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WATER-IN-OIL AND OIL-IN-WATER EMULSIONS STABILIZED BY OCTENYLSUCCINIC ANHYDRIDE MODIFIED STARCH AND ADSORPTION OF MODIFIED STARCH AT EMULSIFIED OIL/WATER INTERFACES

by

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Abstract

Emulsions are utilized to help control phase separation and are found in many products ranging from food to pharmaceuticals. Because of the hydrophobic properties of its functional group, octenylsuccinic anhydride (OSA) modified starch is commonly used in oil in water (o/w) emulsions. The first objective of this study was to investigate if OSA modified starch could be used in water in oil (w/o) emulsions. Experiments were designed to determine the effects of concentrations of OS starch, mineral oil and water on the stability of emulsions. High shear homogenizers and a microfluidizer were used to create stable o/w and w/o emulsions. The stability of the emulsions was examined by optical microscopy, gravitational separation, and electrical conductivity.

The microfluidized samples always had a longer stability (days), no gravitational separation and did not exceed three microns, compared to the unmicrofluidized (o/w and w/o) samples. Stable (over 100 days of stability) o/w emulsions could be made without a microfluidizer if the emulsion was made of 2, 60, 38% (w/w) oil, water, starch, respectively. Stable o/w emulsions prepared with a microfluidizer were stable for over 100 days. The o/w emulsion prepared by 8, 66, 26% oil, water, and starch, respectively, was stable for over 600 days. The most stable w/o unmicrofluidized sample was made of 52, 22, 26% oil, water, starch, respectively, with a stability of 240 days. For the w/o emulsions from the microfluidizer, the most stable emulsion was made of 52, 34, 14% oil, water, starch, respectively, with a stability of 250 days. The most stable emulsion that could flow (under the 30,000 cP) was 56, 38, 6% oil, water, starch, respectively, with a stability of 150 days. The statistical mixture experiments models successfully predicted the stability for other ratios of oil, water, and starch for o/w and w/o emulsions.

The second objective of the study was to determine the concentration of modified OS starch adsorbed to the mineral oil and the water phases for oil-in-water (o/w) emulsions. The percentage of the starch adsorbed at the mineral oil phase was determined and compared when different ratios of starch to oil and water were used. When the ratio of oil:starch was decreased, the emulsion particle size decreased. As the starch content increased, the percent starch adsorbed onto oil based on total oil increased. The adsorption yield and the level of starch in the emulsion did not show a trend. The surface load ranged from 1.6 to 6.98 mg/m^2. The sample with the

highest concentration of starch (26 g/ml) had the highest surface load (6.98 mg/m^2) and samples with low concentrations of starch (0.84 and 1.68 g/ml) had the second and third highest surface loads (6.82 and 4.70 mg/m^2, respectively). The ratio of oil:starch was increased to determine the emulsifying capacity. A high emulsifying capacity was achieved. Samples with an oil:starch ratio of 3:1 were stable for over 80 days while other samples with oil:starch ratios of 5:1 and 6:1 could be stable for one week.

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Dedication

To my family and friends who have supported me throughout this journey. They have taught me that research, like life, is a journey; there are detours, various paths, some paths that you do not expect, dips and hills along the way.

Chapter 1 - Water-in-oil and oil-in-water emulsions stabilized by OSA modified starch

Abstract

Emulsions are utilized to help control phase separation and are found in many products ranging from food to pharmaceuticals. Because of the hydrophobic properties of its functional group, octenylsuccinic anhydride (OSA) modified starch is commonly used in oil in water (o/w) emulsions. The first objective of this study was to investigate if OSA modified starch could be used in water in oil (w/o) emulsions. Experiments were designed to determine the effects of concentrations of OS starch, mineral oil and water on the stability of emulsions. High shear homogenizers and a microfluidizer were used to create stable o/w and w/o emulsions. The stability of the emulsions was examined by optical microscopy, gravitational separation, and electrical conductivity.

The microfluidized samples always had a longer stability (days), no gravitational separation and did not exceed three microns, compared to the unmicrofluidized (o/w and w/o) samples. Stable (over 100 days of stability) o/w emulsions could be made without a microfluidizer if the emulsion was made of 2, 60, 38% oil, water, starch, respectively. Stable o/w emulsions prepared with a microfluidizer were stable for over 100 days. The o/w emulsion prepared by 8, 66, 26% oil, water, and starch, respectively, was stable for over 600 days. The most stable w/o unmicrofluidized sample was made of 52, 22, 26% oil, water, starch, respectively, with a stability of 240 days. For the w/o emulsions from the microfluidizer, the most stable emulsion was made of 52, 34, 14% oil, water, starch, respectively, with a stability of 250 days. The most stable emulsion that could flow (under the 30,000 cP) was 56, 38, 6% oil, water, starch, respectively, with a stability of 150 days. The statistical mixture experiments models successfully predicted the stability for other ratios of oil, water, and starch for o/w and w/o emulsions.

Introduction

Emulsions are used in food, beverage, industrial, and pharmaceutical applications to bring two substances that are usually not miscible together and typically one ingredient is dispersed in another. The two most common types of emulsions are oil-in-water (o/w) and water-in-oil (w/o) emulsions, with w/o being the most common in food applications (Stauffer, 1999). By nature, oil and water tend to separate after mixing; therefore, there is a great need for emulsifiers to keep them from separating. The classification of an emulsion depends upon which substance is in the dispersed phase or the continuous phase. In a w/o emulsion, water is dispersed in the oil; thus, water is the dispersed phase and oil is the continuous phase. Emulsions are formed by applying mechanical force to a system composed of oil, water, and an emulsifier. Some types of mechanical force include high-pressure homogenizers, high-shear homogenizers, microfluidizers, and sonicators.

The emulsion may become unstable due to internal and external factors such as time, temperature, and makeup of the emulsion (McClements, 1999). Two types of instability are physical and chemical instability. Physical instability is more common than chemical instability for emulsions using starch. When molecules are distributed, placed, or organized differently compared to its preferred state, this is an example of physical instability (McClements, 2005). Physical instability includes coalescence, flocculation, sedimentation, creaming, Oswald Ripening, or phase inversion (McClements, 1999, 2005 & 2007). Oswald Ripening tends to happen for flavor emulsions and the emulsions prepared in this study are cloud emulsions, which use non-flavor oils (Buffo & Reineccius, 2001; McClements, 2005).

Understanding the interactions of the ingredients is important to determine the kinetic stability of the emulsion, the rate of the process/change that happens (McClements, 2005). The emulsifier attached to the oil droplet prevents it from adhering to another oil droplet (McClements, 2005). According to McClements (2007), creaming is defined as particles of lower density (ie, oil) coming together and floating to the top, packing together to form a creamed layer because they cannot rise anymore. If the dispersed phase particles are of higher density than the continuous phase, the droplets will sink to the bottom through sedimentation (McClements, 1999). Creaming is common for o/w emulsions while sedimentation is common for w/o emulsions. Creaming may have been caused by flocculation or coalescence (McClements, 2005). A particle size analyzer cannot tell if the emulsion has undergone flocculation or coalescence. Instead, a microscopic image of the emulsion, before and after aggregation, shows the droplets in either the flocculated or coalesced form. If the particles are observed to have increased in size, the emulsion is undergoing coalescence. If the particles stay the same size, but are aggregated, the emulsion is undergoing flocculation (McClements, 2005). Another way to distinguish between flocculation and coalescence is viscosity of the emulsion. Higher emulsion viscosity is usually due to flocculation, while a lower emulsion viscosity is usually coalescence (McClements, 2005). The occurrence of flocculation, which is considered a quality failure factor in the food industry, can be inhibited by lowering the droplet size, radius, and critical concentration (Chanamai & McClements, 2002).

Modified starch may be utilized as an emulsifier by means of its functional groups, which bridge the interface of two immiscible phases. One way to create an amphiphilic starch is to react the starch (hydrophilic) with octenylsuccinic anhydride, or OSA (hydrophobic) (Trubiano, 1986).

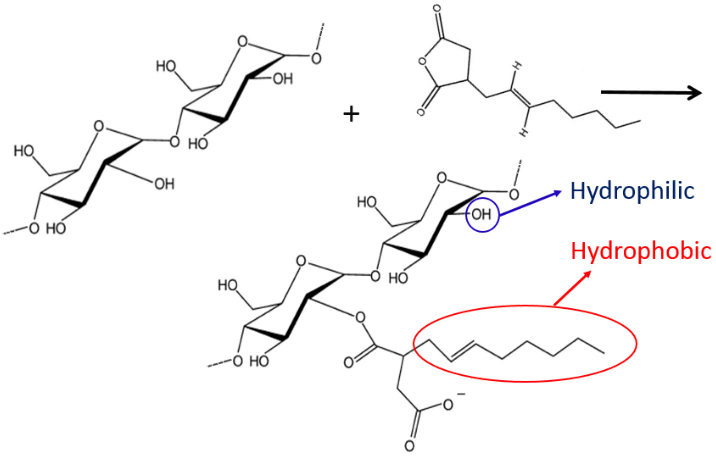

Figure 1.1 Substitution reaction for octenylsuccinic anhydride (OSA) modification (Bai, 2007; Nilsson & Bergenstahl, 2007)

The starch's hydroxyl groups provide the hydrophilic component while the octenylsuccinate group provides the hydrophobic component (Figure 1.1). The amphiphilic nature of the OSA modified starch helps the starch act as an emulsifier that can be used in beverages (Trubiano, 1995). The OSA reacts with hydroxyl groups attached to carbons 2, 3, and 6 on the starch's glucose units and undergo a substitution reaction (Trubiano, 1986; Bai, 2007). FDA guidelines dictate that up to 3% OSA can be added to react with starch for food applications and the product must be labeled "Food Starch Modified" (Trubiano, 1986). The degree of substitution (DS) for 3% OSA modified starch is usually about 0.018 (Nilsson, Leeman, Wahund, & Bergenstahl, 2006). OSA modified starches have shown to be good stabilizers and emulsifiers by inhibiting and slowing the natural process of instability. The main stabilization mechanism is through steric hindrance where the droplets are protected from aggregation (Chanamai & McClements, 2001; Tesch, Gerhards, & Schubert, 2002). Steric hindrance works to stabilize molecules using nonionic molecules and help inhibit particle aggregation, but cannot directly inhibit sedimentation and creaming (Chanamai & McClements, 2001; Napper, 1976; Tesch, Gerhards, & Schubert, 2002). In addition, OS starch increases the viscosity of the continuous phase which inhibits the movement of molecules and therefore, reduces the interaction of particles and stabilizes the emulsions (McClements, 2005).

The OS starch can reach the interface of the system because it has a high molar mass and is easily adsorbed (Nilsson et al., 2006). Based on kinetic factors, polymers with a greater radius have a decreased adsorption time (the time it takes for the particles to adsorb to the surface) when the sample is processed under turbulent flow (Nilsson et al., 2006). With a larger sized molecule, there is a higher substituent density and higher adsorption energy. Therefore, there will be a larger surface load, which is the amount absorbed to an interphase and measured in units of macromolecule per surface area (milligrams per square meter) (Nilsson et al., 2006). This concept is related to overrepresentation of large molecules at the surface and stronger kinetic adsorption factors (Nilsson & Bergenstahl, 2007). Therefore, the larger size of the OS starch molecules, and their larger radius, causes the OSA modified starch to be adsorbed into the interfacial region faster and so can be used as efficient emulsifiers (Nilsson et al., 2006).

OS starch can function as a surfactant. When the surfactants are at the interfacial region (area between the hydrophilic and hydrophobic regions, where the emulsifier acts), they lower the interfacial or surface tension (γ). This lower surface tension helps stabilize emulsions (Prochaska, Kedziora, Thanh, & Lewandowicz, 2007).

