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Effect of Organic Germanium, Oligosaccharide and Starters on Fermentation of Fresh *Kimchi* Juice

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김치즙액의 발효에 미치는 유기게르마늄, 올리고당 및 Starter의 영향

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Abstract

Changes in pH, titratable acidity, CO_2 production, reducing sugar, and lactic acid bacteria of fresh *kimchi* juice supplemented with combinations of 3 lactic acid bacteria and germanium(Ge)-132 or fructooligosaccharide(FO) during fermentation at 30℃ were investigated to assess the potential for extending the shelf life and enhancing the functional properties in *kimchi*. In *kimchi* juice containing Ge-132, sample(I) (inoculated with a mixture of bacteriocin-producing SNF-13 strain and E. faecium) exhibited that the amounts of organic acid and evolved CO_2 gas were lower than those of the other starter samples(Ⅱ-Ⅳ). The growth of lactic acid bacteria naturally present in *kimchi* juice, particularly *Lb. plantarum and Leu. mesentroides*, may be inhibited due to competition of the isolated SNF-13 strain and *E. faecium by Ge-132*. During fermentation of *kimchi* juice containing FO sugar, the contents of organic acid and evolved CO_2 gas in juice broth with 4 starters were predominantly higher than those of control and Ge-132 groups, and then the growth of lactic acid bacteria originated from *kimchi* ingredients was thought to be markedly accelerated. Our results indicated that functional properties like the extension of shelf life and increase of biological activity in *kimchi* were enhanced by adding Ge-132 and bacteriocin-producing lactic acid bacterium, which were resistant to organic acid and stimulated by Ge-132.

Key words : lactic acid bacteria, Ge-132, oligosaccharide, fresh *kimchi* juice, fermentation

Introduction

Kimchi is a traditional Korean fermented food. In Korea, it is probably the most widely consumed food together with fermented soy products and has also been noted for long time as one of the most indigenous foods fermented from vegetable. It contains various nutrients and bioactive compounds from minor ingredients including red pepper, garlic, green onion and ginger, and then a series of bacteriocin, enzymes, vitamins, volatile flavor compounds, lactic acid and lactic acid bacteria were produced by *kimchi*-related microorganisms from fermentation process(1). It was known that the antimutagenic/anticancer effects were more increased in the optimally ripened *kimchi* than the fresh *kimchi*(1,2). More recently many people of several countries including the United State of America as well as Japan

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are interested in facts that *kimchi* is good for health as a acid-fermented vegetable dish and has a considerable potential as a unique fermented food in world markets.

On the other hand, the fermentation period and quality of *kimchi* depend upon salt concentration and temperature as a main factors. The former reduces the aerobic putrefactive microorganisms and stimulate the growth of favorable lactic acid bacteria that are mainly responsible for *kimchi* fermentation. The latter plays an important factor in formation of desirable flavor and good taste in *kimchi*. The other important factors were a minor ingredients such as spices, seasonings, protein and carbohydrate materials. In *kimchi* microflora originated naturally from raw materials, *Leuconostoc mesenteroides* and *Lactobacillus plantarum* are the predominant microorganisms, followed by *Streptococcus faecalis, Pediococcus cerevisiae* and *Lb. brevis*. In the later stage, small number of yeasts and molds that can cause softening and off-odors in *kimchi* are present. During fermentation of *kimchi*, *Leu. mesenteroides* is grown from the initial to the intermediate stage and produce organic acids and CO_2 to impart refreshment and weak acid taste to *kimchi*. *Lb. plantarum*, an acid-tolerant bacteria responsible for acidification of *kimchi*, is predominated at later or final stage in *kimchi* fermentation and not inhibited relatively by most of natural antimicrobials occurring naturally in food as compared with the other lactic acid bacteria.

In recently years, although many studies have also been reported on the preservation of *kimchi*, there were still some problems like excess acidification, softening of cabbage texture and CO_2 inflation during storage of *kimchi*(3-6). In the *kimchi* industry, such problems usually come to light as a result of consumer's complaints and can involve substantial financial loss to the *kimchi* company. Therefore, the application of starter and natural biopreservatives for enlarging the preservation period in fermented food such as *kimchi* and milk has been receiving more and more attention(7-11). In addition, it is well known that oligosaccharide plays an important role in *Bifidus* growth factor, prevention of caries and diabetes(12-15). Ge-132(carboxyethylgermanium sesquioxide) is a novel organogermanium compound now being applied to the field of medicine and has extremely low toxicity by many toxicological and pharmacological studies(16,17).

