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Effect of Temperature on the Production of Free Organic Acids during Kimchi Fermentation

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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 α-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 acids 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 and the Kimchi fermented at low temperature produced more acetic acid than that fermented at high temperature.

There are many kinds of Kimchi (traditional Korean pickles) in Korea. The following factors determine what kind of Kimchi will be: the raw materials; the preparation method; the season in which it was made; and the area at which it was made. And the Kimchi which has gone through a proper fermentation gives the best taste. The sour taste of Kimchi is one of the important factors related to palatability, and the pH and the acidity of Kimchi at the optimum ripening time is 4.2 and 0.6%, respectively (11). The organic acids is Kimchi which impart the sour taste are formed either from microbial action or from raw materials.

There have been many reports concerning the organic acids in Kimchi. So far, those studies have been carried out on qualitative and quantitative detection of the acid compositions (1, 3-8, 11-13), changes in the production of the acids during fermentation (3, 8, 12, 13), and the effect of temperature (1, 6, 11), salt concentrations (8, 11) and raw materials (12) on the acid production. Among them, only few papers have described the changes in the production of organic acids in Kimchi during fermentation at certain temperatures (3, 8, 12, 13), and the difference in the acid production caused by different temperatures during fermentation have not been stated in detail. In this article, we report the effect of different temperatures on the production of free non-volatile and volatile organic acids during Kimchi fermentation.

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MATERIALS AND METHODS

Preparation of Kimchi

The Kimchi from our previous experiment (9) was fermented at 30, 20 and 5℃ for 8, 16 and 60 days, respectively.

Analysis of Non-volatile Acids

Gas chromatography (GC) was used for the analysis of non-volatile acids (2). One-hundred grams of samples were extracted with three 150 ml portions of methanol by blending and centrifugation (5,000×g, 15 min). The combined extract was concentrated with a rotary vacuum evaporator at 80℃. Two mililiters of 14% BF_3 methanol (boron trifluoride methanol complex, Sigma Co.) was added and the mixture was heated at 60℃ for 30 min for methylation. Saturated ammonium sulfate (4 ml) and chloroform (2 ml) were mixed and allowed to stand at room temperature. Then the chloroform layer was separated. After small amount of Na_2SO_4 was added to remove moisture, the obtained filtrate was used for the GC analysis. A gas chromatograph (GC-8A, Shimazu Corp.) with a flame ionization detector was used. The GC was performed with a glass column (3 m×3 mm) packed with 5% DEG and 1% H_3PO_4 on Chromosorb WAW 60/80 mesh. The temperatures of both the injector and the detector was 230℃ and column temperature was programmed to increase from 80℃ to 200℃ at 6℃/min. The N_2 carrier gas flow was 30 ml/min. The peak of each organic acid was identified by comparing the retention time of each with those from the standard mixture, and the non-volatile organic acid composition was measured by calculating the area of each peak and applying correlation factors obtained from chromatogram of known acids.

Analysis of Volatile Organic Acids

The method of Yamashita *et al.* (14) was used for the analysis of volatile organic acids.

Fifty grams of Kimchi sample was blended and distilled by steam. The distillate was neutralized by adding NaOH, and concentrated with a vacuum rotary evaporator at 45℃. For butylation, n-butanol (2 ml), conc. H_2SO_4 (0.2 ml) and anhydrous Na_2SO_4 (2 g) were added and the mixture was boiled for 30 min. After cooling, the esters were extracted with 5 ml of hexane three times. To remove the remaining water and H_2SO_4 in extract, a small amount of Na_2CO_3 was added, and the filtrate was used for the following GC analysis. The same gas chromatograph with glass column (1 m×3 mm) packed with silicon D.C. 550 on Chromosorb WAW 100/200 mesh was used. The temperature was programmed to increase from 60℃ to 220℃ at 6℃/min. The N_2 carrier gas flow was 30 ml/min. The peak of each acid was identified and the acid composition was measured by the same way as in the case of non-volatile acid analysis described above.

RESULTS AND DISCUSSION

The production of major organic acids at 30, 20 and 5℃ during fermentation is described in Fig. 1~3.

