INTERFACIAL RHEOLOGICAL PROPERTIES OF PROTEIN EMULSIFIERS, DEVELOPMENT OF WATER SOLUBLE β-CAROTENE POWDER AND FOOD SCIENCE ENGAGEMENT (EMULSIFIER EXPLORATION)

by

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For the dancing and the dreaming…

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π	Surface pressure
γ_0	Surface tension of pure water
γ	Surface tension of monolayer coated interface
σ	Surface tension
F	Force
θ	Contact angle
E	Surface dilatational modulus
σ_0	Stress amplitude
A_0	Strain amplitude
δ	Phase angle between stress and strain
ω	angular frequency
$E' = E_d$	Dilatational elasticity
$E'' = E_v$	Dilatational viscosity
G^*	Complex dynamic interfacial shear modulus
G'	Shear Storage (elastic) modulus
G''	Shear Loss (viscous) modulus
B_o	Boussinesq number
P_c	Contact perimeter between rheological probe and the interface
A_c	Contact area between the probe and the subphase.
L_c' and L_c''	Characteristic length scales in which the velocity decays in the surface and subphase respectively.
C	β–carotene mass (mg) at time t (days), with C-Spec and C-HPLC representing spectrophotometric and HPLC data respectively,
C_0	Initial β–carotene mass (mg)
k	Rate constant (day^{-1}) for the reaction
$t_{1/2}$	Half life time
E_a	Activation energy (kJ/mol)
k_0	Pre-exponential constant (day^{-1}),
R	Universal gas constant (0.008314 kJ/molK),
T	Absolute temperature (K).
L	Lightness parameter
a	a red/ green parameter
b	b blue/yellow parameter
H°	Hue
Ch	Chroma
ΔE	Total Color Difference

ABSTRACT

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Title: Interfacial Rheological Properties of Protein Emulsifiers, Development of Water Soluble β-Carotene Powder and Food Science Engagement (Emulsifier Exploration)
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Interfacial rheology describes the functional relationship between the deformation of an interface, the stresses exerted in and on it, and the resulting flows in the adjacent fluid phases. These interfacial properties are purported to influence emulsion stability. Protein emulsifiers tend to adsorb to the interface of immiscible phases, reduce interfacial tension as well as generate repulsive interactions. A magnetic interfacial shear rheometer was used to characterize the surface pressure-area isotherms as well as interfacial rheological properties of two proteins- sodium caseinate and β-lactoglobulin. Then, sodium caseinate was used as a carrier for β-carotene encapsulation.

β-carotene is a carotenoid that exhibits pro-vitamin A activity, antioxidant capacity and is widely used as a food colorant. It is however, highly hydrophobic and sensitive to heat, oxygen and light exposure. Thus β-carotene as food ingredient is mainly available as purified crystals or as oil-in-water emulsions. In this study, β-carotene stability, and solubility in water for application as a natural colorant was improved by preparation of a sodium caseinate/ β–carotene powder using high pressure homogenization, solvent evaporation and spray drying. The powders thus prepared showed good solubility in water and yielded an orange coloration from β-carotene. The effect of medium chain triglyceride concentration (1%, 10%) and incorporation of a natural antioxidant (Duralox, Kalsec) on powder stability was studied as a function of storage time and temperature. β-carotene stability was reduced at higher storage temperature ($4^oC > 21^oC > 50^oC$) over 60 days and followed first order degradation kinetics at all temperatures. Incorporation of natural antioxidant improved β-carotene stability and resulted in a second first order degradation period at 50^oC. As β–carotene content decreased, Hunter Lab color values denoting lightness increased, while those for redness and yellowness of the powder decreased. This sodium caseinate based β-

carotene powder could be used as a food ingredient to deliver natural β-carotene to primarily aqueous food formulations.

In the last part of this work, an engagement workshop was developed as a means to educate young consumers about the function of emulsifiers in foods. Food additives are important for food product development, however to consumers, a discord between their objective purpose and subjective quality has led to confusion. Food emulsifiers, in particular, are associated with lower healthiness perception due to their unfamiliar names. In collaboration with the 4H Academy at Purdue, a workshop high school student was conducted to develop an increased understanding of emulsions and emulsifiers. A survey was conducted with the participants who self-evaluated their gain in knowledge and tendency to perform certain behaviors with regards to food ingredient labels. The participants reported a gain in knowledge in response to four key questions on emulsions and emulsifiers, as well as an increased likelihood to read ingredients on a food label and look up information on unfamiliar ingredients.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Food emulsions, colloids, and foams are closely related to the evolution of animal species. Today, different emulsions are regularly encountered in everyday life, with perhaps the oldest one being milk (Becher 1991). Historically, an important source of nourishment for humans has been milk, which as a complex emulsion and colloidal sol, consists of fat droplets dispersed in an aqueous phase containing protein. Various dairy products such as cream, butter, cheese, and ice cream could therefore be produced, due to destabilization of dispersed phase fat droplets or the aqueous phase proteins. Early food formulations used the natural emulsifiers in the system, but today synthetic emulsifiers are also widely used. Today, emulsifiers are involved in the preparation of products such as dairy products and substitutes, baked foods, confectionery, margarines and spreads, salad dressings, and beverages (Hasenheuttl and Hartel 1997).

Emulsion and foam based food products often rely on proteins and low molecular weight surfactants for stabilization (Bos and van Vliet 2001). Proteins in particular, adsorb to the interface of immiscible phases in emulsions, reduce interfacial tension as well as generate repulsive interactions. Further development of protein stabilized emulsions however, depends on increased understanding of their physicochemical properties, interfacial behavior and the relationship between interfacial characteristics and bulk physicochemical properties (McClements 2004). Interfacial rheology is one such developing field that tries to elucidate the effect of particles, surface active materials and proteins on the stability and microstructure of the interface of multiphase materials often found in the food industry (Dickinson 2001; Bos and van Vliet 2001). Accordingly, the use of magnetic interfacial shear rheometer to characterize interfacial rheological properties of two milk proteins at the air/water interface has been studied in this dissertation.

Apart from the wide variety of food products that are emulsions, there is an interest in using available knowledge regarding these multiphase systems to develop more sophisticated emulsions (nanoemulsions, multiple emulsions, multilayer emulsions, solid lipid nanoparticles, filled hydrogel particles) for novel applications in the food industry. These applications involve the incorporation and release of functional molecules such as flavors, antimicrobials or even nutraceuticals (McClements 2010). Emulsions can also be employed to encapsulate natural

colorants such as carotenoids by improving their chemical stability and dispersant state , as well as to possibly improve their bioavailability upon ingestion (Mao et al. 2018a). Accordingly, one particular method of development of a water-soluble β-carotene and sodium caseinate powder for natural colorant application in beverages was studied in this work.

Emulsions and emulsifiers are also widely encountered in daily life, however consumer perception for food with additive names that are difficult to pronounce result in a perception that they are harmful (Varela and Fiszman 2013). Extension programs from land grant institutions can be harnessed to not only educate consumers and dispel these misperceptions, but also to expose and educate graduate students to the scholarship of engagement (Schaffner 1991; O'Meara and Jaeger 2006; USDA 2014). Therefore, in collaboration with Purdue University Office of Engagement and the 4H Academy at Purdue, an extension workshop on emulsions and emulsifiers was performed and knowledge gain among participants recorded, as the last part of this study.

1.2 Emulsions and Emulsifiers

Emulsions are systems that consist of two immiscible liquids such that one is dispersed in the other, respectively referred to as the dispersed phase and continuous phase. These may be classified as oil-in-water (O/W), water-in-oil (W/O) or oil-in-oil (O/O) (Tadros 2013).
Emulsifiers are surface active substances that assist in the creation and stabilization of an emulsion. These can also be amphiphilic polymers and small particles. These compounds must reduce the oil-water interfacial tension to low values and also rapidly diffuse to the newly created interface to assist with emulsion preparation. Low molecular weight surfactants, therefore, are more effective than high molecular weight polymers, proteins, and hydrophobic particles because of faster diffusion properties (Kronberg, Bengt, Krister Holmberg 2014). Surface active substances typically orient such that the hydrophilic (or water-loving) group is oriented to the hydrophilic/aqueous phase while the lipophilic (or oil-loving) tail orients towards the oil/hydrophobic phase. Proteins may consist of lipophilic and hydrophilic amino acid groups, and these amino acids will fold and orient towards the oil and aqueous phases respectively. Charged proteins may also cause repulsion of like charged droplets and thus stabilize emulsions. The solubility of an emulsifier in water or oil phase is governed by the number and relative polarity of polar groups in the molecule. This is represented by the HLB value. Conventional practice involves

dispersing the surfactant in the continuous phase and emulsifiers with high HLB values are associated with easy water dispersibility (Table1) therefore high HLB emulsifiers are used for O/W emulsions and low HLB ones for W/O emulsions (Hasenheuttl and Hartel 1997)

Table 1 HLB Values for emulsifiers and their applications (Griffin 1949)

HLB Range	Application
3-6	W/O emulsifier
7-9	Wetting agent
8-18	O/W emulsifier
13-15	Detergent
15-18	Solubilizer

Emulsions typically vary depending on the size of the droplets of the dispersed phase and in their thermodynamic stability. Accordingly, they may be classified as emulsions or macroemulsions, nanoemulsions or microemulsions (Table 2). Thermodynamic instability implies that the free energy of the individual emulsion phases is lower than the free energy of the emulsion, hence there is a tendency for the emulsion to break down over time, except in the case of microemulsions. Droplets that have similar dimensions to the wavelength of light will result in opaque or turbid emulsions however when they are lower than that, the emulsions tend to be transparent or only slightly turbid. Reduced particle size also implies better stability to gravitational separation (McClements and Rao 2011).

Table 2 Comparison of emulsion types Source: (McClements and Rao 2011)

System	Droplet Radius	Thermodynamic Stability	Surface to mass ratio m^2/g particles	Optical properties
Emulsion	100nm-100 μm	Unstable	0.07-70	Turbid/Opaque
Nanoemulsion	10-100 nm	Unstable	70-330	Clear/Turbid
Microemulsion	2-100 nm	Stable	330-1300	Clear/Turbid

The preparation of kinetically stable emulsions (macroemulsions) involves the use of high energy approaches to create disruptive forces that result in disruption and mixing of the oil and aqueous phase droplets. The emulsifier is typically dissolved in the phase where it is most soluble, the second phase is added, and shear is applied to the mixture. Turbulent mixing is crucial to the formation of small droplets for O/W emulsions and an additional mixing step with high shear force is typically required. These include the use of high-pressure valve homogenizers, microfluidizers and sonication methods. Alternatively, low energy approaches depend on spontaneous emulsion formation due to changes in environmental conditions, leading to thermodynamically stable microemulsions which are of little relevance for food systems due to the high concentration of surfactants needed. Thus, high pressure homogenization is the most commonly used method for the preparation of food emulsions. A coarse emulsion is passed through a homogenizing valve, slit or orifice and turbulence, cavitation and velocity gradients result in droplet size reduction. Variations in pressure conditions, and number of cycles can be used to control mean particle size and droplet size distributions (Schramm 2005b; Tadros 2013).

Emulsion breakdown can occur through various processes that may occur simultaneously. These include coalescence or the irreversible joining of two or more droplets with the formation of a larger droplet, flocculation or the aggregation of two or more emulsion droplets, creaming or sedimentation which is the motion of droplets to the top or bottom of the system respectively due to gravitational or centrifugal forces, Ostwald ripening which results due to diffusion of molecules from smaller droplets to larger ones shifting the droplet size distribution over time and phase inversion where the disperse phase an continuous phase exchange or invert (Tadros 2013).

Emulsions are ubiquitous, with several examples occurring in food and agricultural, pharmaceutical and cosmetic products. Milk, cream, ice cream and coffee whiteners and toppings are examples of some O/W food emulsions stabilized by proteins. Cream liqueurs are emulsions with high alcohol content that must remain stable for years and are therefore formulated to limit the breakdown processes (creaming, flocculation, coalescence) while maintaining the cream like appearance. Carbonated soft drinks are typically formulated using syrups made of O/W emulsions of flavoring oils in solutions of sugars, food colorants and preservatives that need to survive shipping, dilution and storage. Butter margarine, spread and some salad dressings are examples of W/O emulsions (Schramm 2005c). Emulsified pharmaceutical products are often used to improve drug solubility, to control the distribution of drugs in the body, and, possibly provide targeted drug

delivery. Emulsions can also be used to develop personal care products with pharmaceutical qualities (anti-wrinkle, sun protection) by encapsulation of active ingredients (Schramm 2005a).

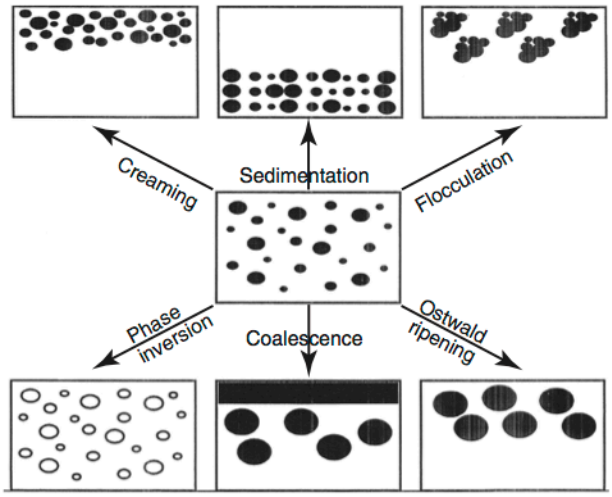

Figure 1 Schematic representation of emulsion breakdown processes (Tadros 2013)

Emulsions can also be used to deliver bioactive ingredients (e.g. vitamins, carotenoids, polyphenols, functional lipids) into food systems and thus address the challenges of poor water solubility and stability, associated with these ingredients. These novel functional foods can therefore help ease or prevent the occurrence of chronic diseases such as diabetes, cardiovascular disease and certain cancers (Mao et al. 2018b). Similarly, emulsions can also be used to deliver flavor, color, antimicrobials, preservatives and antioxidants, each of these being associated with their own challenges due to the different molecular weights, polarities, charges and conformations involved that can affect their physicochemical properties and the type of delivery system required for each functional ingredient (McClements et al. 2009).

1.3 Protein Emulsifiers

Proteins are biopolymers made of amino acids linked together by peptide bonds and which have a strong tendency to adsorb at interfaces. Their complex structure can be broken down into four levels: primary, secondary, tertiary and quarternary. Amino acid sequence in the polypeptide chain is the primary structure, whereas the ordering of the polypeptide chain establishes the

secondary structure, giving rise to alpha helices and β-sheets. Tertiary structure is defined by the twists and bends of the whole polypeptide. Quaternary structure is related to the interchain interactions when a protein comprises more than one polypeptide chain. The spatial conformation of the protein is dependent on the secondary, tertiary and quarternary structures that are in turn dependent on electrostatic interactions, hydrogen bonds, hydrophobic interactions and covalent bonds. Proteins can also be divided into flexible random-coil type molecules and the more rigid globular molecules (Benjamins 2000). Similar to low molecular weight surfactants and polymeric surfactants, proteins are surface active materials. They provide kinetic stability to dispersant by adsorbing at the interface, reducing interfacial tension, providing the interface with surface elasticity and viscosity, causing smaller droplets to be formed and keeping them small by retarding coalescence. The interfacial viscoelastic properties of the adsorbed interfacial layer will determine the stabilizing effect of the emulsifier, and are therefore relevant to foam and emulsion formation and stabilization in food processing, cosmetics and the pharmaceutical industry (Jaishankar et al.; Benjamins 2000).

One of the most common protein-based emulsifiers is sodium caseinate. Sodium caseinate is a milk protein that is used in a variety of food emulsions such as coffee whitener, cream liqueur and whipped toppings (Srinivasan et al. 1996). This protein is widely used as an emulsion stabilizing agent due to its good solubility, surface activity, heat resistance and water holding properties. This milk protein is made by acidification of casein micelles. It is a random coil protein mixture consisting of α_{s1}-casein, α_{s2}-casein, β-casein, κ-casein and γ-casein of which α_{s1} and β caseins are the most abundant and most surface active. The average molecular weight is 2.33×10^4 g/mol. All caseins contain 35-45 % apolar amino acids which make them relatively hydrophobic. The hydrophobic amino acids are not uniformly distributed over the molecule but are present in hydrophobic clusters which can adsorb at the air-water interface (Otter 2003). This partially aggregated mixture of caseins, especially α_{s1}-casein and α_{s2}-casein have strong tendency to bind calcium ions due to presence of phosphoseryl residues (Ye and Singh 2001). During emulsion formation using high pressure homogenization, casein molecules and aggregates, are rapidly associated with the surface of the oil droplets, thus limiting recoalescence and providing emulsion stability. O/W emulsions can be prepared with both, α_{s1} and β-caseins, with similar protein surface coverages, and they both have adsorbed layers of low surface shear viscosity (Dickinson 1999).

Another commonly used protein in food emulsions is β-lactoglobulin. β-lactoglobulin is a milk protein that accounts for 50% of the whey protein in ruminants and 10% of cow's milk protein. Its biological function has not been clarified but its presence in the milk of only certain species implies it may not be essential for all species (Creamer et al. 2011). This protein has a globular, well-defined secondary structure with 162 amino acids and a molecular weight of 18kDa (Cicuta 2007). Its amphiphilic nature affects whey functionality such as adsorption at water/oil and air/water interfaces. This molecule has two disulfide bonds, a free sulfhydryl group and no phosphorus which affects molecular flexibility and/or stability. (Bouaouina et al. 2006).

1.4 Proteins at the Interface

Protein adsorption at an interface will result in a conformational change, which is affected by the protein type, surface type, pH and ionic strength (Table 3). As opposed to solid surfaces, conformational changes at liquid interfaces have not been as extensively studied due to limitations in methods (such as circular dichroism). These changes however, are important for stability of food emulsions and therefore further work to better understand protein structure and changes at the interface, and the role it plays on emulsification is needed (Benjamins 2000).

Emulsion and foam-based food products have very large interfaces and the stabilization of these systems depends largely on controlling these interfaces. The physical properties of the fluids as well as the surface-active species in the interface between the fluids are the two important aspects for stability. Viscosity of the continuous and dispersed phases, their densities, purity and polarity of the phases, pH and ionic strength of the continuous phase are some of the most important physical properties of the fluids. With respect to the interfacial surface-active molecules, their interfacial tension lowering capabilities (as well as the rate of lowering), amount adsorbed, ability to desorb, changes in conformation due to adsorption, adsorbed layer thickness, interactions between these adsorbed molecules and their lateral mobility are important. The static/dynamic interfacial or surface tension however, is the most important factor because when this is reduced, the mechanical work for emulsion/foam formation is also reduced. When emulsions/foams are formed there is a large increase in interfacial area at a certain rate. So the dynamic surface tension at the relevant expansion and/or compression rate proves important and can be determined with surface rheological methods (Bos and van Vliet 2001). In addition, biological systems such as membrane systems, alveolar fluid in lungs, dispersions of fat in milk and blood are controlled by

adsorbed proteins. Various foods such as dairy products, dressings, ice cream, bakery products, foam on beer depend on stabilizing ability of proteins adsorbed at air/water and oil/water interfaces, so the study of proteinaceous interfacial films has been carried out for a long time (Benjamins 2000)

Table 3 Select examples and typical values for adsorbed amount and surface pressures obtained for certain proteins under various conditions (Bos and van Vliet 2001)

Protein	Condition Conc. (g l^{-1})	pH	Surface pressure (mN m^{-1})	Adsorbed amount (mg m^{-2})
BSA	0.1	6.7	17.8	2.4
	0.01	7.1	16.7	1.95
β-casein	0.01	6.7	19.8	2.95
	0.1	7.1	22	4.2
Sodium Caseinate	0.3	6.7	25	3.3
Ovalbumin	0.1	6.7	16.3	1.52

1.4.1 Surface Pressure-Area Isotherms

Surface pressure-area isotherms are used to characterize the interfacial properties of an amphiphilic material determined by measuring surface pressure as a function of area of water surface available to each molecule at a constant temperature. For an air/water interface, the surface pressure π (Eq.1) is measured as the difference between surface tension of the pure water γ_0 and surface tension of monolayer coated air-water interface γ (Duncan and Larson 2008).

$$\pi = \gamma_0 - \gamma \quad \text{.....(Eq. 1)}$$

The surface pressure is continuously monitored as the area (a) of the film is reduced at a constant rate. A schematic π-a isotherm typically has distinct regions or phases (Figure 2). The monolayers exist in the gaseous state (G), on compression transitioning to the liquid-expanded state (L1), then the liquid-condensed state (L2), then at higher densities reaching the solid state (S), after which further compression will result in collapse often observed as a rapid decrease in surface pressure or as a horizontal break in the isotherm if in the liquid state. Critical points in a π-a isotherm include the molecular area at which rapid increase in surface pressure is observed and the transition surface

pressures between L1, L2 and between L2 and S. The long plateau (shown in inset) corresponds to the gaseous- liquid expanded region (Andelman et al. 1994; NIMA 2013).

Surface pressure area isotherms can be measured using a Langmuir trough (a teflon rectangular trough with movable barriers) and Wilhelmy plate (roughened platinum plate). The substrate liquid is filled in the trough and thus constitutes the sub-phase.

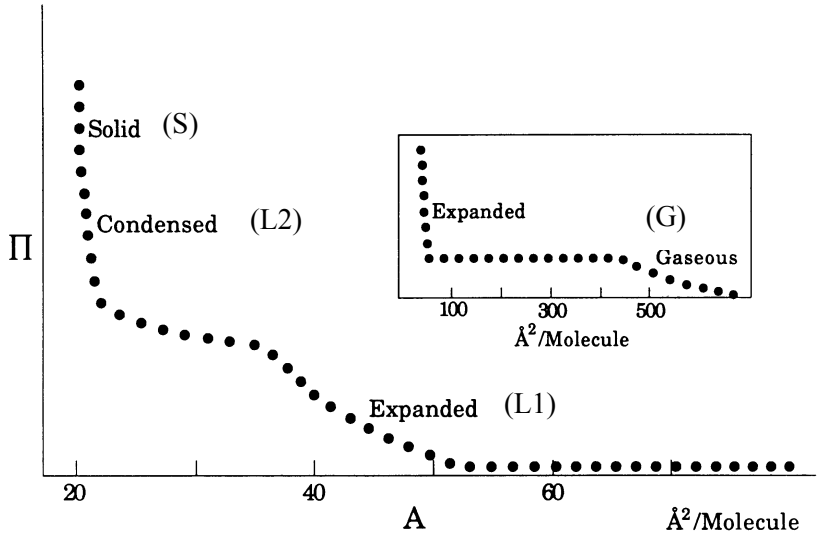

Figure 2 Schematic π-a isotherm (Andelman et al. 1994)

The Wilhelmy plate is suspended from a pressure sensor and contacts the sub-phase and thus a force (F) that is correlated with the wetted length (L), surface tension (σ) and contact angle (θ) acts on the plate (Eq. 2).

$$\sigma = \frac{F}{L.cos\theta} \quad \text{.....(Eq 2)}$$

The Wilhelmy plate is made of platinum since it is chemically inert, easy to clean, optimally wetted and generally forms a contact angle of 0° with liquids. The sub-phase pH can be varied using buffers and the trough area is controlled by compressing the barriers. Compression rate is typically 3.3 cm/min which has been shown to be the highest value for isotherms that give reproducible measurements (Nino et al. 1999; Patino and Fernández 2004; Rodríguez Patino et al. 2007)

Measurement techniques for proteins are generally either via spreading or adsorption. In the former, aliquots of protein solutions are spread on a sub-phase using a micrometric syringe and then sufficient time is permitted for adsorption and rearrangement of the proteins before measurements are taken. The advantage of this method is that the concentration of the film forming

component can be controlled (Cejudo Fernández et al. 2007). In the adsorption mode, proteins can be left in the trough and allowed to adsorb at the interface; however the protein concentration should be such that sufficient migration to the interface occurs (Patino and Fernández 2004). For instance, β-casein measurements of surface pressure vs. average area per molecule performed by spreading β-casein solution (200 μL with spread protein amount of 0.0284 mg) at the interface, between 5 and 40 °C and with sub-phase pH values of 5 and 7, showed that β-casein monolayers had a liquid expanded like structure under all experimental conditions, that the π-a isotherms were displaced towards surface pressure axis and monolayer was more condensed in acidic sub-phase (Nino et al. 1999). In another study, π-a isotherms of sodium caseinate monolayers at pH 7 and 20°C at air-water interface, formed by adsorption of proteins from am aqueous solution ($7.5x10^{-6}$ to $1x10^{-5}$ wt%) showed a liquid expanded like structure at every surface pressure (Rodríguez Patino et al. 2007). These properties are however dependent on the material and environment of measurement. Surface pressure isotherms for dipalmitoylphosphatidylcholine (DPPC), typically display a variety of phase transitions from liquid condensed, coexistence between liquid condensed and liquid expanded phases, liquid expanded and coexistence between liquid expanded and gaseous phases. At higher temperatures, the isotherms shift to higher surface areas or higher surface pressure at a fixed area (Duncan and Larson 2008).

1.4.2 Interfacial Rheology

Emulsions are made of two immiscible liquids such that one is distributed in the other using surface active agents. Their stability is influenced by droplet size and polydispersity, droplet volume fraction, solubility of dispersed phase in continuous phase and additives in the dispersed phase. The role of surface rheology has been hypothesized as being an important parameter to understand the stability of the emulsion. Surfactant molecules in particular, adsorb spontaneously at the interface of the two immiscible liquids and thus reduce interfacial tension. This molecular layer can then resist compression deformation and has viscoelastic properties (Georgieva et al. 2009).

Interfacial rheology therefore is the "study of the relationship between interfacial stress and resultant deformation of the interface" (Murray 2002). Interfacial rheology can be measured with two types of deformation: surface compression/dilation (at constant shape, response to

changes in area are measured) and surface shear (at constant area, response of the surface to changes in shape are measured). These can be determined with small periodic deformation or under continuous expansion or shear (Benjamins and Lucassen-Reynders 2009).

Interfacial shear viscosity (η_s) is the "ratio between shear stress (σ) and shear rate (γ) in the plane of the interface", therefore has a unit of $Nm^{-1}s$ or surface Pa.s (i.e. it is a two dimensional viscosity). In the presence of an adsorbed surfactant or polymer therefore, interfacial shear viscosity observed can be orders of magnitude higher than bulk viscosity of the film. Surfactant molecules tend to form a monolayer of vertically oriented molecules such that the hydrophobic regions point to the oil phase while the polar head points to the aqueous phase. In the case of macromolecules that form loops and tails at the interface, resistance to compression arises from lateral repulsion between the loops and tails.

Interfacial rheological properties due to surfactant, polymer or their mixtures can be studied by understanding viscosity and elasticity of the interfacial film. Shear applied to an interfacial film will cause constituent molecules and those of the oil and water phases in the immediate vicinity to be displaced from equilibrium. The molecular rearrangement will affect the stress developed which in turn affects the interfacial viscosity of the film and therefore the bulk emulsion rheology, specifically if droplets are large and deformable. If emulsion droplets are small, then deformation is less likely and interfacial rheology is less significant. The solubility and distribution of the emulsifier in both phases also has an effect on rheology of the system (Tadros 1994)

In the case of small periodic deformations, the surface dilatational modulus E is obtained from change of surface tension σ (dilatational stress) caused by a small change in surface area (dilatational strain) where σ_0 and A_0 are the stress and strain amplitudes and δ is the phase angle between stress and strain and ω is the angular frequency (Eq. 3, 4, and 5). It is composed of real and imaginary parts where the real part $E' = E_d = |E|cos\delta$ is the storage component or dilatational elasticity and the imaginary part $E'' = E_v = |E|sin\delta$ is the loss component or dilatational viscosity such that the dilatational modulus represents total dilatational resistance to deformation (von Staszewski et al. 2014).

$$\sigma = \sigma_0 \sin(\omega\theta + \delta) \quad \text{.....(Eq. 3)}$$

$$A = A_0 \sin w\theta \quad \text{.....(Eq. 4)}$$

$$E = \frac{d\sigma}{dA/A} = E_d + iE_v \quad \text{.....(Eq. 5)}$$

Similarly for surface shear rheology the complex dynamic interfacial shear modulus G^* is a combination of a real part storage modulus $G' = (|\sigma|/|\gamma|)cos\phi$ (elastic) and imaginary part $G'' = (|\sigma|/|\gamma|)sin\phi$ loss modulus (viscous) such that $G^* = G' + iG''$.

Several devices and instruments to measure interfacial shear rheological parameters of fluids and semisolid dispersions exist with each having their own advantages or limitations related to operating range, sensitivity and suitability for material. They can be divided into direct and indirect techniques. Indirect techniques use inert visible particles to measure shear interfacial characteristic, with examples such as the deep channel surface viscometer and the channel surface viscometer. The deep channel surface viscometer consists of two stationary concentric cylinders and a rotating dish filled with test sample. Talc or Teflon particles are placed within the fluid interface and along with rotation of the chamber at a known angular velocity, measurement of surface motion between the cylinders is possible, thus making this suitable for liquid-liquid interfaces. The channel surface viscometer is limited to insoluble monolayers at gas-liquid interfaces and involves determining flow rate of a film through a narrow channel at an applied pressure differential. Direct methods, on the other hand, involve measurement of shear stress by contact with a foreign rotating or oscillating body that is connected to a motor which can then determine torque and displacement while the sample is in a stationary container. These include torsion pendulum surface viscometers (sharp edge geometry for gas-liquid interface, biconical disk geometry for liquid-liquid interfaces). Another example is the magnetic rod viscometer or magnetic interfacial shear rheometer which is the most sensitive instruments for measuring interfacial shear rheological characteristics (Pelipenko et al. 2012). This instrument consists of a of a Langmuir trough which contains the sample and is equipped with a surface pressure measurement via Wilhelmy balance. A smaller petri dish may replace the Langmuir trough to reduce sample volume when required. Two Helmholtz coils are used to produce a uniform magnetic field gradient and the measurement probe is a magnetized rod supported at the interface by surface tension, placed in a rectangular channel in between the coils (Brooks, Carlton F, Fuller, Gerald G, Frank, Curtis W., Robertson 1999; Reynaert et al. 2008)

The sensitivity of interfacial rheometers is limited by their ability to detect stresses in the surface film in presence of stresses from the underlying sub-phase. Accordingly, measurement geometries of probes used in devices must be designed to provide adequate sensitivity. This is

characterized by the Boussinesq number which indicates relative importance of surface and sub-phase contributions. This is the ratio of surface drag to sub-phase drag.

$$B_o = \frac{surface\ drag}{subphase\ drag} = \frac{\eta_s . P_c L_c''}{\eta . A_c L_c'} = \frac{\eta_s}{\eta . a} \text{.....(Eq 6)}$$

Where P_c is contact perimeter between rheological probe and the interface and A_c is the contact area between the probe and the subphase. L_c' and L_c'' are the characteristic length scales in which the velocity decays in the surface and subphase respectively. When B_o >>1 surface stresses dominate and when B_o<<1 sub-phase stresses dominate. The parameter a has units of length and therefore for instruments that use the disk, bicone or ring probes, this value is related to the radius of the geometry, while for the magnetic needle it is related to the radius of the needle (Reynaert et al. 2008)

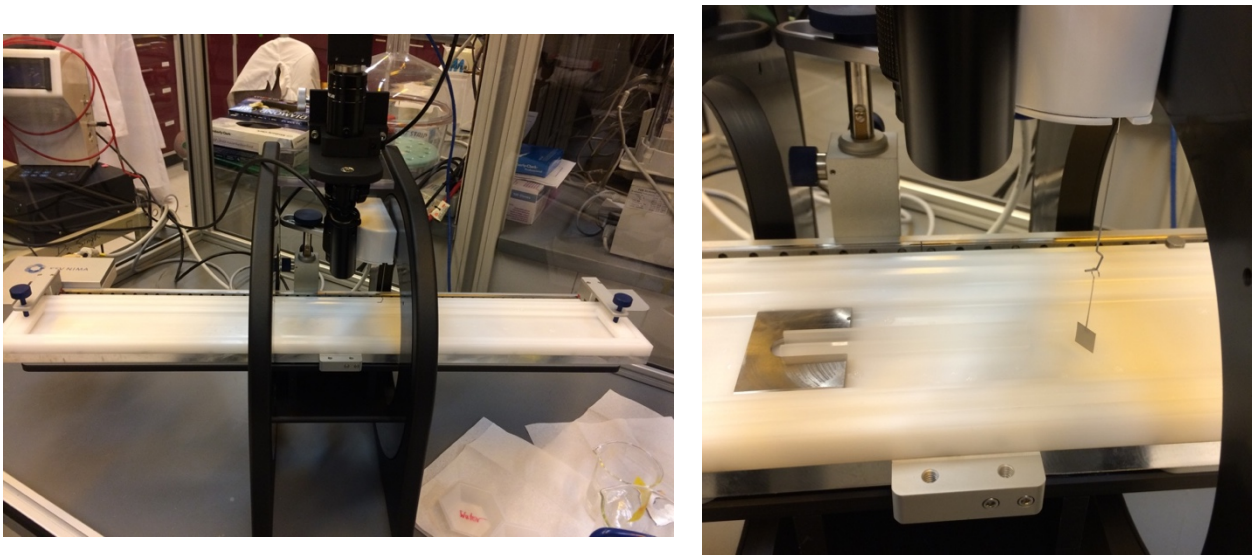

Figure 3 Interfacial Shear Rheometer (KSV NIMA) whole instrument with Langmuir trough, Helmholtz coils and camera (left); Close up of rectangular channel for magnetic needle probe and Wilhelmy Plate (right)

The magnetic interfacial shear rheometer (ISR) was developed to measure time dependent response of rheological probe when shear stress was applied to the Langmuir monolayer. The advantages of this instrument are 1) Real time measurement of interfacial stress and thus elasticity and viscosity of the monolayer, 2) Easy changes to frequency of applied force without changes to rheometer itself, 3) Position of rod is used to determine strain rate and no need for tracer particles, 4) Non-Newtonian surfaces can be studied, 5) The high P/A ratio for the magnetic rod means there

is high sensitivity to surface stress in presence of bulk sub-phase stress and 5) it is easy to change surface pressure and temperature (Pelipenko et al. 2012). This was the instrument used in this work.

1.4.3 Effect of High-Pressure Homogenization on Protein Functional Properties

Functional properties of food proteins can be classified as hydration, interfacial, aggregation and gelation properties. Hydration is driven by protein-water interactions and affect phenomena such as wettability, swelling, adhesion, dispersibility, solubility, viscosity, water absorption/holding. Interfacial properties affect emulsion and foam formation, while protein-protein interactions influence aggregation and gelation. Dynamic high-pressure treatment or high-pressure homogenization can improve these functional protein properties. This is different from static high-pressure systems, since cavitation, shear, turbulence and temperature rise occur simultaneously over short periods of time. Factors affecting changes in protein functionality when subjected to high pressure include extrinsic ones such as pH, temperature, ionic environment, as well as intrinsic factors like disulfide bonds in proteins that determine molecular flexibility and stability (less disulfide bonds result in greater susceptibility of the protein to pressure). In general, high pressure affects intramolecular hydrophobic and electrostatic interactions in protein molecules, and therefore disrupts quaternary and tertiary structure of globular proteins but have little influence on secondary structure. There may exist therefore an optimum pressure at which emulsifying properties of proteins are improved (Bouaouina et al. 2006).

The effect of pressure on surface functional properties of proteins has shown that in some cases, these properties were improved while in others they were impaired. Additionally, previous work involving preparation of emulsions with static pressure treated β-lactoglobulin in the range of 200-800 MPa at 10, 20 and 40 minutes, showed that with increasing pressure and treatment time there was a general trend of larger average droplet sizes thus reducing this protein's emulsifying capacity. The rate of creaming was also faster and storage of emulsions over time resulted in significant increase in droplet sizes, however high pressure treatment of prepared emulsions did not result in any such changes. (Galazka et al. 1996)

Dynamic high-pressure treatment, such as in homogenization results in different effects such as shear stress, turbulence, cavitation and temperature rise. These effects occur

simultaneously and for very short periods of time, hence their influence on protein conformation and functionality is not comparable to hydrostatic pressure treatments and are not as widely characterized. There are only few studies on the influence of dynamic high-pressure treatment on proteins. Some studies report that functionality of globular proteins can be enhanced up to pressures of 150 to 200 MPa (Bader et al. 2011). Treatment of whey protein isolate solutions with dynamic pressure ultra-high-pressure homogenizer at 50,100, 150, 200, 250 or 300MPa did not affect whey protein solubility, native structure nor resulted in denaturation. With increase in pressure, however, the particle size distribution of the protein decreased, as did surface hydrophobicity due to disruption of large protein aggregates to reveal buried hydrophobic groups. Higher pressure treatment also gave higher foam stability, and the elastic modulus E' also increased with increase in pressure thus implying that decreasing size of protein aggregates resulted in increase in elasticity of interface (Bouaouina et al. 2006). High pressure homogenization of sodium caseinate on the other hand, has been shown to result in an increase in molecular weight with increase in pressure. This has been attributed to increased aggregation as a result of cross linking in the random coil structure, also resulting in an increase in sedimentation coefficients (Phoon et al. 2014).

1.4.4 Cosurfactant Effect on Protein Emulsifiers

Polyphenol use in functional foods has been of interest due to their antioxidant and antimicrobial properties. Phenolic molecules, however, have been shown to have surface activity and thus affect the dispersion degree and physical stability of the dispersed phase (Richards et al. 2002). In addition, the interactions between proteins and polyphenols and their effect on protein adsorption, interfacial and colloidal properties, however remains to be elucidated (Sausse et al. 2003; von Staszewski et al. 2014). In one study, epigallocatechin gallate was found to prevent adsorption of β–casein at the air/water interface possibly due to changes in protein conformation that would occur during adsorption or by affecting the protein's hydrophobic/hydrophilic balance. The layer structure at the interface is also affected by polyphenol, and adsorption of polyphenol-protein association also affected surface tension and dilatational modulus of the adsorption layer of β-casein. Polyphenol structure also plays an important role in these interactions since oxidation can enhance the effect of the polyphenol on protein adsorption (Sausse et al. 2003). Green tea

polyphenols and β-lactoglobulin form nanocomplexes which behave differently from pure proteins during diffusion, penetration and rearrangement at oil/water interface, by reducing the surface activity and also reducing dilatational elasticity of interfacial films over time, as compared to pure β-lactoglobulin films. The binding in these nanocomplexes are likely dominated by stacking of polyphenolic rings against hydrophobic surfaces. Oil in water emulsions prepared with these nanocomplexes, also showed a reduction in droplet size and reduced creaming rate thus indicating greater stability, possibly due to charge effects of the nanocomplexes (von Staszewski et al. 2014).

