Heat induced conformational changes of whey proteins in model infant formulae: effect of casein and inulin

Mariana RODRÍGUEZ-ARZUAGA*[1,2], María C. AÑÓN[2], Analía G. ABRAHAM[2]

(1) Latitud, LATU Foundation, Uruguay. (2) CIDCA (National University of La Plata, CONICET, CIC-Bs.As.), Argentina.

Introduction

Infant milk formula (IMF) and cow milk have compositional differences, such as whey protein-to-casein and protein-to-lactose ratios and presence of components with prebiotic function. Whey proteins (WP) are sensitive to heat-induced denaturation and aggregation, which could have techno-functional and nutritional implications. This study investigated how the presence of casein and inulin, at ratios relevant to IMF processing, affect the heat-induced conformational changes of WP.

Materials and methods

- **MODEL SYSTEMS** were prepared as aqueous dispersions of WPI (WP), calcium caseinate (CAS) and/or inulin (INUL), at the levels presented in Table 1.

Table 1. Model systems composition.

Model systems	WP (%, w/w)	CAS (%, w/w)	INUL (%, w/w)
WP	1.8	0.0	0.0
WP-CAS	1.8	1.2	0.0
WP-INUL	1.8	0.0	1.5
WP-CAS-INUL	1.8	1.2	1.5

- **HEAT TREATMENTS (HT)** were applied to all model systems in a temperature-controlled water bath at 66, 70 and 75 ºC for 30 min. Heated and unheated (UH) systems were freeze-dried for further analyses.

- **TEMPERATURE (Td) AND DEGREE OF DENATURATION** were determined by DSC in the freeze-dried systems reconstituted in distilled water to 30 % TS. Td was defined as the minimum of the endotherm peak and degree of denaturation as the % of the area of the endotherm obtained for the same model system but UH.

- **CHANGES IN THE SECONDARY STRUCTURE** were evaluated by ATR-FTIR in the amida I region (1700-1600 cm^{-1}).

- **SDS-PAGE under reducing and non-reducing conditions** were performed for all samples.

- **PARTICLE SIZE DISTRIBUTIONS** were obtained by means of dynamic light scattering in the freeze-dried samples reconstituted at 1 % (w/v) in simulated milk ultrafiltrate (SMUF).

- **PROTEIN SOLUBILITY (%, w/w)** was calculated in the model systems before freeze-drying as: (soluble protein/total protein)x100. Soluble protein was determined by Bradford method in the supernatant obtained after centrifugation at 20.000 × g for 20 min at 25 ºC. Total protein in the model systems was determined by Kjeldahl.

Conclusions

- HT induced denaturation, aggregation and loss of solubility of WP.
- Before HT, CAS addition reduced protein solubility but did not modify Td. WP-CAS solubility increased with HT. Particle size and FTIR results suggested that CAS inhibited WP heat-induced aggregation.
- INUL did not affect aggregation or solubility but increased Td.

Results

- **EFFECT ON DENATURATION**

Table 2. Denaturation temperature (Td) of the unheated model systems.

Model systems	Td (ºC)
WP	74.6 ± 0.2[a]
WP-CAS	74.8 ± 0.3[ab]
WP-INUL	75.7 ± 0.4[c]
WP-CAS-INUL	75.4 ± 0.2[bc]

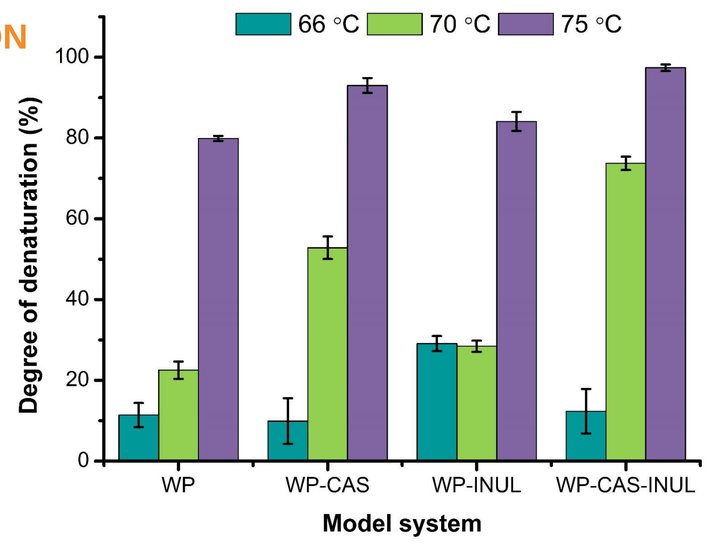

Fig. 1. Degree of denaturation of model systems.

- **EFFECT ON AGGREGATION**

WP: under non-reducing conditions, SDS-PAGE showed a band of high MW that decreased with temperature due to aggregation. Particle size increased from 258 (UH) to 932 nm at 75ºC (Fig. 2). FTIR spectra showed higher proportions of intermolecular β-sheet structures.
EFFECT OF CAS: Particle size was constant with HT and less intermolecular β-sheets were obtained by FTIR.
EFFECT OF INUL: did not prevent increase in particle size (Fig. 2).

