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ARTICLE

Investigation on the Microbiological and Biochemical Properties of *Kimchi* in the Solid-state 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 *kimchi* LAB as starter culture in the production of fermented sausages. For this, the solid-state model media composed to simulate the substantial conditions of meat mixtures were fermented for 120 h after the treatment with different concentrations of *kimchi* (0.5, 1.0, 1.5, 3.0, and 5.0%) and lyophilized *kimchi*-powder (0.2 % and 0.5%). During the fermentation period, the growth of total viable cells and LAB, and the changes of pH and titratable acidity were investigated. The initial LAB counts ranged from 7.18 to 8.34 Log CFU/mL for *kimchi* media and from 6.93 to 6.94 Log CFU/mL for *kimchi*-powder media depending on the added concentrations. The *kimchi* LAB in this study were not influenced by the immobilized condition for their adaptation and growth by showing no lag phase and thus acted similar as in the submerged medium. The initially increased counts reached around 9 Log CFU/mL in 12 h independent of the concentrations of added *kimchi*. However, the growth and metabolic activity of *kimchi*-powder LAB were influenced by the immobilized condition. Supposedly, as the nutrient supply in solid-state depended solely on diffusion, these differences in the souring properties were caused by the LAB topography in the medium matrix. Nevertheless, the differences in the numbers of LAB between two media were less than 0.5 Log units and the pH drop in the solid-state batches was quite rapid and reached low values. Therefore, it can be assumed that *kimchi* and *kimchi*-powder LAB showed the utility as the substitute of commercial starter culture even without a rehydrating pretreatment.

Key words: Fermented sausage, solid-state fermentation, *kimchi*, *kimchi*-powder, lactic acid bacteria, starter culture

Introduction

At the area of fermented sausage production, the requirements of large-scale, low-cost industrial production with short ripening times and highly standardized end-products has made research intensive, 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 deal with the history and the physiological and technological aspects of starter cultures (Bacus and Brown, 1981; Geisen *et al*., 1992; Kunz, 1994; Luecke and Hechelmann, 1986; Smith and Palumbo, 1983). Among the microbial components of meat starter cultures that are commercially available, the application of lactic acid bacteria (LAB) as starter culture 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 (Hammes and Knauf, 1994; Kunz 1989; Roca and Incze, 1990). The beneficial property of LAB to decrease pH resulted from sugar utilization and production of organic acids (mainly lactic acid) is essential for the desired achievement of texture, color, flavor, and above all hygienic safety in sausages (Kunz, 1989).

The most used and commercially available LAB as starter culture 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. Indeed, as shown in the previous study, *L. brevis*, *L. sake*, *L. plantarum, L. curvatus,* and *Leuconostoc mes.mes./dent* were the main LAB isolated and identified from *kimchi* (Lee *et al*., 2006). Although the environmental conditions in *kimchi* and fermenting sausages are different, the LAB emerged in *kimchi* during

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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; Lee *et al*., 2006).

The utility potential of *kimchi* LAB as starter culture in the production of fermented sausages was investigated at the previous study using submerged model-medium, in which the conditions were composed to simulate those of meat mixture (Lee and Kunz, 2009). A real sausage mixture, however, has different features from the submerged medium. The nutrients are not distributed evenly and the mobility of microorganisms is limited in sausage mixtures due to insufficient water content as compared to the liquid medium. Microorganisms cannot be evenly distributed and their growth can only take place in small groups, so-called "nests". The nests are usually enclosed in small "cavities" within the sausage mixture. As the fermentation progresses, bacteria are captured in these "cavities" and they are immobilized there. Thus, sausage ripening can be regarded as 'solid-state fermentation' (Katsaras and Leistner, 1988). In the beginning of the fermentation, the nutrients are distributed almost homogeneously in mixed media. In a solid-state mixture, bacteria start to consume nutrients from their surroundings. As the fermentation progress, continues a depletion of nutrients in their surroundings develops. In such a condition, the nutrients are supplied to the bacteria via diffusion. As a result, diffusion is a decisive factor in the determination of fermentation velocity.

