The impact of Moderate Electric Fields on amyloid fibril aggregate formation

R Leala, RM Rodriguesa,b and RN Pereira a,b

*a CEB - Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal   
b LABBELS—Associate Laboratory, 4710-057 Braga/Guimarães, Portugal*

Amyloid fibril aggregates (AFA), are protein superstructures with versatile applications, including drug delivery systems and food hydrogels enhancement. Thue to their amyloid nature, they can also serve as models for studying and treat protein misfolding diseases such as Parkinson's and Alzheimer's[1]. The use of high temperatures can naturally promote the formation of these fibrils in acidic, protein-based foods during thermal processing[2]. Food proteins, such as β-lactoglobulin (BLG), can act as semi-conductors in aqueous solutions, allowing alternating and regulated electrical currents to flow. Depending on the strength and frequency of the electrical waveform, electrochemical reactions and internal heat dissipation—also referred to as the ohmic heating (OH) effect—occur when subjected to moderate electric fields (MEF). According to recent research, MEF and the resulting OH can change the unfolding, denaturation, and molecular interactions of globular proteins like BLG, changing their dynamic behavior and playing a crucial role in AFAs formation[3]. MEF provides rapid and volumetric heating with precise temperature control at high temperatures, offering the potential to induce structural changes in proteins under conditions that have not yet been thoroughly explored. This work aims to evaluate the impact of MEF-induced OH at a temperature beyond traditional applications on AFA development, with a focus on its effects on protein behavior, aggregation, and fibril formation, to uncover potential applications and limitations. Under a MEF (< 10 V/cm) at 20 kHz, OH was delivered to WPI aqueous solutions (1% m/v, pH 2) for 0.5h–6 hours at 100°C.The development of BLG fibrils was tracked and characterized through advanced spectroscopic methods, including intrinsic and extrinsic Thioflavin T (ThT) fluorescence, circular dichroism, and detection of degree hydrolysis. Results show that MEF affects unfolding, denaturation, and molecular interactions—all of which are essential for the synthesis of AFA—and thus the dynamic behavior of BLG. The study shows that MEF after 3h of heating at 100 ºC, the maximum amount of AFA was produced, which was marked by a reduction in intrinsic fluorescence and surface hydrophobicity as a result of tertiary structure disruption. Structural analysis revealed an increase in random coil structures along with partial loss of β-sheet, α-helix, and turn formations. These findings demonstrate MEF's ability to rapidly promote AFA formation under specific thermal conditions, highlighting its potential in biotechnological applications such as food texture enhancement, drug delivery systems, and therapeutic approaches for protein misfolding diseases.

*References:*

1. Vetri, V.; Foderà, V. The Route to Protein Aggregate Superstructures: Particulates and Amyloid-like Spherulites. *FEBS Lett.* **2015**, *589*, 2448–2463, doi:10.1016/j.febslet.2015.07.006.

2. Mohammadian, M.; Madadlou, A. Technological Functionality and Biological Properties of Food Protein Nanofibrils Formed by Heating at Acidic Condition. *Trends Food Sci. Technol.* **2018**, *75*, 115–128, doi:10.1016/j.tifs.2018.03.013.

3. Joeres, E.; Schölzel, H.; Drusch, S.; Töpfl, S.; Heinz, V.; Terjung, N. Ohmic vs. Conventional Heating: Influence of Moderate Electric Fields on Properties of Egg White Protein Gels. *Food Hydrocoll.* **2022**, *127*, doi:10.1016/j.foodhyd.2022.107519.