colloids and interfaces

MDPI

Supplementary Materials

Influence of casein and milk phospholipid emulsifiers on the digestion and self-assembled structures of milk lipids

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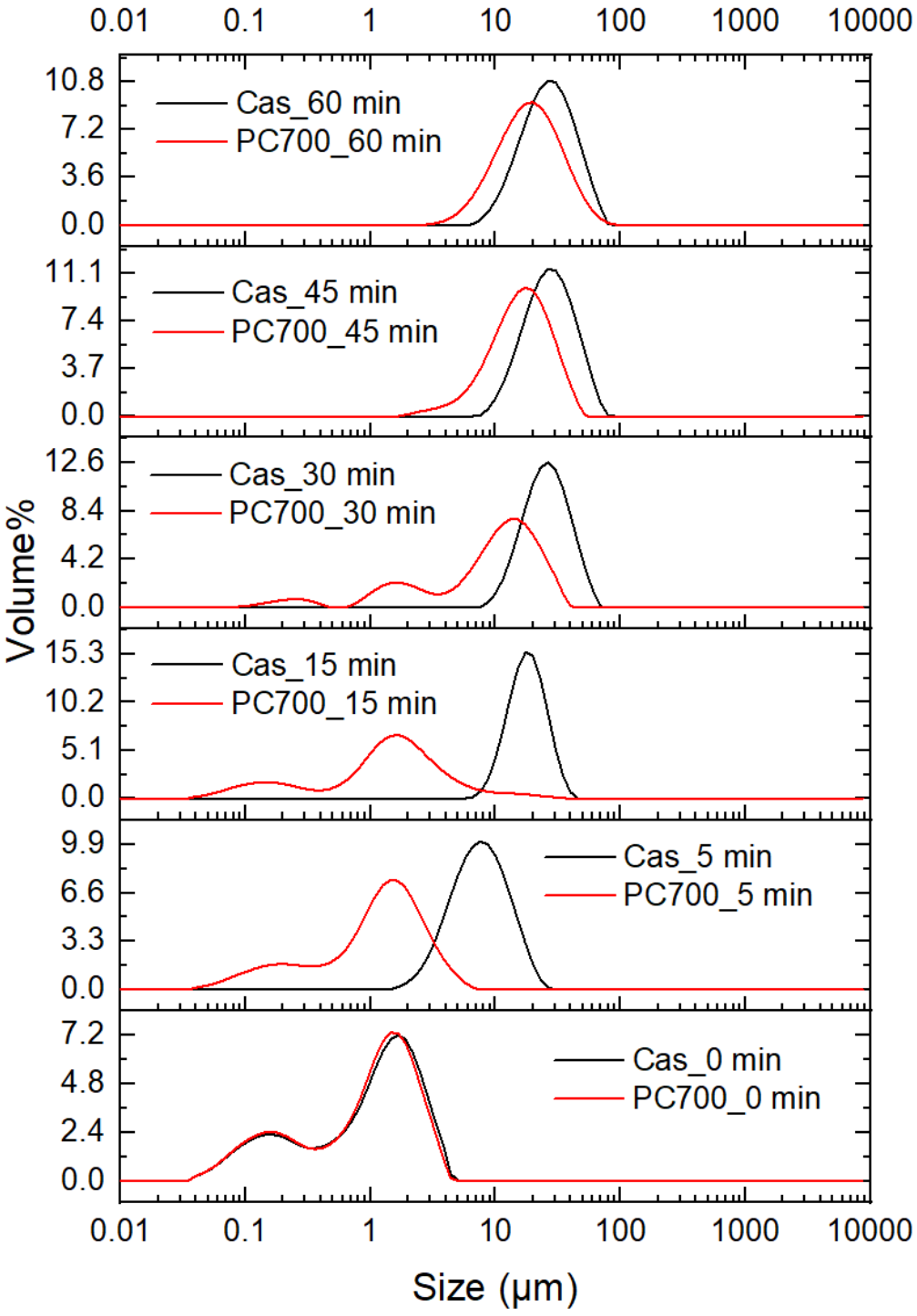

Figure S1. Particle size distributions of 2 w/v% casein+AMF and 2 w/v% PC700+AMF emulsions at pH 6.5 before digestion (0 min) and during digestion (up to 60 min) by pancreatic lipase.