At the air/water interface gallic acid showed no surface activity, an increase in catechin resulted in an increase in surface pressure, while quercetin caused a decrease in surface pressure. In comparison, at the olive oil/water interface both catechin and quercetin resulted in an increase in surface pressure while gallic acid resulted in a constant reduction in interfacial tension that was not concentration dependent. The effect of these phenolic compounds on the stability of olive oil/water emulsions prepared with a mixture of Tween20/β-lactoglobulin as emulsifier showed that the gallic acid emulsion droplets were not different probably due to its poor surface activity, while catechin showed an increase in droplet size trend (reducing emulsifying activity) possibly by inducing protein-protein interactions and thus interfering with protein rearrangement at the interface. Quercetin at low concentrations also impaired emulsifying activity but at higher concentrations, the droplet size showed a decreasing trend and therefore improved emulsifying capacity (Di Mattia et al. 2010).

1.5 Encapsulation of natural colorants with proteins for food applications

1.5.1 Introduction

Food proteins (whey, soybean and milk proteins) have unique physicochemical properties to facilitate formation and stability of food emulsions by adsorbing at the interface and forming layers to stabilize oil droplets from flocculation and coalescence (Kiokias et al. 2017). Milk proteins such as sodium caseinate and β-lactoglobulin, in particular, can interact with biopolymers, form gels and retard lipid oxidation by chelating metal ions or free radical scavenging (Sáiz-Abajo et al. 2013; Kiokias et al. 2017). They can therefore be used for the encapsulation of ingredients in emulsions, gels and powders and protect them from degradation, improve solubility in water, and control ingredients' release thus affecting their bioavailability (Mao et al. 2018b). These

encapsulation methods are employed in the development of food ingredients like vitamins, flavors, probiotics and natural colors such as carotenoids, and more specifically β-carotene (Hasenheuttl and Hartel 1997; Chen et al. 2006; Champagne and Fustier 2007; Acosta 2009; Wackerbarth et al. 2009).

1.5.2 Food Colorants

Food color additives include dyes, pigments and other materials incorporated in foods to provide an aesthetic, appetizing and informative quality (Harp and Barrows 2015). This is important because food color is an important sensory character that influences flavor perception, food acceptability, choice and preference (Clydesdale 1993). It has been shown that the brain associates color with flavor, the initial food perception occurs within the first 90s of observation, and a majority of the assessment is dependent on color (Singh 2006). Consequently, an unacceptable or unappealing color in a food product may result in other aspects such as flavor and texture not being judged at all (Francis 1995).

Color additives are regulated by the US Food and Drug Administration (FDA) under the authority of the Federal Food, Drug and Cosmetic Act (Chapter VII, section 721). This means that all such additives must be approved by the FDA before being used in "food, drugs or cosmetics or medical devices that are in contact with bodies of people or animals for a significant period of time". The definition of Color Additive according to this act is therefore, " a material which is : a) dye, pigment or other substance made by a process of synthesis or similar artifice or extracted, isolated or otherwise derived with or without intermediate or final change of identity from a vegetable, animal, mineral or other source b) when added or applied to a food, drug or cosmetic or to the human body or any part thereof is capable (alone or through reaction with other substance) of imparting color thereto; except that such does not include a material which the Secretary, by regulation, determines is used (or intended to be used) solely for a purpose of purposes other than coloring… the term "color" includes black, white and intermediate grays". These color additives are listed in Title 21 of the Code of Federal regulations (U.S. Government Printing Office, Washington 2002; Harp and Barrows 2015).

In general, as mentioned by (Hutchings 1999) there are several reasons to add colors to food, such as, **reinforcement**: to intensify the colors already present in food to match consumer

expectation (e.g. sauces, soft drinks), **uniformity**: avoid batch to batch color variation of food, **restoration**: counteract the effects of processing which may have resulted in color loss, **addition**: provide colorless products such as sugar confectionery, ice lollies soft drinks, gelatin-based jelly with a more attractive appearance, **protection**: to assist in protection of flavor and light sensitive vitamins during storage and **quality**: provide visual indication of quality (Henry 1996; Hutchings 1999)

1.5.3 Classification of Food Colorants

Food colorants are classified as synthetic, nature-identical or natural. Synthetic colors are produced through chemical synthesis and do not occur naturally e.g. sunset yellow, tartrazine. Nature-identical colors are manufactured such that they are chemically identical to natural colorants (occurring in nature) with common examples being β-carotene and canthaxanthin. Natural colors are derived from natural edible sources e.g. chlorophyll, curcumin from turmeric, anthocyanins from red fruits, bixin from annatto seeds, beetroot red (Henry 1996).

In addition, the US FDA classification of food colorants specifies color additives exempt from certification, color additives subject to certification, color additive lakes, and color additive mixtures. Color additives exempt from certification are “derived from plant or mineral sources and one insect source”. Color additives subject to certification are “synthetic organic dyes, pigments and their lakes”. These are synthesized using materials acquired from either coal or petroleum. These are known as the FD&C colors listed as follows: FD&C Blue No.1 (Brilliant Blue FCT), FD&C Blue No. 2 (Indigotine) , FD&C Green No. 3 (Fast Green FCT) , FD&C Red No. 3 (Erythrosine), FD&C Red No. 40 (Allura Red), FD&C Yellow No. 5 (Tartrazine) and FD&C Yellow No. 6 (Sunset Yellow). Citrus Red No. 2 and Orange B are restricted to use in certain foods. These colorants are straight color or dyes that have not been mixed with other substances, while “lakes” refer to insoluble pigments produced by reacting these straight colors with precipitants (such as aluminum cations) and substrata (aluminum hydroxide) and mixtures are combinations of the straight colors (Harp and Barrows 2015).

More recently, the trend towards the use of natural colors has been observed to be rising steadily. Several reporting agencies have been noticing the projected rise of the food colors market, with natural colorants playing a major role. According to a Research and Markets report, the US

Food Colorants market is projected reach $2.5 billion by 2020. Increasing awareness on suggested ill effects of synthetic colors has led to a greater global demand for natural colors, which are more costly than synthetic ones. The “food safety norms and increased production of natural colors in North America, are ultimately driving the market for food colors”. In 2013, Hansen, the world’s largest producer of natural food colorants, noted that the natural color segment accounted for 25% of total sales revenues for the year to end August 2012. They also reported that the sale of natural food colorants increased by 3.4% (Research and Markets 2016)

1.5.4 Natural Food Colorants

The criteria for natural food colorants is that they must exist in nature, the raw material must be natural and the processes used to extract them must be nonchemical (Bomgardner 2014). Plants, plant extracts as well as other sources such as animals (mostly insects), algae, fungi and bacteria are known as traditional sources for natural food colorants (Mortensen 2006). Natural colors are widely permitted and used, however, a universally accepted definition is lacking and varies from country to country. Some countries exclude substances that have both flavoring and coloring properties from their list of permitted colors. For instance, in Sweden, turmeric, paprika, saffron and sandalwood are not to be considered colorants, so long as the flavoring components have not been removed. In Italy, these same substances must not be classified as colors, but as ingredients (Henry 1996). In the United States of America, the FDA does not consider color additives as “natural” food constituents since they are added ingredients, however those ingredients that provide their own color are not considered color additives (e.g. strawberries, green or red pepper). The USA classification of ‘natural’ colors categorizes them as certification exempt color additives that can be used for food or animal feed. Accordingly, the list of certification exempt color additives for use in “foods generally” are as shown in Table 4.

Table 4 Certification exempt color additives permitted for use in food (Harp and Barrows 2015)

21 CFR Section	Color Additive	Restrictions
73.30	Annatto extract	
73.40	Dehydrated beets (beet powder)	
73.75	Canthaxanthin	NTE 30 mg/lb of solid or semisolid food or per pint of liquid food;
73.85	Caramel	
73.90	β–Apo-8' -carotenal	NTE 15 mg/lb solid, 15 mg/pt liquid
73.95	β-Carotene	
73.100	Cochineal extract, carmine	
73.125	Sodium copper chlorophyllin	Citrus-based dry beverage mixed, NTE 0.2% dry mix
73.140	Toasted partially defatted cooked cottonseed flour	
73.160	Ferrous gluconate	Ripe olives
73.165	Ferrous lactate	Ripe olives
73.169	Grape color extract	Nonbeverage food
73.170	Grape skin extract (enocianina)	Still and carbonated drinks and ades; beverage bases; alcoholic beverages
73.200	Synthetic iron oxide	Sausage casings, NTE 0.1% (by weight); dog and cat food, NTE 0.25% (by weight)
73.250	Fruit juice	
73.260	Vegetable juice	
73.300	Carrot oil	
73.340	Paprika	
73.345	Paprika oleoresin	

Table 4 continued

21 CFR Section	Color Additive	Restrictions
73.450	Riboflavin	
73.500	Saffron	
73.530	Spirulina extract	Confections (including candy and chewing gum), frostings, ice cream and frozen desserts, dessert coatings and toppings, beverage mixes and powders, yogurts, custards, puddings, cottage cheese, gelatin, breadcrumbs, and ready-to-eat cereals (excluding extruded cereals)
73.575	Titanium dioxide	NTE 1% by weight
73.585	Tomato lycopene extract; tomato lycopene concentrate	
73.600/615	Turmeric/ oleoresin	

Natural colors (and colors of natural origin) as listed by EU are shown in the Table 5. Some of these colorants however, are only commercially available as nature-identical products, with β-carotene being available both as natural extract and nature identical, the latter being used more prevalently.

The factors affecting implementation of these natural colorants in foods include

1. Solubility: Some colorants are naturally water soluble while others are oil soluble thus limiting their applicability.
2. Physical form: Food colorants are typically present as liquids, powders, pastes and suspensions, thus affecting their method of incorporation in foods and the possibility of color changes during processing.
3. pH: The addition of colorants to unbuffered solutions may cause change in pH of that solution since the colorants are manufactured with pH close to maximum stability.

4. Microbiological quality: There is a possibility that a pigment or color additive may spoil due to microbiological growth. This is more likely in water soluble extracts or color additives, as the moisture content of oil-soluble pigments is low.
5. Other ingredients: The inclusion of gums, stabilizers or emulsifiers can be used to make oil soluble color additives water miscible. (Hendy and Houghton 1996)

Table 5 Natural colorants numbers as listed by the EU (Henry 1996)

Number	**Color**
E100	Curcumin
E101	Riboflavin, riboflavin-5 ' -phosphate
E120	Cochineal, carminic acid, carmines
E140	Chlorophylls and chlorophyllins
E141	Copper complexes of chlorophylls and chlorophyllins
E150a	Plain caramel
E153	Vegetable carbon
E160a	Mixed carotenes and β-carotene
E160b	Annatto, bixin, norbixin
E160c	Paprika extract, capsanthin, capsorubin
E160d	Lycopene
E160e	β-Apo-8'-carotenal (C30)
E161b	Lutein
E161g	Canthaxanthin
E162	Beetroot red, betanin
E163	Anthocyanins

1.5.5 Carotenoids

Carotenoids are a group of over six hundred fat soluble red, orange and yellow compounds found naturally occurring in plants, algae and many microorganisms. They cause the red color of tomatoes, the orange color of carrots, as well as the red to yellow color in the feathers, flesh and exoskeletons of certain animals, with only a few of these compounds being found in human blood

and tissue, and only two in the retina and lens of the eye. (Simpson 1983; Krinsky and Johnson 2005; Boon et al. 2010)

These compounds are tetraterpenes, formed from eight isoprene (C_5H_8 units). With the exception of lycopene, the end groups are typically modified into six membered rings. Carotenoids are also highly hydrophobic unless the backbone is esterified with strongly polar oligopolysaccharides (Solymosi et al. 2015) In general, food coloring agents, such as carotenoids, have the property to absorb light due to conjugated double bonds, which create a delocalized electron system. The wavelength of the absorbed light is related to the number of conjugated double bonds (Schoefs 2002). Accordingly, depending on the wavelength ranges, carotenoids in solution can vary from pale yellow (ζ-carotene), yellow (xanthophylls), orange (β-carotene) or red (lycopene). Their observed colors are also dependent on pigment concentration i.e. with an increase in concentration the yellow β-carotene turns orange and then red (Wrolstad and Culver 2012).

Due to the presence of the conjugated system of double bonds, carotenoids are unstable. They are sensitive to acids, the presence of light, presence of oxygen (easily oxidized and further catalyzed by fluorescent light and lipoxygenase). Carotenoids will form cis isomers from the all-trans structure, with the initial site of attack being the 5,6 double bond or in-chain double bonds. This results in epoxide formation and chain cleavage. Processing and cooking steps, therefore cause degradative changes to these provitamin A compounds in food. Drying and extrusion are more destructive than cooking and canning, and in general low processing time and temperatures can help minimize losses (Simpson 1983).

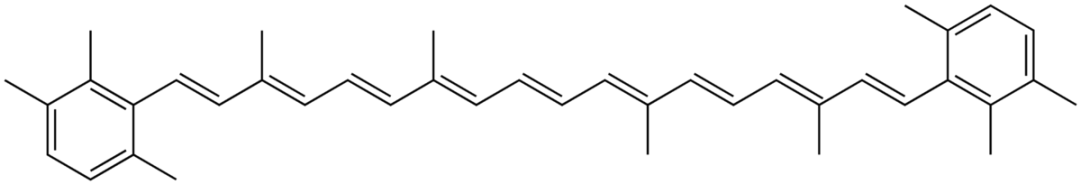

Figure 4 General carotenoid structure, Ben Mills, Own work, Public Domain, https://commons.wikimedia.org/w/index.php?curid=6084479

Carotenoids are associated with provitamin A activity because when assimilated they split into two retinal molecules, which are precursors for vitamin A. Vitamin A is important for vision, skin protection and cell growth. This is important since animal tissues (except in aphids) cannot produce carotenoids, therefore they are only obtained through consumption (Solymosi et al. 2015). Specifically, β-carotene, α-carotene and lycopene as well as xanthophylls and oxygen-containing carotenoids (β-crypotxanthin, lutein and zeaxanthin) are the major dietary carotenoids (Krinsky and Johnson 2005). It has been found that α-carotene, β-carotene, lycopene, lutein and β-cryptoxanthin have the highest known blood concentrations of more than 600 carotenoids that have been identified (Holden et al. 1999).

1.5.6 β-carotene

β-carotene ($C_{40}H_{56}$) is the most extensively reviewed carotenoid having the highest provitamin A activity (Krinsky and Johnson 2005). Due to its symmetrical structure, all trans-β-carotene will undergo oxidative cleavage along the 15,15' carbon-carbon bond and produce two molecules of all-trans-retinal. This reaction is catalyzed by β-carotene monooxygenase (Grune et al. 2010). In comparison, γ-carotene, α-carotene and xanthophyll have 50% of the activity of β-carotene (Simpson 1983; Ferreira and Rodriguez-Amaya 2008).

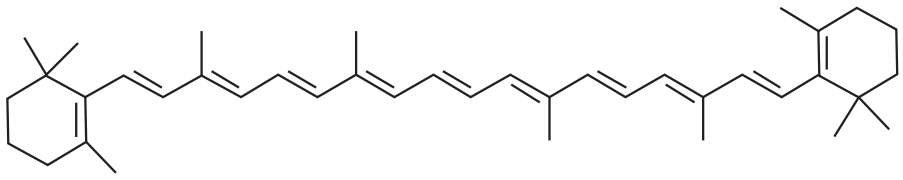

Figure 5 Structure of β-carotene

1.5.6.1 Sources of β-carotene

Dietary β-carotene sources (Figure 6) include green leafy vegetables (collard, turnip, spinach, lettuce), as well as orange and yellow fruits and vegetables (mangoes, cantaloupe melons, apricot, mandarin, peach, pumpkin, carrots and sweet potatoes) (Holden et al. 1999; Sass-Kiss et al. 2005). It can also be found in several microroganisms such as fungi *(Phycomyces blakesleanus,*

B. trispora, Rhodotorula sp.) and microalgae *(D. salina)* (Johnson and Schroeder 1996). β-carotene can be obtained from these various sources, however a large proportion of β-carotene is produced by synthesis (nature-identical form). The pharmaceutical or health food industry primarily uses β-carotene extracted from natural sources, but it is more expensive (Henry 1996).

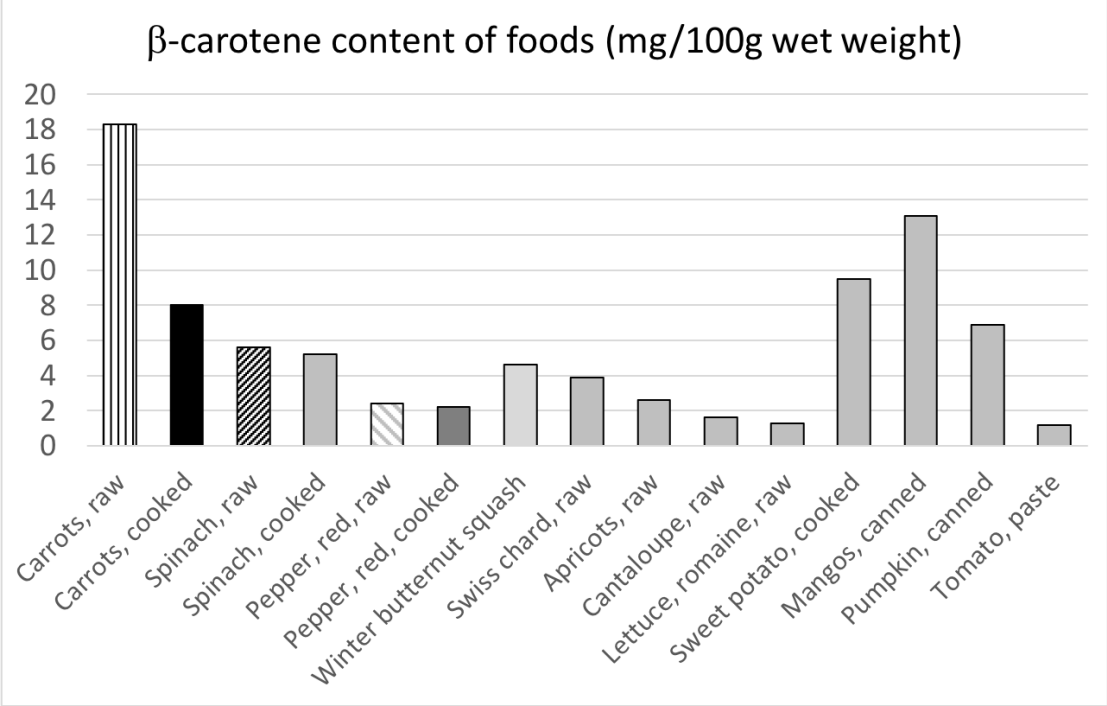

Figure 6 β-carotene content of certain foods (Krinsky and Johnson 2005)

1.5.6.2 β-carotene production

β-carotene can be produced by physicochemical methods, chemical synthesis or microbiological biosynthesis. Physicochemical methods involve extraction from green parts of plants, flowers, fruits, seeds, roots and tubers, however, the most popular source is orange carrot. Disadvantages of β-carotene extraction from natural sources include high cost, geographic determinants and seasonality of raw material.

Chemical synthesis was developed in 1950 and used largely to produce synthetic β-carotene on an industrial scale for feed and food dye purposes. Few other naturally occurring carotenoids are also synthesized industrially such as lycopene, canthaxanthin, astaxanthin, β,β–carotene, β-apo-8'-carotenal, β-apo-8'-carotene and cytranaxanthin. The chemical reaction used are either Wittig reactions producing aldehydes from aldehydes or ketones, or by use of

organometallic Grignard compounds. Microbiological biosynthesis of β-carotene (Figure7) is of interest due to the need for commercial production of natural pigment. Species of interest include *Dunaliella* spp. *Algae*, *Eustigmatos* cf. *Polyphem alga*, bacteria such as *Rhodococcus maris* and *Rhodobacter sphaeroides* as well as certain species of yeast that can synthesize β-carotene (Bogacz-Radomska and Harasym 2018).

Industrial production of natural carotenoids can involve biotechnological processes or solid-liquid extraction from plants. The major sources of industrial production of carotenoids, approved by the United States and the EU legislation involve fermentation using algae (*Dunaliella salina*) and fungi (*Blakeslea trispora*) (Additives 2002). A major advantage of algal β-carotene is that it is present in a nearly equal mixture of all-trans and 9-cis stereoisomers, as opposed to the cheaper and synthetic all trans β-carotene, since the naturally occurring mixture of isomers is claimed to favor biological and health effects and have higher bioavailaibility (Ben-Amotz and Levy 1996; Leach et al. 1998)

The major industrial application of β-carotene is as a food coloring agent but it also finds use in human food and animal feed, vitamin and antioxidant preparation and cosmetics due to its provitamin A activity (Del Campo et al. 2007). Nature identical forms of β-carotene have been used extensively in the soft drink and dairy industries in the form of suspensions, dissolved in vegetable oil, in emulsified water-soluble forms, and as spray dried powders (Henry 1996).

1.5.6.3 Health Effects of β-carotene

An important source of vitamin A for humans is β-carotene. It is very widespread in nature, with both halves of the structure being related to retinol. It therefore possesses 100% provitamin A activity. In addition, carotenes are considered antioxidants and several studies show they may be an important factor in reducing the incidence of cancer and several degenerative diseases (Ben-Amotz and Levy 1996; Del Campo et al. 2007; Tanaka et al. 2012; Solymosi et al. 2015; Sharoni et al. 2016) While these effects such as antioxidant activity, singlet oxygen quenching and radical scavenging, that go beyond this establish provitamin A function, have been studied they have not been completely proven in humans (Grune et al. 2010).

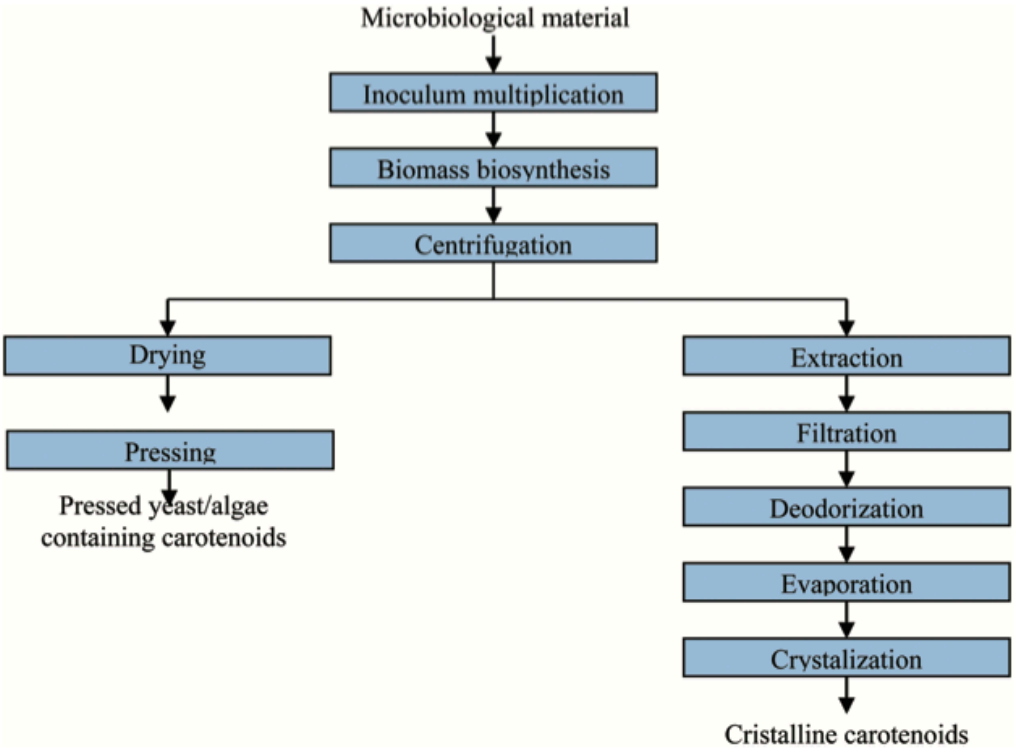

Figure 7 Simplified schema of technological process of production of β-carotene from microbiological origin (Bogacz-Radomska and Harasym 2018)

The chemical structure of conjugated carbon bonds in the β-carotene structure makes it an efficient singlet oxygen quencher. This is important in light-harvesting plants where it prevents photooxidative damage; however, in people suffering from erythropoetic protoporphyria, a condition where a genetic defect results in high levels of photosensitizer circulation in the organism, it has been shown that symptoms improved with high doses of β-carotene supplementation (Mathews-Roth et al. 1977) The general mechanism of photoprotection is an electron exchange energy transfer between singlet oxygen and carotenoid. This results in a triplet state carotenoid and ground state oxygen. Through rotational and vibration interactions with the solvent energy, the triplet state carotenoid returns to ground state as shown in the following reactions: $^1O_2 + CAR \rightarrow {}^3O_2 + {}^3CAR$; $^3CAR \rightarrow CAR + heat$ (Krinsky and Johnson 2005)

β-carotene has been proposed to act as a radical scavenger reacting with lipid oxide and lipid peroxide radicals under conditions of oxidative stress. In general, this reaction would involve donation of a hydrogen atom or electron, forming a carotenoid radical or carotenoid radical cation,

the carotenoid radical anion being formed with acceptance of an electron. The delocalization of electrons over the double bonds in the structure of β-carotene stabilizes these species, but in further reactions β-carotene decomposes and cannot be regenerated. Several studies have also shown that under conditions of high oxidative stress prooxidant activities of β-carotene can be observed (Mortensen et al. 2001; Grune et al. 2010)

Observational epidemiological studies have consistently demonstrated that individuals with high fruit and vegetable consumption are associated with reduced cancer risk, more so for lung and stomach cancer. The chemopreventive action of carotenoids in fruits and vegetables could occur due to its provitamin A activity, antioxidant function or immunomodulatory effects (Krinsky and Johnson 2005). Higher intakes of vitamin A and carotenoids was shown to have an opposite relation with breast cancer in women (ages 26-46 years) that were premenopausal and also smokers (Cho et al. 2003). Additionally, the association between lung cancer and β-carotene has been the most studied with several studies having shown a significant inverse relation of β-carotene intake and/or plasma level and a risk of lung cancer (Krinsky and Johnson 2005).

A reduced risk of cardiovascular disease has also been associated with β-carotene intake through several prospective cohort studies. In one of these studies (the Nurses' Health Study) a group of 121,000 female nurses in the United States of America aged 30-55 were followed after eight years, and it was seen that those within the highest quintile of β-carotene consumption had a 22% risk reduction of coronary events compared to those in the lowest quintile. The Health Professionals Follow-Up Study examined dietary antioxidants in 39,000 men based on four years follow up and found that those with the highest quintile of β-carotene intake had a relative risk of 0.75 when compared to those in the lowest quintile. Similarly, the Massachusetts Elderly Cohort Study showed that for individuals followed for an average of 4.75 years, the relative risk of cardiovascular deaths was 0.57 when comparing lowest and highest quartile of β-carotene intake. A relative risk of less than one signifying a reduced likelihood for the event to occur (Gazianoa and Hennekens 1993).

Blood-based observational studies have also shown an inverse correlation between baseline plasma carotenoid level and risk of myocardial infarction. The Physicians' Health Study (randomized trial) showed that subjects who received β-carotene had a 51% risk reduction in of major coronary events (coronary revascularization, fatal coronary disease and nonfatal myocardial

infarction) and a 54% risk reduction of major vascular events (nonfatal and fatal stroke). In addition, oxidized LDL may promote atherogenicity which is associated with an increased risk of cardiovascular disease. The effect of dietary antioxidants such as β-carotene to protect against LDL oxidation has been shown via *in vitro* supplementation of LDL with β-carotene (Jialal et al. 1991)

Protective mechanism of carotenoids may involve quenching free radicals that could attack lipid membranes causing chain reactions that cause lipid peroxidation and subsequently damage to membranes, enzymes and nucleic acids. β-carotene has been shown to have these protective effects both *in vivo* and *in vitro* (Bendich and Olson 1928). In addition, β-carotene is an important carotenoid in the skin and has also shown moderate UV protective effects in human intervention studies, however the exact mechanism (singlet oxygen quenching, antioxidant activity, interference with inflammatory pathways) is unknown (Sies and Stahl 2004; Kopcke and Krutmann 2008)

1.5.7 Challenges Affecting Stability of Natural Colorants

The high degree of unsaturation inherent in its chemical structure make carotenoids such as β-carotene susceptible to degradation processes such as thermal and chemical oxidation, isomerization and photosensitization upon exposure to oxygen, light and high temperature (Ferreira and Rodriguez-Amaya 2008). In addition, the molecule is lipophilic or insoluble in water and marginally oil-soluble at room temperature (Mattea et al. 2009; Liang et al. 2013)

When exposed to radicals or oxidizing species carotenoids tend to "bleach" or lose their color due to cleavage reaction or by addition to one of the double bonds in the conjugated double bond system (Krinsky and Johnson 2005). The presence of oxygen in the headspace, even at low levels of 1-2%, has also been shown to be significant during study of the storage of β-carotene model systems simulating dehydrated foods (Goldman et al. 1983). Due to its potential health effects, there is interest in incorporating β-carotene in foods and beverages as a natural pigment or nutraceutical however its inherent chemical properties present a challenge.

1.5.8 Strategies to Improve Natural Colorant Stability and Application

Methods to overcome the challenges associated with solubility, chemical stability in foods and bioavailability of β-carotene upon ingestion include encapsulation of β-carotene in different delivery systems such as gels, emulsions and powders (Mao et al. 2018b)

In one study galactomannan (locust bean gum) was used to disperse and stabilize oil-solubilized β-carotene at a mass ratio of 20:1:13 (locust bean gum : β–carotene: corn oil) producing a dry hydrocolloid powder with a vibrant orange color that resisted degradation by thermal, light and oxidative stress (Selig et al. 2018). Another convenient and effective methods of incorporating β-carotene into food includes **emulsion delivery systems** wherein the active component is solubilized in the oil or hydrophobic phase and then homogenized with the water soluble emulsifier containing aqueous phase (Qian et al. 2012a). The advantages of nanoemulsion delivery systems include the ability to be incorporated in optically transparent products, stability to aggregation or separation and possibly increased bioavailability of the lipophilic bioactive components (Acosta 2009).

Previous research has also shown that co-ingestion with lipids can improve β-carotene bioavailability since the digested products of lipids form mixed micelles, that can solubilize and transport β-carotene to epithelium cells (Hof et al. 2000; Borel 2003). Delivery of β-carotene as a nanoemulsion can therefore further enhance its absorption and in fact (Wang et al. 2012) showed that in emulsions prepared with soybean oil and decaglycerol monolaurate as the emulsifier, the bioaccessibility of β-carotene increases as the droplet size of the β-carotene containing dispersions decreases. Food-grade biopolymer emulsifiers such as octenylsuccinic anhydride (OSA) modified starch may also be used and was used to prepare oil in water nanoemulsions that resulted in an approximately 30% increase in bioaccessibility of β-carotene as assessed by in vitro digestion (Liang et al. 2013).

Another approach includes the use of **emulsification evaporation** techniques. This involves the use of a solvent to assist in dissolution of the carotenoid in the organic phase during emulsion preparation. After homogenization with the aqueous phase, the solvent is then removed by rotary evaporation. This method has been used to prepare lycopene nanoemulsions with the objective of being suitable for applications in beverages (Kim et al. 2014) as well as β-carotene nanoemulsions that showed good physical stability during 21 days of storage but had inconclusive

results for β-carotene degradation during that time (Silva et al. 2011). One approach involves the use of high temperature, high pressure emulsification and antisolvent precipitation. In this process a homogeneous solution of β-carotene in ethyl acetate is prepared at 60 bar and 145°C, subsequently mixed with a cold solution of octenyl-succinate starch in water to produce an oil in water emulsion, and solvent is thereafter removed with a rotary evaporator under vacuum to obtain an aqueous sample (Paz et al. 2014).

Drying is also often used to convert liquid forms of these delivery systems into the powdered form, to reduce storage and transportation costs, as well as to simplify handling and utilization, and to increase shelf life (Maher et al. 2014). The use of drying methods such as spray drying and freeze drying further assists in encapsulation of sensitive ingredients such as β-carotene. Of these, spray drying is a popular encapsulation method in the food industry and can be applied for the encapsulation of enzymes, flavors, antioxidants, preservatives as well as bioactive compounds (Donhowe and Kong 2014). Spray drying is 30-50 times less expensive compared to freeze drying, provides a large surface area and therefore the wall materials used must provide good oxygen barriers to protect against oxidation (Desobry et al. 1997). It can be described as a unit operation where the moisture from a liquid stream such as a solution, suspension or emulsion is evaporated by mixing the atomized liquid feed (a spray of finely divided droplets) with the drying medium (typically air). Contact between the hot drying medium and the spray occurs in the drying chamber, producing dried fine particles which are then separated using a cyclone or bag filter. The open cycle mode is used when the initial liquid stream is primarily aqueous, but a closed loop mode is preferred for organic based systems. The short exposure times to thermal conditions make spray drying a moderate drying technology. Critical parameters for consideration during spray drying are the aspirator rate, solid content of the liquid stream, humidity of drying gas, feed rate, spray gas flow, inlet temperature and use of organic solvent. Optimization of parameters for each system are usually performed depending on desired output by trial and error (Buchi Labortechnik AG 2002; Patel et al. 2015).

For bioactive ingredients such as β-carotene, the compound is first distributed in a solution of encapsulating material, which upon quick drying will encapsulate and protect the active compound. β–carotene spray dried powders include algae, carbohydrate and water dispersible powders (Donhowe and Kong 2014).

1. Algal powders: Orange, dry, free flowing algae powders of *Dunaliella salina* were obtained by spray drying concentrated feed suspensions as well as by microencapsulation of the feed with a mixture of maltodextrin dextrose equivalent 12 and gum Arabic (3.5:1). Rapid degradation of β-carotene in spray dried algal powders was observed but there were no changes in isomer proportions from the drying process and under appropriate conditions β-carotene recoveries exceeded 90%. Microencapsulated powders were more stable due to protective action of the polymer coat and first order degradation constants of 0.06 day^{-1} (r^2=0.98) for 200°C inlet temperature and 0.10 day^{-1} (r^2=0.99) for 265°C were obtained (Leach et al. 1998).
2. Carbohydrate powders: Production of spray dried carbohydrate powders involves drying of a homogenized or mixed solution of the bioactive ingredient with a specific wall material such as gum arabic, emulsifying starches and hydrolyzed starches. Each of these have their own advantages and disadvantages. In one study acid modified tapioca starch, native tapioca starch and maltodextrin were used to prepare spray dried powders and analyzed for their total carotene and surface carotene contents. Modified tapioca starch had the highest total carotene content, and the lowest surface carotene content, therefore indicating that it had the highest encapsulation efficiency among the three studied wall materials (Loksuwan 2007). Other researchers have investigated the use of octenylsuccinic anhydride modified starch alone or in combination with soy protein isolate for the effective microencapsulation of β-carotene. Prepared emulsions were spray dried and the total β-carotene and surface β-carotene contents were used to determine encapsulation efficiencies, with the modified starch showing much lower encapsulation efficiency compared to the protein isolate and the blends of the two wall materials (Deng et al. 2014).
3. Water dispersible powders: The nature of the emulsifier or wall material plays an important role in physicochemical properties of powders produced by spray drying of emulsions. Applications of β-carotene in functional foods is limited by its poor water dispersibility, chemical stability and bioavailability. Development of water dispersible β-carotene powders therefore is an area of interest. This approach is common for the pharmaceutical industry where preparation of spray dried oil in water emulsions that are dispersible in water are used to improve bioavailability of certain drugs that have

low solubility in aqueous systems. For instance, an oil in water emulsion using oil:water and maltodextrin:water ratios of 10% (w/w) and a load of solid material of 20% (w/w) was selected to deliver the a highly lipophilic model drug 5-phenyl-1,2-dithiole-3-thione (5-PDTT) (Dollo et al. 2003). In addition, milk proteins such as casein and its sodium salt have excellent functional proteins and have been studied for their use as wall materials in micro and nanoencapsulation of drugs and vitamins in the pharmaceutical field either alone or with other encapsulant materials (Jarunglumlert and Nakagawa 2013). Accordingly, this approach can also be used for bioactive ingredients such as carotenoids by preparation of a β-carotene enriched emulsion via microfluidization using casein as an emulsifier followed by mixing with maltodextrin and dehydration via spray drying and freeze drying. This study showed that higher β-carotene retention, lower moisture content and higher water dispersibility was obtained with the freeze dried powder (Chen et al. 2017). Similarly, β-carotene encapsulation with sodium salt of casein was achieved by spray drying a mixture of the protein in water and β-carotene dissolved in acetone (Jarunglumlert and Nakagawa 2013)

Further stability can be achieved by combining spray drying with a fluidized bed coating to produce a uniform layer around the particles and improve barrier properties thus protecting the sensitive ingredients such as described by (Coronel-Aguilera and San-Martín Gonzalez 2015) where β-carotene emulsions prepared using sodium caseinate and maltodextrin were spray dried to produce powders that were coated with hydropropyl cellulose and evaluated for color and carotenoid stability over time.

The use of water and oil soluble **antioxidants** in emulsion formulations can enhance stability of β-carotene. Four antioxidants approved for use in food specifically ascorbic acid, EDTA, coenzyme Q10 and vitamin E acetate were shown to have protective effects on β-carotene decay by acting as free radical quenchers (Qian et al. 2012b). β-carotene emulsions stabilized by gum Arabic also showed improved thermal stability in presence of antioxidants in the general order of α-tocopherol> TBHQ> ascorbyl palmitate (Liu et al. 2015).

1.5.9 Protein Encapsulation Methods for β-carotene

Encapsulation of β-carotene specifically with proteins is commonly achieved through the preparation of oil in water emulsions, nanodispersions or nanoparticles and dried powders (Champagne and Fustier 2007). In comparison to low molecular weight emulsifiers, protein emulsifiers have also been shown to be more effective in preventing bioactive degradation (Laakso 1984; Sáiz-Abajo et al. 2013). As a result of these encapsulation methods, the dispersibility, water solubility and chemical stability of these bioactive compounds like β-carotene can be improved, and therefore they can be used as functional ingredients in foods and beverages (Patel et al. 2015; Chen et al. 2017). Additional drying steps like freeze drying and spray drying then subsequently reduce the cost of storage and transportation, extend shelf life and provide ease of use (Maher et al. 2014).