Many emulsifiers are available on the market, including gum arabic, mono- and di-glycerides, hydrocolloids, methyl cellulose, and modified starches (McClements, 2007; Saunders, 1968). All of these have benefits and drawbacks. Gum arabic has been used in many applications as an emulsifier; however, gum arabic is not always consistent in its quality (Chanamai & McClements, 2002). Gum arabic has been shown to be adequately replaced by hydrophobically modified starches, such as OS starch, in carbonated beverages (Trubiano, 1986

& 1995). The beverages may be stabilized with a lower concentration of OS starch than the concentration of gum arabic for an equivalent purpose with no change in particle size, clouding efficiency, or emulsion stability (Trubiano, 1986). OS starch, compared to gum Arabic, has a higher oil load (National Starch Bulletin) and a high surface load (Nilsson and Bergenstahl, 2006 and 2007). OSA modified starch has been used in the beverage emulsions, flavors, and clouding agents (Prochaska et al., 2007). OSA modified starch has been used for emulsifying and encapsulating flavors and nutrients such as vitamin E (Qiu, Yang, & Shi, 2015). Reiner, Reineccius, & Peppard (2010) compared gum arabic and starch-based emulsifiers for cloud emulsions, also called o/w emulsions, and found that the native and modified gum acacia is more stable than the modified starches when used in orange terpene based beverages. The modified starches and hydrocolloid emulsifiers examined were commercial samples.

Emulsion stability is defined by McClements (2005 & 2007) as resisting physiochemical change. There are various ways to measure emulsion stability, depending on the sample. Optical microscopy, gravitational separation, rheology, and electrical conductivity are used to measure the stability of the emulsions (McClements, 2007). Bessoles, Duccini, & Trouve (2011) noted that emulsions are stable if "no breakage is noticed after 6 months at room temperature."

Emulsions should flow; otherwise, they may be more of a gel and not a flowable emulsion (McClements, 2007). Prochaska et al. (2007) found that the viscosity of the starch solution changes from the pH of the system. Using a Brookfield viscometer, they found increased viscosity (48, 200, 225, 275, and 350 mPas) with increased pH (2.5, 3.5, 4.5, 5.5, and 7 pH respectively) of their starch solutions. The degree of substitution and the pasting viscosity is compromised with less than ideal pH conditions, which is outside of the pH of 5.5 to 7.0 range (Prochaska et al., 2007). OS starch is not stable at high pH (Chung, Lee, Han, & Lim, 2010). Changes in OS starch can influence the rheological properties and the gelling process of OS starch and is affected by the temperature and the time of the processing (Martinez, Partal, Munoz, & Gallegos, 2003).

Conductivity can be used to determine if the emulsion is o/w or w/o by determining the electrical potential of the sample (McClements, 2007). The conductivity of a sample can also help determine if the sample is going through phase inversion. Oil in water emulsions have an aqueous continuous phase; therefore, they have a high conductivity. A lipid continuous phase from a w/o emulsion creates a lower conductivity. If the o/w emulsions were to destabilize, this would result in a lower conductivity. If the emulsion has a high conductivity (o/w emulsions) and reduces in conductivity over time to the range that’s common for w/o emulsions, it may have gone through a phase inversion.

Hydrophilic-Lipophilic Balance (HLB) has been calculated and used to predict if the emulsifier is a lipophilic emulsifier (HLB value < 10) or a hydrophilic emulsifier (HLB value > 10) (Wang et al., 2011). HLB is a good predictor, but it alone cannot predict if the emulsifier is good in a w/o or o/w system. OS starch has a HLB hydrophilic emulsifier value of 12.7 (Wang et al., 2011); however, the w/o has not been evaluated so this needs to be checked.

OS starch is usually used for o/w emulsions, however there are emulsifiers used for w/o emulsions as well (Fingas & Fieldhouse, 2003; Iyer, Hayes, & Harris, 2001; Yan, Gray, & Masliyah, 2000). Iyer et al. (2001) made w/o emulsions as small as 0.004 micrometers using methoxypolyethylene glycol, triethylamine, mesyl chloride, p-toluenesulfonic acid, 3-hexadecanone, lysozyme, N-acetylglucosamine, and glycol chitin (degree of polymerication of 2500). Those are all non-starch based w/o emulsions.

Yan et al. (2000) used kaolinite clay particles treated with hydrophobic polystyrene latex miceospheres and could make w/o emulsions as small as two microns. However, most of the surfactants they used did not allow them to create stable emulsions. Fingas & Fieldhouse (2003) studied w/o emulsions (called “chocolate mousse” in the oil industry) stabilized by asphaltene, resin, and oil emulsions for the oil industry where emulsification slows oil spills at sea. They found that stable emulsions have between 60 and 80% water. They did not examine OS starch.

Objective

OSA modified starch is commonly used in oil in water (o/w) emulsions. The objective of this study was to investigate if OS starch could be used in w/o as well as o/w emulsions. Experiments were designed to determine the effects of the levels of OS starch, oil, and water on o/w and w/o emulsions. High shear homogenizers and a microfluidizer were used and compared. The stability of the emulsions was determined using multiple methods including optical microscopy, viscosity, gravitational separation, and electrical conductivity.

Materials and Methods

OS starch, HI-CAP® 100, was obtained from Ingredion (Bridgewater, NJ) and had a DS of 0.02, and 6.0% moisture. Mineral oil (cat. No. BP2629-1) and sodium benzoate (cat. No. S224-500) were purchased from Fisher Scientific (New Jersey, USA). Distilled water was made in the laboratory.

Experimental Design

For experiments with three ingredients, the experimental domain is within an equilateral triangle (Fig. 1.2). The vertices represent the pure components, the edges of the triangle represent the two-component blends, and points within the triangle represent the three-component blends. This is called the Mixture Emulsion Design. A completely randomized design (CRD) following the mixtures experiments with a centroid design were used (Figure 1.2). This mixture design was chosen because this research uses more than two ingredients in a mixture where the levels of one factor are dependent of the other factor's levels (Mason, Gunst, & Hess, 2003). The design was used for both un-microfluidized and microfluidized samples. To reduce sample pool size, MiniTab Mixtures Experiment Triangle Statistical Software (Minitab, Inc., Pennsylvania) predicted the stability of all possible emulsions by using only 50 samples (plus replicates and other preliminary samples). The samples start with two and not zero percent so every ingredient will be included equally and due to preliminary research, at least 2 percent starch was needed. There were two areas of compositional interest, o/w and w/o, as shown in Figure 1.2 as bold smaller triangles.

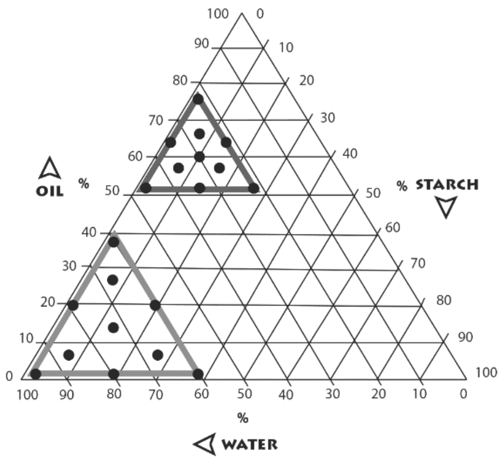

Figure 1.2 Emulsions Mixture Triangle depicting area of largest triangle. The smaller upper left triangle are w/o emulsions and the smaller lower left triangle are o/w emulsions. The amount of each ingredient are shown as true percentages.

These areas were determined by preliminary data, previous methods, and data from Shah, Tsong, Sathe, & Liu (1998) and McClements (2007). The design was formed with ten points each. The augmented simplex centroid design was used for the component mixture sample. The response variable was days of stability. Figure 1.2 shows those parts of the emulsion mixture triangle focused on in this study. The other areas are explained in the discussion section.

Emulsion Preparation

Water and the OS starch (Figure 1.2, Table 1.1 and Table 1.3) were stirred at 25°C in a 150 mL screw cap glass jar on a magnetic stirring plate for 1.5 h. The samples that were not transparent after 1.5 h of stirring (due to higher concentration of starch) were stirred for an additional hour. Once the starch solution was semi-translucent, sodium benzoate was added at a level of 0.1% of the starch concentration. The mixture was then stirred in a water bath at 60°C for 15 min to ensure complete dissolution. Mineral oil was added slowly while a High Shear Homogenizer (Bamix Biohomogenizer, Switzerland) mixed the sample at high speed (10,000 rpm) for 6 min at 25°C, until there were no oil droplets on the surface. The total weight of each emulsion was 150 g. Due to the high viscosity (around 30,000 cP at a shear rate of 2 s^{-1}), the w/o emulsions also were mixed with a Laboratory Bench Top Homogenizer (PRO Scientific Inc., Oxford, CT, USA) for 4 min at 8,000 rpm. For comparison purposes, samples were also made without the lab bench top homogenizer.

Part of each sample was saved for stability testing via optical microscopy, viscosity, electrical conductivity, and gravitational separation measurements. The remainder of the sample was microfluidized (Microfluidizer M110PII, Microfluidics, Westwood, MA) at 18,000 psi for 5 passes. The chambers used were Auxilary Processing Module (APM = H30Z), 200 microns followed by the Interaction Chamber (G10Z), 87 microns. Cold tap water surrounding the coil of the microfluidizer ensured sample temperature was maintained at 30°C. Care was taken to insure that only the emulsion was collected from the microfluidizer. The microfluidizer originally had water in its piping; therefore, the initial output from the microfluidizer was discarded. This ensured that the collected emulsion represented the undiluted original sample. Due to the high viscosity of the w/o emulsions, some samples (M and Q) were made with only 3 passes, instead of 5 because the emulsions were too thick by the third pass and would have occluded the microfluidizer if they were subjected to a fourth or fifth pass. The same stability tests were conducted on the non-microfluidized (and microfluidized) samples. The samples were stored at 4°C for subsequent stability measurements.

Characterization of Emulsions

Particle Size Distribution of Emulsions

A Laser Scattering Particle Size Analyzer LA-910 (Horiba Ltd., Kyoto, Japan) was used to determine the particle size (volume diameter) of the o/w emulsions at room temperature (~25°C), but was not used for w/o emulsions because the samples would be diluted by water. The pipette was inserted into the middle of the o/w emulsion and when the tip was withdrawn from the sample, the pipette tip was wiped with a Kimwipe. Due to possibility of oil droplets on the surface of the emulsions, only the middle of the emulsion was sampled for this test. This was injected into the reservoir tank using distilled water as the dispersant. Only o/w emulsions can be tested with the particle size analyzer. To ensure a homogenous sample, each sample and dispersing liquid was agitated at 400 rpm. The sample was then circulated and sonicated with ultrasonic vibrations (39 kHz). The particle size (assuming all particles were spherical) was determined by the instrument's software equations that were based on the light scattering off the particles. The light sources were a He-Ne laser and tungsten lamp and the software produced a particle size distribution. The particle size was evaluated on fresh and aged emulsions, depending on the results from the gravitational separation stability testing. Therefore, particle size was tested on day 0, 7, and if there was a difference in gravitational separation. The samples were run in duplicates.

Optical Microscopy

Each emulsion was viewed by an optical microscope (Olympus BX51 microscope, Olympus America Inc., Melville, NY). The emulsion sample varied between 0.1 to 2 mL depending on the original starch concentration and opacity of the sample. The sample was also taken by a pipette and placed on a plain microscope slide and covered with a cover slip (Fisher Sci. International Inc.). A 40x objective lens was used for all samples. The SPOT 4.6 Windows software (Diagnostic Instrument Inc., Sterling Heights, MI, USA) captured the images of the emulsions. Calibration markers were added to the images, with the software, to give the particle sizes of the droplets. The samples were run in duplicates.

