Whey protein coating efficiency on surfactant-modified hydrophobic surfaces

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Whey protein oxygen-barrier coatings on peanuts are not effective, due to incomplete peanut-surface coverage, as well as some cracking and flaking of the coating. Addition of sorbitan laurate (Span 20) in the whey protein coating solution up to the critical micelle concentration (cmc) of 0.05% (w/w) significantly improved coating coverage to 88% of the peanut surface. Increasing the Span 20 concentration in the coating solution to 3 times the cmc (0.15% w/w) produced a substantial increase in peanut surface energy (>70 dyn/cm), indicating adsorption of the surfactant to the peanut surface. With this level of Span 20, the whey protein coating coverage on peanuts increased to 95%. These results suggest that a concentration of surfactant above the cmc in the coating solution is required for formation of self-assembled structures of surfactant molecules on peanut surfaces, which significantly increases the hydrophilicity, and thus coatability, of peanut surfaces.

KEYWORDS: Whey protein; edible coating; adhesion; surfactant; peanuts

INTRODUCTION

Whey protein films and coatings possess excellent oxygen-barrier properties, comparable to those of synthetic polymer polyvinylidene chloride (PVDC) and ethylene vinyl alcohol (EVOH) films (1). Whey protein films are also excellent flavor and aroma barriers (2) as well as good oil barriers (3, 4).

Nuts are rich in polyunsaturated fatty acids (PUFAs). Diets with a high ratio of polyunsaturated to saturated fats can reduce the risk of cardiovascular diseases (5). However, the high polyunsaturated lipid content of many nuts makes them especially susceptible to oxidative rancidity (6). The oxygen-barrier properties of whey protein coatings have potential for increasing the shelf life of nuts by decreasing their rate of lipid oxidation. However, adhesion of the hydrophilic whey protein coatings to hydrophobic foods such as nuts is inherently poor, due to differences in the chemical nature of the two surfaces. During the coating of nuts, dewetting of the solution from nuts dipped in the coating solution occurs. Shrinkage and cracking of the coating may occur during drying, as well as flaking and de-adherence of the coating after drying.

The number and strength of attractive interactions across a coating–substrate phase interface determine the adhesion between the two phases. The number of attractive interactions across the interface can be increased by improving intermolecular contact between the coating and substrate. The criterion of good adhesion is essentially a criterion of wettability. Wetting is improved when the contact angle (θ) of a coating liquid on the solid to be coated is lowered. To lower θ, surfactants are typically added to the liquid phase to lower its surface tension (energy). A liquid perfectly wets the solid when the critical surface tension of a solid is greater than or equal to the surface tension of the liquid. When a liquid and substrates are in perfect contact, short-distance intermolecular interaction forces generate the adhesion.

At low surfactant concentrations, only individual amphiphilic molecules exist in aqueous solution. Above the critical micelle concentration (cmc), surfactant molecule aggregation occurs by cooperative self-association, and both individual molecules and micellar clusters coexist in dynamic equilibrium. At still higher solution concentrations, micelles of various sizes and shapes pack together and form characteristic symmetries (7). As the concentration of surfactant in the bulk solution exceeds the cmc, the solution surface energy remains fairly constant, but there is a tendency for the surfactant molecules to adsorb and aggregate at a solid–liquid interface. The time taken to reach equilibrium for a surfactant to be adsorbed from the bulk solution onto a solid surface generally decreases with increasing concentration of surfactant. The adsorption of surfactants at the solid/solution interface modifies the solid surface energetics and wettability (7).

Previous studies have shown that addition of sorbitan monolaurate (Span 20) as a surfactant in a whey protein coating solution successfully reduced the surface tension of coating solution to slightly below the surface energy of peanuts (8). Observing the pan-coated peanut surfaces with an imaging technique indicated that the addition of Span 20 in the coating solution significantly improved coating coverage (8). In some applications, it would be desirable to replace the synthetic Span 20 with a natural surfactant. Addition of lecithin in the whey protein coating solution up to the cmc of 0.05% (w/w) also reduced solution surface tension to slightly below the peanut surface energy and significantly improved coating coverage (8).
However, coating coverage improvement on the peanut surface using lecithin was not as great as with Span 20. Furthermore, a significant amount of the coating flaked off during stress testing, indicating poor coating adhesion.

