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Impact of AAPH-derived Peroxyl Radical-Induced Oxidation on the Chemical Crosslinking of Pea Protein (*Pisum sativum*) and Its Influence on the Formation of Fibrous Structures During High-Moisture Extrusion

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Introduction

The global population, projected to reach 10 billion by 2025 (FAO, 2022), poses challenges to food security. Plant-based meat analogs are sustainable alternatives, requiring fewer resources and supporting the UN Sustainable Development Goals (Jarunglumlert, 2023). However, replicating meat-like characteristics remains a challenge. High-moisture extrusion is a key technology in the formulation of meat analogs. This process combines high temperatures, pressure, and shear to transform plant proteins, such as pea proteins, from a globular structure to a fibrous one, achieving meat-like textures. During this process, peroxyl radicals (ROO·) trigger protein oxidation, impacting structure and functionality. Controlled oxidation can promote covalent bond formation, essential for fibrous structures. AAPH, a stable generator of peroxyl radicals, simulates oxidation in complex systems, aiding in the development of more sustainable meat analogs. The objective was to evaluate the effect of controlled oxidation of plant proteins using peroxyl radicals generated from the thermolysis of AAPH in a commercial pea protein concentrate for future assessments of fibrous structure formation during high-moisture extrusion processing.

Materials & Methods

Peroxyl radical (ROO·)-mediated oxidation was induced using AAPH (2,2'-azobis(2-methylpropionamidine) dihydrochloride). Briefly, the protein was solubilized in phosphate buffer (P) at pH 8 (3 mg mL^{-1}). The solutions were incubated with 10 and 100 mM AAPH at 37 °C, with oxygen supplied every 15 seconds. After 3 hours of incubation, the oxidation reaction was promptly stopped. AAPH was removed by size-exclusion chromatography using a Bio-Gel P-6 column (1–6 kDa). Control solutions (incubated without AAPH) were processed identically.

Molecular mass changes were analyzed by SDS-PAGE, and the degree of oxidation was estimated by measuring tryptophan intensity through fluorimetry and quantifying free thiols by spectrophotometry.

