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# β-lactoglobulin aggregation triggered by pulsed electric fields

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## 1. Introduction

The thermal denaturation kinetics of whey protein, in particular β-lactoglobulin (BLG), has been extensively investigated.<sup>1</sup> However, studies on combining thermal and pulsed electric field (PEF) effects to ensure a denaturation of the quaternary and tertiary structure in a controlled manner are lacking, when reviewing published work, especially which mechanisms (Fig. 2) exert the strongest effect.<sup>2</sup> The charge environment plays a crucial role in this modification process. Achieving a complete controllability of the heat, electric fields and the environment allows a techno-functional fine-tuning of the proteins.

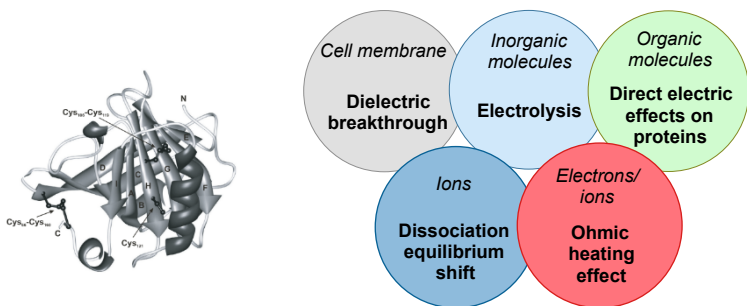


Fig. 1: Molecular structure of BLG.<sup>1</sup>

Fig. 2: Mechanisms involved when applying PEF.

## 2. Method overview

### Treatment:

#### Lab-scale:

- T<sub>IN</sub>: 25°C
- Plate-plate cell
- Square wave pulse
- 10-16 kV/cm
- 23-30 μs
- PRF: 3, 6, 9 Hz
- 2% dw WP

#### Pilot-plant:

- T<sub>IN</sub>: 70°C
- Tubular heat exchanger (THE)
- Colinear cell
- Square wave pulse
- 10-20 kV/cm
- 18 μs
- PRF: 10-50 Hz
- 2% dw WP

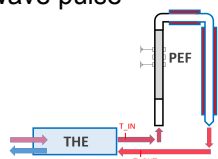


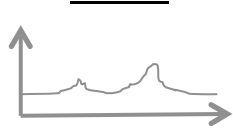
Fig. 3: Schematic setup of THE and PEF on a pilot-plant scale.



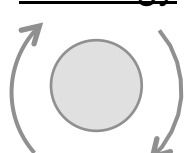
Fig. 4: Lab-scale (A) and pilot-plant (B) PEF chambers.

### Analyses:

#### HPLC



#### Rheology



#### Simulations



## 3. Results

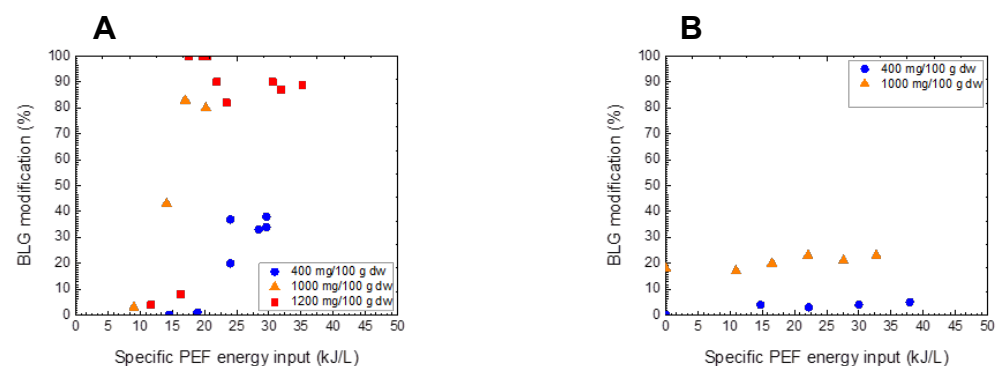


Fig. 5: Different BLG modification rates (determined with HPLC) on a lab-scale (A) and pilot-plant-scale (B) for 2% WP and changing ion environment (400 mg Ca<sup>2+</sup>/100 g dw, 1000 mg Ca<sup>2+</sup>/100 g dw, 1200 mg Ca<sup>2+</sup>/100 g dw; latter only shown for lab-scale).

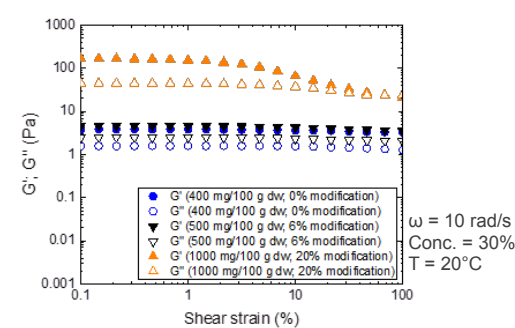


Fig. 6: Change of elastic (G') and viscous (G'') moduli as a function of BLG modification and Ca<sup>2+</sup> addition. Colinear cell, pilot-plant.

- Reference, no pulse
- 23.1 kJ/L, 18 μs, 30 Hz
- 22.1 kJ/L, 18 μs, 20 Hz

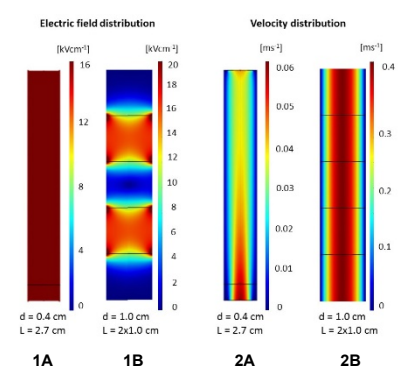


Fig. 7: E-field (1) and velocity (2) distributions for a plate-plate lab-scale (A) and colinear pilot-plant (B) geometry. Calculated for 400 mg Ca<sup>2+</sup>/100 g dw.

## 4. Conclusions

- On a lab scale, electric and thermal effects led to BLG modifications at lower calculated and measured temperatures than expected from thermal kinetics.
- If the energy input per surface area was too high, instabilities (electrolysis, BLG deposit on electrodes) were detected.
- With the given geometries and setups, BLG modification occurred stepwise due to inhomogeneities. In order to achieve a successful fine-tuning of the proteins, new designs of cells might potentially be required.
- Calcium addition increased the BLG modification and thus the visco-elastic properties per energy input. The increased modification might be a consequence of an increased ohmic heating effect due to a higher conductivity.

## 5. References

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- 2 Giteru, S. G., Oey, I. & Ali, M. A. Feasibility of using pulsed electric fields to modify biomacromolecules: A review. *Trends Food Sci. Technol.* **72**, 91–113 (2018).