Supplementary Materials

1. Supplementary Investigation on the Hempseed Protein Extraction Kinetic

In this study, the main goal is to check if the protein extractability would change after increasing the extraction pH and whether introducing the H+ is the key to ceasing the extraction system. Herein, the hempseed protein was extracted at pH 9.0 for 10 min (0–10 min), then we adjusted the system pH back to 7.0 and kept extracting at pH 7.0 for 20 min (10–30min). In the second stage, we increased the extraction pH to 10.0 and extracted for 20 min (30–50 min), then the system pH was adjusted to pH = 7.0 again and we kept extracting at this pH for 20 min (50–70 min). Ultimately, the extraction pH was brought to 11.0 and we extracted for another 20 min (70–90 min). The protein concentration and free -SH content were measured at each key time point. The protein yield can be enhanced at any extraction time once the extraction pH is further improved, which indicates that the reduction in the extraction efficiency could be attributed to the pH drop during the extraction. In addition, there were no significant changes observed when we adjusted the pH to neutral. Similar trends can be depicted in the free -SH content changes. Results suggested that H+ can act as a switch of extraction in the current system. It can temporarily cease the extraction until the pH is further away from its isoelectric point.

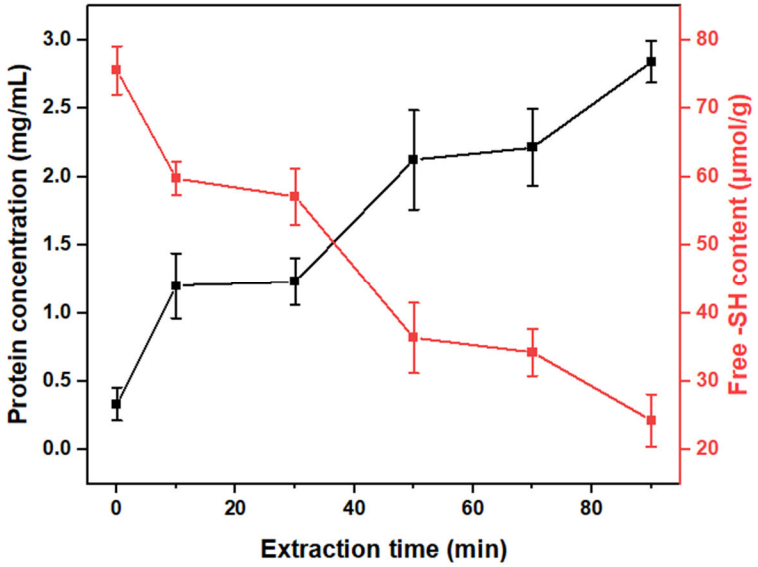

Figure S1. The hempseed protein concentration and free -SH content change after a series of extraction pH adjustments. The extraction temperature was fixed at 37 °C.

2. Supplementary Investigation on the Hempseed Protein Extraction Mechanism

This study aims to investigate whether the antioxidant agent impacted the extraction kinetics. The efficiency of sodium L-ascorbate in inhibiting SS formation was carried out. A similar kinetic (protein yield and free -SH content) of sodium-L-ascorbate-involved extraction was determined. The difference in the extraction kinetic was negligible after introducing the antioxidant to the system. The findings have good agreement with the previous study. Giustarini et al. [1] reported that the efficiency of ascorbate in reducing the disulfide bridge was considerably low, where the second-order rate constant was only 4.72 $\times 10^{-5}$ M^{-1} s^{-1} for cysteine disulfide.

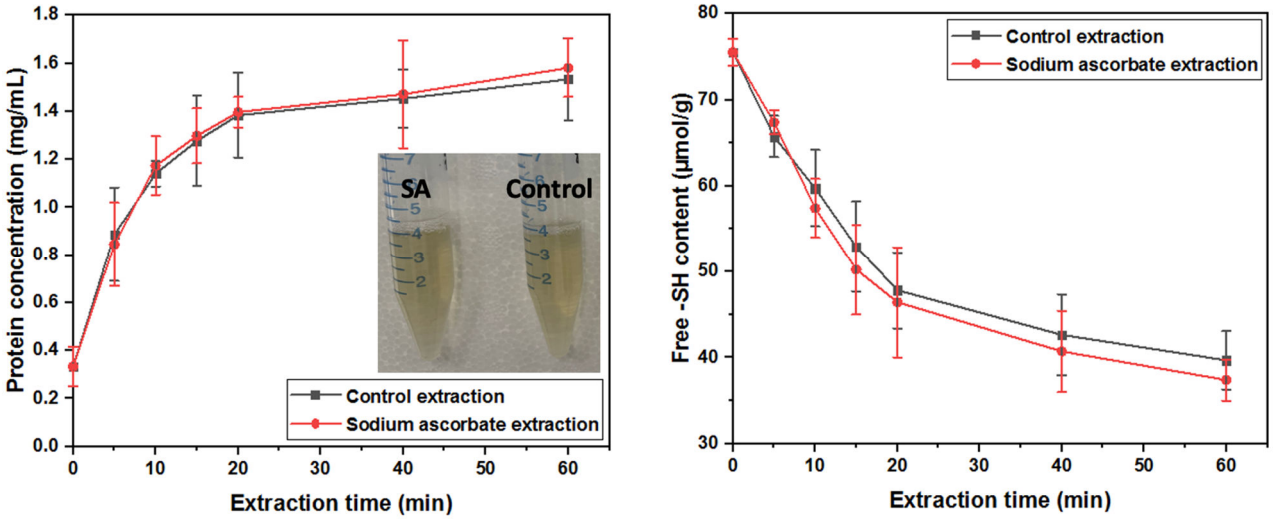

Figure S2. The impact of antioxidant agent introduction to the system on the protein concentration and free -SH content. The extraction conditions were fixed at pH 9 and 37 °C, and the sodium L-ascorbate concentration was 20 mM.

Reference

1. Giustarini, D.; Dalle-Donne, I.; Colombo, R.; Milzani, A.; Rossi, R. Is ascorbate able to reduce disulfide bridges? A cautionary note. *Nitric Oxide* **2008**, *19*, 252–258.