In this study, the microbiological and biochemical properties of LAB originated from *kimchi* under the fermented sausage condition designed as a fixed phase ("solid-state") were investigated to verify the utility potential of *kimchi* LAB as starter culture for the production of fermented sausages. The type of *kimchi* used was verified as fresh one as well as lyophilized *kimchi*-powder.

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 in length and soaked in 15% (w/v) brine for 30 min. The soaked cabbage was washed twice with fresh water and then drained for 30 min. The prepared ingredients were mixed well and then distributed evenly on the Chinese cabbage. The *kimchi* mixture was put into a polyethylene bag (iUL, Spain) and closed under vacuum. It was fermented at 20°C for 6 d, respectively. For the production of *kimchi*-powder, fermented *kimchi* (at 20°C for 5 d and at 7°C for 10 d) was frozen in a round flask at -72°C and later dried in vacuum using a freeze-dryer (Bench Top Shell Bath Freezeer, VIRTIS, England). After freeze-drying, the *kimchi* was pulverized with a blender (KRUPS, Germany). The powder was kept under sterile conditions in a plastic bag at -72°C until further used.

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

Preparation of solid-state 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 100 mL beakers. 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 (Hechelmann, 1985; Koch, 1982; 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. As a gelling agent for a fixed phase ("solid phase") agar was added to the fermentation broth in a concentration of 3.5 g agar/200 mL before autoclaving.

Fermentation after treatment with *kimchi* or *kimchi*-powder

Fifty mL solid-state medium was prepared in a 100 mL

Table 2. Composition of model-medium

Materials and ingredients	Concentration (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
Agar	17.5

beaker. To avoid the influence of sampling on the fermentation characteristics of *kimchi* under solid-state, 77 beakers in total (7 batches with 11 samples each) were prepared. Prepared media were cooled to 40°C after autoclaving. Prior to coagulation, *kimchi* (0.5, 1.0, 1.5, 3.0, and 5.0%) and *kimchi*-powder (0.2 and 0.5%) were added. For cooling further and to solidify the treated medium was left at room temperature for 40 min. Then the agar block was smashed with a spatula and cut into small cubes of approximately 0.5 cm in diameter. The samples were incubated at 25°C for 5 d.

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 and then every 24 h during the fermentation period of 120 h. Ten g of each sample were homogenized in 90 mL sterile physiological saline solution (0.9% NaCl) for 2 min with a stomacher (Masticator, iUL, Spain). After sampling 1 mL from this suspension for the determination of CFU, the residue was filtered using a strainer and used to investigate pH and titratable acidity. 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.

Results and Discussion

***Kimchi* in the solid-state fermentation**

To evaluate the possibilities of alternative use of *kimchi* microorganisms as a substitute for the commercial starter cultures applied in sausage production, a series of microbiological and chemical experiments were carried out. The adaptation of LAB from *kimchi* to the special sausage conditions was investigated by evaluating their growth and ability to utilize fermenting sugar resulting in production of acids and lowering pH. However, such investigations are very difficult to be surveyed in the real sausage because of an inevitable contamination with unidentified microorganisms deriving from the raw materials or the environment (Liepe *et al*., 1989). Furthermore, it is also very difficult to obtain reproducible and constant fermentation conditions in sausages (Katsaras and Leistner, 1988). Therefore, experiments were carried out using solid-state model media in which substantial conditions of a meat mixture prepared for sausage production were devised. The results are compared with those of previous trials carried out in submerged model medium (Lee and Kunz, 2009) to investigate the effect of fixed phase on the microbiological and chemical characteristics of *kimchi* LAB.

The tendency of microbial changes in both TVC and LAB counts in the solid-state model medium was shown in Figs. 1 and 2. The initial TVC (Fig. 1) ranged from 6.95 Log CFU/g to 7.49 Log CFU/g depending on the concentration of added *kimchi*. The highest TVCs reached around 8.90 Log CFU/g in 12-24 h comparable with that in the submerged medium (around 9 Log CFU/mL) (Lee and Kunz, 2009). The initial TVC as well as the TVC during the fermentation period in each sample were closed to the counts of *lactobacilli*.