Gravitational Separation

The gravitational separation was performed by the method of McClements (2007) using graduated transparent centrifuge tubes. The creaming index was calculated using the serum layer value and the total height of the emulsion. The samples were run in duplicates.

A known homogenous concentration of the sample (40 ml) was poured into the 50 ml plastic screwcap tubes and sealed. The tube was inverted once back and forth to ensure a homogenous sample. The samples were kept undisturbed at 4°C to ensure a constant temperature and observations were taken daily. Changes in appearance or if layers appeared were measured and the time noted. The creaming index (CI) was calculated by the following formula:

$CI = 100 \times H_S/H_E$

Where H_S is the serum layer and H_E is the emulsion layer.

McClements (1999) and Dickinson (1992) describe the Stokes' law for the creaming rate of a particle in a liquid and how it can predict an emulsion's stability. If the original particle size of the oil droplet is 1 micron and has a density ρ of 910 kg/m^3, suspended in water (shear viscosity η_1 = 1 mPa s, density ρ = 1000 kg/m^3) then the creaming rate will be 17 mm/day. Dickinson (1992) determined that if an emulsion has a creaming rate less than 1 mm/day it can be called an emulsion stable against creaming.

Emulsions' Viscosity

The viscosity of emulsion was measured at room temperature (~ 25°C) by DV-II+Pro Brookfield viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA) with the #21 spindle. The samples were tested at different rpms in order to have a torque value between 10 and 100%. The viscosity, shear stress and shear rate were recorded. The samples were run in duplicates.

Electrical Conductivity

The conductivity of a sample was measured by an electrical conductivity meter (Fisher Scientific, accumet®, Pittsburgh, PA, USA) based on the method of McClements (2007). The output number was in mS/cm or μS/cm. The samples were run in duplicates.

Statistical Analysis

Every sample was produced in triplicate. The samples for all of the analysis methods were run in duplicates. The results were analyzed using Microsoft Excel (Redmond, WA) where calculations were conducted for the mean, standard deviations, and other statistical analysis. For experiments with three ingredients, the experimental domain is within an equilateral triangle. The vertices represent the pure components, the edges of the triangle represent the two-component blends, and points within the triangle represent the three-component blends. This is called the Mixture Emulsion Design. MiniTab (State College, PA) was used to produce the Mixture Emulsion Design. The results were also analyzed using SAS Program Software MiniTab using the Mixture Emulsion Design results.

Results and Discussion

Emulsion Stability

The stability (in days) of the emulsions was determined by gravitational separation, particle size growth/expansion in microns, conductivity change, and creaming index. An emulsion was deemed stable if it was stable for at least 100 days. It is stable if there is no separation of phases. The stability of the other parts of the emulsion triangle that are not completed in the lab was estimated by inputting the results for the selected mixtures (see Methods section).

O/W Emulsions

Table 1.1 Stability of o/w emulsions, where the amount of water, oil, and starch are in percent, on a 100 g basis.

				Stable emulsion?	
				w/out	with
ID	oil	water	starch	microfluidizer	
A	38	60	2	No	No
B	20	78	2	No	Yes
C	26	66	8	No	Yes
D	20	60	20	No	Yes
E	8	84	8	No	Yes
F	14	72	14	No	Yes
G	8	66	26	No	Yes
H	2	96	2	No	Yes
I	2	78	20	No	Yes
J	2	60	38	Yes	Yes

All of the o/w emulsions without microfluidizing were not stable for the 100 days except for sample J (Table 1.1). This may be due to the large amount of emulsifier needed to create o/w at low mechanical energy (without the microfluidizer). All of the o/w emulsions created using the microfluidizer were stable except for sample A (Table 1.1). Sample A may not have enough starch (emulsifier) needed to emulsify the sample.

Table 1.2 Stability and particle size of o/w emulsions. The amount of water, oil, and starch are in percent, on a 100 g basis. "X" indicates that the sample is not stable.

		Particle size (microns)			Particle size (microns)	
		w/out microfluidizer			w/ microfluidizer	
ID	Stable in days	Day 0 average	Day 100 average	Stable in days	Day 0 average	Day 100 average
A	25	2.5±0.5 a	x	80	1.5±0.5 a	x
B	30	2.5±0.5 a	x	160	0.2±0.0 b	1.0±0.0 b c
C	45	3.0±1.0 a	x	180	1.5±0.1 a	2.5±0.1 a
D	8	1.0±0.5 a	x	200	0.5±0.0 b	2.0±0.5 a b
E	38	2.5±1.0 a	x	365	0.15±0.0 b	0.2±0.0 c
F	15	2.5±1.0 a	x	365	0.15±0.0 b	0.15±0.0 c
G	55	2.5±1.0 a	x	365	0.15±0.0 b	0.9±0.0 b c
H	20	2.0±1.0 a	x	365	0.15±0.0 b	0.15±0.0 c
I	35	3.0±0.5 a	x	365	0.15±0.0 b	2.5±0.9 a b
J	120	1.0±0.5 a	2.0±0.5	140	0.5±0.0 b	3.0±0.9 a
	Means that do not share a letter are significantly different.					

The emulsions that were not microfluidized had initial particle sizes around 2 microns, whereas the microfluidized samples had particle sizes between 0.15 and 1.5 microns (Table 1.2). The particle size increased over time (0 to 100 days) for all the samples. There was no trend relating particle size to amount of starch for the unmicrofluidized and microfluidized samples. The microfluidized samples with the largest particle size (A and C with particle sizes of 1.5 microns) had the highest amount of oil (38 and 26%, respectively) (Table 1.2). Samples A and C were unstable emulsions because they had the highest oil content.

For the o/w emulsions that were not microfluidized, the most stable emulsions were ones with more starch and water (Table 1.2). The sample with 2% oil, 60% water, and 38% starch had an average of 120 days of stability (2.0 +/- 0.5 microns for particle size) (Table 1.1 and 1.2). The main reason is the high viscosity contributed by high percentage of starch. The movement of oil droplets in the system was reduced, thus the chance of droplets getting close is low. This means that this OSA modified starch can be used for o/w emulsions with common laboratory equipment (hand held homogenizer to make a crude emulsion and bench top high shear mixing homogenizer), and does not have to go through microfluidization.

For the o/w emulsions that were microfluidized, the most stable emulsions were almost evenly spread through various starch: oil: water combinations conducted in these experiments. The most stable samples were 14% oil, 72% water, and 14% starch, which was stable for over

365 days (3.0 +/- 0.9 microns for particle size); and the sample with 2% oil, 96% water, and 2% starch, which was also stable for over 365 days (0.15 +/- 0 microns particle size) (Table 1.2).

Microfluidized emulsions were more stable than those not undergoing this process. These particle sizes were smaller and stayed smaller for a longer period of time (Table 1.2). This agrees with the results from Taherian, Fustier, & Ramaswamy (2005). Many of the o/w emulsions had particle sizes less than 1 micron. When the emulsion was visibly unstable by the gravitational separation test its particle size was larger (usually by tenfold) than a fresh emulsion (Appendix A). Most of the emulsions had a large increase in particle size at the point of their instability. Some emulsions had an initial particle size greater than 2 microns and some as high as 9 microns after 100 days. Samples D (20, 60, 20; oil, water, starch, respectively) and F (14, 72, 14; oil, water, starch, respectively) had very short shelf lives. Both had a ratio of starch to oil of 1:1.

Viscosity of Emulsions

The emulsions with higher concentrations of starch and oil (samples A and J), making the emulsion very viscous, had a lower particle size when going through 3 passes instead of 5 passes. Some of the viscous samples (A and J) could not pass through the 87 micron size channel more than 3 passes and instead the emulsion was broken. Therefore, the w/o samples had 3 passes; however these same samples are made with 5 passes as well in order for comparison.

The oil in water emulsions did not have a viscosity value due to being not viscous enough for the Brookfield Viscometer (due to the equipment, the value is under 2 cP at 100 rpm, SS and SR are estimated at 2.33 and 93.0, respectively.

Creaming Index

The creaming index results did not show a clear trend (appendix A). The samples became unstable in three different ways. The w/o emulsions tended to form two layers while the o/w emulsions formed three layers, unless the samples had component ratios close to the phase inversion line. The two layers were composed of cream and serum layers. The three layer system was composed of oil, emulsion, and serum layers. The creaming index was similar for the majority of the samples. It stayed at zero until the emulsion was unstable. The trends of the remaining emulsion samples are in appendix A. Once the samples showed signs of a layer of oil on the surface, (i.e. a creaming index above zero) the samples were declared unstable. This determination in conjunction with the other analyses allowed the estimation of stability in days. This value was used in the subsequent statistical models.

Electrical Conductivity

Conductivity tended to increase over time for all samples except 38, 60, 2; 20, 78, 2; and 2, 96, 2 (percent oil, water, starch, respectively) (Appendix A). The conductivity of these samples was constant. The increase in conductivity may be due to the way these emulsions become unstable. When these emulsions are in their unstable state, as determined by gravitational separation, an oil layer was present on the surface, then an emulsion layer (middle), and finally a serum layer (bottom). When the sample was assessed for conductivity, the probe was submerged in the middle of the sample and the sample stirred slightly. If there were a few oil droplets coalescing and rising to the surface, these may not have been detected due to slight stirring of the sample. Therefore, the sample in contact with the probe had a higher water content. Water (conductivity of 7.18 µS for distilled water and 0.41 µS for deionized water), but not oil (0 µS), can conduct electricity. Therefore, the samples had a higher ms conductivity over time.

Overall Stability

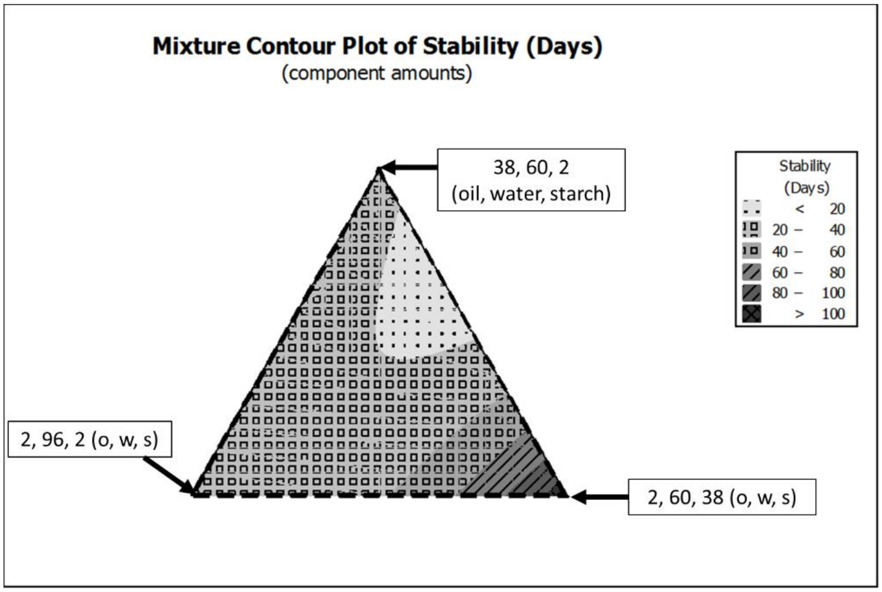

Figure 1.3 Contour plot of stability (days) of o/w emulsions made without microfluidizer

Figure 1.3 is the contour plot of stability of o/w emulsions made without the microfluidizer. The R^2 (adj) was 72%, suggesting that the stability for o/w emulsions without the microfluidizer may be reasonably predicted with this model. A higher R^2 indicates a better fit. The Lack of Fit (LOF) was low (Figure 1.3). This was a good indicator that this model accurately estimated stability for the remaining emulsions in the plot. Figure 1.3 shows the stability in days using darker gray and lines representing longer stability. This contour plot of stability shows MiniTab results estimating the days of stability for every point in the mixtures triangle. The most stable emulsion was 2, 60, 38 (percent oil, water, starch, respectively), (the low right hand corner of the triangle, and marked with the darkest gray). Figure 1.3 shows that the more stable emulsions are those with more starch and less oil.

