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HEAT-SET GELS BASED ON OIL/WATER EMULSIONS: AN APPLICATION OF WHEY PROTEIN FUNCTIONALITY

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Abstract

The microstructure of protein/lipid gels produced by heat treatment of whey protein stabilized oil-in-water (O/W) emulsions was studied. Scanning electron microscopy as well as transmission electron microscopy was performed on glutaraldehyde/osmium tetroxide fixed, and critical point-dried samples. Microstructure analysis showed that in the case of homogenized O/W emulsions, extensive coating of the fat globule surface with coagulated protein led to a "cauliflower-like" structure. In such gels, uncoated fat globules having smooth surfaces were not present. This specific microstructure was not obtained with protein/lipid gels in which whey protein was added in the continuous phase and mixed with a O/W emulsion stabilized by lecithin.

Introduction

The most important functional property of whole egg is its ability to form heat-set gels. This "functionality" is exploited to form textural properties in culinary applications of egg. To design a functional egg substitute from whey protein, we developed a process in which whey protein concentrates are combined with selected lipids of vegetable or animal origin. High pressure homogenization of such compositions leads to stable O/W emulsions in which whey protein acts as emulsifier and, at a subsequent process stage, as the gelling agent (Jost et al., 1986).

A remarkable feature of the gelling O/W emulsion is the fact that lipids, although having no gelation capacity on their own, contribute to the firmness of the gelled texture, under the condition that they are present as a homogeneous population of small droplets. Critical droplet size and size distribution parameters were, e.g., 700 nm diameter for the mode of the distribution as an upper limit and a polydispersity index of < 0.35 (Baechler et al., 1986).

Another feature of gels produced from such O/W emulsions is their smooth texture and lipid-like "mouth-feel". Microstructure studies of such protein/lipid gels were aimed at finding characteristics in their fine structure which might explain textural properties and elucidate the role of high pressure homogenization.

Materials and Methods

O/W emulsions were produced from whey protein concentrates (aqueous phase with 5-10 % w/v of WPC solids at pH ranging from 5.5 to 8.0) and from sunflower oil as previously described (Jost et al., 1986). The composition used for microstructure studies was an emulsion with 15 % (v/v) of sunflower oil and 7.5 % WPC solids, corresponding to 6 % of protein. The pH of our emulsions was adjusted to 7.0 by addition of KOH(1 N) or NaOH(1 N), prior to gelation. Heat-induced gelation of the degassed O/W emulsions was performed in hermetically closed glass beakers (25 ml total volume, 27 mm inner diameter) placed in a metal rack for the incubation in a water bath at 90°C.
for 30 min. Following cooling to room temperature, the gels were aged 24 h at 4°C prior to further processing.

Measurement of particle size distribution in emulsion was done by dynamic light-scattering, using a Malvern photon correlation spectrometer (Masson and Jost, 1986).

Scanning electron microscopy (SEM)

Gel slices (1 mm x 2 mm x 6 mm) were fixed in phosphate (0.1 M, pH 7.2) buffered 3% glutaraldehyde. Fixation was performed for about 12 h and was followed by post-fixation in 2.5% osmium tetroxide for 2 h. Glutaraldehyde fixation had been shown to preserve particularly well the original structure of protein gels (Heertje and van Kleef, 1986). Critical point-drying in CO2 following the dehydration in alcohol was preferred to freeze-drying, thus minimizing the risk of ice crystal formation (Woodward and Cotterill, 1985).

Particles which had been freshly dry-fractured were mounted on SEM stubs with a conductive carbon cement and coated with a 20 nanometer layer of gold in a Polaron sputter coating unit. The preparations were observed in a Philips 505 SEM at an accelerating voltage of 30 kV.

For fixation of the (liquid) O/W emulsion, "deep-well" stubs were used. A drop of the emulsion was placed in the cavity of the stubs and covered with a Nuclepore filter membrane (0.4 μm), and sealed with a metal ring. At this stage, the stubs were processed in an analogous manner as the gels.

