ISSN: 2586-7342
KJFHC website: http://accesson.kr/kjfhc
doi: http://doi.org/10.13106/kjfhc.2024.vol10.no3.19

The High-throughput Solid-Phase Extraction in the Field of Synthetic Biology: Applications for the Food Industry and Food Managements

Hyeri SEONG[1], Min-Kyu KWAK[2]

Received: May 24, 2024. Revised: June 05, 2024. Accepted: June 05, 2024.

Abstract

The field of synthetic biology has emerged in response to the ongoing progress in the life sciences. Advances have been made in medicine, farming, eating, making materials, and more. Synthetic biology is the exploration of using living organisms to create new organisms. By manipulating specific genes to express targeted proteins, proteins can be created that are both productive and cost-effective. Solid-phase extraction (SPE) and liquid-liquid extraction (LLE) are employed for protein separation during the production process involving microorganisms. This study centers on Scanning Probe Microscopy (SPM) to showcase its utility in the food industry and food management. SPE is predominantly utilized as a pretreatment method to eliminate impurities from samples. In comparison to LLE, this method presents benefits such as decreased time and labor requirements, streamlined solvent extraction, automation capabilities, and compatibility with various other analytical instruments. Anion exchange chromatography (AEC) utilizes a similar methodology. Pharmaceutical companies utilize these technologies to improve the purity of biopharmaceuticals, thereby guaranteeing their quality. Used in the food and beverage industry to test chemical properties of raw materials and finished products. This exemplifies the potential of these technologies to enhance industrial development and broaden the scope of applications in synthetic biology.

Keywords: Synthetic biology, Solid-phase extraction, Scanning Probe Microscopy

Major Classifications: Food industry, Synthetic biology, Solid-Phase Extraction, Food Science (Food Nutrition, Healthy Food)

1. Introduction

The background of this study emphasizes the fact that high-efficiency solid-phase extraction techniques are opening up new applications in synthetic biology and protein separation. The field of biological research is diverse and ongoing, with rapid advances in knowledge and technology in genomics, proteomics, and biotechnology (Meng & Ellis, 2020). Among these areas, synthetic biology is recognized as an effective technological approach to the design and engineering of biological systems (Benner & Sismour, 2005). It reflects research into understanding complex systems problems and improving accuracy, robustness, speed, and affordability. As a consequence of

1 First Author. Laboratory of Microbial Physiology and Biotechnology, Department of Food and Nutrition, College of Bio-Convergence, and Institute of Food and Nutrition Science, Eulji University, Republic of Korea, Email: seonghyeri2@naver.com

2 Corresponding Author. Professor, Laboratory of Microbial Physiology and Biotechnology, Department of Food and Nutrition, College of Bio-Convergence, and Institute of Food and Nutrition Science, Eulji University, Republic of Korea, Email: genie6@eulji.ac.kr

this research and investment, synthetic biology is being transformed into commercially viable services and products with potential applications (Clarke & Kitney, 2020). Protein isolation is an important part of synthetic biology, requiring effective isolation and purification of the desired protein (Lee & Kim, 2013). Techniques used in this process often include solid-phase extraction and liquid-liquid extraction. Prior to the 1970s, liquid-liquid extraction was a common technique employed in research, but it was time-consuming and expensive. As a result, solid-phase extraction (SPE) was developed as an alternative to this technique to adapt to automation. SPE has since become a widely used method and is still used today in a variety of ways (Thurman & Mills, 1998). Currently, it is the most developed and applied technique in the food industry and represents the latest analytical method that is still being developed (Ötles & Kartal, 2016). This underscores the significance and applicability of highly efficient solid phase extraction techniques in the food industry and food control. The applications of SPE in the food industry are diverse. It is used for the analysis of trace residues and contaminants in food, as a sample preparation step, for the quantification of analytes and for the analysis of disease biomarkers. (Badawy et al., 2022, Ridgway et al., 2007, Silva et al., 2022).

