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EFFECT OF SWINE SLAUGHTER BY–PRODUCT *as a substitute of FBS FOR CELL CULTURE*

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I. INTRODUCTION

Fetal bovine serum (FBS) is an essential supplement used in cell and tissue culture systems for cell culture. FBS contains amino acids, fatty acids, growth factors, and cytokines that provides an appropriate environment for cell growth and proliferation [1]. However, the production of FBS has animal welfare and ethical issues, and there are disadvantages in that the consistency of ingredients is not maintained, which may affect cell growth depending on the production source [2]. In this study, the efficiency of FBS replacement was evaluated using lungs, hearts, stomachs, and livers, which are often discarded in swine slaughterhouses. Swine slaughterhouse by-products are known to be rich in amino acids helpful for cell culture and contain many kinds of trace elements and minerals [3][4]. Accordingly, the culture efficiency of C2C12 cells according to the material and substitute production method using swine slaughter by-products was compared to confirm its effectiveness as an FBS substitute materials.

II. MATERIALS AND METHODS

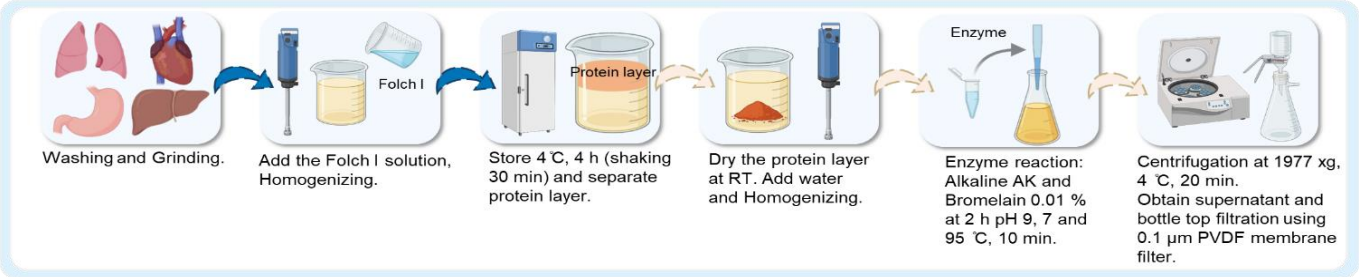


Figure 1. Manufacturing procedures of FBS substitute

1. Preparation of FBS substitute materials from swine slaughterhouse by-products

Swine by-products were purchased from Gyeongdong Livestock located in Hwaseong, and experiments were conducted using four types of visceral by-products (lung, heart, stomach, liver). The swine slughterhouse by-products were used after defatting and enzyme digestion. Alkalain AK (Sigma-Aldrich, P7000-100g) and Bromelain (Nutricost) were used as enzymes, and all enzyme extracts were filtered, and the detailed method is shown in Figure 1.

2. Cell culture with FBS substitute materials from swine slaughterhouse by-products

The C2C12 mouse myoblast cell line was obtained from ATCC (Cat. No. CRL-1772, USA). C2C12 cells were used for experiment. The treatments used DMEM as basal media and a mixture of 5% FBS and 40% enzyme extracts. MTT assay was used to confirm the viability of the cell with enzyme extracts.