The initial LAB counts (Fig. 2) varied widely between 7.18 Log CFU/g and 8.34 Log CFU/g depending on the concentration of added *kimchi*. Smith and Palumbo (1983) suggested that an addition of large numbers (7-9 Log CFU/g) of desirable microorganisms would inhibit the growth of undesirable species, thereby preventing or

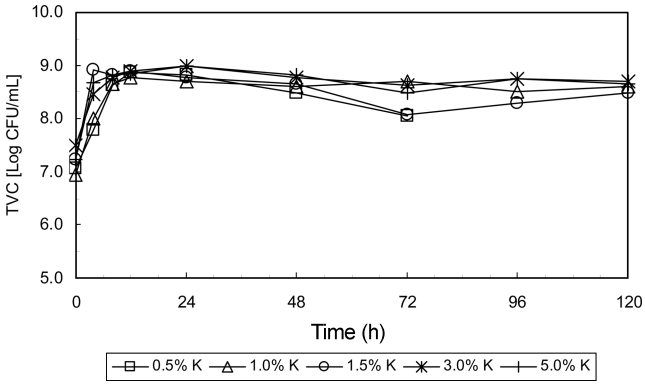

Fig. 1. Changes in total viable count introduced by the addition of *kimchi* at different concentrations (w/v) into the solid-state model medium as a function of fermentation time (K=*kimchi*).

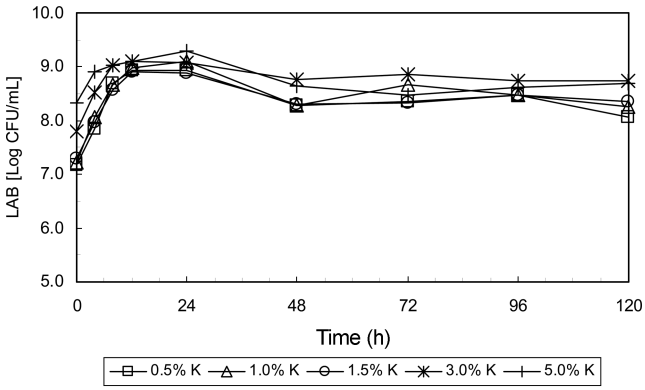

Fig. 2. Growth of lactic acid bacteria introduced by the addition of *kimchi* at different concentrations into the immobilized model medium as a function of fermentation time.

reducing meat fermentation failures. After that, Luecke and Hechelmann (1985) stressed the importance of increasing the inoculation counts of LAB, contributing to the competitiveness against 'spontaneous microflora' and development of optimal sensory characteristics with more predictable and more rapid pH decrease. In the present work, the use of *kimchi* as an additive for the production of fermented sausages was resulted in the accompanying inoculums of LAB in the concentrations of more than 7 Log CFU/g.

An organism usually needs time to adapt to the new environment when inoculated, so called lag phase, in which it needs the time for the synthesis of enzymes to utilize the nutrients available (Garbutt, 1997). Such an adaptation time of LAB under the sausage condition is not needed by the use of *kimchi* under the solid-state fermentation. LAB population inoculated into the model medium had no lag phase but showed a logarithmical growth from the beginning of the fermentation.

