2004 MAFMA Final Report

Project Title:	**Preparation and Characterization of Neutrally Buoyant Particles in Fortified Foods**
PI:	**Ganesan Narsimhan**
Academic Institution	**Department of Agricultural and Biological Engineering Purdue University**
Award Date:	**9/1/2004 to 5/31/2006**

Objective Summary:

Quantification of the contact angle and dispersability of neutrally buoyant wax coated particles modified by adsorbed food emulsifier/proteins of different concentrations.

Objective Accomplishments:

Abstract:

To study the wettability of wax coated particles in fortified beverage, contact angle of wax surface at different pH, salt concentration, protein concentration, emulsifier type and concentration was measured. Contact angle decreased with an increase in the emulsifer conecentration when the concentration was low, and reached a fairly constant value at higher concentration. Whey protein was more efficient compared to other emulsifierss and decreased the contact angle from 100° to 40° at a concentration of 0.05% or higher in water. Tween 20 was more efficient than other tween surfactants and it decreased the contact angle from 100° to 67° at a concentration of 0.1% in water. In a model beverage system (5% citrate buffer, pH 2.5) Contact angle was found to be high (above 70°) without NaCl and decreased dramatically to around 10° for 0.5% whey protein in the presence of 0.5 M NaCl. Π-A isotherm of 150-80SV wax particle at air-water interface was obtained using Langmuir trough. Contact angle of the particles at the air-liquid interface was inferred from the critical surface pressure in the isotherm. The contact angle from this technique agreed well with the contact angle of planar surface that was measured using goniometer. Dispersibility of 150-80SV wax particles in aqueous solution was characterized by the measurement of bulk particle concentration upon suspension. At pH 2.5, 0.5% whey protein in the presence of 0.5 M NaCl in 5% citric buffer showed highest dispersability, while 0.1% whey protein solution with 0.5 M NaCl and Tween-20 solution showed much poorer dispersability.

Materials

Tween 20, Tween 40, Tween 60, Tween 80 and 2-propanol (Sigma-Aldrich, USA); whey protein (SCIFIT, PA); NaCl, citric acid and Na-citrate (Mallincktodt Baker, NJ), 150-80VS neutrally buoyant wax particles (Loders Croklaan, IL)

Experiment Procedure

Microscopic glass slides were coated with wax by dipping the slide in hot molten wax and cooling thereafter. Wax-coated slides were immersed in surfactant solutions overnight (16 hours) in order to allow the surfactants to adsorb onto the wax surface and attain equilibrium. The slides were then removed from the solution and dried. A 2mm diameter droplet of surfactant solution of the same concentration as that employed for treating the wax surface was formed on the modified slides. The slide with the droplet was placed in a humid chamber in order to prevent evaporation of solvent from the droplet. Contact angle was measured by contact angle meter (Ramehart 50-00) at different times. These experiments were repeated several times. 5% citrate buffer (pH4.0) was prepared. 0.1% of whey protein was dissolved in the buffer. 120 mL of this solution was poured into a 400 mL beaker (ID =73 mm). Desired amount of 150-80VS particle was added into the solution. After magnetic stirring for 5 min, the dispersion was allowed to rest for 5 min. A layer of particles was observed at the air-liquid interface. Bulk dispersion was taken out from the middle of the dispersion using a 5 mL pipet. 40 mL solution was taken out and filtered with S&S quantitative filter paper (Aldrich, Milwaukee, USA). The dry mass of filter paper was measured before filtration. The wet paper with particles was allowed to dry overnight and the total mass of filter paper with particles was measured. A control experiment was performed without whey protein in the solution.

Results and Discussion

Screening of emulsifiers: The effect of Tween 20 and whey protein concentrations on contact angle are shown in Figs.1 and 2 respectively. As expected, the contact angle decreased at higher surfactant concentration. This decrease was pronounced only at lower Tween 20 concentrations (up to 0.1%). At higher Tween 20 concentrations, however, the contact angle became fairly constant. In case of whey protein, most of the decrease in contact angle occurred at much lower concentrations (Fig.2). Fig.3 compares the contact angles of wax surface treated with different surfactant solutions for 16 h. Among the surfactants, Tween 20 reduced the contact angle the most whereas whey was the most efficient in that it resulted in the minimum contact angle.

