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D-amino acid profile would be varied depending on the storage time of fermented food

Although the natural amino acids are dominated by l-amino acids, various fermented foods are known to contain many d-amino acids. The enantiospecific modulation of amino acids might be caused by fermentation bacterial metabolism during manufacturing and storage processes. Particularly d-amino acids alternation might act important roles to construct the special food functions. The time-dependent alternation of d-amino acids profile have been thought as interested. Nevertheless, very few studies have been reported due to the lack of practical analytical methods. The present best method for enantioselective profiling of d,l-amino acid required several hundred minutes for one sample analysis. To elucidate d-amino acids contribution to food functions, easy handling high throughput analytical method are essential. Recently, a novel enantioselective d,l-amino acid profiling method has been developed by our group. The method using a combination of enantioselective column and liquid chromatogram- time of flight mass spectrometer (LC-TOFMS) requires only ten minutes for one sample analysis.

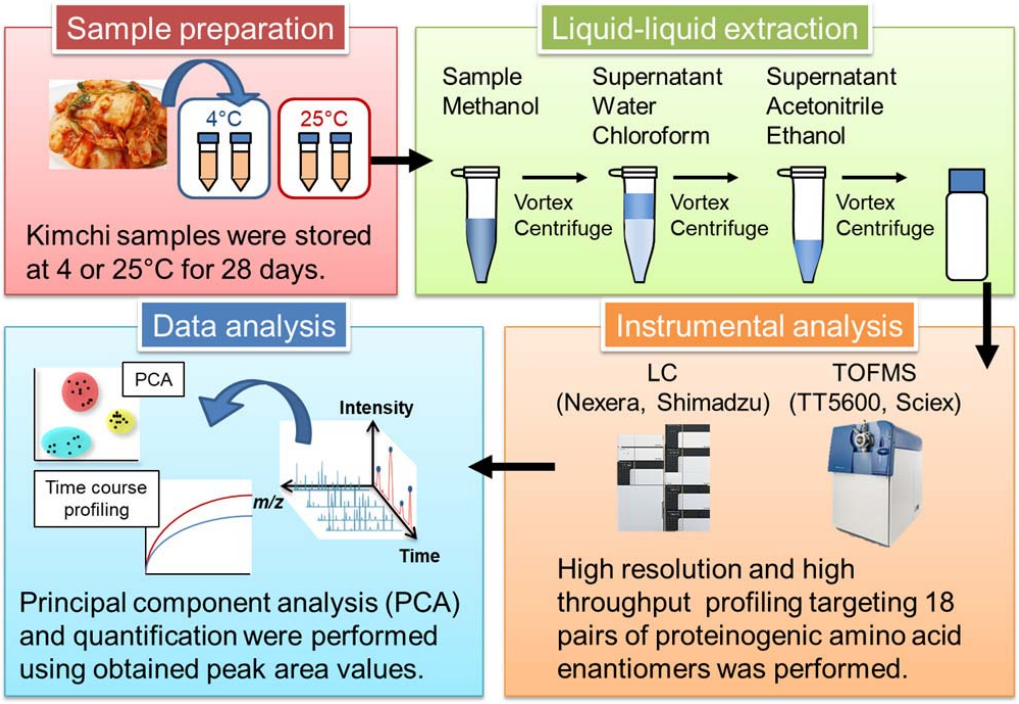

Fig. 1. Experimental work flow.

In this study, time-dependent d- and l- amino acid enantioselective alternation was investigated by our novel high throughput analytical method. Kimchi, a Korean traditional food made *via* fermentation of vegetables, was selected as a target fermented food. Previously, we revealed that kimchi contains comparatively various types and high amounts of d-amino acids. Since kimchi products contain living microorganisms, time-dependent alternation of d-amino acids profile during

storage of kimchi was expected. Commercially available kimchis were stored at 4 or 25°C, and the time-course samples along to the storage period were subjected to our developed analytical method and subsequent principle component analysis (PCA) (Fig. 1).