The objective of this study was to assess the applicability of SPE in the food industry and food control. In particular, we were interested in protein isolation and purification in the field of synthetic biology. To this end, we compared two techniques, SPE and liquid-liquid extraction (LLE). Anion exchange chromatography(AEC), which can be considered technically equivalent to SPE, is employed in a variety of experiments and utilizes diethylaminoetyl cellulose-Sepharose CL-6B resin (DEAE-Sepharose CL-6B) to separate small molecules such as carbohydrates and proteins(Kwak *et al.*, 2018). AEC uses an ion exchange resin packed in a column to separate or extract anions, while SPE uses the solid phase material in the column to adsorb and separate specific compounds. AEC has been used to quantify ions and ionisable compounds for over 50 years and has a wide range of applications in food analysis and bioanalysis (Bigard et al., 2023). We will demonstrate the similar principles of SPE and AEC to illustrate their value in experimental and industrial applications. The present study aims to demonstrate the similar principles of SPE and AEC, with a view to elucidating their respective applications in experiments and industry. In order to achieve this, a comparison will be made between SPE and LLE, with a view to demonstrating the applicability of SPE. The two methods were used to cultivate lactic acid bacteria, separate the culture and cells, and use the supernatant to perform experiments. Through this comparison, the advantages and disadvantages of both technologies will be identified and evaluated, with a view to determining the industrial application value of SPE. The principles, advantages, and disadvantages of SPE and LLE are distinct, and the objective of this study was to compare them in order to ascertain which technology is more effective for separating and purifying proteins. This comparison was conducted with the aim of gaining a deeper understanding of their applicability in the food industry and food management field and to confirm their industrial application value. Therefore, the objective of this study was to evaluate the industrial application potential of protein separation and purification through SPE. To achieve this objective, a convergent research approach was employed, integrating insights from the fields of synthetic biology and the food industry.

2. Materials and Methods

2.1. Strains and Culture condition

The bacterial strains used in this study are listed in the table (Table 1). The *Lactobacillus plantarum* LBP-K10 strain was consistently maintained in beef extract. broth without modified MRS (mMRS)(Kwak *et al.*, 2018). For the cultivation of LAB strains, overnight-grown inoculum was used, which represented 0.1% of the total volume of the mMRS liquid medium.

The bacterial inoculum was spread on 4 L of mMRS liquid medium and incubated in a stationary incubator at 30 degrees for 72 hours. The composition of the culture medium is 2% D-glucose, 0.5% yeast extract, 0.5% sodium acetate (CH_3COONa), 0.2% ammonium citrate dibasic ($C_6H_{14}N_2O_7$), 0.2% dipotassium hydrogen phosphate (K_2HPO_4), 0.005% manganese sulfate monohydrate ($MnSO_4$-H2O), and 0.01% magnesium sulfate anhydrous ($MgSO_4$), and no peptone. C18-based SPE and LLE were prepared using *Lb. plantarum* LBP-K10 cultured under these conditions for 3 days.

Table 1: Strains in this study

Strain	Type or strains	Source or reference
LAB Strain		
LB. plantarum	Original isolate from Chinese cabbage	This study, (Kwak *et al.*, 2018)

2.2. Sample Preparation for SPE, LLE

Prepare a sample suitable for SPE. Centrifuge a culture of Lb. plantarum LBP-K10 at 8000 rpm for 15 minutes to separate the culture into two components. One is the culture supernatant and the other is the cell pellet. Filter the CS through a 0.22 um nitrocellulose membrane to obtain the culture filtrates. Concentrate 4 L to 1 L using a rotary

evaporator to reduce the amount of organic solvent required for the metabolites. Methylene chloride (MC, CH_2Cl_2) is used in four times the amount in the concentrated CF. After 16 h of LLE, the aqueous and organic solvent layers were separated using a separating funnel. The separated organic solvent layer was evaporated and the residual metabolites were dissolved in TDW. 1 ml of silver was dissolved for SPE and 10 ml for the final sample via LLE. This solution was also filtered through a 0.22 um acetate cellulose filter.