Effect of pH: The results of contact angle measurements for wax coated glass slides at different pH in the presence of 0.05% whey protein solution are shown in Fig.4. The contact angle reduces dramatically from a high value of around 70° at pH 2.5 and below to a limiting low value of around 10 degrees for pH 4.5 and above. Therefore, whey protein in the presence of citric acid buffer is found to be very efficient in increasing the wettability of wax surface. Fig.5 compares the contact angle of wax coated glass slide with different treatments. Citric acid did not reduce the contact angle of wax coated glass slide significantly. However, 0.05 % whey protein solution in citric acid buffer at pH 3.5 did considerably reduce the contact angle. Interestingly, 0.05% whey protein solution in water was able to reduce the contact angle only to around 40°. Also, the contact angle of the surface was even higher (around 50°) when exposed to 0.05% whey protein solution in phosphate buffer at pH 3.5.

Effect of ionic strength: At low pH, e.g. pH 2.5, even high concentration of whey protein does not decrease the contact angle very much. At higher pH, however, the contact angle is found to be much lower. This may be due to the effect of ionic strength. At higher pH, higher concentration of Na-citrate in the buffer may contribute to higher ionic strength which may play a role in the reduction of contact angle. The results of contact angle measurements for wax coated glass slides at different ionic strength in the presence of different concentration of whey protein solution at pH 2.5 are shown in Fig.6. The contact angle decreased with increasing ionic strength at each protein concentration. At each ionic strength, contact angle decreased with increasing protein concentration. Therefore, high protein concentration and high ionic strength are favorable for wettability of the hydrophobic surface. When the whey protein concentration is 0.5% and the NaCl concentration is 0.5, the contact angle decreases to 10.6±2.7°.

Contact angle by Langmuir-trough: Π-A isotherm of the hydrophobic particles is shown in Fig.7. Applying Clint and Taylor's method, critical surface pressure Π_c was obtained and related to contact angle by

$$\Pi_c = \frac{\pi\gamma_{LA}\left(1 \pm \cos\theta\right)^2}{2\sqrt{3}}$$

Where γ_{LA} is the air-liquid surface tension and θ is the contact angle. The "±" means there are two possible situations to give same Π_c, which depends on whether the particles are compressed to move into air phase or liquid phase. The contact angle for the wax coated particles obtained by the Π-A isotherm is 95.8±0.9° assuming that particles are compressed into air. This value agreed well with that of planar surface as measured by goniometer. The contact angle obtained using goniometer is the contact angle of a small droplet on a planar solid surface, while that obtained by Langmuir trough is the average contact angle of small particles at a liquid surface. It is impossible to get the Π-A isotherm of particles when protein/surfactant is present in the liquid phase, because the protein/surfactant would also adsorb at the interface. The compression of protein/surfactant adsorption layer would also contribute to the surface pressure variation. Therefore, the variation of surface pressure is not solely due to the compression of particles. The good agreement between the contact angles from goniometer and Langmuir suggests that the contact angle of a particle is close to that of a planar surface.