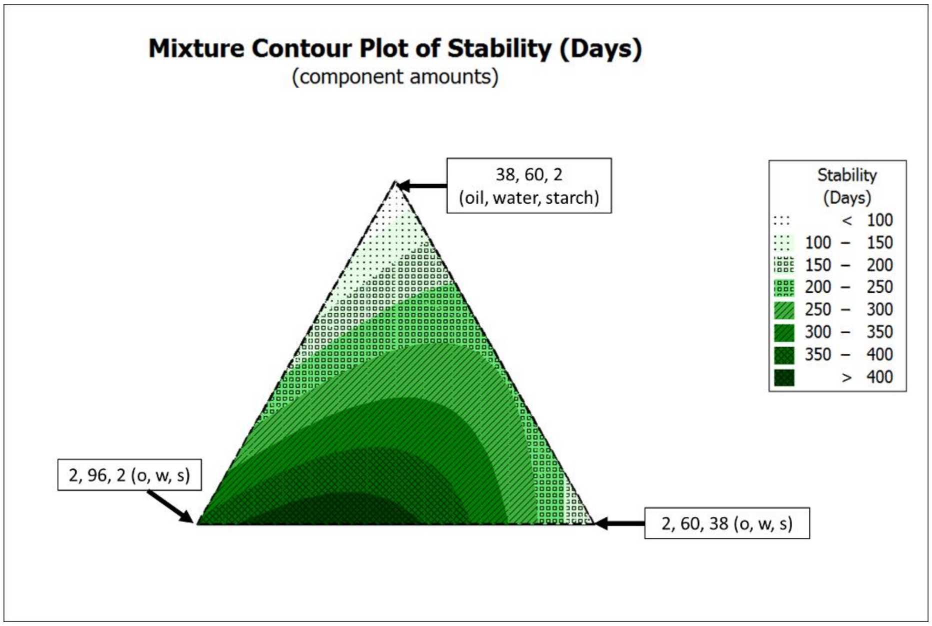

Figure 1.4 Contour plot of stability of o/w emulsions made with microfluidizer in days

Figure 1.4 shows the contour plot of stability of o/w emulsions made with the microfluidizer. The R^2 (adj) value of 83%, was sufficient to make accurate estimations of stability for the remainder of the emulsions in the triangle. Figure 1.4 shows the stability in days using a darker green and checkered representing longer stability. The most stable emulsion was sample H (2% oil, 96% water, 2% starch). The more stable emulsions are those with more water (Figure 1.4). When comparing Figure 1.4 to Figure 1.3, microfluidized emulsions are more stable.

W/O Emulsions

Table 1.3 Stability and viscosity of w/o emulsions, where the amount of water, oil, and starch are in percent, on a 100 g basis. Note: the emulsion is called stable if it is stable for at least 100 days, without separation. "Thick" indicates that the emulsion was too viscous for the viscometer. "X" indicates that the emulsion was not prepared due to being too thick for the microfluidizer. SR is the shear rate and SS is the shear stress.

						Viscosity Day 0					
				Stable emulsion?		w/out microfluidizer			w/ microfluidizer		
				w/out	with	SR	SS	viscosity	SR	SS	viscosity
ID	oil	water	starch	microfluidizer		(s^{-1})	(Pa)	(cP)	(s^{-1})	(Pa)	(cP)
K	76	22	2	No	No	20	720±2 a	1500±5 c	93	40±1 b	43±2 d
L	64	34	2	No	Yes	20	90±1 d	440±3 d	0.3	212±5	76333±7
L									0.5	260±3	55900±9
L									1	331±3	36050±7
L									1.5	410±5 a	29467±8 a
M	64	22	14	No	X	thick	thick	thick	X	X	X
N	60	30	10	No	Yes	20	500±6 b	1500±4 c	thick	thick	thick
O	52	46	2	No	No	93	30±2 e	31±2 f	20	17±2 c	90±3 c
P	52	34	14	Yes	Yes	20	110±6 d	509±4 d	thick	thick	thick
Q	52	22	26	Yes	X	thick	thick	thick	X	X	X
R	68	26	6	No	No	1	239±3	25750±7	thick	thick	thick
R						1.5	303±4	16300±7			
R						5	427±3 c	9200±9 b			
S	56	38	6	No	Yes	20	40±3 e	200±3 e	1	259±4	27950±7
S									1.5	343±7	18475±8
S									3	406±3 a	14583±5 b
T	56	26	18	Yes	Yes	1	134±6	14450±3	thick	thick	thick
T						3	300±5	10767±8			
T						5	439±3 c	9440±4 a			
	Means that do not share a letter are significantly different.										

For the w/o emulsions created without the microfluidizer, the most stable contained more starch and water (Table 1.3). Thus, OSA modified starch can be used for w/o emulsions made with common laboratory equipment, and does not have to go through microfluidization. Specifically, the area with 52% oil, 22% water, and 26% starch had an average of 240 days of stability. All of the w/o emulsions made without microfluidization were not stable except for samples P, Q, and T, potentially due to the large amount of emulsifier needed to emulsify the sample with low mechanical energy (without the microfluidizer) (Table 1.3). Samples P and M had the same amount of starch, but M was not stable. This is possibly due to the higher amount of oil in sample M. All of the w/o emulsions made with the microfluidizer were stable except for samples K, O, and R. This may be due to the sample not having enough starch (emulsifier) to emulsify the sample.

Except for sample O, all other samples were viscous and some (samples M and Q) too thick for the Brookfield viscosity measurement. The emulsion viscosity over 50,000 cP could not be determined with the Brookfield. Because the samples were tested at different rpms in order to have a torque value between 10 and 100%, some of the samples were too thick or too thin in order to be measured at 20 SR. Table 1.3 shows the viscosity of the samples around 20 SR. Sample L microfluidized could not get up to a SR of 20 and therefore the table shows the results that were obtainable. Sample O unmicrofluidized only had one viscosity measurement because everything else was out of range. But, sample O microfluidized had results at multiple SR and therefore, the viscosity at 20 SR was calculated. Other samples could not go through the microfluidizer (labeled "thick" on Table 1.3), most likely because they were too thick and clogged the instrument. Thus if the system has high amount of oil, a lower starch content is needed, to lower viscosity.

Table 1.4 Stability and particle size of w/o emulsions, where the amount of water, oil, and starch are in percent, on a 100 g basis. "X" indicates that the sample is not stable. "N/A" indicates that the emulsion was not prepared due to being too thick for the microfluidizer.

		Particle size (microns) w/out microfluidizer			Particle size (microns) w/ microfluidizer	
ID	Day unstable	Day 0 average	Day 100 average	Day unstable	Day 0 average	Day 100 average
K	4	2.5±1.0 a b	X	10	0.7±0.1 a	X
L	20	2.5±0.5 a	X	100	1±1 a	3±1.5 a
M	90	0.5±0.5 c	X	N/A	N/A	N/A
N	80	0.5±0.1 c	X	170	0.6±0.5 a	3±1 a
O	10	1±0.5 b c	X	50	0.9±0.5 a	X
P	100	0.4±0.1 c	3±1 a	250	0.3±0.1 a	2±1 a
Q	240	0.2±0.1 c	0.75±0.5 a	N/A	N/A	N/A
R	7	0.8±0.1 b c	X	15	1±0.5 a	X
S	25	2.5±0.5 a	X	150	0.09±0.01 a	3±1.5 a
T	120	0.1±0.01 c	2±1 a	140	0.08±0.01 a	X
	Means that do not share a letter are significantly different.					

W/o emulsions could be created without the use of a microfluidizer (Table 1.4). The best sample was P (52, 34, 14; percent oil, water, starch, respectively) which was stable for 100 days and had a viscosity of 505 cP (30 rpm, 27.9 s^{-1} SR, 140.9 Pa SS). An even more stable sample was sample Q (52, 22, 26; percent oil, water, starch, respectively). It was stable for 240 days, but could not flow (over the max viscosity limit for the Brookfield).

OS starch could be used to create stable w/o emulsions, best with a microfluidizer (Table 1.4). However, some of these stable emulsions such as the most stable (250 days of stability) sample P (52, 34, 14% oil, water, starch, respectively) could not flow (14,583 cP, 3 rpm, 12.79 s-1 SR, 406.7 Pa SS) (Tables 1.3 and 1.4). Sample O (52, 46, 2% oil, water, starch, respectively) was stable for 50 days and could flow (Tables 1.3 and 1.4). Sample L (64, 34, 2% oil, water, starch, respectively) was stable for 100 days, but would flow very slowly, 29,467 cP (1.5 rpm, 1.39 s-1 SR, 410.1 Pa SS). Sample S (56, 38, 6% oil, water, starch, respectively) was stable for 150 days, but was viscous and could only flow only very slowly (Tables 1.3 and 1.4).

There was no trend for the conductivity of these samples. Emulsions created without microfluidizing had initial particle sizes between 0.2 and 2.5 microns as measured by microscopy. The particle size increased over time (0 to 100 days) for all the samples. By 100 days, the microfluidized samples had particle sizes between 0.08 and 1 microns. The nonmicrofluidized samples that had higher amounts of starch (samples M, N, P, Q, and T) had initial original particles sizes (0.1-0.5 microns), but no such trend was not observed for the microfluidized samples.

Overall Stability

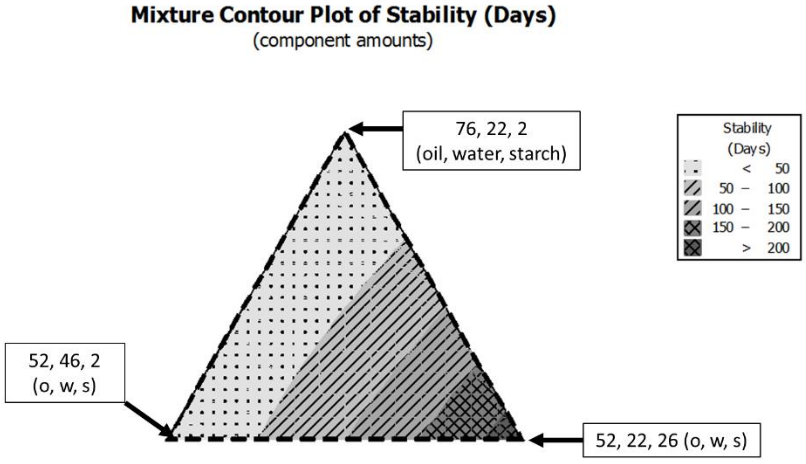

Figure 1.5 Contour plot of stability (days) of w/o emulsions made without microfluidization

Figure 1.5 shows the contour plot of stability of w/o emulsions made without microfluidization. The R^2 (adj) value was 96% so this model was accurate to estimate the days of stability for the remainder of the emulsions in the triangle. The Lack of Fit (LOF) was zero. Figure 1.5 shows the stability in days using darker gray and checkered representing longer stability. This mixture contour plot of stability shows the results from MiniTab results estimating the days of stability for every point in the mixtures triangle. The lower right hand corner of the triangle (darkest gray and checkered) represents Sample G, which had 52, 22, 26% oil, water, starch, respectively. The more stable emulsions are those with more starch and less oil. The lightest gray dotted areas are the least favorable combinations of oil, water, and starch if made without using a microfluidization (Figure 1.5). The light gray region was where there was too little starch and more oil, making the sample difficult to stir during the homogenization steps and being too viscous to pass through the microfluidizer, without clogging.

