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Changes of Free Sugars in *Kimchi* during Fermentation

Jae-Ho Ha, Wooderck S. Hawer, Young-Jin Kim and Young-Jung Nam
Korea Food Research Institute, Banwol, Kyonggi

Abstract

The changes of free sugars in *Kimchi* during fermentation were analyzed by GC. The major sugars in *Kimchi* were mannose, fructose, glucose, and galactose and they were reduced gradually with fermentation, whereas mannitol appeared in the middle stage of fermentation and reduced slowly. The presence of mannitol in *Kimchi* was identified by GC and GC/MS for the first time. Most of free sugars were stemmed from chinese cabbage and radish, and reduced with fermentation. These patterns of change of free sugars were almost the same in *Kimchi*. It could be concluded that regardless of kinds of *Kimchi* the fermentation mechanism of *Kimchi* was very similar on the basis of the changes of free sugars.

Key words: *Kimchi*, free sugars, mannitol

Introduction

Kimchis are a group of fermented pickled vegetables in Korea, originating at least three thousand years ago. Chinese cabbage or radish is generally used as a source vegetable for *Kimchi*, adding various ingredients such as red pepper, garlic, onion, ginger and jeotkal (salted and fermented seafoods)[1]. It has been known that complicated mechanisms are involved in fermenting *Kimchi*, producing lots of organic acids. it was reported that after the fermenting of *Kimchi*, in certain cases, yeasts and moulds made it unfit for consumption by softening the texture and imparting undesirable odour[2].

Many researches have been carried out on the changing patterns of microflora involved in fermentation of *Kimchi* and the taste and odour produced during fermentation[3,4,5]. Major microflora isolated from *Kimchi* were *Lactobacillus plantarum, Lactobacillus brevis, Streptococcus faecalis, Leuconostoc mesenteroids, Pediococcus cerevisiae* as anaerobic, and *Achromobacter, Flavobacterium,* and *Pseudomonas* species as aerobic. In the meantime, major taste compounds were also reported, including free amino acids, organic acids, carbon dioxide. However, a few systematic studies have been done on the free sugars exuded from *Kimchi*, which may play an important role as a taste compound[6,7,8].

It is believed that only a few studies have been done on the free sugars exuded from *Kimchi* because no successful analyzing method has been developed up to date. The liquid chromatographic method applied to other kinds of foods has been tried with unsatisfactory results due to not only poor detector response but also the inhibition of the polar compounds such as organic acids exuded from cabbage, radish as well as various ingredients added[8]. In the present study, *Kimchis* without ingredients as well as with ingredients were prepared to estimate the changes of free sugars in them and the effect of ingredients on the changes of free sugars. The free sugars in *Kimchis* during fermentation were analyzed and determined with gas chromatographic methods.

Corresponding author; Jae-Ho Ha, Korea Food Research Institute, 148-1 Dangsu, Banwol, Whasung, Kyonggi 445-820

Materials and Methods

Preparation and fermentation of *Kimchi*

The outer layer of chinese cabbage was removed, perpendicularly cut into 4 pieces, salted with 2 volumes of 15% brine for 24 hours, and washed twice with city water. After draining, final salt concentration was adjusted to 2.5% by adding re-

quired amout of salt. The concentration of salt was determined by the Mohr method[9]. *Kimchi* was put into plastic can (21 cm in diameter, 15 cm in height) and fermented in room temperature up to analysis. In order to compare the effect of ingredients on the change of free sugars, the other minor ingredients were added and mixed. The minor ingredients were blended to paste before addition. *Kimchi* contained chinese cabbage 100g, green onion 1.5g, garlic 1.5g, red pepper 1.5g. Final salt concentration was adjusted to 2.5% by adding required amount of salt. The radish was washed with city water, cut into small pieces (2.5 cm × 2.5 cm), and fermented with 2% brine in plastic can at room temperature.

Sample preparation for free sugars

Fifty grams of cabbage *Kimchi* was weighed, homogenized with 50 m*l* of distilled water, and centrifuged at 5,000 × g for 10 minutes. The supernant was taken and the residue was treated twice as the same procedure described as above. All the supernants obtained were combined to make up 200 m*l* with distilled water, and 100 m*l* was taken to evaporate to about 20 m*l* at 70 °C under the reduced pressure. The concentrated samples were frozen in a freezer at -20 °C and freeze-dried with a freeze-drier (Korea Food Research Institute made). Fifty grams of radish solid was weighed and 50 m*l* of radish liquid was taken to folow the similar procedure to cabbage *Kimchi*.