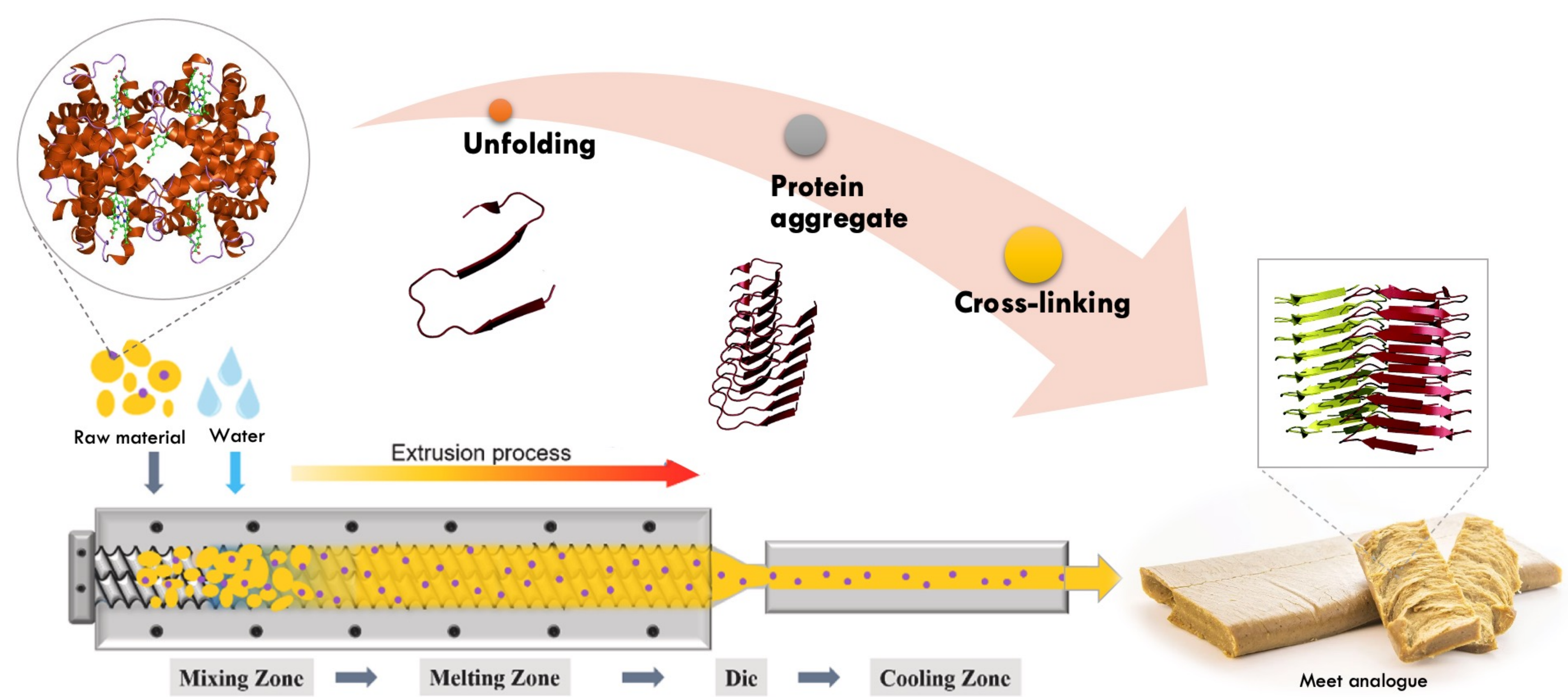

Figure 1. Abstract graph.