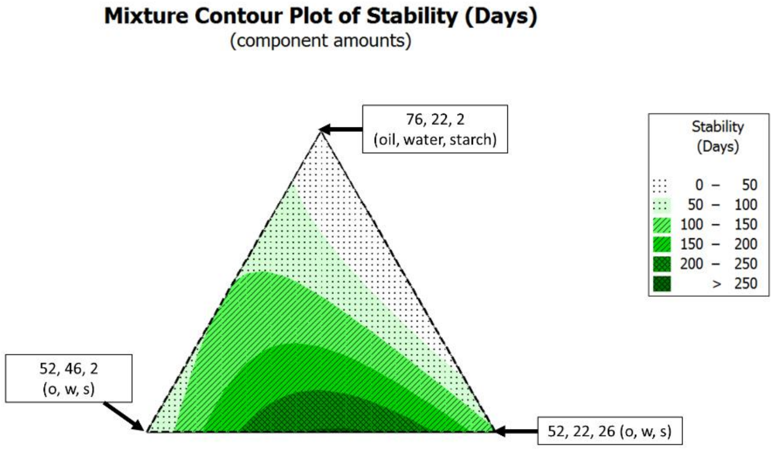

Figure 1.6 Contour plot of stability (days) of w/o emulsions made with microfluidization

Figure 1.6 shows the contour plot of stability of w/o emulsions made with the microfluidizer. There was still a problem with the two lower points samples (2 samples, each having a duplicate), which are omitted from this analysis. This was due to that the samples were not able to go through the microfluidizer. The overall model was significant. The R^2 (adj) value was 91%, which was sufficient for this study, to make accurate estimations of stability for the remainder of the emulsions in the triangle. This means that the regression model fits well. The Lack of Fit (LOF) was high. Figure 1.6 shows the stability in days using a darker green representing a longer stability. This mixture contour plot of stability shows the results from MiniTab results estimating the days of stability for every point in the mixtures triangle. To read the graph, here was an example: the lower left hand corner of the triangle was the darkest green and checkered. This point was representing the 52, 34, 14 (percent oil, water, starch, respectively) sample. The more stable emulsions are those with less oil and a balance between water and starch (Figure 1.6). In Figure 1.6, the white and dotted area shows the least favorable combinations of oil, water, and starch. The lower right region, are combinations with too much starch, making the sample difficult to stir during the homogenization steps and very viscous to pass through the microfluidizer, which causes clogging. Due to clogging for the too thick samples, these are left out of the analysis.

Overall Stability for o/w & w/o Microfluidized Samples

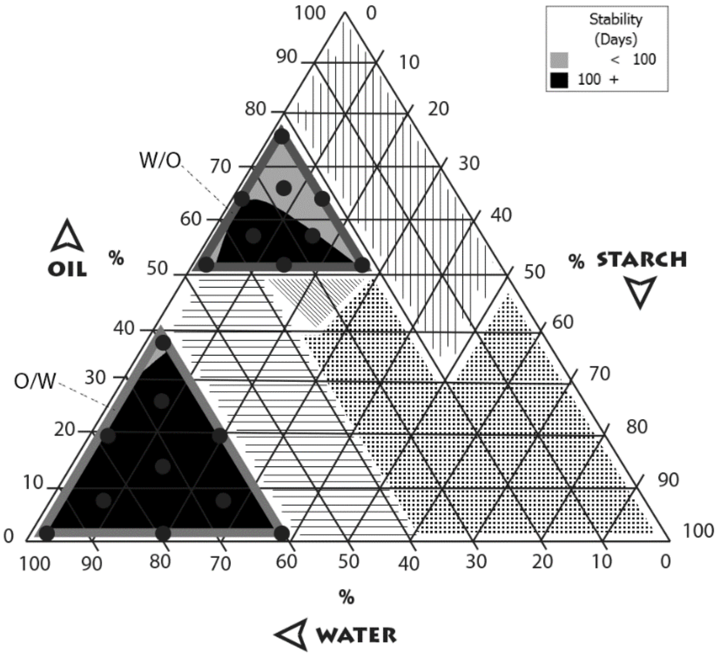

Figure 1.7 Emulsions Mixture Triangle of the amount of water, oil, and starch are in percent, on a 100 g basis.

This study focused on microfluidized samples: w/o emulsions (smaller upper left triangle) and o/w emulsions (smaller lower left triangle) where the key indicates that the solid black areas are stable over 100 days stable and the gray solid areas are unstable under 100 days stable. The other areas were not prepared and thus cannot be distinguished as o/w or w/o emulsions. The upper right area (vertical lines) are samples that cannot go through the microfluidizer. The lower right area (dots) are samples where the starch:water ratio is too high. The lower left area (horizontal lines) are samples where results from the contour plots show low stability, and the final middle area (diagonal lines) are samples where it is not clear if the emulsion is o/w or w/o.

The area outside of the small o/w and w/o triangles (Figure 1.7) were not the focus of this study. The samples in the upper right hand corner have too much oil which results in a very unstable emulsion while using this modified starch and the sample will be very viscous and not fluid (usually over 30,000 cP at 1.5 rpm, shear stress around 410 Pa and a shear rate around 1 s^{-1}; more viscosity results in Table 1.3). The instrument chosen for this experiment had limitations as to sample viscosity (high concentration of starch and/or oil). It was very difficult to get a sample with a high concentration of oil through the microfluidizer. The largest area in Figure 1.8, the area in the lower right corner, was too thick and due to the high viscosity caused by the high ratio of starch to water. The solid (filled in) area would result in emulsions that would be hard to determine if they are o/w or w/o emulsions. This is an area for future study. The area that is in the top point of the largest triangle does not have enough starch to stabilize the emulsion and has too much oil to be emulsified.

Emulsions from the upper left triangle in Fig. 1.7 would have more water, less oil and more starch and be stable. Any instability may be due to a lack of sufficient starch (emulsifier) to deal with the high oil level. The lower left triangle in Fig. 1.7 (the o/w triangle) indicates that the samples with more oil are less stable. The modified starch used in this study is advertised to be used for o/w emulsions (National Starch Bulletin). Even though this OS starch has an HLB value that indicates that it is used for o/w emulsions, this study shows that this OS starch can also be used for w/o emulsions.

Conclusions

Stable o/w emulsions (over 100 days in this case) could be made without a microfluidizer if a large amount of starch is used. This may be due to the large amount of emulsifier needed for the emulsions to emulsify the sample with low mechanical energy (without the microfluidizer). Stable o/w emulsions prepared with a microfluidizer had stability for over 100 days, some even were stable for over 600 days. The o/w microfluidized samples were all stable except for sample A with a very low amount of starch (2%). This may be due to the sample not having enough starch (emulsifier) to emulsify the sample. These are similar findings for the w/o samples, where the unmicrofluidized samples were stable with a high amount of starch (14-26%) and the microfluidized samples were not stable with the lowest amount of starch (2-6%). The microfluidized samples always had a higher stability compared to the unmicrofluidized (o/w and w/o) samples. The o/w emulsions were more stable than the w/o emulsions. For the o/w emulsions that were not microfluidized, the most stable emulsions were ones with more starch and water. This means that this OSA modified starch can be used for o/w emulsions with common laboratory equipment (hand held homogenizer to make a crude emulsion and bench top high shear mixing homogenizer), and does not have to go through microfluidization. For the o/w emulsions that were microfluidized, the most stable emulsions were almost evenly spread through various starch: oil: water combinations conducted in these experiments, but the sample with the most oil was not stable.

W/o emulsions were created without a microfluidizer. The most stable emulsions were ones with more starch and water. OSA modified starch can be used for w/o emulsions with common laboratory equipment, and does not have to go through microfluidization. Some samples could not go through the microfluidizer, most likely because they were too thick and clogged up the equipment. This means that if there is a high amount of oil, then a lower starch content is needed, so the sample won't be too thick.

Stable w/o emulsions are more difficult to prepare compared to o/w emulsions, as expected from previous work; however, microfluidizing helped to increase stability. Two of the unmicrofluidized samples (76, 22, 2 and 68, 26, 6; percent oil, water, starch, respectively) had stability of 4 and 7 days, respectively. These samples had the least amount of starch and also higher amount of oil. This confirms that emulsions using OS starch are less stable if a large amount of oil is used, while the amount of OS starch is low. The most stable w/o unmicrofluidized may have been the most stable due to its high viscosity. Therefore, to adequately compare the remainder of the w/o microfluidized results, the most stable emulsion that could flow had a stability of 150 days. This sample had the second lowest amount of oil.

The statistical mixture experiments models using MiniTab was successful at predicting the stability for the other emulsions in the o/w and w/o emulsion triangles.

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Chapter 2 - Adsorption of modified starch at emulsified oil/water interfaces

Abstract

The concentration of modified OS starch adsorbed to the mineral oil and the water phases was determined for oil-in-water (o/w) emulsions. The percentage of the starch adsorbed at the mineral oil phase was determined and compared when different ratios of starch to oil and water were used. When the ratio of oil:starch was decreased, the emulsion particle size decreased. As the starch content increased, the percent starch adsorbed onto oil based on total oil increased. The adsorption yield and the level of starch in the emulsion did not show a trend. The surface load ranged from 1.6 to 6.98 mg/m^2. The sample with the highest concentration of starch (26 g/ml) had the highest surface load (6.98 mg/m^2) and samples with low concentrations of starch (0.84 and 1.68 g/ml) had the second and third highest surface loads (6.82 and 4.70 mg/m^2, respectively). The ratio of oil:starch was increased to determine the emulsifying capacity. A high emulsifying capacity was achieved. Samples with an oil:starch ratio of 3:1 were stable for over 80 days while other samples with oil:starch ratios of 5:1 and 6:1 were stable for one week.

Introduction

Emulsions are used in the food, industrial, and pharmaceutical industries to bring two substances that are usually not miscible together and typically one ingredient is dispersed in another (McClements, 2007b). By nature, oil and water tend to separate by coalescence when mixed; therefore, there is a great need for emulsifiers to keep them from separating. Modified starch may be utilized as an emulsifier by means of its functional groups, which bridge the interface of two immiscible phases (McClements, 2007b). One way to create an amphiphilic starch is to react the starch (hydrophilic) with OS (hydrophobic) (Trubiano, 1986). OS starch is advertised for use for o/w emulsions and encapsulation (National Starch Food Innovation Technical Service Bulletin). Its main stabilization mechanism is steric hindrance where the droplets are protected from aggregation (Chanamai & McClements 2001; Tesch, Gerhards, & Schubert, 2002). Steric hindrance works to stabilize particles using nonionic particles, help inhibit particle aggregation, but cannot directly inhibit sedimentation and creaming (Chanamai & McClements, 2001; Napper, 1976; Tesch, Gerhards, & Schubert, 2002).

Prochaska et al. (2007) determined that the adsorption process for modified starches is different compared to the adsorption process for unmodified starches. One of the reasons why there were differences was due to the hydrophobically modified starch that could adsorb better at the air/water interface. Some methods to determine the surface activity of a sample include conducting equilibrium surface/interfacial tension experiments (Prochaska et al., 2007). The modified starches were more surface-active and had lower equilibrium surface tensions. Prochaska et al. (2007) also determined that the air/water surface tension was lowered from 70 mN/m to 55 mN/m. Compared to cross-linked starch, oxidized starch and other modified starches, starch sodium octenylsuccinate resulted with the best adsorption activity for toluene/water and air/water interfaces (Prochaska et al., 2007). Modified starches with a higher degree of substitution had higher surface activity (Prochaska et al., 2007).