Under the primary solid-state fermentation, as mentioned before, the nutrients are distributed almost homogeneously in mixed media. In a solid-state mixture, bacteria start to consume nutrients from their surroundings. As the fermentation progress, continues a depletion of nutrients in their surroundings. As the fermentation progress, continues a depletion of nutrients in their surroundings develops. In such a condition, the nutrients are supplied to the bacteria via diffusion. As a result, diffusion is a decisive factor in the determination of fermentation velocity. A slower metabolism and growth of bacteria caused by such conditions due to the solid-state fermentation was already observed in the works of Katsaras and Leistner (1988), Liepe (1987), and Liepe *et al*. (1989). In the work of Liepe (1987), for example, the numbers of viable bacterial counts in the immobilized condition were reduced for about 1 Log unit as compared to the liquid medium. According to the experimental results of the present work, the adaptation and growth of *kimchi* LAB was not influenced by the immobilized condition by showing no lag phase and thus acted similar as in the submerged medium (Lee and Kunz, 2009). The initially increased LAB counts reached the higher values of 8.93-9.11 Log CFU/mL in 12 h. These counts were comparable with or even higher than those in submerged medium (8.85-8.99 Log CFU/mL) although a decrease in the number of the LAB was observed after 24 h reaching below 9 Log CFU/mL (Lee and Kunz, 2009). After that, however, the counts were nearly maintained until the end of the fermentation period reaching final values of 8.08-8.70 Log CFU/mL. This indicates that neither the mobility of the LAB nor the supply of fermenting sugar was limited by the immobilization. Such a result could be contributed to the enriched water by *kimchi* addition since the water content of *kimchi* is more than 80% (Hwang, 1991; Park *et al.*, 1996). This water from *kimchi* might have been located in the space between the particles ("cavities") and may have acted as a means of transport of the LAB and also of the nutrients. Such an effect provoked by the *kimchi* addition should be also expected in the real sausage system and it might negatively influence the sensorial development and hygienic stability (Leistner, 1985). It must be taken into account that the *kimchi* addition was also accompanied by an enrichment of the mixture with salt (3.0% in *kimchi*). Kastaras and Leistner (1998) observed that any additional salt have penetrated the inside of medium particles and caused a swelling of particle owing to an absorption of nearby water. In this process, the molecules of the medium have been released from the inside of the medium particles to surroundings and supplied to the LAB. This effect could have positively influenced on the metabolism and growth of the LAB also in the present work.

Figs. 3 and 4 show the evolution of pH and lactic acid of the solid-state model medium treated with different concentrations of *kimchi* during the fermentation period of 120 h. Although the initial pH values varied from 5.54 to 6.06 depending on the added amount of *kimchi*, a remarkable decrease in pH was observed in all batches during the initial 24 h. The immobilized condition did not affect the growth of LAB from *kimchi* in the environment of fermented sausage, however it did affect the metabolism of LAB showing the longer time for reaching the lowest pH value and higher pH value than the submerged condition (Lee and Kunz, 2009). This tendency could be

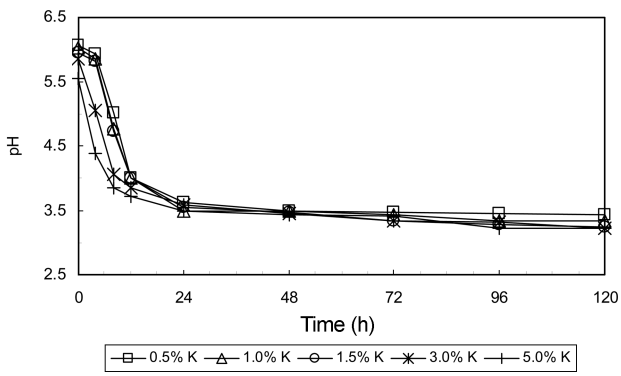

Fig. 3. Changes in pH of *kimchi*/medium-mixture under the immobilized condition as a function of fermentation time at different concentrations of added *kimchi*.

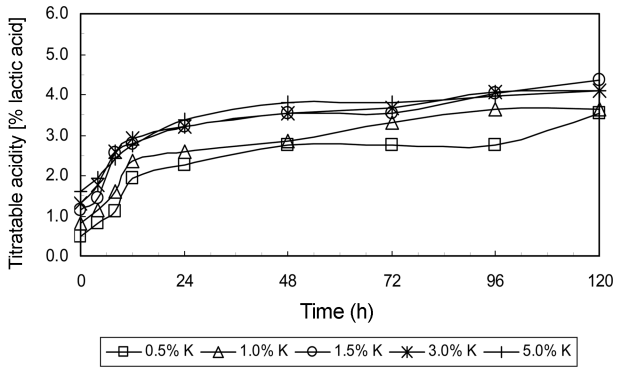

Fig. 4. Changes in lactic acid contents in the *kimchi*/medium-mixture under the immobilized condition as a function of fermentation time at different concentrations of added *kimchi*.