Derivatization of samples

One hundred milligrams of freeze-dried sample was weighed into 2 m*l* of a disposable centrifuge tube. 300 μl of pyridine containing methyl stearate as an internal standard, 270 μl of hexamethyldisilizane (HMDS), and 30 μl of trifluroacetic acid(TFA) were added and reacted in a oven at 40 °C for 30 minutes to make trimethylsilyl derivatives of sugars. The standard sugars were treated in the same way as samples in order to identify and quantify.

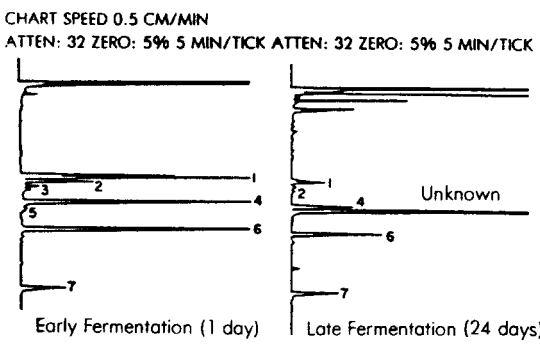

Fig. 1. GC chromatograms of free sugars in cabbage *Kimchi*.
1. Mannose, 2. Fructose, 3,5. Galactose, 4,6. Glucose, 7. Methyl Stearate (Internal standard)

Gas chromatography and mass spectroscopy

Varian VISTA capillary gas chromatograph was used to analyze sugars in *Kimchi* and BP-10 (0.33 mm × 30m) as a column. Initial oven temperature was 170 °C, held for 1 min, increased to 230 °C at the ratio of 5 °C per minute, and finally held for 8 minutes. Carrier gas was nitrogen at 12 *psi* and flame ionization detector(FID) was used. At this time injector temperature was adjusted to 300 °C, and detector to 320 °C. Shimadzu QP-1000 Gas chromatograph/Mass spectroscopy (GC/MS) was used to identify an unknown component with ion temperature at 270 °C; mass range, 30-440 m/e; EI voltage, 70 eV; separator temperature, 270 °C, and helium as carrier gas. The conditions of gas chromatograph and column used were the same as above.

Results and Discussions

Chromatograms of free sugars in cabbage *Kimchi* by GC were presented in Fig. 1. Free sugars present in the early stage of *Kimchi* were, for the most part, reducing sugars such as mannose, fructose, glucose, and galactose. However those in the late stage were a small amount of mannose, fructose, and glucose with little galactose. It was characteristic that in the late stage of fermentation an

Table 1. Changes in free sugars of cabbage *Kimchi* without ingredients during fermentation at 20°C for 24 days (mg/100g)

	Days								
	0	1	3	7	10	14	17	21	24
Mannose	5.2	4.7	3.6	1.5	1.1	1.1	1.1	1.1	0.6
Fructose	3.1	1.3	0.8	0.7	0.4	0.3	0.2	0.2	0.1
Galactose	0.8	0.5	0.3	0.3	0.0	0.0	0.0	—	—
Glucose	21.6	19.5	14.7	10.3	9.4	8.2	6.1	5.4	4.4
Unknown	—	—	1.2	1.8	5.5	9.2	9.5	10.5	9.5
Total	30.7	26.0	20.6	14.6	16.4	18.8	16.9	17.1	14.6

unknown peak appeared. The changes of each sugar in cabbage *Kimchi* during fermentation were shown in Table 1. The content of mannose was 4.7 mg/100g in the early stage (1 day) of fermentation and reduced sharply to 0.6 mg/100g at the late stage (24 days of fermentation). The same pattern was observed in the content of fructose, galactose and glucose as 1.3 mg/100g, 0.8 mg/100g, and 19.5 mg/100g after 1 day of fermentation and 0.1 mg/100g, trace and 4.4 mg/100g after 1 day of fermentation and 0.1 mg/100g, trace and 4.4 mg/100g after 24 days of fermentation respectively. These results showed that microorganisms present in *Kimchi* used reducing sugars in cabbage as energy sources.

On the other hand, the unknown peak appeared after 3 days of fermentation. The content of the unknown component was 1.2 mg/100g at the early stage (3 days of fermentation), reached to the maximum as 10.5 mg/100g after 24 days, and reduced slowly thereafter when the response of the unknown was calculated on the basis of that of glucose. In order to identify the unknown compound, freeze-dried free sugars of *Kimchi* was trimethylsilylated to inject into GC/MS and obtained the mass fragments as shown in Fig. 2. The possible candidate components were glucitol, galactitol, and mannitol. Thus, the standards of those sugar alcohol were trimethylsilylated to inject into GC/MS and the retention time of each trimethylsilylated sugar were compared. It could be concluded that the unknown component was man-