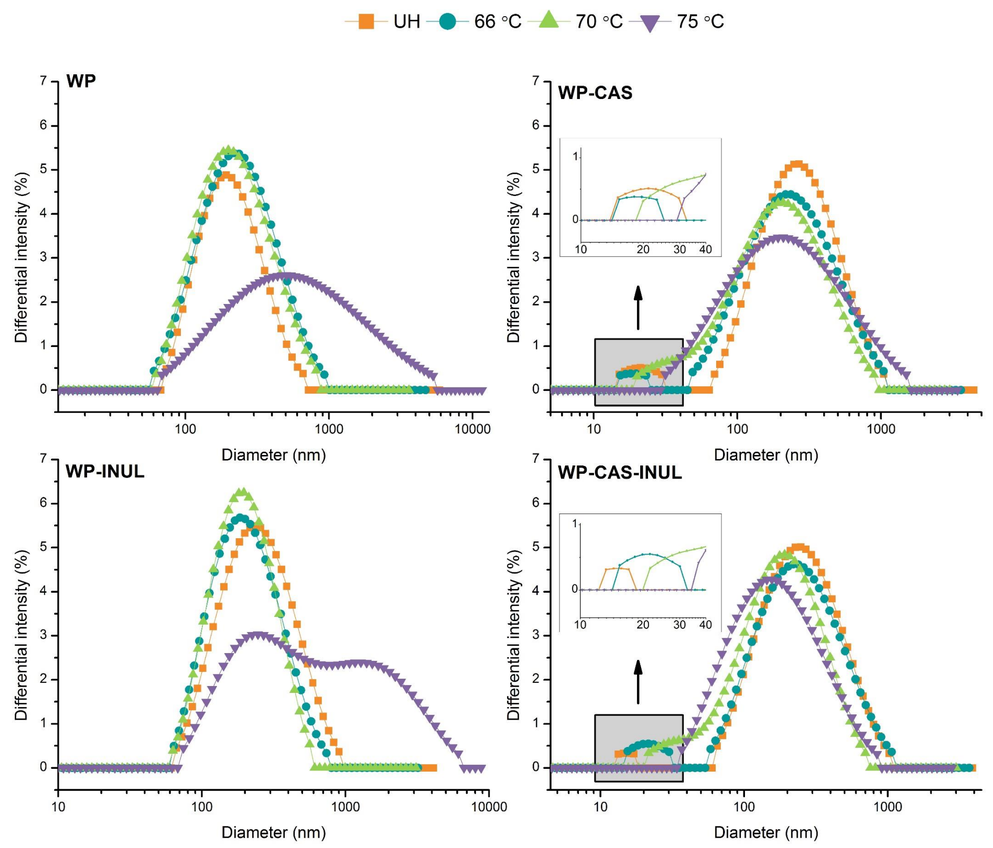

Fig. 2. Particle size distribution of model systems.

- **EFFECT ON PROTEIN SOLUBILITY**

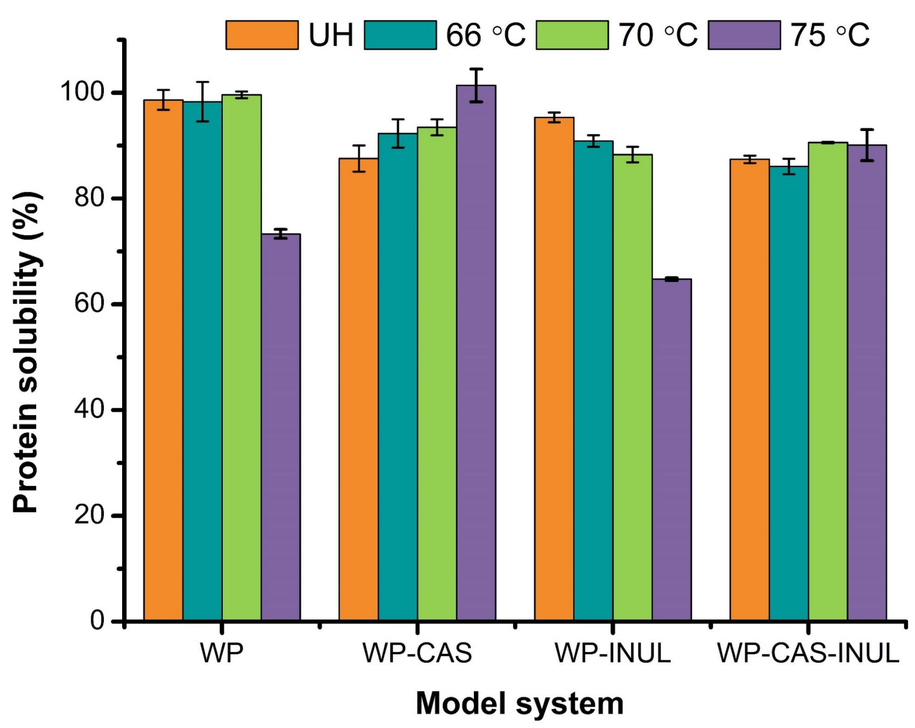

Fig. 3. Protein solubility of model systems.