Oil in water emulsions of β-carotene prepared with either β-lactoglobulin or Tween20 showed that emulsions prepared with the protein emulsifier had less color fading, suggesting that it was more effective in preventing carotenoid degradation due to antioxidative properties (Laakso 1984; Mao et al. 2009; Qian et al. 2012b). In another study, casein and β-carotene aggregates were formed by mixing of casein solutions (5% w/v) with 2mL of β-carotene solution (β-carotene dissolved in acetone with a β-carotene : CAS ratio of 1:200) followed by spray drying to produce powders. The encapsulation efficiency of casein aggregates was determined by measured release rate of β-casein and it was reported that pH and storage time influenced surface loading and encapsulation properties. Acidic pH conditions (pH=5.5) showed highest surface β-carotene loading, suggesting that rapid aggregation conditions assisted in β-carotene entrapment. With storage, there was also an increase in total and inner loading efficiency possibly due to relocation of surface β-carotene that was associated with casein to inner particle space (Jarunglumlert and Nakagawa 2013). The stability of β-carotene in oil and water emulsions of β-carotene can also vary with protein emulsifier and lipid carrier used. Whey protein isolate and sodium caseinate used to prepare oil in water emulsions with 10% w/w lipid phase containing 0.05% (w/w) β-carotene and water phase of 30% sucrose along with different protein concentrations, showed that with whey protein isolate a multilayer structure was formed while sodium caseinate gave a monolayer around the oil droplets. Emulsion stability influenced β-carotene degradation and the solid state of hydrogenated palm kernel oil in one of the formulations was purported to enhance β-carotene

protection as compared to liquid sunflower oil. In addition, the monolayer of sodium caseinate was also more effective in protecting β-carotene, possibly due to different amino acid composition and radical scavenging property of the protein (Cornacchia and Roos 2011b).

Nanodispersions can be prepared by solvent displacement wherein an organic solvent containing bioactive is mixed with aqueous phase containing emulsifier, and rapid diffusion of solvent to the interface causes formation of nanoparticles. Further evaporation of solvent will then cause nucleation and crystal growth and precipitation of nanoparticles. For instance, this method was used to prepare β-carotene nanodispersions by dropwise addition of acetone (0.015% w/v β-carotene) to aqueous phase at 1% wt emulsifier. Sodium caseinate was reported to produce the largest particle size but also had best stability to oxidation (4 °C for 8 weeks), as compared to Tween20, decaglycerol monolaurate and sucrose fatty acid ester (Yin et al. 2009). A combination of emulsification and evaporation methods can also produce nanodispersions/nanoparticles. This involves formation of oil-in water emulsion with active compound in lipophilic solvent and emulsifier in aqueous phase, followed by evaporation of solvent such that the active compound crystallizes in the oil in water emulsion droplets, resulting in a decreased particle size. The bioactive compound is then stabilized by emulsifiers or a combination of emulsifiers that are at interface of the two phases. For instance, β-carotene nanodispersions were prepared by emulsification-evaporation using various proteins and hexane as the initial carrier solvent. Sodium caseinate, as compared to soy protein isolate, whey protein concentrate, whey protein isolate and whey protein hydrolysates, was reported as the most suitable emulsifier. This was due to its low molecular weight, structural flexibility that allowed rapid adsorption onto surfaces of droplets or particles, its strong electrostatic and steric stabilization that helps prevent coalescence and its ability to lower interfacial tension. An increase in sodium caseinate concentration also gave reduction in mean particle size, while higher β-carotene concentrations and organic phase ratios gave larger β-carotene particles (Chu et al. 2007). Other proteins such as soy protein (Deng et al. 2016) and soy protein isolate (Deng et al. 2014) have also been used to prepare β-carotene powders. Accordingly, a combination of emulsification-evaporation using sodium caseinate as an emulsifier and a food grade solvent followed by spray drying to prepare a water dispersible β-carotene powder is of interest. Natural antioxidants such as tocopherol could also be incorporated to improve β–carotene stability in this functional ingredient system.

1.5.10 Applications of β-carotene Color

Historical applications of β-carotene as a natural colorant include butter, margarine and shortening. It has also been used to add color to baked goods, natural and processed cheese, ice cream, yogurt, dairy product substitutes as well as juices and beverages. Incorporation of β-carotene oily suspensions in the preparation of salad oils, dressings and spreads have also been used to impart bright colors. The method of application varies depending on the product but in certain instances the water dispersible beadlet form is necessary. A vanilla ice cream can be colored with water dispersible β-carotene to give a French vanilla type or golden vanilla ice cream, while in dairy product substitutes consistency in color can also be achieved for imitation milk, whipped toppings, fluid and dry coffee whiteners, sour cream (Kläui and Bauernfeind 1981). In yogurt low concentrations of water dispersible forms can be used to give colors such as lemon yellow, apricot, peach to redder tints, and in one study the encapsulated β-carotene demonstrated stability in the acidic environment of a yogurt, giving a color similar to that of peach yogurt (Coronel-Aguilera and San-Martín Gonzalez 2015).

Water dispersible beadlets used in above mentioned examples were typically prepared with β-carotene in vegetable oil suspension that was heated to dissolve β-carotene and then emulsified in colloid-plasticizer combination like gelatin-sugar solution. When dispersed in water they give cloudy yellow to orange pulplike solution resembling orange juice that may not always be desirable. Some water dispersible formulations used gelatin as a carrier which could react with other components and cause flocculation or precipitation. To limit creaming of flavor oils in beverages often weighted oils like brominated vegetable oil that would increase specific weight of oil phase and add stability were used but FDA restrictions have limited their use. These and other substances used to match the specific weight of the oil phase to that of the aqueous phase may also act as clouding agents. In addition the type of hydrocolloid or protein used as an emulsifier is important, as well as pH conditions of the beverage (Kläui and Bauernfeind 1981). Development of water dispersible powders and nanoemulsions in particular that can be used in beverages and provide transparency in appearance is therefore an area of interest. This is possible by preparing emulsions or powders that resolubilize to produce droplets less than 100 nm in size (Kim et al. 2014). These ingredients may also improve bioavailability of the natural colorants being used. The bioavailability of carotenoids such as β-carotene is dependent on the source and formulation, and

it has been shown that plasma β-carotene concentrations are higher after intake of beverage including β-carotene dispersed from a water dispersible powder as compared to carrot juice (Thürmann et al. 2002).

1.6 Food Science Extension

1.6.1 Land Grant Universities and Extension

The system of land grant universities began in 1862 with the Morrill Act that provided states with public lands to be sold or used for profit such that at least one college that would provide education in agriculture and the mechanical arts would be established, thus extending higher education to large segments of the United States population. Over time many of these colleges developed into universities and expanded into additional areas of higher education. Further legislative acts also endowed these colleges with a three part function of teaching, research and extension which became the hallmark of land grant universities (Committee on the Future of Land Grant Colleges of Agriculture, Board on Agriculture, National Research Council 1995).

The concept of university extension was developed so that knowledge, education and research developments achieved through public funding was made available to those individuals otherwise unable to attend these institutions. This is achieved through lectures, community development and consumer education programs through the efforts of extension staff (National Research Council 2018). In 1999, however, The Kellogg Commission on the future of State and Land Grant Universities strongly suggested the need to go beyond outreach and service and to start investing in engagement, wherein engagement exceeds conventional outreach, extension and public service and is strongly committed to sharing and reciprocity via partnerships. Accordingly, an engaged institution therefore, is one that responds to students' present and future needs, enriches their experiences with research and engagement as part of the curriculum and uses its resources to influence problems of the communities. In addition seven guiding characteristics for an engaged institution were elucidated as responsiveness, respect for partners, academic neutrality, accessibility, integration, coordination and resource partnerships. (Kellog Commission and Land-Grant Universities 1999). Purdue University is a land grant university for the state of Indiana and as per the Purdue University Engagement Strategic Plan has a vision to be "the partner of choice

for engagement that effectively leverages the University's resources to advance the economy, education and quality of life" and a mission "to design, guide and lead collaborations that drive innovation, prosperity and an improved quality of life throughout Indiana and beyond".

1.6.2 Engagement in Graduate School Education

It has been observed that graduate education and doctoral programs in particular do little to introduce graduate students to the public service role of faculty via community engagement. Given the tripartite nature of land grant universities in particular, integration of engagement into graduate education will promote deeper understanding and learning and allow development of connections with public agencies thus enriching the graduate education experience (O'Meara and Jaeger 2006). Limited research in this area exists however accounts of service learning and community based research being incorporated into a graduate program do exist with positive outcomes such as improving team skills, written and presentation skills, improved ability to gather, interpret and disseminate community based information, positive influences on interpersonal development and critical thinking (Hyde and Meyer 2004; Coffey and Wang 2006). In response to this criticism however, the Council of Graduate Schools and Graduate Record Examination Board recommended the need for deliberate and significant work outside university walls with the hope that the social implications of projected research, would be considered wherever possible thus directing academic projects towards accomplishing meaningful social change (Council of Graduate Schools in the U.S. and Service 1973). Historically however, the pursuit of a specialized area of scientific research has held more value promoting students to become more insular and disconnected from communities. This is accompanied by limited exposure to the variety of roles that faculty members undertake, particularly in extension and engagement. In response however, programs such as the Carnegie Initiative on the Doctorate, the University of Washington's Re-Envisioning the PhD program, the Woodrow Wilson National Fellowship Foundation's Responsive PhD initiative and the Preparing Future Faculty Program have been developed to reform doctoral programs to address the need to prepare students with knowledge about the scholarship of teaching and learning as well as the ability to connect their disciplines to the needs of communities and societies (Applegate 2002). As a result of the Purdue University Preparing Future Faculty program, this graduate student was exposed to the concepts of the Scholarship of

Engagement and the basic tools and methodologies of developing an extension/engagement program.

1.6.3 Logic Models and Extension Plans

One of the challenges for integration of engagement into graduate education, is that each department and discipline must determine the best approach. The experiences and opportunities are highly dependent on the content and framework of each discipline. General methodological approaches such as the development of a logic model and an extension plan thereafter can, however be applied. The logic model framework is generally adopted by extension programs and is a systematic and visual way to represent "how your program works, the theory and assumptions, the road map of the program, the activities and desired outcomes". Its basic components therefore are the situation, the inputs, the activities, the outputs, the outcomes and the assumptions and external factors that are involved in the program (Figure 8). Logic models help individuals and stakeholders the human and financial investments needed to achieve the desired goals (W K Kellogg Foundation 2004). Logic models also enhance the case for investment and are increasingly being used by funding agencies to evaluate grant applications.

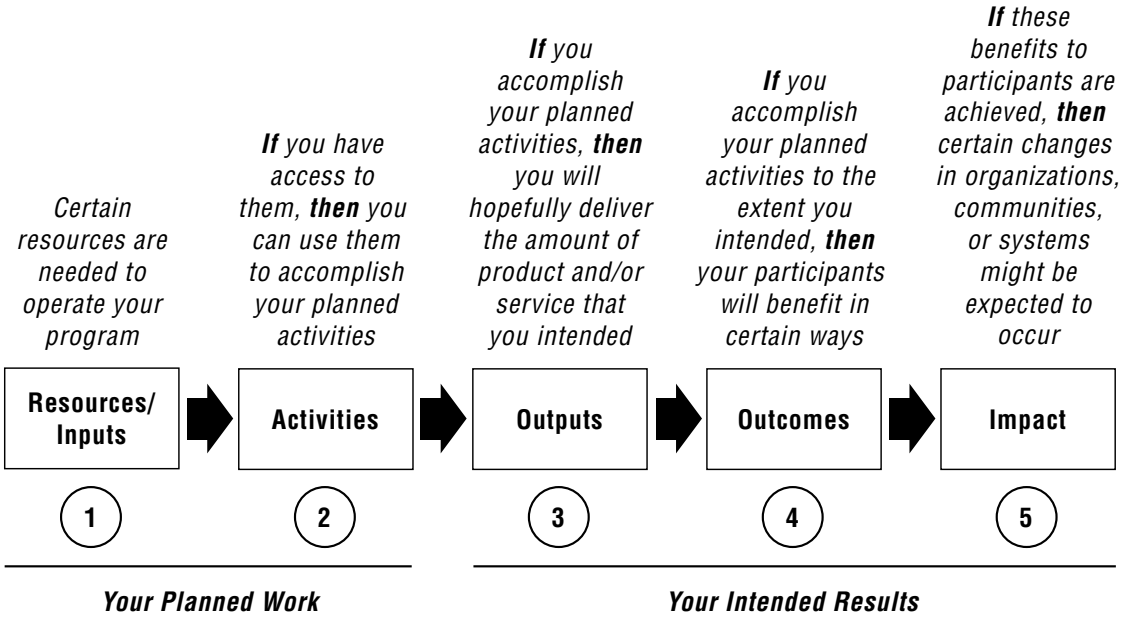

Figure 8 The basic logic model and how to read it (W K Kellogg Foundation 2004)

1.6.4 Food Science Extension

Consumer surveys and questionnaires are often used to determine factors that influence decisions regarding the purchase of a product, product acceptability, as well as consumer attitudes and preferences. Similar methods have been also used to determine consumer knowledge around food additives that are used in the food industry. A Canadian study with 0.1% of the country's population showed that the role of additives in food was not well known , that people had different opinions as to the definition of a food additive, and that respondents were concerned about the presence of additives in the diet (Knox and Pope 1980). The International Food Information Council Foundation's 2018 Food and Health Survey showed that familiarity of a product weighs heavily and there is a strong preference (7 in 10 people) for no artificial ingredients in foods, and that people would also give up a familiar product for one with no artificial ingredients, however, confusion amongst consumer remains due to conflicting information being provided (International Food Information Council Foundation 2018).

In addition, a consumer's definition of what constitutes natural ingredients or natural food also varies widely and can range from a preference for no antibiotics, no hormones or no preservatives to foods that have improved animal welfare practices, improved nutritional value or improved food safety. In one study, it was shown that food ingredients with chemical names had a greater tendency to be characterized as non-natural even if they are manufactured or derived from natural sources (e.g. xanthan gum). Other inconsistencies observed were a much greater tendency to view wheat flour as natural as compared to gluten which is major protein in wheat flour and that baking soda as viewed as natural as compared to sodium bicarbonate which is its chemical name. Corn and soybeans were also viewed as natural by a majority of the consumers despite more than 90% of each of these crops being genetically modified in the United States (Chambers et al. 2018). Similar studies in various countries like Turkey, Korea, Mauritius, and Ethiopia have also shown a general lack of awareness about the functions and advantages of food additives (Altug and Elmaci 1995; Shim et al. 2011; Koyratty et al. 2014; Legesse et al. 2016)

Programs aimed to educate consumers, however, have been shown to improve consumer recognition and awareness of food additives with the development of appropriate training and consumer education (Lee et al. 2014). Previous studies have also shown that the ability of consumers to identify the correct role of additives in food production is dependent on the education level of the respondents (Kozelová et al. 2012).

Extension is a means to provide non-formal education to individuals from both rural and urban areas using information obtained through teaching and research and to create a positive change (USDA 2014). Food Science extension programs typically focus on food safety and nutrition/wellness information. Resources related to Food and Nutrition at the Purdue University Extension Education are categorized as Entrepreneurship, Food Preservation and Storage, Food Safety, Human Nutrition, MyPlate, MyPyramid and Wine (Purdue University 2016). Food science extension can however, be also be used to expose people to food science at an early age by developing educational programs with food science specialists in collaboration with county extension agents. This material can include demonstrations with experiments and thus help improve food science literacy and potentially create a pool of talented food scientists for the future (Schaffner 1991)

A USDA report on employment opportunities for college graduates in food, agriculture, renewable natural resources or the environment projects that between 2015-2020 only 61% of the expected average annual job openings will be satisfied by US graduates with expertise in these specific areas (Goecker et al. 2015). This coupled with diminishing science literacy, and negative public perceptions about food science and technology raises the importance of food science education, extension and outreach programs. These challenges are being approached in several different ways by different organizations.

National standards for Food Science, Dietetics and Nutrition education have been developed by the National Association of State Administrators of Family and Consumer Sciences and are designed with the intention of accommodating various state philosophies. These standards were developed with the intention of providing structure for identifying program outcomes and what learners should know and be able to do at the end of completing the sequence of courses in the specific area of study (National Association of State Administrators of Family and Consumer Sciences 2018). Certain states also have established academic standards for food science and other agricultural based areas of study. Indiana Academic Standards include a section for agriculture that has standards for Introduction to Agriculture, Food and Natural Resources, Food Science and Advanced Life Science-Foods to name a few (Indiana Department of Education 2014a; Indiana Department of Education 2014b; Indiana Department of Education 2014c; Indiana Department of Education 2017).

FDA/NSTA Partnership in Food Science was established in 1999 to develop curriculum called Science and Our Food Supply for middle and high school science teachers. Online lesson plan and resources, tutorials and the opportunity to attend a weeklong professional development program in food science is provided (NSTA 2006). The Institute of Food Technologists (IFT), the IFT Foundation and Discovery Education partnered to create materials to educate high school students about food science as well as to create awareness about potential careers and opportunities in food science and technology. The first kit meant for science teachers contained scientific content such as experiments and an informative video. The other kit was developed for high school career counselors and also included a poster that had career related information, description of food science, IFT approved undergraduate food science programs and coursework typical to food science programs, scholarship and salary survey information (Mcentire and Rollins 2007). IFT also developed the IFT Food Science Activity Guide containing activities for food chemistry, nutrition, food processing, product development, sensory evaluation and more (Institute of Food Technologists 2007).

Food science demonstrations are an effective tool towards improving STEM teaching and learning. Familiarity with food materials, the strong public interest and awareness in food and health and the intrinsic interdisciplinary nature of food science such that it involves areas such as chemistry, microbiology, engineering, nutrition and sensory sciences makes it an advantageous choice to expose and engage students to STEM disciplines. This in turn is advantageous for the creation of more capable STEM workforce, more awareness about food science and possibly more knowledgeable selection of food science as a future career (Schmidt et al. 2012). For instance, Food MASTER (Food, Math and Science Teaching Enhancement Resource) initiative is one such compilation of projects aimed at using food as a tool to teach multidisciplinary science, and was shown to be effective for 4th grade students' in schools in Ohio and North Carolina (Hovland et al. 2013). In another instance, activities around caramel sauce, maraschino cherries and ice cream have been used to explain colligative properties to students from 5th grade to high school level and when their educational impact was evaluated with a group of 7th grade students, they showed a 36% increase in score on a test related to the workshop (Wickware et al. 2017).

Accordingly, in partnership with the Office of Engagement at Purdue University, the basics of extension programming were studied, and a logic model and extension plan were developed and implemented (Chapter4).

1.7 References

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CHAPTER 2. INTERFACIAL RHEOLOGICAL PROPERTIES OF SODIUM CASEINATE AND β-LACTOGLOBULIN

2.1 Introduction

Rheology is defined as the study of the deformation and flow of matter (Young 2011). Foods can be classified as solids, gels, homogeneous liquids, suspensions of solids in liquids and emulsions; and depending on their nature will respond in different ways in response to applied forces or deformation. Many foods exhibit viscous and elastic properties and are therefore classified as viscoelastic materials. Rheometry is thus used to understand these responses to determine the structure, composition and interaction between components of the foods (Miri 2011).

Interfacial rheology is the "study of the relationship between interfacial stress and resultant deformation of the interface" (Murray 2002). The region of finite thickness where two homogeneous bulk phases meet is known as the interface. The properties of the interface are involved in the stability of systems where one phase is dispersed in the other (Barnes and Gentle 2010). Interfacial rheology describes the functional relationship between the deformation of an interface, the stresses exerted in and on it, and the resulting flows in the adjacent fluid phases (Krägel and Derkatch 2010). Compressional deformation of the interface (dilatational rheology) is performed by measuring the variation of area while maintaining constant shape and is proposed to be related to the short-term stability of dispersions. On the other hand, shearing motion of the interface (shear rheology) involves change in shape at constant area provided that the interface is in thermodynamic equilibrium with the bulk solution, and has been suggested to be associated with the long-term stability of the dispersion. These two complementary approaches are typically used to study the interfacial layer and facilitate a correlation between its structure and its mechanical properties (Maldonado-Valderrama and Patino 2010; Torcello-Gómez et al. 2011).

The effect of particles, surfactants or proteins at interfaces of multiphase materials are important for food, biomedical and oil recovery industries to name a few. Proteins, specifically, can effectively produce stabilized dispersed systems like emulsions and foams through reduction of interfacial tension or viscoelastic film formation (Prins et al. 1998).

To study interfacial rheology several devices have been proposed such as canal devices, channel flow devices, rotating disks and rings and knife-edge devices. Each has its own advantages

and disadvantages specifically related to the small forces and torques associated with deformation of the surface and due to the fact that flow and deformation of the surface will involve the surrounding bulk phases to some extent. Accordingly, the Bousinesq Number B_o (Eq.7) is used to assess relative contributions of surface to that of the surrounding phases in an interfacial measurement. It is represented by ratio of surface drag to subphase drag.

$$B_o = \frac{surface\ drag}{subphase\ drag} = \frac{\eta_s . P_c L_c''}{\eta . A_c L_c'} \quad \text{.....(Eq. 7)}$$

Where P_c is the contact perimeter between rheological probe and the interface and A_c is the contact area between the probe and the subphase. L_c' and L_c'' are the characteristic length scales in which the velocity decays in the surface and subphase respectively. When B_o >>1 surface stresses dominate and when B_o <<1 subphase stresses dominate. To avoid contribution of the surface therefore, it is important to have minimal contact area per perimeter with the sub-phase thus presenting the need for a sensitive measurement device. The ratio of P_c/A_c can be varied depending on the device geometry to increase the sensitivity to surface stresses. For a rotating disk this ratio is minimum (4/D) where D is diameter of the disk, while for the magnetic rod probe it can be as high as 2.8 mm^{-1} (for probe 33 mm long and 0.45 mm in diameter) (Brooks, Carlton F, Fuller, Gerald G, Frank, Curtis W., Robertson 1999). This equation can be further simplified to ratio of surface viscosity (η_s) to bulk viscosity (η) of the sub-phase multiplied by characteristic experimental length scale *a* which is related to the radius of the entire geometry (ratio of area Ac to perimeter Pc) as shown in Eq. 8.

$$B_o = \frac{\eta_s}{\eta . a} \quad \text{..... (Eq. 8)}$$

Proteins such as β-casein, β-lactoglobulin, ovalbumin and bovine serum albumin can be used to decrease the surface tension and impart kinetic stability in colloidal systems. They are amphiphilic, are able to form films, are bulkier than low molecular weight surfactants and tend to diffuse into the interface at a relatively slower rate. In addition, proteins can also form strong viscoelastic films that resist mechanical stresses and provide either electrostatic or steric stabilization of dispersed oil droplets, making them useful for emulsion formation (Bos and van Vliet 2001; Rodríguez Patino et al. 2008; Lam and Nickerson 2013). Various studies have elucidated that protein foam

and/or emulsion stability "appears to be linked to protein adsorption and interfacial unfolding, simultaneous increase of elasticity and decrease of viscosity, compressibility of adsorbed protein layers, formation of a 2D interfacial gel or to the increase of the dilatational modulus*"*(Maldonado-Valderrama et al. 2008). Thus, studies of dilatational and shear rheology of interfacial proteins can help to understand their role in emulsion stabilization.

High-pressure homogenization is commonly used to prepare emulsions. This process can modify the functional aspects of proteins due to the combined effects of cavitation, turbulence, shear stress, friction and heat. For example, casein molecules exist as micelles held together by extension of the hydrophilic C-terminal region of κ-casein from the surface of the casein micelle into the solution. In addition, colloidal calcium phosphate and hydrophobic interactions play a role. Particle size reduction occurs when casein micelles and casein micelle isolates are treated with high pressure in the range of 100-250 MPa, while in the presence of excess calcium in solution, large aggregates of casein are formed at pressures exceeding 300 MPa (Roach and Harte 2008). Previous work has shown that high-pressure homogenization improved the oxidative stability of an emulsion stabilized with sodium caseinate, possibly due to increased interfacial cross-linking and this effect was dependent on applied pressure. This cross-linking effect may cause a change in the interfacial rheological properties of the emulsifier, and affect emulsion oxidative stability (Phoon et al. 2014). In the case of globular proteins, high pressure disrupts the tertiary and quaternary structure, and protein unfolding occurs due to changes in the α-helices and β-sheets (secondary structure). This leads to aggregation, in a similar way that partial denaturation caused by thermal treatment does (Floury et al. 2000). Pressure induced (450 MPa, 25°C, 15 min) changes in rheological properties of emulsions due to aggregation of β-lactoglobulin have also been noted (Dumay et al. 1994) . Decreased stability due to pressure treatment has also been shown. High pressure treatment (upto 800 MPa) of industrial β-lactoglobulin protein isolate, led to a reduction in overall emulsion stability (Galazka et al. 1996). In another study, heat treatment and high-pressure homogenization resulted in changes in secondary and tertiary structures of the β-lactoglobulin protein, which affected oxidative stability of the menhaden oil-in-water emulsions stabilized with them (Phoon et al. 2013). Characterization of the interfacial rheological properties of casein and β–lactoglobulin before and after high-pressure treatment will therefore help elucidate the contribution of these properties to overall emulsion stability.

Protein molecules may also be either displaced from the surface by other proteins (globular proteins replaced by flexible ones) or by surfactants. They may even interact with other components such that their interfacial properties would be altered. Carvacrol is a phenolic terpene with antimicrobial properties and encapsulation of this phytophenol using emulsions has been studied as potential method to prevent microbial contamination of food. The addition of carvacrol at various concentrations (5, 15, 30 and 50 wt%) to emulsions prepared with triacylglyceride (Miglyol 812N) with 2 wt% Tween 80 in the aqueous phase, showed an initial decrease in droplet size. Above 30% carvacrol concentration, the droplet diameters increased and loss of stability on exceeding this critical concentration was attributed to Ostwald ripening effect. Emulsions that were prepared without carvacrol also had larger oil droplets, suggesting that the phytophenol may act as a co-surfactant and reduce the interfacial tension (Terjung et al. 2012). Previous work involving the preparation of emulsions with coconut oil and a soy lecithin Ultralec P (phosphatidylcholine 23%, phosphatidylethanolamine 18%, and phosphatidylinositol 15%, HLB 7) acquired from ADM (Decatur IL, USA) showed higher stability (smaller droplet size, more negative surface charge) with an increase in carvacrol concentration (up to 2.5% w/w of total emulsions), while emulsions prepared with Tween 20, were in the nanoemulsion range only in the absence of carvacrol (Rodriguez Martinez 2014). The effect of carvacrol on the interfacial properties of emulsions prepared with protein emulsifiers sodium caseinate and β-lactoglobulin is therefore of interest.

The objectives of this project therefore, were to characterize interfacial rheological properties of sodium caseinate and β-lactoglobulin at the air/water and oil/water interface, to determine the effect of high-pressure homogenization and presence of carvacrol on these properties using the magnetic interfacial shear rheometer or ISR.

2.2 Materials and Methods

2.2.1 Materials

Proteins used were sodium caseinate (Miprodan 30) that was generously donated from Arla Foods (Viby J, Denmark) and β-lactoglobulin (Davisco Foods International, Inc, Le Sueur, MN). Buffer salts were sodium phosphate dibasic (Na_2HPO_4) sodium phosphate monobasic ($NaH_2PO_4.H_2O$) from VWR Life Science (Radnor, PA). Double distilled water (18 megaohm-cm)

was prepared in the lab with a distillation unit. Stearic acid (grade I, >98.5%) and carvacrol were obtained from Sigma Aldrich (St Louis, MO) and chloroform from J.T. Baker-Avantor (Center Valley PA, USA). Phosphate buffer stock solution (100 mM) was prepared with 10.9 g of Sodium phosphate dibasic (Na_2HPO_4) and 3.1 g of sodium phosphate monobasic ($NaH_2PO_4.H_2O$) mixed in double distilled and diluted to desired concentration. This stock solution was stored at 4°C for one month at a time diluted to 12 mM for protein spreading experiments. Chloride /phosphate buffer (pH 7, 0.05 M NaCl, 0.05 M Na_2HPO_4) was prepared by mixing 29.22 g sodium chloride and 71 g disodium phosphate in 1 L water to prepare a 0.5 M buffer stock solution which was then diluted ten times to a 0.05 M buffer solution. Stock solution of stearic acid in chloroform was prepared by dissolving 250 mg stearic acid in 25 mL chloroform and then diluting to 1 mg/mL for use during calibration for surface pressure-area isotherms. Protein solutions of sodium caseinate and β-lactoglobulin, were prepared by continuous stirring in buffer for 2 hours at room temperature and then stored for 24 hours at 4°C to ensure complete hydration of protein.

2.2.2 Methods

The KSV NIMA Magnetic Interfacial Shear Rheometer (KSV, NIMA, Finland) was used to determine surface pressure-area isotherms as well as interfacial rheological properties. This instrument consists of a Langmuir trough with a Wilhelmy balance for measurement of surface pressure, barriers for compression of the interface and Helmholtz coils to produce a uniform magnetic field in the measuring area. The working principle is that the magnetized probe or needle floats at the interface inside a quartz channel. This channel creates a small meniscus on both sides of the surface and guides the probe to move in a straight line, thus ensuring uniform flow geometry. A metal channel holder is used to ensure that the channel is centered for every measurement. The probe moves due to the magnetic field created by the Helmholtz coil and a light source with a camera is used to monitor the position of the needle. The drag experienced by the rod is assumed to arise primarily from interfacial shear stresses developed at the interface. The position of the rod and its movement is recorded optically with the camera. Accordingly, the complex surface modulus is calculated from strain and signal phase shift which can be separated into elastic and viscous properties of the interfacial film.

Clean Surface Preparation: Pure ion exchanged water with resistivity of (18 $M\Omega cm^{-1}$) was filled in the Langmuir trough so that the surface of the water sub-phase was a couple of millimeters above the edge of the trough. A vacuum pump was used to aspirate the surface during barrier compression. The tip of the hose on the vacuum pump was cleaned with ethanol and water when the pump was on to ensure contaminants were not added to the sub-phase. The barriers on the trough were closed at 20% of maximum speed (54.15 mm/min) and as they moved the surface of the water was aspirated with the vacuum pump until the barriers reached the maximum closed position (138 cm^2). The barriers were then opened (moved to zero position) and the procedure was repeated two-three times with more water being added as needed. During the last aspiration, the water level was adjusted to be just a little higher than first step on the trough edge such that the meniscus of water curved up to the holes on the barriers, but not so high that water flowed through. The barriers were then moved back to the open (zero) position before proceeding. The Wilhelmy plate was rinsed with pure ethanol and ion exchanged water and then exposed to strong flame of a Bunsen burner and then hung on the balance such that two-thirds of the plate was above the surface. With the Wilhelmy plate in contact with the sub-phase the balance was set to zero and barriers were compressed. If the surface pressure value stayed below 0.2-0.3 mN/m it indicated that the sub-phase was clean and ready for further analysis, otherwise the surface aspiration procedure was repeated.

Protein Analysis: In the *spreading* experiments, aliquots of protein solutions were deposited on the clean sub-phase dropwise using a microliter syringe. To allow for processes of spreading, adsorption and structural rearrangement the samples were left to stand for 30 minutes and then measurements were performed. In the *migration* experiments, prepared protein solutions were filled in the trough and 2 hours were allowed for protein migration and structural rearrangement at the surface, prior to analysis.

1. Surface Pressure and Area Isotherms (air/water interface): The fully automated Langmuir type film balance on the KSV NIMA ISR was used to determine surface pressure (π)-surface area (a) isotherms using a compression cycle with two movable barriers and a Wilhelmy plate for surface pressure measurement. Barrier speed compression during measurement was 33 mm/min and area of the trough was reduced from 401 cm^2 to 138 cm^2

and at least three isotherms were measured to ensure reproducibility. Stearic acid (molecular weight 284.5 g/mol) π-a isotherm was used as a standard for calibration of the equipment. A volume (50 μL) of stearic acid solution (1 mg/mL in chloroform) was deposited dropwise on the clean sub-phase using a microliter syringe, 10 minutes were allowed for chloroform evaporation and then the π-a isotherm was obtained by compression of the barriers at 20 mm/min. π-a isotherm measurements were performed with sodium caseinate as well as combinations of sodium caseinate and carvacrol on a buffer sub-phase using a microliter syringe to control surface concentrations.

2. Interfacial Rheology: The metal channel holder and a quartz channel were placed in the middle of the trough in the line of sight of the camera above the trough. Protein solutions were poured in the Langmuir trough (migration experiments) for the π-a isotherm. The magnetic ISR probe (Length: 44.30 mm, weight 17.20 mg and diameter 0.4 mm) was placed in the quartz channel such that it was floating at the air-water interface and oscillating in the magnetic field produced by the Helmholtz coils. The position of the needle was set by finding the edge of the needle, using a frequency of 0.5 Hz, amplitude 0.5 V, offset voltage to adjust needle position in the line of sight of the camera as needed and threshold value of 150 to indicate dark and light regions. Calibration of the magnetic rod was performed via a frequency sweep from 0.5-1.5 Hz, initial amplitude of 0.5 V for a 50 micron movement and in ramp down mode. Amplitude sweeps were also performed to determine the linear viscoelastic range for each material. Frequency sweep from 0.5-1.5Hz, initial amplitude of 0.5V for 50 micron movement in ramp down mode was performed to determine rheological parameters G' (storage or elastic modulus) and G'' (loss or viscous modulus).
3. High pressure homogenization treatment: Protein solutions 1% (w/v) in buffer were prepared and passed through a high-pressure homogenizer (Nano DeBee, BEE International Inc., South Easton, MA, USA) at 20 kpsi (137.85 MPa) for 3 passes. The treated solutions were then diluted to the desired concentration for analysis and compared with the control (0 passes).

Statistical analysis: Three replicates of each measurement were performed and t-test and one-way analysis of variance ($p<0.05$) was used to determine statistical differences using Minitab18. Standard deviations for certain parameters are also shown.

2.3 Results

2.3.1 Surface Pressure-Area Isotherms at Air/Water Interface

Calibration: Surface pressure versus area (π-a) isotherm of stearic acid in chloroform (1 mg/mL) was performed during equipment installation and training and was repeated prior to further surface pressure-area isotherm experiments (Figure 9). Verification of the two distinctive points specifically, an increase in surface pressure at mean molecular area around 25 $Å^2$/molecule and a clear change in slope of surface pressure vs. area curve at surface pressure of 25-26 mN/m were used to determine suitability of calibration. The isotherm of stearic acid is also divided into following three regions as determined by molecular area. Gaseous state where molecular area > 30 $Å^2$ and distance between molecules is large, liquid state where molecular area 20-30 $Å^2$ and molecules start to interact and solid state where molecular area <20 $Å^2$ and molecules are packed together. Maximum surface pressure before monolayer collapse was 52.171, 51.649, 52.011 and 52.788 for A, C, D (replicates using the same stock solution of stearic acid in chloroform) and the training experiment respectively.

Sodium caseinate: The results of (π-a) isotherm spreading experiments using sodium caseinate (1mg/mL in DD water) deposited at 10, 20 and 50 μL to obtain different surface concentrations of 0.01, 0.02, 0.03 and 0.07 mg on a subphase of 12 mM sodium chloride and phosphate buffer are shown in Figure 10. Only compression curves where performed and the average of three for each concentration are shown. Barrier speed for compression of 33 mm/min was selected as it was seen to be the highest value for reproducible isotherms as reported previously (Patino and Fernández 2004; Rodríguez Patino et al. 2007). Sodium caseinate showed a liquid expanded like structure with a slight region of coexistence between liquid-expanded and liquid condensed. The surface pressure before compression varied with protein mass at the surface and increased as the surface area was continuously reduced. At maximum compression the surface pressure values varied from 15.517- 20.847 mN/m with 0.07 mg of sodium caseinate being

significantly different from 0.01 mg sodium caseinate, however, not different from the other two concentrations (Table 6).

Sodium caseinate and Carvacrol: (π-a) isotherms of sodium caseinate and carvacrol are shown in Figure 11. Sodium caseinate to carvacrol surface concentration ratios of 2:1 and 1:1 were studied by spreading different volumes (50, 100 or 200 μL) of sodium caseinate solution (0.2 mg/mL in DD water) and carvacrol solution (0.1 mg/mL in acetone) on a clean air/water sub-phase. Sodium caseinate showed liquid expanded like structure with carvacrol showing liquid expanded and the commencement of a region of liquid expanded-liquid condensed.