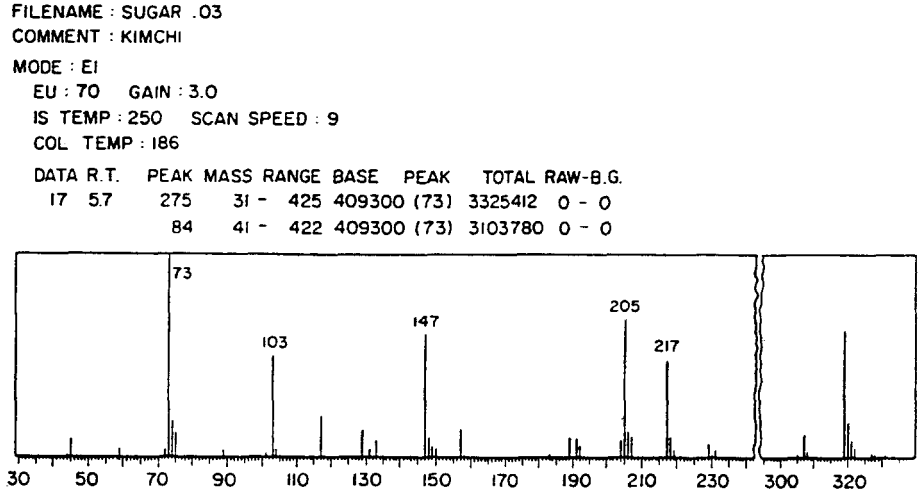

Fig. 2. Mass spectrum of the unknown component of free sugar in *Kimchi*.

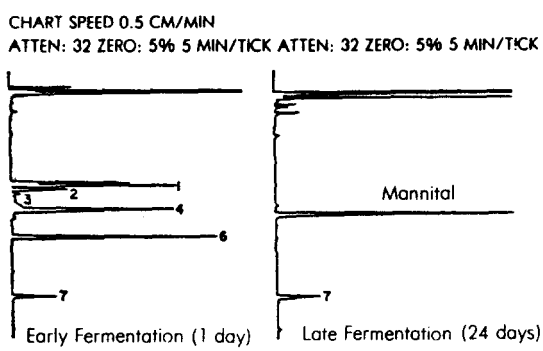

Fig. 3. GC chromatograms of free sugars in radish *Kimchi* solid.

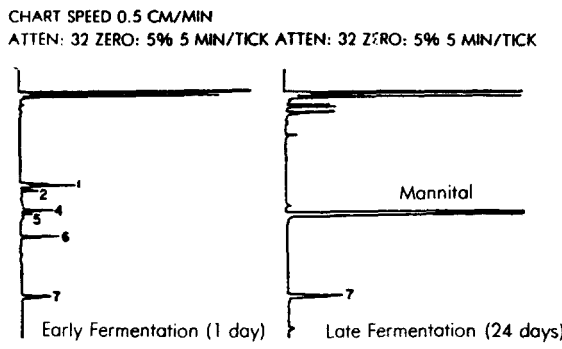

Fig. 4. GC chromatograms of free sugars in radish *Kimchi* juice.

nitol by GC and GC/MS with the conformation of coninjection technique.

GC chromatograms of free sugars in radish *Kimchi* solid at the early and late stage of fermentation were shown in Fig. 3 and that of radish *Kimchi* liquid in Fig. 4. The similar pattern of the contents in free sugars was observed in radish *Kimchi* solid and liquid at the early stage as well as at the late. However, a small amount of galactose was detected in 1 day of fermentation, while little in solid. As large amount of mannitol was appeared in both solid and liquid after 10 days, it seemed that mannitol might influence the taste of *Kimchi* at the ripened stage. The changes of free sugars in solid were presented in Table 2. While the contents of mannose, fructose, and glucose after 1 day of fermentation was 3.0 mg/100g, 1.1 mg/100g, and 10.6 mg/100g, respectively, mannose disappeared after 17 days and glucose after 21 days. Mannitol, which appeared after 3 days, reached the maximum of 18.9 mg/100g, and then reduced slowly to 10.9 mg/100g after 24 days. It was observed that the period of the maximum contents of total free sugars was corresponded to that of mannitol. It has been known that mannose yield mannitol and that such reductions can be carried out by enzymes[10]. At the same time, fructose also can yield mannitol throught hetero fermentation[11]. But the detailed mechanisms and reasons for mannitol present in fermentated *Kimchi* are not discussed here and further researches on the mechanisms will be continued in our laboratories.