According to Nilsson & Bergenstahl (2007b), a larger droplet size results from a smaller emulsion surface area. The surface load is calculated using the adsorbed concentration and the specific surface area of the emulsion. It is also influenced by the original concentration of OS starch (Eliasson, Bergenstahl, Nilsson, & Sjoo, 2013; Nilsson & Bergenstahl, 2006, 2007a, 2007b, 2013). It is the concentration absorbed to an interphase and measured in units of macromolecule per surface area (milligrams per square meter) (Nilsson, Leeman, Wahund, &

Bergenstahl, 2006). The surface load can be high if there are many molecules present and the emulsion surface area is small (Eliasson, Bergenstahl, Nilsson, & Sjoo, 2013; Nilsson & Bergenstahl, 2006, 2007b). Nilsson & Bergenstahl (2007b) concluded that both the total surface area created during emulsification and amount of OS starch available for adsorption at the surface affect the adsorption of the starch. Based on kinetic factors, polymers with a larger radius will cause decreased adsorption time (the time it takes for the particles to adsorb to the surface) when the sample is processed under turbulent flow (Gerhard, 2002; Napper, 1977 Nilsson, Leeman, Wahund, & Bergenstahl, 2006; Nilsson & Bergenstahl, 2007b). With a larger sized molecule, there is a higher substituent density and higher adsorption energy. The substituent density influences the surface load (Nilsson & Bergenstahl, 2006, 2007a, 2007b, 2013). This is related to overrepresentation of large molecules at the surface and stronger kinetic adsorption factors (Nilsson & Bergenstahl, 2007b). Therefore, the larger size of the OS starch molecules, and thus a larger radius, causes the OSA modified starch to be adsorbed into the interfacial region faster and can be used as efficient emulsifiers (Nilsson, Leeman, Wahund, & Bergenstahl, 2006). Nilsson & Bergenstahl (2007a) suggested that the adsorption yield could be higher if there is enough OS starch available because the adsorption yield depends on interfacial charge density. The adsorption yield is calculated by the concentration of starch adsorbed divided by the original concentration of starch (Nilsson & Bergenstahl, 2007a).

The emulsifying capacity (EC) is how much oil can be emulsified with a certain amount of emulsifier (McClements, 1999). The EC is important to determine for a food manufacturer because it helps determine how much emulsifier is needed to create a stable emulsion (McClements, 1999). The EC is for o/w emulsions using water soluble emulsifiers. This value is determined by having a fixed amount of emulsifier and continually increasing the amount of oil until the emulsion breaks down (McClements, 1999 and Sherman, 1995).

The objective of this study was to determine the concentration of starch adsorbed to mineral oil and the water phases for o/w emulsions. The concentration of starch adsorbed to the water phase and the oil phase was calculated by using known ratios of the concentration of ingredients and by determining the total starch content in the original and the test sample. The calculations are in the following procedures. The following Figure 2.1 depicts the original and the test sample.

The emulsion goes through centrifugation for serum depletion, where the serum (subnatant) is collected. The concentration of starch in the serum layer of the sample is determined from total starch analysis. The concentration of starch in the original sample is the concentration in the original sample (the formula amount).

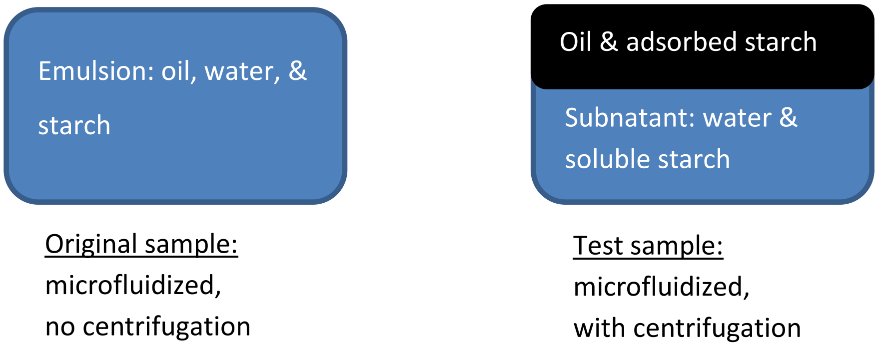

Figure 2.1 Depicting the reference (original) and test samples, with and without centrifugation

Materials & Methods

OS starch, HI-CAP® 100, was obtained from Ingredion (Bridgewater, NJ) and had a DS of 0.02, and 6.0% moisture. Mineral oil (cat. No. BP2629-1) and the ingredients to make the phosphate buffer [sodium azide (cat. No. S227I-1); monosodium phosphate, monohydrate (cat. No. S369-500); and disodium phosphate, heptahydrate (cat. No. S373-500)] were purchased from Fisher Scientific (Bridgewater, New Jersey). Deionized water was prepared in the lab. The total starch assay kits (cat. No. K-TSTA; AACC International Method 76-13.01) were purchased from Megazyme International Ireland (Wicklow, Ireland).

Procedure

Phosphate buffer, 100 mM pH 6.0, and Hi Cap 100 OS starch (concentrations shown in Table 2.1), were stirred at 25°C in a 150 mL screw cap glass jar on a magnetic stirring plate for 1.5 h. The mixture was stirred in a water bath at 60°C for 15 min, to ensure complete hydration. The samples were mixed in a high shear homogenizer (Bamix Biohomogenizer, Switzerland) set at high speed (10,000rpm) while mineral oil was added slowly over a 6 min period at 25°C, until there were no oil droplets seen on the surface. Due to the high viscosity, the w/o emulsions also were mixed with a Laboratory Bench Top Homogenizer (PRO Scientific Inc., Oxford, CT, USA) for 4 min, at 8,000 rpm. The samples were then microfluidized (Microfluidics M110PII) at 18,000 psi for 5 passes. The chambers used were Auxilary Processing Module (APM = H30Z), 200 microns followed by the Interaction Chamber (G10Z), 87 microns. Cold tap water surrounding the coil of the microfluidizer ensured temperature was maintained. Care was taken to insure that only the emulsion was collected from the microfluidizer. The microfluidizer originally had water in its piping; therefore, the initial output from the microfluidizer was discarded. This ensured that the collected emulsion represented the undiluted original sample. The samples were tested for the particle size using a Laser Scattering Particle Size Analyzer LA-910 (Horiba Ltd., Kyoto, Japan). The particle size (volume diameter) of the emulsions measured at room temperature (~25°C) was used to determine if the emulsions were stable for the entire test (Nilsson & Bergenstahl, 2006). The pipette was inserted into the middle of the emulsion and when the tip was withdrawn from the sample, the pipette tip was wiped with a Kimwipe. Due to possibility of oil droplets on the surface of the emulsions, only the middle of the emulsion was sampled for this test. This was injected into the reservoir tank using distilled water as the dispersant. To ensure a homogenous sample, each sample and dispersing liquid was agitated at 400 rpm. The sample was then circulated and sonicated with ultrasonic vibrations (39 kHz). The particle size (assuming all particles were spherical) was determined by the instrument's software equations that were based on the light scattering off the particles. The light sources were a He-Ne laser and tungsten lamp and the software produced a particle size distribution. The samples were run in duplicates.

Two-mL aliquots of the emulsion were taken and put into a 2 mL plastic microcentrifuge tube. The emulsions were centrifuged at 3,400 g for 10 min, 5,500 g for 25 min, and 7,000 g for 40 min. The subnatant was pipetted out and placed in new microcentrifuge tubes and centrifuged for an additional 40 min at 7,000 g (Nilsson & Bergenstahl, 2006). One gram of the subnatant was pipetted out and analyzed using the Megazyme kit.

The ratios of emulsions studied are displayed in Tables 2.1 and 2.2. Samples 8-16 were chosen because they demonstrated a good stability during preliminary experiments due to the low ratio of oil to starch. Samples 8, 10-13 and 15 all had a fixed concentration of water while varying the levels of oil and starch (Table 2.1). Samples 4-7 had the same concentration of water while increasing the ratio of oil to starch. Samples 1-3 had the highest emulsifying capacity with ratios of oil to starch as high as 6:1 (sample 1) (Table 2.1).

Calculations of the percent total starch and other raw data are in appendix C. The original concentrations of the ingredients ($c_{o\ total}$, $c_{o\ oil}$, $c_{o\ starch}$) were known. The $c_{starch\ in\ oil\ phase}$ and $c_{starch\ in\ water\ phase}$ ($c_{subnatant}$) were unknown. The concentration of starch in water phase (subnatant phase) is calculated with Equation 1.

<u>Equation 1:</u> $c_{subnatant} = [Starch\ concentration\ ] \times v_{o\ water}$

Where the starch concentration (g/g) was the test sample's total starch results and vo water (g/ml) was the original volume of water in the emulsion. The concentration of the starch in the oil and the concentration adsorbed is calculated with Equation 2.

<u>Equation 2:</u> $c_{starch\ in\ oil} = c_{o\ starch} - c_{subnatant} = c_{adsorbed}$

Where $c_{o\ starch}$ (g/g) was the concentration of starch in the original sample and $c_{subnatant}$ (g/g) was the concentration of starch in the subnatant after the emulsion was separated by centrifugation.

<u>Equation 3:</u> $Adsorption\ yield = \frac{c_{adsorbed}}{c_{o\ starch}}$

Where $c_{adsorbed}$ was the concentration of starch adsorbed as calculated in Equation 3 and $c_{o\ starch}$ was the original starch concentration (Nillson et al., 2006).

Equation 4: $\Gamma = \frac{(c_{adsorbed})(d_{32})}{6\varphi}$

Where Γ was the surface load (mg/m^2) obtained by relating the adsorbed concentration to the specific surface area of the emulsion; d_{32} was the area-weighted droplet diameter; $c_{adsorbed}$ was the concentration of starch adsorbed; and the φ was the dispersed phase volume fraction.

By comparing the amount of starch determined and the weight of starch in the oil phase of the test sample, the concentration of starch based on the weight of oil is calculated with Equation 5.

Equation 5: $\frac{g\ starch\ in\ oil\ of\ test\ sample}{g\ original\ oil} \times 100 =$

$\%\ starch\ adsorbed\ onto\ oil\ based\ on\ total\ oil$

Results & Discussion

The particle size of the emulsions was higher for Samples 1-5 (with values of 6.5, 3.9, 2.9, 5.9, 3.9 µm with 0.84, 1.0, 1.68, 2.0, 2.3 g/ml starch, respectively) (Table 2.1). This was due to the samples becoming quickly unstable (first 3 samples with the high emulsifying capacity were stable for one day). Other samples with a high emulsifying capacity, such as samples 4 (6:1, oil:starch) and 5 (5:1, oil:starch), were stable for 1 week. When oil:starch was 3:1 and lower (samples 6-16, Table 2.1), the samples were more stable (at least 80 days), as reflected with their smaller particle size.

The adsorption yield and the level of starch in the emulsion did not show a trend. The adsorption yield was the highest for Samples 3, 7, 9, 15, and 16 (with values of 0.46, 0.42, 0.46, 0.44, 0.46 with 1.68, 4.66, 5.60, 18.75, 26 g/ml starch, respectively) (Table 2.1). The adsorption yield for the remainder of the samples was on average 0.33.