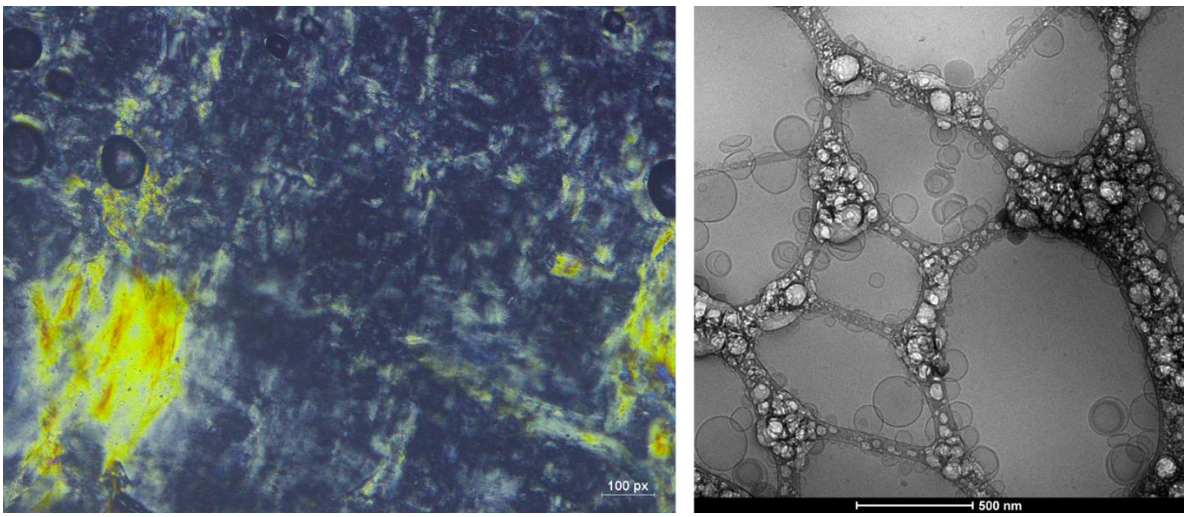

Figure S2. Left: Polarised light microscopy image of hydrated bulk milk phospholipids (PC700) in water at 37 °C indicating birefringence lamellar textures. Scale bar: 100 px = 50 μm. Right: Cryogenic electron microscopy image of dispersed PC700 showing presence of vesicles.

Table S1. Average volume weighted mean particle size ($D_{4,3}$) of anhydrous milk fat (AMF) emulsified with casein and milk phospholipids (PC700) under different conditions: pre-gastric emulsions; emulsions post-pepsin incubation with pH adjusted to pH 6.5 (post-pepsin, pH 6.5); and emulsions post-pepsin and fungal gastric lipase incubation with pH adjusted to 6.5 (post-pepsin + FGL, pH 6.5). Particle size of PC700 emulsified in tris buffer, i.e. no AMF, was also included for comparison. SD is standard deviation from three measurements.

Sample	Condition	Volume weighted mean ($D_{4,3}$) ± SD, μm
Casein+AMF	Pre-gastric (emulsion in pH 6.5 buffer)	1.8 ± 0.4
	Post-pepsin, pH 6.5	2.3 ± 0.9
	Post-pepsin + FGL, pH 6.5	6.4 ± 3.3
PC700+AMF	Pre-gastric (emulsion in pH 6.5 buffer)	1.5 ± 0.4
	Post-pepsin, pH 6.5	2.0 ± 0.0
	Post-pepsin + FGL, pH 6.5	3.3 ± 1.0
Casein+PC700+AMF	Pre-gastric (emulsion in pH 6.5 buffer)	1.4 ± 0.4
	Post-pepsin, pH 6.5	3.9 ± 0.1
	Post-pepsin + FGL, pH 6.5	46.0 ± 22.6
PC700 only (no AMF)	Pre-gastric (emulsion in pH 6.5 buffer)	1.0 ± 0.0

