DESIGN OF MICRO- AND NANOSTRUCTURES FROM β-LACTOGLOBULIN UNDER SELECTED ENVIRONMENTAL CONDITIONS

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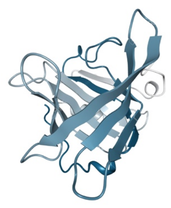
Introduction

Bovine β-lactoglobulin (β-Lg) is a globular protein and the major component of whey proteins (ca. 50 % of its protein content). Besides the high nutritional value, the biological properties and resistance to proteolytic degradation in the stomach, its gelation capacity is particularly important allowing the formation of bio-based micro- and nanostructures (e.g. particles and hydrogels).[1] β-Lg when heated above a critical temperature (i.e. denaturation temperature: 76 ºC) undergoes conformational changes followed by subsequent protein-protein interactions. The order and rates of aggregation is highly dependent on the temperature, pH and protein concentration and can result in the formation of micro- and nanostructures with different properties and morphologies[2]. This work intends to understand the heat-induced aggregation of β-Lg, affected by combined environmental conditions (various pH, heating temperature and protein concentrations) that lead to the formation of β-Lg bio-based micro- and nanostructures.

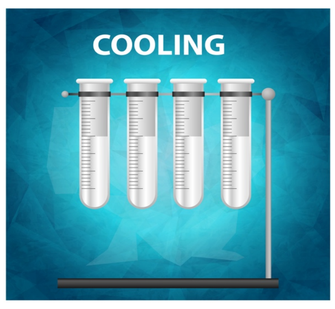
Methods



β-Lg at 0.5, 1, and 1.5 %(w/v) was solubilized in phosphate buffer (0.025 M)

Adjustment of pH: 3, 4, 6 and 7

Heating treatments: 60, 70 and 80 ºC



Cooling in ice for 10 min

Protein structures

Characterization

Dynamic Light Scattering

- Particle size
- Polydispersity index

Adapted from [3]

Results

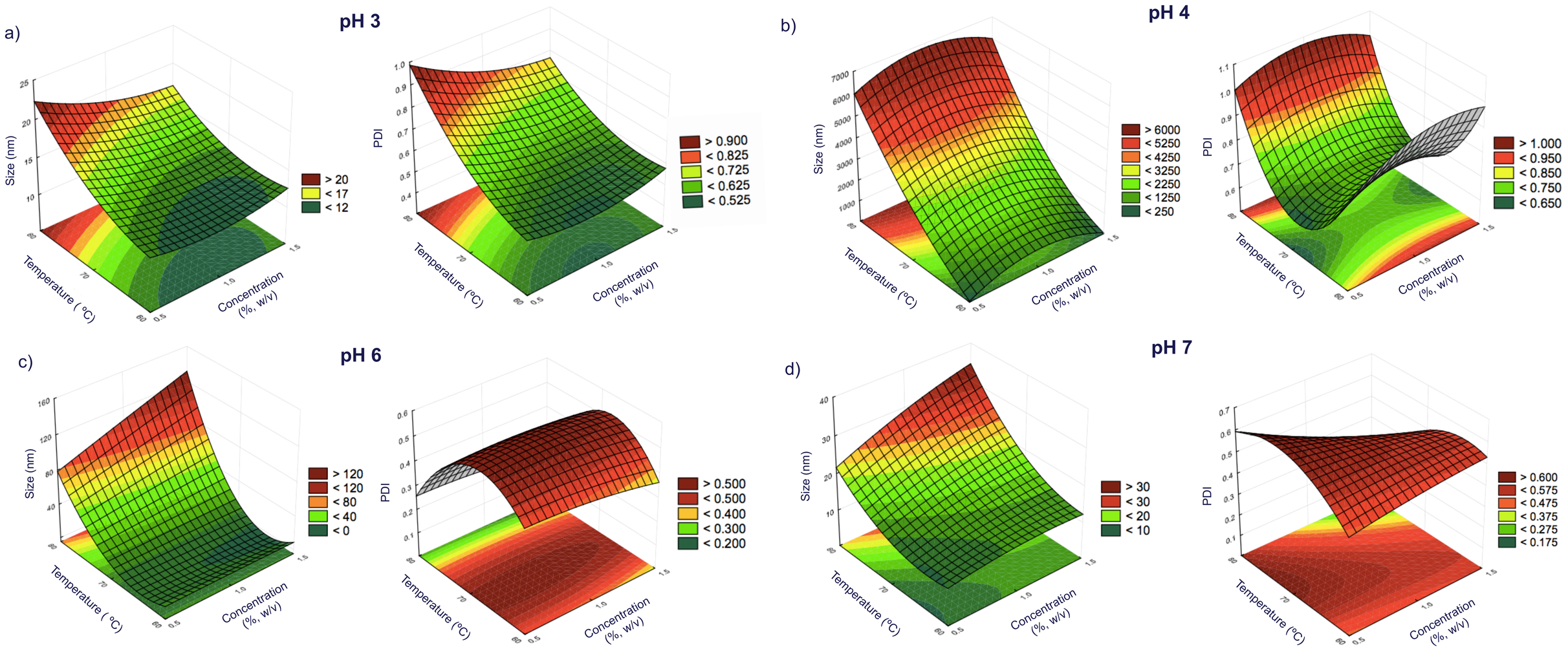


Fig. 1. Particle size and polydispersity index (PDI) of β-Lg structures prepared at various protein concentrations (from 0.5 to 1.5 %, w/v) and temperatures (from 60 to 80 ºC), as function of pH 3, 4, 6 and 7: a), b), c) and d), respectively.

- β-Lg nanostructures were formed at pH 3 and 7 independently of the β-Lg concentration and heating temperature employed, displaying particle sizes below 50 nm, but high PDI values (≥ 0.5).
- β-Lg structures ranging from ca. 76 to 140 nm were obtained at pH 6, depending of β-Lg concentration used (from 0.5 to 1.5 %, w/v), for heating temperatures of 80 ºC (i.e. above the denaturation temperature of β-Lg). At these conditions the structures showed the lowest PDI values (≤ 0.2).
- At pH 4, it was possible to obtain structures at the microscale (i.e. ≥ 3 μm) independent of the β-Lg concentration used for heating temperature of 70 and 80 °C. At this pH, which is relatively close to the isoelectric point of β-Lg (i.e. 5.2), the net charge of proteins is close to zero, so the protein structures tend to aggregate, thus showing higher size values.

Conclusions

- β-Lg structures can be formed at sizes above or below 100 nm, at pH 6 and for heating temperature of 80 ºC, by changing the protein concentration (i.e. from 0.5 to 1.5 %, w/v), which can be very useful for the development of bio-based delivery systems of bioactive compounds (e.g. antimicrobials, antioxidants and nutraceuticals) for food and pharmaceutical industries.
- Protein aggregation mechanisms appear to be controlled by the environmental conditions applied; therefore, an understanding of the quantitative effect of these conditions is crucial for the rational design of protein structures at micro- or nanoscale with tailored functionalities.

References

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