DEVELOPMENT OF CHICKEN MEAT-LIKE BROTH FOR MICROBIAL CULTIVATION

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I. INTRODUCTION

Chicken meat is popular and widely consumed due to its low cost, universal appeal, and nutritional value. However, it is highly perishable and can be easily contaminated by foodborne pathogens. Various methods have been applied to detect foodborne pathogens, including enrichment methods, polymerase chain reaction, enzyme immunochemical assay, and other techniques. In recent years, omics-based approaches have been increasingly utilized for rapid detection of foodborne pathogens [1]. Extracellular metabolites have shown potential as biomarkers for specific foodborne pathogens. However, bacterial metabolites can be strongly influenced by the environment [2], especially in complex food matrix such as meat. Therefore, it is important to investigate changes in the metabolites of foodborne pathogens in meat. While several nutritional broths and media have been developed for the growth of microorganisms, there is currently no broth that accurately mimics the composition of chicken meat. Additionally, sterilizing meat is a time-consuming and expensive process. Thus, the aim of this study was to develop a method for creating chicken meat-like broth (CMB) for the growth of microorganisms and verified its similarity to chicken meat in terms of metabolites.

II. MATERIALS AND METHODS

Fresh chicken breast was obtained from a local supermarket in Korea. The chicken extract was prepared by homogenizing chopped meat with distilled water (1:10, w/v) and filtering it using No.4 filter paper. The extract was sterilized by three methods: (1) F - filtering using 0.22 um syringe filter; (2) A - autoclaving at 121°C for 15 min; (3) B - boiling at 100°C; (4) FP - filtering using 0.22 um syringe filter and pasteurizing at 65°C for 25 min. The sterilized extracts were lyophilized and used to make CMB (56 g/L). The bacterial counts and stability of CMB was evaluated during incubation. The metabolites in CMB were extracted using methanol (1:3, v/v) [3] and non-targeted metabolomics analysis was conducted by liquid chromatography-mass spectrometry (LC-MS, Q-Exactive, Thermo Scientific, USA). The metabolomic similarity between CMB and chicken meat was validated using multivariate analysis by MetaboAnalyst. Finally, the growth of *Escherichia coli* and *Listeria monocytogenes* in CMB was observed.

III. RESULTS AND DISCUSSION

The stability of CMB was evlauted through microbial counts and delta optical density (OD) at 600 nm. The fresh chicken bresat meat had 3.28 Log CFU/mL of microbial counts. After sterilizing, microbial counts in all CMB by different sterilization were not detected (data not shown). During the incuabation at 37°C for 24 h, microbial counts in non-treated chicken meat were increased up to 8.03 Log CFU/mL whereas microbial growth was not detected in any CMB samples (A, B, FP, F). However, delta OD_{600} signifcanlty increased in C and F treatments, indicating that while microorganims did not grow in F treatment, it was chemically altered. Therfore, only A, B, and FP treatment were selected for validating the metabolomic similiarity.

To compare the metabolomics similarity of CMB, we used non-treated chicken meat (C) as the positive control and commercial broth such as Mueller Hinton Broth (MHB), Tryptic Soy Broth (TSB) as the negative controls. A total of 77 metabolites were identified from samples and 62 metabolites

were commonly detected in every samples. The partial least squares-discriminant analysis represented the 82.8% of total data, and the C and F treatment were clustered differently with A and B treatment or commercial broth (TSB and MHB) (Fig. 1a). Inosinic acid, carnosine, arabinonic acid, creatinine, and hydroxyproline were not detected in commercial broth and were higher in A and B treatment compared to C and F treatment (Fig. 1b). However, some small amino acids and di-peptides were highly detected in commercial broth. When compared to subgroup 1 (C and FP) and subgroup 2 (A and B), they were totally separated by orthogonal partial least squares discriminant analysis (data not shown), which was contributed by low some peptides and amino acids in subgroup 2 (data not shown). This result suggested that destruction due to heat treatment of A and B led to significant metabolomic difference compared to C. Finally, CMB (FP) was selected because of the highest metabolomic similarity of it, and its role as a broth for microbial cultivation was validated (data not shown).

(a) (b)

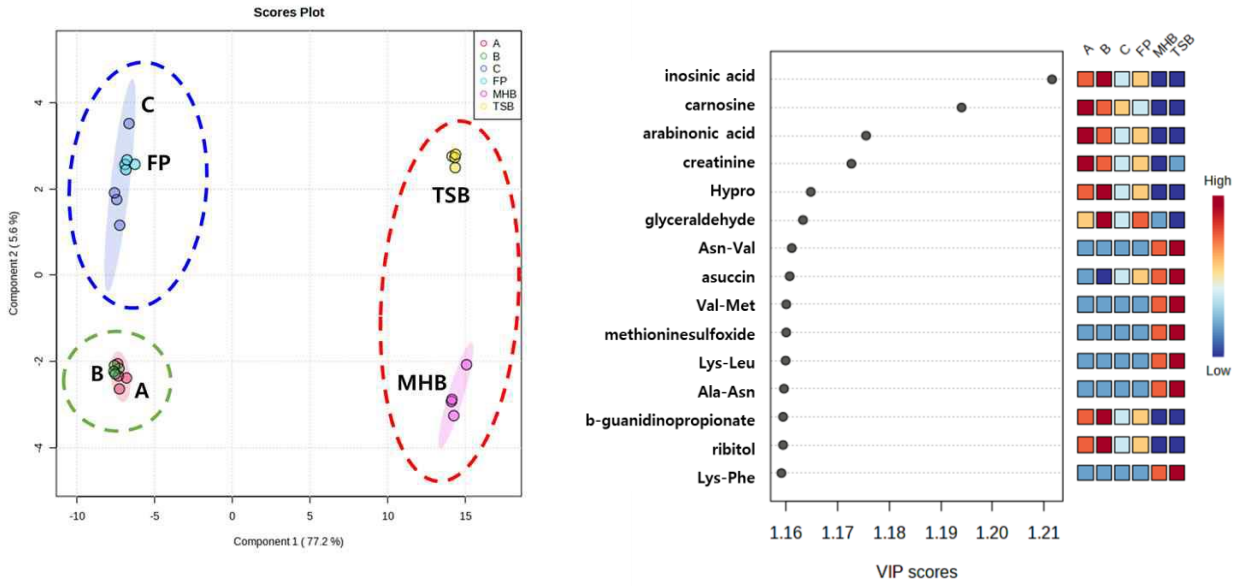

Figure 1. Multivariate analyses (a-b) in chicken meat-like broths and commercial broth. C, non-treated chicken meat broth; A, autoclaved chicken meat-like broth; B, boiled chicken meat-like broth; FP, filtered and pasteurized chicken meat-like broth, MHB, mueller hinton broth; TSB, tryptic soy broth.

IV. CONCLUSION

CMB developed in this study using the filtering and pasteurizing method showed the highest metabolomic similarity with chicken meat, and its role as a broth for microbial cultivation was validated. Although further study for similarity of intracellular metabolites of bacteria in CMB will be necessary, this new CMB offers advantages in terms of similarity, time and space efficiency for various studies aimed at evaluating properties of bacteria or investigating biomarkers for bacteria in meat.

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