To receive the benefit of whey protein coatings on peanuts in commercial applications, the coating must adhere to the food surface during processing, storage, and transportation. We hypothesized that if the surfactant concentration in a coating solution were increased sufficiently above the cmc, micelles would form on the peanut surface. The result would be that, in addition to lowering the surface energy of the coating solution, the surfactant would raise the surface energy of the peanuts sufficiently above the surface energy of the coating solution to enhance coating efficiency. Thus, the surface energy of peanuts modified by surfactant adsorption onto the peanut surface was investigated for improvement of the adhesion of whey protein coatings.

The objectives of this study were to determine (1) the increase of peanut surface energy by adsorption of surfactants used in this study, (2) the concentration and structure of adsorbed surfactant molecules on the peanut surface, and (3) the whey protein coating coverage on surfactant-modified peanut surface. The surfactants studied were from natural and renewable resources. Sugar ester (SE) is a nonionic surface active agent manufactured from a pure sugar and vegetable oils. It is an ester compound consisting of sucrose and fatty acids. Because of their low to nonexistent toxicity, biocompatibility, and excellent biodegradability, SEs have the potential of food and pharmaceutical applications (9). Soy lecithin is a natural surfactant that can be readily extracted from soybeans. Our study investigated the potential of these surfactants as alternatives for Span 20.

**MATERIALS AND METHODS**

**Materials.** Whey protein isolate (WPI) was supplied by Davisco Foods International (Le Sueur, MN). Liquid lecithin (Centrolecane A) and deoiled lecithin (Centrolex PF-40) were supplied by Central Soya (Fort Wayne, IN). Sugar esters of sucrose laurate (L-1695), sucrose myristate (M-1695), and sucrose oleate (O-1570) were supplied by Mitsubishi Chemical America, Inc. (White Plains, NY). Sorbitan monolaurate (Span 20) was purchased from Sigma-Aldrich (St. Louis, MO). Glycerol (Gly) and lithium chloride were purchased from Fisher Scientific Co. (Fair Lawn, NJ). Brilliant Blue dye (FD&C Blue No. 1 powder) was purchased from Warner Jenkinson Co., Inc. (St. Louis, MO). Raw peanuts and split blanched dry-roasted peanuts (Flavor Runner variety) were supplied by Hershey Foods Corp. (Hershey, PA). Peanuts were divided into 1000 g batches and stored at −40 °C. One 1000 g bag of peanuts was taken from the −40 °C freezer and stored in a laboratory freezer (−17 °C) for our experimental use.

**Measurement of cmc of Aqueous Surfactant Solutions.** Surfactants were prepared as aqueous solutions over a range of concentrations. Surface tensions of the solutions were measured with a digital tensiometer (model K 10 ST, Kruss USA, Charlotte, NC). The measurements were taken at 25 °C using the Wilhelmy plate method (10, 11). Measurements were taken when the surface tension readings reached a constant value, which occurred ~30 min after insertion of the plate in the aqueous surfactant solutions. The Wilhelmy plate method requires the use of a small rectangular plate of platinum, which is attached to a force-measuring system. The bottom edge of the plate is held parallel to the liquid surface, and the liquid is raised until it touches the plate. Force on the plate increases due to wetting of the liquid against the plate and is used to determine the surface tension. The plate method gives a continuous reading of surface tension, unlike the Du Nuoy ring method (22).