Particle dispersion: A set of experiments were conducted to investigate the effect of whey protein in the prevention of flotation of wax particles. Wax particles were suspended in 0.1% whey protein solution in citric acid buffer at pH 4 for 5 min by stirring. Stirring was then stopped and samples were then taken from the bulk solution after 5 min. to determine the particle concentration. Since all the particles were not neutrally buoyant, particles that were lighter rose to the top to form a cream layer. As a result of this loss of particles, there was a decrease in particle concentration in the bulk. The final bulk particle concentration was found to be about $1/7^{th}$ of the initial concentration for low as well as high initial particle concentrations indicating thereby that the particles were not really neutrally buoyant. Interestingly, upon creaming, wax particles in the presence of whey formed a cream layer in which they were fully wetted. However, in case of wax particles suspended in citric acid buffer (control), most of the particles were lost to the air-water interface (Fig. 8). Also, in this case, the particles resided at the interface since they were not fully wetted. In fact, at higher initial particle concentration, one could observe dry particles floating at the surface. In another set of experiments (Fig.9), particles of 0.5% initial concentration were suspended for 5 min by stirring. In one set of experiments, a sample was withdrawn from the bulk. In the other set, stirring was stopped and a sample was withdrawn from the bulk after 5 min. In case of control, there was insignificant difference in the particle concentration between the two sets. However, in case of particles in 0.1% whey solution, stirring during sampling gave a much higher particle concentration. This difference can be attributed to the fact that whey protein solution wets the particles.

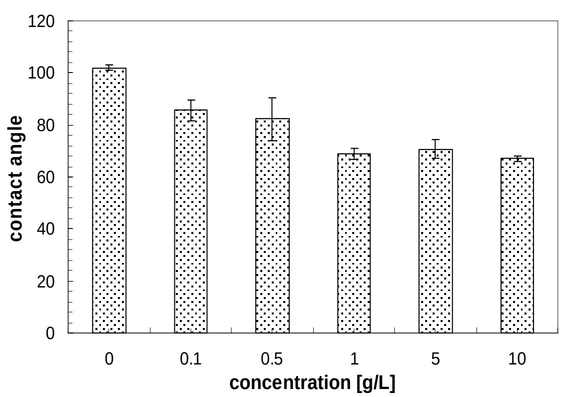

Fig.1 Contact angle of wax coated slides treated with different concentrations of Tween 20 solutions.

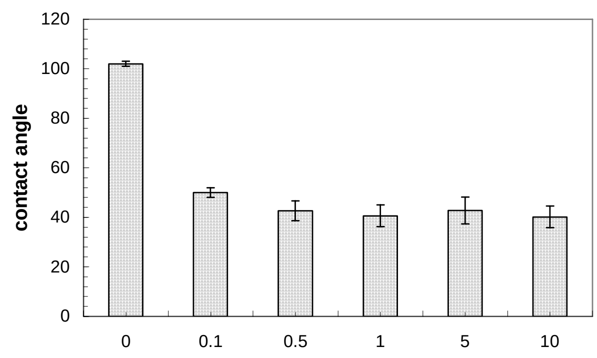

Fig.2 Contact angle of wax coated glass slide treated with whey protein of different concentrations.

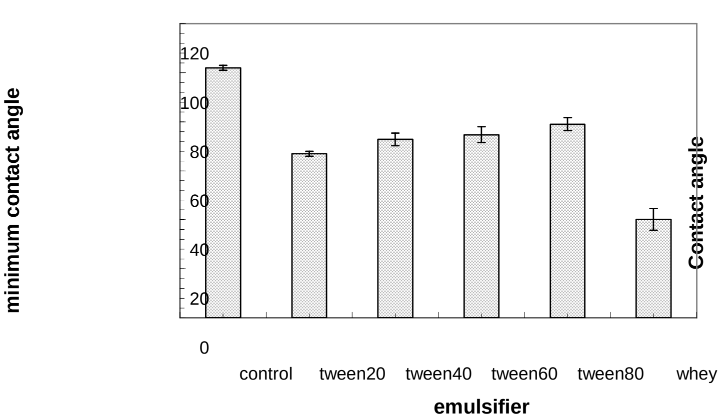

Fig.3 Minimum contact angle of wax coated glass slide treated with different surfactant solutions.