Consequently, twelve d-amino acids were detected from stored kimchi, and time-dependent alternation of d-amino acids profile in kimchi was successfully observed. PCA revealed d- and l-amino acids profile was drastically altered in kimchi at 25 °C along to the storage time. (Fig. 2 (A)). Moreover, as shown in the PCA loading plot (Fig. 2 (B)), many d-amino acids were plotted far from the origin, and these contributed to clustering of kimchis stored at 25°C in the score plot. The result of PCA demonstrated d-amino acids in kimchi stored at 25°C significantly increased during storage. In more detail, d-amino acids in kimchi collected at several sampling time points during storage were determined (Fig. 2 (C)). Consequently, concentrations of d-Ala and d-Glu dramatically increased to 1293.5 nmol/mL and 746.3 nmol/mL, respectively. Moreover, for several amino acid enantiomers, %D ratio (the abundance ratio of d-enantiomer to the total of d- and l-enantiomers) increased during storage at 25 °C. In particular, %D of d-Ala, d-Glu, and d-Arg reached 22.2%, 23.4%, and 34.3%, respectively. Concentrations of some d-amino acids (d-Ala, d-Glu and d-Arg) were found to increase during storage at 4 °C as well. However, they were very small changes compared with kimchi stored at 25 °C.

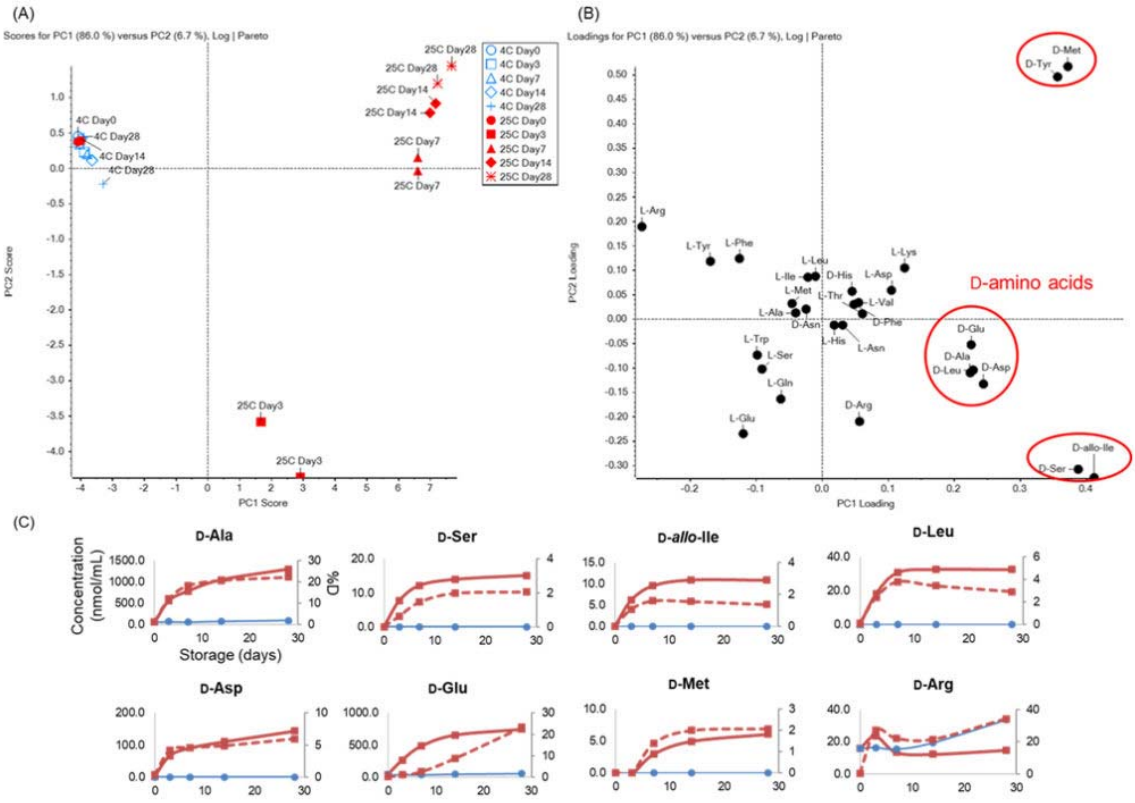

Fig. 2. Principle component analysis of D,L-amino acid profiles (A,B) and determination of D-amino acids in stored kimchi samples (C).

High resolution and high throughput screening targeting d-amino acids in a fermented food was successfully performed by our novel LC-TOFMS method. It can be expected to advance the food functional analysis of d-amino acids in future.

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Publication

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