Casein Micelle- kappa-Carrageenan Interactions in Milk Systems

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Introduction

The functionality of κ-carrageenan (κ-carr) in dairy systems has been well known and studied for many years. One aspect of "milk reactivity" of κ-carr is its ability to inhibit phase separation between milk proteins and polysaccharides at very low concentrations (0.015-0.02%, in 4% protein/0.14% locust bean gum (LBG) systems) [1], although we have previously shown that such "stable" systems remain microscopically phase separated into water-in-water emulsion structures, with discrete protein enriched domains in a continuous protein depleted phase [2,3]. Furthermore, it has also been determined that the κ-carr is associated with the protein enriched phase [3].

It has been proposed that the negatively charged κ-carr interacts with a positively charged region of para-κ-casein, thus adsorbing to the surface of the casein micelle [4,5]. However, the surface of the micelle is negatively charged and stabilized sterically by the the glycomacropeptide portion of κ-casein, the so-called hairy layer. Thus, for both steric and electrostatic reasons, stabilization of the micelle by κ-carr through surface adsorption seems unusual. It has also been proposed that stabilization is via κ-carr weak gel formation, which holds casein micelles suspended [6]. The purpose of this research was to elucidate on the mechanisms of interaction between κ-carr and casein micelles.

Materials and Methods

•Solutions: skim milk powder (SMP), LBG, guar gum, λ-carrageenan, κ-carr from Kappaphycus alverezii "cottonii" (Danisco), and agarose (Sigma Chemicals), weight ratio equivalent to 3.2% casein/0.015% κ-carr as control.
•Field emission scanning electron microscopy (FE-SEM): samples adsorbed to carbon planchets, fixed with glutaraldehyde and osmium tetroxide, dehydrated with ethanol, critical point dried, viewed with Hitachi S4800.
•Dynamic light scattering (DLS, Malvern): Model systems adjusted to maintain casein: κ-carr ratio's as above.

References

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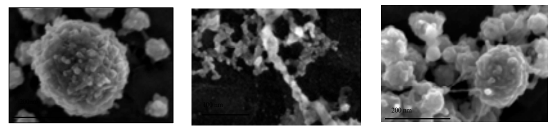
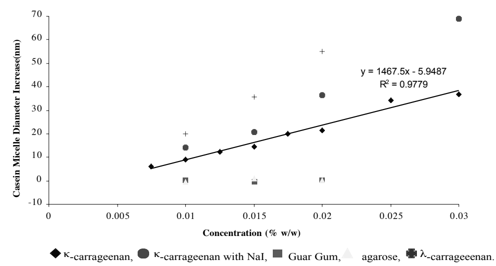
Results and Discussion



The structures of casein micelles (left), κ-carr (middle) and mixtures of the two (right) have been examined by FE-SEM. It appears that κ-carr has interacted with the casein micelle, but no information is gained about the nature of the interaction.



Casein micelle diameter increases upon addition of either κ-carr or λ-carr. There is no increase in diameter with the addition of either guar or agarose.

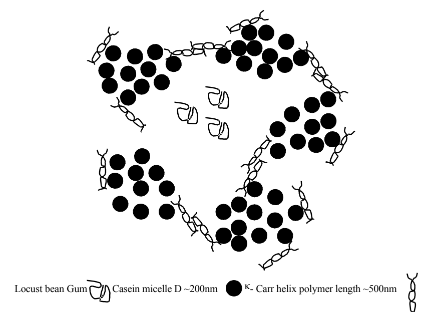
We conducted several phase separation experiments with SMP and LBG systems to determine the conditions under which κ-carr is effective at inhibiting separation. Above 60°C there was separation whereas below 60°C there was none indicating that the helical form of κ-carr is required to inhibit separation. λ-carr was not effective at inhibiting separation. It was shown to interact with casein micelles but does not form helices or aggregate and this implies that helix formation is necessary to inhibit phase separation. Sodium iodide blocks κ-carr helix formation. In the presence of NaI, κ-carr did not inhibit phase separation. DLS showed casein micelle diameter increase in the presence of NaI, which implies that κ-carr/casein micelle interaction is not sufficient to inhibit phase separation, helix aggregation also required. Agarose addition resulted in no increase in casein micelle diameter and was unable to prevent phase separation, which suggests that interaction is also necessary to achieve macroscopic stability.

Conclusions

•κ-carr appears to adsorb to the surface of the casein micelle.
•DLS shows that the increase in casein micelle diameter is highly linear with κ-carr and shows increase with κ-carr in presence of NaI as well as with λ-carr
•Helix form of κ-carr and helix aggregation are required for κ-carr to inhibit phase separation between casein micelles and LBG, in addition to adsorption.

Proposed Stabilization Mechanism

Our objective was to explain how κ-carr stabilizes milk protein:polysaccharide mixed systems from macroscopically phase separating. Based on these results, together with those of our earlier work, we envision a mechanism whereby κ-carr adsorbs to casein micelles at the periphery of the microscopically-separated, casein-enriched domains. κ-carr helix aggregation is then able to prevent these domains from coalescing and creaming. A schematic diagram is illustrated below.



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