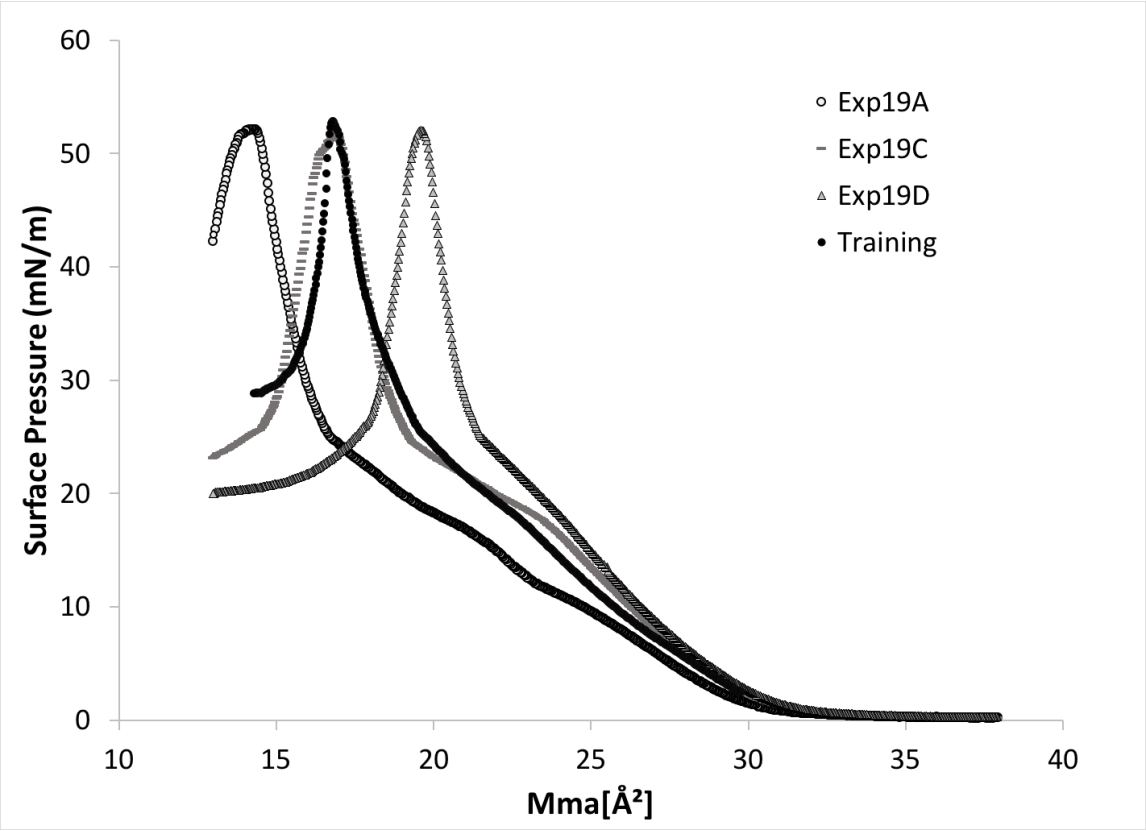

Figure 9 Stearic acid π-a isotherm for calibration. Training refers to calibration performed during instrument setup. 19 A, C, D are replicates using the same stock solution of stearic acid in chloroform

Table 6 Sodium caseinate π-a isotherms: Initial and maximum surface pressure values at different sodium caseinate surface mass values

Sodium caseinate mass (mg)	Initial surface pressure (mN/m)	Maximum surface pressure (mN/m)
0.01	2.20 ± 0.44^{A}	17.64 ± 1.83^{A}
0.02	4.92 ± 0.24^{B}	$20.01 \pm 0.65^{A,B}$
0.03	7.05 ± 0.80^{C}	$20.62 \pm 0.32^{A,B}$
0.07	11.32 ± 0.17^{D}	20.63 ± 0.24^{B}

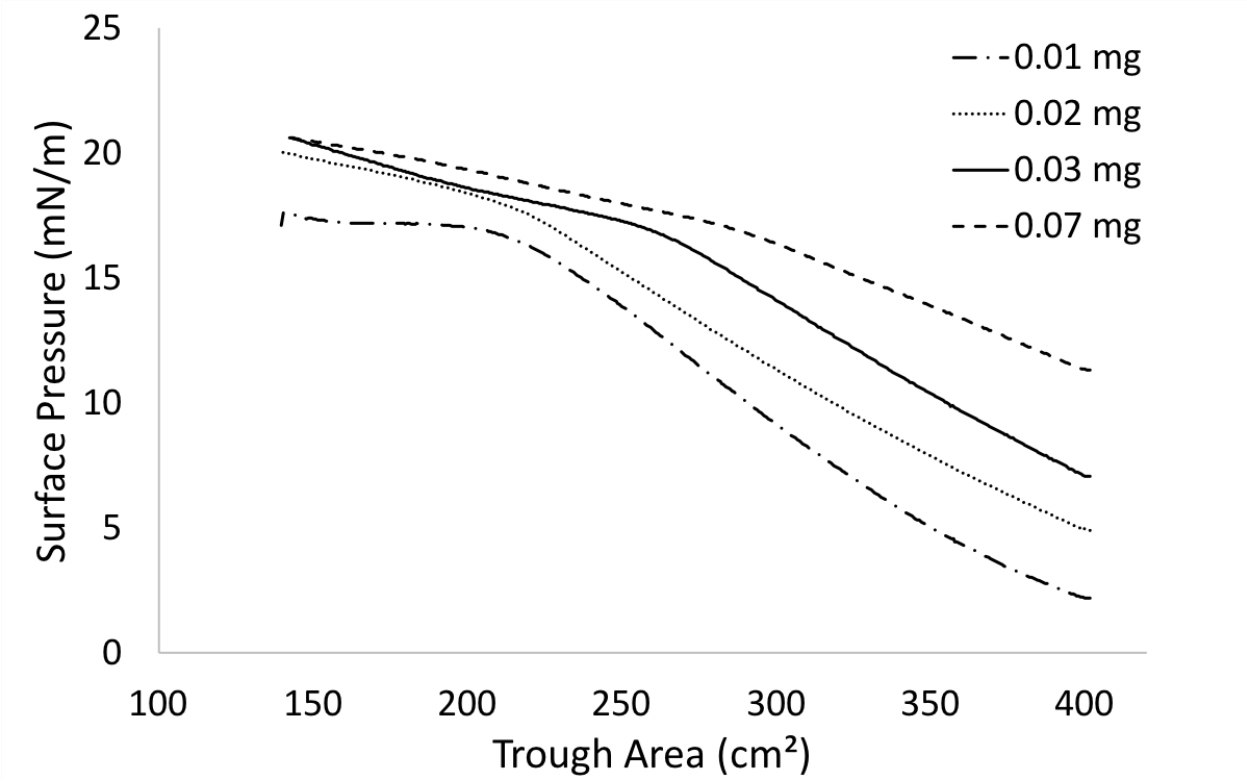

Figure 10 Sodium caseinate π-a isotherm at 0.01, 0.02, 0.03 and 0.07 mg surface mass values. Measurements were performed in triplicate, average data shown

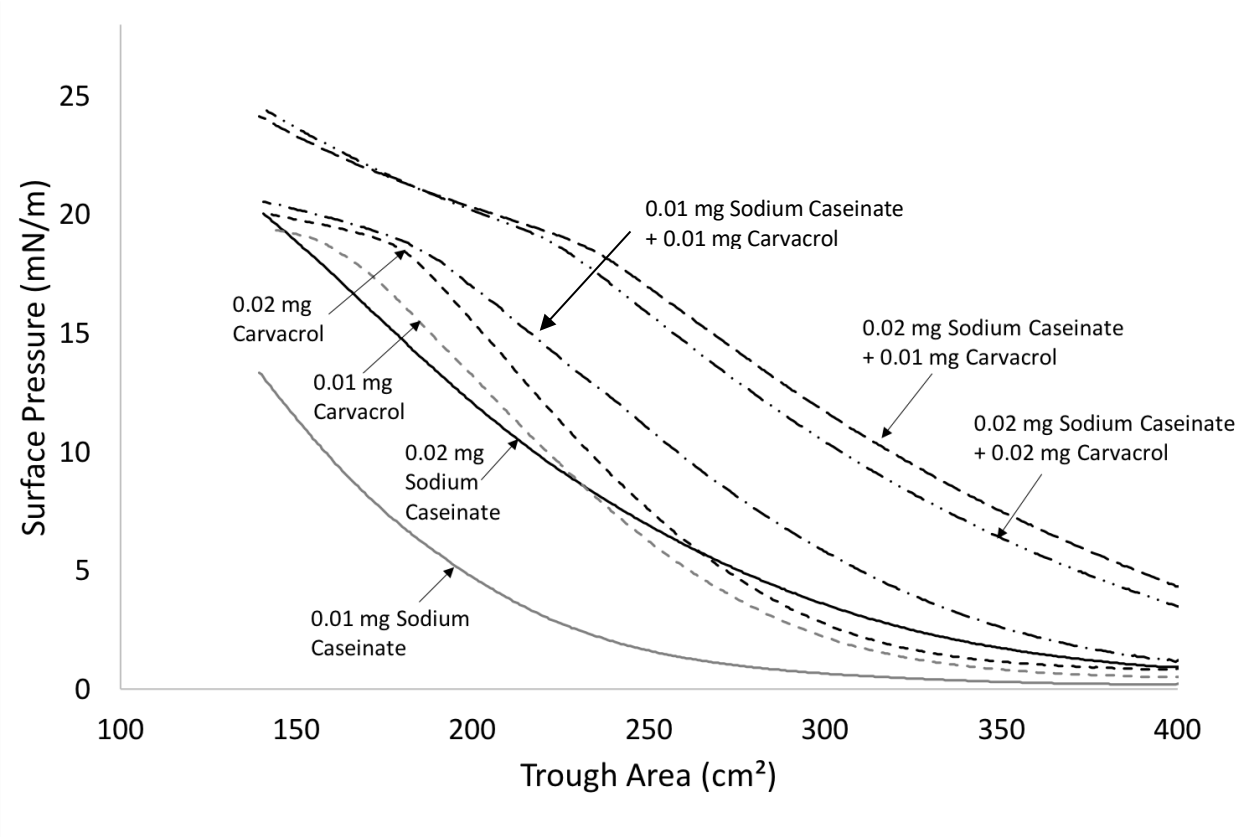

Figure 11 Sodium caseinate and carvacrol π-a isotherm with 2:1 and 1:1 ratio of protein to carvacrol. Measurements were performed in triplicate, average data shown.

At maximum compression the surface pressure values for sodium caseinate were significantly higher with increase in protein mass. The maximum surface pressure for carvacrol did not significantly differ between the two concentrations tested or from that of sodium caseinate at 0.02 mg. At 0.02 mg of sodium caseinate, combinations of sodium caseinate and carvacrol were significantly higher than all other treatments but did not significantly differ due to different masses of carvacrol. Initial surface pressure values for combinations of protein and carvacrol were also significantly higher as compared to that for protein or carvacrol alone (Table 7).

β-lactoglobulin and Sodium Caseinate: (π-a) isotherms of sodium caseinate and β-lactoglobulin are shown in Figure 12. The same volume (100 μL) of sodium caseinate and β-lactoglobulin solutions (0.2 mg/mL) were spread on a clean DD water subphase. Both proteins

demonstrated liquid expanded like structure with β-lactoglobulin showing a higher maximum surface pressure (18.19 ± 0.51 mN/m) as compared to sodium caseinate (14.12 ± 1.2 mN/m).

Table 7 Sodium caseinate and carvacrol π-a isotherms: Initial and maximum surface pressure values at different surface mass values for each substance.

Sodium caseinate mass (mg)	Carvacrol (mg)	Initial surface pressure (mN/m)	Maximum surface pressure (mN/m)
0.01	-	0.22 ± 0.03^{c}	13.46 ± 0.36^{C}
0.02	-	$0.94 \pm 0.24^{b,c}$	20.31 ± 1.13^{B}
-	0.01	$0.51 \pm 0.12^{b,c}$	19.75 ± 0.84^{B}
-	0.02	$0.86 \pm 0.05^{b,c}$	20.02 ± 0.19^{B}
0.01	0.01	1.19 ± 0.4^{b}	20.55 ± 0.68^{B}
0.02	0.01	4.32 ± 0.75^{a}	24.15 ± 1.02^{A}
0.02	0.02	3.51 ± 0.23^{a}	24.45 ± 0.14^{A}

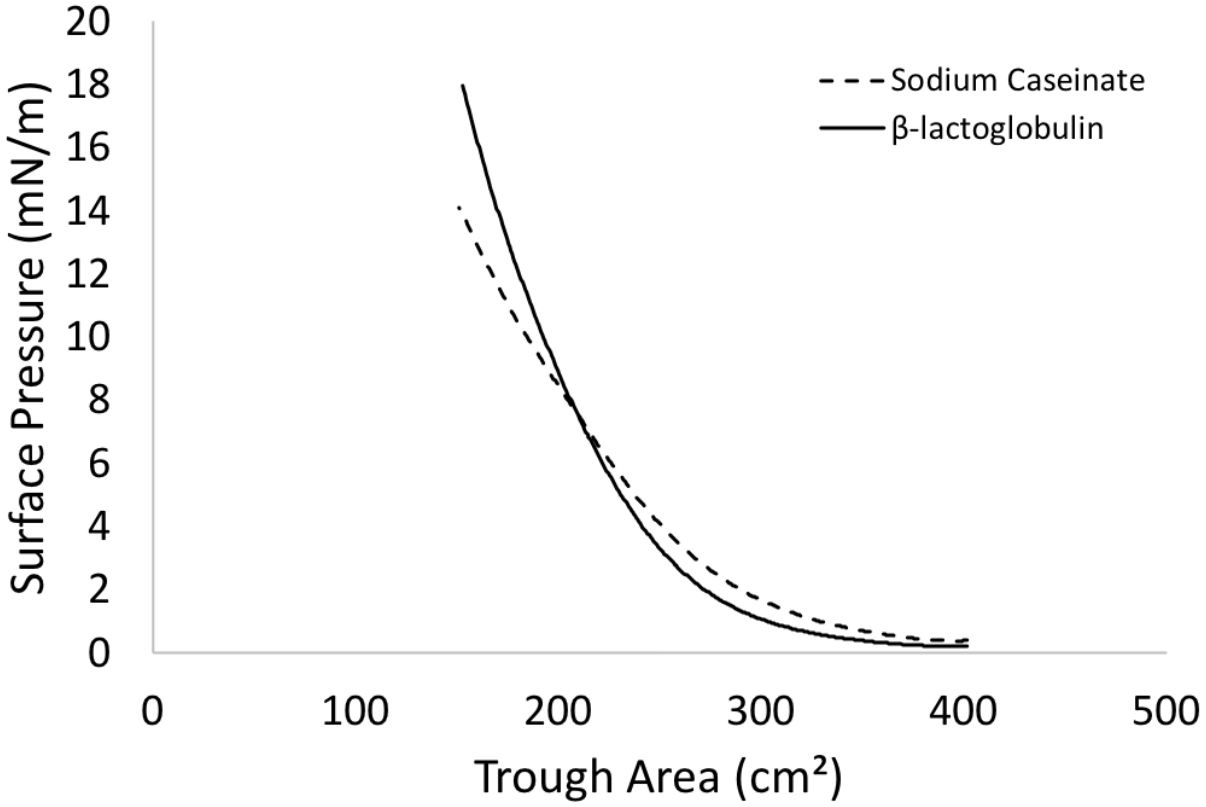

Figure 12 β–lactoglobulin and sodium caseinate π-a isotherms: comparison at 0.02 mg surface mass

Discussion: Sodium caseinate and β-lactoglobulin (π-a) isotherms in previous work also demonstrated liquid expanded like structure as observed here (Cornec and Narsimhan 2000; Fernández et al. 2007; Rodríguez Patino et al. 2007). The occurrence of liquid expanded and some coexistence between liquid expanded and liquid condensed regions on the isotherm indicate that with compression, there is first the crowding together of protein molecules and possibly some self-assembly and aggregate formation (Marichal 2014).Unlike small molecular weight surfactants protein molecules adsorption at the interface is time dependent and may occur with or without change in protein conformation (Walstra and Deroos 1993) and furthermore, upon compression the molecules may also be subject to compression induced immersion of polar groups in the sub phase (Nieto-Suárez et al. 2008). Carvacrol deposition on the interface also resulted in measurable surface pressure values that increased with barrier compression and in presence of carvacrol, sodium caseinate π-a isotherm also showed a significant shift to higher surface pressure values, thus indicating surface activity of these molecules. Carvacrol is a phenolic antimicrobial compound that has been shown to affect droplet size in emulsions and has therefore been demonstrated to exhibit a co-surfactant effect by causing an increase in surface pressure values when used in combination with sodium caseinate. Previous work with mixtures of Miglyol 812N and carvacrol at the air-water interface has shown that as carvacrol concentration increased, interfacial tension also decreased linearly which suggests it has a strong affinity for air-water and oil–water interfaces (Terjung et al. 2012). No dependence of carvacrol concentration on surface pressure in this study was observed, possibly due to partial solubilization of carvacrol in the aqueous subphase due to the method of preparation using acetone as a solvent for delivery of carvacrol to the air-water interface. The solubility of carvacrol in water has been reported as 0.83 g/liter at 25°C and this can also be increased in the presence of solvents such as ethanol (Chen et al. 2014). Other phenolic compounds such as catechin and quercetin have also demonstrated surface activity at the air/water interface. Catechin accumulation at the air/water interface resulted in an increase in surface pressure values that were found to increase with its concentration in the aqueous phase, while quercetin exhibited the opposite effect such that an increase in concentration led to decrease in surface pressure (Di Mattia et al. 2010).

Two methods of preparing materials for analysis at the air/water interface have been outlined previously: spreading and adsorption/migration from solution (Cejudo Fernández et al. 2007; Rodríguez Patino et al. 2007). For further experiments to measure interfacial rheological

properties, the migration/adsorption from solution method was used to study proteins at the air/water interface. This is primarily because establishing the position of the magnetic needle probe as well as using the spreading method of deposition was impractical and could compromise the integrity of the interface, thus affecting reproducibility.

2.3.2 Interfacial Rheology

The magnetic interfacial shear rheometer is a very sensitive instrument therefore optimal protein concentrations for measurement were found to vary with the type of protein. Calibration at the air/water interface was performed prior to every measurement to verify calibration constants in the range of 2-3 x 10^{-8} N/Amp. Representative calibration experiment data performed prior to measurements with sodium caseinate is shown in Figure 13.

Sodium Caseinate: Sodium caseinate solution (1% w/v in buffer pH 7, 0.05 M NaCl, 0.05 M Na_2HPO_4) was used to prepare different bulk concentrations of 116 mg/L, 200 mg/L and 582.5 mg/L. These were analyzed for G' and G'' at the air/water interface using migration method (bulk solutions poured into the trough directly). Calibration at the air/water interface was performed (Calibration constant 2.711- 3.419x10^{-8} N/Amp) and an amplitude sweep of 0.5-4.0 V at 1 Hz with 5 pts per amplitude was used to verify the linear viscoelastic region (representative graph shown in Figure 14). A frequency sweep (0.5-2 Hz, 0.5 V, 50 microns) was used to determine G' and G'' values after 2 hours were allowed for the protein solution to rest in the trough. The effect of protein solution homogenization on G' and G'' was measured with a frequency sweep under the same conditions (Figure 15).

Figure 15 shows that the G'' values for sodium caseinate was higher than G' implying the interface was more viscous than elastic. High pressure homogenization treatment caused a significant reduction in storage modulus G' for 116 mg/L sodium caseinate solution, but there was no significant difference at 200 mg/L and 582.5 mg/L. There was also no significant difference between G' values at each frequency studied, for both 200 mg/L and 582 mg/L and both before and after high pressure homogenization treatment. G'' (loss modulus) increased with an increase in frequency for 116 mg/L and was significantly reduced by high pressure homogenization treatment at all frequencies studied. At 200 mg/L there was no significant difference between

control and homogenized solutions at each respective frequency. At 582 mg/L, a significant reduction in G'' values were observed at 2Hz, 1.59Hz and 1.26 Hz but there was no significant difference between values at other frequencies.

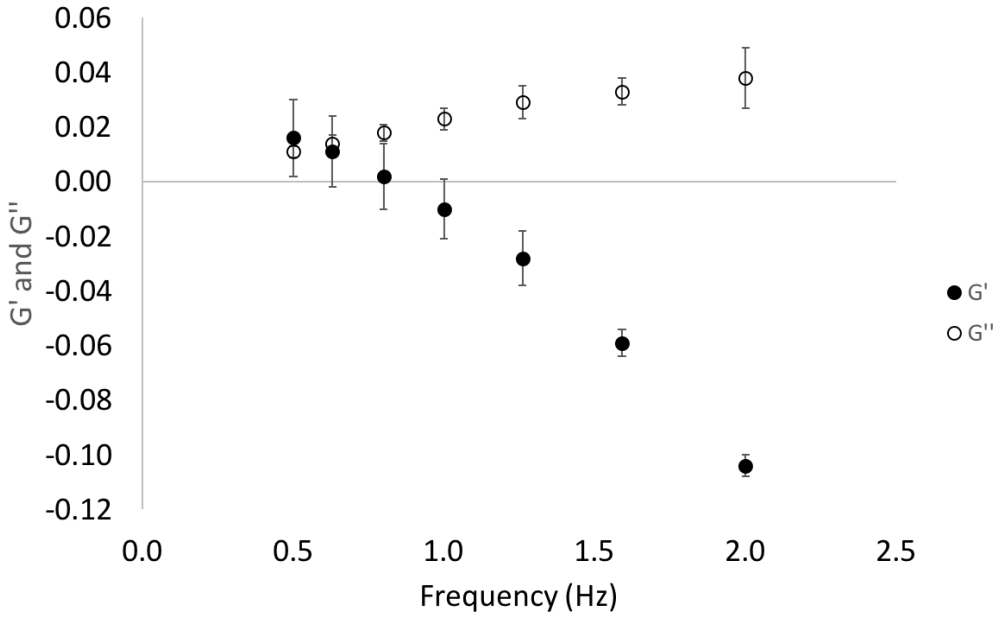

Figure 13 Magnetic ISR Calibration at air/water interface for G' and G'' (mN/m) with a frequency sweep (0.5-2.0 Hz)

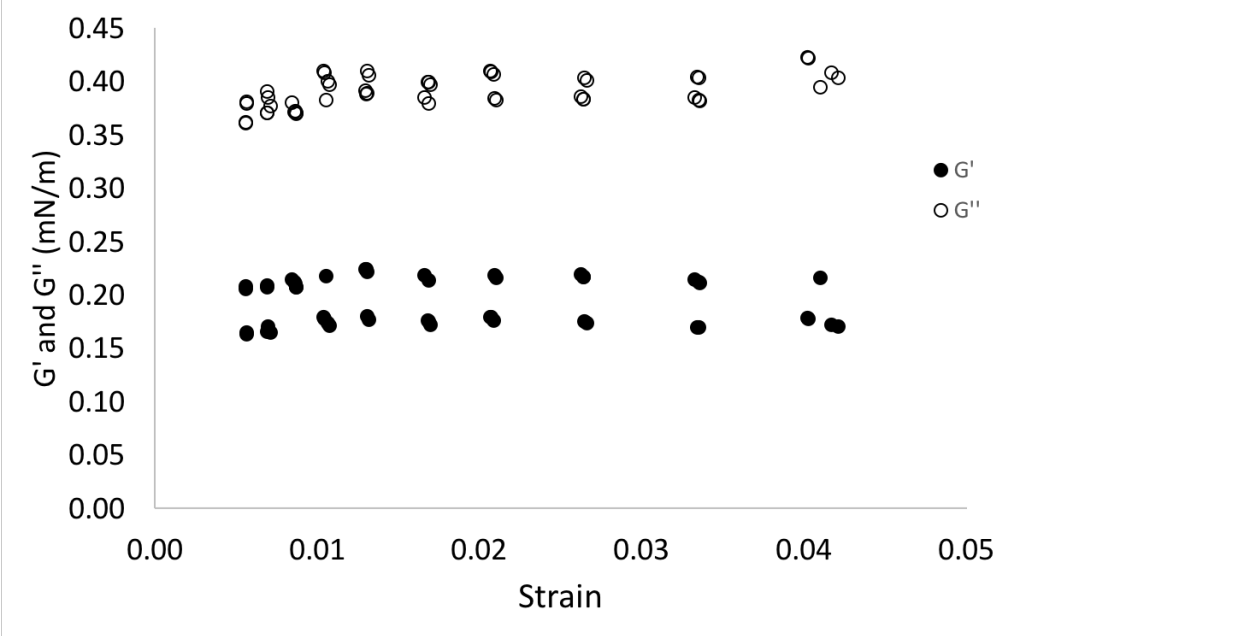

Figure 14 Example amplitude sweep at air/water interface for magnetic ISR to determine LVER of a sodium caseinate solution (200 mg/L)

β-lactoglobulin: Measurement of different concentrations of β-lactoglobulin were attempted (0.9 g/L, 0.45 g/L, 10 mg/L) however after allowing for protein unfolding and migration to the interface, these concentrations were too high for measurement by the ISR. On the other hand, 1 mg/L (0.001 mg/mL) did not show any difference from air/water calibration frequency sweep values and therefore was too low for measurement (as seen in Figure 16). Accordingly, 2 mg/L (0.002 mg/mL) β-lactoglobulin solution was measured for comparison with the sodium caseinate 200 mg/L (0.2 mg/mL). β-lactoglobulin solution (1% w/v in buffer pH 7, 0.05 M NaCl, 0.05 M Na_2HPO_4) was prepared and subjected to high pressure homogenization treatment (20 kpsi or 137.85 MPa, 3 passes). It was subsequently diluted to 2 mg/L and measurements were performed via protein migration/adsorption method (bulk solution poured into trough) with a 2 hour wait time (Figure 17). Amplitude sweep (0.5-4V at 1 Hz with 5 pts per amplitude) was used to verify the linear viscoelastic region and a frequency sweep (0.5-2 Hz, 0.5 V, 50 microns) was used to determine G' and G''. G'' was higher than G' for β-lactoglobulin implying the interface was more viscous than elastic. Storage modulus G' was significantly reduced at 1Hz with homogenization treatment, but did not vary at the other frequencies studied, while G'' was significantly reduced at all frequencies studied.

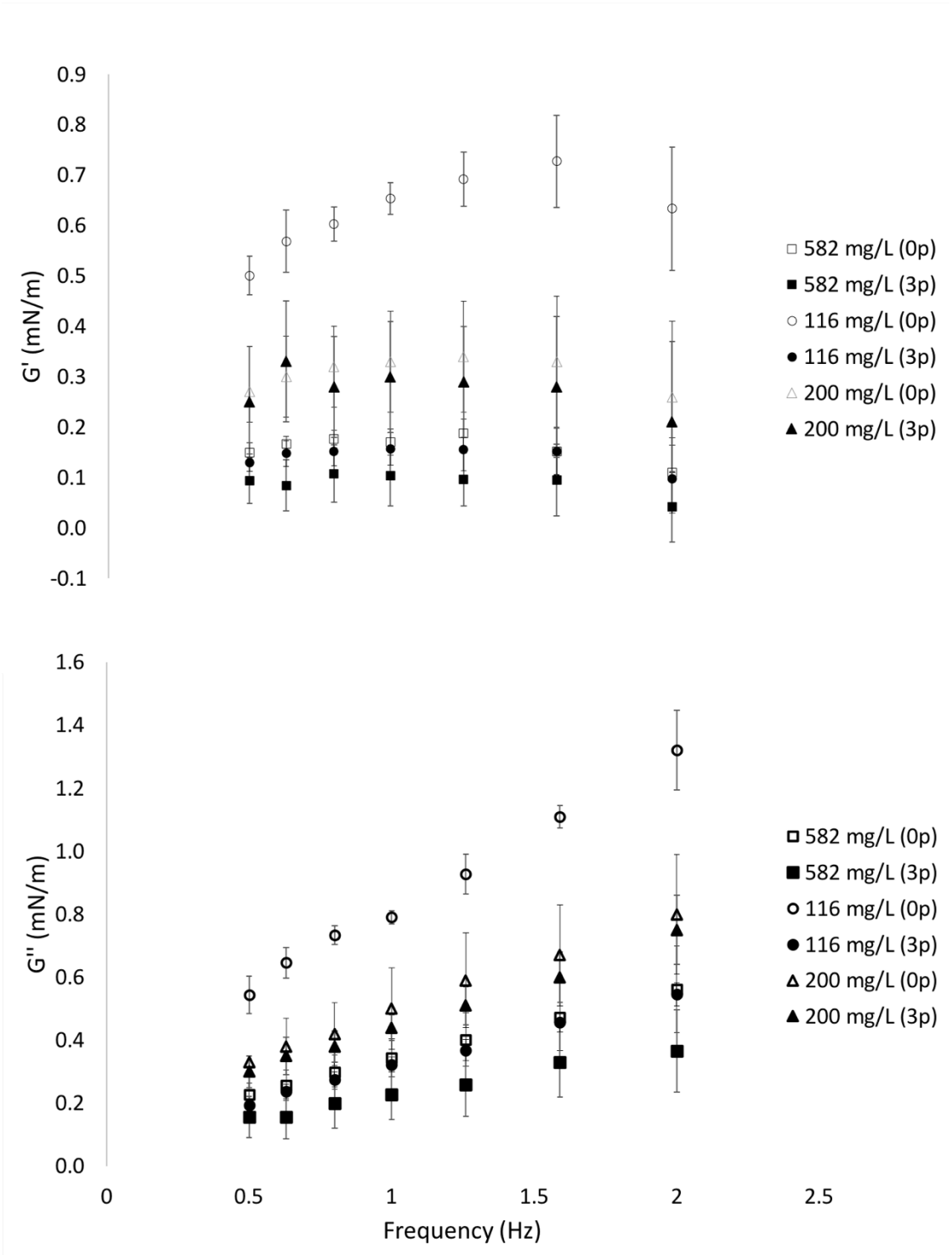

Figure 15 Effect of high-pressure homogenization (20 kpsi, 3passes) on interfacial storage modulus G'(mN/m) and interfacial loss modulus G'' (mN/m) of sodium caseinate at 116, 200, 582 mg/L (pH 7, 0.05 M NaCl, 0.05 M Na_2HPO_4). Measurements performed in triplicate, average values with standard deviation are shown. Empty symbols denote 0passes (control), filled symbols denote 3passes.

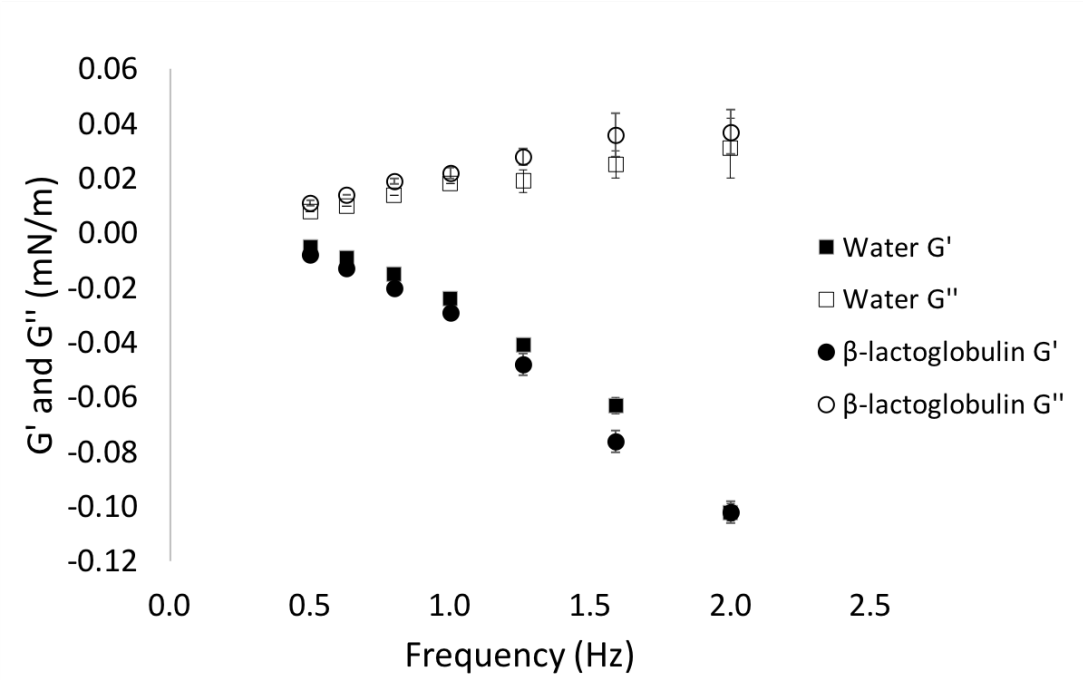

Figure 16 Comparison of β-lactoglobulin (1 mg/L, pH 7, 0.05 M NaCl, 0.05 M Na_2HPO_4) and water determinations for calibration frequency sweep. No difference in rheological values G' and G'' (mN/m) observed, indicating protein solution is too dilute for measurement

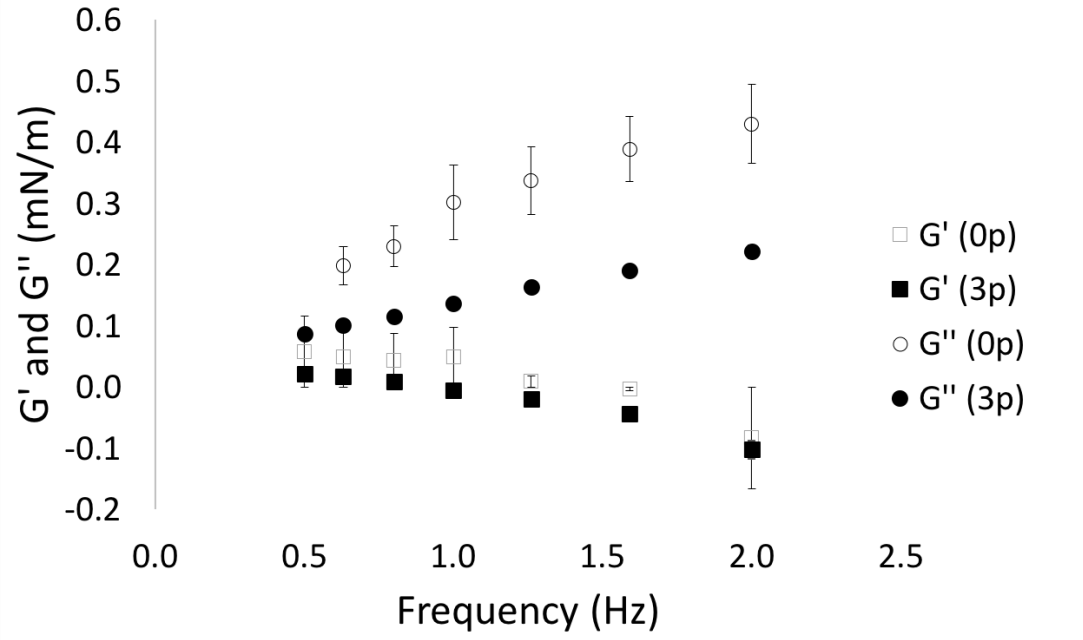

Figure 17 Effect of high- pressure homogenization (0passes or 3 passes) on β-lactoglobulin (2 mg/L, pH 7, 0.05 M NaCl, 0.05 M Na_2HPO_4) interfacial storage modulus G' (mN/m) and interfacial loss modulus G'' (mN/m) at the air/water interface. Measurements performed in triplicate, average values with standard deviation are shown. Square symbols denote storage modulus G' and circles denote loss modulus G''

Discussion: In comparison to sodium caseinate, the interfacial shear rheological properties of very low concentrations of β-lactoglobulin (100 times) were measured due to the limitations of the ISR. β-lactoglobulin is a globular protein while sodium caseinate has an unordered flexible structure. These differences in protein structure, could explain the large differences in interfacial rheological behavior at the air/water interface, with the globular protein possibly creating a more viscous interface. Adsorption of proteins at the air/water interface can cause change in their conformation and this is dependent on protein concentration, temperature, pH, nature of the protein. The proteins can remain in their native structure or unfold to expose sections of the peptide chain and allow for lateral interactions that can create a network structure resulting in measurable surface shear elasticity and viscosity. β-lactoglobulin is a globular protein, and is thought to form a strong cohesive network within the interface, without fully unfolding at the interface (Prins et al. 1998). Although, globular proteins have intricate secondary and tertiary structures at hydrophobic surfaces the hydrophobic groups become adsorbed, thus implying that protein conformation is changed (Walstra and Deroos 1993).

High pressure homogenization conditions in this study only had a significant effect on sodium caseinate rheological properties at one concentration (116 mg/L) while the other concentrations didn't show significant reductions in values. It is possible that homogenization resulted in aggregation of sodium caseinate, resulting in less surface hydrophobicity and the effect was more pronounced in the case of the more dilute protein solution. Previous work has also shown that high pressure homogenization of sodium caseinate has resulted in the formation of dimensionally larger aggregates possibly due to cross-linking in its random coil structure, and this in turn has shown increased values for sedimentation coefficients (Phoon et al. 2014). For β-lactoglobulin the loss modulus G'' was reduced after high pressure treatment, while G' only showed a significant reduction at 1Hz, suggesting the surface viscosity was reduced with negligible change in surface elasticity, and that therefore it is likely that foam formation capabilities of treated proteins was reduced. Previous work involving β-lactoglobulin showed that under static high pressure treatment the protein had reduced emulsifying capacity (Galazka et al. 1996). However dynamic pressure treatment of whey protein isolate (70% b-lactoglobulin, 24% a-lactalbumin, 4% bovine serum albumin, 2% casein, 10% lactose and 5% mineral matter) with an ultra-high pressure homogenizer resulted in improved elasticity, foam forming capacity and increased surface hydrophobicity of the protein which was attributed to a decrease in size of protein

aggregates resulting in exposure of buried hydrophobic groups (Bouaouina et al. 2006). The effect of high hydrostatic pressure on β-lactoglobulin solutions was also found to vary with pressure, such that above 300 MPa b-lactoglobulin solutions exhibited an increase in viscosity due to structural changes such as unfolding and aggregation (Marjanović and Jovanović 2011). In comparison to hydrostatic high pressure treatments, however, the duration of dynamic high pressure treatments is shorter and simultaneous effects such as shear stress, turbulence, cavitation and temperature rise occur, therefore it is difficult to compare the influence of hydrostatic and high pressure homogenization on protein conformation at the interface (Bader et al. 2011).

2.4 Limitations and Future Work

The objectives of this project were to characterize interfacial rheological properties of sodium caseinate and β-lactoglobulin at the air/water and oil/water interface as well as to determine the effect of high-pressure homogenization and presence of carvacrol on these properties using the magnetic interfacial shear rheometer or ISR.

Preliminary experiments with oil/water interface proved to be practically challenging. Attempts to create an oil/water interface with protein solution in the sub-phase and oil phase (medium chain triglycerides) being poured on top proved futile. Confirmation that the magnetic needle/probe is situated at the oil/water interface and not the air/oil interface without a side viewfinder was not possible and confining the probe to the measuring area while setting up the interface for measurement proved very challenging. Initial measurements also showed no difference between water/MCT and protein solution/MCT G' and G'' values. The KSV NIMA company was contacted for assistance and the communication received was as follows: "*You NEED to pour the oil on top of the sub-phase with the needle in place. This has to be done in this order. First fill the trough with clean water – just high enough so there is a hemisphere in the channel. Turn on the ISR and place the needle at air/water interface. Make sure the fields are on to help keep the needle in place. Pour oil on top of needle – you may need a large amount to fully cover the needle and make sure it is resting at the interface. This is critical – if it is not the results will be meaningless.*"

This method was attempted, however while pouring the oil on top of the sub-phase with needle in place, the oil would not distribute rapidly and equally, the needle would float out of the measuring area or drop to the sub-phase. As a result, further experiments to complete the objectives

of this study did not proceed and the final status of the project showed the effect of high-pressure homogenization on G' and G'' for sodium caseinate at three different concentrations, for β-lactoglobulin at one concentration as well as pressure-area isotherms for different surface concentrations of sodium caseinate and carvacrol.

Future suggested work for this project is additional interfacial rheological measurements with different concentrations of β-lactoglobulin, studying the rheological properties of sodium caseinate and carvacrol at the air/water interface as well as more protein characterization experiments before and after high pressure homogenization treatment (particle size distribution, surface hydrophobicity, size exclusion high performance liquid chromatography and dilatational measurements).

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CHAPTER 3. DEVELOPMENT OF PROTEIN BASED WATER SOLUBLE β-CAROTENE POWDER

3.1 Introduction

Food color additives include dyes, pigments and other substances incorporated in food products to provide an aesthetic, appetizing and informative quality (Harp and Barrows 2015). This is important because food color is an important sensory character that influences flavor perception, food acceptability, choice and preference (Clydesdale 1993).