Non-volatile Organic Acids

The initial amount of each non-volatile organic acid in Kimchi, soon after preparation, was: malic acid, 64.4 mg%; citric acid, 55.5 mg%; tartaric acid, 46.3 mg%; pyroglutamic acid, 17.5 mg%; lactic acid, 10.6 mg%; oxalic acid, 10.2 mg%; succinic acid, 8.8 mg%; and α-ketoglutaric acid, 2.2 mg%. The patterns of changes in the amount of acid at each temperature were similar, while the production rate accelerated with an increase in the temperature.

The production of lactic acid gave the highest increase rate during the fermentation process. The amounts of malic, citric and tartaric acids increased successively after that of lactic acid. They reached their maximum after the optimum ripening time, and then decreased quickly. Succinic acid was also produced and then lessened. Oxalic acid increased moderately in its amount during the fermentation process. The amount of pyroglutamic acid decreased after a gradual increase, and fumaric acid was produced very slowly. Production of α-ketoglutaric, maleic and malonic acids increased only slightly, and

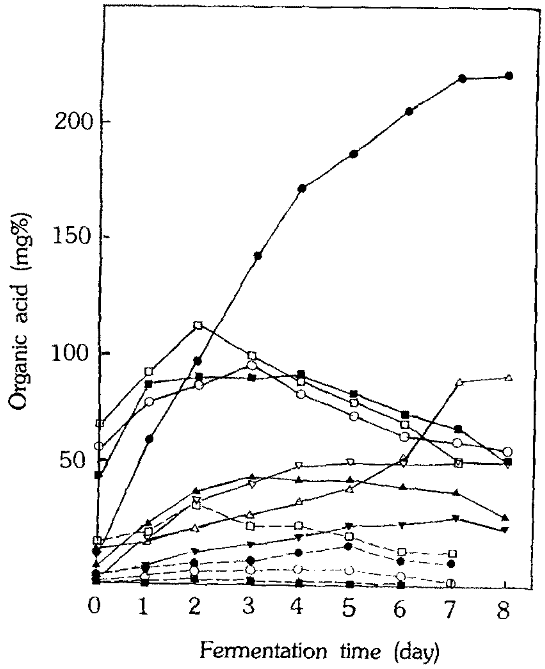

Fig. 1. Changes of the production of free organic acids during Kimchi fermentation at 30°C.
●–●, lactic acid; □–□, malic acid; ○–○, citric acid; ■–■, tartaric acid; ▲–▲, succinic acid; △–△, oxalic acid; □··□, pyroglutamic acid; ▼–▼, fumalic acid; ●··●, α-ketoglutaric acid; ○··○, maleic acid; ■··■, malonic acid; ▽–▽, acetic acid.

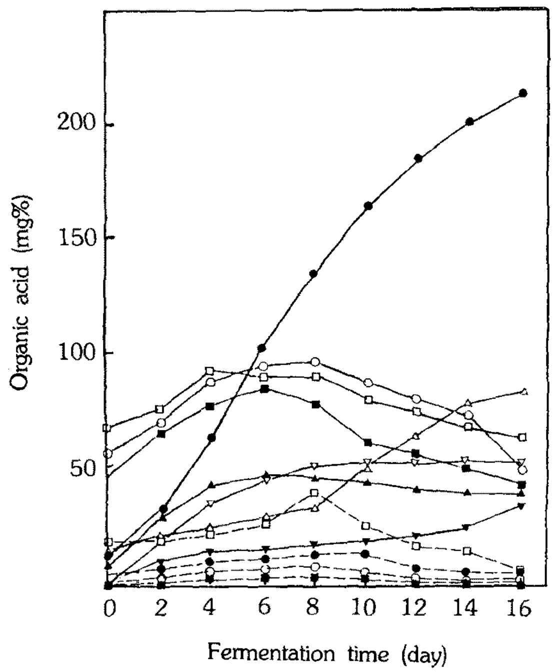

Fig. 2. Changes of the production of free organic acids during fermentation at 20℃.
Symbols are the same as in Fig. 1.