In previous paper, we reported that Ge-132 stimulated the cell growth and production of viscouse material in some lactic acid bacteria(18). The purpose of this study is to investigate the possibility of extending shelf life and enhancing functional properties in *kimchi* throughout fermentation of fresh *kimchi* juice supplemented with combinations of 3 lactic acid bacteria and Ge-132 or fructooligosaccharide.

Materials and Methods

Materials

For preparation of baechu(oriental cabbage) *kimchi*, baechu and minor ingredients including red pepper powder, garlic, ginger, green onion and salt were purchased from local market in Sunchon. Ge-132 powder was obtained from Asahi Germanium Research Institute, Tokyo, Japan. It was dissolved to 1%(w/v) in deionized water containing 10M NaOH(pH 8.0) and adjusted to pH 6.5 with 10M HCl. Fructooligosaccharide (purity 58%) was purchased from Jeil Jedang company in Korea.

Preparation of fresh *kimchi* juice

The baechu was cut, brined with saturated salt solution for 1hr, rinsed with fresh tap water and drained. The salting baechu(100g) was mixed with basic ingredients of red pepper powder(2%), chopped green onion(2%), crushed garlic(1%) and crushed ginger(1%). The final salt content in the *kimchi* was adjusted to about 2.5%(w/w) with the same salt. The mixture was homogenized and immediately filtered with sterilized gauze. The liquid was centrifuged at 6,000 rpm at 2℃ for 10min, and then the supernatant fluid was used as a fresh *kimchi* juice broth just after centrifuging.

Fermentation of fresh *kimchi* juice

Fresh *kimchi* juice broth (50ml) was taken into 100ml Erlnmeyer flask. Ge-132 and fructooligosaccharide solutions were added into the flask in the concentration of 0.5%(w/v) and 3%(w/v), respectively. Tested cultures (3 strains) were paired in dual or triple combinations: sample (I), SNF + *Enterococcus faecium* ; (II), SNF + *S. facalis* ; (III), *E. faecium* + *S. faecalis* ; (IV), SNF + *E. faecium* + *S. faecalis*. The test flasks inoculated with 0.2%(v/v) starter samples(1×108cells/ml) were incubated at 30℃ for 48h. *Kimchi* juice broths were taken and analyzed for microbiological quality and some sensory components as fermentation indicators of *kimchi.*

Starter and microbial analysis

Strains used in this study were obtained from Korea Fermented Food Research Institute(KOFRI) and National Food Research Institute(NFRI) of Japan. Lactic acid bacteria included *Enterococcus faecium*(JCM 5804), *Streptococcus faecalis*(ATCC 2580) and the isolated SNF-13 strain having a bacteriocin-producing ability(19). A loopful of cell from litmus milk culture broth stored in cold chamber was transferred to 5ml of MRS broth. These organisms were incubated at 30℃ and maintained by daily transfer in MRS broth(Difco, Detroit, USA). After cultivation in MRS broth at 30℃ for 24h, the cells were harvested by centrifuging at 6,000rpm for 10 min and harvested cells were suspended in distilled water at 1×10^{8}cells/ml. Each starter samples was mixed to the same ratio in the cell number of lactic acid bacteria. Cell viability of lactic acid bacteria during fermentation was determined by the plate dilution method using MRS agar plate. Serial diluted solutions of each culture broths were plated in duplicate and were incubated at 30℃ for 36-48h. Results were expressed as colony forming units(logCFU/ml).

Determination of pH and titratable acidity

The culture broths from each sample were taken into small beaker. The pH was measured using a pH meter (Fisher Accumet, USA). Titratable acidity expressed as lactic acid percentage was measured with 0.1 N NaOH as standard solution.

Determination of gas production

The amount of CO_2 gas evolved in a flask was measured by trapping gas in a mass cylinder placed reversely placed in water bath of incubator and was expressed as ratio of amount(ml) of CO_2 of tested juice broths to amount(ml) of CO_2 generated when incubated *kimchi* juice alone at 30℃ for 24h.

Determination of reducing sugar

To analyze the contents of residual reducing sugar, *kimchi* juice broths were filtered and diluted with distilled water. Reducing sugars were determined by the 3,5-dinitrosalicylic acid(DNS) method according to the procedure of Miller using glucose as a standard(20).

Results and discussion

Changes in pH of fresh *kimchi* juice supplemented with starters, Ge-132, oligosaccharide and starters during fermentation

To obtain basic data for extending the shelf life of *kimchi*, the changes of pH in fresh *kimchi* juice were investigated when incubated juice broths supplemented with combinations of 3 lactic acid bacteria, Ge-132 and oligosaccharide at 30℃ for 48h(Table 1).