In general there are several reasons to add colors to food, such as, **reinforcement**: to intensify the colors already present in food to match consumer expectation (e.g. sauces, soft drinks), **uniformity**: avoid batch to batch color variation of food, **restoration**: counteract the effects of processing which may have resulted in color loss, **addition**: provide colorless products such as sugar confectionery, ice lollies soft drinks, gelatin-based jelly with a more attractive appearance, **protection**: to assist in protection of flavor and light sensitive vitamins during storage and **quality**: provide visual indication of quality (Henry 1996; Hutchings 1999). Food colorants are classified as synthetic, nature-identical or natural. Synthetic colors are not found in nature and are prepared by chemical synthesis e.g. sunset yellow, tartrazine. Nature-identical colors are manufactured chemically identical to natural colorants occurring in nature e.g β-carotene, canthaxanthin while natural colors are derived from natural edible sources e.g. curcumin from turmeric, bixin from annatto seeds, anthocyanins from red fruits, chlorophyll, beetroot red (Henry 1996). More recently, the trend towards natural and nature-identical colors has been rising steadily. Several reporting agencies have noticed the projected rise of the food colors market, with natural colorants playing a major role. According to a Research and Markets report (2016), the US Food Colorants market is projected reach $2.5 billion by 2020. Increasing awareness on purported ill effects of synthetic colors has led to a greater global demand for natural colors, which are more costly than synthetic ones. The “food safety norms and increased production of natural colors in North America, are ultimately driving the market for food colors”. In 2013, Hansen, the world’s largest producer of natural food colorants, noted that the natural color segment accounted for 25% of total sales revenues for the year to end August 2012. They also reported that the sale of natural food colorants increased by 3.4% (Research and Markets 2016).

Carotenoids are a group of over six hundred fat soluble red, orange and yellow compounds found naturally occurring in plants, algae and many microorganisms. They are responsible for the red color of tomatoes, the orange color of carrots, as well as the red to yellow color in the feathers, flesh and exoskeletons of certain animals, with only a few of these compounds being found in human blood and tissue, and only two in the retina and lens of the eye (Simpson 1983; Krinsky and Johnson 2005; Boon et al. 2010). β-carotene ($C_{40}H_{56}$) is the most widely studied carotenoid having the highest provitamin A activity (100%) due to its symmetrical structure, which on oxidative cleavage along the 15,15' carbon-carbon bond yields two molecules of all-trans-retinal. It is therefore an important vitamin A source for humans. In addition, carotenes are considered antioxidants and several studies have demonstrated that they may be an important factor in reducing the incidence of cancer and several degenerative diseases (Ben-Amotz and Levy 1996; Del Campo et al. 2007; Tanaka et al. 2012; Solymosi et al. 2015; Sharoni et al. 2016).

The high degree of unsaturation inherent in its chemical structure make carotenoids such as β-carotene susceptible to degradation processes such as thermal and chemical oxidation, isomerization and photosensitization upon exposure to oxygen, light and high temperature (Ferreira and Rodriguez-Amaya 2008). In addition, the molecule is lipophilic or insoluble in water and marginally oil-soluble at room temperature (Mattea et al. 2009; Liang et al. 2013). Methods to overcome the challenges associated with solubility, chemical stability in foods and bioavailability of β–carotene upon ingestion include encapsulation of β-carotene in different delivery systems such as gels, emulsions and powders (Mao et al. 2018b). One such approach is the emulsification-evaporation method which involves the use of a solvent to assist in dissolution of the carotenoid in the organic phase during emulsion preparation. After homogenization with the aqueous phase, the solvent is then removed by rotary evaporation. Spray drying is often used to convert liquid forms of these delivery systems into the powdered form, to reduce storage and transportation costs, to simplify handling and utilization, and to increase shelf life (Maher et al. 2014). The use of water and oil soluble antioxidants in emulsion formulations can further enhance the stability of β–carotene. In one study four antioxidants approved for use in food: ascorbic acid, EDTA, coenzyme Q10 and vitamin E acetate, were shown to have protective effects on β-carotene decay by acting as free radical quenchers (Qian et al. 2012b). β-carotene emulsions stabilized by gum arabic also showed improved thermal stability in presence of antioxidants in the general order of α-tocopherol> TBHQ> ascorbyl palmitate (Liu et al. 2015). Previous studies have also reported

the preparation of β-carotene emulsions and their conversion into powders using various drying technologies and various emulsifiers such as gum Arabic (Lim et al. 2016), almond gum (Mahfoudhi and Hamdi 2014), modified starch (Liang et al. 2013), hydrolyzed starch (Wagner and Warthesen 1995), soy protein(Deng et al. 2014), soy protein-gum Arabic complex (Nakagawa and Fujii 2015), cyclodextrin (Durante et al. 2016), sodium caseinate (Coronel-Aguilera and San-Martín Gonzalez 2015), casein (Jarunglumlert and Nakagawa 2013), have been used. Development of a simpler processing methods to prepare stable β-carotene powders with casein as an emulsifier, through spray drying and freeze drying has also been studied, however, with the use of artificial antioxidants like TBHQ and BHT (Chen et al. 2017). The use of simpler formulations and processing methods, food grade solvents, and natural antioxidants for emulsification and stabilization of β-carotene is therefore still under study. Sodium caseinate has been shown to be an effective emulsifier with reported antioxidant properties, and Duralox is a natural antioxidant consisting of a blend of tocopherols, with potential applications for β-carotene stabilization in emulsions and spray dried powders. The objectives of this study therefore, were to use sodium caseinate as a carrier material to improve solubility of β-carotene in aqueous systems using emulsification-evaporation and spray drying techniques, and to analyze the degradation of β–carotene in the powder during storage at 4, 21 and 50 °C for a period of 60 days. The effect of different levels of medium chain triglycerides and the incorporation of a natural antioxidant were also evaluated.

3.2 Materials and Methods

3.2.1 Materials

Pepsin from porcine gastric mucosa, β-carotene (>97%) and butylated hydroxytoluene were purchased from Sigma Aldrich (St Louis, MO). HPLC grade ethyl acetate, hexane and methanol were purchased from Thermofisher Scientific (Waltham, MA). Other reagents purchased include sodium phosphate dibasic, sodium phosphate monobasic anhydrous, potassium hydroxide from VWR Life Science (Radnor, PA) and ammonium acetate HPLC reagent from JT Baker (Phillipsburg, NJ). Hydrochloric acid (37%) from Sigma-Aldrich (St. Louis, MO) was used to prepare 0.1 M HCl solution using double distilled water (18 megaohm-cm). Duralox oxidation

management system Kalsec (Kalamazoo, MI), NEOBEE 1053 medium chain triglycerides (MCT) from Stepan Company (Northfield, IL) and Miprodan 30 Sodium caseinate from Arla Foods (Viby J, Denmark) were generously donated by each company. For HPLC analysis, reconstituted samples were filtered using 3 mL BD Disposable Syringes with Luer-Lok™ Tips BD (Franklin Lakes, NJ) and Acrodisc 13 mm, 0.45 um PTFE syringe filters from Pall Corporation (New York) into amber vials sealed with blue 9mm PTFE screw caps from Agilent (Santa Clara, California). Spray dried powders were stored in 2 oz straight sided amber jars with PTFE faced, PE lined caps purchased from VWR (Radnor, PA), 15 mL polypropylene centrifuge tubes from Corning (Corning, NY) and disposable culture tubes from VWR (Radnor, PA) were also used during analysis of β–carotene content.

3.2.2 Powder Preparation

Dispersion Preparation: A 0.05 M sodium phosphate dibasic and sodium phosphate monobasic buffer at pH 7 was prepared using ethyl acetate saturated water (0.8% w/w). The aqueous phase consisted of 2% w/w solution of sodium caseinate dissolved in this buffer (4 hours mixing, 12 hours hydration at 4 °C). The organic phase of MCT (1% or 10% w/w), β–carotene (0.1% w/w), ethyl acetate (98.9% or 89.9% w/w) and Duralox (0% or 0.1% w/w) was sonicated for 15 minutes in a Branson 1510 ultrasonic water bath (Branson, Danbury, CT) to aid in dissolution. A coarse emulsion was prepared by homogenization of aqueous and organic phases (8:2 by weight) at 11,000 rpm for 15 seconds with a Polytron (Kinematica AG, Switzerland) high shear mixer. The coarse emulsion was further homogenized using a high-pressure homogenizer NanoDeBee (B.E.E. International, Easton, MA) at 20 kpsi (137.85 MPa) with one pass, and emulsion from the outlet port was cooled at 4°C.

Solvent Evaporation: Ethyl acetate was removed from the emulsion by rotary evaporation under vacuum using an RE121 Rotary Evaporator and 461 Water Bath (Buchi, New Castle, USA) for 25 minutes at 50°C with rotation speed of 50-60 rpm. An aliquot of the final emulsion was used for β-carotene analysis, while the rest was stored in a glass bottle covered with aluminum foil to limit light exposure, flushed with nitrogen and stored at 4°C until spray drying the following day (<10 hours). Solvent evaporation was conducted under yellow light.

Spray Drying: The dispersion was spray dried using a Buchi (New Castle, USA) B-290 mini spray dryer with a nozzle atomizer in open mode with the outlet vented to a fume hood for residual ethyl acetate volatiles. The inlet temperature was maintained at 135°C, outlet temperature 89±1 °C, pump setting 15% and Qflow 40-50. During spray drying the cyclone, sample collector and the glass bottle containing the emulsion were covered with aluminum foil to limit light exposure. Total spray drying time was 2.25 hours for approximately 650 mL of emulsion, after which powder was collected and the total powder mass was recorded.

3.2.3 Moisture Content and Water Activity

The moisture content of the powder was determined gravimetrically after drying in a vacuum oven at 75-80 °C for 24 hours. The water activity of the powder was determined using an Aqualab Water Activity Meter (Decagon Devices, Pullman, WA). Water activity and moisture measurements were performed on the day of preparation hereby referred to as Day0.

3.2.4 Storage Stability

The powder was equally distributed (mass basis) into three amber glass vials. Each vial was flushed with nitrogen and stored at a different temperature (4, 21, 50 °C) for 60 days. Degradation of β-carotene and changes in color were measured every 2 days for the first 12 days and every 6 days until day 60. All measurements were done in triplicate and the mean and standard deviation values were reported.

3.2.5 Determination of β-carotene

Extraction: β–carotene was extracted from the spray dried powders by slight modification of method previously described (Cornacchia and Roos 2011b). Spray dried powder (0.01-0.015 g) was mixed with 0.5 mL double distilled water in 15 mL centrifuge tubes and sonicated for 10 minutes in a Branson 1510 ultrasonic water bath (Branson, Danbury, CT). It was then mixed with 1 mL of pepsin solution (10 mg/mL in 0.1 M HCl), vortexed and sonicated for 10 minutes before being nitrogen flushed and incubated in a Forma Scientifica (Marietta, OH) Model 2568 shaking water bath at 37°C for 15 minutes. This mixture was then treated with 1 mL of 50% KOH in

methanol (w/v), vortexed, flushed with nitrogen and incubated in shaking water bath (37°C, 30 minutes) again. Repeated extractions (3 mL x 8) of β-carotene from the final mixture was performed using hexane containing 0.1% BHT under yellow light, using a Pasteur pipette to collect the upper hexane phase in a disposable glass culture tube (VWR, Radnor, PA). The final hexane extract was brought to a final volume of 25 mL with hexane as the diluent.

Spectrophotometric quantification: Aliquots (1 mL) of the hexane extract were read at 450 nm absorbance using UV-Vis Spectrophotometer (Beckman Coulter DU-800, Fullerton, CA) to determine the total carotenoid content using molar extinction coefficient 139,500 M^{-1} cm^{-1} as described previously (Britton, Liaaen-Jensen, & Pfander, 2004).

HPLC: In addition to spectrophotometric quantification, to permit detection of lower concentrations, β-carotene content was determined using HPLC-DAD based on a method previously described (Kean et al. 2008). Aliquots (2 mL) of the hexane extract were dried under nitrogen, reconstituted with ethyl acetate (1mL) and methanol (1 mL), and filtered into amber HPLC vials. The HPLC (Agilent 1200 Series) was equipped with a diode array detector, a YMC Carotenoid S-3 C-30 column (2.0 x 150 mm, 3 um particle size). At a flow rate of 0.37 mL/min, a binary mobile phase of methanol with 2% aqueous ammonium acetate (pH 4.5) and ethyl acetate was used with a gradient method (0% B-0 min, 80% B-6 min, 100% B-12 min, 0% B-14 min). Sample was injected (10 μL) and β-carotene quantified at 450 nm with retention time of 8.0 minutes on the basis of peak areas analyzed with Chemstation software (Agilent Technologies, Santa Clara, CA). Calibration was performed by solubilizing a small amount of β-carotene in hexane with absorbance ~0.8 and measuring absorbance of this solution and subsequent dilutions with UV-Vis spectrophotometer. Absorbance measurements in triplicate were used for stock and diluted solutions and concentration determined using a molar extinction coefficient 139,500 M^{-1} cm^{-1}. Each dilution was dried under nitrogen and analyzed with HPLC-DAD to correlate peak area with β-carotene concentration. Calibration curve coefficient (R^2) of determination was 0.993, limit of detection (LOD) was 0.88 uM and limit of quantitation (LOQ) was 2.68 uM as determined by the standard deviation of the intercept and slope, based on Guidance for Industry: Q2B Validation of Analytical Procedures (1996).

3.2.6 Calculation of Kinetic parameters

The kinetics of β-carotene degradation in the prepared powders at 4, 21 and 50 °C storage temperatures was determined using first order kinetics (Eq. 9). C is β–carotene mass (mg) at time t (days), with C-Spec and C-HPLC representing spectrophotometric and HPLC data respectively, C_0 is initial mass (mg) and k is rate constant (day^{-1}) for the reaction at a particular temperature.

$$C = C_0\, e^{-kt} \quad \text{..... (Eq. 9)}$$

The half-life time ($t_{1/2}$) is the time required for the β-carotene content to decrease to half of its initial value (Eq. 10) where k is the reaction rate constant:

$$t_{1/2} = -\ln(0.5)\, k^{-1} \quad \text{..... (Eq. 10)}$$

Activation energy was determined from the Arrhenius equation (Eq. 11) where k is the reaction rate constant (day^{-1}), k_0 is the pre-exponential constant (day^{-1}), R is the universal gas constant (0.008314 kJ/molK), E_a is the activation energy ($kJ.mol^{-1}$), and T is the absolute temperature (K).

$$k = k_0\, e^{-Ea/RT} \quad \text{..... (Eq. 11)}$$

3.2.7 Color Stability

Color of prepared powder was measured using LabScan XE colorimeter (HunterLab, Reston, Va., USA) using Iluminant D65 and 10° observer angle. A standard calibration with white and black reference tiles was performed for each sample. Hunter Lab scale parameters of L (lightness), +a (redness) and +b (yellowness) were measured with port size 0.125 mm. Sample color was indicated by Hue angle (H°), color intensity indicated by Chroma (Ch) and total color difference by Δ E, as shown in Eq. 12, 13 and 14, where L_0, $a_0$and $b_0$are values of sample at zero time (Day0) and L, a and b are values at given incubation time.

$$H = tan^{-1}(b/a) \quad \text{..... (Eq. 12)}$$

$$Ch = \sqrt{a^2 + b^2} \quad \text{..... (Eq. 13)}$$

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad \text{..... (Eq. 14)}$$

3.2.8 Water Solubility Index

Water solubility index of powder in water was determined by modification of methods previously described by (Schuck et al. 2012; Jafari et al. 2017) such that 0.05 g of powder was mixed with 5 mL of water, sonicated for 15 minutes in an ultrasonic water bath and then centrifuged at 1560xg (3000 RPM) for 20 minutes in Eppendorf Centrifuge 5804 (Hamburg, Germany). The liquid supernatant was poured into a pre-weighed dish and dried at 105°C to a constant weight. Water solubility index was then calculated as in Eq. 15.

$$\%Water\ Solubility\ Index = \frac{Dried\ supernatant}{Initial\ sample\ weight} \times 100 \quad (Eq.\ 15)$$

3.2.9 Redispersed Powder Properties

Particle size, polydispersity and zeta potential of redispersed powder was recorded with Zetasizer Nano ZS (Malvern Instruments Inc., Malvern, UK) using a 1000x dilution of redispersed powder with double distilled water as the diluent. Briefly, 0.025 g of powder was dissolved in 1 mL of DD water for 20 minutes (A: 12.5 mg powder/ mL), 20 μL of A was then mixed in 4 mL DD water (B: 0.0625 mg/mL, and then 0.5 mL of B was mixed with 2 mL of DD water (C: 0.0125 mg/mL) and the final dilution (C) measured. Measurements were conducted after 2 minutes of equilibration at 25 °C with a backscatter detection angle of 173° and refractive indices of 1.330 and 1.447 for water and MCT respectively. Particle size was reported as $d_{3,2}$ (nm) which represents volume weighted mean and was the average of three independent measurements, each of which were the average of three measurements. Polydispersity index represents uniformity of the distribution and is calculated by particle size measurement software using PDI= $\sigma^2/2R_H^2$ where σ is standard deviation of hypothetical Gaussian distribution centered on Z average size and R_H is the hydrodynamic radius (Malvern Instruments Ltd 2016)

3.2.10 Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) profiles were obtained using differential scanning calorimeter Q2000 (TA Instrument, New Castle, DE). Powder (5 mg) was placed in hermetically sealed aluminum pan, an empty pan was used as a reference and the sample was equilibrated at 30

°C for 5 minutes then heated from 30 to 250°C at a rate of 5°C/min. Nitrogen was used as the transfer gas at 10 mL/min.

3.3 Results

3.3.1 Powder Preparation

For two levels of MCT (1% and 10%) and two levels of antioxidant (AO: 0% and 0.1%) independent powder preparations were performed in triplicate as shown in Table 8. Powder yields varied from 71% to 83% with significant difference in yield noted between 1% MCT with AO and 10% MCT with AO. Total powder mass was higher for higher lipid level (10%MCT>1%MCT). Spray drying yields obtained varied from 71-83% which agrees with other examples of similar spray dried powder preparations. In one study of a redispersible oil in water emulsion to improve bioavailability of a model dug of lipophilic 5-phenyl-1,2-dithiole-3-thione (5-PDTT) using maltodextrin as carrier and sodium caseinate as emulsifying agent, the powder yields varied from 44-74%, and the selected formulation with oil:water and maltodetrin:water ratios of 10% w/w and load of solid material of 20% (w/w) gave a yield of 68% (Dollo et al. 2003). In this study a bulking agent such as maltodextrin was not used and therefore the yield obtained in comparison to other spray dried milk systems such as spray dried camel's milk and cow's milk (68.84-88.20%t) the yields obtained were similar (Sulieman et al. 2014).

The total β-carotene content in the final dispersion prior to spray drying varied from 95.75-108.3 mg which compared to the 165 mg possible represented an approximately 40% loss in β-carotene during dispersion preparation (Table 9). A significant difference in the β-carotene mass in dispersions with 10% MCT with and without antioxidant were noted. After spray drying the Day0 β-carotene content of the powders varied from 59.14±3.69 to 65.60 ± 6.01 mg with no significant differences between them. Thus total β-carotene loss during powder preparation was approximately 60-65%. The concentration of β-carotene (0.1%) in the organic phase for development of the protein-based spray dried emulsions was determined based on maximum solubility that could be achieved in organic phase (ethyl acetate + MCT) in combination with brief sonication.

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Table 8 Spray dried β–carotene sodium caseinate powder quantities and yields. AO indicates presence of Duralox antioxidant. Same letters in each column indicate no significant difference in values for that column.

Powder	Aqueous Phase		Organic phase			Final emulsion (g)	Spray dried powder (g)	%Yield
	Sodium caseinate (g)	Buffer (g)	MCT (g)	Ethyl acetate (g)	β-carotene (g)			
1% MCT	12.82 ± 0.02	643.67 ± 4.69	2.022 ± 0.011	199.6 ± 0.53	0.2048 ± 0.0042	632.33 ± 20.21^{A,B}	11.57 ± 0.57^{A}	80.25 ± 3.47^{C,D}
1% MCT AO	12.81 ± 0.02	647.80 ± 1.64	2.042 ± 0.020	197.97 ± 0.16	0.2035 ± 0.0202	618.67 ± 7.09^{B}	11.91 ± 0.55^{A}	83.30 ± 3.86^{C}
10% MCT	12.83 ± 0.01	643.40 ± 3.16	20.23 ± 0.33	179.97 ± 0.12	0.2103 ± 0.0106	617.10 ± 6.71^{A,B}	21.67 ± 0.55^{B}	74.49 ± 2.04^{C,D}
10% MCT AO	12.83 ± 0.03	648.68 ± 10.08	20.05 ± 0.05	180.42 ± 0.44	0.2059 ± 0.0006	623.06 ± 3.39^{A}	20.71 ± 1.42^{B}	71.91 ± 5.12^{D}

The solubility of β-carotene in ethyl acetate at 20 °C is 0.627 mg/mL (0.06%), 1.153 mg/mL at 30°C (0.12%), 4.733 mg/mL at 40°C (0.5%), 12.06 mg/mL at 50°C (~1.5%), 15.18 mg/mL at 60°C (~2%) and therefore could have been increased by heating the solvent as discussed by (Paz et al. 2014). Additionally researchers have dispersed β-carotene in corn oil at 0.1% by stirring for 10 minutes at 50 °C then for 1 hour at room temperature to ensure solubilization (Yi et al. 2014) while others have used β–carotene at 0.5% w/w in corn oil by sonicating and mild heating (<50°C for 5 min) to ensure dissolution (Salvia-Trujillo et al. 2013). Increasing the temperature of organic phase in a controlled safe environment to increase solubility could however be used to improve β-carotene content in the powder and thus produce a darker colorant if β-carotene degradation during exposure to thermal energy during preparation is limited.

In general, spray drying operating conditions were adapted from several sources and determined based on best operating conditions for available Buchi Mini Spray Dryer. For instance (Jarunglumlert and Nakagawa 2013) used a similar lab scale spray dryer (Buchi B-290) to encapsulate β-carotene using casein aggregates with operating conditions of 0.7 mm diameter nozzle, Inlet drying temperature: 110 °C, Outlet temperature: 80 °C, Pump rate: 145 mL/h, Aspirator rate: 90% and Spray drying air flow rate: 350 mL/h. Another study used Niro pilot-scale

spray drier (Niro Atomizers, Ltd., Copenhagen, Denmark) with a rotary atomizer to spray dry an emulsion prepared using sodium caseinate as the emulsifier along with maltodextrin as a bulking agent at an inlet air temperature of 160 ± 2 °C, with air pressure of 0.5 MPa and feeding rate 18 mL/min (Coronel-Aguilera and San-Martín Gonzalez 2015).

Table 9 Changes in total β-carotene mass during powder preparation

Powder	Maximum β-carotene (mg)	β-carotene in Dispersion (mg)	%Loss (dispersion)	β-carotene Day0 powder (mg)	β-carotene Day0 powder (mg/g)
1% MCT	164.92 ± 4.11	105.63 ± 9.17 ^{A,B}	36.78 ± 5.49	65.6 ± 6.01	5.46 ± 1.02
1% MCT, AO	165.85 ± 0.06	97.11 ± 10.23 ^{A,B}	41.47 ± 6.17	64.21 ± 6.06	5.39 ± 0.74
10% MCT	169.32 ± 9.27	108.3 ± 7.21 ^{A}	35.15 ± 4.32	59.14 ± 3.69	2.63 ± 0.51
10% MCT, AO	165.35 ± 0.45	95.75 ± 9.53 ^{B}	41.95 ± 5.78	59.63 ± 4.89	2.88 ± 0.22

3.3.2 Moisture Content and Water Activity

Moisture content and water activity of powders was measured on Day0 (Table 10). Powder prepared with 1% MCT and no antioxidant had significantly higher moisture and water activity compared to the remaining powders. For the same level of MCT, powders with antioxidant had significantly lower water activity. Moisture content represents the water composition of the system and affects the growth and development of microorganisms in the system. The moisture contents of powders varied from 1.53 to 5.26% and decreased as the lipid level increased. The water content of the feed in the case of 10% MCT emulsions was lower and solid content was higher thus reducing the amount of water for evaporation and producing powders with reduced moisture content under equal drying conditions.

Table 10 Moisture and water activity of powders on Day0

	Moisture (%dry basis)	Water activity
1% MCT	5.26 ± 0.50^{A}	0.2609 ± 0.0036^{C}
1% MCT, AO	2.23 ± 0.26^{B}	0.1064 ± 0.0286^{D}
10% MCT	1.53 ± 0.16^{B}	0.1680 ± 0.0023^{D}
10% MCT, AO	1.79 ± 0.06^{B}	0.1511 ± 0.0036^{D}

Water activity measures the availability of free water in a food system and is defined as the "ratio of vapor pressure of water in a food system to vapor pressure of pure water at the same temperature". High water activity is usually correlated with higher rates of biochemical reactions and hence a reduction in shelf life of a spray dried powder. In general, foods with water activity<0.6 are considered microbiologically safe (Quek et al. 2007). The water activity of powders prepared in this study was 0.1064-0.2609 thus the powders are microbiologically safe and any degradation that could occur would be attributed to chemical reactions.

In comparison, other studies have shown that water activity in spray dried camel's milk and cow's milk was low varying from 0.154 to 0.208 (Sulieman et al. 2014). Similarly, a spray dried β-carotene emulsion prepared with sodium caseinate and maltodextrin and coated with hydroxyproplyl cellulose had moisture content varying from 1.69-8.53 g/100g with the lowest value observed for uncoated (no HPC) powder. Water activity of the prepared powders ranged from 0.117 for uncoated powder to 0.423 for a coated powder (Coronel-Aguilera and San-Martín Gonzalez 2015).

3.3.3 Storage Stability (β-carotene)

The spray dried powders were stored for 60 days in nitrogen flushed amber vials at 4, 21 and 50 °C. Degradation of β-carotene over time was measured by spectrophotometric and HPLC-DAD methods (Figure 18 and 19). In general, the stability of β-carotene was reduced at a higher storage temperature (4>21>50 °C) while the incorporation of antioxidant improved the stability of β-carotene. For 1% and 10% MCT at (4 and 21 °C) with and without treatment with antioxidant, for the 60 days period of the study the total β-carotene content was higher at a lower lipid level

(1%MCT> 10% MCT). This was also true at 50°C in the absence of antioxidant, however when treated with antioxidant, the β-carotene content at 10% MCT was higher compared to 1% MCT and measurable levels of β-carotene were detected for 60 days as opposed to 12 days in the 10% MCT powder without antioxidant.

For powders prepared with 1% MCT significant differences in β-carotene amounts from Day0 were noted at Day30 (4°C), Day10 (21°C) and Day2 (50°C.). With the inclusion of the antioxidant, the stability was improved such that at 4°C, no significant difference from initial content was noticed for 60 days, while at 21°C difference from initial was observed at Day24 and at 50°C a rapid initial decline still occurred at Day2, however measurable levels of β-carotene remained until the end of the study.

For powders prepared with 10% MCT significant difference from initial content was noted at Day 12 (4 °C), Day6 (21°C) and Day2 (50°C). With the addition of antioxidant (AO) at 4°C no significant decrease occurred for 60 days while at 21°C at Day24 β-carotene was significantly lower than initial. Similar to 1% MCT powders, at 50°C, a rapid decrease was seen at Day2, but measurable levels of β-carotene were observed throughout the remainder of the storage period.

3.3.4 β-carotene Degradation Kinetics

The kinetics of β-carotene degradation for powders prepared with 1% MCT and 10% MCT followed a first order reaction. Zero order and second order reaction rates did not give a good fit to the data for conditions tested. Previous work has also described the degradation of carotenoids in spray dried powders where different carbohydrates and protein have been used as the wall materials and most of the fitting models include first order kinetics or pseudo first order kinetics (Deng et al. 2014). The degradation of β-carotene due to light exposure was monitored in spray dried carrot carotenes prepared using hydrolyzed starches with various dextrose equivalents and was described by first order degradation. Rate constants at 21°C varied depending on the dextrose equivalent with 36.5DE providing the best protection giving a half-life of 431 days at 21 °C (Wagner and Warthesen 1995). Additionally, photodegradation of β-carotene in a vegetable juice system (Pesek and Warthesen 1987) followed first order kinetics when exposed to 210 ft-c (2260 lux) of light at 4°C with a rate constant of 0.309 ± 0.044 day^{-1}. Model systems have also been used

to study β–carotene degradation. The oxidative discoloration of β-carotene studied using a model system of microcrystalline cellulose and 0.5% β-carotene at 20°C, at two water activities and in the presence and absence of antioxidant BHT (butylated hydroxy toluene) reported first order kinetics for β-carotene loss. Observed rate constants varied depending on temperature ranging from 2.87 x 10^{-2} (5°C), 10.5 x 10^{-2} (20°C) and 21 x10^{-2} (35°C) (Chou and Breene 1972). Furthermore, β-carotene degradation has been shown to follow first order kinetics in various other systems as well (Chou and Breene 1972; Goldman et al. 1983; Pesek and Warthesen 1987; Achir et al. 2010; Aparicio-Ruiz et al. 2011; Mahfoudhi and Hamdi 2015; Xiao et al. 2018).

The reaction rate constants were determined by plotting natural logarithm of β-carotene mass (mg) against the storage time (days) for each temperature of storage (4, 21 and 50 °C). The plots were linear (with R^2>0.90) confirming the first order kinetics and the slope of each plot was used to determine the rate constant for that degradation reaction (Figure 20, 21, 22 and 23). For 1% MCT powders the calculated rate constants varied from (1.4± 0.3) x 10^{-3} to (334.6 ± 75) x 10^{-3} day^{-1} while for 10% MCT powders they varied from (1.0 ± 0.7) x 10^{-3} to (264.4 ± 85) x 10^{-3} day^{-1}, and were significantly affected by temperature (4<21<50 °C) which in turn affected half-life for β-carotene degradation at the three temperatures. The calculated rate constants for powders stored at 4°C were nearly zero and were further decreased in the presence of antioxidant, thus indicating a very slow decay in β-carotene under refrigerated conditions (Table 11 and Table 12).

Powders prepared with antioxidant, also followed first order kinetics, however at 50°C, two periods of first order kinetics were observed with a cutoff point being observed at Day8. Accordingly, at 50°C, the reaction rate constants and half-life from the corresponding Day0 and Day10 were calculated (representing conditions before and after the cutoff point of Day8). In general, at 50°C the rate constant decreased by approximately ten times before and after the Day8 cutoff point indicating a decrease in the rate of β-carotene decay in the presence of antioxidant even under these high temperature conditions.

In this study, for 1% MCT powders with antioxidant, the half-life of β-carotene was increased as compared to powders without antioxidant. At 4°C, this increase was approximately three times from 173 days (0.47 years or 5 1/2 months) to 510 days (1.40 years or 1 year and 5 months), while at 21°C the increase was from 31 days (1 month) to 58 days (almost two months). At 50°C a rapid decrease in β-carotene content was observed within the first 2-3 days both with

and without antioxidant, however for antioxidant treated powders a second first order degradation with a lower rate constant was observed after Day8. The rate constant for this reaction was approximately a tenth of that for the first period and thus the calculated half-life for further β-carotene degradation after Day8 was 23 days. Similarly, at 10% MCT, addition of antioxidant improved shelf life of the protein based β-carotene powder. The half-life at 4°C was increased approximately six times from 108 days (0.29 years or 3.5 months) to 662 days (1.82 years or 1 year and 10 months). At room temperature the half-life also improved from 36days (~1 month) to 83 (~2.5 months) days.

Two first order periods of degradation were also observed for powders stored at 50°C, with initial half-life of 3 days followed by 74 day period after Day8 for another 50% decrease in β-carotene mass in the presence of antioxidant. β-carotene content as determined by HPLC did not reflect the same magnitude of increase in half life, however slight increases were still noted. For 1% MCT powder, at 4°C half-life increased from 217 days (7.13 months) to 261 days (8.5 months), while at 21°C the shift was from 33 days to 44 days. At 50°C, β-carotene content was rapidly reduced by Day2, however in presence of antioxidant an additional 16 days would produce another 50% decrease. Similarly, 10% MCT powder, at 4°C the half-life increased from 103 days to 222 days while at 21°C the increase was from 33 to 55 days. At 50°C the same rapid decline in β-carotene content was noted however the first period had a half-life of 4 days in presence of antioxidant, and further decrease was calculated as occurring after an additional 74 days.

To determine activation energy (E_a) a plot of natural logarithm of rate constant against inverse of temperature was used to determine the slope which was in turn used to calculate E_a (Figure 24). Activation energy before cutoff point and after cutoff point was affected by the change in the rate constant after Day8 at 50°C.

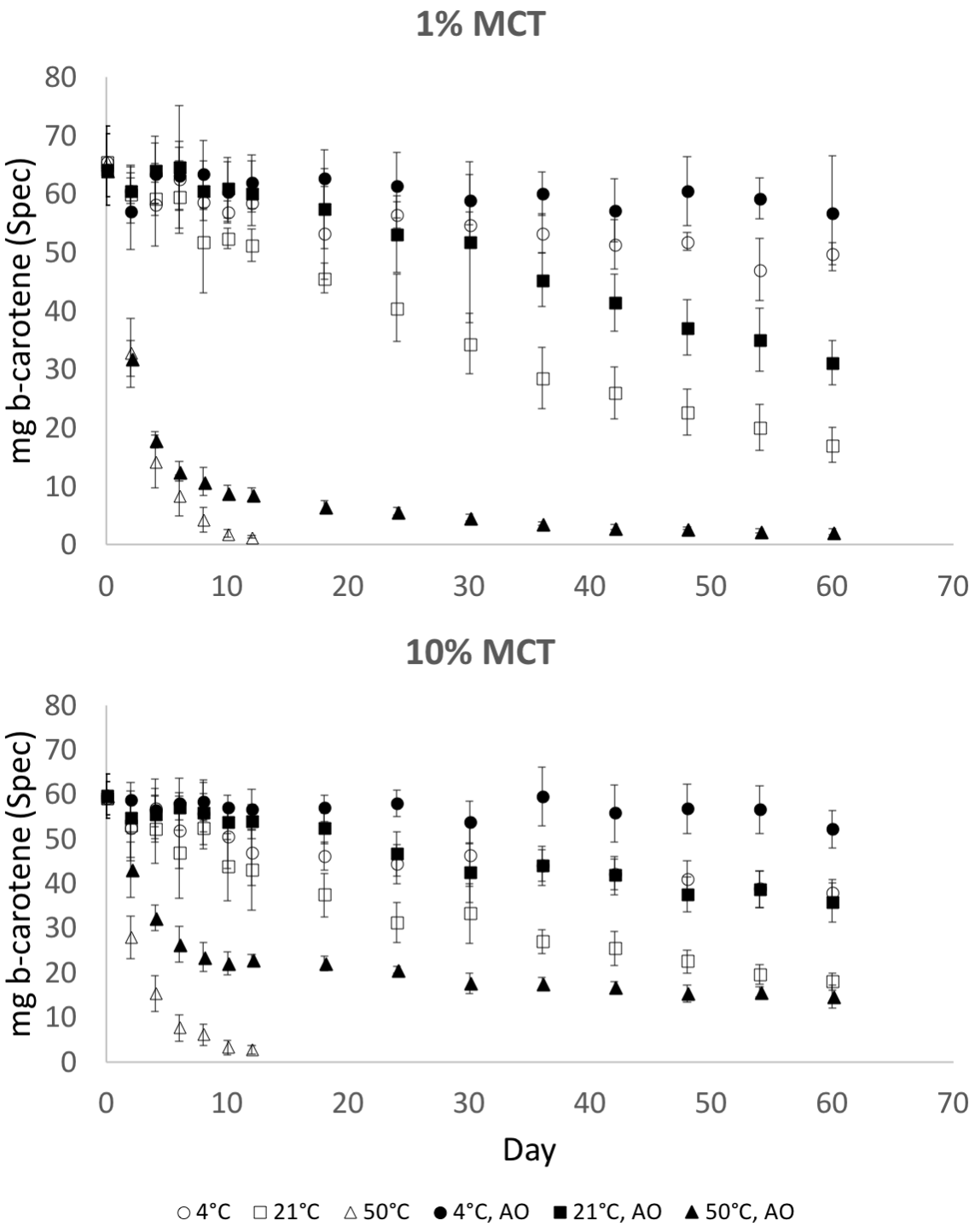

Figure 18 β-carotene degradation over 60 days for powders prepared with 1% MCT and 10% MCT stored at 4, 21 and 50° C determined by spectrophotometric measurement. Filled symbols represent treatment with antioxidant (AO).

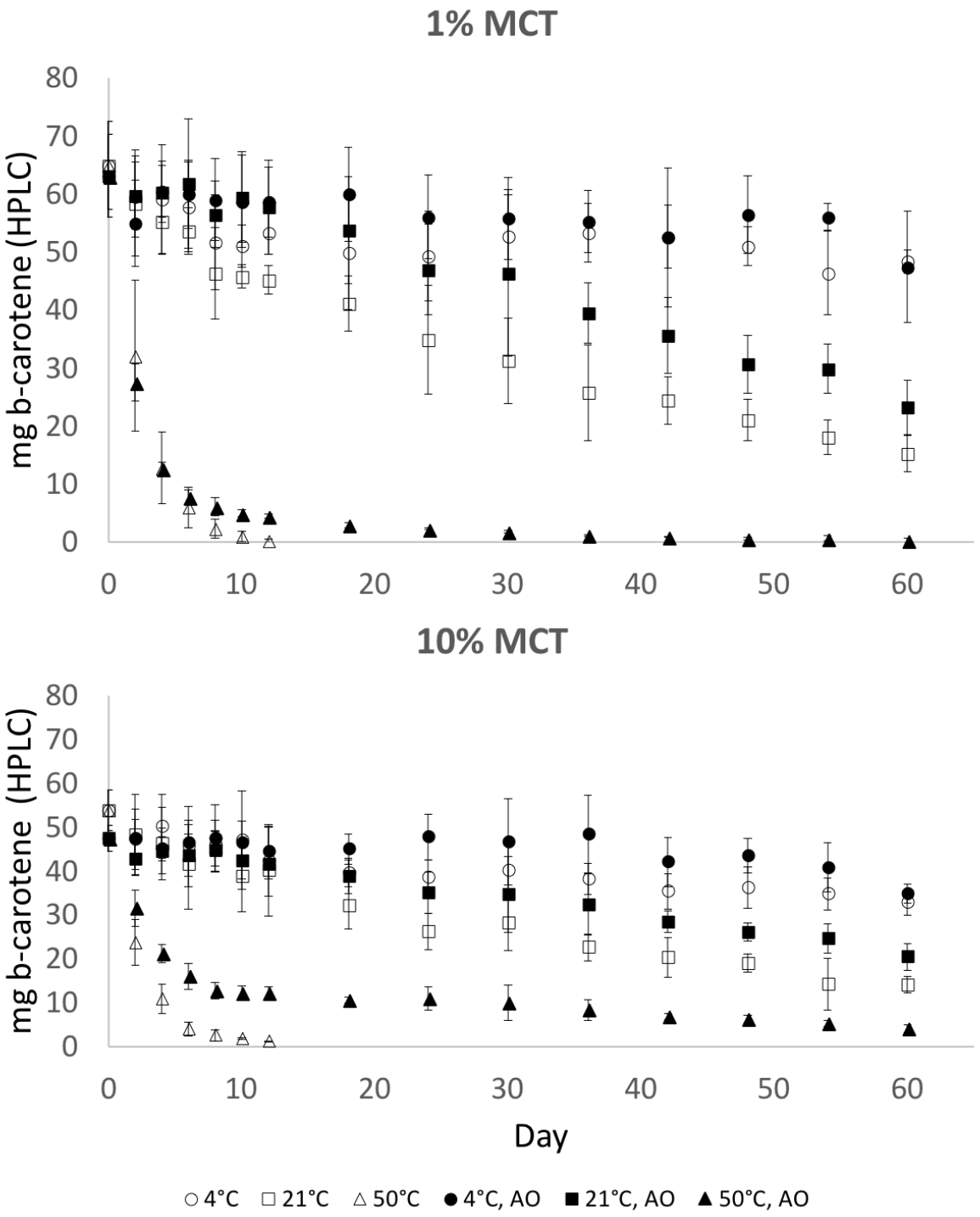

Figure 19 β-carotene degradation over 60 days for powders prepared with 1% MCT and 10% MCT stored at 4, 21 and 50°C determined via HPLC-DAD. Filled symbols represent treatment with antioxidant (AO).