Transmission electron microscopy (TEM)

Small cubes of gel (1 mm3) were fixed as described for SEM. Dehydration was accomplished in a graded alcohol series up to 95% ethanol concentration. Infiltration was with mixtures of 95% alcohol and LR White (2/1 v/v overnight and 1/2 v/v for another 12 h). Subsequent infiltration of pure LR White in gelatin capsules was performed overnight at 4°C, followed by polymerization at 52°C. Thin sections (60 nm) stained with uranyl acetate and lead citrate were examined in a Philips 300 TEM at an accelerating voltage of 80 kV.

Results and Discussion

SEM of an unheated O/W emulsion (15% v/v of dispersed oil and 6% w/v of whey protein, pH = 7) shows primarily the fat globules in a perfectly spherical shape. The mean diameter established by light scattering analysis on the liquid parent emulsion, roughly 400 nm, corresponds well with the projected globule diameters as revealed by SEM (Fig. 1a). The protein which is essentially present in a soluble state is of rather discrete appearance or not visible at all.

Following heat-induced gelation of the emulsion, SEM shows an entirely different structure. Again, the lipid droplets are clearly visible but, in the gel, their surface is rough and structured (Fig. 1b) and at a higher magnification, a "cauliflower-like" structure is evident (Fig. 1c). The granulated surface structure of the fat globules is thought to result from extensive protein coagulation on the lipid surface. Smooth lipid globules are not seen nor can we distinguish protein coagulum not associated with the lipid globules, at least not in considerable amounts.
Whey protein gels based on O/W emulsions

It is very instructive to compare the fine structure of the gels based on the emulsion with a whey protein gel produced under the same heating conditions using a mere aqueous dispersion of whey protein concentrate (12% w/v of total solids or 9.6% of protein). Such a protein gel appears in SEM as a dense but porous structure composed of strands of protein granules (Fig. 2). The diameter of the protein granules which are roughly spherical, is near 100 nm. We can imagine that the same kind of protein granules melted together on the fat surface, leads to a micro-structure similar to that shown in Figs. 1b and 1c.

We learn from this comparison that in an O/W emulsion stabilized by whey protein and possibly other proteins, the dispersed lipids when coated with the protein, act as a matrix on which further protein will preferentially coagulate during the heating step. TEM of the gelled emulsions brings additional strong evidence in favour of a massive interfacial protein coagulation, resulting from the preceding homogenization step during and after which protein "migrated" from the continuous phase to the interface.

Thin sections show the protein coating of the lipid globules (Fig. 3a) and the higher magnification clearly shows how gelled protein crosslinks the coated fat globules (Fig. 3b). Additional experiments were done to highlight the particularity of the gelled emulsions and the role of homogenization in the presence of the protein for the formation of this structure. It appeared that the formation of a uniform and integrated protein/lipid microstructure could not be obtained unless homogenization at relatively high pressures (20-35 MPa, 2-5 passages) in the presence of the protein, preceded the gelation step. Stabilization of the emulsion resulting from protein migration to the interface is necessary to favour protein coagulation at the lipid surface. Varying the proportions of lipids and proteins will certainly modify the fine structure of the gel. As we increased the protein content of the emulsion and consequently the protein/lipid ratio, more protein coagulation took place in the continuous phase, while at protein/lipid ratios < 0.5, most of the protein was associated with the fat globules.

A good demonstration that interfacial protein coagulation depends on the presence of the protein during the homogenization step was obtained from SEM analysis of gels produced from a lecithin-stabilized O/W emulsion, to which whey protein was added. The protein was simply well
dispersed in the emulsion and the combined emulsion subjected to heat treatment. Gels obtained according to such a procedure were considerably weaker than their counterparts produced with protein stabilized emulsions. Weak gels were formed provided that the lecithin concentration was <1%, whereas at higher lecithin concentrations, the emulsion remained completely liquid after heating. SEM of lecithin-based gels show smooth lipid spheres, devoid of adsorbed protein, randomly distributed in the gel matrix made of whey protein (Fig. 4).