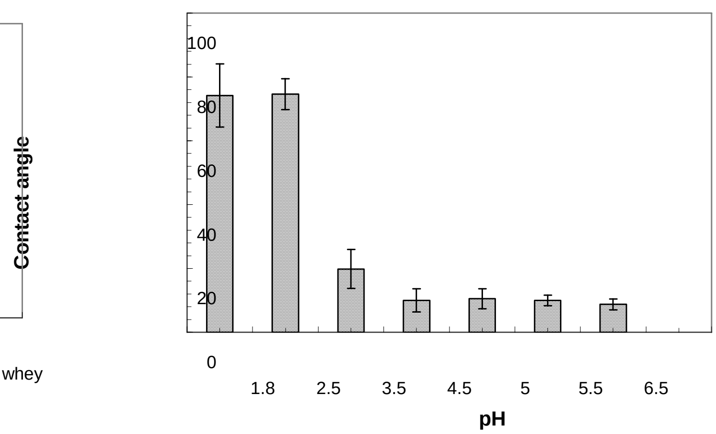

Fig.4 Contact angle of wax coated slides treated with 0.05% whey protein solution (5% citrate buffer)

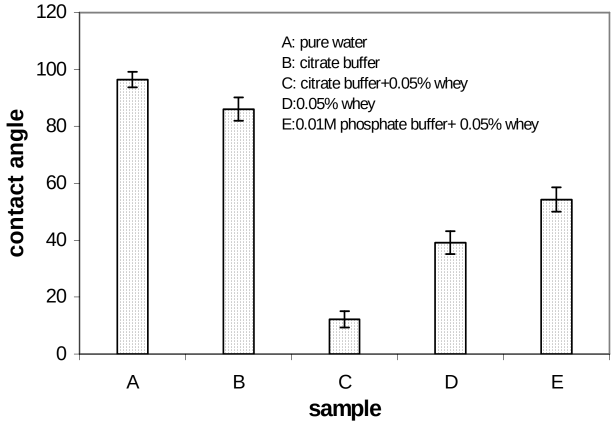

Fig.5 Contact angle of wax coated slides treated with different solutions

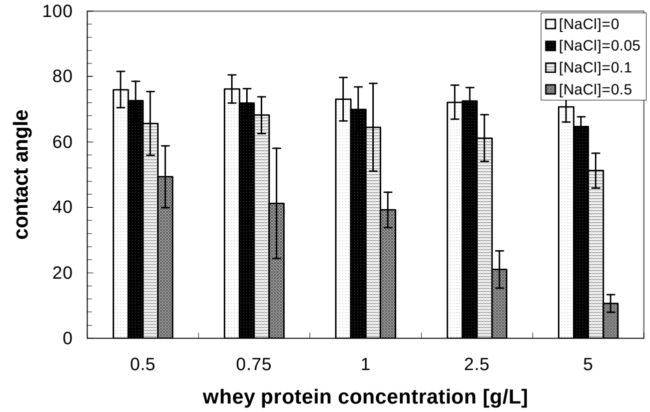

Fig.6 Contact angle of wax coated slides treated with whey protein solution at different concentration and different NaCl concentration (5% citate buffer, pH 2.5)

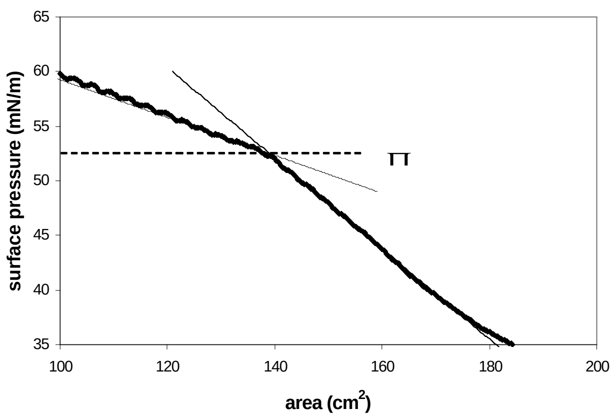

Fig.7 Π-A isotherm of particle 150-80VS at air-water interface. Liquid phase is 5% citrate buffer (pH4.0).