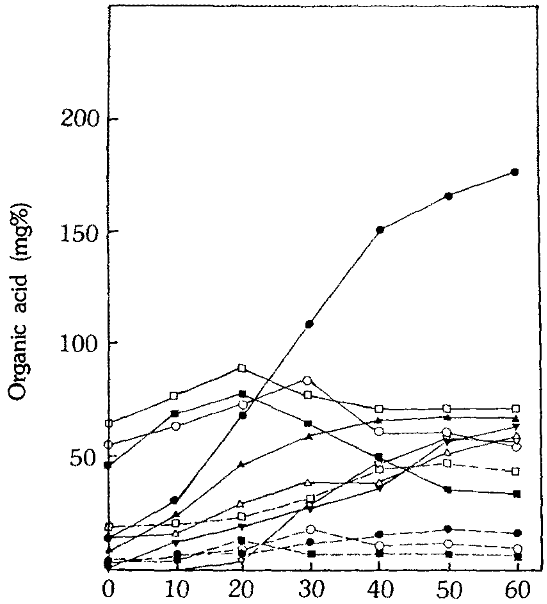

Fig. 3. Changes of the production of free organic acids during Kimchi fermentation at 50℃.
Symbols are the same as in Fig. 1.

then decreased. These patterns of changes were similar to those reported by Tsuyuki and Abe who described the changes in the production of organic acids in Kimchi prepared during winter time (13). But the pattern of the production of oxalic acid from our experiment differed significantly from their result in which the amount of oxalic acid reached its maxmum at the optimum ripening time.

The composition of major non-volatile acids at the optimum ripening time varied depending on the fermentation temperature. At 30℃, it was: malic acid, 93.9 mg%; tartaric acid, 85.3% mg%; citric acid, 77.3 mg%; lactic acid, 66.1 mg%; and succinic acid, 26.1 mg%. At 20℃, it was: malic acid, 91.8 mg%; citric acid, 89.1 mg%; tartaric acid, 74.2 mg%; lactic acid, 69.5 mg%; and succinic acid, 39.5 mg%. At 5℃, it was: lactic acid, 111.8 mg%; citric acid, 84.6 mg%; malic acid, 79.6 mg%; tartaric acid, 64.4 mg%; and succinic acid, 61.2 mg%. These results indicate that as the temperature is lowered, the production of lactic and succinic acids are increased to a great extent at the optimum ripening time, and that of oxalic, pyroglutamic and fumaric acids which are present in relatively small amount, are also increased moderately, whereas the production of malic and tartaric acids are reduced. The differences in the production of lactic, succinic, malic and tartaric acids at different temperatures were similar to the results given by Kim and Rhee (6), who reported the production of organic acide at 6~7℃ and 22~23℃. However, the production of oxalic and fumaric acids, were different from them.

Volatile Organic Acids

The amount of each volatile organic acid in Kimchi, soon after preparation, was: acetic acid, 0.5 mg%; butyric acid, 0.1 mg%; propionic and formic acids, <0.1 mg%. The pattern of the production of acids during fermentation at different temperatures was analogous, and it changes rapidly with a increase in the temperatures. Among the volatile acids, acetic acid was produced in an appreciable amount during fermentation and its production decreased to a small extent after over-ripening. Meanwhile, the production of other acids were increased very slightly.

The composition of volatile acid at the optimum ripening time varied depending on the fermentation temperature. At 30℃, it was: acetic acid, 18.6 mg%; and bytyric acid, 0.2 mg%. At 20℃, it was: acetic acid, 25.1 mg%; and butyric acid, 0.2 mg%. At 5℃, it was: acetic acid, 30.6 mg%; and butyric acid, 0.2 mg%. The production of acetic acid increased as the temperature was lowered, and this was comparable with the result made by Chyun and Rhee (1). This is assumed to be caused by dominance of *Leuconostoc mesenteroides* which grow well at lower temperature (9), and to be related with better taste of Kimchi prepared in winter time. Meanwhile, there was no apparent relationship between the temperature and other acids.

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