III. RESULTS AND DISCUSSION

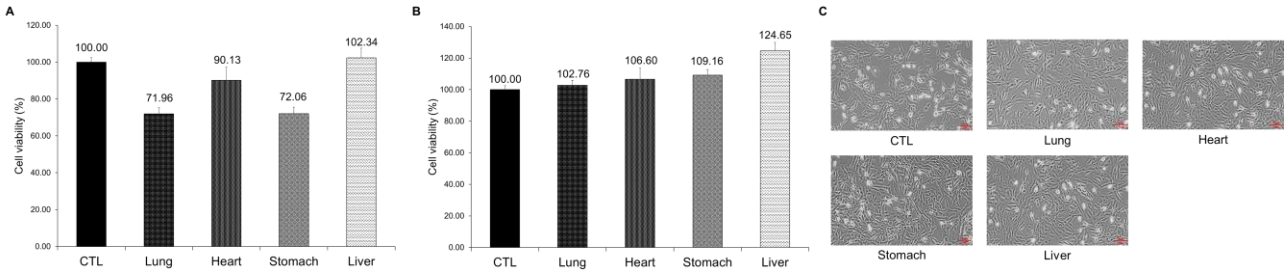


Figure 2. Effect of proliferation on C2C12 cells by enzyme extracts of swine slaughterhouse by-products.

A: Comparison of cell viability by Enzyme extracts using Alkaline AK, B: Comparison of cell viability by Enzyme extracts using Bromelain, C: Morphology of C2C12 cells 3 d after adding Bromelain enzyme extracts

As shown in Figure 2, among the Alkaline AK enzyme extracts, the lung, heart, stomach, and treatment groups were diluted 30-fold, and the liver was diluted 100-fold and used in cell experiments. When 5% FBS and 40% enzyme extracts were mixed and added to C2C12 cell, the liver showed a cell viability similar to that of the control group with 10% FBS. In addition, 40% of the 100-fold diluted bromelain enzyme extracts and 5% FBS were mixed and added to the cells. As a result, the liver showed the highest cell viability, and all treatment groups showed cell viability similar to that of the control group with 10% FBS. Therefore, based on the results of cell morphology and cell viability, when the enzyme extract was added at an appropriate dilution ratio, the cell proliferation rate and cell viability showed values similar to or higher than those of the control group with 10% FBS.

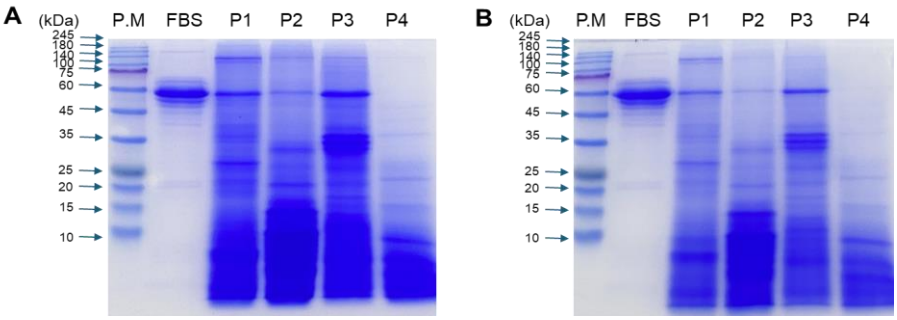


Figure 3. Comparison of protein components of the enzyme extracts of swine slaughterhouse by-products. A: Enzyme extracts using Alkaline AK, B: Enzyme extracts using Bromelain

The protein composition of the enzyme extracts is presented in Figure 3. In both enzyme extracts, proteins of similar size to FBS were identified around the 60 kDa band, which is presumed to be albumin. Previous studies have shown that albumin promotes cell adhesion, survival, and proliferation [2]. In addition, the Alkaline AK enzyme extracts showed a large amount of proteins with larger molecular weights than the Bromelain enzyme extracts. This indicates that the protein hydrolysis rate is higher when the Bromelain enzyme is used. Previous research reported that the shorter the peptide length, the more positively it affects cell proliferation, and similar results were observed in this study [5].

IV. CONCLUSION

Comparing the protein composition of the enzyme extracts, a protein of a similar size to FBS was observed around 60 kDa, but it was confirmed that there were not many bands similar to FBS. In addition, the highest cell viability was shown in the liver. When Bromelain enzyme was used rather than Alkaline AK enzyme, the cell viability was confirmed to be higher, owing to the higher protein hydrolysis rate when using Bromelain.

ACKNOWLEDGEMENTS

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