Table 1. Changes in pH of fresh *kimchi* juice supplemented with combinations of 3 strains and Ge-132, oligosaccharide and starters during fermentation

Starters	Control		Ge-132		OS	
	24h	48h	24h	48h	24h	48h
Juice	5.01	4.20	4.94	4.17	4.74	3.79
(Ⅰ) SNF+EF	4.83	4.54	4.91	4.72	4.69	4.31
(Ⅱ) SNF+SC	4.71	4.43	4.82	4.68	4.51	4.18
(Ⅲ) EF+SC	4.57	4.05	4.68	4.36	4.21	3.77
(Ⅳ) SNF+EF+SC	4.66	4.29	4.74	4.53	4.32	3.95

Cultures were grown at 30℃ for 48h in fresh *kimchi* juice containing 0.5%(w/v) Ge-132 and 3%(w/v) fructooligosaccharide. Each value represents the average of triplicate measurements. Abbreviations: SNF, bacteriocin-producing lactic acid bacteria isolated from Dolsan leaf mustard *kimchi*: Enterococcus faecium : SC, Streptococcus faecalis.

In the cultivation of control group(no addition of Ge-132 and oligosaccharide) for 48h, the pH value in starter(I) composed of SNF-EF pair was shown to be higher than that in the other mixture starters as well as juice broth alone, indicating that the isolated SNF-13 strain, which was isolated from Dolsan leaf mustard *kimchi* and pro어ced the proteinous bacteriocin, inhibited the lactic acid bacteria such as *Lb. plantarum and Leu. mesenteroides.* that are of great importance in the fermentation of *kimchi.* However, pH values of juice broth with various starters during fermentation of 24h were lower than that of *kimchi* juice broth alone due to growth of starters and little production of bacteriocin produced from SNF-13.

When Ge-132 was added to fresh *kimchi* juice, pH value in juice broth with starter (I) cultivated for 48h was higher than that of control group because of stimulant growth of SNF strain and *E. faecium* by Ge-132. Perhaps it seemed that these strains inhibited the lactic acid bacteria such as *Lb. plantarum* and the other lactic acid-producing bacteria present at medium or late stage of *kimchi* fermentation. However, change of pH in juice broth without starter was not affected by addition of Ge-132. In previous paper, *E. faecium* could grow to a high degree(about 2 times) by addition of Ge-132 (10mg/ml) on GPYS medium. the growth of *Leu. mesenteroides* and *P. pentosaceus* were inhibited in the presence of 10mg/ml Ge-132. In *Lb. plantarum*, it was not affected by Ge-132(18).

In contrast to Ge-132, it was thought that fructooligosaccharide stimulated the growth of lactic acid bacteria present naturally present in *kimchi* ingredients and all the starter strains tested. Among combination of starters tested, starter (I) was shown to be the most inhibitory effect against acid- producing bacteria like *Lb. plantarum* throughout the slow pH decline of *kimchi* juice broth during fermentation of 48h. In the other studies, Lee and Kim(29) and Choi et al.(18) reported that pH value was lower by around 3.0 during fermentation of baechu *kimchi* supplemented with several starter cultures. Our results indicated that pH decline of juice by adding Ge-132 and SNF-EF starter pair was more inhibited than that of the other studies.

Changes in titratable acidity of fresh *kimchi* juice supplemented with starters, Ge-132, oligosaccharide and starters during fermentation

Titratable acidity and pH were usually used as simple indicators of *kimchi* quality during fermentation. To obtain basic data for extending the shelf life of *kimchi*, when incubated juice broths with combinations of 3 lactic acid bacteria and Ge-132 or oligosaccahride at 30℃ for 48h, the changes of titratable acidity in fresh *kimchi* juice were investigated(Table 2).

In the cultivation of control group for 48h, the acidity values in starter(I) composed of 'SNF-EF' pair was shown to be lower than that in the other mixture starter as well as juice broth alone. Perhaps the isolated SNF-13 strain, a acid-tolerant and bacteriocin-producing lactic acid bacteria, was thought to play an important role in inhibition of lactic acid bacteria originated from raw materials during fermentation of *kimchi* juice broth. However, acidity values of juice broth with various starters during fermentation of 24h were higher than that of *kimchi* juice broth alone due to growth of starters and little production of bacteriocin produced from SNF-13 strains.