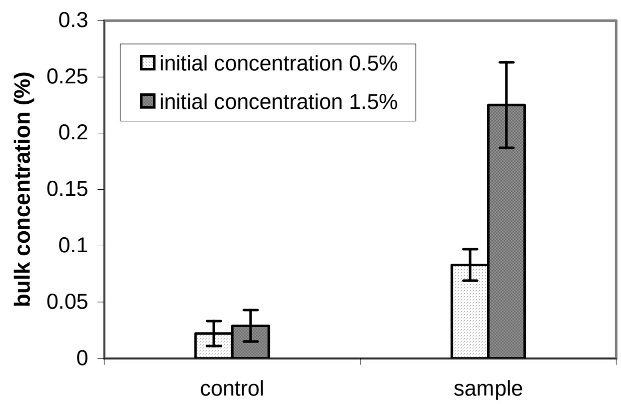

Fig.8 Bulk concentration of particle dispersion with low (0.5%) and high (1.5%) initial concentration.

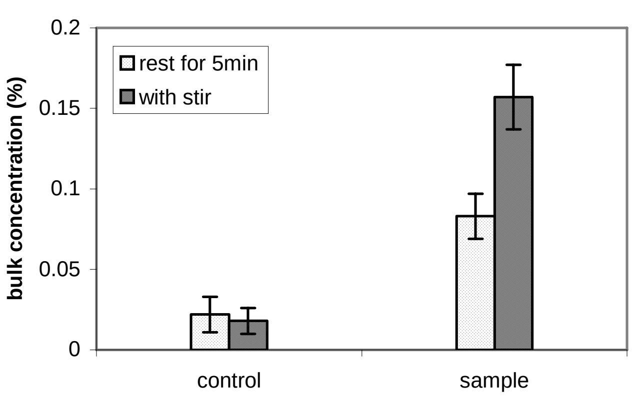

Fig.9 Bulk concentration of particle dispersion with stir and 5min rest after stir was stopped. Initial particle concentration is 0.5%.

Unexpected findings:
Whey proteins were found to be more efficient than food emulsifier such as Tween 20 in increasing the wettability of wax coated particles. Also, the performance of whey in improving the wettability was pH sensitive in that the efficiency decreased considerably at pH lower than 3.

Practical impacts:
Micronutrient fortified foods, including beverages, are becoming increasingly important in many countries. Iodine on salts, iron and vitamin C in milk or drink yogurt, and calcium in orange juice are typical examples of micronutrient fortification in various foods. The contribution to micronutrient intakes from fortified foods in the U.S. ranged from 6% for vitamin B6 and folic acid to 24% for iron and vitamin (Lachance, 1989). Iron deficiency affects approximately 20% of the world population and is considered to be the commonest nutritional deficiency (Martinez-Navarrete et al., 2002). Iron deficiency persists although it is abundant in the food supply and the nutritional requirements for it are low. The surgeon general (HHS 1988) and the National Research Council (1988) recommended that adolescents and women increase their intake of iron rich foods (Martinez-Navarrete et al., 2002). Iron fortification could increase nutritive value and consumer appeal of dairy and other food products. Main barriers of iron fortification are finding an iron compound that adequately absorbed but no sensory changes to the food systems and overcoming the inhibitory effect on iron absorption of dietary components such as phytic acid, phenolic compounds and calcium (Hurrell, 2004). Water-soluble iron compounds, which are readily bioavailable, often lead to the development of unacceptable color and flavor changes in the food system, while insoluble iron powders give little or no nutritional benefit with very poor absorption causing no sensory changes. Ferrous sulphate and ferrous fumarate can be used instead of elemental iron for better absorption.

Success of iron fortification in dairy and other food products depends on encapsulation of ferrous sulphate and/or ferrous fumarate to prevent lipid unacceptable color and flavor change. In addition, it is necessary to ensure that iron particles are uniformly suspended in the food system. Microencapsulation of iron by an inert would alleviate these problems by first minimizing the effects of lipid oxidation and secondly making the particles neutrally buoyant. However, the inert coating material should be sufficiently hydrophilic to ensure wettability of coated iron particles so that they are easily dispersed in the aqueous medium. The proposed research would address these two issues, namely (i) make neutrally buoyant iron particles by coating these particles with an inert such as wax of required thickness and (ii) minimize the surface energy of coated wax surface with appropriate emulsifier/protein to make the particle wettable.

