Fractionation of liquid egg yolk: 
Influence of chemical and structural characteristics of egg yolk granular and plasma fraction on the continuous centrifugal separation process  
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ABSTRACT 
Egg yolk is an essential ingredient for the preparation of a large variety of food emulsions. Thereby the emulsifying properties of egg yolk depend mainly on the physiological status of the two egg yolk fractions, granules (HDL) and plasma (LDL), which can be influenced by environmental conditions. Recent studies substantiate that a separate use of each fraction in innovative food products will offer new ways in emulsification technology. It became evident that the granula fraction represents no homogeneous component, but consists of different subclasses with different size, density and structure. This inhomogeneity leads to a subclass depending sedimentation behaviour of the whole granula fraction in egg yolk. A variation within the egg yolk pH to acidic milieu conditions results in changes of each subclass constitution. Especially sub fractions with small particle size and high protein content incorporate a higher amount of granular LDL. Therefore, the particle density is decreased, which leads to a much lower sedimentation velocity. Based on these findings, the impact of variable rheological properties on the sedimentation behaviour of polydisperse colloidal suspensions with high particle concentrations is discussed. It was detected that if a limiting particle concentration is reached all particles within the polydisperse particle collective are sedimenting with the same velocity. At the so called zone-sedimentation the high fluid viscosity as well as strong particle-particle interactions are resulting in constrained separation effects. The results allow a deeper insight in the structural complexity of the LDL and HDL egg yolk fraction and offer therefore new information for the techno-functional properties of egg yolk fractions. 

Keywords: egg yolk; LDL; HDL; fractionation; continuous centrifugation;  

INTRODUCTION  
Protein stabilised emulsions play a major role in many areas of the food industry [1, 2]. One of the most complex protein-based emulsifier systems is hen’s egg yolk. Fresh egg yolk contains about 48-50% total dry matter, of which 80% is the water-soluble plasma fraction and 20% is present in the form of insoluble granules. Plasma contains mainly low-density lipoproteins (LDLs) and globular glycoproteins. LDL, whith apoproteins called lipovitellenins, is the main constituent of egg yolk. Granules contain 16% phosvitin, a phosphoprotein, and 70% high-density lipoprotein (HDL). In addition, 12% LDL can be found in insoluble granule aggregates. The complex between HDL and phosvitin is held together by phosphocalcic bridges. The emulsifying properties of the two main egg yolk fractions, egg yolk granules and LDL micelles, are highly dependent on environmental conditions. Changes in pH or salt concentration influence the chemical and structural characteristics for each fraction in a different way. Many studies [3, 4] have shown that LDL is likely to play primary roles in the formation and stabilisation of yolk-based emulsions. It is reported that the emulsifying activity of LDL is due to the apoproteins rather than the phospholipids. The apoproteins of yolk LDL have a great capacity to adsorb at the oil–water interface in emulsions, due to their amphipathic character and flexibility. Yolk granular proteins also have the ability to contribute in the building of the adsorbed layer although their adsorption and stabilizing potential seem to depend much more on the extent of structure disorganization [5, 6]. Granules can be dissociated by increasing the ionic strength to values above 0.3 M because of the rupture of phosphocalcic bridges. Dependent on their structure intact granules adsorb in a higher amount than dissociated granules, but form less stable emulsions [7, 8]. Furthermore, at pH 4 the interface formed with egg yolk is dominated by HDL–apoproteins, whereas the interface at pH 6.4 and high ionic strength is
dominated by LDL–apoproteins and phosvitin, which becomes available due to a breakdown of the granule structure [9, 10]. Recent studies substantiate that a separate use of each fraction in innovative food products will offer new ways in emulsification technology. However, so far no systematic study deals with the separation process as a function of physico-chemical factors. Therefore the present study applies a wide range of compositional conditions and centrifugal separation parameters enabling the determination of the optimal operating conditions for the centrifugation of liquid egg yolk. A pilot scale bowl centrifuge is used to evaluate the process parameters varying centrifugal acceleration, temperature and dilution. The influences of different pH and salt concentrations, where granules are in their native and a disintegrated form, are investigated. As well the impact of variable rheological properties on the sedimentation behaviour of polydisperse colloidal suspensions with high particle concentrations is discussed.

MATERIALS & METHODS

Materials
Commercial pasteurised refrigerated liquid egg yolk (pH 6.35 ± 0.1) was purchased from Ovobest, Neunrichten-Vörden, Germany. The total dry matter was 46% (± 1.0). Egg yolk suspensions were diluted by an isotonic sodium chloride solution (0.15 M NaCl) to total dry matters varying between 34% and 10%. Hydrochloric acid and sodium hydroxid were used to adjust the pH-values to 4.0 or pH 10.0, respectively. NaCl was added to obtain an ionic strength of 0.55 M. The separation studies were performed on a lab scale centrifuge “contifuge stratos” (Heraeus, Thermo scientific, Langenselbold, Germany) equipped with a titanium continuous flow rotor.

