STRUCTURAL CHARACTERIZATION OF HEAT-INDUCED β-LACTOGLOBULIN NANOHYDROGELS UNDER THE EFFECTS OF SELECTED PHYSICAL CONDITIONS

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Introduction

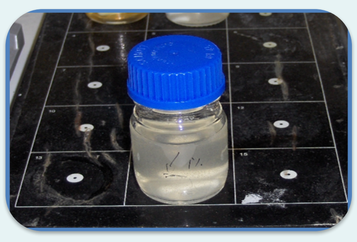
β-Lactoglobulin (β-Lg) is the major protein fraction in bovine whey serum (ca. 50% of its protein content). It is a bio-based and a Generally Recognized As Safe (GRAS) material, with a high nutritional value, that can be used to encapsulate nutraceuticals essentially due to its gelation capacity, which allows the formation of nanohydrogels. Furthermore, β-Lg displays a high binding capacity, under specific environmental conditions and it is resistant to proteolytic degradation in the stomach. These features make of β-Lg an excellent bio-based material to be used as carrier of nutraceuticals[1,2]. This study aims at understanding the impact of different conditions (β-Lg concentration and heating times) in the physical properties of β-Lg nanohydrogels.

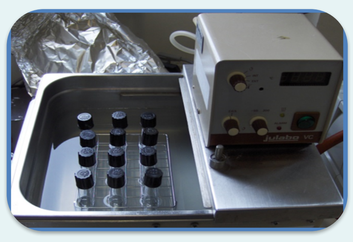
Methods

CONDITIONS
β-Lg (5, 10 and 15 mg mL^{-1}) was solubilized in phosphate buffer (0.025 M)

pH ADJUSTMENT
The pH of β-Lg solutions were adjusted at 3, 4, 6 and 7

HEATING PROCESS
β-Lg solutions were heated at 80 ºC for 5, 15 and 15 min.





Cooling in ice For 10 min

Characterization

Dynamic Light Scattering
- Particle size
- Polydispersity index (PDI)
- Surface charge

Spectrofluorimeter
- Intrinsic and extrinsic fluorescence intensity determined by means of tryptophan and ANS tools, respectively

