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Investigation of the Microbiological and Biochemical Properties of Kimchi in the Submerged Model System Designed for Fermented Sausages

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Abstract

The objective of this study was to investigate the potential of the application of lactic acid bacteria (LAB) from kimchi as a starter culture in the production of fermented sausages. To achieve this, a submerged model medium that contained LAB as part of a complex system of kimchi (0.5, 1.0, 1.0, 3.0, and 5.0%) and lyophilized kimchi powder (0.2 and 0.5%) was fermented for 120 h. During the fermentation period, the growth of total viable organisms and LAB, and the changes in the pH and the titratable acidity, were investigated. The initial LAB counts ranged from 6.4 to 7.7 Log CFU/mL for the kimchi media, and from 6.9 to 6.9 Log CFU/mL for the kimchi powder media. In all the kimchi batches, the LAB increased logarithmically, and the highest LAB counts (around 9 Log CFU/mL) were reached in 24 h. An evident lag phase of the LAB was observed in the kimchi powder samples and reached 8.8 Log CFU/mL in 8 h. The decrease in the pH and the formation of lactic acid were rapid in the kimchi batches, and reached pH values of 3.4-3.5 in 12 h. With these results, the LAB that was integrated with the addition of kimchi or kimchi powder demonstrated its potential utility as a substitute for starter culture.

Key words: fermented sausages, kimchi, lactic acid bacteria, starter culture

Introduction

The application of lactic acid bacteria (LAB) as starter culture in association with catalase-positive cocci, yeasts, and molds in the production of fermented sausages is ubiquitous due to their technological and hygienic advantages compared to "natural fermentation" that is governed by the microflora derived from the raw materials or during processing (Kunz 1989; Roca and Incze, 1990; Hammes and Knauf, 1994). In principle, excellent fermented sausages can be produced traditionally by spontaneous ripening with LAB or 'back-slopping' method (Daly *et al*., 1973). However, the requirements of large-scale, low-cost industrial production with short ripening time and highly standardized end products has made research in sausage fermentation intensive, as seen, for example, in the USA (in the 1930s) and in Europe (in the 1950s) when the first systematic studies on the microbiology and the production of fermented sausage were published (Kroeckel, 1995). Since then, the interest in starter cultures concerning meat fermentation has greatly increased, and numerous excellent reviews mention the history and the physiological and technological aspects of starter cultures (Bacus and Brown, 1981; Smith and Palumbo, 1983; Luecke and Hechelmann, 1986; Hammes and Knauf, 1994; Kunz, 1994; Kroeckel, 1995).

Among starter microorganisms, LAB are the most important bacteria in meat fermentation because these bacteria are applicable to all types of sausages that are to be fermented and contribute to all aims of the process (Hammes *et al*., 1990). The beneficial property of LAB to decrease pH by utilizing carbohydrates and producing organic acids (mainly lactic acid) is essential for the desired achievement of texture, color, flavor, and above all hygienic safety in sausages (Kunz, 1989). At low pH values (4.6-5.9), the isoelectric point of proteins is approached and the muscle protein coagulates, resulting in sliceability and firmness found in the final product. The acidic condition is also favorable for the development of curing color. They can be also used as "protective cultures" to suppress the emergence of undesirable microorganisms in virtue of their varied desirable antagonistic activity, such as competition for the available nutrients, decrease in redox potential, production of lactic acid and the resulting decrease in pH.

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The most used and commercially available LAB in the production of fermented sausages are *Lactobacillus curvatus, L. sake, L. plantarum,* and *Pediococcus acidilactici*, since they are highly competitive in and well adapted to the environment of fermented sausages. These species of LAB are also the common ones that emerge in kimchi and continuously participate in its fermentation. During fermentation of kimchi at 20°C to 30°C, the strains of LAB *Leuconostoc*, *Lactococcus*, and *Pediococcus* increase initially and then decrease rapidly after the optimum ripening period has been reached. *Lactobacillus* is distributed throughout the whole process and continuously participates in the fermentation (Lee *et al*., 1992). Indeed, as shown in the previous study, *L. brevis*, *L. sake*, *L. plantarum*, *L. curvatus* and *Leuconostoc mesenteroides* were the main LAB isolated and identified from kimchi fermented at 20°C (Lee *et al*., 2006). Although the environmental conditions in kimchi and fermenting sausages are different, the LAB emerged in kimchi during fermentation at 20-30°C are expected to have the potential utility as a substitute of starter culture in the production of fermented sausages (Hwang *et al*., 1960; Lim *et al*., 1989; Lee *et al*., 2006). Hence, the objective of this study was to investigate the microbiological and biochemical properties of kimchi-originated LAB under the modified fermented sausage condition. Fermented raw sausages vary according to different factors such as meat quality, manufacturing process, as well as the use of auxiliary materials. Since the handling of these factors is very complicated, a submerged model system was developed and used for the investigations.