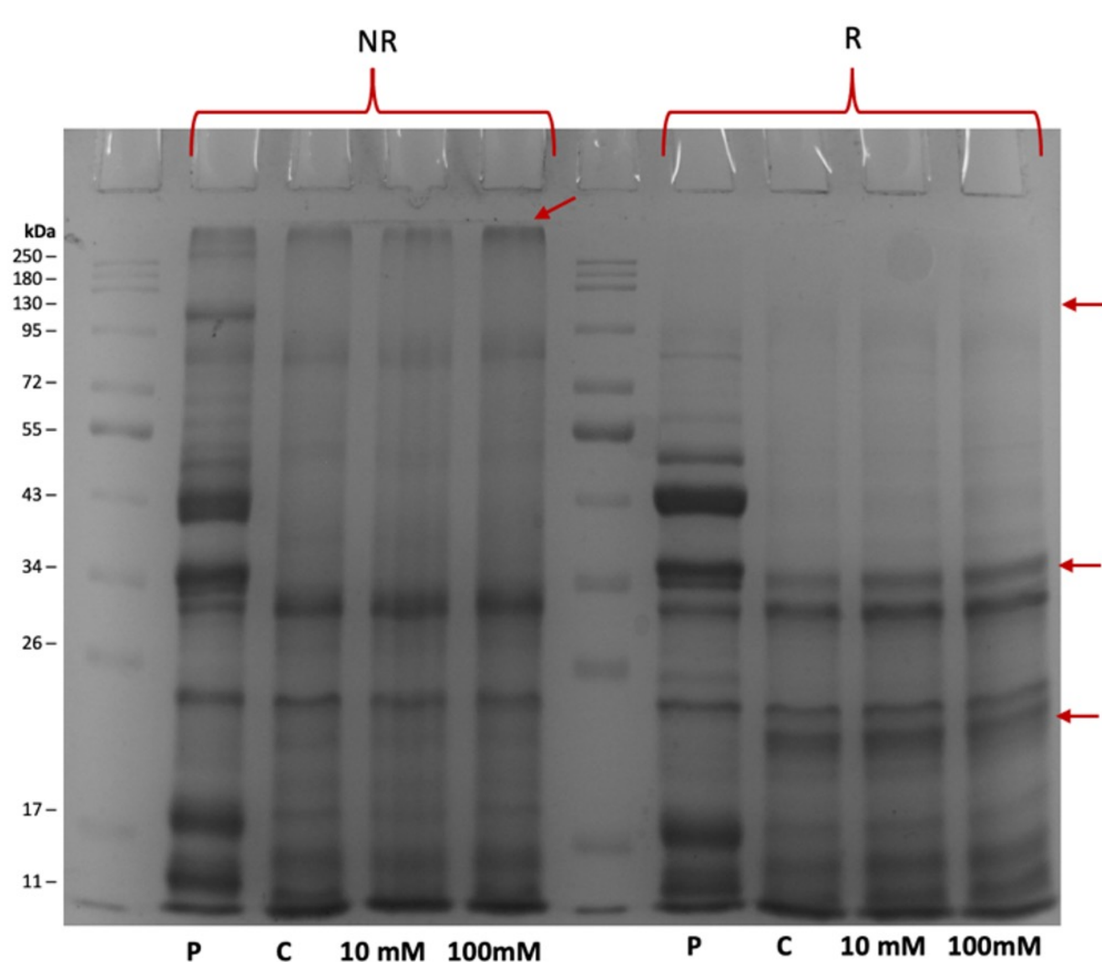

Figure 2. SDS-PAGE profile of oxidized and non-oxidized pea proteins (15 µg), evaluated under reducing (R) and non-reducing conditions (NR).

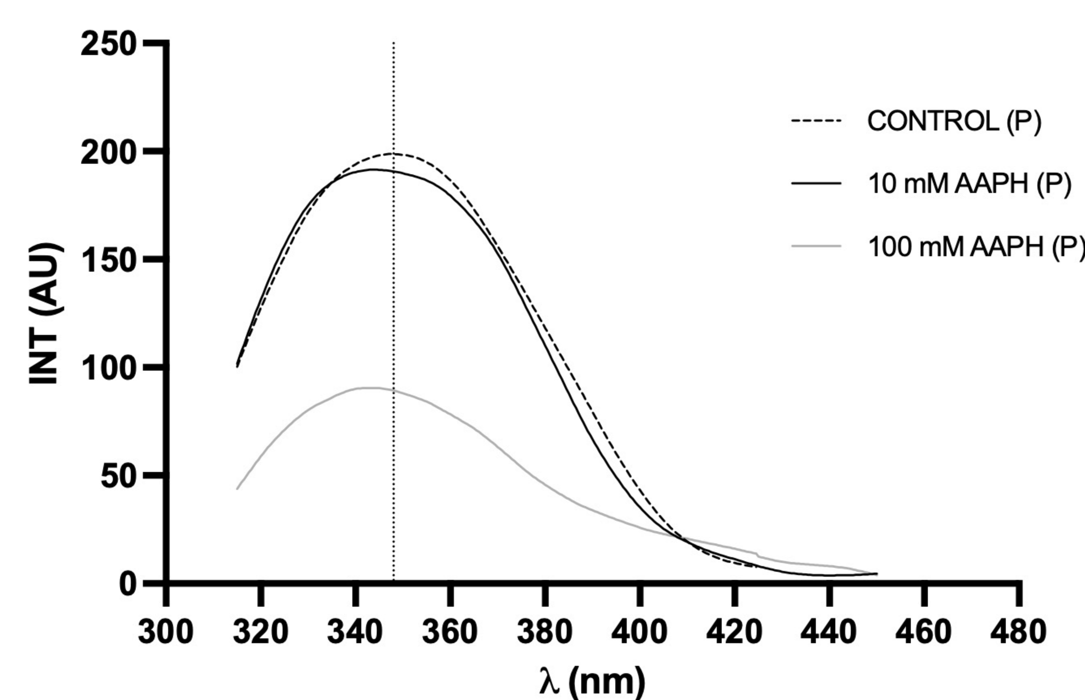

Figure 4. Effect of non-oxidized and AAPH-oxidized pea proteins at different concentrations (10 and 100 mM) on tryptophan intensity, as determined by fluorescence spectroscopy. (P): 0.1 M phosphate buffer, pH 8.

