BIOL7040 -- Article Review 2 -- Peter Huang -- 45367115

Case study

Pi, X. *et al.* (2023) 'Characterization of the improved functionality in soybean protein-proanthocyanidins conjugates prepared by the alkali treatment', *Food Hydrocolloids*, 134, p. 108107.

Introduction

Soy Protein Isolate (SPI) is frequently incorporated in an assortment of food products. However, a pressing concern associated with SPI is its allergenicity, manifested through its capacity to bind Immunoglobulin E (IgE). Given the rising demand for soy products and the health concerns linked with food allergies, there's an increasing interest in understanding the allergenicity of SPI. Historically, researchers have been keen on characterizing SPI to harness its potential and address its drawbacks. Prior studies, Zhou et al. (2020) and Chung & Reed (2012), have hinted at altering the IgE binding capacity by conjugating SPI with different molecules like EGCG and tannic acid, showed promising results pointing towards reduced allergenicity. These revelations underlined the potential of modulating SPI's properties by introducing specific conjugates, setting the stage for further exploration. The case study Pi et al., (2023) embarked on the journey to characterize SPI when conjugated with Proanthocyanidin (PC) and to ascertain whether such conjugation could offer a solution to the allergenicity properties of SPI. The aim was to pave the way for producing hypoallergenic soybean products, catering to a wider audience and upholding food safety.

Description of the Characterization Technique

The study focuses on the creation of Soy Protein Isolate-Proanthocyanidin (SPI-PC) conjugates using the alkali technique. SPI was first mixed with varying concentrations of PC. A PC-free SPI was maintained as a control to provide a reference point. To analyze the formation of SPI-PC conjugates, SDS-PAGE was executed following Sun et al. (2021)'s protocol. Samples were prepared by mixing with a loading buffer, boiled, and then loaded into SDS-PAGE gels. The gel electrophoresis procedure was followed by staining and imaging, which shed light on the molecular weight distribution of the formed conjugates. Then, ELISA was used to measure antigen concentration. The SPI-PC is captured, an enzyme-tied antibody is added, and a substrate is used to produce a visible color change. The intensity of this change provides an indication of the initial protein concentration (Li et al., 2018). Subsequently, Western Blotting is used to separate proteins via electrophoresis, and these proteins are then transferred onto a specific membrane. Specific antibodies are utilized to detect and spotlight the target protein. The appearance of distinct bands on the membrane reveals the protein's size and abundance, as described by Wu et al. (2007). Collectively, the array of analytical tools utilized ensures a comprehensive understanding of the conjugates. Each technique provides valuable insights into various aspects of the conjugate, ranging from its genesis to its makeup.

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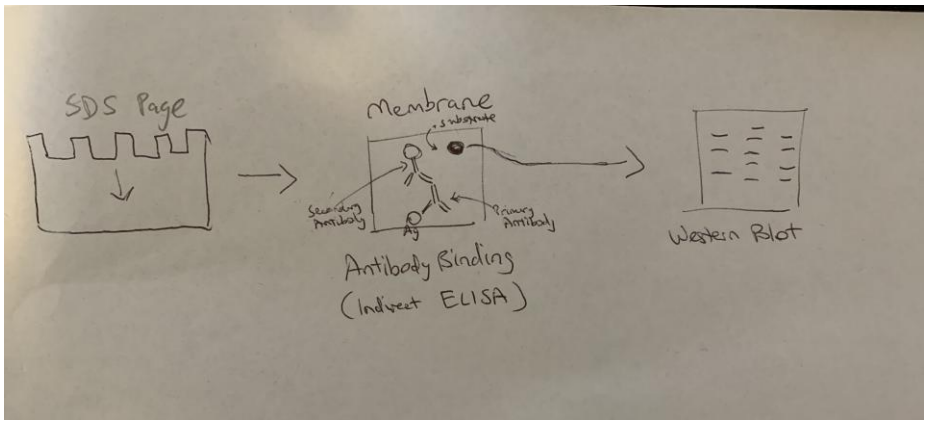
Schematic Figure:



Description of what was done

Selecting the right characterization technique was no small task, as they had to juggle a myriad of essential factors. Both ELISA and Western Blotting shine due to their use of antibodies in these techniques guarantee pinpoint accuracy in characterising the SPI-PC conjugates. SPI-PC conjugates were characterized using ELISA to evaluate their IgE binding capacity, crucial for understanding their allergenicity. The decrease in IgE binding capacity with increasing PC levels would indicate reduced allergenicity, a desirable trait for many consumers. Western Blotting was employed to assess changes in protein structure and size after conjugation with PC. This technique provides a visual representation of protein bands, offering both qualitative and quantitative data about the protein.

The results provide a clear understanding of the effects of conjugating SPI with PC. The SDS-PAGE for visualizing molecular weight changes proved effective. Notably, the the conjugation process increases molecular weight and leads to the formation of high-molecular-weight polymers offers valuable insights into the nature of SPI-PC interactions. The findings (Fig. 1) showed that after conjugating SPI with varying levels of PC, there was a reduction in the density of molecular weight bands. New bands, specifically above 180 kDa, appeared when SPI was conjugated with different PC concentrations. An increase in PC levels corresponded with the appearance of more such bands.The results suggest that the conjugation increased the molecular weight of the samples, implying that PC was attached to SPI through covalent bonds.

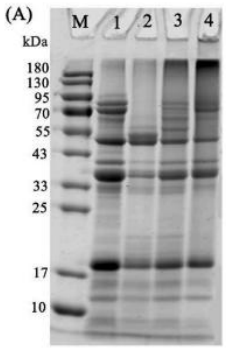


Figure 1

The ELISA data (Fig. 2) revealed that as PC concentration increased in the SPI-PC conjugates, the IgE binding capacity significantly decreased, indicating reduced allergenicity.
Western Blotting analysis (Fig. 3) further confirmed these findings, showing a diminished intensity of protein bands in SPI-PC conjugates compared to control SPI. This reduced intensity, especially at specific protein sizes (e.g., 17, 43–55, and 130 kDa), corroborates the ELISA findings, suggesting reduced IgE binding with increased PC levels.

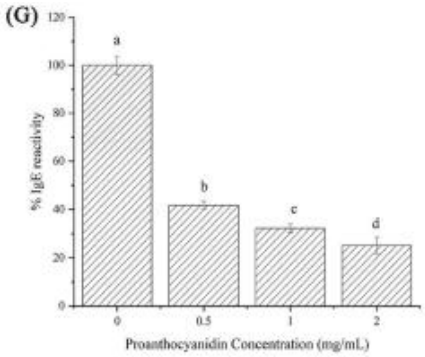


Figure 2

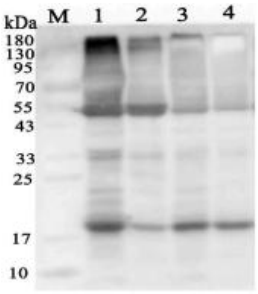


Figure 3

The results offer insights into the benefits of SPI-PC conjugation. The reduced IgE binding capacity suggests a lower allergenicity of the conjugated protein, addressing soy protein's common allergenic issues. This could pave the way to produce hypoallergenic soy products.

Conclusion

The study investigated how different levels of PC in SPI-PC conjugates influenced their functional attributes like allergenic potential. Conjugating PC to SPI brought changes in its molecular weight and structure. This led to enhanced antioxidant activity in the conjugates, given the high PC content. The capacity of SPI-PC conjugates to bind IgE, an indicator of allergenicity, reduced due to the increased exposure of PC to these epitopes. The work hinted at a method to diminish SPI's allergenic potential, paving the way for hypoallergenic soybean products. One potential improvement would be to delve deeper into the mechanisms underlying the observed changes or to provide more context on the real-world applications and implications of these results for food processing and manufacturing. Overall, the study is a significant contribution to understanding the potential benefits of SPI-PC conjugates in the food sector.

BIOL7040 -- Article Review 2 -- Peter Huang -- 45367115

Annotated Bibliography

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