Results

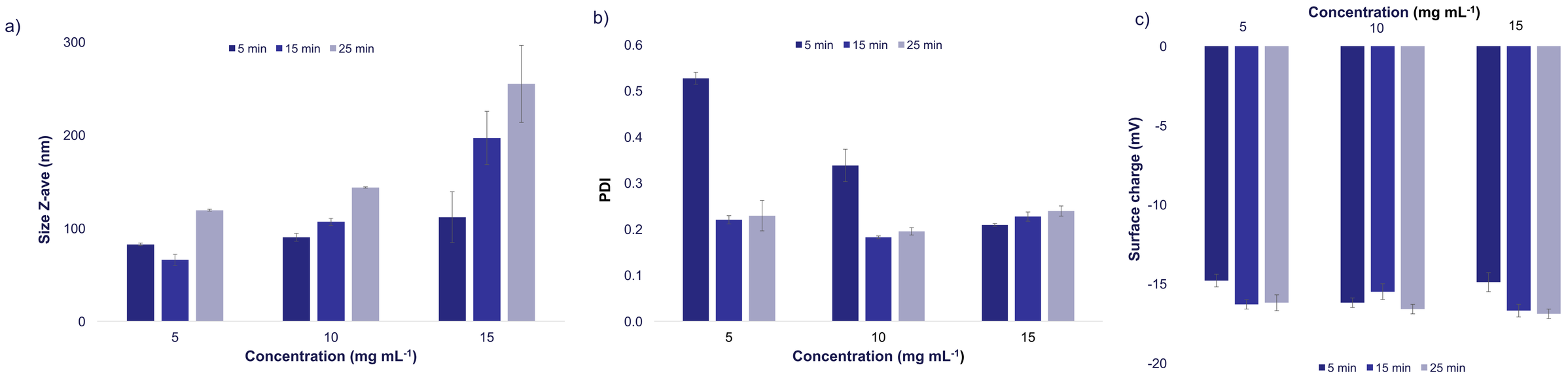
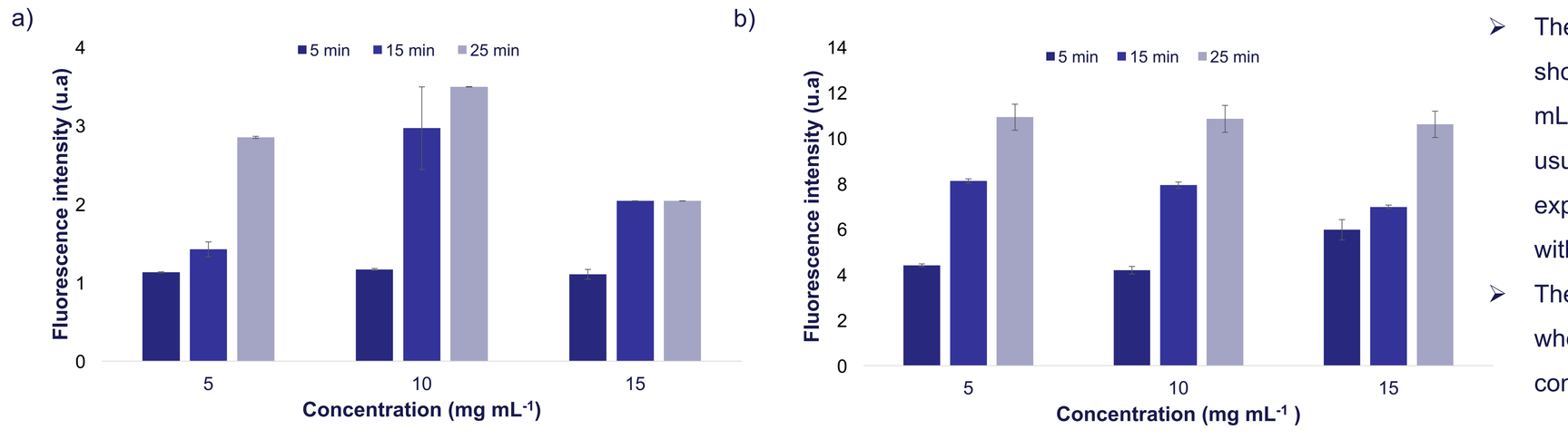


Fig. 1. Particle size a), polydispersity index (PDI) b) and surface charge c) of β-Lg nanohydrogels prepared at various concentrations of proteins (5, 10, 15 mg mL^{-1}) and heated at 80 ºC for 5, 15 and 25 min.

- Stable β-Lg nanohydrogels were obtained for heating periods longer than 15 min, characterized by a low polydispersity (< 0.2), independent of the protein concentration used.
- β-Lg nanohydrogels showed increasing particle size values, ranging from 50 nm to 260 nm, and relative constant surface charge, ranging from -15 mV to -17 mV, as β-Lg concentration increased.
- β-Lg nanohydrogels exhibited particle size values below 100 nm for protein concentration of 5 and 10 mg mL^{-1} when heated at 80 ºC for holding periods up to 15 min.



- The intrinsic fluorescence intensity of β-Lg nanohydrogels showed the highest value for protein concentration of 10 mg mL^{-1} heated for 15 and 25 min; this suggests that tryptophan, usually buried in the native structure, undergoes a partial exposure and, under these conditions, it is able to interact with bioactive compounds[3].
- The extrinsic fluorescence intensity showed the highest value when heated for 25 min, independently of the protein concentration used.

Fig. 2. Normalized maximum intrinsic and extrinsic fluorescence intensity determined by means of tryptophan a) and ANS b) tools, respectively.

Conclusions

- The size of β-Lg nanohydrogels is highly dependent of both, protein concentrations and holding time employed.
- The results obtained represent a significant contribute to enrich the knowledge about the impact of several environmental conditions on β-Lg nanohydrogels characteristics and thus in the desired properties intended for their final application, either in the food and pharmaceutical industries.

References

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