Table 3 showed the changes of free sugars in radish *Kimchi* liquid and the similar pattern to solid was observed. All the sugars except glucose and mannitol were disappeared after 10 days, so little was detected after 14 days. On the other hand, lots of free sugars exuded from radish solid so that abundant sugars was present in liquid after 3 days of fermentation. Mannitol reached the

Table 2. Changes in free sugars in radish *Kimchi* soid without ingredients during fermentation at 20°C for 24 days (mg/100g)

	Days								
	0	1	3	7	10	14	17	21	24
Mannose	4.0	3.0	2.6	1.0	0.8	0.0	0.1	—	—
Fructose	2.7	1.1	0.5	0.1	0.1	—	—	—	—
Glucose	13.4	10.6	7.7	4.5	2.4	1.1	0.7	0.3	0.1
Mannitol	—	—	7.3	18.9	17.7	16.5	14.7	10.5	10.9
Total	20.1	14.7	18.1	24.5	20.7	17.6	15.5	10.8	11.0

Table 3. Changes in free sugars in radish *Kimchi* juice without ingredients during fermentation at 20°C for 24 days (mg/100g)

	Days								
	0	1	3	7	10	14	17	21	24
Mannose	0.1	1.7	4.3	1.3	0.2	—	—	—	—
Fructose	0.1	0.5	0.5	0.1	0.0	—	—	—	—
Galactose	—	0.3	0.2	0.1	0.0	—	—	—	—
Glucose	0.2	3.1	11.5	6.0	2.0	0.1	0.1	0.1	0.1
Mannitol	—	0.5	9.2	17.1	18.3	16.5	14.9	12.8	11.8
Total	0.4	6.1	25.7	24.6	20.5	16.6	15.0	12.9	11.9

Table 4. Changes of free sugars in *Kimchi* juice with ingredients during fermentation at 25°C for 4.5 days (mg/100g)

	Fermentation time (days)									
	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5
Mannose	0.5	0.9	0.8	0.7	0.6	0.2	0.2	0.1	0.1	—
Frutose	0.5	0.4	0.4	0.3	0.2	0.1	0.9	—	—	—
Galactose	0.3	0.3	0.2	0.2	0.1	—	—	—	—	—
Glucose	3.2	2.8	2.7	2.6	2.5	2.1	1.8	1.4	1.3	1.1
Mannitol	—	—	—	5.1	7.9	5.9	5.4	3.7	2.7	2.5
Total	4.9	4.4	4.1	8.9	11.3	8.3	8.3	5.2	4.1	3.6

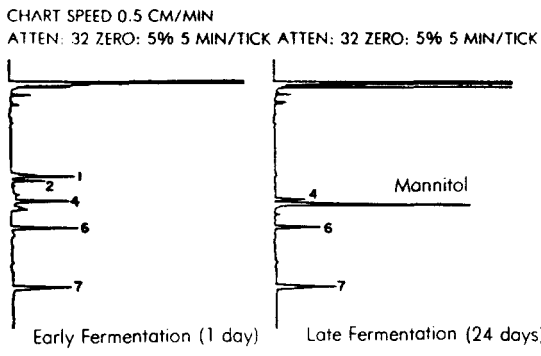

Fig. 5. GC chromatograms of free sugars in cabbage *Kimchi* liquid with ingredients.

maximum after 10 days, and reduced slowly thereafter. The total contents of sugars were principally affected by that of mannitol. The changes of free sugars in *Kimchi* with various ingredients (Fig. 5 and Table 4) showed the similar patterns to those of *Kimchi* prepared in the absence of ingredients. In other words, *Kimchi* made with chinese cabbage 100g, green onion 1.5g, garlic 1.5g, red pepper 1.5g in 2.3% brine contained mannose, fructose, glucose, galactose, and mannitol. The contents of sugars decreased with the fermentating periods. Mannitol in *Kimchi* with ingredients came into existance after about 1.5 days, reached to the maximum of 5.9 mg/100g after about 2 days, and then reduced slowly.

Therefore, it could be concluded that most free sugars were stemmed from cabbage and radish, and used as the energy source for microorganisms, whereas mannitol developed in the fermented stage might play an important role of *Kimchi* taste at the late stage. In addition, it seemed that the fermentation mechanism of *Kimchi* is almost the same regardless of kinds of *Kimchi*.

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김치숙성 중 유리당의 변화

하재호·허우덕·김영진·남영중
한국식품개발연구원 이화학연구실

김치숙성 중 유리당의 변화를 GC로서 분석하였다. 김치에 존재하는 주요 유리당은 mannose, fructose, glucose, galactose 등이었고 이들은 숙성이 진행됨에 따라 점차 감소한 반면 mannitol은 숙성이 진행됨에 따라 생성되었다가 서서히 감소하였다. 여러종류의 김치 중 유리당의 변화를 비교한 결과 그 변화양상이 비슷하였으며 이로부터 대부분의 김치는 비슷한 발효기작을 따르는 것 같았다.