2.3. SPE Fraction Preparation

Perform SPE using C18 SPE resin (Waters Sep-Pak C18 Plus cartridge, Millipore, UK). Rinse with 100 % methanol and 15 ml TDW before continuing. After sample loading, elute using a methanol gradient of 5-50% in 5% increments. Each eluent is evaporated and dissolved in TDW for HPLC fractionation.

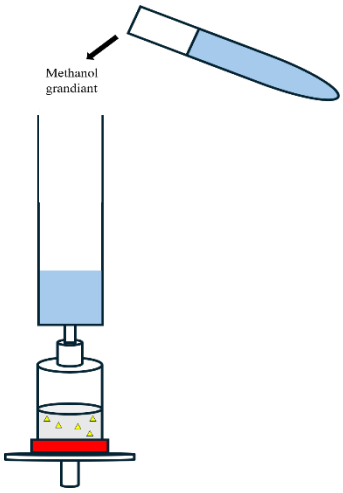

Figure 1: Methanol gradient with SPE

3. Results and Discussion

The culture supernatant of *Lb. plantarum* LBP-K10 was utilized to concentrate and MC extraction was performed. Following the evaporation of the solvent fraction, silver for SPE was dissolved in 1 ml, and the LLE was dissolved in 10 ml. Subsequently, 1 ml was employed for SPE in accordance with the experimental objective. From this experiment alone, it may appear that SPE requires a longer preparation time than LLE, given that it was pretreated with LLE before SPE sample preparation. However, in many other experiments, it is not uncommon to skip LLE when preparing SPE samples. With SPE alone, fractions can be obtained in a single day, and samples can be obtained in less time and in a simpler way. LLE requires a significant quantity of solvents and is time-consuming due to the necessity of manual handling of the samples. The variability introduced by manual procedures reduces the reproducibility of the results and may necessitate the repetition of experiments. Each extraction step requires the mixing, separation, and removal of solvents, which collectively results in a significantly longer total time than that required for SPE. The time required to obtain a sample of LLE, excluding the incubation period, is approximately 2-3 days. While LLE has its advantages, SPE has many more, including cleaner sample separation and easier access for subsequent work. SPE demonstrates that it is possible to purify a substance with only a small amount of sample. In summary, the high consumption of organic solvent solutions, labour and time required by SPE pre-treatment methods demonstrates the superiority of SPE pre-treatment(Hamidi *et al.*, 2021). The principle of SPE is divided into three parts: first, the sample is added and the analyte is adsorbed on the stationary phase. After the adsorption step, the sample is washed with an appropriate solvent to remove impurities. Finally, an appropriate solvent for the sample is used to elute the analyte from the stationary phase. This has the advantage of selectively eluting the analyte from the stationary phase.

In previous studies, protein fractionation using DEAE-Sepharose CL-6B anion exchanging chromatography required approximately six hours. The incubation conditions employed in this study are identical to those used in the previous investigation. It is evident that AEC, in accordance with the fundamental principles of SPE, requires a considerably shorter time than LLE. Due to its numerous advantages, SPE has been a well-established technique for a considerable period of time and is still employed in a multitude of applications. LLE is less efficient in separating analytes through solvent interactions and is less reproducible, making SPE a more preferable technique(Li *et al.*, 2006). The results of these experiments demonstrate that SPE exhibits a notable advantage in comparison. This allows SPM to be utilized as well, depending on the specific application. It can be employed to ascertain the surface properties of nanoparticles, nanostructures, or specific chemicals. Nanoparticles obtained by SPE can be analyzed for surface structure, morphology, size, and other characteristics using SPM(Bian *et al.*, 2021).