Materials and Methods

Preparation of kimchi and kimchi-powder

The preparation of kimchi was carried out based on the "baechu-kimchi" recipe. The ingredients are listed in Table 1. Previously, garlic, ginger, and leek were chopped. Paprika, purchased from Korea, and sugar were weighed. The Chinese cabbage was cut into pieces of 3×3 cm and soaked in 15% (w/v) brine for 30 min. The soaked cabbage was then washed twice with fresh water and drained for 30 min. The prepared ingredients were mixed well, and cabbage was coated with the seasoning mixture. The kimchi was placed in a polyethylene bag (iUL) and vacuum-packaged, and it was fermented at 20°C for 6 d. For the production of kimchi-powder, fermented kimchi (at 20°C for 6 d) was frozen in a round flask at -72°C and later dried in vacuum using a freeze-dryer (WIRTIS). After freeze-drying, the kimchi was pulverized with a blender (KRUPS). The powder was kept under sterile conditions in a plastic bag at -72°C until further use.

Preparation of model-media

The fermentation medium used in this study was composed to simulate the substantial conditions of meat mixtures employed for the sausage production. The medium samples were prepared in 250 mL Erlenmeyer flasks. The ingredients are listed in Table 2. The start conditions of the model-media were adjusted by the addition of corresponding salt concentrations as well as by setting appropriate pH values as much as the common condition of fermented sausages is (Koch, 1982; Hechelmann, 1985, Liepe, 1985). The pH value was set with 0.5 N HCl to 5.8 and then the medium was autoclaved for 20 min at 121°C and 1.2 bar. To avoid maillard reactions owing to heat treatment, glucose was sterilized separately and added aseptically to the medium after cooling

Fermentation after treatment with kimchi or kimchi-powder

After autoclaving, the medium (200 mL in a 250 mL flask) was cooled at room temperature. After cooling, the medium was divided into 7 batches in total according to the concentrations of kimchi and kimchi-powder added. Kimchi fermented at 20°C for 6 d was added in concentrations of 0.5, 1.5, 3.0, and 5.0%(w/v) under aseptic conditions. Kimchi-powder was added in concentrations of 0.2 and 0.5%(w/v). The inoculated samples were incubated at 25°C for 120 h.

Table 1. Composition of kimchi materials

Materials and ingredients	Percentage share (%)
Salted Chinese cabbage	90
Leek	4
Paprika powder (Korean)	2
Garlic	2
Ginger	1
Sugar	1

Table 2. Composition of model-medium

Materials and ingredients	g/L
Meat extracts	12.0
Glucose	10.0
NaCl	20.0
Dipotassium hydrophosphate	2.0
$MgSO_4 \cdot 7H_2O$	0.15
Glutamate	0.5

Biochemical characterization of fermentation with kimchi and kimchi-powder

The sampling of all experiments were carried out in duplicate after 0, 4, 8, 12, 16, 24, 36, 48 h and then every 24 h during the fermentation period of 120 h. The medium was shortly mixed with a sterile magnetic stirrer and then 1 mL or 2 mL was taken out from them for the determination of CFU and pH as well as titratable acidity, respectively. The determination of the microbiological changes were investigated by the numbers of total viable counts (TVC) using Plate Count agar and lactic acid bacteria using MRS agar (MERCK).