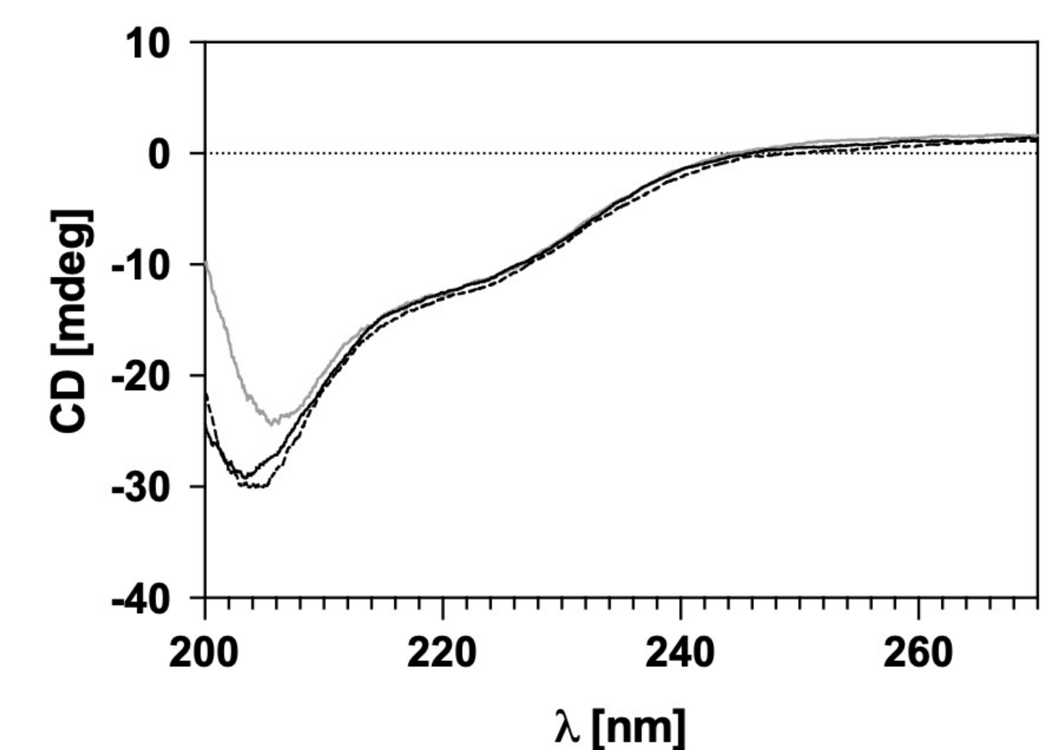

Figure 3. Circular dichroism spectra of non-oxidized and AAPH-oxidized pea proteins at different concentrations (10 and 100 mM) to assess their secondary structure. (P): 0.1 M phosphate buffer, pH 8.

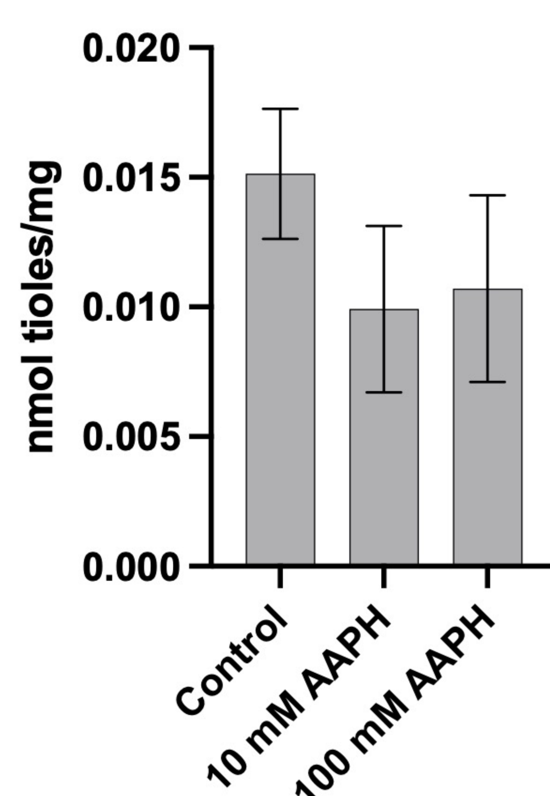

Figure 5. Oxidation markers induced by AAPH exposure in pea proteins solubilized in 0.1 M phosphate buffer, pH 8. Free thiol quantification.

Conclusions

Incubation of pea proteins with AAPH at concentrations of 10 and 100 mM resulted in oxidation, reflected by an increase in thiol content and a decrease in tryptophan fluorescence. Sulfhydryl groups may be involved in disulfide bond formation, leading to aggregate formation, as shown by SDS-PAGE. These effects induced changes in the molecular arrangement of the proteins, as indicated by the reduced endogenous fluorescence intensity (related to tryptophan content), and oxidation at 100 mM affected the secondary structure, assessed by CD.

References

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