The surface load was highest for sample 16 (6.98 mg/m^2 with 26 g/ml starch) (Equation 4 and Table 2.1). Sample 3 (1.68 g/ml starch) had the second highest surface load (6.82 mg/m^2) and sample 1 (0.84 g/ml starch) had the third highest surface load (4.70 mg/m^2) (Table 2.1). The surface load's equation includes the concentration adsorbed (dependent on the original concentration of starch, thus not discussed in the comparison) and the particle size, as numerators, and the dispersed phase volume fraction, as the denominator (equation 4). The sample with the second and third highest surface load (samples 3 and 1, respectively) had a high particle size and a low dispersed phase volume fraction. The sample with the highest surface load (sample 16) had a low particle size and a dispersed phase volume fraction about twice as high as sample 3 and 1.

The percent starch adsorbed onto oil based on total oil increased when there was a higher concentration of starch in the sample, except for the sample with 18.75g of starch, which had a lower percent starch adsorbed onto oil based on total oil value (64%) compared to the third highest concentration of starch (108% adsorbed onto oil based on total oil value) (Table 2.2). The percent starch in water phase based on total water increased when there was a higher concentration of starch in the sample (Table 2.2). The samples in the mid-range of starch concentration were fairly similar for percent starch adsorbed onto oil based on total oil (40 and 45%) and for the percent starch adsorbed onto water based on total water (1.5, 2.7, and 3.5%) (Table 2.2).

Table 2.2 indicated that the samples with the highest original concentration of starch (18.75 and 26 g/ml) had the highest percent of starch adsorbed onto oil based on total starch (64.8 and 64.2%, respectively), which means lowest percent starch adsorbed onto water based on total starch (35.2 and 35.8%, respectively). The remainder of the samples all had similar percent starch adsorbed onto oil based on total starch (32-53%). This may have to do with the optimal ratio of ingredients needed to create an emulsion that will adsorb to one layer over the other. Therefore, as the starch content increased, the percent starch adsorbed onto oil based on total oil increased, the percent starch adsorbed onto water based on total water increased, the percent starch adsorbed onto oil based on total starch did not support a concrete trend but mostly increased and the percent starch adsorbed onto water based on total starch mostly decreased (Table 2.2).

Nilsson, Leeman, Wahund, & Bergenstahl (2006) reported that the adsorption yield was 45-81%. The authors found that the adsorption yield was lower with more OS starch. The findings from this research found no trend (Table 2.1). Nilsson, Leeman, Wahund, & Bergenstahl (2006) found that the adsorption yield was about the same amongst their three OS starch samples (0.68, 0.71, 0.54 g/ml) and was not influenced of the differences in degree of substitution among the samples. The original concentrations of starch that they used (0.84, 1.26, 1.68 g/ml) were fairly low concentration of starch compared to this study. The results in Tables 2.1 and 2.2 display samples with varying concentrations of starch.

The surface load results for this study (1.6-6.98 mg/m^2) (Table 2.1) were slightly lower than Nilsson, Leeman, Wahund, & Bergenstahl's (2006) study (1.3-15.9 mg/m^2). For one of the Nilsson samples (DS 0.0224, degree of branching 0.0548, initial molar mass 39 x 10^9 g/mol, homogenized molar mass 12 x 10^6 g/mol and root mean square radii of 38 nm), had very high surface loads (1.8-15.9 mg/m^2). This may have been due to multilayers at the interface, polymer polydispersity, or polymer orientation at the interface. The other two samples had similar degree of branching, molar mass, but one sample had a lower DS. These other two samples had surface loads similar to the results in this study (1-3 mg/m^2) and had a large variation in the surface area. Nilsson et al. (2006) found that the surface load was correlated to the thickness of the interface layer (density of substituents on the OS starch molecule's surface), which was then correlated to the degree of substitution, radius, and molar mass. With a higher density of surface substituents, there was a higher surface charge density, and therefore, resulting in a higher surface load (Nilsson & Bergenstahl, 2007).

Nilsson, Leeman, Wahund, & Bergenstahl (2006)'s samples with the lowest concentration of starch (0.84 g/ml) had the highest surface load values (average 8.36 mg/m^2). For our study, the sample with a low concentration of starch (1.68 g/ml), but not the lowest, had the second highest surface load (6.82 mg/m^2) (Table 2.1). These results may be due to the calculation, which included particle size of the droplets. This may also be due to the lower concentration of oil in that sample.

Nilsson, Leeman, Wahund, & Bergenstahl (2006)'s samples had a higher percent starch adsorbed onto oil based on total oil, which was the trend found in this study (Table 2.2). Nilsson et al. (2006)'s samples had a higher percent starch adsorbed onto water based on total water, with an increase in initial starch, which was the trend found in this study (Table 2.2). The percent starch adsorbed onto oil based on total starch and the percent starch adsorbed onto water based on total starch did not show a trend for the Nilsson, Leeman, Wahund, & Bergenstahl (2006)'s samples, which was also similar to the findings in this study (Table 2.2).

Conclusions

The concentration of OS starch adsorbed in the water and the oil layer depended on the ratio of the ingredients. As the starch content increased from 0.8 to 26 g/ml, the percent starch adsorbed onto oil based on total oil increased from 5 to 208% and the percent starch adsorbed onto water based on total water increased from 0.6 to 14%.

The majority of the samples with high surface loads had the lowest concentration of starch. A high emulsifying capacity was achieved where samples with an oil:starch ratio of 3:1 were stable for over 80 days while other samples with oil:starch ratios of 5:1 and 6:1 were stable for one week. When oil:starch decreased, the emulsion particle size decreased.

Tables

Table 2.1 Surface load and adsorption yield compared to Nilsson, Leeman, Wahund, & Bergenstahl (2006) results with samples in order from lowest to highest concentration of starch.

	ratio oil: starch		oil	water	c_o	φ	d_{32}	r	adsorption
sample		oil,water,starch(db)	(g/ml)	(g/ml)	(g/ml)	(v/v)	(µm)	(mg/m^2)	yield
1	6:1	5,94.16,0.84	5	94.16	0.84	0.055	6.50±4.51	4.70±0.02 b	0.29±0.01 b
2	5:1	5,94,1	5	94	1	0.056	3.92±0.93	3.54±0.07 b c	0.30±0.03 b
3	3:1	5,93.32,1.68	5	93.32	1.68	0.056	2.91±0.12	6.82±0.01 a	0.46±0.01 a
4	6:1	12,86,2	12	86	2.00	0.132	5.86±2.21	3.10±0.02 b c	0.21±0.03 c
5	5:1	11.7,86,2.3	11.7	86	2.30	0.129	3.85±3.17	4.02±0.01 b	0.35±0.02 b
6	3:1	10.5,86,3.5	10.5	86	3.50	0.117	0.65±0.04	1.11±0.03 e	0.34±0.01 b
7	2:1	9.33,86,4.66	9.33	86	4.66	0.106	0.39±0.03	1.22±0.06 e	0.42±0.02 a
8	1.5:1	8.4,86,5.6	8.4	86	5.60	0.093	0.35±0.06	1.61±0.04 d e	0.46±0.02 a
9	1:1	6,100,6	5.36	86.6	5.36	0.062	0.35±0.02	1.77±0.04 d e	0.34±0.01 b
10	1:1	8,100,8	6.9	86.21	6.90	0.078	0.35±0.01	1.60±0.26 d e	0.31±0.04 b
11	1:1	10,100,10	8.33	83.33	8.33	0.094	0.34±0.01	1.74±0.09 d e	0.34±0.01 b
12	1:1	12,100,12	9.68	80.65	9.68	0.110	0.36±0.03	1.59±0.08 d e	0.30±0.02 b
13	0.7:1	8,100,12	6.67	83.33	10	0.076	0.27±0.04	1.96±0.04 d e	0.33±0.01 b
14	0.4:1	4,86,10	4	86	10	0.046	0.37±0.01	4.61±0.05 b	0.34±0.01 b
15	1:1	30,100,30	18.75	62.5	18.75	0.217	0.38±0.02	2.44±0.06 c d	0.44±0.01 a
16	0.3:1	8,66,26	8	66	26	0.097	0.34±0.04	6.98±0.06 a	0.46±0.04 a
	Means that do not share a letter are significantly different (p<0.05).								
The following from Nilsson et al. (2006)									
similar to sample 1		**5,94.16,0.84**			0.84	0.05	6.9	15.9	0.81
					0.84	0.05	6.5	9.1	0.7
					0.84	0.05	4.6	13.3	0.72
					0.84	0.05	0.75	1.4	0.71
					0.84	0.05	1.6	2.1	0.45
similar to sample 2		**5,94,1**			1.26	0.05	1.9	6.1	0.74
					1.26	0.05	4.5	14.3	0.74
					1.26	0.05	0.6	1.5	0.62
					1.26	0.05	0.86	2.7	0.74
similar to sample 3		**5,93.32,1.68**			1.68	0.05	0.6	2.1	0.61
					1.68	0.05	0.6	1.8	0.49
					1.68	0.05	0.55	1.5	0.51
					1.68	0.05	0.42	1.3	0.56

The samples are listed in concentration of starch, from lowest to highest. Where, c_o was the initial OS starch concentration (dry basis); φ was the dispersed phase volume fraction; d_{32} was the area-weighted droplet diameter; the r was the surface load of OS starch; and the adsorption yield is equal to $\frac{c_{adsorbed}}{c_o}$ where the $c_{adsorbed}$ was the concentration of starch adsorbed.

Table 2.2 Adsorption of starch in water and oil phases

Sample	grams starch in water	grams starch in oil	% starch adsorbed onto oil based on total oil	% starch adsorbed onto water based on total water	% starch adsorbed onto oil based on total starch	% starch adsorbed onto water based on total starch
1	0.56±0.004	0.28±0.01	5.50±0.73 j	0.60±0.00 a	32.74±0.28 h	67.26±0.28 a
2	0.66±0.01	0.34±0.01	6.84±0.04 I j	0.70±0.01 a	34.20±0.23 g	65.80±0.18 a
3	0.84±0.01	0.84±0.02	16.80±0.24 h	0.9±0.01 a	50.01±0.72 c	49.99±0.72 f
4	1.36±0.02	0.64±0.03	5.34±0.25 j	1.58±0.02 a	32.06±0.73 h	67.94±0.73 a
5	1.28±0.03	1.02±0.04	8.71±0.26 i	1.49±0.03 a	44.29±0.74 d	55.71±0.74 e
6	1.98±0.02	1.52±0.03	14.50±0.25 h	2.30±0.02 a	43.49±0.73 d e	56.51±0.73 d e
7	2.32±0.03	2.34±0.04	25.06±0.26 g	2.70±0.03 a	50.17±0.74 c	49.83±0.74 f
8	2.62±0.04	2.98±0.05	35.44±0.27 f	3.05±0.04 a	53.16±0.75 b	46.84±0.75 g
9	3.14±0.4	2.26±0.03	41.85±0.54 e	3.52±0.04 a	41.85±0.54 e f	58.15±0.54 b c
10	4.12±0.03	2.78±0.3	40.25±0.9 e	4.78±0.01 a	40.25±0.9 f	59.75±0.9 b
11	4.58±0.12	3.75±0.12	45.0±0.8 d	5.50±0.13 a	45.0±0.8 d	55.0±0.8 e
12	5.45±0.1	4.23±0.15	43.67±1.2 d	6.76±0.16 a	43.67±1.2 d e	56.33±1.2 c d
13	5.56±1.1	4.44±0.24	66.63±2.0 c	6.67±0.9 a	44.42±0.9 d	55.58±0.9 e
14	5.67±0.27	4.33±0.03	108.32±0.8 b	6.59±0.33 a	43.33±0.26 d e	56.67±0.26 c d
15	6.59±0.3	12.16±0.01	64.83±1.2 c	10.55±0.08 b	64.83±1.2 a	35.17±2.1 h
16	9.32±0.62	16.68±0.8	208.51±0.12 a	14.12±0.14 b	64.16±0.18 a	35.84±0.18 h
	Means that do not share a letter are significantly different (p<0.05).					
The following from Nilsson et al. (2006)						
similar to sample 1	0.14	0.7	14.04	0.16	83.55	16.45
	0.22	0.62	12.44	0.25	74.02	25.98
	0.20	0.64	12.73	0.24	75.75	24.25
	0.21	0.63	12.58	0.24	74.89	25.11
	0.40	0.44	8.80	0.46	52.37	47.63
similar to sample 2	0.27	0.99	19.8	0.33	78.58	21.42
	0.27	0.99	19.80	0.33	78.58	21.42
	0.39	0.87	17.31	0.48	68.69	31.31
	0.27	0.99	19.80	0.33	78.58	21.42
similar to sample 3	0.51	1.17	23.35	0.66	69.5	30.5
	0.67	1.01	20.20	0.86	60.12	39.88
	0.64	1.04	20.73	0.82	61.68	38.32
	0.58	1.10	22.04	0.74	65.59	34.41

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Appendix A: Creaming Index and Conductivity

Appendix A.1 – Creaming Index and Conductivity of o/w emulsions

Table A.1 Creaming Index, conductivity, and starch in water percent of unmicrofluidized and microfluidized o/w samples. "X" indicates that the sample is not stable.