Results and Discussion

Kimchi in the submerged fermentation

Meat as a research material is very difficult to examine. In contrast to it, in a liquid condition the nutrients are distributed almost homogeneously and microorganisms can move actively. Accordingly, the microbial metabolism may be facilitated as compared to the real sausage conditions (Liepe *et al*., 1989). This is of benefit to shorten the experimental period than that needed for fermentation and ripening as compared with sausages. Therefore, the experiments were carried out using submerged medium.

Fig. 1 and 2 present the effects of the concentration of added kimchi on the changes in (Fig. 1) and the growth of LAB (Fig. 2). The initial TVC varied from around 6.5 Log CFU/mL to 7.7 Log CFU/mL depending on the added kimchi concentration (Fig. 1). Smith and Palumbo (1983) suggested that an addition of large numbers (7-9 Log CFU/g) of desirable bacteria would inhibit the growth of undesirable species, thereby preventing or reducing meat fermentation failures. According to Luecke and Hechelmann (1985), the presence of 6-7 Log CFU

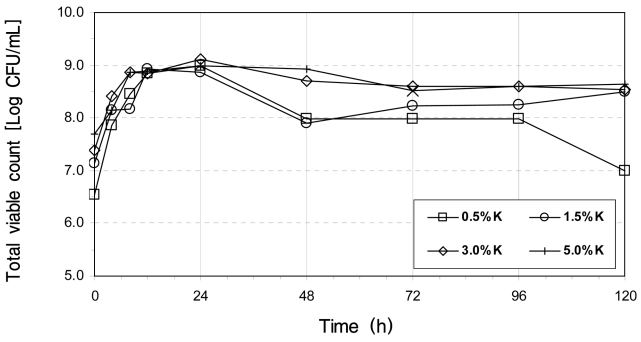

Fig. 1. Changes in total viable counts introduced by the addition of kimchi at different concentrations (w/v) into the liquid model-medium as a function of fermentation time (K=kimchi).

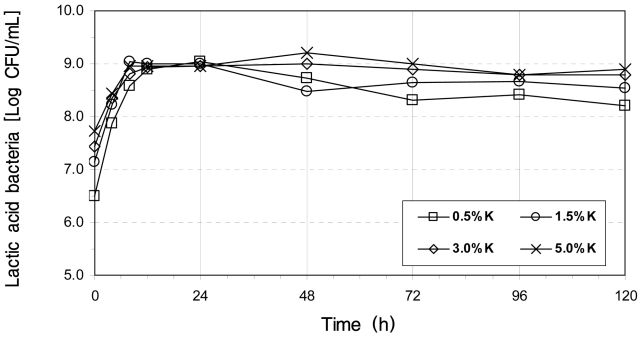

Fig. 2. Growth of lactic acid bacteria introduced by the addition of kimchi at different concentrations into the liquid model-medium as a function of fermentation time.

LAB per gram in the fresh sausage mixture leads to a more predictable and more rapid pH decrease and to earlier development of firmness. They stressed the importance of increasing the inoculation counts of LAB according to their competitiveness against 'spontaneous microflora'. In the present work, 6-7 Log CFU/mL of LAB were inoculated (Fig. 2) by the addition of different concentrations of kimchi (0.5-5.0%) into the submerged model medium.

The highest TVC (around 9 Log CFU/mL) was observed after 12 h in all batches. This value was maintained until the end of the investigated fermentation time of 120 h in the batches 3.0% K and 5.0% K, whereas the TVC in the batches with kimchi in lower concentrations slightly decreased after 24 h (Fig. 1).

The initial TVC as well as the TVC during the fermentation in each sample were closed to the counts of lactobacilli (Fig. 1 and 2).