Spray drying accounts for the majority of commercial encapsulated materials in food products. Coacervation, coating with fat and spray chilling are the other techniques employed for encapsulation (Shafer and Shafer, 2003). Encapsulation has been shown to result in ease of handling, stability against oxidation, retention of volatile ingredients and flavor,taste masking and enhanced bioavailability and efficacy. Microencapsulation has been evaluated by several investigators (Pekarek et. al., 1994; Teipel et. al., 2001) and a comprehensive review of this technique is given by Clark (2002). An excellent discussion of various factors that influence adhesion of particles onto air-liquid interface as related to flotation are given by Gaudin (1957) and Fuerstenau (1980). Wettability and flotability of minerals as a function of surface tension were investigated by Kelebek et. al. (1986). Theoretical models for the prediction of flotability of minerals at air-solution interfaces are discussed by Jowett (1980) and Clarke and Wilson(1983).

Without proper treatment, the inert surface of food particle would adhere to the air-aqueous phase interface thus compromising the texture of food beverage. This research identified the efficiency of different treatments with food emulsifiers/proteins in order to modify the surface to minimize adhesion to air interface. The results of this research is valuable for the development of guidelines for formulations in fortified beverage systems.

REFERENCES:

Clark, P., Food Encapsulation: Capturing One Substance by Another, *Food Tech*., **56**(11),63 (2002)

Clarke,A.N. and Wilson, D.J., Foam Flotation-Theory and Applications, Marcel-Dekker, Inc.(1983)

Clint,J.H. and Taylor, S.E., Particle Size and Interparticle Forces of Overbased Detergents: a Langmuir Trough Study, Colloids and Surfaces, 65,61-67(1992)

Fuerstenau, D.W., Fine Particle Flotation, in Fine Particle Processing, Vol. I. P. Somasundaran (ed), p 669-705 (1980)

Gaudin, A.M., Flotation, Second Edition, McGraw Hill Book Co. (1957)

HHS. The Surgeon General's Reprot on Nutrion and Health. Public Health Service. U.S. govt. Printing Office, Washington, D.C. (1988)

Hurrell, R.F. Fortification: overcoming technical and practical barriers. J. Nutr. 132:806S-812S (2002)

Jowett,A., Formation and Disruption of Particle-Bubble Aggregates in Flotation, in Fine Particle Processing, Vol. I. P. Somasundaran (ed), p 722-754 (1980)

Kelebek, S., Finch, J.A., Yoruk,S. and Smith,G.W., Wettability and Floatability of Galena-Xanthate System as a Function of Solution Surface Tension, Colloids and Surfaces, 20,89-100(1986)

Kumar, N., Varanasi, K., Tilton, R.D. and Garoff, S., Surfactant Self-Assembly ahead of the Contact Line on a Hydrophobic Surface and Its Implications, *Langmuir*, **19**,5366(2003)

Lachance, P.A., Nutritional responsibilities of food companies in the next century. *Food Tech.*, **43**,144(1989)

Martinez-Navarrete, N., Camacho, M.M., Martinez-Lahuerta, J., Martinez-Monzo, J., Fito, P. Iron deficiency and iron fortified foods- a review. Food Research International 35:225-231 (2002)

National Research Council. Designing Foods. National Academy Press. Washington D.C.(1988)

Pakarek, K,J., Jacob, J.S. and Mathiowitz,E., One-Step Preparation of Double-Walled Microspheres, Adv. Mater., 6(9),684-687(1994)

Schafer,A. and Schafer, S., Novel Encapsulation System Provides Controlled Release of Ingredients, Food Techn., 57(11), 40-42 (2003)

Teipel,U., Heintz,T. and Krober, H., Microencapsulation of Particulate Materials, *Powder handling & processing.*, **13**,283(2001)