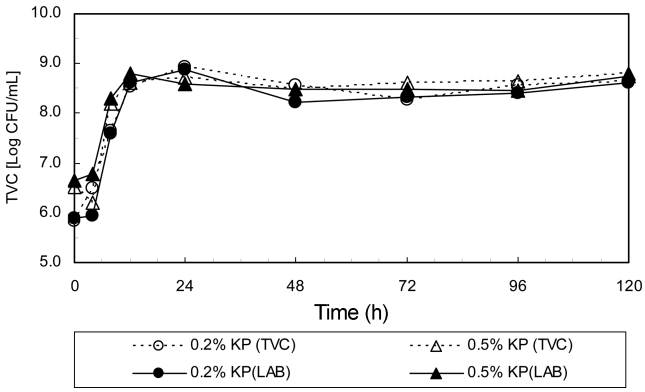

Fig. 5. Changes in total viable count (TVC) and lactic acid bacteria (LAB) count introduced by the addition of *kimchi*-powder (KP) at different concentrations into the immbolizied model medium as a function of fermentation time.

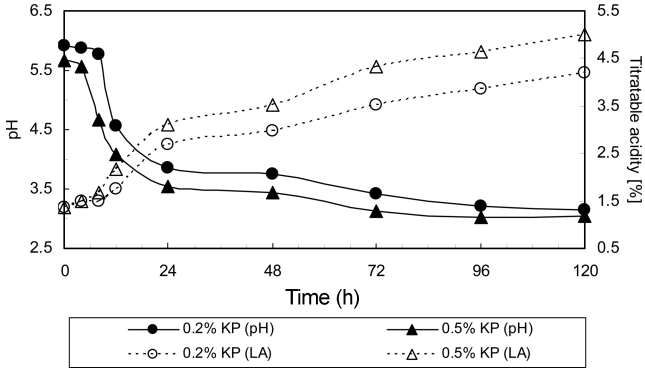

Fig. 6. Changes in pH and lactic acid contents in the *kimchi*-powder/medium-mixture under the immobilized condition as a function of fermentation time at different concentrations of added *kimchi*-powder (LA=lactic acid).

also found in the evolution of lactic acid contents. The solid-state medium had the effect to lower the production of lactic acid by showing reduced lactic acid contents as compared with those in the submerged medium throughout the fermentation period (Fig. 4) (Lee and Kunz, 2009). After the pH values of the *kimchi* samples under the immobilized condition reached the deepest points, they underwent very little variation and reached final values of 3.43-3.22.

Kimchi-powder in the submerged fermentation

The microbial and physiological activities of the LAB in *kimchi*-powder were investigated using solid-state model media in the same manner used for *kimchi* LAB (Figs. 5 and 6). Even though the utility of *kimchi* LAB as a substitute for commercial starters in the production of sausages is advantageous due to their vital condition (Crueger, 1984), *kimchi* can present a handicap in its use due to its high water content. For example, an addition of 10% *kimchi* to the sausage mixture causes a moisture addition of approximately 8% of the sausage weight. For this reason, a dried form of *kimchi* (*kimchi*-powder) by using lyophilization was chosen to be studied for the properties of its LAB as starter culture for the production of fermented sausages. The lyophilized materials almost maintain their original volume, shape, aroma, color, enzyme and different kind of nutrients, and can be maintained for a long time (Jennings, 1997). Furthermore, lyophilization is regarded as the best method for preserving bacterial cultures. The metabolism of bacteria is interrupted by lyophilization but they can multiply again even after some years of storage (Chemie Lexikon, 1995). Therefore, the utility of freeze-dried *kimchi*-powder might compensate the disadvantageous features of *kimchi* but with comparable microbiological and physiological values.

The changes in TVC and LAB counts introduced by the addition of *kimchi*-powder during fermentation are presented in Fig. 5. The TVCs initially increased logarithmically with the exception of the 0.5% KP that had a decrease in the first 4 h. The counts reached 8.53 log CFU/mL (0.2% KP) and 8.61 log CFU/mL (0.5% KP) in 12 h. After that the same values were remained more or less up to the end.