An organism usually needs time to adapt to the new environment when inoculated, called lag phase. This time is needed for the synthesis of enzymes to utilize the nutrients available (Garbutt, 1997). Such an adaptation time of LAB could be omitted by the use of kimchi. LAB population inoculated into the submerged media by kimchi addition had no lag phase but showed good adaptation to the new environmental conditions with rapid increase in their number (log phase) right from the beginning of the fermentation (Fig. 2). Although the initial counts (from 6.4 Log CFU/mL to 7.7 Log CFU/mL) of LAB counts ranged widely depending on the added concentrations of kimchi, LAB in all batches increased logarithmically and the highest LAB counts (around 9 Log CFU/mL) were observed in 24 h. Regarding the concentration of added kimchi, there were differences in the initial numbers of LAB counts between batches depending on the concentrations of added kimchi, but the differences became very small after the growth phase was finished. Thus, the

count of LAB reached to a high value (about 9 Log CFU/mL) even with a least concentration of kimchi (0.5%). Such a good growth of kimchi LAB under the sausage condition can be due to the typical characteristics of kimchi, in which a spice mixture (red pepper, ginger, garlic and leek) was simultaneously enriched and it may accelerate the sugar metabolism of the bacteria. The properties of spices to stimulate the fermentation of carbohydrate to lactate of several lactobacilli were already found in previous works (Zaika *et al*., 1978).

The most important task of the LAB in the production of sausages is the formation of lactic acid from added carbohydrate (Kunz, 1989). The immediate and fast acid formation at the beginning of the fermentation is regarded as an essential requirement for lactic acid starter culture. In the present work, the liquid model medium was actively acidified by the kimchi bacteria, which showed a rapid decrease in pH reaching 3.4-3.5 after 48 h (Fig. 3). Since the medium was treated with only kimchi, the pH drop might be due to the capability of kimchi LAB to produce organic acids during their metabolization of sugar, which is needed for their growth and survival. Indeed, the pH drop was accompanied by a fast increase in the LAB count as well as in the production of lactic acid (Fig. 3 and 4). The rate of the pH drop in the kimchi batches was comparable to that of the medium inoculated with a series of lactic acid starter cultures in the study by Liepe *et al*. (1989). This indicates that the LAB of kimchi had a capability of using glucose and producing lactic acid comparable to that of the bacteria usually used as starter cultures in the production of sausages. Furthermore, the investigations by Liepe *et al*. (1989) were carried out in MRS broth, which is an optimal medium for growth of lactobacilli. Therefore, the comparable pH drops in both studies demonstrate the excellent souring properties of the kimchi LAB applied in the present work. As mentioned, the

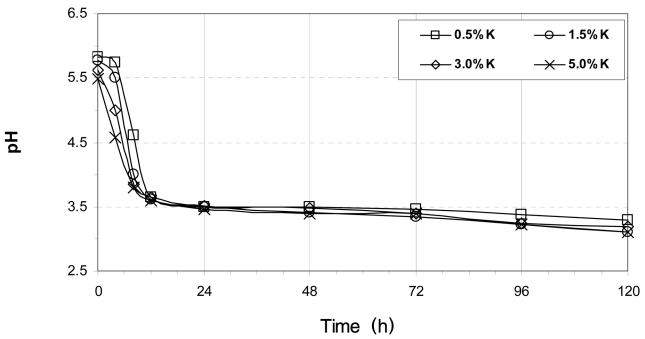

Fig. 3. Changes in pH of kimchi/medium-mixture as a function of fermentation time at different concentrations of added kimchi.

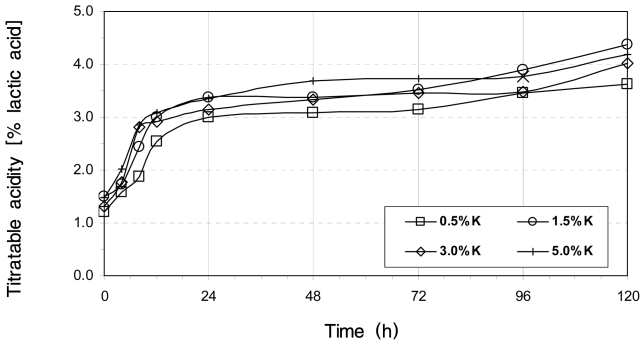

Fig. 4. Changes in titratable acid contents in the kimchi/medium-mixture as a function of fermentation time at different concentrations of added kimchi.

spice mixture added with kimchi might have attributed to such good souring properties of kimchi LAB in the new environment.