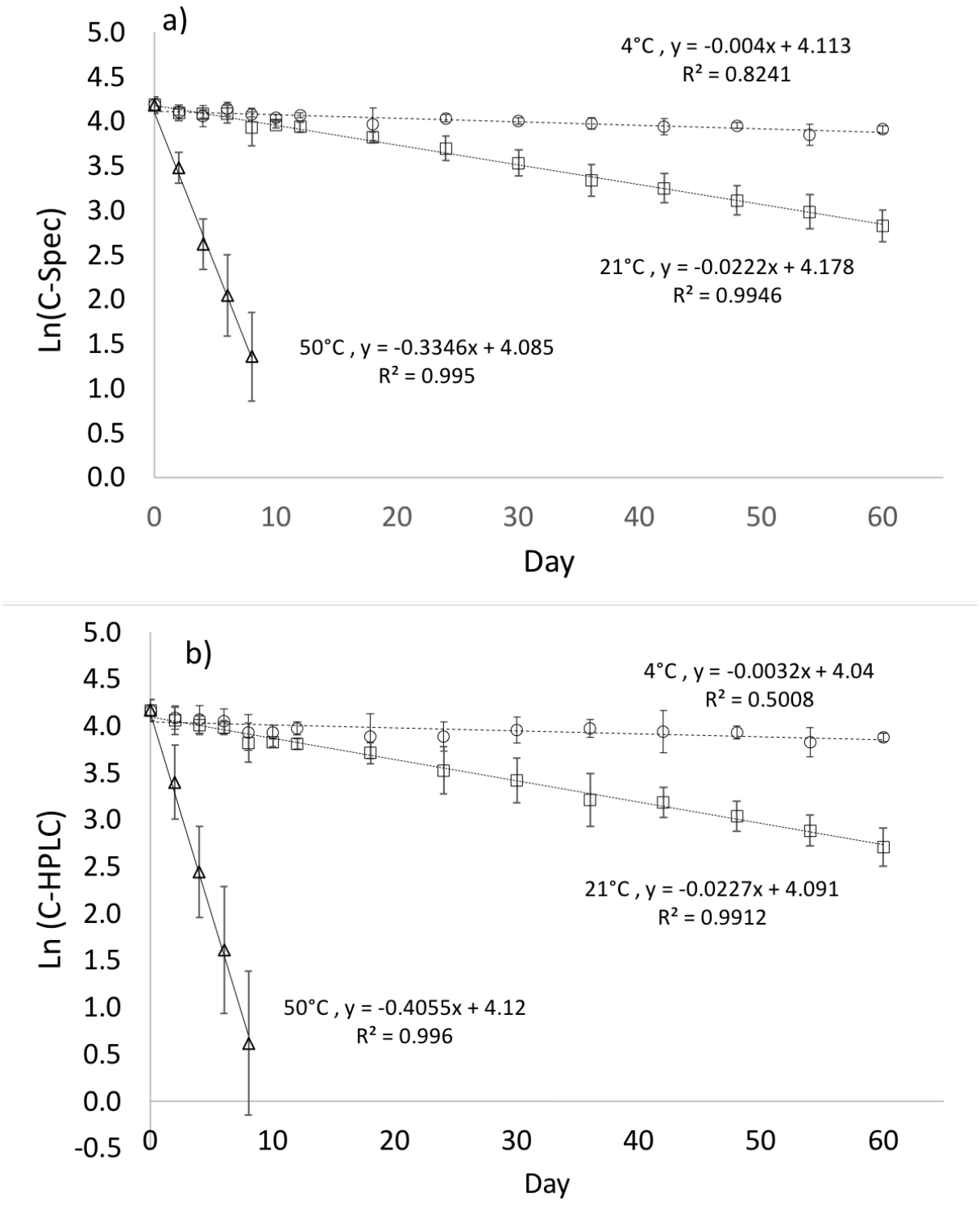

Figure 20 Determination of β-carotene degradation kinetic parameters for powders prepared with 1% MCT stored at 4, 21 and 50°C for 60 days by a) spectrophotometry b) HPLC-DAD

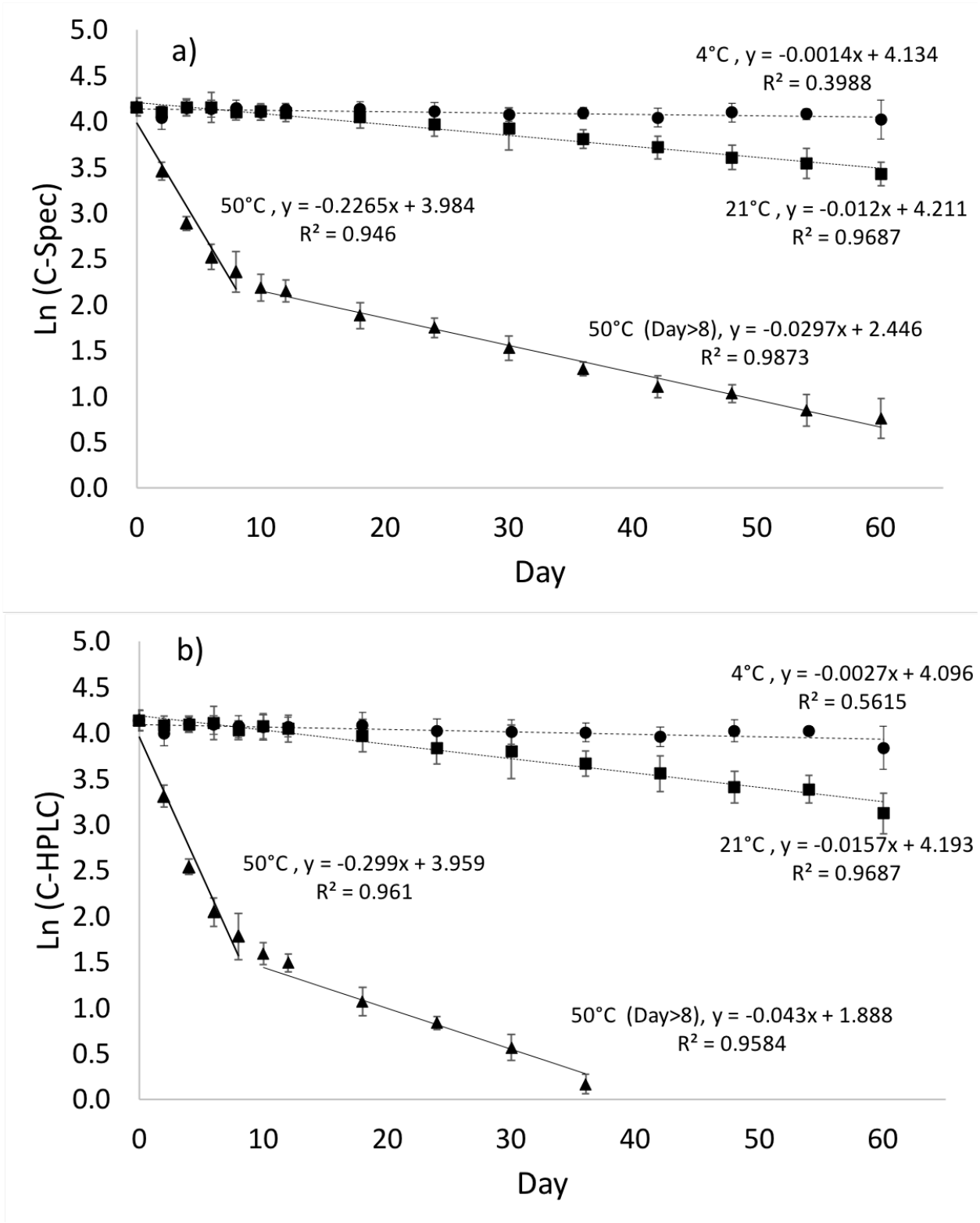

Figure 21 Determination of β-carotene degradation kinetic parameters for powders prepared with 1% MCT + antioxidant, stored at 4, 21 and 50°C for 60 days by a) spectrophotometry b)HPLC-DAD.

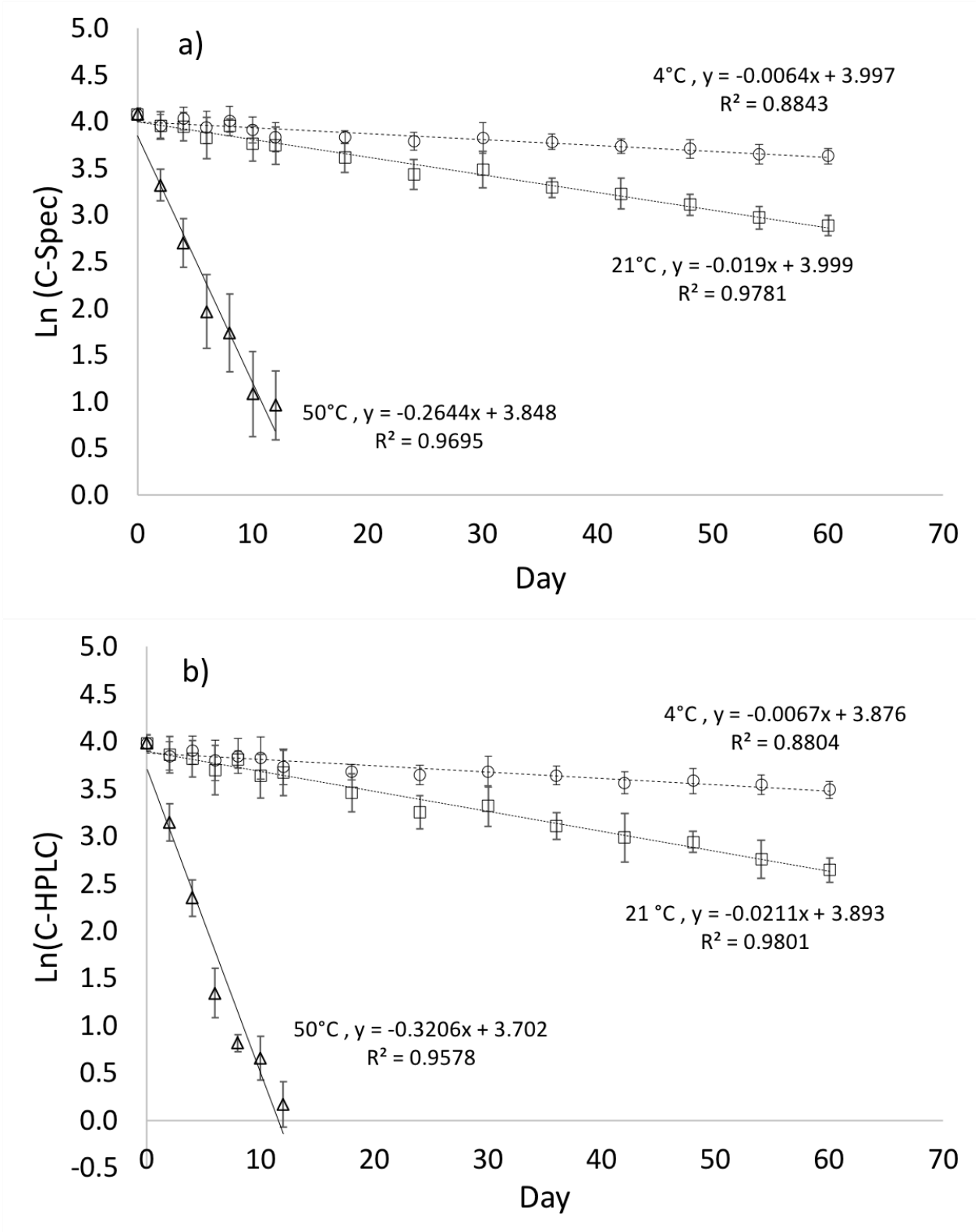

Figure 22 Determination of β-carotene degradation kinetic parameters for powders prepared with 10% MCT, stored at 4, 21 and 50°C for 60 days by a) spectrophotometry b) HPLC-DAD

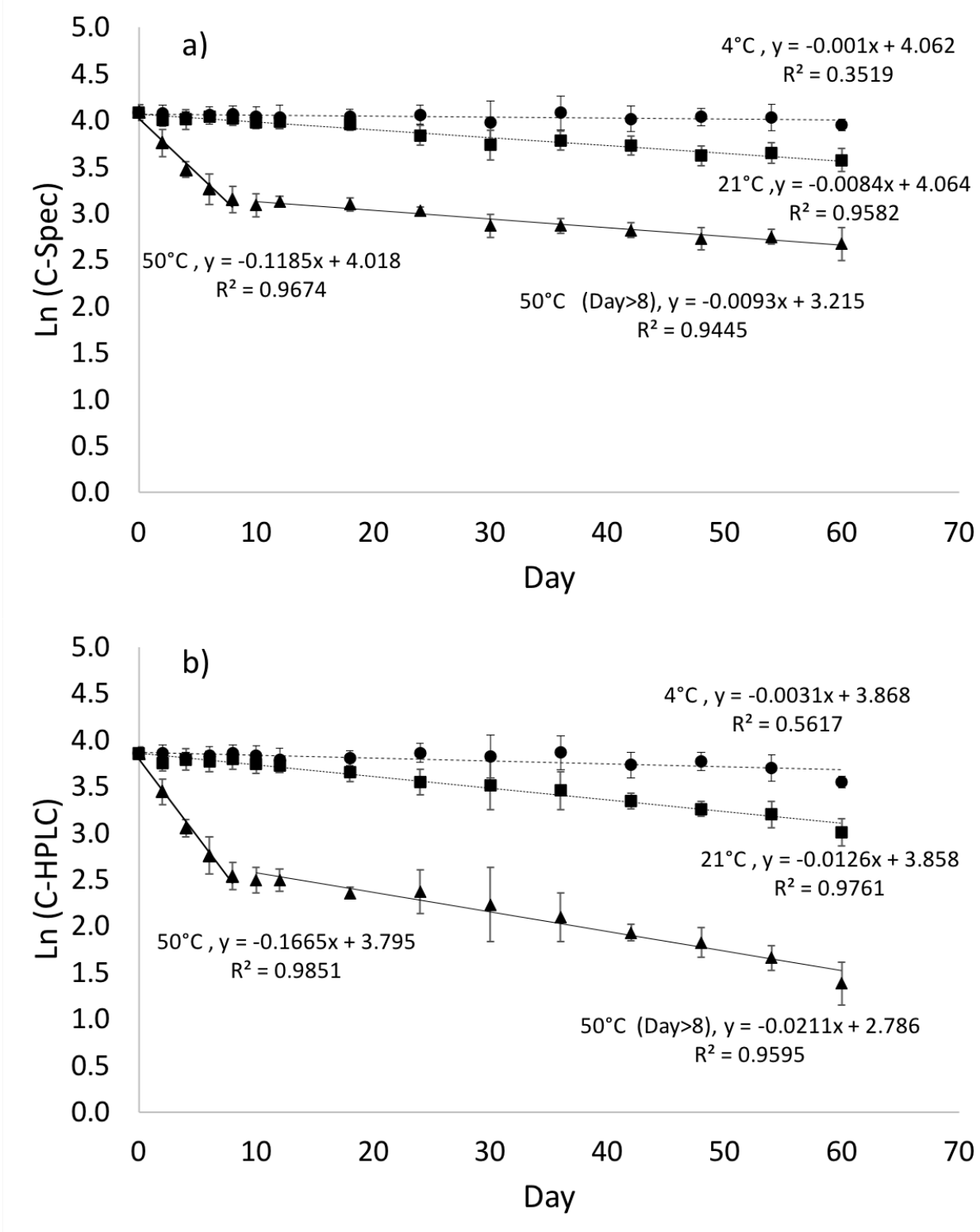

Figure 23 Determination of β-carotene degradation kinetic parameters for powders prepared with 10% MCT + antioxidant stored at 4, 21 and 50°C for 60 days

Table 11 Kinetic parameters for β-carotene degradation in powders at 1% MCT and 10% MCT, with and without antioxidant determined spectrophotometrically. *(Day>8)

Temp (°C)	Rate constant k x 10^{-3} (day^{-1})	Half life $t_{1/2}$ (day)	Activation Energy Ea (kJ/mol)	Rate constant k x 10^{-3} (day^{-1})	Half life $t_{1/2}$ (day)	Activation Energy Ea (kJ/mol)
1% MCT				10% MCT		
4	4.0 ± 1.0	173	73.80	6.4 ± 0.8	108	62.75
21	22.2 ± 4	31		19 ± 1	36	
50	334.6 ±75	2		264.4 ± 85	3	
1% MCT with AO				10% MCT with AO		
4	1.4 ± 0.3	510	84.67	1 ± 0.7	662	78.24
21	12.0 ± 1	58		8.4 ± 1	83	
50	226.5 ± 36	3		118.4 ± 13	6	
	29.71 ± 2 *	23 *	49.14*	9.3 ± 2*å	74 *	33.78*

Table 12 Kinetic parameters for β-carotene degradation in powders at 1% MCT and 10% MCT, with and without antioxidant determined via HPLC. *(Day>8)

Temp (°C)	Rate constant k x 10^{-3} (day^{-1}) HPLC	Half life $t_{1/2}$ (day)	Activation Energy Ea (kJ/mol)	Rate constant k x 10^{-3} (day^{-1}) HPLC	Half life $t_{1/2}$ (day)	Activation Energy Ea (kJ/mol)
1% MCT				10% MCT		
4	3.2 ± 2	217	80.45	6.7 ± 0.7	103	65.22
21	22.7 ± 5	31		21.1 ± 1	33	
50	405.5 ± 63	2		320.6 ± 97	2	
1% MCT with AO				10% MCT with AO		
4	2.7 ± 0.1	261	78.72	3.1 ± 0.7	222	66.60
21	15.7 ± 2	44		12.6 ± 1.7	55	
50	298.9 ± 43	2		166.5 ± 18	4	
	43.0 ± 8*	16*	44.76*	21.1 ± 5*	33*	30.36*

Oxidation of β-carotene primarily occurs through free radical mediated reactions (Xu et al. 2013) such as the presence of reactive peroxyl radicals which results in formation of cleavage products of the initial carotene (Mordi et al. 1991; Mordi 1993). Duralox consists of a mixture of tocopherols that act as an oxidation management system. Tocopherols are derivatives of 6-chromanol that act as peroxyl radical scavengers and inhibit fatty acid oxidation chain reactions. Each tocopherol molecule can neutralize two peroxyl radicals (Kamal-Eldin and Budilarto 2015). Previous work has shown that β-carotene is protected from deterioration by α-tocopherol, possibly by inhibiting reaction singlet oxygen or free radical attack on β-carotene (Palozza and Krinsky 1991). The free radical scavenging activity of α-tocopherol also inhibits lipid oxidation in emulsions and therefore, may further inhibit oxidation of β-carotene by preventing generation of lipid oxidation products that would cause the reaction of β-carotene with itself or other chemical species (Liu et al. 2015).

Emulsions can be divided into three distinct regions, the droplet interior, the continuous phase and the interfacial region. This interfacial region consists of surface-active molecules but may also contain oil, water molecules and other surface-active substances or counter ions attracted to the charged interface, such that partitioning of molecules in the three different regions depend on their polarity and surface activity. The interface characteristics depend on type and concentration of molecules present and if antioxidants are present in low amounts, then a significant fraction will partition into the interface (McClements and Decker 2000). Partitioning of antioxidants at the interface, such as tocopherols used in this study, exerts an important effect on antioxidant lipid interactions and antioxidant protection, since oxidation of lipids in emulsions is initiated at the interface (Jacobsen and Sørensen 2015; Kamal-Eldin and Budilarto 2015). During spray drying of emulsions rapid dehydration results in formation of a powder with an interior core protected by wall or shell material. Several studies have shown that binding of proteins and β-carotene can produce stable protein-carotenoid complexes (Dufour and Haertle 1991; Wackerbarth et al. 2009; Jarunglumlert and Nakagawa 2013). It is likely therefore that after spray drying of the emulsion, a portion of β-carotene was bound at the interface to sodium caseinate and a portion remained in the interior core with the oil phase and tocopherol molecules (ntioxidant Duralox). In the presence of Duralox therefore, the initial decrease in β-carotene at 50°C was likely due to

oxidation of interfacial β-carotene and remaining β–carotene was stabilized such that tocopherols were preferentially oxidized and thus exhibited a protective effect on β-carotene.

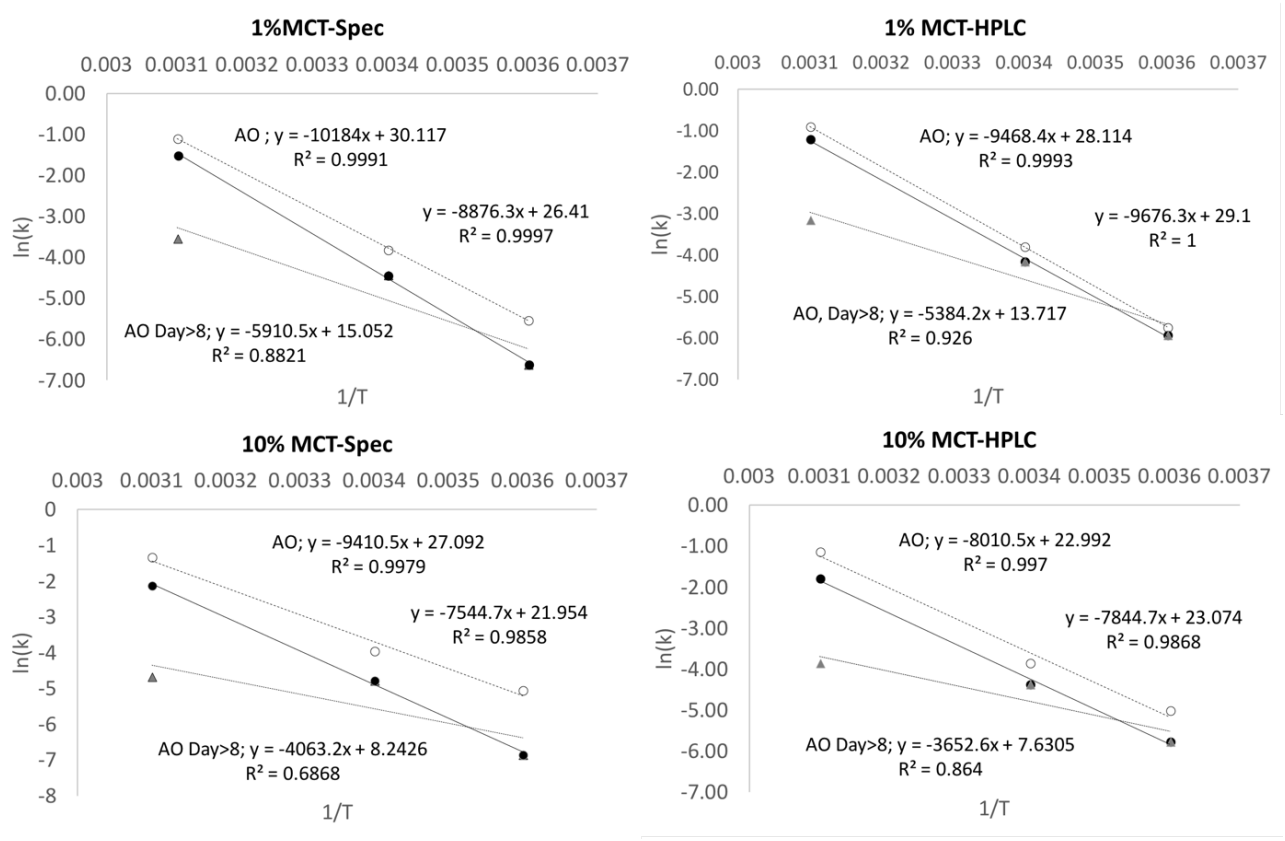

Figure 24 Plots of ln(k) vs. 1/T to determine activation energy for powders prepared with 1% MCT, 10% MCT with and without antioxidant treatment determined by spectrophotometric and HPLC data.

At 10% MCT, an improvement in β-carotene retention possibly suggests that encapsulation of β-carotene in this system was improved and therefore greater retention of β-carotene was possible due to presence of the antioxidant Duralox. Further analysis of powders at the other two temperatures of storage (4°C and 21°C) would likely result in similar two first order degradation periods for β-carotene.

While most studies have shown first order kinetics for β-carotene decay over time, two periods of decay and subsequent changes in rate constants have also been noted. In one study, encapsulation of β-carotene with 25DE maltodextrin using different drying methods (spray drying, drum drying and freeze drying) was compared, and two periods of first order kinetics for β-

carotene degradation were observed. The second first order period had a much slower degradation rate and a change in activation energy was noted after the defined cutoff point, which also varied with temperature and relative humidity during storage. This change in degradation rate and retention of β-carotene was also affected by the drying procedure used. Since this study only used maltodextrin to encapsulate the β-carotene, it was suggested that the spray dried powders had greater surface/volume ratio and were more prone to oxidation of carotene that remained present on the surface of the powder. Therefore, the initial period of degradation was attributed to fast oxidation of surface carotene content (Desobry et al. 1997).

It can also be hypothesized that increased stability seen at higher level of MCT could possibly be attributed to naturally occurring tocopherols in the MCT itself that could have provided additional antioxidative protective effects. This effect has been previously observed during heating of canola oil enriched with α-tocopherol and a mixture of carotenoids (Romero et al. 2007). A similar mechanism of thermal degradation of β-carotene in palm olein and vegetaline ® (a hydrogenated coprah based fat containing 93% saturated fatty acids) at elevated temperatures of 120-180 ºC has also been observed. In this instance, a sudden decrease in initial degradation rates after a certain time point was dependent on saturation level of the lipid, total naturally occurring tocol content of the two lipids and temperature (Achir et al. 2010).

Sodium caseinate is used as an emulsifier to help stabilize dispersions and is preferential to whey proteins due to higher thermal stability, possibly as a result of its structure. It consists of a soluble mixture of flexible surface-active caseins that absorb at the oil-water interface and are less susceptible to heat induced conformational changes unlike globular whey proteins. Sodium caseinate also protects emulsified oils from oxidation by iron chelation and by producing thick interfacial layers around the droplets (Kanafusa et al. 2007). β-carotene emulsions stabilized with sodium caseinate have also been shown to have better stability against oxidation during storage at 4 ºC for 8 weeks due to thicker interfacial membrane and antioxidative effects of casein (Yin et al. 2009; Cornacchia and Roos 2011a). In this study therefore, it is possible that sodium caseinate also assisted in stabilization of β-carotene powders during storage.

3.3.5 Color

The effect of temperature on the lightness (L), redness (a) and yellowness (b) of each of the powders prepared with and without antioxidant at 1% MCT and 10% MCT was measured then used to calculate Hue, Chroma and Total Color Difference with respect to Day0 to determine the level of decoloring caused by β-carotene degradation over time. In general, the powder became lighter, less red and less yellow over time. Higher temperatures had a greater bleaching effect and in the presence of antioxidant this effect was reduced. L, a, b color values for 1% MCT and 10% MCT powders are shown in Figure 25 and 26 respectively, while Hue, Chroma and Total Color Difference for the corresponding powders are shown in Figure 27 and 28.

Hue angle is a basic unit of color and a measure of how color is perceived with 0º = red and 90 º = yellow (Itle and Kabelka 2009). In general, the hue angle increased the most for powders stored at 50°C. For both 1% MCT and 10% MCT powders no significant difference in hue was seen at 4°C and 21°C both with and without antioxidant for the entire storage period of 60 days. Hue values for these temperatures also did not significantly differ from each other. For 1% MCT powders at 50 °C a significant difference from Day0 was observed at Day8 (without antioxidant) and Day6 (with antioxidant). Hue for powders stored at 50°C was significantly different from those stored at 4°C and 21°C after 10 days, and difference between hue values with antioxidant and without antioxidant at 50°C was observed at Day12. For powders prepared with 10% MCT, without antioxidant, and stored at 50°C hue value at Day6 was significantly higher than at Day0, while in the presence of antioxidant this difference was seen at Day8.

Chroma is an indicator of color intensity or saturation and for 1% MCT powders with antioxidant, stored at 4°C and 21°C no significant difference in values was observed for 60 days. At 4°C without antioxidant also no significant difference was observed over 60 days, however at 21°C without antioxidant, Day24 showed significant difference from Day0. Powders prepared with antioxidant and stored at 50°C, showed significant difference from initial value at Day30 while in the absence of antioxidant treatment this difference was noted at Day2. For 10% MCT powders with antioxidant, all three temps (4, 21, 50°C) of storage showed no difference in Chroma over 60 days and were not significantly different from each other as well. Without antioxidant however significant differences from Day0 were seen at Day30 (4°C), at Day18 (21°C) and at Day4 (50°C).

The total color difference between powder at a particular storage time and Day0 was calculated. These values were not significantly different from each other over the 60 days of storage at 4°C both with and without antioxidant and at 21°C with antioxidant. A significant increase in color difference was noted at Day24 for powders at 21°C without antioxidant, at Day6 for 50°C without antioxidant and at Day30 for 50°C with antioxidant. For 10% MCT powders with antioxidant no significant change in color difference was observed for 60 days at all three temperatures of storage. Without antioxidant however, the values increased significantly at Day30 (4°C), Day18 (21°C) and Day6 (50°C). A smaller color difference value denotes that the colors are more similar and in the analysis of fish minces, color difference values used by the textile industry were used to interpret and match different fish samples. Accordingly a color difference of 0.3 was excellent match, 1 was a fair match, 2 a poor match and 3 or greater an unsatisfactory match (Young and Whittle 1985). Similarly in the case of Korean red pepper powders, values used to denote differences were as follows: 0-0.5 imperceptible difference, 0.5-1.4 slight difference, 1.5-3.0 noticeable difference, 3-6 remarkable difference, 6-12 extremely remarkable difference, above 12 different shade (Kim et al. 2002) Based on the values used for red pepper powders, slight differences in color for 1% MCT powder without antioxidant would be noticed at Day2 and an extremely remarkable difference at Day36 followed by a different shade observed at Day60, while for similar powder with antioxidant only a slight difference would occur after 60 days. Similarly, for 10% MCT powders without antioxidant a slight color difference would be noted by Day4, a noticeable difference by Day8-10 and a different shade at Day36, in the presence of antioxidant, however, only a noticeable difference would be seen even after 60 days.

β-carotene oxidative degradation follows an initial cis-trans isomerization step followed by formation of a single diradical, and then subsequent breakdown to apocarotenals and then further on to aldehydes and ketones (Mordi 1993). It was also observed that on direct exposure of β-carotene to molecular oxygen in the dark, after 24 hours all β-carotene was consumed but oxygen uptake continued and after 48 hours remaining compounds were primarily β-ionone and 5,6 epoxy β-ionone (Mordi et al. 1991). It has also been shown that β-carotene dissolved in thermal oils (140-180 ºC) undergoes an initial isomerization reaction from all trans to cis isomer followed by decay of isomers and β-carotene content in a matter of hours (Qiu et al. 2012). In this study, temperature conditions of storage were lower, but β-carotene decay was also monitored every 2 days till Day12

and subsequently every 6 days after that and no isomers were detected during HPLC analysis at any point. It is therefore likely that initial isomerization reactions occurred within the first 48 hours and further β-carotene content decay was observed during the storage study thus forming non-colored compounds resulting in the observed changes in color values (L, a, b) and therefore hue, chroma and saturation values as well. The correlation between carotene content and color Lab values shown in Table 13. The L value showed weak to medium negative correlation with β-carotene content for all treatments. This implies that as β-carotene content decreases the L value increases which is expected because as carotenoids content decreases the lightness of the powder is increased and the L value would increase. The 'a' value indicates the color direction red or green, with positive 'a' value indicating more redness. There was a strong positive correlation between carotene mass and 'a' value for most of the treatments, but the strength of the correlation was reduced in the presence of antioxidant and in one instance (1% MCT at 21°C) a low negative correlation was observed. Yellowness of the powders was indicated by positive b values which showed positive correlation with β–carotene in the absence of antioxidant, however, in the presence of antioxidant a weak negative correlation was observed for powders at 4°C and 21°C. At 50 °C the strong positive correlation was maintained but slightly reduced. This suggests that in presence of antioxidant as β-carotene content increases, the 'b' value tends to decrease (less yellow and more blue).

Hue (tint of color, angular measure) showed a mostly negative correlation with β–carotene at 21°C and 50°C. At 4°C the slight positive correlation was shifted to a negative correlation in presence of antioxidant implies that as hue angle decreases carotenoids concentration would increase. Chroma (saturation or vividness of color) showed a positive correlation with β–carotene content, however in presence of antioxidant it became a weak negative correlation for powders at 4°C and 21°C. At higher temperature, the positive correlation was maintained but slightly reduced. Addition of antioxidant therefore, improved the stability of β-carotene over time and this is reflected in the stability of color values as well with reduced correlation coefficients. Accordingly, in several studies, the chemical stability of β-carotene is monitored by color fading and this has been shown to correlate well with spectrophotometric methods of measuring β-carotene degradation (Qian et al. 2012b).

