DETERNIMATION OF ALDOSES IN BEEF SAMPLES BY APPLYING AN ALDONONITRILE ACETATE DERIVATIZATION METHOD

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I. INTRODUCTION

The aroma of beef is known to be caused by the perception of odor-active volatiles, which are formed during cooking and then released from beef during eating. In making process of corned beef products, a sterilization of sealed can contain beef and other ingredients is conducted with high-temperature and high-pressure steam. The heating treatment might contribute to generate its characteristic roast odor from beef. Our previous study showed that pyrazines, roast meat-like aroma, were suggested to be a candidate responsible for the aroma [1]. Pyrazines would be formed by an amino-carbonyl reaction between amino acids and sugars in beef, however, the underlying mechanisms of aroma generation in beef with heating have not been clarified enough. To date, a variety of analytical techniques have been employed to determine the monosaccharide composition. In metabolomics studies, metabolites are usually derivatized with an oximation reagent followed by silylation. One drawback of this method is the silylated derivatizes exist in anomeric forms, which leads to multiple peaks on GC/MS analysis. The aldononitrile acetate derivatization (AND) has been also used for a monosaccharide composition analysis from soil or plant samples [2,3]. The derivatized aldose produces a unique single peak in a GC/MS analysis. Therefore, in this study, we tried to quantify aldose component in beef sample by using AND approach.

II. MATERIALS AND METHODS

The AND was performed as previously described [2,3] with some modifications. Briefly, the standard monosaccharides were incubated with a derivatization reagent (30 mg/mL hydroxylamine hydrochloride in pyridine) at 60^{o}C for 30 min. Then, for acetylation of aldononitrile derivatives, acetic anhydride was subsequently added and incubated at 30^{o}C for 10 min. After the derivatization step, dichloromethane was added to extract the acetylated derivatives. The solution was washed with deionized water for two times. Finally, 1 µL of the dichloromethane layer was subjected to GC/MS analysis. Sirloin cuts of commercial Australian beef obtained from three individuals (unknown strains) were used. To extract aldoses in beef, the sample was mixed with a reagent (water:methanol: chloroform = 1:2.5:1, V/V) and incubated at 37^{o}C for 30 min. After centrifugation, the supernatants were freeze-dried. The derivatization method of beef sample was the same as described above. All experiments were performed in triplicate.

III. RESULTS AND DISCUSSION

We successfully identified 10 monosaccharides (ribose, ribitol, arabinose, xylose, xylitol, lyxose, allose, glucose, mannose and galactose) in a single GC/MS analysis (Figure 1). In this study, ribitol was added to each sample as internal standard and the standard curves of aldose were calculated. The curves of all analytes show good linearity within the concentration range of 1-20 µg/sample. In Figure 2, the representative curves of 3 monosaccharides (ribose, glucose and mannose) were summarized. Therefore, this analytical method used here might be sufficiently sensitive.

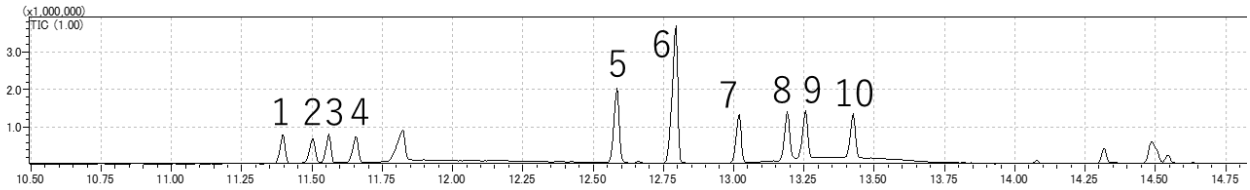

Figure 1. GC/MS chromatogram of aldononitrile acetate derivatized aldoses. Peak No.1: ribose, 2: lyxose, 3: arabinose, 4: xylose, 5: ribitol, 6: xylitol, 7: allose, 8: mannose, 9: glucose, 10: galactose.

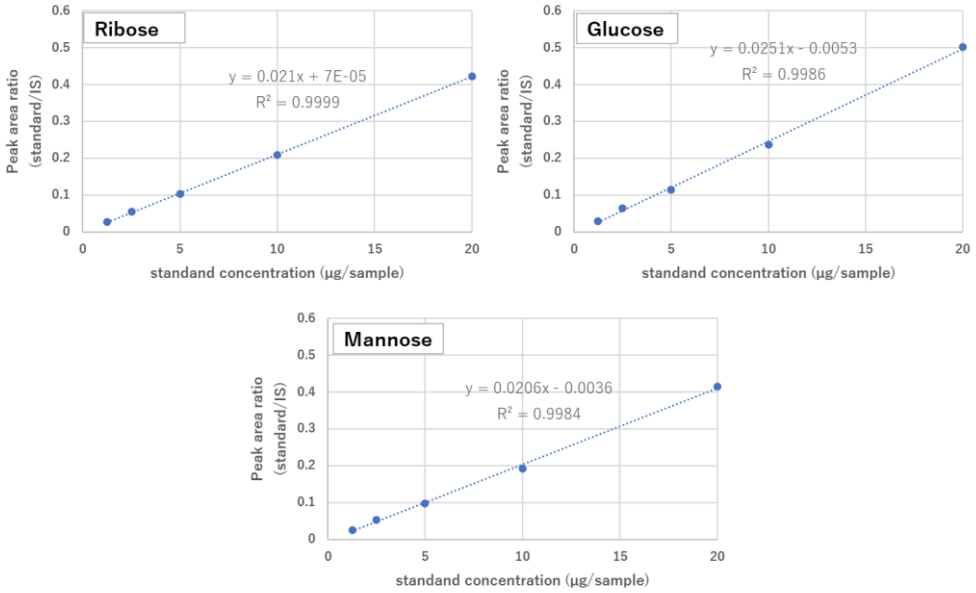

Figure 2. Standard curve of monosaccharides

Furthermore, the aldose concentrations of raw lean beef were measured by using AND. The beef sample contains 1.34 g/kg-beef of glucose, 0.38 g/kg of mannose and 0.17 g/kg of ribose. These results are similar to those of other report using a standard metabolomics approach [4].

IV. CONCLUSION

In the present study, our analytical methods using AND and GC/MS seems to be enough to quantify the concentration of aldoses in beef. We'll try to determine the aldoses in a heat-treated beef sample. Further studies are needed for clarifying which sugar compounds would be a candidate precursor of "retort beef aroma".

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