The pH drop caused by kimchi LAB was quite rapid and the extent of the pH drop was considerable. An initially rapid decrease in pH by accumulation of produced acids in sausage mixtures is very important for the microbial stability. In addition, a pH drop below the isoelectric point of proteins (< 5.3) is important for the development of sliceability, firmness, cohesiveness and color as well (Hugas and Monfort, 1997). Nevertheless, a too rapid pH drop can cause unbalanced sour flavor and a lack of fine aroma by preventing the development of important aroma-forming microorganisms (Roedel and Stiebing, 1988). Hence, the results of the present work pose a question about the negative effect of the rapid and strong souring properties of the LAB from kimchi on the sensorial development of sausages. However, it must be considered that the experiments were carried out in a liquid medium where nutrients are distributed almost homogeneously and the mobility of microorganisms is ensured unlike in real meat mixture (Liepe *et al*., 1989). Furthermore, the medium was treated with glucose as the only fermenting substrate. These factors should have attributed to the accelerated pH drop in the experimental medium. Considering the real condition of sausage mixtures where the nutrients are unevenly distributed, the movement of microorganisms is restricted, and different kind of sugars exist together, it may be supposed that the souring performance of the kimchi LAB would be less.

Kimchi-powder in the submerged fermentation

The utility of kimchi LAB as a starter is advantageous due to their vital condition (inoculum culture) unlike commercial starter cultures, and no need of time for rehydration. In commercial starter cultures, which is needed

for the microorganisms that are provided as freezing or freeze-dried forms (lyophilized) like commercial starter cultures. However, kimchi has high moisture contents (approximately > 80%), and this may cause possible problems regarding the hygienic stability and sensory characteristics in the sausage production. Furthermore, it is difficult to maintain desired conditions of kimchi during preservation and transportation since microbial metabolism is taking place continuously in fresh kimchi. As an alternative form of kimchi, freeze-dried (lyophilized) kimchi-powder was chosen to be studied for the properties of its LAB. The lyophilized materials almost maintain their original volume, shape, aroma, color, enzyme, and different kind of nutrients (Jennings, 1997).

The microbial and physiological activities of the LAB in kimchi-powder in concentrations of 0.2 and 0.5% were investigated using model media in the same manner used for kimchi (Figs. 5 and 6).

The initial counts of LAB were significantly different

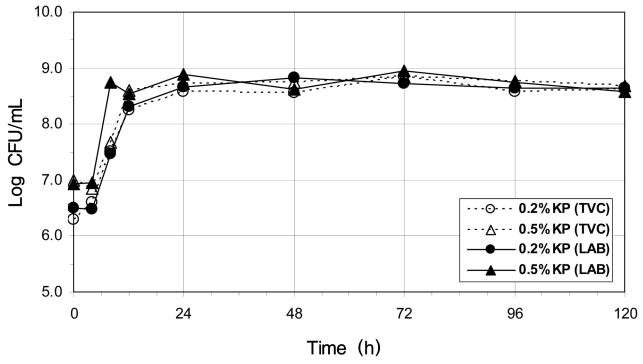

Fig. 5. Changes in total viable counts (TVC) and lactic acid bacteria (LAB) counts introduced by the addition of kimchi-powder (KP) at different concentrations into the submerged model-medium as a function of fermentation time.

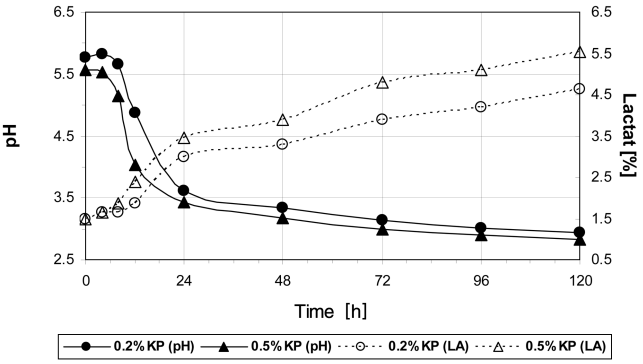

Fig. 6. Changes in pH and titratable acid contents in the kimchi-powder/medium-mixture under the submerged condition as a function of fermentation time at different concentrations of added kimchi (LA=lactic acid).