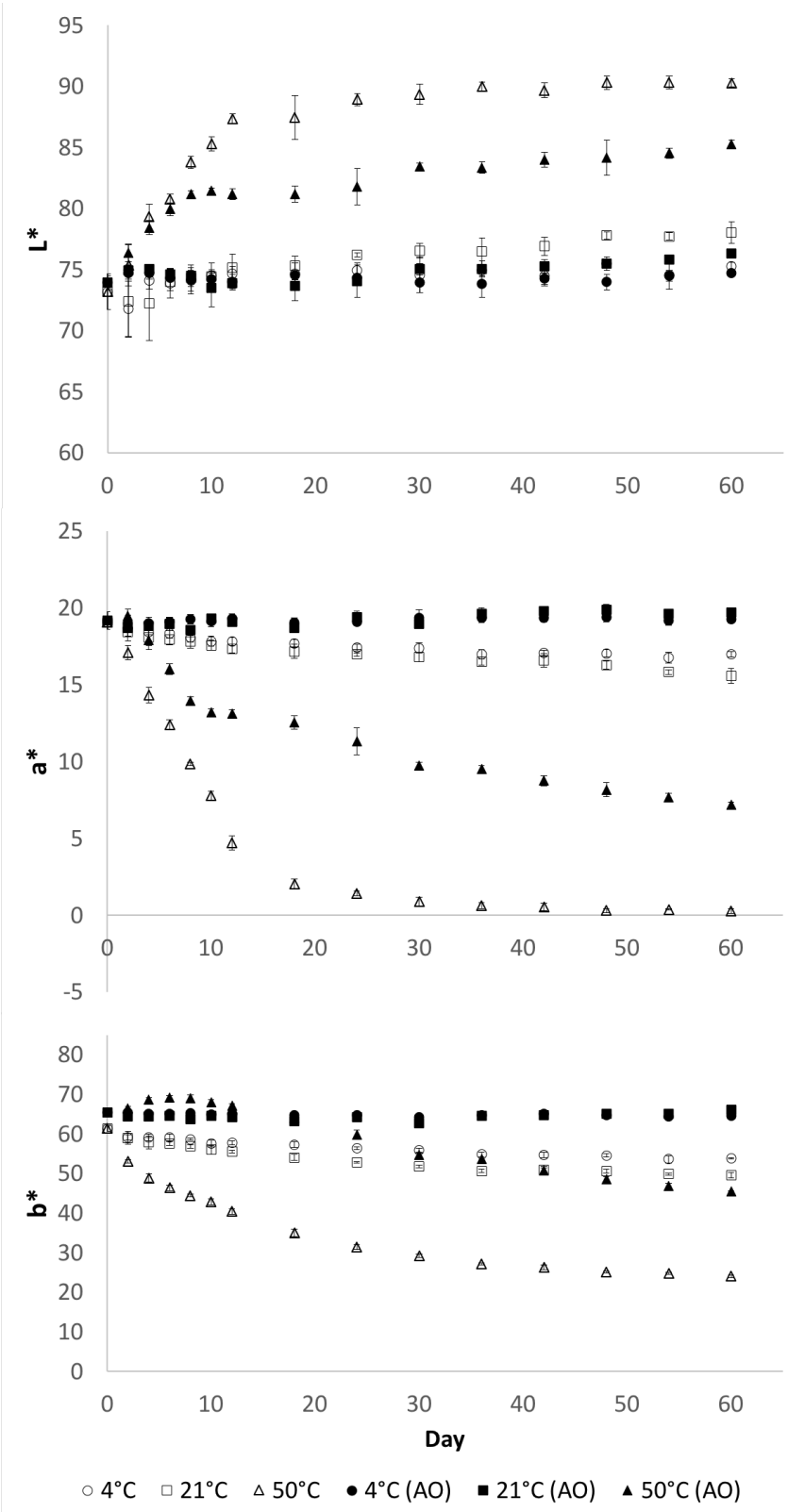

Figure 25 L*, a*, b* values during 60 days of storage for 1% MCT powders at 4°C, 21°C and 50°C with and without antioxidant treatment

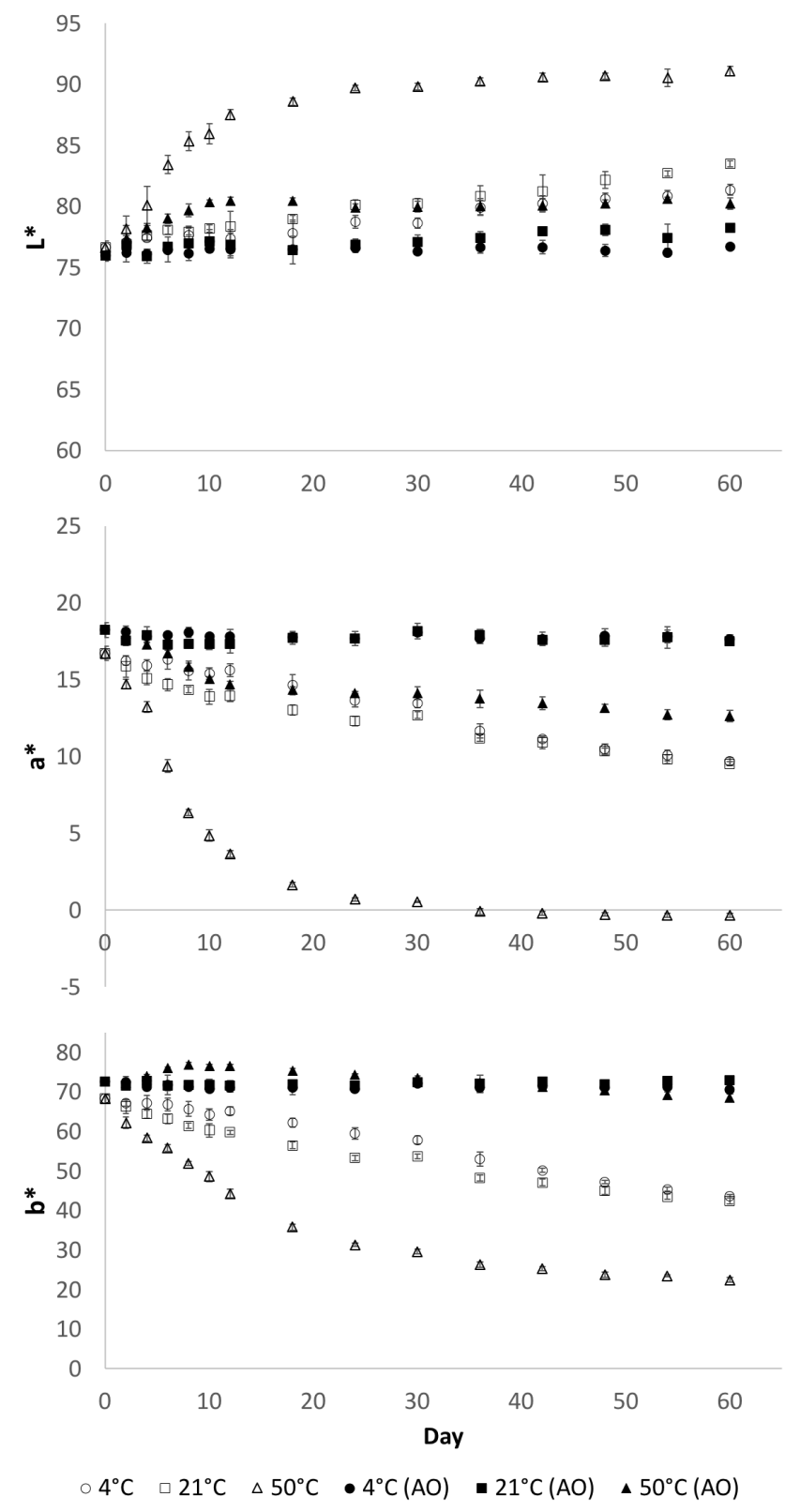

Figure 26 L*, a*, b* values during 60 days of storage for 10% MCT powders at 4°C, 21°C and 50°C with and without antioxidant treatment

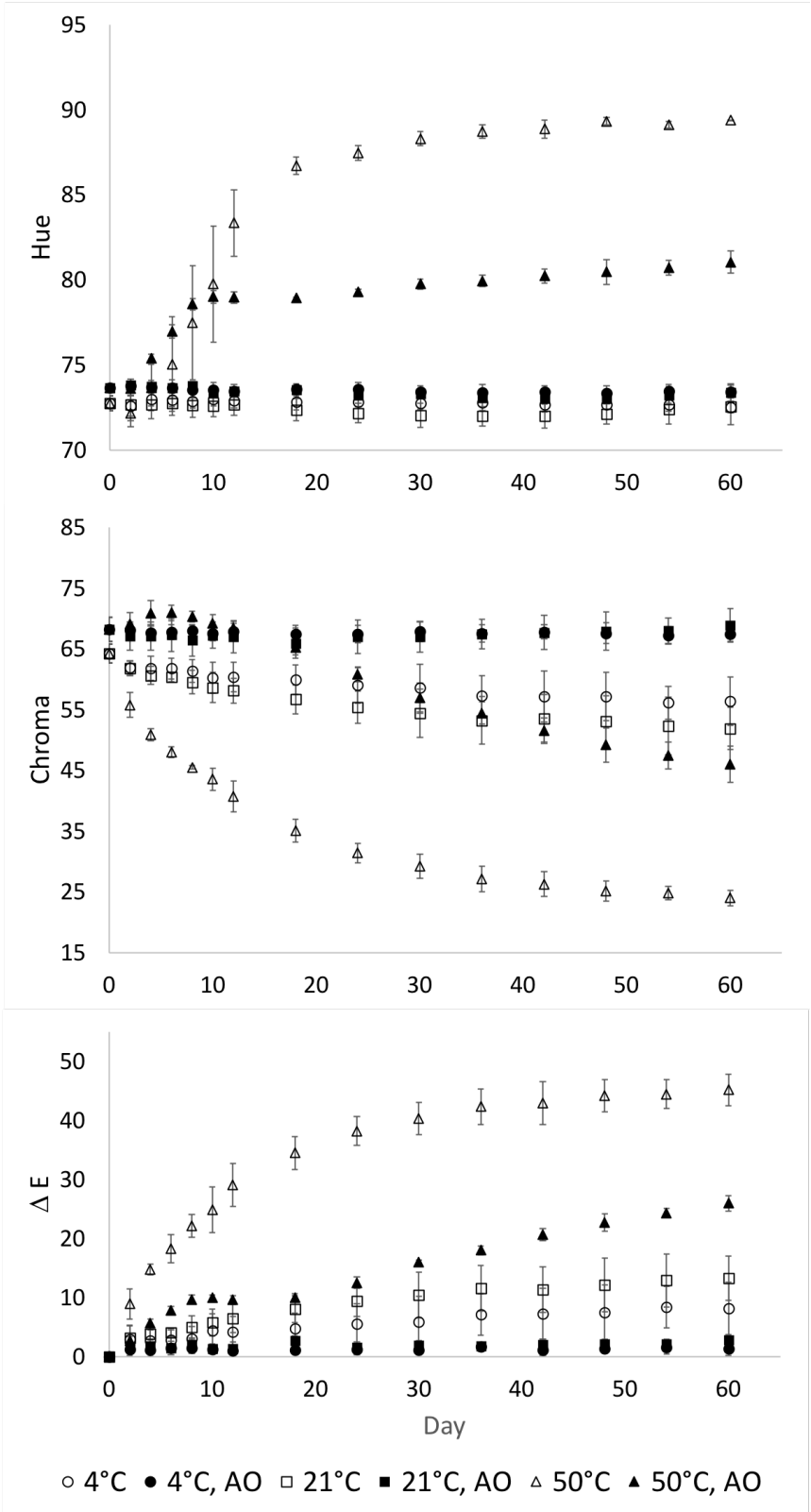

Figure 27 Hue, Chroma and ΔE during 60 day storage period for powders prepared with 1% MCT at 4°C, 21°C and 50°C with and without antioxidant

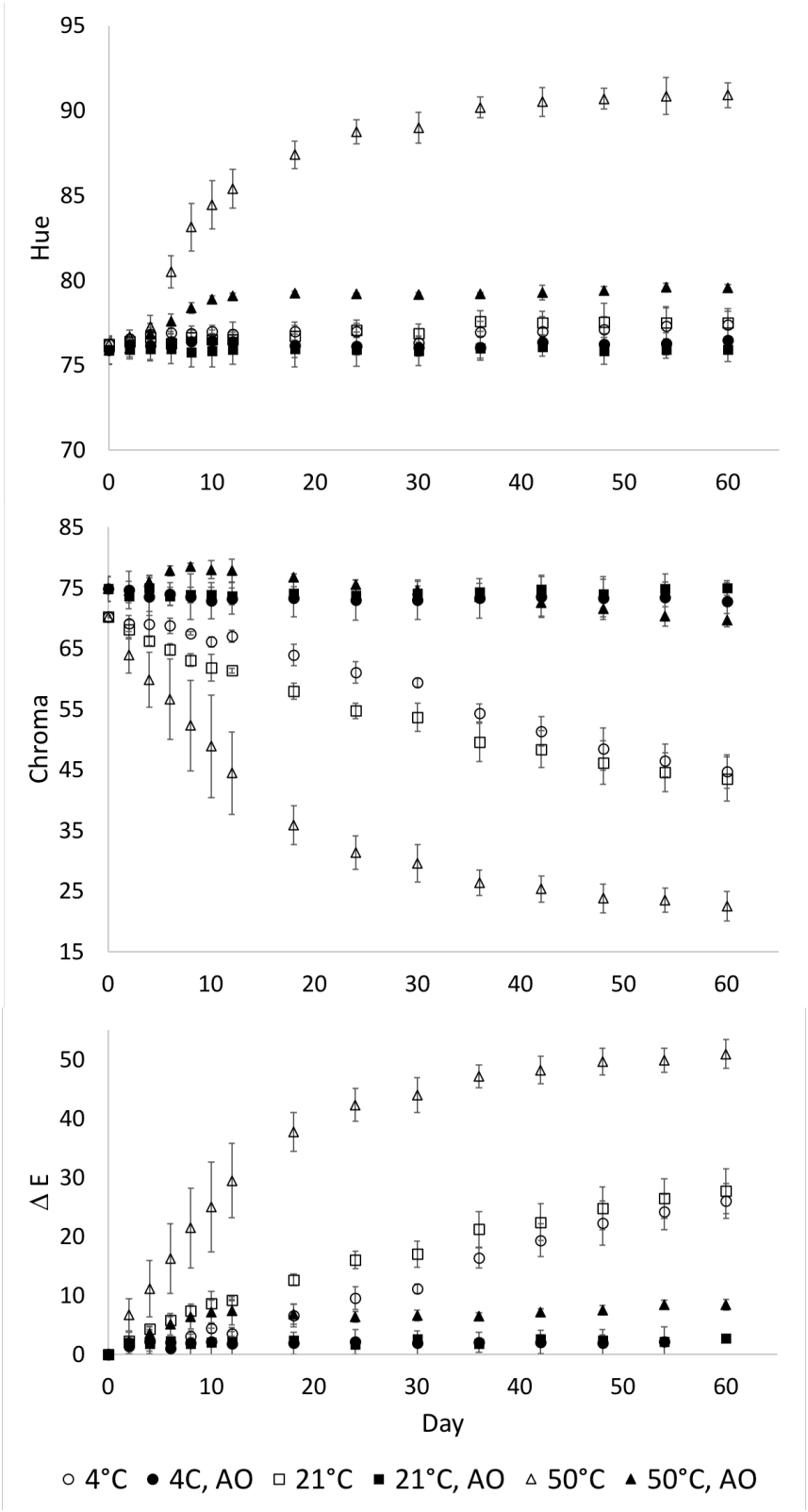

Figure 28 Hue, Chroma and ΔE during 60 day storage period for powders prepared with 10% MCT at 4°C, 21°C and 50°C with and without antioxidant

Table 13 Pearson correlation coefficient and p-values: a) Spectrophotometric data and b) HPLC Data for β-carotene and color values L, a, b, Hue and Chroma

°C	a) Spec Data	L		*a*		*b*		Hue		Chroma	
		r	pval	*r*	pval	*r*	pval	*r*	pval	*r*	pval
4	1% MCT	-0.298	0	0.478	0	0.568	0	0.166	0.054	0.565	0
	1% MCT /AO	-0.344	0	0.172	0.047	-0.33	0	-0.43	0	-0.303	0
	10% MCT	-0.702	0	0.742	0	0.701	0	-0.658	0	0.705	0
	10% MCT/AO	-0.293	0.001	0.128	0.138	-0.498	0	-0.477	0	-0.486	0
21	1% MCT	-0.744	0	0.773	0	0.86	0	0.264	0.002	0.862	0
	1% MCT /AO	-0.415	0	-0.641	0	-0.448	0	0.303	0	-0.474	0
	10% MCT	-0.863	0	0.878	0	0.875	0	-0.426	0	0.877	0
	10% MCT/AO	-0.59	0	0.11	0.203	-0.324	0	-0.285	0.001	-0.303	0
50	1% MCT	-0.866	0	0.828	0	0.942	0	-0.645	0	0.94	0
	1% MCT /AO	-0.637	0	0.724	0	0.789	0	-0.617	0	0.793	0
	10% MCT	-0.847	0	0.836	0	0.784	0	-0.768	0	0.796	0
	10% MCT/AO	-0.859	0	0.827	0	0.118	0.174	-0.883	0	0.221	0.01
°C	b) HPLC Data	L		*a*		*b*		Hue		Chroma	
		r	pval	*r*	pval	*r*	pval	*r*	pval	*r*	pval
4	1% MCT	-0.234	0.006	0.385	0	0.397	0	-0.016	0.856	0.397	0
	1% MCT /AO	-0.381	0	0.152	0.08	-0.361	0	-0.438	0	-0.335	0
	10% MCT	-0.7	0	0.709	0	0.651	0	-0.67	0	0.656	0
	10% MCT/AO	-0.293	0.001	0.192	0.026	-0.414	0	-0.461	0	-0.399	0
21	1% MCT	-0.757	0	0.775	0	0.848	0	-0.238	0.005	0.85	0
	1% MCT /AO	-0.417	0	-0.654	0	-0.466	0	0.297	0	-0.492	0
	10% MCT	-0.861	0	0.867	0	0.853	0	-0.436	0	0.855	0
	10% MCT/AO	-0.588	0	0.15	0.083	-0.259	0.002	-0.289	0.001	-0.236	0.006
50	1% MCT	-0.864	0	0.84	0	0.938	0	-0.623	0	0.937	0
	1% MCT /AO	-0.66	0	0.738	0	0.784	0	-0.639	0	0.79	0
	10% MCT	-0.825	0	0.814	0	0.757	0	-0.725	0	0.773	0
	10% MCT/AO	-0.861	0	0.835	0	0.135	0.119	-0.885	0	0.238	0.006

3.3.6 Redispersed Powder Properties

All powders prepared showed >90% water solubility index in water regardless of lipid level and were not significantly different from each other ($p>0.05$). For redispersed powders, at the same level of MCT, the Z-ave values for powders prepared with antioxidant, were significantly lower compared to without antioxidant, while the 1% MCT without antioxidant and 10% with antioxidant were not significantly different from each other. The presence of antioxidant can affect droplet size as observed in the case of β–carotene emulsions stabilized with gum Arabic, and three kinds of antioxidants (ascorbyl palmitate, α-tocopherol and TBHQ). In this case, α-tocopherol resulted in slightly larger droplet size as compared to TBHQ and ascorbyl palmitate (Liu et al. 2015). The droplet size produced during homogenization can decrease with decreasing interfacial tension which may be produced by presence of antioxidant (Qian and McClements 2011). This effect may also be influenced by the type of emulsifier. Nanoemulsions prepared with Tween20 and loaded with tocopherol showed increasing particle size with increasing tocopherol concentration (Teixeira et al. 2017). On the other hand, in the case of carvacrol loaded nanoemulsions increasing particle size was observed with Tween20 as an emulsifier and decreasing particle size was observed when Ultralec P Lecithin was used (Rodriguez Martinez 2014).

The polydispersity index for the redispersed powders at 1% MCT were not significantly different when treated with antioxidant, however a difference was observed for the 10% MCT powders. The zeta potential did not vary at 1% MCT, however at 10% MCT in presence of antioxidant a more negative value was observed compared to without (Table14). A more negative zeta potential value was also observed at a higher MCT levels. This could possibly be attributed to the greater free fatty acid content of these powders owing to greater percentage of MCT. Free fatty acids are surface active compounds that can concentrate at the oil-water interface and potentially make the surface more negatively charged (Waraho et al. 2011).

As a potential application for preparation of transparent beverages the 1% MCT β-carotene powders with antioxidant would give the droplet size less than 100 nm and potentially show high β-carotene stability over time. In the preparation of cloudy beverages (droplet size>100 nm) the 10% MCT powders would be better implemented. The magnitude of zeta potential indicates stability of the colloidal system and it is widely accepted that the dividing line for stability is +30

or -30 mV (Malvern Instruments 2011). The redispersed powders with less lipid produced systems with low magnitude of zeta potential values (<30 in magnitude) while those with higher lipid level gave more potentially stable systems. It is important to note, that spray drying of emulsions can affect the particle size observed in the reconstituted system. The particle size can increase as compared to the original emulsion as reported for β-carotene encapsulated using almond gum or gum Arabic emulsions (Mahfoudhi and Hamdi 2015) or with using soy protein isolate or OSA-modified starch (Deng et al. 2014). Particle size may also remain unchanged as in the case of spray dried β-carotene nanoemulsions prepared with modified starch (Liang et al. 2013). Similarly the zeta potential value may change as in the case of reconstituted spray dried β-carotene emulsions that exhibited a reduced zeta-potential value as compared to the original emulsion, suggesting that irreversible particle aggregation occurred due to exposure to elevated temperatures during the drying process (Chen et al. 2017).

The water solubility index of powders in waters is important for their application in beverages. Typical values of water solubility index for select dairy and food powders are shown in Table 15. Powders prepared in this study therefore were highly dispersible in water.

Table 14 %Water Solubility Index, particle size, polydispersity index and zeta potential of redispersed powders

Powder	% Water Solubility Index	Z-ave	PDI	Zeta potential (mV)
1% MCT	93.17 ± 2.08	160.9 ± 81.8^{A}	0.345 ± 0.089^{A}	-13.36 ± 3.75 ^{A}
1% MCT, AO	92.63 ± 2.76	89.79 ± 11.23^{B}	0.301 ± 0.71 $^{A, B}$	-15.30 ± 6.12 ^{A}
10% MCT	92.12 ± 1.50	202.42 ± 28.3^{C}	0.265 ± 0.091 ^{B}	-22.59 ± 13.73 ^{B}
10% MCT, AO	89.25 ±3.18	166.285 ± 4.289^{A}	0.167 ± 0.014 ^{C}	-34.91 ± 9.01 ^{C}

Table 15 Water solubility index for select food and dairy powders adapted from Schuck et al. 2012

Solubility index	**Mean**
Skimmed milk	99.8
Milk 26% fat	99.5
Sodium caseinate	99.8
Calcium caseinate	99.5
Micellar casein	64.6
Whey	99.5
β-carotene water soluble powder – in this work	89-93%

3.3.7 Differential Scanning Calorimetry

Sodium caseinate showed a pronounced peak at 208°C with another transition temperature at 149°C. Control powder (no β-carotene) showed a similar transition peaks as did all prepared powders for both levels of MCT with and without antioxidant with transition temperatures of 149.74 ± 4.85 and 206.04 ± 13.96°C.

Pure β-carotene showed a pronounced peak at 180°C which represented its thermal degradation and melting point, however this transition was not observed in any of the powders. Since the β-carotene powders, control powder and sodium caseinate protein had similar temperature profiles it is possible that the quantity of β–carotene in the prepared powders was too low to show a significant effect on the thermal properties of the powder as compared to sodium caseinate. Representative graphs of 1% MCT powder and 10% MCT powder compared to β–carotene and sodium caseinate are shown in Figure 29.

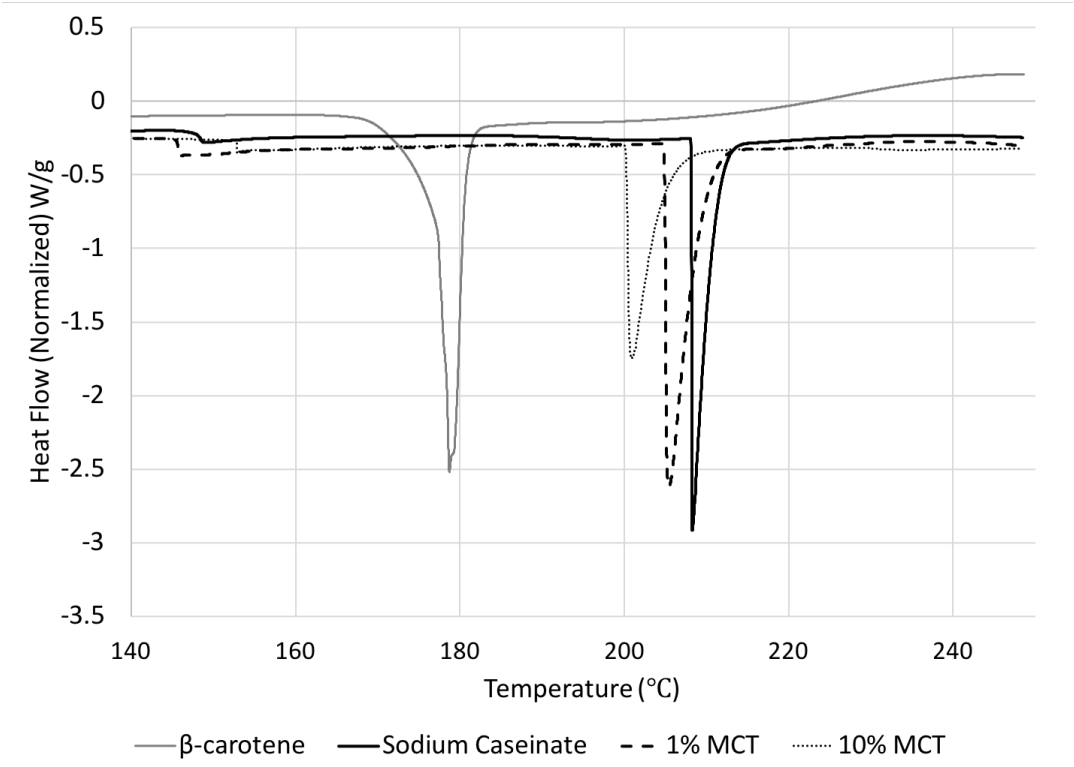

Figure 29 DSC analysis of β-carotene, Sodium caseinate and 1% MCT powder, 10% MCT powder

3.4 Conclusions

Emulsification-evaporation and spray drying was successfully used to prepare natural colorant powders of β-carotene and protein emulsifier. These powders were comparable to dairy powders upon re-dispersion in water (>90% water solubility index), had low moisture and low water activity and were microbiologically safe for storage. β-carotene degradation was studied for 60 days and followed first order kinetics. Incorporation of a natural antioxidant (Duralox, Kalsec) in the powder formulation had a significant effect. It improved the stability of β-carotene such that the rate constant of degradation was reduced, with refrigerated conditions being the most stable. At the highest thermal stress of 50°C, presence of antioxidant resulted in the creation of two first order kinetic decay periods with the second period showing a lower rate constant, thus prolonging stability of β-carotene under these conditions. The appearance of a second degradation period is unique to the powder systems prepared in this study as most previous research has only noted

direct first order kinetic decrease in β-carotene when encapsulated using a variety of other emulsifiers and ingredients. The efficacy of Duralox as an antioxidant ingredient for formulation product development is therefore, also noted in this work. The concentration of lipid in the formulation was significant. 1% MCT powders gave transparent solutions on re-dispersion in water with smaller droplet size but also had low zeta potential and thus reduced stability, while 10% MCT powders would produce cloudy system (higher droplet size) that were more stable according to zeta potential. This information can be used, therefore, to design natural colorant ingredients for use in beverages with lipid level as a determinant for clarity or cloudiness in the final beverage. In addition, β-carotene concentration during the storage study was generally lower at 4°C and room temperature conditions, however at 50°C in the presence of antioxidant higher values of β-carotene were observed compared to the powder prepared with less lipid.

3.5 Future Work

Optimization of powder preparation conditions by including more passes through the homogenizer at the same operating pressure could yield powders that give more stable systems when re-dispersed. The possibility of more β-carotene loss during preparation however would need to be accounted for in order to avoid a loss in color intensity. At the same time, the β-carotene content of the powders could be increased by increasing mass of β-carotene used during emulsion preparation. This could be achieved by heating or pressurizing the solvent as discussed by (Paz et al. 2014).

Other natural proteins such as β-lactoglobulin or a mixture of β-lactoglobulin and sodium caseinate could be used as protein emulsifiers for the formulation and the effect of the globular nature of the protein on β-carotene stability over time could be studied. The effect of including ascorbic acid or other secondary antioxidants in the spray dried emulsion formulation could be studied. Ascorbic acid, ascorbyl palmitate and catechins work synergistically with tocopherols. They can regenerate the tocopheroxyl radical to its parent pheonol thus improving its antioxidative effects and subsequently protecting β–carotene from oxidation(Kamal-Eldin and Budilarto 2015) This emulsification-evaporation and spray drying method could also potentially be used to prepare water dispersible powders of other carotenoids such as lycopene, xanthophylls, lutein, zeaxanthin

or astaxanthin. Industrial production of these powders would also involve scale up studying to determine appropriate ratios and concentrations of the various materials. In addition, a shelf life study of the rehydrated powders in a simulated beverage system exposed to light, oxidative and thermal stress would help determine optimal conditions for preparation and implementation as well as color stability over time. Comparative effect of other natural antioxidants could also be explored to further develop this powder formulation.

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CHAPTER 4. FOOD SCIENCE ENGAGEMENT-EMULSIFIER EXPLORATION

4.1 Introduction

Food additives play an important role in the development of foods, however in recent years consumers have become increasingly concerned about their safety. A discord between the objective purpose of these food ingredients and the subjective quality as perceived by consumers has led to confusion and controversy. Food ingredients with longer names, or those that are difficult to pronounce have a lower healthiness perception which in turn relates to perceptions of greater health risk (Varela and Fiszman 2013). Food science extension and outreach programs can be used to educate consumers (Lee et al. 2014) however, engagement with young people is also important to develop an increased understanding of the food production system as well as the skills needed to act as informed producers, purchasers and preparers of food in the future (Jones et al. 2012).

An emulsion is a mixture of immiscible fluids such that one is divided into fine droplets and dispersed in the other. This mixture is temporarily stable and require emulsifiers which are surface active agents or polymeric compounds to help from a barrier to prevent droplets from coming together. Emulsifiers are typically named such that they represent the chemical structure and therefore tend to sound more like “chemicals” to the layperson. Emulsions however are ubiquitous in daily life therefore they can provide a large spectrum of activities to promote food science (Bravo-Diaz and Gonzalez-Romero 1997).

Common food emulsions are milk (oil in water), margarine (water in oil), ice cream (oil and air in water with solid ice particles). Other examples include salad dressings, mayonnaise, Hollandaise sauce. Food emulsifiers typically consist of hydrophilic (water-attracting) and lipophilic (oil-attracting) ends that then orient themselves at the interface of the two immiscible phases such that they reduce interfacial tension. These food emulsifiers can be synthetic or derived from natural sources. Common food emulsifiers include proteins, gums, esters of fatty acids and polyhydroxyl substrates (lactic acid, sucrose, polysorbates) (Peter Clark 2013). The lipophilic tails of emulsifiers usually comprised of longer fatty acids such as palmitic, while shorter chains are avoided due soapy or undesirable flavors. They can also consist of unsaturated fatty acids (C18-oleic) or a mixture of saturated and unsaturated fatty acids. The polar head groups may be anionic,

cationic, amphoteric or nonionic in nature. For instance the -OH functional group is present in mono and diglycerides (nonionic emulsifier), while a mixture of phosphatides in lecithin can be amphoteric or cationic depending on product pH (Hasenheuttl and Hartel 1997). Emulsion formation is dependent primarily on the emulsifier type. In the case of O/W emulsions, the more water soluble emulsifiers are preferred while for W/O emulsions the more oil soluble emulsifiers are needed (Bravo-Diaz and Gonzalez-Romero 1997). In the United States, food emulsifiers are either categorized as GRAS ("Generally Recognized As Safe"- 21CFR184) or as direct food additives (21CFR172) (Table 16 and 17). Regulations for emulsifiers between the United States and the European Economic Community (EEC) are for the most part similar, however, for some specific emulsifiers, differences do exist. Similarly, other parts of the world have different perspectives on emulsifiers permitted in food products.

Table 16 Emulsifiers affirmed as GRAS (Hasenheuttl and Hartel 1997)

Emulsifier	**21CFR No.**	**Functionalities**	**Typical applications**
Lecithin	184.1400	Coemulsifier, viscosity reducer	Margarine, chocolate products
Monoglycerides	184.1505	Emulsifier, aerator, crystal stabilizer	Margarines, whipped toppings, peanut butter stabilizers
Diacetyltartaric acid esters of monoglycerides	184.1101	Emulsifier, film former	Baked goods, confections, dairy product analogs
Monosodium salt of analogs, phosphate monoglycerides	184.1521	Emulsifier, lubricant, release agent	Dairy products, soft candy

For this particular study, a logic model (Page 143) with specific inputs, outputs as well as short, medium and long term impacts was developed and used to create an extension plan. (Page 144-145). Accordingly the specific audience chosen was high school students in collaboration with the 4H Academy @ Purdue. The 4H Organization was established as part of the Cooperative Extension System and serves youth in rural, urban and suburban communities in every state across the United States of America.

Table 17 Emulsifiers classified as direct food additives (Hasenheuttl and Hartel 1997)

Emulsifier	**21CFR No.**	**Functionalities**	**Typical applications**
Lactylated monoglycerides	172.850	Emulsifier, plasticizer, surface-active agent	Baked products, whipped toppings
Acetylated monoglycerides	172.828	Film former, moisture barrier	Fruits, nuts, pizza
Succinylated monoglycerides	172.830	Emulsifier, dough strengthener	Shortenings, bread
Ethoxylatd monoglycerides	172.834	Emulsifier, stabilizer	Cakes, whipped toppings, frozen desserts
Sorbitan monostearate	172.842	Emulsifier, rehydrating agent	Confectionery coatings, yeast, cakes, icings
Polysorbates	172.836 172.838 172.840	Emulsifier, opacifier, solubilizer, wetting agent	Salad dressings, coffee whiteners, gelatins, ice cream
Polyglycerol esters	172.854	Emulsifier, aerator, cloud inhibitor	Icings, salad oils, peanut butter, fillings
Sucrose esters of fatty acids	172.859	Emulsifier, texturizer, film former	Baked goods, fruit coatings, confectionery
Calcium and sodium stearoyl lactylates	172.844 172.846	Emulsifier, dough conditioner, whipping agent	Bread, coffee whiteners, icings, dehydrated potatoes
Propylene glycol esters	172.858	Emulsifier, aerator	Cake mixes, whipped toppings

The 4H Academy @Purdue is an annual program coordinated by Purdue University Extension and is designed to provide 9-12 grade students who are 4H members from across Indiana an opportunity to learn about a variety of subjects and careers. The participants reside on the Purdue University campus for 3 days and choose workshops of interest. The Food Science and Nutrition workshop is coordinated between staff members at Purdue University Department of Food Science and the Department of Nutrition. Students choose workshops based on areas of their own interest. The allotted time for the Food Science and Nutrition workshop was June 14, 2018 from 8:30 am to 3 pm and the day was divided such that food science activities were in the morning at the Phil E Nelson Hall of Food Science, Purdue University and the Nutrition workshop and activities were in the afternoon at the Nutrition department at Purdue University. As part of the morning's activities a 45 to 60-minute section was allotted for Emulsion Exploration activity represented here (presentation slides are included in Appendix D).

The objectives of the Emulsion Exploration activities were to increase participant understanding about emulsions and emulsifiers, develop ability to recognize emulsifiers in their food items and reduce the perception of risk associated "chemical" sounding names of emulsifiers.

Your Name: Simran Kaur **TITLE: Emulsifiers-The Food Science Mediators**

Situation: *The food choices that individuals make are influenced by the names of food ingredients that they encounter. The perceived "chemical-like" names lead to misinformed choices and erroneous comparisons due to lack of food science knowledge, awareness and education. Food emulsions, for instance, are encountered in everyday life and their ingredients are often misunderstood for this reason. The Indiana department of education includes standards for food science education at the high school (grades 9-12) level. These standards can be supported through the 4H Academy @ Purdue: Food Science and Nutrition Workshop, where food emulsions can serve as an educational tool for promoting the importance of understanding food ingredients and making informed choices.*

Inputs	**Outputs**		**Outcomes -- Impact**		
	Activities	*Participation*	*Short*	*Medium*	*Long*
Self Faculty- Dr. Fernanda San Martin (knowledge support) 4-H Purdue extension contact (Tony Carrell) for workshop coordination Food science PhD Graduate student/s (workshop assistance) Workshop supplies (to demonstrate emulsions, natural color-applications) Knowledge - Food emulsions - Emulsifiers - Color and flavor application	Publication containing curriculum and information related to food emulsions, identifying everyday food emulsions and common emulsifiers found on food labels. 4-H Academy @Purdue (Science Workshops): Food Science and Nutrition Workshop with high school students in Summer 2018 for high school students with focus on food emulsions. Workshop will include information about emulsions, common emulsifier ingredients and how emulsions are processed. Workshop participant recruitment in association with 4-H Science Academy.	High school students from Tippecanoe County coordinated with 4-H Academy@Purdue	1. Increased understanding of what an emulsion is and the role of emulsifiers. 2. They will be able to identify at least the five most common food emulsifiers. 3. The students will be able to recognize common emulsion applications and identify everyday food emulsions. 4. The students will have increased awareness of the link between processing and food chemistry of emulsions.	The students will recognize food emulsifiers/emulsions in their everyday life and make informed choices about the food they consume.	An increased understanding for food ingredients and their roles and a reduced perception of harm from unfamiliar food ingredients.

Assumptions

4-H nutrition and food science workshop not already designated. At least one graduate student can be identified with the time, availability and interest in supporting the workshops.

External Factors

Graduation deadline of August 2018. Research time commitment may extend into timeline for extension output.

Extension Plan- **Emulsifiers: The Food Science Mediators**

November 3rd, 2017

The food choices that individuals make are influenced by their familiarity with the food ingredients that they encounter. The perceived "chemical-like" names of many such substances lead to misinformed choices and erroneous comparisons due to a lack of food science knowledge, awareness and education. This lack of information, familiarity and understanding, leads to fear and perceived harm from foods that contain food additives. These "food fears" may be tackled with effective communication of the ingredient's history, background and general usage. While there are many good examples, food emulsions and emulsifiers would be a logical place to start. Food emulsions are often encountered in everyday life and the complicated names of emulsifiers, which are crucial to their stability, can lead to apprehension and concern. In addition, the Indiana department of education has standards for food science education for high school students (grades 9-12). These standards can be supported through the 4H Academy @ Purdue: Food Science and Nutrition Workshop, where food emulsions can serve as an educational tool for promoting the importance of understanding food ingredients. Developing an awareness about these ingredients can impact the food choices made by an individual throughout their life.

The Purdue Food Science Department's emulsion and encapsulation lab works primarily in the area of developing food emulsions for different applications. The 4H Academy @ Purdue is a program for high school 4-H members, wherein they are given the opportunity to interact with professors, graduate students and other experts at Purdue University to learn about various subjects through interactive, hands on activities. As a member of the emulsion lab at Purdue, and with the help of my advisor, Dr. Fernanda San Martin, I will use our knowledge and resources to develop a workshop that will be implemented through the 4H Academy @Purdue: Food Science and Nutrition Workshop. This workshop will be forty-five minutes long, with one primary presenter (myself) and carried out in the teaching laboratory of the Food Science building with the assistance of another graduate student. This workshop will be repeated three times in the same afternoon for three different groups of approximately ten students that will rotate through this workshop and others that are implemented by the 4H Science Academy.

The workshop will include an introduction to emulsions using water soluble and oil soluble dyes to help distinguish between oil/water and water/oil emulsions. The role of emulsifiers as "mediators" in emulsions such that they allow water and oil to mix will be supported by common

examples of food emulsions seen in daily life. Common household emulsifiers as well as a few common ones often seen on food labels will then be used to make emulsions using water and oil, to show the impact that each has on final emulsion stability. The names of five most common food emulsifiers seen in foods will be highlighted with emphasis on how their names are simply a reflection of their structure, and their general purpose in foods will be highlighted. Additionally, the role of processing in emulsion formation will be showcased with a quick demonstration, and the use of emulsions to deliver color, flavor and health ingredients will be discussed. Additional information about resources available regarding information on food emulsifiers, and other such food additives will be provided in a leaflet or handout. Workshop information will also be used to develop a short extension publication that will be made available for download through the Purdue Education Store. This will include identifying a food emulsion, common everyday food emulsions and common emulsifiers found on food labels. It will be at the high school student grade level in support of Indiana DoE education standards ALSF-1.8 Analyze food products to identify food constituents, ALSF-1.9 Identify common food additives (e.g. preservatives, antioxidants, buffers, stabilizers, colors, flavors), ALSF-1.10 Formulate and explain incorporation of additives into food products.

In the short term, the students will be able to identify an emulsion, be able to state the role of food emulsifiers, they will be able to identify common food emulsifiers and food emulsions and have an increased awareness about the link between food processing and chemistry and stability of food emulsions. The intermediate goal is that students will recognize emulsifiers and food emulsions in their everyday life, and use that information to make informed choices related to the food that they consume. The long-term goal is that the students will have an increased understanding for food ingredients and their roles, along with a reduced perception of harm from unfamiliar food ingredients.

The effectiveness of the workshop will be evaluated using pre-and post-workshop surveys asking questions related to knowledge acquired about food emulsions and emulsifiers, increased awareness of food emulsifier ingredients, and the inclination to think about food emulsions encountered in everyday life. The publication with workshop information and activities will be made available on the Education Store through Purdue Extension and the number of downloads monitored.

4.2 Methods

To allow for survey data collection from the participants the CITI human research Group2 social behavioral research investigator and key personnel program was completed and a Category 1 exemption determination form was submitted for which approval was received on 06/06/18 (IRB Protocol # 1806020682, Study Title: Emulsifiers- The Food Science Mediators, Principal Investigator: Dr. Maria Fernanda San Martin-Gonzalez)

A short introduction to emulsifiers was followed by an activity in the Skidmore Product Development Lab. The students were provided with personal protective equipment such as lab coats, safety glasses and hair nets as they would be required for working in the Skidmore Product Development Lab as well as in the Pilot Plant later for other parts of the morning's activities. The students were provided with workshop information sheet that outlined instructions, timers as well as pen and paper (Figure 30 and 31). The students used materials provided to prepare emulsions and note the time for separation. Food colors were used to make the separation easily visible. Commons food products, such as salad dressings, pudding mix, bakery products were also passed around and students were asked to identify emulsifiers in the ingredient lists.