The values of TVC were similar to those of the LAB count over the fermentation period. An initial lag phase was observed in the *kimchi*-powder batches as shown in the submerged model medium (Lee and Kunz, 2009). This adaptation phase of LAB from *kimchi*-powder may due to the time needed for rehydration since they subsisted as lyophilized culture in freeze-dried form (Luecke

and Hechelmann, 1986). As the metabolism and enzyme functions of the LAB in lyophilized *kimchi*-powder were at a standstill, and therefore the adaptation phase in the present work may have been necessary. Such a standstill of biological activity and chemical reactions can be also found in the initial period of lactic acid production and pH changes in the present work (Fig. 6).

The difference in the numbers of LAB counts between the batches (2% KP and 5% KP) was continued during the growth phase, but after they entered into the stationary phase the difference began to narrow. The highest counts were 8.88 Log CFU/mL (0.2% KP) and 8.58 Log CFU/mL (0.5% KP) after 24 h and these values remained relatively constant until the end of the investigation period. The values in this period (8.23-8.88) were similar to those of the *kimchi*-powder batches under the submerged condition (8.32-8.96) (Lee and Kunz, 2009). Although there was not much difference in their counts, the viable cell numbers in the solid-state batch treated with *kimchi*-powder were lower than those in the liquid medium. This is due to the fact that in solid-state condition the content of freely available water is very low as compared to the liquid medium (Katsaras and Leistner, 1988). In the case of *kimchi*-powder, the rehydration is an essential process for the recovery of its biological growth and chemical reaction (Luecke and Hechelmann, 1986). In the solid-state fermentation, this process may have been retarded or limited as compared to the submerged fermentation. Since the *kimchi*-powder was added into the solid-state model medium without any water treatment, the microorganisms in the eutectic mixture had to rely on the moisture that existed in cracks as well as on the surface of the medium particles for their rehydration (Luecke and Hechelmann, 1986). Accordingly, the water in the cracks and on the surface of the medium particles must have been absorbed by the *kimchi*-powder resulting in an enhanced drying of the mixture. As this should have slowed down the metabolic activity in the *kimchi*-powder batches, the reduced viable cell counts in the solid-state medium can be explained. However, the difference of two media was not too much with 0.08-0.09 Log CFU/mL.

The souring properties of the LAB have also been influenced by the consistency of the medium as both solid-state batches showed higher pH values and lower lactic acid productions throughout the fermentation than the liquid medium batches. The decrease of pH in *kimchi*-powder batches under the solid-state condition was slow during the initial 4-8 h, being consistent with a slow increase in the LAB counts. After an initially stationary phase of pH, a sharp decrease followed up to 24 h and the value decreased slightly until the end of the fermentation. Final values were 3.14 (0.2%) and 3.04 (0.5%) and thus were slightly higher than the values in the submerged model medium (2.94 and 2.83, respectively) (Lee and Kunz, 2009). This coincided with a lower formation of lactic acid with final values of 4.19-5.00% in the solid-state model medium as compared with those in the submerged model medium of 4.65-5.55%. Nevertheless, the pH drop in the solid-state batches was quite rapid and reached low values (< 4.0), demonstrating the excellent souring properties of *kimchi*-powder LAB. Furthermore, the slow-down of pH values of *kimchi*-powder samples in the solid-state media could contribute to a satisfactory aroma formation which can be impaired by preventing the growth of important aroma-forming microorganisms or by interfering with their nitrate reducing activities with the rapid reduction of pH (Roedel and stiebing, 1988).

In conclusion, the LAB integrated by the addition of *kimchi* or *kimchi*-powder under the solid-state model medium were able to fulfill the basic criteria for fermented sausage starter cultures by showing salt tolerance, good fermentation of sugar and metabolic activities at 25°C (Buckenhueskes, 1993; Hammes *et al.*, 1985; Luecke and Hechelmann, 1985). In particular, the fermentation of *kimchi*-powder LAB under the real condition of sausages (solid-state immobilized condition) may make up the weak points of too much production of lactic acid and rapid pH reduction.

Acknowledgments

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