Table 2. Changes in titratable acidity of fresh *kimchi* juice supplemented with Ge-132, oligosaccharide and starters during fermentation

Starters	Control		Ge-132		OS	
	24h	48h	24h	48h	24h	48h
Juice	0.42	0.68	0.40	0.74	0.56	0.87
(Ⅰ) SNF+EF	0.49	0.58	0.45	0.52	0.61	0.72
(Ⅱ) SNF+SC	0.51	0.64	0.47	0.57	0.69	0.77
(Ⅲ) EF+SC	0.62	0.74	0.56	0.64	0.73	0.94
(Ⅳ) SNF+EF+SC	0.55	0.66	0.51	0.60	0.71	0.79

Each value represents the average of triplicate measurements. See the legend in Table 1 for the strain combinations of each sample(I~IV).

When Ge-132 was added to fresh kimch juice with starter(Ⅰ), acidity value in juice broth cultivated for 48th was lower than that of control group because of stimulant growth of SNF strain and *E. faecium* by Ge-132. However, changes of titratable acidity in juice broth without starter was not affected by addition of Ge-132. In contrast to Ge-132, fructooligosaccharide stimulated the growth of lactic acid bacteria present naturally present in *kimchi* ingredients and all the starter strains tested. Among combinations of starters tested, starter (I) was shown to be the most inhibitory effect on titratable acidity of juice broth during fermentation of 48h.

Changes in CO_2 gas of fresh *kimchi* juice supplemented with Ge-132, oligosaccharide and starters during fermentation

The initial microflora of *kimchi* consists of a wide rage of microorganisms, but the increase of acidity and evolved CO_2 gas affect the microbial ecology and quality of *kimchi*. Therefore, the evolved CO_2 was important for the formation of anaerobic state and stability of storage in *kimchi*. The CO_2 evolution in starter(I) composed of SNF-EF pair with no addition of Ge-132 and oligosaccharide was shown to be lower than that in the other mixture starters as well as juice broth alone(Table 3), indicating that the isolated SNF-13 strain, which produce the proteinous bacteriocin, inhibited the heterofermentative lactic acid bacteria like *Leu. mesenteroides* and a few *kimchi*-fermenting bacteria. Carbon dioxide in *kimchi* fermentation was mainly produced by *Leu. mesenteroides*, which is relatively sensitive against salt and lactic acid as a heterofermentative lactic acid bacterium, start to decrease in around pH 4.5 and rapidly disappeared in *kimchi* juice below pH 4.0(1-3). The production of CO_2 gas and growth of this strain is performed in lower temperature as compared of the other lactic acid bacteria in *kimchi* fermentation. To extend shelf life of *kimchi* during storage, the evolved CO_2 gas must be maximally decreased because the dissolved CO_2 gas contributes to the desirable fresh carbonated taste. Therefore, it has been known that more acceptable *kimchi* may be prepared by fermentation with lower salt concentrations at lower temperatures for longer times(1).

Table 3. Changes in CO_2 production of fresh *kimchi* juice supplemented with of Ge132, oligosaccharide and starters during fermentation

Starters	Control		Ge-132		OS	
	24h	48h	24h	48h	24h	48h
Juice	1.00	1.65	0.94	1.33	1.35	1.93
(I) SNF+EF	0.81	1.02	0.62	0.79	0.88	1.07
(Ⅱ) SNF+SC	0.85	1.18	0.78	0.87	0.92	1.24
(Ⅲ) EF+SC	0.96	1.31	0.87	1.12	1.11	1.47
(Ⅳ) SNF+EF+SC	0.92	1.25	0.73	0.84	0.96	1.36

Each value represents the average of triplicate measurements. See the legend in Table 1 for the strain combinations of each sample(I~IV). Gas production was expressed as ratio of amount(ml) of CO_2 of tested juice broths to amount(ml) of CO_2 generated when incubated *kimchi* juice alone at 30℃ for 24h.

Changes in reducing sugar of fresh *kimchi* juice supplemented with Ge-132, oligosaccharide and starters during fermentation

The contents of residual reducing sugar in starters of control and Ge-132 groups were decreased by approximately 1.7% during fermentation of 24h and then remained at between 1.2% and 1.8% throughout fermentation of 48h(Table 4). In Ge-132 group, the addition of starters rapidly reduced the content of reducing sugar during fermentation. For reducing sugar, there were obvious differences between *kimchi* juice broth added with and without Ge-132 whereas samples added with fructooligosaccharide had significantly higher content throughout fermentation. Addition of fructooligosaccharide stimulated a increase in the contents of organic acid during fermentation. The utilization degree of fructooligosaccharide increased with fermentation time in all samples; the rate of increase was faster at starter(III) than at starter(I).

