

## **Destabilization of protein-based emulsions caused by bacteriostatic emulsifiers**

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For milk-based emulsion products such as canned coffee or tea, the addition of bacteriostatic emulsifiers is necessary to inhibiting the growth of heat-resistant sporeformers. Since bacteriostatic emulsifiers often cause the destabilization of emulsions, other type of emulsifiers, such as stability-enhancing ones, are necessary for the long-term stability of emulsions. Four milk-based emulsions were prepared from powdered milk combined with several types of emulsifiers. The long-term stability of emulsions, which was detected by the occurrence of a creaming layer after 3 months of storage, differed according to the composition of emulsifiers. To understand the reason for the difference in the stability of emulsions, particle size, distribution,  $\zeta$ -potential, and the amount of proteins and phospholipids present in the cream layer (separated oil droplets) in the emulsions were measured. Only the amount of proteins adsorbed onto oil droplets was found to be closely related to the difference in emulsion stability, that is, the more proteins adsorbed, the higher the emulsion stability. SDS-PAGE analyses revealed that  $\kappa$ -casein and  $\beta$ -lactoglobulin play an important role in emulsion stability by adsorbing onto the oil droplet surface.

Diglycerol esters of fatty acids, a representative of the bacteriostatic emulsifiers used for the canned beverages promotes not only creaming and aggregation but also coalescence of oil droplets stabilized by milk proteins under certain conditions. As a systematic and fundamental approach to clarify relationships between the structure and the destabilizing effects of the emulsifiers concerning oil phase types, a wide variety of polyglycerol esters of fatty acids with different polymerization of glycerols and various chain lengths of fatty acid residues were provided. The author focused on the effects of the molecular structural similarity between oil phase and fatty

acid residues of the emulsifiers, and the size of polar head of the emulsifiers on the destabilization of the protein-based emulsions.

Destabilizing effects of diglycerol esters of different mono-saturated or unsaturated fatty acids (DF) on protein-based emulsions prepared with various types of oil were examined by visual observations and particle size analyses. By diglycerol esters of mono-oleic acid (DO), hydrocarbon emulsions were more obviously destabilized than food oil emulsions under static conditions. Interfacial tension measurements indicated that the adsorbed protein on oil droplet surfaces of hydrocarbon emulsions can be more easily displaced by DO compared to the case of food oil emulsions. The degree of hydrocarbon emulsion destabilization by DO varied with the chain length of hydrocarbon molecules. From the results of combination tests of five hydrocarbons varying in chain length in oil phase and five DF having different mono-fatty acid residue, it was revealed that DF could most effectively destabilize the hydrocarbon emulsion when the chain length of fatty acid residue of DF was similar to that of hydrocarbon molecules.

Under agitating conditions, DO causes severe coalescence of droplets consisting of food-grade oil stabilized by milk proteins and phase separation between oil and aqueous phase. In order to clarify the destabilizing mechanism of the emulsifier, physicochemical and colloidal properties of the emulsifier were compared to similar emulsifiers without destabilizing effects. DO, that is dispersible both in oil and water, adsorbed to the oil-water interface to reduce the interfacial tension, and migrated from oil phase to aqueous phase and vice versa in a plane interface system. Experiments performed in an emulsion system revealed that DO had little ability to emulsify food-grade oil, but displaced milk proteins from the oil droplet surface. These data indicate that DO with little emulsifying ability predominantly occupies the oil droplet surfaces via emulsifier-protein competitive interactions to promote severe coalescence of emulsion oil droplets probably because DO actively migrates between oil and aqueous phase and it favors to form the planar oil-water

interface.

Lastly, the author developed a novel method for rapid determination of aggregation forces between colloidal particles such as emulsion oil droplets and suspension particles to evaluate the long-term storage of the milk beverages rapidly and simply. Long-term stability of milk-based emulsions in canned coffee or tea varies with the formulation of small-molecule emulsifiers aiming at bacteriostatic effects or stability-enhancing effects. To predict the long-term stability of the emulsions in short-time period, we developed a novel method for rapid determination of aggregation forces between colloidal particles using model emulsions and suspensions with different stability to aggregation. The novel method was based on an idea that particles with stronger aggregation forces tend to form aggregates and cannot be readily redispersed. While the milk-based emulsions were subjected to long-term storage test with coffee extract, the same emulsions, but not including coffee extract, were rapidly evaluated by both the novel method and a common method, Turbiscan analysis often applied to the evaluation of emulsion stability. Statistical regression analysis according to the datasets obtained by the two rapid assays revealed that the long-term stability of the milk-based emulsions can be better predicted by the aggregation forces evaluated by the newly developed method than the initial aggregating process evaluated by Turbiscan method. The author named the novel method “vibration-redispersion method”.