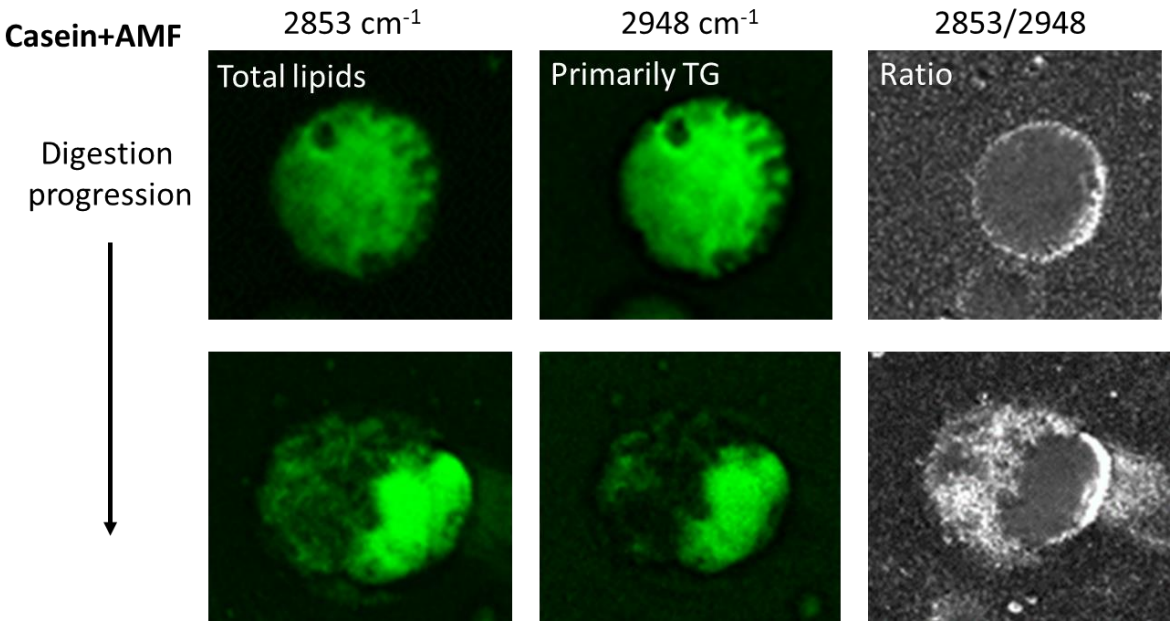

Figure S3. CARS microscopy images of casein-emulsified fat globules during digestion by pancreatic lipase, showing distributions of triglycerides (TG, middle column) and all lipids including the digestion products (left column); and the ratio between the two signals (right column).