A pre/post survey (Figure 32) related to topics discussed in the presentation and during the workshop was developed using template obtained during the FNR 50600: Theory and Application of Natural Resource Extension Programming class. It was divided into three sections to assess general knowledge, expected future behavior and a general assessment of the workshop. The survey responses were voluntary and anonymous. The % Knowledge Gain was calculated as per Eq. 16 and average knowledge gain pre/post as per Eq. 17. The %likelihood of participants to read ingredients on a food product, look up information on unfamiliar ingredients and think that an unfamiliar ingredient is harmful was determined based on number of individuals that stated they were either not likely, somewhat likely, likely, or very likely, to exhibit these behaviors in response to these questions listed as questions 5,6 and 7 on the survey.

$$\%Knowledge\ Gain = \frac{(Average\ post\ score - Average\ pre\ score) \times 100}{Average\ Post\ Score} \quad \ldots(\text{Eq } 16)$$

All of the workshop participants opted to participate in the survey, therefore 16 responses were collected. In general, the participants indicated a 60-65% gain in knowledge on four key questions related to emulsions and emulsifiers that were discussed during introduction seminar and workshop activities (Figure 33 and 34).

4.3 Results and Discussion

Approximately 44% (n=7) stated that they would likely/very likely read the ingredients on a food product, 50% (n=8) said they were somewhat likely to do so while 6.25% (n=1) said that they would likely not. When faced with an unfamiliar ingredient, 19% (n=3) stated they would likely/very likely look up information about it, while 43.75% (n=7) said they were somewhat likely to do so and 37.5% (n=6) said they were not likely to do so. In addition, 68.75% (n=11) reported they were likely/very likely to think an unfamiliar ingredient was not harmful while 25% (n=4) said they were somewhat likely to think it would be harmful. On the other hand, 6% (n=1) said they were likely/very likely to think an unfamiliar ingredient is harmful, however they also reported that they would be only somewhat likely to read ingredients on a product and to look up more information on an unfamiliar ingredient (Figure 35). Additional comments about the workshop were submitted by two participants and were complimentary and thankful in nature.

Emulsion Exploration Worksheet
June 14, 2018

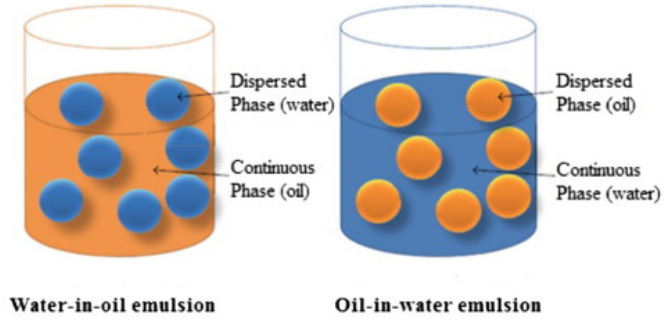
Introduction

Emulsions are temporarily stable mixtures of two immiscible liquids. These are prepared by dividing one phase into small droplets and dispersing them in the other phase. For example, oil in water emulsions have droplets of oil (dispersed phase) suspended in water (continuous phase).

Emulsifiers are molecules used to help make emulsions. Emulsifiers are molecules with water loving (hydrophilic) and oil-loving (lipophilic) regions on the same molecule. The oil loving end is often a long hydrocarbon chain such as a fatty acid and the water loving end is often ionic.

To make an emulsion we must add energy to make the small droplets, and to have the droplets coated with the appropriate emulsifier. This can be done with vigorous beating with a whisk or a hand mixer. In the food processing facility, specialized equipment is used. Emulsifiers will behave in different ways depending on their chemical structure and are obtained from different sources.

Emulsifier	*Source*
Lecithin	Egg yolk, soybeans
Sucrose ester	Reaction between sucrose and fatty acids. Fatty acid from animal or vegetable fat.
Mono and diglycerides	Animal fats, vegetable oils



Learning Objectives

1. Understanding the role of emulsifiers in food.
2. Discuss the behavior of different emulsifiers.

Figure 30 Worksheet provided to participants (page1 of 2)

Materials

1. 4 plastic tubes with 3 mL of soybean oil
2. 1 plastic tube with 6 mL soybean oil+ mono and diglycerides
3. 5 plastic tubes that have
    a. Vinegar
    b. Egg yolk mixture in water
    c. Sucro (Sucrose ester) in water
    d. Soy lecithin in water
    e. Vinegar
4. Food coloring solutions + plastic pipette droppers
5. Timers/stopwatches

Method

1. To make it easier for us to see what is happening we will use a little bit of food coloring. Add a drop of the color of your choice to the tubes that have **vinegar, egg yolk with water, sucrose ester +water, lecithin +water (front row)**
2. Now take plastic #1 with oil and add all the oil to the first tube in the front row on the rack (vinegar).Put the cap back on the tube with the mixture and seal it shut tight.
3. Shake this tube with your hands very quickly for 15 seconds.
4. After shaking, put the tube back on the rack and immediately start the stopwatch or timer.
5. Watch as the oil and water separate again and record how long it takes for you to see a complete separation. Write down the time in the table on your worksheet.
6. Repeat the same steps but now use the second tube with egg yolk mixture. Continue with the third and fourth tube.
7. For the last tube (#5 with a red sticker) you will repeat the same procedure except you will **add the water to the oil.**

Experiment	Time for complete separation (min, s)
Vinegar	
Egg yolk mixture in water	
Sucrose ester	
Soy lecithin	
Mono+diglycerides	

Things to think about

1. Which emulsifier gave the fastest separation?
2. Which emulsifier gave the slowest separation?
3. Why did we add the water to the oil for the last tube?
4. What can emulsions be used for? Why are they important?

Figure 31 Worksheet provided to participants (page2 of 2)

DO NOT WRITE YOUR NAME

MARKING INSTRUCTIONS
Correct ● Incorrect ⊘ ⊗ ⊖ ⊜

Thank you for participating in the 4H Academy@Purdue Food Science and Nutrition workshop on **Emulsion Exploration**. Please take the time to let us know what you learnt!
Your responses are voluntary and anonymous.

1. Please rate your knowledge levels on the following topics before today and now:

TOPICS	**Before today**					**Now**				
	Not at all 1	2	3	4	Very much 5	Not at all 1	2	3	4	Very much 5
a. What is an emulsion?	O	O	O	O	O	O	O	O	O	O
b. What is an emulsifier?	O	O	O	O	O	O	O	O	O	O
c. Examples of foods that are emulsions.	O	O	O	O	O	O	O	O	O	O
e. Names of at least two food emulsifiers.	O	O	O	O	O	O	O	O	O	O

2. Based on the information presented in this workshop, if you were given a packaged food product, please mark the things that you will do within the next 12 months:

	Not Likely	**Somewhat Likely**	**Likely**	**Very Likely**
I will read the list of ingredients on a packaged food product	O	O	O	O
If I see an ingredient on a label that I do not know anything about, I will look up more information about it	O	O	O	O
If I see an ingredient on a label that I do not know anything about, I will think that the food is bad for me because it has an ingredient that I do not know.	O	O	O	O

3. How useful was the Emulsion Exploration part of today's workshop in providing new knowledge to help you understand the food you eat?

Not Useful	**Somewhat Useful**	**Useful**	**Very Useful**
O	O	O	O

4. Any additional comments or suggestions:

Thank you for participating and for providing this helpful information!
Please return this form face down before you leave.

Figure 32 Survey sheet provided to participants

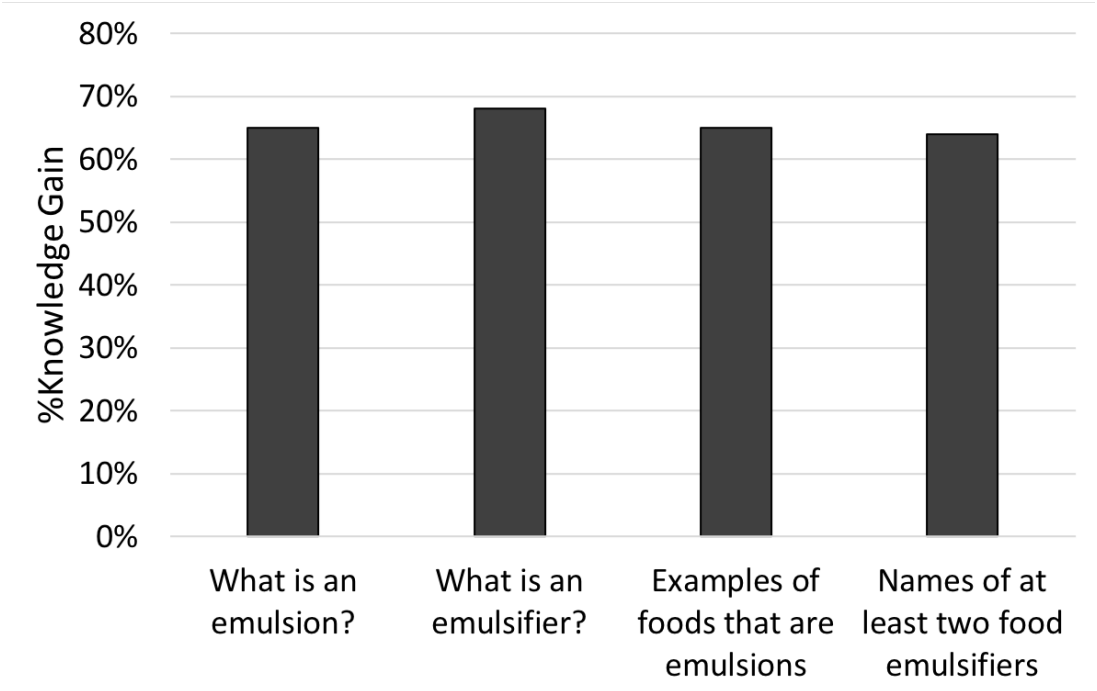


Figure 33 %Knowledge gain from workshop as self-evaluated by participants (average values)

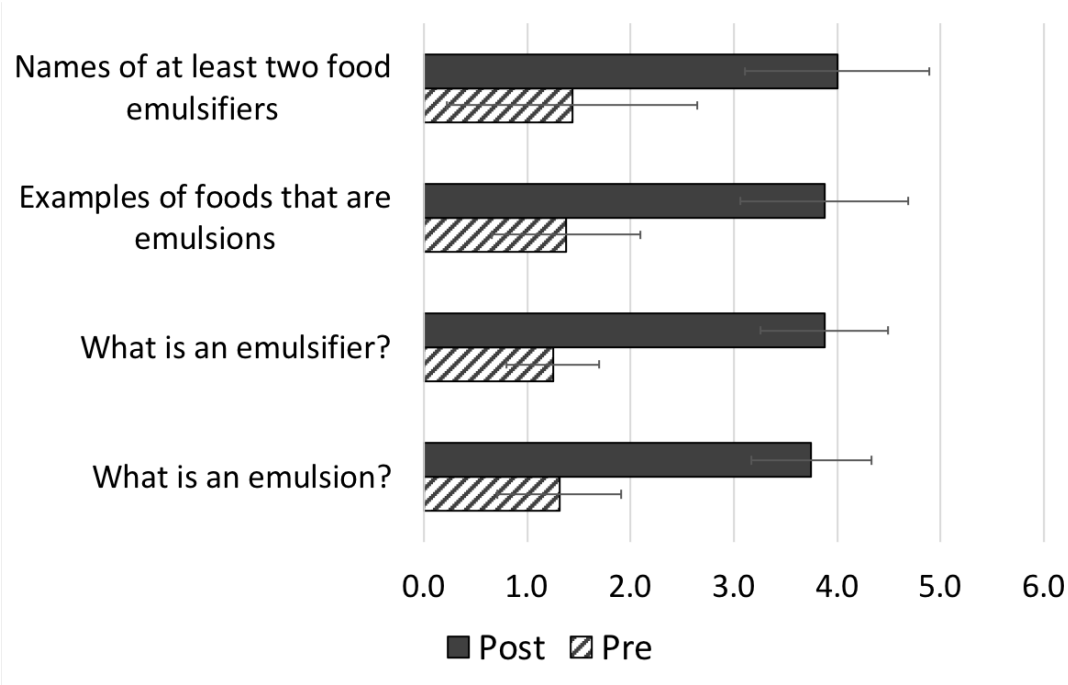


Figure 34 Self evaluation of knowledge of specific topics pre and post-workshop (average and std. dev values shown for n=16)

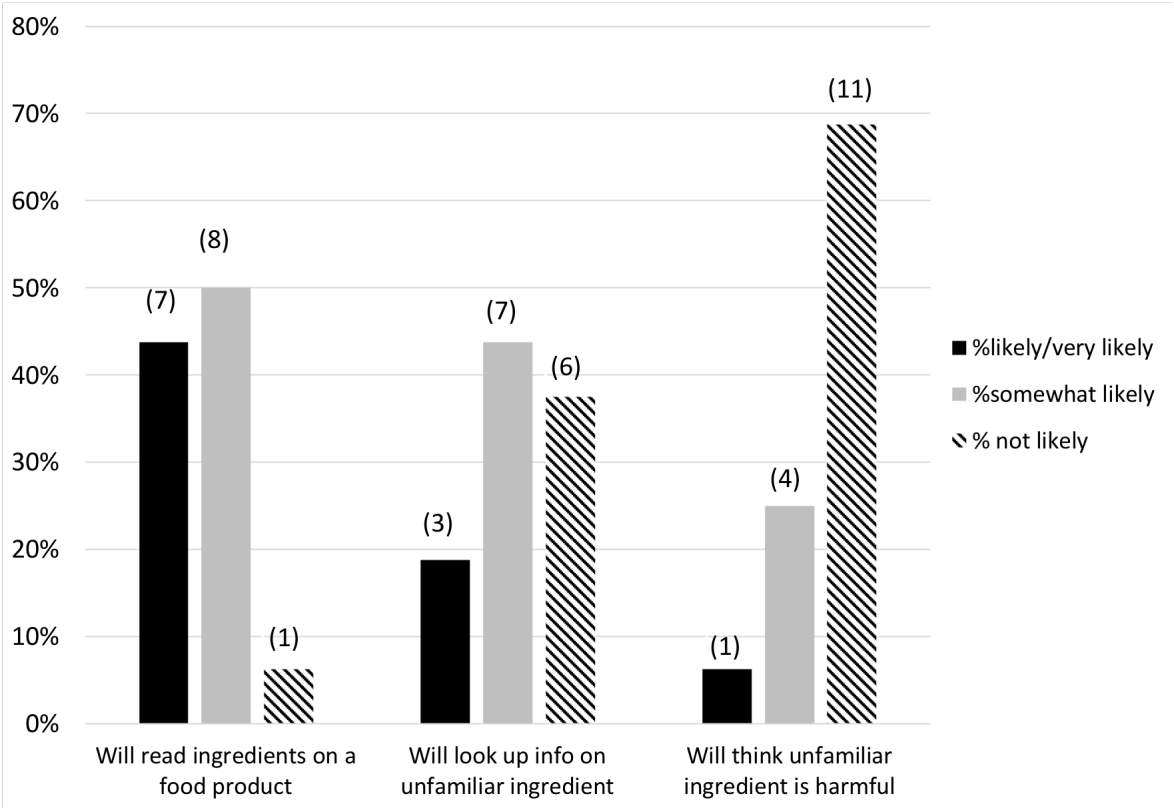


Figure 35 %likelihood responses to anticipated behavioral questions (Q2 on survey) calculated as number of responses to %likely/very likely, % somewhat likely, % not likely divided by total number of responses.

In the short term, the participants showed an increase in knowledge about emulsifiers and a significant percentage anticipated future behaviors that would provide them with information about a food product. This program could be repeated as part of the 4H Academy @ Purdue Food Science workshops and the same analysis conducted to report trends over time. The workshop didn't include demonstration of emulsion preparation equipment, as was originally planned, but they were discussed, and diagrams shown as part of the initial presentation. Given the short timeline of this graduate student's involvement developing a mutual partnership and improving this workshop and its activities by collaborating with the 4H Academy every year was not in the scope of this project. However, this project has the potential to be adapted such that an in the long term an increased understanding for food ingredients and their roles, along with a reduced perception of harm from unfamiliar food ingredients among high school students that attend these workshops could be achieved.

4.4 References

Bravo-Diaz C, Gonzalez-Romero E (1997) Showing Food Foams Properties with Common Dairy Foods. J Chem Educ 74:1133. doi: 10.1021/ed074p1133

Hasenheuttl GL, Hartel RW (1997) Food Emulsifiers and Their Applications.

Jones M, Dailami N, Weitkamp E, et al (2012) Engaging Secondary School Students in Food-Related Citizenship: Achievements and Challenges of A Multi-Component Programme. Educ Sci 2:77–90. doi: 10.3390/educsci2020077

Lee JS, Park JM, Wi SH, et al (2014) Improving consumer recognition and awareness of food additives through consumer education in South Korea. Food Sci Biotechnol 23:653–660. doi: 10.1007/s10068-014-0089-1

Peter Clark J (2013) Emulsions: When Oil and Water Do Mix. Food Technol 67:1–8.

Varela P, Fiszman SM (2013) Exploring consumers' knowledge and perceptions of hydrocolloids used as food additives and ingredients. Food Hydrocoll 30:477–484. doi: 10.1016/j.foodhyd.2012.07.001

APPENDIX A. PHOSPHATE BUFFER PREPARATION

Purpose: To make a phosphate buffer (0.01 M at pH=7) to be used in emulsions

Materials:

- Milli Q water
- Sodium phosphate (dibasic)
- Sodium phosphate (monobasic)
- Volumetric flasks
- pH meter

Methods:

1. In a 250 mL volumetric flask, make a 0.1 M solution of sodium phosphate dibasic.
2. In another 250 mL volumetric flask, make a 0.1 M solution of sodium phosphate monobasic.
3. Transfer the dibasic solution to a beaker and measure the pH of the solution.
4. Gradually add the monobasic solution until pH=7. *Note: Approximately, 61.3% of the total solution will be dibasic solution, while the remaining 38.65% will be comprised of monobasic solution.*
5. Dilute the total solution in 10x volume of MilliQ water to obtain a solution of 0.01 M.
6. Store in the refrigerator until use. For official experiments, fresh buffers should be remade every 2 weeks.

**Note: Ensure pH meter is calibrated (pH 7 standard then pH 4 standard) with a curve of 96 % or greater. Also, ensure that the hole is exposed so that the internal solution can reach equilibrium. Be sure to close the hole when done and that the electrode is submerged in KCl solution for storage.*

Buffer salt	Molar mass (g/mol)	Desired concentration (Mol/L)	Volume (L)	Mass of salt needed (g)
Sodium phosphate dibasic	141.96	0.1	0.25	3.549
Sodium phosphate monobasic	156.01	0.1	0.25	3.90025

.

APPENDIX B. HPLC CALIBRATION CURVE (β-CAROTENE)

1. Turn on UV/vis spec lamps.
2. Scrape standard into culture tube with glass pipette.
3. Add 10-15mL hexane and sonicate for 5 minutes.
4. Read absorbance, adjust to ~1.
5. Filter with 0.45um and label tube A.
6. Dilute following the below dilution scheme for A-D. Vortex and cap.

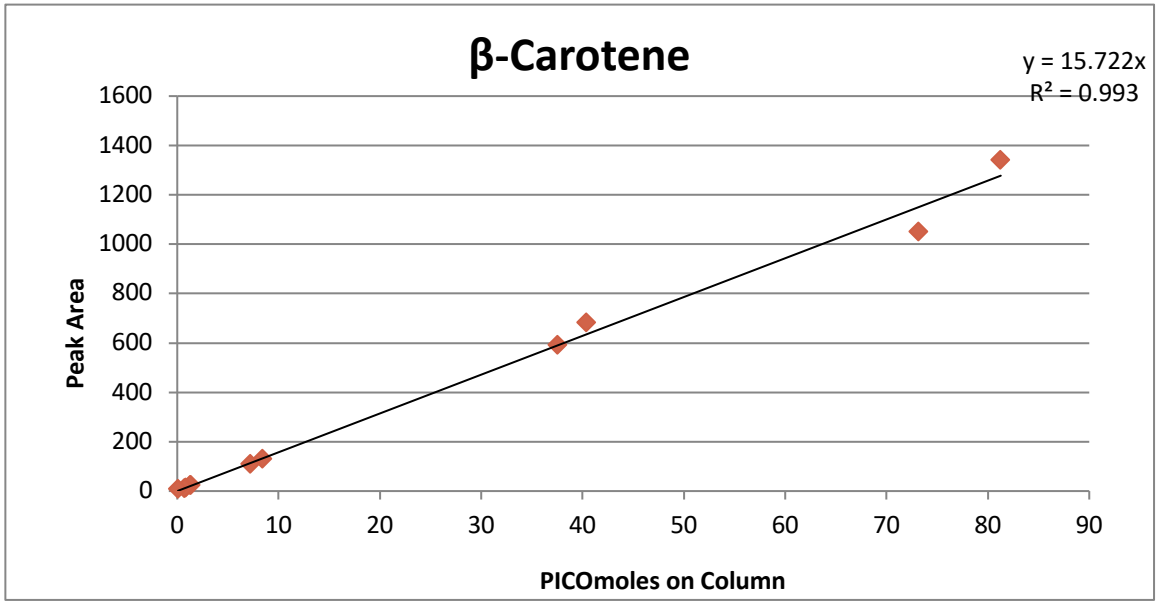
Sample	Dilution	Sample added	Solvent
A	1		
B	1/2	5mL A	5mL
C	1/10	2mL B	8mL
D	1/50	2mL C	8mL
E	1/100	1mL C volumetrically	In 10mL volumetric flasks
F	1/500	1mL D volumetrically	In 10mL volumetric flasks
G	1/1000	1mL E volumetrically	In 10mL volumetric flasks

7. Read absorbance of three different 1 ml aliquots each with new cuvette
8. Prepare E-G volumetrically.
9. If solvent is hexane transfer 2mL volumetrically of each sample into a new culture tube
    a. dry with nitrogen
    b. Resolubilize volumetrically 1mL Ethyl Acetate and 1mL Methanol.
10. If solvent is methanol, transfer directly to HPC vial.
11. Inject 10uL.
12. Integrate at 450nm. Sum area of ALL isomers
13. Save curve on an excel spreadsheet.

14. To determine retention time against other carotenoids:

15. Add 100 uL “B” and 200 uL test salad to 2mL tube, vortex. Inject 10 uL

Carotenoid	**Wavelength(nm)**	**E (M^{-1} cm^{-1})**	**Solvent**
β-carotene	451	139,500	Hexanes
Lycopene	471	185,000	Hexanes
α-carotene	445	145,000	Hexanes
β-cryptoxanthin	450	136,000	Hexanes
α-cryptoxanthin	445	145,737	Hexanes
Lutein	445	145,000	Methanol
Zeaxanthin	450	141,000	Methanol
Phytoene	290	49,776	hexanes



APPENDIX C. IRB EXEMPTION APPROVAL

PURDUE UNIVERSITY

HUMAN RESEARCH PROTECTION PROGRAM
INSTITUTIONAL REVIEW BOARDS

To:	SAN MARTIN-GONZALEZ, MARIA FERNANDA
From:	DICLEMENTI, JEANNIE D, Chair Social Science IRB
Date:	06/06/2018
Committee Action:(1)	Determined Exempt, Category **(1)**
IRB Action Date:	06 / 06 / 2018
IRB Protocol #:	1806020682
Study Title:	Emulsifiers- The Food Science Mediators

The Institutional Review Board (IRB) has reviewed the above-referenced study application and has determined that it meets the criteria for exemption under 45 CFR 46.101(b).

Before making changes to the study procedures, please submit an Amendment to ensure that the regulatory status of the study has not changed. Changes in key research personnel should also be submitted to the IRB through an amendment.

General

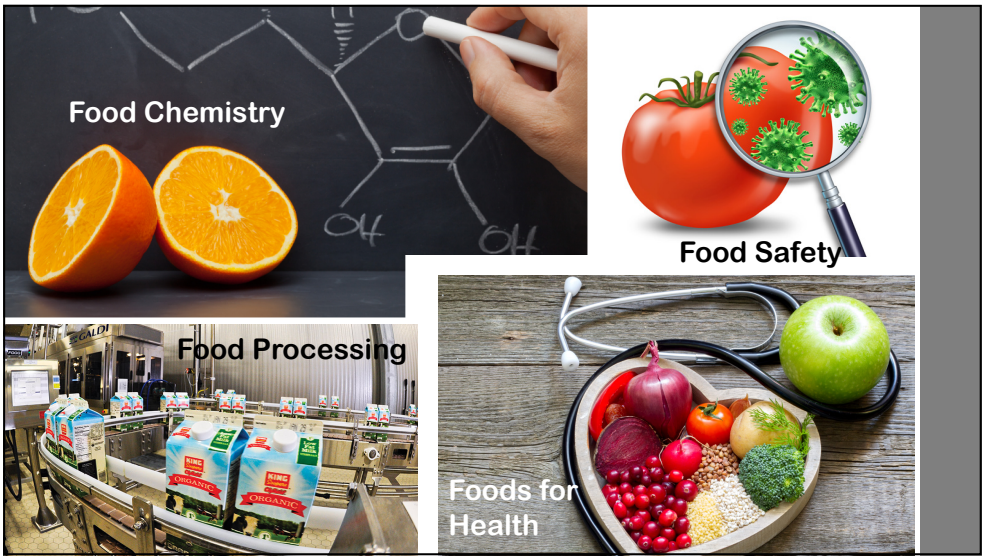
- To recruit from Purdue University classrooms, the instructor and all others associated with conduct of the course (e.g., teaching assistants) must not be present during announcement of the research opportunity or any recruitment activity. This may be accomplished by announcing, in advance, that class will either start later than usual or end earlier than usual so this activity may occur. It should be emphasized that attendance at the announcement and recruitment are voluntary and the student's attendance and enrollment decision will not be shared with those administering the course.
- If students earn extra credit towards their course grade through participation in a research project conducted by someone other than the course instructor(s), such as in the example above, the students participation should only be shared with the course instructor(s) at the end of the semester. Additionally, instructors who allow extra credit to be earned through participation in research must also provide an opportunity for students to earn comparable extra credit through a non-research activity requiring an amount of time and effort comparable to the research option.
- When conducting human subjects research at a non-Purdue college/university, investigators are urged to contact that institution's IRB to determine requirements for conducting research at that institution.
- When human subjects research will be conducted in schools or places of business, investigators must obtain written permission from an appropriate authority within the organization. If the written permission was not submitted with the study application at the time of IRB review (e.g., the school would not issue the letter without proof of IRB approval, etc.), the investigator must submit the written permission to the IRB prior to engaging in the research activities (e.g., recruitment, study procedures, etc.). Submit this documentation as an FYI through Coeus. This is an institutional requirement.

APPENDIX D. PRESENTATION SLIDES FOR EMULSIFIER EXPLORATION WORKSHOP

PURDUE EXTENSION

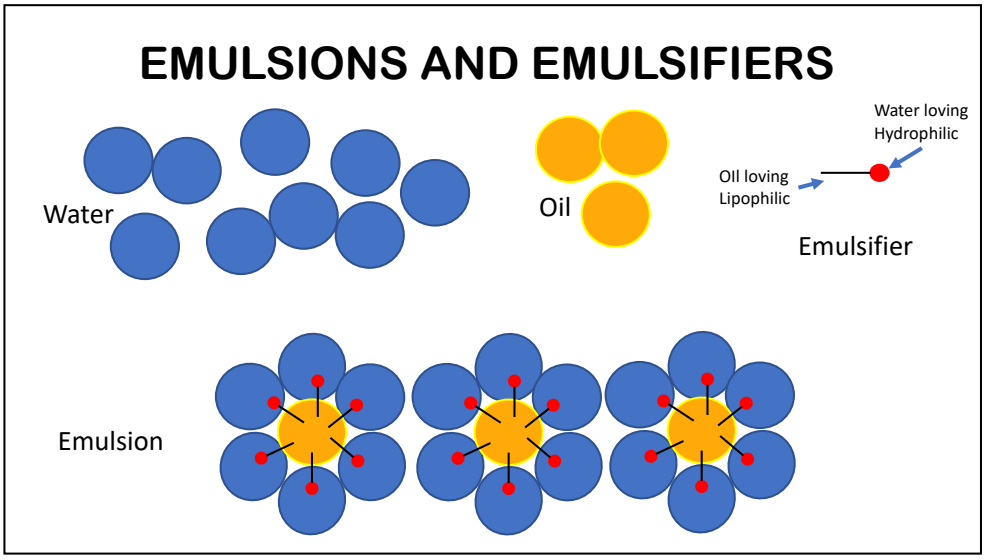
4-H Academy @ Purdue

FOOD SCIENCE


Food Chemistry
Food Safety
Food Processing
Foods for Health

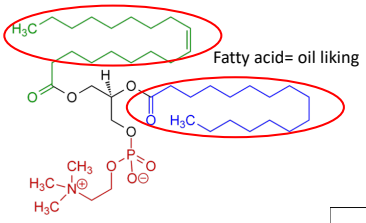

Activities this morning
Emulsion Exploration
Kale Processing
Salad Building
What's Growing in my Kale?


Emulsion
Exploration


EMULSIONS AND EMULSIFIERS
Water
Oil
Water loving
Hydrophilic
Oil loving
Lipophilic
Emulsifier
Emulsion

EMULSIFIERS IN MY FOOD!

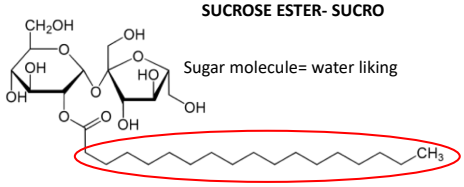
LECITHIN



Fatty acid= oil liking

An example of a phosphatidylcholine, a type of phospholipid in lecithin. Red - choline and phosphate group; Black - glycerol; Green - monounsaturated fatty acid; Blue - saturated fatty acid

SUCROSE ESTER- SUCRO

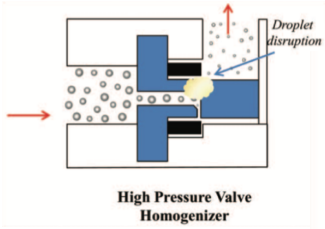


Sugar molecule= water liking

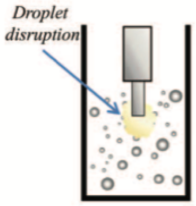
Fatty acid= oil liking

Monoglyceride	Diglyceride
CH_2-OH CH-OH CH_2-O-C(CH_2)$_{14}CH_3$	CH_2-O-C(CH_2)$_7$CH=CH(CH_2)$_7CH_3$ CH-OH CH_2-O-C(CH_2)$_{14}CH_3$

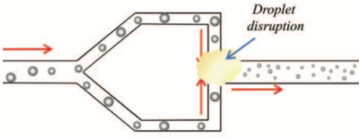
MAKING EMULSIONS



High Pressure Valve Homogenizer



Ultrasonic Probe Homogenizer



Microfluidizer

Emulsifier Rescue

Image source: https://lifestyle.howstuffworks.com/crafts/science-projects/science-projects-for-kids-chemical-reactions11.htm

VITA

Simran Kaur

EDUCATION

- **PhD Food Science,** Purdue University, West Lafayette, IN — Jan.2014-May2019

  Major Professor: Dr. M. Fernanda San Martin-Gonzalez

  Dissertation Title: Interfacial rheological properties of protein emulsifiers, development of water-soluble β-carotene powder and food science engagement (Emulsifier Exploration)

- **MS Food Science**, Purdue University, West Lafayette, IN — Aug. 2011-Dec. 2013

  Major Professor: Dr. Mark Morgan

  Thesis title: Chlorine dioxide gas treatment of cantaloupes and residue analysis

- **BTech Food Engineering and Technology**, Institute of Chemical Technology (ICT), Mumbai, India — 2007-2011

INTERNSHIPS

- **Nestle-Gerber, Fremont, Michigan, USA** — June 2015-Dec. 2015
  Rheological Method Competency at the Nestle Fremont, developed a rheological toolkit, was an active member of the Rheology Core Group, studied different cereal + puree recipes rheological behavior, directed pilot plant trials, initiated a rheology knowledge database to be used as a resource for the Nestle, Fremont product technology center.

- **General Mills India Pvt. Ltd., Mumbai, India** — May-June 2010
  Studied literature on problems of inactivation and analysis of peroxidase activity in oats for granola bar formulation, investigated literature on in vitro Glycemic index testing and worked on formulation of Masala (spice) mix.

- **Godrej Industries Ltd. (Veg. Oils Division) & National** — May-July 2009
  Investigated spectrophotometric and NMR based methods for quantitative determination of tertiarybutylhydroquinone (TBHQ) in refined groundnut oil.

CERTIFICATIONS

- **Graduate Teaching Certificate** 2018
  Center for Instructional Excellence, Purdue Teaching Academy
- **Graduate Instructional Development Certificate** 2016
  Center for Instructional Excellence, Purdue Teaching Academy
- **Better Process Control School** 2011

ACADEMIC DISTINCTIONS, HONORS AND AWARDS

- **Bilsland Dissertation Fellowship** 2018
  This fellowship provides support to outstanding Ph.D. candidates in their final year of doctoral degree completion.
- **Phi Tau Sigma Student Achievement Scholarship** 2016
  The Phi Tau Sigma Student Achievement Scholarship is given to a student Member of Phi Tau Sigma who has shown exceptional scholastic achievement and a dedication to Phi Tau Sigma (Food Science Honor Society)
- **IFTSA Food Science College Bowl 2nd Place at Nationals and Midwest Regional Champions (Purdue University Team)** 2016
  The annual IFTSA College Bowl Competition tests the knowledge of student teams from across the United States in the areas of food science and technology, history of foods and food processing, food law, and general IFT/food-related trivia
- **3rd Place Technical Presentation, J. Mac Geopfert Developing Scientist Competition, International Association for Food Protection** 2014
  Awarded to students (enrolled or recent graduates) in the field of food safety research at accredited universities or colleges.
- **Certificate of Excellence in Food Science Research** 2013
  Office of Interdisciplinary Graduate Programs, Spring Reception, Purdue University. Annual reception in celebration of interdisciplinary graduate student research, award is given to one participant from the graduate students in the Food Science category
- **Ross Fellowship**, Purdue University 2011
  100% support towards graduate studies awarded to outstanding PhD track students admitted to Purdue University
- **JN Tata Endowment for the Higher Education of Indians** 2011
  Scholarship awarded to select Indian nationals to support higher education in newly emerging fields of relevance to India
- **Shri. Ashwin J. Desai Prize for Best All Rounder Day Scholar, ICT, Mumbai** 2011
  Awarded to one student from the BTech graduating class for demonstrating excellence in academics and extra-curricular activities
- **1st rank in BTech Food Engineering and Technology, ICT, Mumbai** 2007-2011

LEADERSHIP

- **Food Science Dept. Representative**, College of Agriculture Graduate Student Advisory Board — 2015-2017
- **Vice President**, Food Science Graduate Student Association (FSGSA), Purdue University — 2016-2017
- **President**, Phi Tau Sigma Hoosier Chapter (Food Science Honor Society), Purdue University — 2015-2016
- **Treasurer**, Phi Tau Sigma Hoosier Chapter (Food Science Honor Society), Purdue University — 2014-2015
- **Purdue College Bowl Team Manager** — 2014-2016
- **Graduate Student Representative**, Food Science Program Graduate Committee, Purdue University — 2013-2015
- **General Mills + Purdue Food Science Leadership and Professional Development Seminar Series** — 2012
- **Judge**, Undergraduate Research Poster Symposium, Purdue University — 2013
- **Literary and Debate Secretary**, ICT, Mumbai — 2009-2010
- **Jr. and Sr. Editor**, "The SPIRIT", ICT College Magazine, ICT, Mumbai — 2008-2010

TEACHING EXPERIENCE

- **Instructor**, FS16200-Introduction to Food Processing
  Department of Food Science, Purdue University, West Lafayette, IN — Spring 2017
- **Teaching Assistant**, FS44300-Food Product Design (Senior Capstone Class) — Spring 2016
- **Lecture**: Fat Characterization (FS46700 Food Analysis) — Spring 2017
- **Lecture**: Shelf Life Assessment (FS44300 Food Product Design) — Spring 2016
- **Lecture**: Freeze Drying (FS44700 Food Processing) — Fall 2016
- **Lecture**: Ohmic, Infrared and Dielectric Heating (FS44700 Food Processing) — Fall 2014

OUTREACH/ENGAGEMENT

- **Speaker/facilitator, 4-H Food Science and Nutrition Workshop**. Sponsors Dept. of Youth Development and Agri. Education & Dept. of Food Science: Interactive workshop for 9-12 grade students about gelling agents used in food — 2016
- **Panel member, 'Is Grad School Right For You?'**
  An information session for juniors in the Deans Scholars Honors program in the College of Agriculture — 2016

- **Women in Engineering, Innovation to Reality Program, Purdue University** 2014
  Planned and guided a 2 hour lesson for 6-8thgraders about living in space in coordination with another graduate student
- **Presenter, Next Generation Scholars, Purdue University** 2012
  Designed a poster and an interactive demonstration to explain M.S. research project to students (age 11-13) from Tecumseh Middle School

SERVICE ACTIVITIES

- **Blue Ribbon Judge**, 2017 Lafayette Regional Science and Engineering Fair 2017
- **Volunteer**, Food Science Dept, Purdue Springfest 2016
- **Volunteer**, Martin Luther King Day of Service, Purdue University 2014, ’16, ‘17
- **Volunteer**, Springification, Winterization 2014,’15,’16
- **Volunteer**, Food Finders Bank, Lafayette IN 2015, ‘16
- **Volunteer**, Next Generation Scholars, Purdue University 2014

DIVERSITY AND INCLUSION

Safe Zone Trainee-Purdue University LGBTQ Center April 20, 2017

PUBLICATIONS

Chloroxyanion Residue Quantification in Cantaloupes Treated with Chlorine Dioxide Gas, Simran Kaur, David J. Smith and Mark T. Morgan, Journal of Food Protection, Vol. 8, No. 9, 2015,1708-1718

Inactivation Kinetics and Mechanism of a Human Norovirus Surrogate on Stainless Steel Coupons via Chlorine Dioxide Gas, Jia Wei Yeap, Simran Kaur, Fangfei Lou, Erin DiCaprio, Mark Morgan, Richard Linton, Jianrong Li, Applied and Environmental Microbiology, Vol 82, No. 1, 2016, 116-123