**Peanut Surface Energy Modification by Surfactant Adsorption.** Aqueous lecithin solutions were prepared at 0.15, 0.3, 0.4, and 1.0% (w/w), aqueous deoiled lecithin at 0.05 and 0.15% (w/w), aqueous SEs at 0.1% (w/w), and aqueous Span 20 solution at 0.05 and 0.15% (w/w). Blanched roasted peanuts that had been stored at −17 °C were reconditioned in a 17% relative humidity (RH) chamber at 25 °C overnight to bring the water activity to 0.25 ± 0.05. Thirty-five grams of blanched roasted peanut halves was filled into an aluminum Petri dish with holes in the lid and the bottom to allow circulation of fluid and immersed in a stirred aqueous surfactant solution for up to 30 s at 25 °C. Peanut halves were also treated individually in the aqueous surfactant solutions. Experiments were carried out at room temperature and at elevated temperatures for every 10 °C increment up to 90 °C.

To assess whether peanut surface energy modification was influenced by removal of the peanut surface waxy layer, peanuts treated at the elevated temperatures were then immersed in circulated water at room temperature. If peanut surface energy change were due to only adsorbed surfactant molecules, removal of the surfactant would return the surface energy to its original value. If all or part of the waxy layer were removed due to the melt and release of peanut surface waxy layer in the hot surfactant solutions, the peanut surface energy would not return to the original value. Lecithin was the only surfactant used in this part of assessment.

Peanuts were removed from the surfactant solution and then dried for 6 min using a hair dryer (Conair Corp., East Windsor, NJ). The temperature and velocity of the drying air were monitored repeatedly using an Ertco anemometer (Fisher Scientific) and a vane-probe anemometer (Fisher Scientific). Drying of peanuts was conducted with forced air at 2–3 m/s at 37 ± 2 °C (measured at peanut surface). Surface-modified peanuts were then conditioned in a chamber at 25 °C and 17% RH for 24 h to bring the water activity to 0.25 ± 0.05, and the surface energies of peanuts were determined immediately afterward. Surface energies of surface-modified peanuts that had not been conditioned to the water activity of 0.25 ± 0.05 were also determined for assessment of surfactant energy effects. The surface energies of peanuts were determined by assessing the compatibility of the peanut surface with a series of Lotar Enterprises (Green Bay, WI) testing inks with surface energies ranging from 32 to 70 dyn/cm. An ink with a surface tension greater than the peanut surface energy tends to retract on the peanut surface, whereas an ink with a surface tension smaller than the peanut surface energy tends to spread on the peanut surface.

**Amount of Adsorbed Surfactant on Surface-Modified Peanuts.** Peanuts were conditioned at 25 °C in a 17% RH chamber overnight to bring the water activity to 0.25 ± 0.05. Fifteen grams of peanuts was filled into an aluminum Petri dish with holes in the lid and the bottom to allow circulation of fluid and immersed in 0.4% (w/w) aqueous lecithin solutions, 0.1% (w/w) SE solutions, or 0.15% (w/w) Span 20 solution for 10 s. After surface modification with the surfactants, the peanuts were then conditioned at 25 °C and 17% RH for 24 h. Adsorbed surfactant on surface-modified peanuts was then individually desorbed from the peanut surfaces into 10 mL of deionized water. Fifteen grams of untreated peanuts also went through the same desorption process as the control. The desorption solution was filtered through a Whatman no. 1 filter paper. The optimum wavelength for determination of the surfactant in the filtrate was determined, a standard curve was developed, and the amount of desorbed surfactant was assessed with a UV spectrophotometer (UV-160, Shimadzu Scientific Instruments, Inc., Columbia, MD). The amount of originally adsorbed surfactant was assumed to be equal to the amount desorbed using this procedure.

**Coating of Peanuts by Addition of Surfactant in the WPI Coating Solution.** The peanuts were conditioned at 25 °C in a 17% RH chamber overnight. Whey protein coating solutions were prepared by dissolving WPI powder in deionized water to make 10% (w/w) solutions. Glycerol was then added (WPI/Gly = 1) as a plasticizer. The solution was denatured in 100 g batches for 30 min in a 90 °C water bath (12). The solution was cooled in an ice bath and then equilibrated to room temperature. A 7% (w/w) aqueous solution of Brilliant Blue dye was added at the level of 0.5 g per 100 g of coating solution to the denatured WPI/Gly coating solution to enhance image analysis, and the coating solution was then degassed under vacuum.

