Light Microscopy and TEM to Study the Effect of Biopolymers on Ice Recrystallization in Ice Cream

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Hydrocolloid stabilizers are widely used in ice cream because they hinder ice crystal growth as temperature fluctuates during storage [1]. However, their mechanisms of action are still uncertain. Light microscopy (LM) and transmission electron microscopy (TEM) techniques have been applied to study microstructure and ice recrystallization in ice cream model solutions. Sucrose solutions with or without stabilizers (carboxymethyl cellulose (CMC), xanthan gum, locust bean gum (LBG), and gelatin) and with or without milk solid-non-fat (MSNF) were frozen in a scraped surface heat exchanger and temperature cycled (5 cycles from -6^{o}C to -20^{o}C). Brightfield images were acquired from samples before and after cycling. Measurements of ice crystal size were made by manually tracing the perimeter of the crystal with a computer mouse, the area of each crystal was automatically calculated by the software *Scion Image 1.62* [2]. Ice crystal size distributions were characterized by the logistic dose response model [1]. In the absence of milk proteins, xanthan and LBG were the most effective stabilizers at retarding recrystallization; while in their presence, only xanthan had an effect.

Cycled samples were freeze-substituted (3% v/v glutaraldehyde) and low-temperature-embedded (LR Gold). Resin blocks were sectioned at a thickness of 0.5μm for LM and 90nm for TEM. Stabilizer gel-like structures were observed in sections from LBG, gelatin and gelatin/MSNF solutions after being subjected to differential staining (leucobasic fuchsin for carbohydrates and amido-black for proteins [3]) in LM and uranyl acetate-lead citrate staining in TEM [4]. Representative LM (Fig. 1) and TEM (Fig. 2) micrographs show thermodynamic incompatibility and phase separation between biopolymers that promotes localized high concentrations of milk proteins located at the ice crystal interface, probably exerting a water-holding action that significantly enhances the stabilizer effect in retarding recrystallization. Phase separation was directly proportional to ice crystal growth inhibition [5].

References

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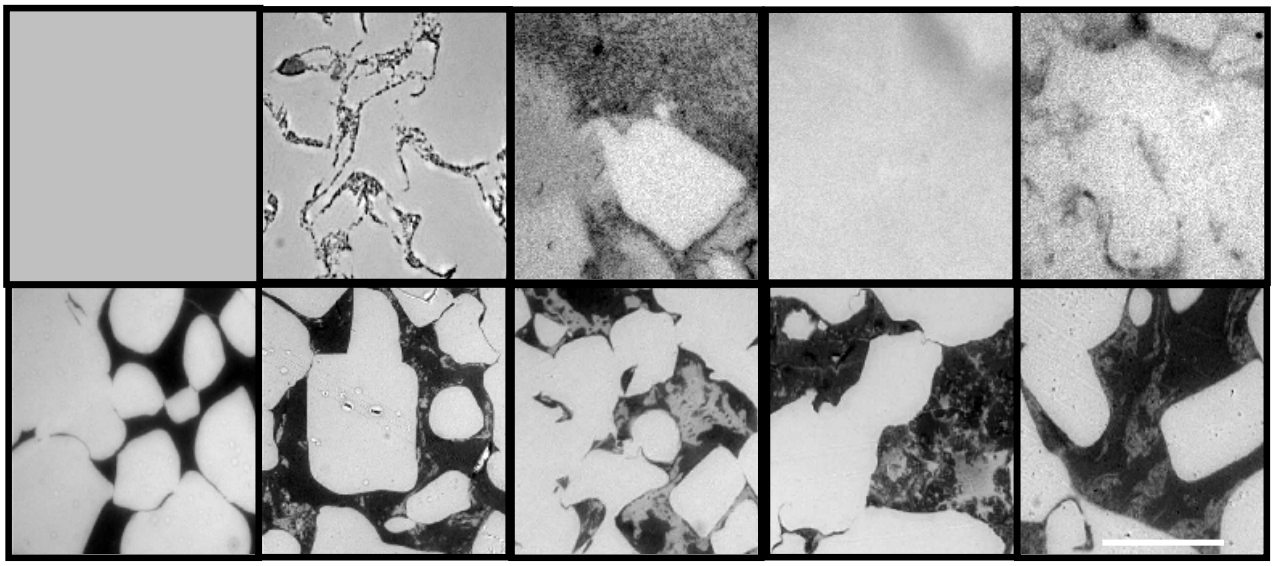

Fig. 1. LM micrographs of freeze substituted and low temperature embedded samples after double staining with leucobasic fuchsin and amido-black. Samples contain sucrose a) without MSNF, b) with MSNF, 1) control, 2) LBG, 3) xanthan 4) CMC, 5) gelatin. *No results.