Determination of the separation efficiency
For biological systems, where dissociation of fine particles due to changed milieu conditions occurs, equation 1 was used to determine the separation efficiency:

\[ \eta = \frac{\dot{m}(DM)_{sed}}{\dot{m}(DM)_{max}} \]  

\(\dot{m}(DM)_{sed}\) = sedimeted granula dry matter mass flow rate

\(\dot{m}(DM)_{max}\) = maximum of separable granula dry matter mass flow rate

The maximum of depositing granula at physiological milieu conditions was assessed by a modified method of McBee and Cotterill [11]. The egg yolk was at first diluted (1:2, w/w) in an isotonic sodium chloride solution (0.15 M NaCl) and stirred gently for 1 h before centrifugation at 10.000 g for 45 min at 10 °C. The supernatant plasma was collected, recentrifuged and the sedimented granules of both steps were weighed. The dry matter DM of the egg yolk solution, the plasma and granula fraction was determined by the sea sand method according to handbook of analysis methods, volume 6 C, 35.3, VDLUFA, 1985.

Rheological Measurements
Rheological measurements were carried out with a controlled shear rate rheometer AR 1000 from TA Instruments (Eschborn, Germany) equipped with cone plate geometry (d=6 cm; angle: 2°, gap width 400 µm). Shear rate \(\gamma\) was increased linearly from 0 to 1000 s\(^{-1}\) over 5 min, then held at 1000 s\(^{-1}\) for 2 min and then decreased linearly back to 0 s\(^{-1}\) over 5 min. Measurements were carried out at 4 °C. The descending part of the flow curve was modelled using the Herschel-Bulkley model (equation (2)), where \(\tau\) is the shear stress, \(\tau_0\) the yield stress, \(K\) the consistency index and \(n\) the flow index.

\[ \tau = \tau_0 + K \gamma^n \]  

The flow index \(n\) is used to characterise the flow behaviour. The consistency index \(K\) is a characteristic criterion for the internal structure of a fluid.
RESULTS & DISCUSSION

Influence of g-force, dilution and temperature

As already known, the sedimentation behaviour of particles in the liquid phase is characterised by its sedimentation velocity [12]. Increasing the solids content results in an increase of hindering effects like counter flow of displaced liquid or rise in density and viscosity of the suspension. To evaluate the effect of the solid content in egg yolk solutions on the sedimentation behaviour of granula particles the dry matter was varied between 10% and 34%.

![Graph showing separation efficiency η of the egg yolk granula fraction in dependency of the g-force for different dry matter concentrations in the egg yolk solution (22 % and 34 %) and product temperatures (4°C and 50°C).]

As can be seen in Fig. 1, an increase in the egg yolk solutions dry matter content from 22% to 34% results in a considerable decrease of the separation efficiency. Also, at a dry matter concentration of 34 %, an increase of the g-force above 5000g does not improve the sedimentation efficiency. If a limiting particle concentration is reached, all particles within the polydisperse particle collective are sedimenting with the same velocity. At the so called zone-sedimentation the high fluid viscosity as well as strong particle-particle interactions are resulting in constrained separation effects.

![Graph showing flow index n as a function of the dry matter DM content in egg yolk solutions (pH 6.4 and 0.15 M NaCl, temperature kept constant at 4°C). Graph B: Viscosity of egg yolk solutions as related to different shear rates for the pH of 4.0 and 6.4 (0.15 M NaCl, temperature kept constant at 4°C).]

Figure 1. Separation efficiency η of the egg yolk granula fraction in dependency of the g-force for different dry matter concentrations in the egg yolk solution (22 % and 34 %) and product temperatures (4°C and 50°C).

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Figure 2. A: Flow index n as a function of the dry matter DM content in egg yolk solutions (pH 6.4 and 0.15 M NaCl, temperature kept constant at 4°C). B: Viscosity of egg yolk solutions as related to different shear rates for the pH of 4.0 and 6.4 (0.15 M NaCl, temperature kept constant at 4°C).
The flow index of egg yolk solutions was calculated and is presented in Fig. 2A. Due to the enhancement of the dry matter content of 22 % to 34 % the flow index $n$ changes from $n=1.0$ to $n=0.78$ (for 4°C). The fluid rheology alters from a Newtonian to a shear-thinning behaviour. The observed effect is reinforced by higher dry matter concentrations. Beiser et al. [13] suggests that the formation of a uniform settling matrix could indicate the formation of a network all over the whole suspension. This interacting network leads to a complete sedimentation blocking.