Conclusions

The particular texture and microstructure observed in whey protein/lipid gels reflects the sequence of operations during processing (Fig. 5). High pressure homogenization in the presence of protein is, in view of the desired gelation properties, to be preferred to the use of an emulsifier such as lecithin during the homogenization step required to produce the parent O/W emulsion. Subsequent heat-induced gelation then results in a particular "integrated" microstructure characterized by an extensive protein coagulation at the surface of the fat globules. Repeated homogenization at pressures of 20-40 MPa increases the fat surface available for protein adsorption and coating and thus favours the subsequent deposition of heat-coagulated protein at the interphase. With an increasing homogeneity of the dispersed lipids and a reduction of the fat globule size, the heat-set gel increases in its strength. Apparently, under these conditions, the fat globules assume the role of filler particles as proposed by Dickinson et al. (1985) who showed this effect with gelatin-based O/W emulsions.

Due to the volume fraction which is occupied by the fat globules in emulsion, the protein concentration is also increased in the continuous phase. This explains why in an emulsion, firm gels can be obtained at comparatively lower protein concentrations than in the case of aqueous dispersions of the protein.

In gelled O/W emulsions, proteins and lipids form an integrated particular microstructure which is macroscopically smooth and gives a pleasant "mouth-feel". This may be favourably exploited in different food applications.

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References


Discussion with Reviewers

Sheng-Chin Yang: Can you explain why you use 50°C as the emulsification temperature? What if the temperature is lower than 50°C?

Authors: By raising the temperature during homogenization, the reduction of fat globule size is facilitated. However, an upper limit is given by the risk of protein denaturation, and, in the case of whey proteins, this limit temperature is 60°C at neutral pH. Homogenization at 50°C is therefore a compromise between a good homogenization effect and the risk of protein denaturation. Homogenization at 50°C avoids unwanted denaturation at the stage of the emulsion.

D. Holcomb: Does whey protein serve as an emulsifier in the unheated emulsion? If so, what happens to the emulsifier whey protein when the system is heated? Is that protein also denatured or coagulated? Does the heating alter the protein's emulsifying capacity and the emulsion stability?

Authors: The whey protein is the only emulsifier present in the system. Following homogenization, a stable emulsion is obtained in which no coalescence nor creaming is observed, nor does the fat globule size distribution change significantly over several days. The experimental conditions selected for gelling the emulsion (pH near 7, temperature 90°C, holding time 30 min) suggest that practically all the protein, adsorbed or not, is fully denatured during the heat treatment. A result of heat denaturation is rearrangement of protein molecules adsorbed to the fat globule surface, metabolically coagulated. In practice it is no more possible to distinguish between adsorbed and "non-adsorbed" protein once the gel is formed. Following heat-induced gelation, the initial emulsion is "frozen" in a gelled structure. It cannot change for this reason, unless the gel is dehydrated or destroyed by other means. Microscopic analysis of the gels indicated projected fat globule diameters which are close to the diameters established by light-scattering on the liquid parent emulsion.

D. Holcomb: It is mentioned that protein coagulation is seen in the outer phase when the protein concentration is increased. At what protein concentration is such coagulation visible?

Authors: The decisive factor is the protein/lipid ratio, not the absolute protein concentration. If this ratio exceeds 0.5, there is an increasing amount of agglomerated protein observed in the space between the protein-covered fat globules (Fig. 6). This agglomerated protein shows a structure resembling the one seen with protein gels produced from aqueous dispersions.
Whey protein gels based on O/W emulsions

Fig. 4. Gel obtained from a lecithin-stabilized emulsion with whey protein used as the gelling agent in the continuous phase.