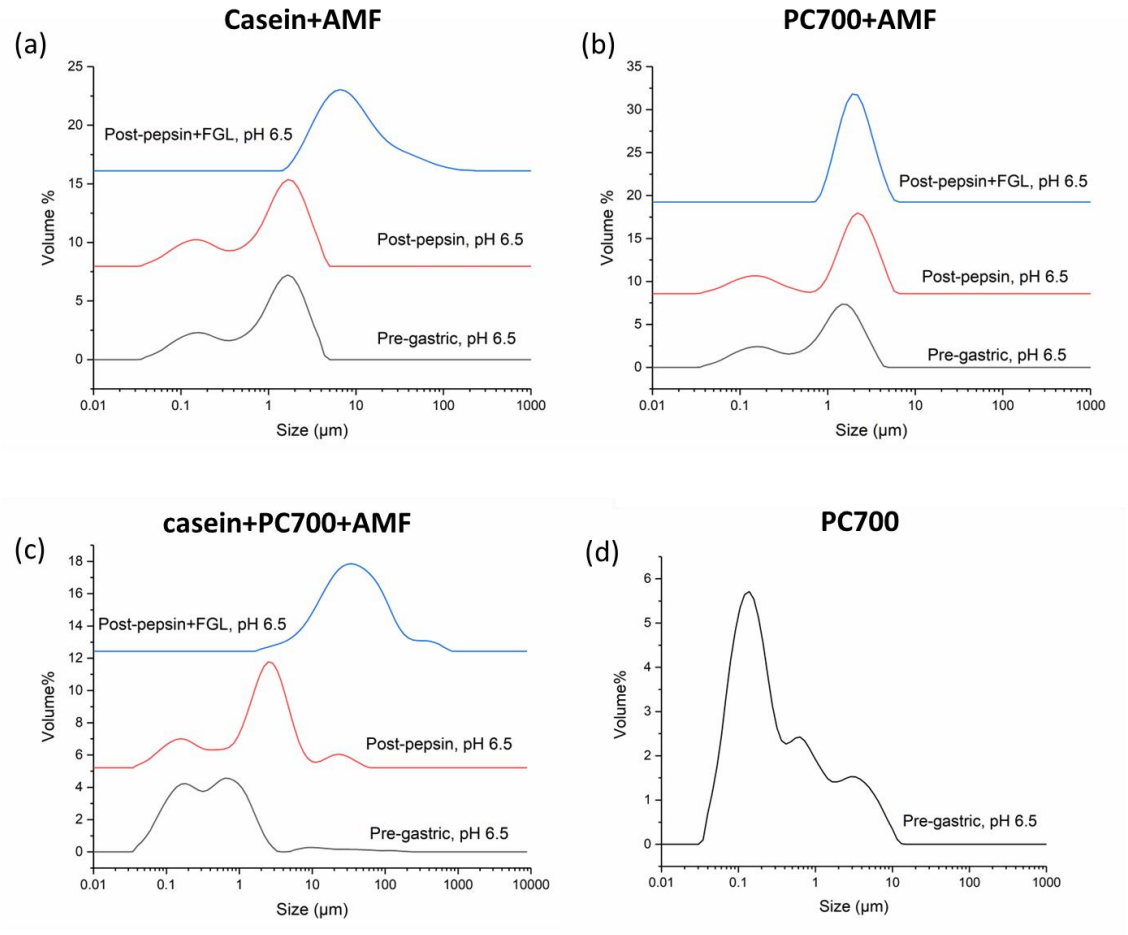

Figure S4. Particle size distributions of (a) 2 w/v% casein+AMF, (b) 2 w/v% PC700+AMF, and (c) 1 w/v% casein+1 w/v% PC700+AMF emulsions at pH 6.5 before and after gastric digestion with pepsin and fungal gastric lipase (FGL); and (d) PC700 in tris buffer.

Table S2. Lists of main liquid crystal structures formed (and their associated lattice parameters) after 60 min or 80 min digestion of casein-emulsified fat globules (casein+AMF) and PC700-emulsified fat globules (PC700+AMF) by pancreatic lipase at pH 6.5, with or without prior gastric step pre-treatment with pepsin and/or fungal gastric lipase (FGL). Milk was included for comparison purposes. Concentration of the emulsifiers was 2 w/v% and AMF was 3.8 w/v%.

Samples	Gastric pre-treatment	Liquid crystal phase formed after digestion by pancreatic lipase	Position of the first peak, q (Å^{-1})	Lattice parameter (Å)
Casein+AMF	None	Lamellar	0.13	47
		Hexagonal H_2	0.11	66
		Bicontinuous cubic *Im*3*m*	0.05	197
	Pepsin	Lamellar	0.13	47
		Hexagonal H_2	0.11	66
		Bicontinuous cubic *Im*3*m*	0.05	197
	FGL+pepsin	Lamellar	0.13	47
		Hexagonal H_2	0.11	66
		Bicontinuous cubic *Im*3*m*	0.05	189
PC700+AMF	None	Lamellar	0.13	47
		Hexagonal H_2	0.10	71
		Bicontinuous cubic *Im*3*m*	0.04	202
	Pepsin	Lamellar	0.13	48
		Hexagonal H_2	0.10	70
		Bicontinuous cubic *Im*3*m*	0.04	202
	FGL+pepsin	Lamellar	0.13	48
		Hexagonal H_2	0.10	70
		Bicontinuous cubic *Im*3*m*	0.04	202
Milk	None	Lamellar	0.14	46
		Hexagonal H_2	0.11	65
		Bicontinuous cubic *Im*3*m*	0.05	185

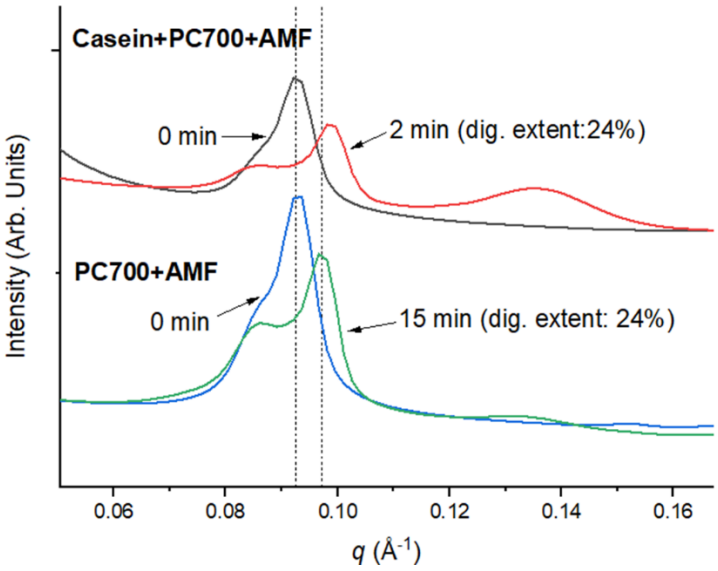

Figure S5. SAXS profiles of PC700- and casein+PC700-emulsified fat globules before digestion (0 min) and after digestion by pancreatic lipase (15 min for PC700 only and 2 min for casein+PC700) at pH 6.5.