In Fig. 1 the influence of the product temperature is also shown. At a dry matter content of 22 % a product temperature of 50 °C leads to a much better separation efficiency of the granula components. As Fig. 1 demonstrates, it was possible to reach a separation efficiency above 95 % at a product temperature of 50 °C and within g-forces higher than 5.000g. For both temperatures, 4°C and 50°, but the same dry matter concentration 22% the flow index $n$ was measured to $n=1$ (results not shown). This indicates that the fluid flow behaviour for 4°C and 50°C was Newtonian although the viscosity decreased by a factor of five. The remarkable increase in the screening of granula particles can be explained by the fact that particle collectives tend to segregate with decreased fluid viscosities due to higher product temperatures. Therefore, the sedimentation velocity of each particle collective is increased.

**Influence of pH and ion concentration**

In Fig. 3 the influence of the pH and electrolyte concentration on the separation efficiency of egg yolk granula is illustrated. At an acidic pH the clearing of granula particles is decreased. Especially at low g-forces (2.000g) only 15 % of the granula fraction could be separated. It became evident that the granula fraction represents no homogeneous component, but consists of different subclasses with different size, density and structure (results not shown). This inhomogeneity leads to a subclass depending sedimentation behaviour of the whole granula fraction in egg yolk. A variation within the egg yolk pH to acidic milieu conditions results in changes of each subclass constitution. Especially sub fractions with small particle size and high protein content incorporate a higher amount of granular LDL. Therefore, the particle density is decreased, which leads to a much lower sedimentation velocity.

![Figure 3](image-url)

**Figure 3.** Separation efficiency $\eta$ of the egg yolk granula fraction in dependency of the g-force for different pH values (6.4 and 4.0) and salt concentrations (0.15 M and 0.55 M NaCl).

The phenomena of the LDL inclusion in HDL complexes can be explained by the isoelectric point IEP of phosvitin which is calculated to pH 4.0. At the physiological pH of egg yolk the negative net charge of phosvitin (about −179 mV) prevents interactions between HDL lipovitellins and LDL apoproteins. At the IEP of phosvitin the strong repulsive forces do not exist. In addition to a pH at acid values the continuous phase viscosity is drastically increased.
Fig. 2B shows the flow behaviour of two egg yolk solutions varying the pH between 6.4 and 4.0. The calculated consistency index K was 154.8 mPas and 14.8 mPas for a pH of 4.0 and a pH of 6.4, respectively. It becomes obvious that the fluid rheology changes completely due to an acidic pH. The high consistency index at acidic pH values is due to an increase of the effective volume of dispersed phase because the continuous phase is trapped between the new-formed HDL and LDL complexes.

Increasing the salt concentration from 0.15 M to 0.55 M NaCl results in a strong decrease of the separation efficiency. Over the whole g-force range between 2.000g to 10.000g the separation efficiency was very low at 8%. The effect of high sodium chloride concentrations (>0.3M) on the granula stability is well known and reported by different authors. At high ionic strength, granules show an increased protein solubility because of the disruption of phosphocalcic bridges, where sodium ions replace calcium ions. The dissociation of granules results in small submicron fragments, which cannot be separated by centrifugal forces.

Results suggest that significant differences in the separation efficiency primarily derive from structural changes in egg yolk granules and LDL micelles. The results also allow a deeper insight in the structural complexity of the LDL and HDL egg yolk fraction.

CONCLUSION

Results suggest that significant differences in the separation efficiency primarily derive from structural changes in egg yolk granules and LDL micelles. At an acidic pH the clearing of granula particles is decreased. It became evident that the granula fraction represents no homogeneous component, but consists of different subclasses with different size, density and structure. This inhomogeneity leads to a subclass depending sedimentation behaviour of the whole granula fraction in egg yolk. A variation within the egg yolk pH to acidic milieu conditions results in changes of each subclass constitution.

Based on these findings, the impact of variable rheological properties on the sedimentation behaviour of polydisperse colloidal suspensions with high particle concentrations is discussed. It was detected that if a limiting particle concentration is reached all particles within the polydisperse particle collective are sedimenting with the same velocity. At the so called zone-sedimentation the high fluid viscosity as well as strong particle-particle interactions are resulting in constrained separation effects.

The study offers a detailed description for the separation of egg yolk granula and plasma fraction under varying parameters in a laboratory scale as well as in a production scale. Additionally, the results allow a deeper insight in the structural complexity of the LDL and HDL egg yolk fraction and offer therefore new information for the techno-functional properties of egg yolk fractions.

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