**Kinetics of amyloid fibril aggregates formation under application of moderate electric fields**

R Leal, RM Rodrigues and RN Pereira

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*

Food proteins can work as semi-conductors in aqueous solution and allow the flow of an alternating and controlled electrical current. When protein solutions are exposed to the application of moderate electric fields (MEF) internal heat dissipation (known as ohmic heating effect) and electrochemical reactions will occur depending on intensity and frequency of the electrical waveform. In recent years, it has been evidenced that internal heating and the presence of MEF interfere with dynamic behaviour of globular proteins such as β-lactoglobulin (Blg) by changing their unfolding, denaturation, and molecular interactions. These events can contribute as critical factor on the production of amyloid fibril aggregates (AFA). AFA are considered protein superstructures enclosing several functional and technological applications once they can be used in the development of food hydrogels as texture and foaming enhancers1. Additionally, AFA can also find important biomedical applications such as for development of nanoencapsulation systems for drugs delivery, design of therapeutics to treat incurable protein misfolding diseases (e.g., Alzheimer’s and Parkinson), or development scaffolds for tissue engineering2. In this study kinetics of Blg AFA formation was followed by combining Thioflavin T (ThT) fluorescence with advanced spectroscopic techniques such as intrinsic fluorescence, surface hydrophobicity (through fluorescent probe binding), and circular dichroism. Results have shown that lag stage of AFA formation under MEF is more noticeable, but nucleation and fibril growth presented higher activation coefficient when compared with control sample (sample heated without MEF but under identical heating kinetics). Maximum yield of Blg AFA formation is attained after 5 h of heating at 90 ºC and characterized by an extensive decrease in surface hydrophobicity and intrinsic fluorescence (due to tryptophan quenching). AFA formation resulted in the partial loss of β-sheet, α-helix and turns with a concomitant increase of random coil structures (> 50 %). For the first time, this work provides information about how MEF appears as a critical factor on aggregation kinetics of Blg AFA, showing potential to increase rate of AFA formation depending on the applied electrical protocol and the way how electrochemical reactions are controlled.

*References:*

1. Peng, D., Yang, J., Li, J., Tang, C. And Li. B. (2017). Foams Stabilized by β-Lactoglobulin Amyloid Fibrils: Effect of pH. *Journal of Agricultural and Food Chemistry* *65 (48)*, 10658-10665.

2. Nyström, G., Fong, W-K, Mezzenga, R. (2017). Ice-Templated and Cross-Linked Amyloid Fibril Aerogel Scaffolds for Cell Growth. *Biomacromolecules*, *18 (9)*, 2858-2865.