The coating solution was prepared as 100 g batches for the addition of surfactants. Lecithins were added at levels of 0.15 and 0.4 g per 100 g of coating solution, SEs were added at a level of 0.1 g per 100 g of coating solution, and Span 20 was added at levels of 0.05 and
Whey Protein Coating Efficiency

0.15 g per 100 g of coating solution. Coating was applied by dipping peanut halves individually in the WPI, WPI–lecithin, WPI–SE, or WPI–Span 20 coating solutions for 10 s. Only the curved side of peanuts was investigated, because it would be more challenging to obtain complete coating coverage on the curved side of peanuts due to gravity. The coated peanut halves were dried with the flat side resting on paperboard under a hair dryer (Conair Corp.) for 15 min with forced air at 2–3 m/s at 37 ± 2 °C. Coated peanuts were then conditioned at 25 °C and 17% RH for 48 h to bring the water activity of coated peanuts to 0.25 ± 0.05.

Image Analysis of WPI-Coated Peanuts. The extent of coating on peanut surfaces was determined by obtaining magnified digital images of the peanuts and then using the Image-J program (Research Services Branch, National Institutes of Health) that was developed for image analysis as described by Sehgal (8). Coating efficiency was determined as the percentage of peanut surface that was covered with whey protein coating after the coating process. Digital images of the peanuts were obtained using a stereomicroscope (Wild M8, Wild Heerbrugg, Switzerland) with an attached camera (Polaroid, model PDMC-2). Figure 1 shows the sample images of uncoated and coated peanuts obtained with the stereomicroscope. Image analysis of each peanut was obtained for only the curved side, because coated peanuts were dried with the flat side sitting on the bench. It would be more challenging to obtain complete coating coverage on the curved side of peanuts due to gravity.

Statistical Analysis. Differences in whey protein coating efficiency on peanuts were analyzed with least standard deviation (LSD) (14) from the SAS statistical program for physical analyses (SAS Institute, Inc., Cary, NC, 1999). The confidence level regarded as significant was P < 0.05.

RESULTS AND DISCUSSION
cmc of Aqueous Surfactant Solutions. The cmc values for crude lecithin, deoiled lecithin, and Span 20 were determined as 0.15, 0.15, and 0.05% (w/w), respectively. However, for the three sucrose fatty acid esters considered in this work (sucrose laureate, sucrose myristate, and sucrose oleate), the cmc values were far too low to permit accurate surface tension data to be obtained in the pre-micelle region. This phenomenon of low cmc preventing accurate measurement was also observed by Herrington and Sahi (15).

Peanut Surface Energy Modification by Surfactant Adsorption. Blanched dry-roasted peanuts had a surface energy of 37−41 dyn/cm on the curved surface. The surface energy of peanuts treated with surfactant solution having a crude lecithin concentration >0.4% (w/w), a deoiled lecithin concentration >0.05% (w/w), a Span 20 concentration >0.15% (w/w), or a sucrose fatty acid esters concentration >0.05% (w/w) was increased to >70 dyn/cm by adsorption of surfactant molecules to the peanut surface (Table 1). A lecithin solution concentration higher than the cmc was required to substantially increase the peanut surface energy. Preliminary results showed no significant effect of peanut water activity on peanut surface energy.

Adsorption of surfactant molecules to the hydrophobic peanut surface increases the affinity of the peanut surface for the coating solution. When coating solution and peanut surface are brought into intimate contact, short-distance intermolecular attractions take place and bonding occurs on minute peanut surface regions (16, 17). The adsorption of surfactants at a liquid/solid interface generally occurs as two types. In the first type, adsorption of the surface-active compound from solutions below the cmc occurs as individual molecules laying flat in a monolayer on the solid surface, with both hydrophobic and hydrophilic domains exposed to the solution. In the second type, adsorption increases dramatically from solutions above the cmc occurring as hemimicelles formed through interaction among the hydrophobic domains of the surface-active compound (7, 18). The hydrophilic domains face outward from the liquid, with a resulting increase in surface energy.

