural preservatives and starter culture influence the quality of kimchi. Among the many factors affecting kimchi fermentation, salt content and temperature are the most important, and the next is the quality of raw materials and microorganisms specially hetero fermentative LAB.

Many homo and hetero fermentative LAB, such as genus \textit{Lactobacillus},
Leuconostoc, Weissella, Lactococcus, Streptococcus and Pediococcus were isolated and identified from various kimchi samples, but genus Leuconostoc, Weissella and other LAB producing organic acids, carbon dioxide and bacteriocins are the most important microorganisms for the controlled fermentation of kimchi. However, further studies are need to understand the real mechanism of those LAB on kimchi fermentation and improved classification techniques using modern taxonomies such as polyphasic and molecular methods are necessary to understand kimchi microorganisms.

After optimum ripening is reached, kimchi fermentation may continue until the product has acidic taste and softened texture, which cause the acid deterioration. So far the preservation of kimchi under refrigeration at around 5°C is known to be the best way for long-term preservation up to 6 months.

Finally, further researches are needed to develop the technology necessary for the controlled fermentation using starters and long-term preservation of commercial kimchi.

And also, further studies are needed to fully understand the mechanisms of action and to gain a complete knowledge, even though systematic research is difficult because of the complexity of kimchi.

VI. References


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