for approximately 0.5 Log unit between the two batches depending on the concentrations of added kimchi-powder (Fig. 5). The initial LAB counts of the batch 0.5% KP (6.9 Log CFU/mL) were comparable with those of the batch 1.0% K (6.9 Log CFU/mL). Considering the moisture content of kimchi (> 80%), it can be estimated that the survival rate of kimchi LAB was less than 40% in a form of freeze-dried kimchi-powder after lyophilization. This survival rate is relatively low compared to those of LAB cultures (Bifidobacteria) having 66.6-83.3% survival rate (Trsic-Milanovi *et al*., 2001). The high survival rates in their work were accomplished by using a new combination of cryoprotectants for lyophilization, which is employed especially in the lyophilization of pure cultures. The treatment with cryoprotectants – such as dextran, gelatine, glucose, etc – as well as their selection is regarded to be very important factors for re-viability of lyophilized organisms (Jennings, 1997; Trisic-Milanovi *et al*., 2001). In the present work, however, no cryoprotectant was treated for kimchi before drying. This might have caused the low survival rate of the kimchi LAB after lyophilization. In spite of the low survival rate the desired number (6 Log CFU/mL) was even achieved by the low kimchi-powder concentration (0.2%). The TVCs were similar with those of lactobacilli. The initial TVCs of the batches were 6.3 Log CFU/mL (0.2% KP) and 7.0 Log CFU/mL (0.5% KP).

Unlike in kimchi batches, an evident lag phase was observed in the kimchi-powder batches (Fig. 5). Such an adaptation phase of the LAB from kimchi-powder may be explained by several reasons. Above all, the microorganisms in kimchi-powder must have been rehydrated since they subsisted as lyophilized culture in freeze-dried kimchi-powder (Luecke and Hechelmann, 1986). The metabolism and enzyme functions of the LAB in kimchi-powder were at a standstill since the biological activity or chemical reactions are no longer supported under the lyophilized condition (Jennings, 1997). Therefore, the adaptation phase in the present work may have been necessary for recovering metabolic and enzyme functions. This could be ascertained by the initial standstill of the lactic acid production resulting in hardly changed pH values during the initial period.

After the lag phase the kimchi-powder LAB exhibited a good growth rate up to 9.0 Log CFU/mL (0.5%) after 8 h, which were maintained until the end of the fermentation period (Fig. 5). The difference in the numbers of LAB between the two batches became smaller as closed to the stationary phase. These results indicate that LAB of kim-

chi-powder were able to fulfill the basic criteria for fermented sausage starter cultures by showing salt tolerance, good fermentation of sugar (glucose) and metabolic activities at a temperature of 25°C (Hammes *et al*., 1985; Luecke and Hechelmann, 1985; Buckenhueskes, 1993).

Fig. 6 shows the evolution of pH and titratable acidity in the kimchi-powder/medium-mixture. The start pH of the model-medium was decreased for 0.2-0.4 units from the original pH (5.8) of the medium by the addition of the kimchi-powder. The start pH of the batch 0.2% KP (5.8) was same as that of the batch 1.5% K (Fig. 3). This agrees with the result that the start lactic acid content of 0.2% KP was the same as that of 1.5% K (Fig. 4). There were little changes in pH during the initial 4 h. The pH of the batch 0.5% KP even increased slightly. In this period, the lactic acid contents were also almost the same as the start values. After an initially unchanged phase of pH level, it decreased remarkably in both batches until 24 h after the start of the experiment reaching values of 3.61 (0.2% KP) and 3.43 (0.5% KP). The initial changes in pH of the kimchi-powder batches were found to be slower than those in the kimchi batches, but the continuous decrease in pH of the kimchi-powder batches during the later half led to the lower final pH values than in the kimchi batches. The final values were ranged between 2.9 (0.2% KP) and 2.8 (0.5% KP). These final values were much lower than those of the kimchi batches, which ranged from 3.3 to 3.1.

In conclusion, the LAB integrated by the addition of kimchi or kimchi-powder have shown the potential utility as a substitute of starter culture by showing good growth and acid forming properties under the fermented sausage conditions. In addition, the use of kimchi-powder may make up the weak points (high water content and low stability of the LAB) of that of kimchi.

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