Increasing lecithin solution concentration to higher than the cmc in our study produced a substantial increase in peanut surface energy (Table 1), indicating a substantial increase in the adsorption of lecithin molecules to the peanut surfaces. A concentration of lecithin solution above the cmc is required for formation of a hemimicelle structure of lecithin molecules on peanut surfaces, which significantly increases the hydrophilicity of peanut surfaces. Modifying the peanut surface by increased lecithin adsorption from a solution above the cmc of lecithin should improve coating coverage and adhesion.

A 4% (w/w) lecithin solution treatment at elevated temperature could potentially increase the peanut surface energy by removal of the surface waxy cuticle layer. The peanut surface energy was increased to >70 dyn/cm after lecithin solution treatment at temperatures in the range of 25−90 °C. However, the peanut surface energy dropped back to 32−35 dyn/cm in each case after subsequent exposure to water at room temperature, indicating extraction of adsorbed lecithin molecules from the peanut surface (Table 2). These results show that the possible removal of the waxy cuticle layer from peanut surfaces with hot lecithin solution does not contribute to increase of surface energy. If it did, the surface energy would remain elevated even after rinsing with water. Furthermore, these results confirm that the increase of peanut surface energy is caused by the adsorption of lecithin molecules to the peanut surfaces.

Table 1. Modification of Peanut Surface Energy by Surfactant Adsorption on Peanut Surface (25 °C, 10 s)

<table>
<thead>
<tr>
<th>solution</th>
<th>surface energy of solution (dyn/cm)</th>
<th>surface energy of peanut (dyn/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated</td>
<td>37−41</td>
<td></td>
</tr>
<tr>
<td>control: H2O</td>
<td>72.8</td>
<td>32−35</td>
</tr>
<tr>
<td>0.15% lecithin (cmc)</td>
<td>35.0</td>
<td>32−35</td>
</tr>
<tr>
<td>0.3% lecithin (&gt; cmc)</td>
<td>32.1</td>
<td>56−60</td>
</tr>
<tr>
<td>0.4% lecithin (&gt; cmc)</td>
<td>28.9</td>
<td>&gt; 70</td>
</tr>
<tr>
<td>0.05% deoiled lecithin (&lt; cmc)</td>
<td>46.0</td>
<td>&gt; 70</td>
</tr>
<tr>
<td>0.15% deoiled lecithin (cmc)</td>
<td>28.4</td>
<td>&gt; 70</td>
</tr>
<tr>
<td>0.05% Span 20 (cmc)</td>
<td>26.2</td>
<td>44−48</td>
</tr>
<tr>
<td>0.15% Span 20 (&gt; cmc)</td>
<td>25.0</td>
<td>&gt; 70</td>
</tr>
<tr>
<td>0.1% sucrose laurate (&gt; cmc)</td>
<td>29.6</td>
<td>&gt; 70</td>
</tr>
<tr>
<td>0.1% sucrose myristate (&gt; cmc)</td>
<td>35.5</td>
<td>&gt; 70</td>
</tr>
<tr>
<td>0.1% sucrose oleate (&gt; cmc)</td>
<td>34.5</td>
<td>&gt; 70</td>
</tr>
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</table>

a cmc, critical micelle concentration.
Lecithin have maximum absorbance at a wavelength of 300 nm. Span 20 and sucrose fatty acid esters have maximum absorbance at a wavelength of 350 nm. However, among the sucrose fatty esters in this study, the absorbances of sucrose myristate (M-1695) and sucrose laurate (L-1695) solutions were too low to give accurate measurement. The untreated peanut extract solutions used as the control also had maximum absorbance at a wavelength of 300 nm. Therefore, the amounts of surfactants desorbed from peanut surfaces were determined at the wavelength for which the absorbance of surfactant solution is relatively higher compared to the absorbance of peanut-soluble solutions. This made it easier to distinguish the desorbed surfactant from peanut solubles. Wavelengths of 400, 380, 390, and 350 nm were selected for the determination of lecithin, deoiled lecithin, Span 20, and sucrose oleate, respectively. It was not possible to quantify the amounts of sucrose myristate or sucrose laurate (Figure 2d,e).