						Creaming Index		Conductivity (mS)			
ID	**oil**	**water**	**starch**	**starch in water %**	**Stable in days**	**Day 50**	**day of unstablity**	**Day 0**	**Day 100**	**Day 300**	**day before unstablity**
A	38	60	2	3	25	x	41±10	2.6±2.4	x	x	0.1±0.0
B	20	78	2	3	30	x	61±3	0.4±0.0	x	x	0.1±0.0
C	26	66	8	11	45	x	63±6	1.3±0.0	x	x	0.9±0.0
D	20	60	20	25	8	x	70±2	1.9±0.1	x	x	1.9±0.1
E	8	84	8	9	38	x	83±7	1.5±0.0	x	x	1.9±0.5
F	14	72	14	16	15	x	76±17	2.5±1.1	x	x	1.6±0.9
G	8	66	26	28	55	x	75±11	2.7±1.0	x	x	2.6±0.9
H	2	96	2	2	20	x	95±6	0.5±0.2	x	x	0.5±0.0
I	2	78	20	20	35	x	88±8	2.6±0.5	x	x	2.3±0.1
J	2	60	38	39	120	0±0	87±6	2.5±0.0	1.6±0.2	x	1.6±0.2
K	38	60	2	3	80	0±0	14±3	0.2±0.1	x	x	0.1±0.0
L	20	78	2	3	160	0±0	7±9	0.3±0.0	0.5±0.2	x	0.2±0.1
M	26	66	8	11	180	0±0	6±2	1.1±0.2	1.1±0.1	x	1.3±0.5
N	20	60	20	25	200	0±0	10±3	1.7±0.2	1.±0.3	x	0.3±0.0
O	8	84	8	9	365	0±0	4±1	2.3±0.1	2.2±0.9	2.2±0.9	2.2±0.6
P	14	72	14	16	365	0±0	3±1	2.4±1.0	2.2±0.5	2.1±0.1	2.1±0.5
Q	8	66	26	28	365	0±0	9±3	4.9±0.9	4.5±1.5	4.1±1.2	3.0±0.0
R	2	96	2	2	365	0±0	9±2	0.4±0.1	0.4±0.3	0.4±0.5	0.4±0.8
S	2	78	20	20	365	0±0	25±6	2.6±0.1	2.8±0.5	2.7±0.2	2.7±0.1
T	2	60	38	39	140	0±0	27±4	3.6±0.3	3.3±0.2	x	3.3±0.1

Appendix B: Adsorption extra calculations

Appendix B.1 – Calculation of total starch concentration using the Megazyme Kit

$$\left(\Delta A \times F \times \frac{FV}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}\right) \times \left(\frac{100}{100 - MC}\right)$$

Where:
$\Delta A =$ Absorbance (reaction) read against reagent blank
$F = \frac{100\ (\mu g\ of\ D-glucose)}{absorbance\ for\ 100\ \mu g\ of\ glucose}$ (conversion from absorbance to µg)
$FV =$ final volume
0.1 = volume of sample analyzed
1/1000 = conversion from µg to mg
100/W = factor to express “starch” as a percentage of sample weight
W = weight in mg “as is basis” of the sample analyzed
162/180 = adjustment from free D-glucose to anhydro D-glucose (as occurs in starch)
$\frac{100}{100-MC}$ = conversion of starch % w/w “as is” to starch % w/w dry weight basis

Appendix B.2 –Adsorption yield and surface load equations

1. W_o = Original (reference) Weight (g)
    a. From total starch (TS) test: test TS= $c_{\text{test starch}}$
2. Unknown: $c_{starch\ in\ oil\ phase}$ and $c_{starch\ in\ water\ phase}$
3. Key values known: $c_{\text{o total}}$, $c_{\text{o oil}}$, $c_{\text{o starch}}$
4. The concentration of starch in water phase for the test sample

    Equation 1: $c_{starch\ in\ waterphase} = [Starch\ concentration\ ] \times c_{o\ water}$

    Where starch concentration was the test sample’s total starch results, $c_{\text{test starch}}$
5. Concentration of starch in the oil

    Equation 2: $c_{starch\ in\ oil} = c_{o\ starch} - c_{starch\ in\ water}$
6. Nillson, et al., 2006 calculated the adsorption yield and surface load as following

    Equation 3: $Adsorption\ yield = \frac{c_{reference} - c_{subnatant}}{c_o} = \frac{c_{adsorbed}}{c_o}$

    Where c_{refernce} was the concentration of starch in the reference sample, with no disperse phase, the $c_{\text{subnatant}}$ was the concentration of starch in the subnatant after the emulsion was separated by centrifugation, c_{adsorbed} was the concentration of starch adsorbed, and c_o was the original starch concentration.

    Equation 4: $\Gamma = \frac{(c_{\text{adsorbed}})(d_{32})}{6\varphi}$

Where г was the surface load obtained by relating the adsorbed concentration to the specific surface area of the emulsion; d_{32} was the area-weighted droplet diameter; c_{adsorbed} was the concentration of starch adsorbed; and the φ was the dispersed phase volume fraction (in this case, the dispersed phase is the oil phase).

Appendix B.3 – Percent starch adsorbed

$$\frac{c_{starch\ in\ oil}}{c_{ref\ oil}} \times 100 = \frac{1.35}{3.068} \times 100 = 44\%\ starch\ adsorbed\ onto\ oil\ based\ on\ total\ oil$$

$$\frac{c_{starch\ in\ water}}{c_{ref\ water}} \times 100 = \frac{6.3197}{89.262} \times 100$$
$$= 7.08\%\ starch\ adsorbed\ onto\ water\ based\ on\ total\ water$$

$$\frac{c_{starch\ in\ oil}}{c_{ref\ starch}} \times 100 = \frac{1.35}{7.67} \times 100 = 17.6\%\ starch\ adsorbed\ onto\ oil\ based\ on\ total\ starch$$

$$\frac{c_{starch\ in\ water}}{c_{ref\ starch}} \times 100 = \frac{6.3197}{7.67} \times 100$$
$$= 82.4\%\ starch\ adsorbed\ onto\ water\ based\ on\ total\ starch$$

Appendix C: Adsorption method issues and alternative calculations

Appendix C.1 – Issues of calculating total starch concentration of emulsions

Starch was lost during the total starch test, similarly to reports in Nilsson, Leeman, Wahund, & Bergenstahl (2006). Such as, 2.33g of starch was lost. (This was calculated by the weight of original concentration of starch added to the sample minus concentration of starch of the reference sample determined by the total starch test.) The starch may have been lost due to it adsorbing to the oil layer, lost during total starch test's boiling step with ethanol and α-amylase, or lost in the microfluizider's micron sized chambers.

If an emulsion is prepared with a microfluidizer, the starch concentration may change due to the dilution of the microfluidizer. The microfluidizer originally has water in its piping; therefore, the initial output from the microfluidizer is diluted. In this case, the total starch in the reference and test samples need to be determined. But there is an issue in determining the total starch in an emulsion.

During the total starch test, there were floating particles/flakes in the reference samples. Hexanes and ethanol were added separately to the flakes to determine their solubility. The flakes only partially dissolved if the samples were vigorously agitated. This indicates that the surface of the flakes was primarily lipid, but the majority of the flakes were most likely starch. Due to the nature of the sample, oil containing, there are problems determining the accurate concentration of starch in the emulsion with this method. This total starch method needs to be improved to determine the starch concentration in emulsions. Future work is needed in this area.

Once the total starch is determined from the reference sample (the entire emulsion), the following calculations can be used to find out how much starch is adsorbed to the oil and the water layer. The following part of this appendix goes through the calculations.

Appendix C.2 –Adsorption yield and surface load equations using total starch test reference sample

1. W_o = Original Weight (g), W_{ref} = Reference Weight (g)
    a. From total starch (TS) test: reference TS =$c_{ref\ starch}$, test TS= $c_{test\ starch}$
2. Unknown: $c_{starch\ in\ oil\ phase}$ and $c_{starch\ in\ water\ phase}$
3. Key values known: $c_{o\ total}$, $c_{o\ oil}$, $c_{o\ starch}$

4. Sample diluted starch with microfluidizer, so find c_{water} using:

   Equation 1: $c_{water} = c_{o\ total} - c_{ref\ oil} - c_{ref\ starch}$

5. Know oil: starch so can find $c_{ref\ oil}$

   Equation 2: $\frac{c_{o\ oil}}{c_{o\ starch}} = \frac{x\ c_{ref\ oil}}{c_{ref\ starch}}$

6. Know $c_{ref\ water}$ was not original because sample was diluted

   Equation 3: $c_{ref\ water} = c_{o\ total} - c_{ref\ oil} - c_{ref\ starch}$

7. The concentration of starch in water phase for the test sample

   Equation 4: $c_{starch\ in\ waterphase} = [Starch\ concentration\ ] \times c_{ref\ water}$

   Where starch concentration was the test sample's total starch results, $c_{test\ starch}$

8. Concentration of starch in the oil

   Equation 5: $c_{starch\ in\ oil} = c_{ref\ starch} - c_{starch\ in\ water}$

9. Nilsson, Leeman, Wahund, & Bergenstahl (2006) calculated the adsorption yield and surface load as following

   Equation 6: $Adsorption\ yield = \frac{c_{reference} - c_{subnatant}}{c_{initial}} = \frac{c_{adsorbed}}{c_{initial}}$

   Where $c_{refernce}$ was the concentration of starch in the reference sample, with no disperse phase, the $c_{subnatant}$ was the concentration of starch in the subnatant after the emulsion was separated by centrifugation, $c_{adsorbed}$ was the concentration of starch adsorbed, and $c_{initial}$ was the original starch concentration.

   Equation 7: $\Gamma = \frac{(c_{adsorbed})(d_{32})}{6\varphi}$

Where Γ was the surface load obtained by relating the adsorbed concentration to the specific surface area of the emulsion; d_{32} was the area-weighted droplet diameter; $c_{adsorbed}$ was the concentration of starch adsorbed; and the φ was the dispersed phase volume fraction.

The same calculations are used to determine the percent of starch adsorbed onto the water and oil layer based on the total oil, starch or water content as explained in the methods section.