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# Depletion flocculation effects in egg-based model salad dressing emulsions

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**Abstract**

The influence of dehydrated egg white (DEW) addition on the droplet aggregation of yolk-based model salad dressing emulsions was studied in an attempt to investigate the role of the egg albumen fraction in the physicochemical changes that take place during the storage of acidic emulsions containing whole egg. Analysis of the adsorbed protein in emulsion by the application of SDS–PAGE provided strong evidence for the almost complete exclusion of the egg albumen from the droplet surfaces by the more flexible yolk lipoproteins. The results on emulsion stability are discussed in terms of possible depletion effects that may arise from the presence of the non-adsorbed egg white proteins in the continuous phase. Observations of emulsions under the light microscope and rheological measurements are presented to support the hypothesis that the extensive droplet–droplet aggregation effects, arising from the presence of unadsorbed albumen proteins, result in emulsion destabilization against creaming.


*Keywords:* Emulsion; Depletion flocculation; Salad dressings; Egg protein; Stability

## 1. Introduction

The preparation and long-term physicochemical stability of salad dressing emulsions depend on the presence of egg yolk in the system. The lipoproteins of yolk rapidly adsorb during the initial stages of emulsion formation and rearrange at the oil droplet surfaces leading to the reduction of surface tension and the development of an adsorbed membrane that favor oil dispersion and droplet stabilization against coalescence (Kiosseoglou, 2003a, b). Salad dressings with a relatively high oil content, e.g. mayonnaise, are practically stable against creaming due to increased hydrodynamic hindrance imposed on oil droplet movement by the very high droplet packing fraction. When the oil content is reduced below 60–65%, the emulsions become unstable and addition of a hydrocolloid is required to prevent undesirable serum and oil separation during storage (Dickinson, 2003).

Although the yolk is a very effective emulsifier, whole egg is often used in its place for the preparation of salad dressings since the white fraction of egg as well as that of yolk is a material rich in proteins of high biological value. Additionally, commercially available yolk is usually contaminated with egg albumen (Powrie & Nakai, 1985). As was reported by Shenton (1979), the egg white proteins, due to their globular nature are competitively displaced from the oil–water interface by the more flexible, surface-active yolk lipoproteins. Similar results were reported by other investigators for the whey proteins or the more flexible caseins (Aluko, Keeratiurai, & Mine, 1998; Mine & Keeratiurai, 2000). On the other hand, Kiosseoglou and Sherman (1983) observed that addition of egg white to yolk-based mayonnaise brought about an enhancement of emulsion viscoelasticity parameter values indicating that the white proteins were somehow involved in droplet interaction of the mayonnaise emulsion. As suggested by Kiosseoglou (2003a, b), these effects should have been the result of the strengthening of the physical interactions between the oil droplets since the non-adsorbing egg white proteins might have enhanced depletion phenomena in the emulsion system by analogy to other proteins. According to Dickinson, Golding, and Povey (1997), unadsorbed casein submicelles in the emulsion continuous phase above

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some critical concentration may induce depletion flocculation to oil droplets and formation of droplet aggregates that rise at a higher rate compared to single droplets, leading to rapid creaming and destabilization of the system. Alternatively, at relatively high oil contents, a very strong droplet network that reorganizes very slowly may form resulting in an emulsion that is very stable against creaming and serum separation during storage (Berli, Quemada, & Parker, 2003).

The present work aims at investigating the role of egg white proteins in influencing the stability against droplet flocculation and creaming of model oil-in-water emulsions based on yolk. As the albumen proteins may become competitively excluded from the droplet surfaces by the more flexible yolk proteins, it would be of interest to determine whether the white fraction of egg influences the long-term physical stability of food emulsion products where both egg fractions are encountered.

## 2. Materials and methods

### 2.1. Materials

Fresh eggs and refined corn oil were bought from the local market. The oil was used without further purification. Dehydrated egg white (DEW) was purchased from Sigma Chemical Co. Its protein content determined by Kjeldahl was 85% (w/w). All the chemicals for the buffers used in the preparation of the emulsion as well as those used for the electrophoretic analysis of proteins were products of Sigma Chemical Co.

### 2.2. Emulsion preparation

The eggs were initially broken manually and the yolks separated. Following removal of adhering albumen from the yolk by rolling on tissue paper, the vitelline membrane was pierced and the liquid yolk was collected. A 10% (w/v) liquid yolk dispersion was produced by adding under continuous stirring, the appropriate amount of yolk in 20 mM citrate buffer (pH 3.8), containing 0.15 M NaCl and 0.01% (w/v) $NaN_3$. A stock emulsion with 60% (v/v) oil content was then prepared by slowly introducing the corn oil into the yolk dispersion while agitating with the aid of a mechanical stirrer. The resulting crude emulsion was finally homogenized for 1 min by employing an Ultra Turrax T25 (IKA Labortechnik, Germany) homogenizer equipped with a S25KG-25F dispersion tool operating at 24,000 rpm. Portions of the stock emulsion were diluted with appropriate volumes of citrate buffer and an egg albumen solution (8% (w/v) in DEW) in citrate buffer to obtain a series of emulsion samples with a final oil volume fraction of 0.3 and containing 0%, 0.5%, 1.0%, 1.5%, 2.5% or 4.0% (w/v) DEW. Three emulsion series were prepared.

### 2.3. Particle size measurements

The emulsion samples were diluted (1:1000) using deionized water and the droplet size distribution was determined by laser light scattering, employing a Malvern Mastersizer 2000 unit (Malvern Instruments, UK). The following optical parameters were applied: refractive indices of corn oil and water 1.4673 and 1.3300, respectively; absorption: 0.002. Application of the laser light-scattering technique was performed following the slow agitation of the diluted emulsion for 1 min or after intense agitation in presence of 0.1% SDS for 1 h to determine the size of the deflocculated emulsion droplets. Particle size data are reported either in the form of particle size distribution curves or as weight-average diameters $D[4,3]$ $(=\Sigma n_i d_i^4/\Sigma n_i d_i^3)$, where $n_i$ is the number of particles with diameter $d_i$. At least three measurements were conducted on each emulsion sample and the mean particle diameter reported was calculated as the average of three measurements conducted on each one of the three different emulsion samples prepared.

### 2.4. Emulsion stability

A quantity (10 mL) of each emulsion was poured into a cylindrical glass container (internal diameter, 18 mm; height, 65 mm), sealed with a plastic cap, and stored at room temperature for a period of a few days. The stability of the emulsion was measured by following the height of the visible serum separation layer, $H_t$, with storage time. The stability of the emulsion was expressed as $\%\text{serum} = (H_t/H_0) \times 100$, where $H_0$ represents the initial emulsion height.

Preliminary droplet size measurements indicated that all the samples were very stable with respect to droplet coalescence as the mean size of the isolated droplets remained unaltered during the time period of the creaming stability observation.

### 2.5. Optical microscopy

An Axiolab A reflected light microscope (Zeiss, Germany) equipped with a Canon Power Shot G 2 photographic camera was employed to determine the structural features of selected emulsion samples. The samples were examined under the microscope without any previous dilution.

### 2.6. Rheological measurements

The emulsion rheological properties were determined with the aid of a Brookfield DVII, LV viscometer (Brookfield Engineering Laboratories Inc., USA) combined with a SC4-18/13R small sample adapter (concentric cylinder geometry). All the measurements were conducted at 25 °C, 30 min after the sample was loaded. Mean viscosity values were calculated as the average of three