Extracts of lecithin-, Span 20-, and sucrose oleate-treated peanuts showed significantly higher absorbance than the extract of untreated peanuts at the selected wavelengths, reflecting the desorption of surfactant previously adsorbed on peanut surfaces (Figure 3). Deoiled lecithin-adsorbed peanut extract showed a higher absorbance than extract of untreated peanuts; however,
The difference is not significant. Theoretically, the amount of lecithin, Span 20, and sucrose oleate adsorbed when laying flat on peanut surface, calculated by dividing the average total peanut surface area by the area occupied by each surfactant molecule laying flat on the surface, would be $1.30 \times 10^{-11}$, $3.46 \times 10^{-11}$, and $2.08 \times 10^{-11}$ mol/g of treated peanuts, respectively. On the basis of our desorption results, the amounts of lecithin, Span 20, and sucrose oleate adsorbed onto peanut surfaces during surfactant solution treatment determined at the selected wavelength were, respectively, $1.02 \times 10^{-7}$, $2.48 \times 10^{-7}$, and $2.75 \times 10^{-6}$ mol/g of treated peanuts, substantially greater than the theoretical amount if the surfactants were laying flat. These results indicate the formation of self-assembled surfactant structures of adsorbed surfactant molecules on the peanut surface. The self-assembled surfactant structure substantially increases the peanut surface energy, which should improve the wetting and adhesion of whey protein coating on peanuts.

Coating Coverage Improvement by Addition of Surfactant in the WPI Coating Solution. With the exception of sucrose laurate, the coating coverage on peanuts was improved by the addition of surfactant in the WPI coating solution (Table 3). The coating efficiency improvement by the addition of lecithin, deoiled lecithin, or sucrose fatty acid esters in the coating solution was significantly smaller than that by the addition of Span 20. Both at the cmc and above the cmc in the WPI coating solution, lecithin, deoiled lecithin, and sucrose fatty acid esters gave coating coverage that varied significantly from peanut to peanut, which is shown by the large standard error (Table 3). Among the sucrose fatty acid esters, sucrose laurate as surfactant showed the smallest standard error; however, the coating coverage was not improved with the addition of sucrose laurate. The addition of Span 20 in the whey protein coating solution gave the greatest improvement of coating coverage on peanuts, and this improvement in coating efficiency was consistent, as indicated by the small standard error. Addition of Span 20 in the coating solution at the cmc (0.05%) increased the average coating coverage to ~88%. Average coating coverage was increased to 95% when Span 20 was added at a concentration above the cmc (0.15%), and fully coated peanuts dominated the samples. The sucrose fatty acid esters used in this study were mixtures of monoesters and diesters. Only Span 20 was a pure compound of sorbitan monolaureate. Because the molecular structure of the surfactant may determine their self-assembled structure on peanut surface, the purity, hydrophilic–lipophilic balance (HLB), and nature of the hydrophilic groups (ionic or nonionic) of the surfactant compound may be important factors for the improvement of coating efficiency and its consistency.

Conclusion. Treating peanuts with surfactant solutions at concentrations higher than the cmc substantially increased the peanut surface energy. The addition of surfactant in a whey protein coating solution gave improved coating efficiency on peanut surfaces. Although all of the surfactants used in this study gave solution surface energies below the peanut surface energy at their cmc values, the improvement of surfactant-incorporated whey protein coating efficiency on peanuts was quite dependent on the surfactant. Span 20 was more effective in improving the coating efficiency than the sucrose fatty acid esters and natural lecithins in the whey protein coating system. The impurity of sucrose fatty esters and lecithins may affect the formation of consistent self-assembled structures of surfactant molecules on peanut surfaces, resulting in the inconsistency of coating efficiency.

The results of this study indicate that further investigation of peanut surface energy modification by adsorption of surfactants and the effect of the modification on whey protein coating coverage on peanut surface is appropriate. This should include the study of other nuts and other surfactants, as well as the translation of bench-scale results to pilot-scale studies.

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