SPE is currently employed in a multitude of industrial sectors, and the greater the diversity of adsorbents, the broader the range of industrial applications, as it can separate a multitude of compounds. Adsorbent development and research is ongoing, as is research into adsorbents for the development of polymeric materials(Płotka-Wasylka *et al.*, 2017). SPEs have the potential to facilitate industrial development in a number of fields, including pharmaceuticals, clinical research, and the food industry. The development of adsorbents for a diverse range of

applications will present a challenge in the future.

Funding

This work was supported by the BK21 Plus project of the National Research Foundation of Korea (NRF) Grant funded by the Korea government. This research was also supported by the National Research Foundation of Korea (NRF) grant funded by the Ministry of Science and ICT of the Korea government (MSIT) (NRF-2021R1F1A1061581) and by a grant from Eulji University in 2023. The funders had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Availability of Data and Materials

All supporting information including table of results and detailed methods is available upon request.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Badawy, M. E., El-Nouby, M. A., Kimani, P. K., Lim, L. W., & Rabea, E. I. (2022). A review of the modern principles and applications of solid-phase extraction techniques in chromatographic analysis. *Analytical Sciences, 38*(11), 1457-1487.

Benner, S. A., & Sismour, A. M. (2005). Synthetic biology. *Nature Reviews Genetics, 6*(7), 533-543.

Bian, K., Gerber, C., Heinrich, A. J., Müller, D. J., Scheuring, S., & Jiang, Y. (2021). Scanning probe microscopy. *Nature Reviews Methods Primers, 1*, 36.

Bigard, A., Cardinael, P., & Agasse, V. (2023). Anion exchange chromatography coupled to electrospray-mass spectrometry: An efficient tool for food, environment, and biological analysis. *Critical Reviews in Analytical Chemistry, 53*(2), 1591-1603.

Clarke, L., & Kitney, R. (2020). Developing synthetic biology for industrial biotechnology applications. *Biochemical Society Transactions, 48*(2), 113-122.

Hamidi, S., Taghvimi, A., & Mazouchi, N. (2021). Micro solid phase extraction using novel adsorbents. *Critical Reviews in Analytical Chemistry, 51*(2), 103-114.

Kwak, M.-K., Liu, R., & Kang, S.-O. (2018). Antimicrobial activity of cyclic dipeptides produced by Lactobacillus plantarum LBP-K10 against multidrug-resistant bacteria, pathogenic fungi, and influenza A virus. *Food Control, 85*, 223-234.

Lee, K. H., & Kim, D. M. (2013). Applications of cell-free protein synthesis in synthetic biology: Interfacing bio-machinery with synthetic environments. *Biotechnology Journal, 8*(11), 1292-1300.

Li, K. M., Rivory, L. P., & Clarke, S. J. (2006). Solid-phase extraction (SPE) techniques for sample preparation in clinical and pharmaceutical analysis: A brief overview. *Current Pharmaceutical Analysis, 2*(1), 95-102.

Meng, F., & Ellis, T. (2020). The second decade of synthetic biology: 2010–2020. *Nature Communications, 11*, 5174.

Ötles, S., & Kartal, C. (2016). Solid-phase extraction (SPE): Principles and applications in food samples. *Acta Scientiarum Polonorum Technologia Alimentaria, 15*(1), 5-15.

Płotka-Wasylka, J., Marć, M., Szczepańska, N., & Namieśnik, J. (2017). New polymeric materials for solid phase extraction. *Critical Reviews in Analytical Chemistry, 47*(4), 373-383.

Ridgway, K., Lalljie, S. P., & Smith, R. M. (2007). Sample preparation techniques for the determination of trace residues and contaminants in foods. *Journal of Chromatography A, 1153*, 36-53.

Silva, M. S., Tavares, A. P., de Faria, H. D., Sales, M. G. F., & Figueiredo, E. C. (2022). Molecularly imprinted solid phase extraction aiding the analysis of disease biomarkers. *Critical Reviews in Analytical Chemistry, 52*(8), 933-948.

Thurman, E. M., & Mills, M. S. (1998). *Solid-phase extraction: Principles and practice*. Wiley.