Fig. 5. Scheme illustrating steps in the formation of a lipid-rich gel based on a protein-stabilized O/W emulsion.

Fig. 6. SEM micrograph of a gel obtained from a whey protein-based O/W emulsion with a protein/lipid weight ratio of 0.6.

Fig. 7. "Deep-well" stub used for processing of liquid O/W emulsion.

D. Holcomb: Please provide an illustration of the "deep-well" stub.

Authors: The stub in which we perform the processing of the liquid emulsion is shown in Fig. 7. The (inner) diameter of the well is 7 mm.

Sheng-Chin Yang: If the pH and homogenization conditions remain constant, would the heated (90°C/30 min) whey protein/lipid emulsion be affected by different heating temperature and time?

Authors: Concerning the effect of temperature, variations within the range of 85-95°C have little effect on the gel-strength, but at temperatures below 85°C the gels become markedly weaker. 70°C is the lower limit to obtain gelation. Higher temperatures (e.g. by autoclaving the emulsion in hermetically sealed tins), can lead to higher gel-strength. The appropriate holding time at a given temperature depends on the heat transfer in the emulsion. Thus, the 30 minutes specified in this work are adequate for 25 ml of emulsion heated in glass beakers of the size specified in the Materials and Methods section.
Based on the levels of $\alpha$-lactalbumin and $\beta$-lactoglobulin in whey, and the area of the oil/water interface produced in this example, is there the possibility of selective adsorption of protein to the surface, thus influencing the composition of the heat-set protein film?

Authors: $\beta$-lactoglobulin accounted for about 50%, $\alpha$-lactalbumin for 20% of the total protein in our whey protein concentrate. These proteins, together with minor whey proteins, will compete for the fat surface which in our example may be near 500 $\text{m}^2/100$ ml of emulsion (oil volume fraction 0.4, mean fat globule diameter 450 nm). We didn't study the selectivity of protein adsorption in our system but may mention the findings of Shimizu et al. (1981) who showed that at neutral pH, the relative abundance of individual whey proteins at the fat surface of an O/W emulsion well reflected their mass proportion in the whey protein concentrate. Thus, $\beta$-lactoglobulin was the most abundant protein in the film at neutral and alkaline pH, but at acidic pH, $\alpha$-lactalbumin was more abundant. We deduce that protein adsorption to fat surfaces is strongly dependent on the pH and the isotonic pH of a given protein. Under the condition of our emulsions, it is likely that $\beta$-lactoglobulin is a major fraction in the primary protein film.

What is believed to be the effect of the added lecithin, and is the order of addition significant? Proteins can displace each other at the interface (Dickinson et al., 1988) and sodium dodecyl sulfate can interpenetrate protein films or possibly displace them (Jaynes and Flood, 1985). Is this what occurs?

Authors: Once the emulsion was produced with lecithin instead of protein, whey protein cannot efficiently adsorb to the fat globules (see fig. 4). This may be due to charge interaction between the lecithin's polar head and ionic sites on the protein, which will keep the protein off the fat surface. In such a case we may still get a stable emulsion, but no gel or only a very weak one. We cannot fully explain this poor gelation behaviour but assume that lecithin also interacts directly with protein and interferes with its gelation properties. Lecithin introduced after homogenization in the presence of whey protein, likewise interfered with gelation. This could indicate that in the unheated emulsion, lecithin is able to displace protein from the fat surface.

Is the high pressure (> 150 bar) required in order to obtain a fine and homogenous emulsion or in order to deposit protein at the interface?

Authors: Although protein migration to the interface takes place at lower homogenization pressure, high homogenization pressure allows, with a limited number of passages, to achieve a low mean lipid globule size and therefore a large fat surface.

Would other proteins show a similar behaviour?

Authors: We have a limited experience with egg white showing that in similar O/W emulsions high pressure homogenization under adequate temperature and pH conditions leads to improved gelation properties.

Additional References