Table 4. Changes in reducing sugar of fresh *kimchi* juice supplemented with Ge-132, oligosaccharide and starters during fermentation

Starters	Control		Ge-132		OS	
	24h	48h	24h	48h	24h	48h
Juice	2.24	1.52	2.08	1.29	2.91	2.04
(I) SNF+EF	2.02	1.77	1.78	1.37	2.77	2.42
(Ⅱ) SNF+SC	1.96	1.63	1.83	1.46	2.59	2.30
(Ⅲ) EF+SC	1.78	1.36	1.61	1.21	2.38	1.89
(Ⅳ) SNF+EF+SC	1.85	1.49	1.72	1.33	2.46	2.29

Each value represents the average of triplicate measurements. See the legend in Table 1 for the strain combinations of each sample(I~IV).

Kang et al. reported that when fructooligosaccharide was added during the preparation of *kimchi* at 25℃ as a beneficial ingredient, mannitol responsible for cooling taste as well as sweet taste was significantly increased(9). In our results, the utilization of fuctooligosccharide by *E. faecium*, were similar to those reported by Oyarzabal and Conner(21)

Table 5. Changes in lactic acid bacteria of fresh *kimchi* juice supplemented with Ge-132 oligosaccharide and starters during fermentation

Starters	Control		Ge-132		OS	
	24h	48h	24h	48h	24h	48h
Juice	6.36	7.66	6.33	7.72	7.04	8.95
(I) SNF+EF	6.43	7.42	7.47	8.33	7.68	8.49
(Ⅱ) SNF+SC	6.52	7.55	7.32	8.12	7.76	8.55
(Ⅲ) EF+SC	6.62	7.89	7.65	8.68	8.23	9.35
(Ⅳ) SNF+EF+SC	6.56	7.62	7.39	8.16	7.81	8.74

Each value represents the average of triplicate measurements. See the legend in Table 1 for the strain combinations of each sample(I~IV).

Changes in lactic acid bacteria of fresh *kimchi* juice supplemented with Ge-132, oligosaccharide and starters during fermentation

Table 5 shows the relationship between growth of lactic acid bacteria and Ge-132 or fructooligosaccharide during fermentation of fresh *kimchi* juice broth supplemented with 4 starter cultures. Among each group tested except for Ge-132, the number of lactic acid bacteria in juice broth supplemented with starter(I) were lower than that of the other starters during fermentation of 48h. However, in the case of Ge-132 group, *kimchi* juice broths(starter I, III) supplemented with *E. faecium* starter contained a large number of lactic acid bacteria during fermentation of 48h. This results suggested that Ge-132 stimulated the growth of a bacteriocin-producing SNF strain and a ropy *E. faecium*(18,19). Furthermore, it seemed that inhibition of acidity and CO_2 were affected that increase is affected by the competition of stater strains for the available microbial nutrients. In particular, fermentation rate of fresh *kimchi* juice tended to be significantly affected by SNF strain and *E. faecium* by Ge-132.

Based on the results of this study, it may be concluded that combination of Ge-132 and starters has the potential for extending the shelf-life of *kimchi* during storage, even though Ge-132 used for *kimchi* preparation can not still be acceptable to the consumer. Perhaps if the cost of Ge-132 will be going down, it may be used as a functional mineral of *kimchi* preparation. Our future studies will be focused on clarifying the effects of Ge-132 and various oligosaccharide on the competition of *kimchi*-fermenting microorganisms with and without individual or combination of some lactic acid bacteria, which are acid or salt tolerant and produced a bacteriocin having a potent antibacterial activity for *Lb. plantarum* and *Leu. mesenteroides*.

요 약

김치의 저장성과 기증성 향상을 위한 기초 연구로서, 유기게르마늄(Ge-132)과 올리고당(프럭토)을 함유하는 배추김치의 생즙액에 3가지 젖산균으로 조합한 스타트를 첨가하여 30℃에서 발효시키면서, pH, 산도, CO_2생성량, 환원당 및 젖산균수의 변화를 조사하였다. 게르마늄 첨가구는 박테리오신을 생산하는 SNF-13 분리 젖산균주와 E. faecium의 혼합배양(I)에서 유기산과 CO_2 생성량이 가장 적었으며, 스타트유래의 젖산균수 증식을 촉진시켰고, 환원당 소비량은 가장 많았다. 올리고당 첨가구는 4가지의 혼합배양에서 모두 각각의 대조구 및 게르마늄 첨가구보다 훨씬 유기산 및 CO_2 생성량이 많았으며, 김치유래의 젖산균수 증식을 촉진시켰다. 유기게르마늄은 박테리오신을 생산하거나 게르마늄 저농도에서 생육이 촉진되는 내산성의 유산균과 혼합병용하면, 김치의 기능성 증진(저장성, 생리활성)의 미네랄로서 활용가능성이 높을 것으로 판단된다.

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