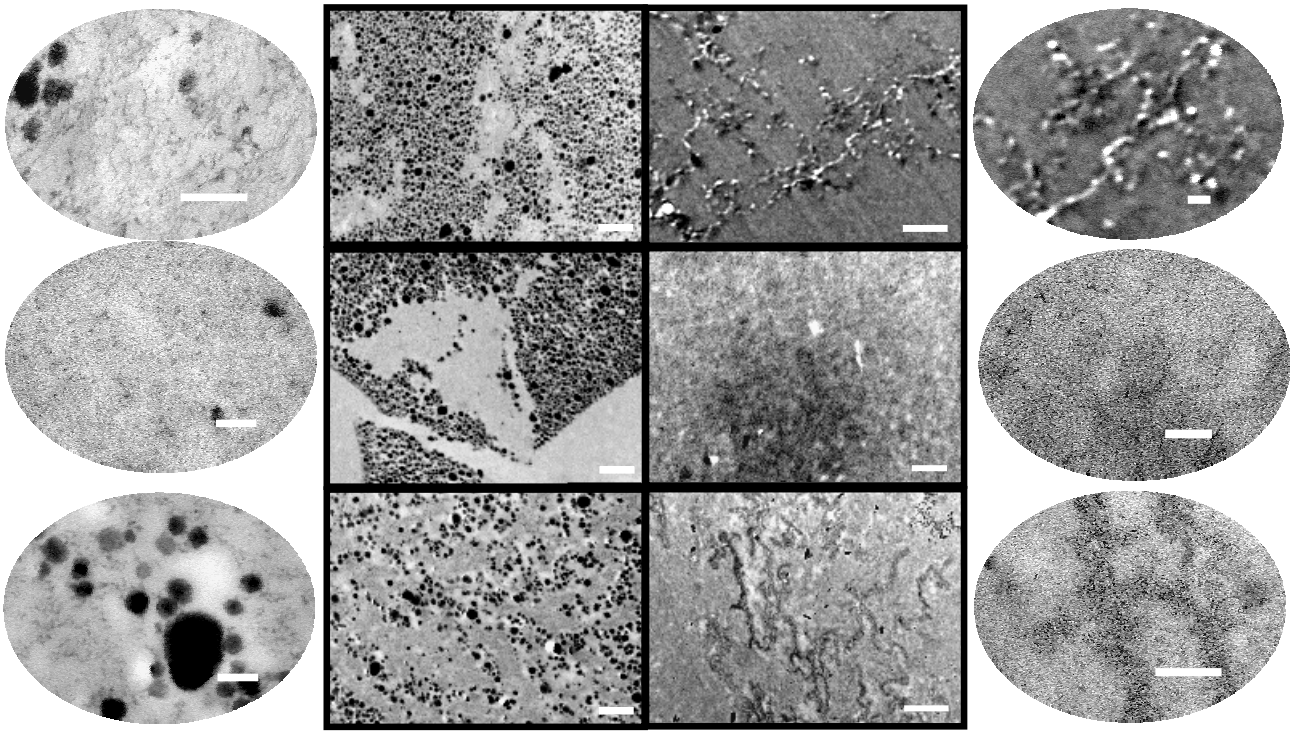

Fig. 2. TEM micrographs of freeze substituted and low temperature embedded samples after uranyl acetate-lead citrate staining. Samples contain sucrose a) with MSNF, b) without MSNF, 1) LBG, 2) xanthan, 3) gelatin. Calibration bar for square images=1µm, for oval images=0.2µm.

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