EFFECT OF FORMULATION AND PH ON RHEOLOGICAL PROPERTIES, PARTICLE SIZE DISTRIBUTION, AND STABILITY OF OIL-IN-WATER BEVERAGE EMULSIONS

BY

Jaideep Kaur Arora

A thesis submitted to McGill University in partial fulfillment of the requirement for the degree of Masters of Science

August, 2009

Department of Food Science and Agricultural Chemistry
Macdonald Campus of McGill University
Montreal, Canada

This thesis is dedicated to my beloved parents

Kamaljit Singh (father) and Harjot Kaur (mother)

ABSTRACT

Beverage emulsions are oil-in-water (o/w) emulsions prepared by dispensing vegetable oils in an aqueous base containing hydrocolloids, preservatives, acid and colors. Stability of such emulsions, in both concentrated forms and diluted final preparations, is a requirement and physical separation (creaming) is a critical problem in the beverage industry. The main objective of this research was to investigate the concentration effects of different hydrocolloids, both individually and in combinations, at two pH levels (neutral and 3.4) on the associated rheological properties, particle size distribution, and stability of prepared o/w emulsions and determine optimal conditions for their stability in both concentrated (2 weeks) and diluted forms (2 months).

Oil-in-water emulsions were made using gelatins (Types "A" and "B"), modified starch and modified Arabic gum alone and with selected viscosity builders (Xanthan gum and propylene glycol alginate), and their rheological properties, and their physico-chemical properties were evaluated. Emulsions demonstrating reasonable stability were selected and incorporated into a simulated juice base and a mimicked dairy beverage. Creaming behavior and stability of simulated beverages, containing 2% emulsion, were evaluated over a storage period of 2 months.

Viscous and elastic properties of the concentrated emulsions as well as their opacity increased with an increase in hydrocolloid concentration. Gelatin type 'A' at neutral pH and type 'B' at pH 3.4 was less stable possibly due to protein aggregation close to their iso-electric points and loss of repulsive force. Modified starch had a smaller average particle size and possessed suitable stability at both pH levels. Modified gum Arabic was more stable at neutral pH. In simulated beverages, those containing modified starch, modified gum Arabic, type 'A' gelatin-modified starch conjugates exhibited stability with no signs of creaming with thermal and high pressure pasteurization. Obtained results provide useful information for the preparation of novel stable juice and milk beverages, without the historically employed weighting agents (brominated vegetable oil, ester gum, sucrose acetate isobutyrate) for stabilizing beverages.

RÉSUMÉ

Les émulsions de boissons huile/eau (o/w) sont préparées en distribuant les huiles végétales dans une base aqueuse contenant des hydrocolloïdes, des agents des agents de conservation, l'acide et des couleurs. La stabilité de telles émulsions, sous les formes concentrées et diluées, est exigent et la séparation physique (écrémage) est un problème critique se posant aux industries des boissons. L'objectif principal de cette recherche était d'étudier les effets de concentration de différents hydrocolloïdes, individuellement et en conjugaisons, à deux niveaux de pH (neutre et 3.4) sur les propriétés rhéologiques, la distribution de dimension particulaire, et la stabilité associées des émulsions huile/eau (o/w) et déterminer des conditions appropriées pour leur stabilité en formes des concentrées et diluées.

Des émulsions huile dans eau ont préparé en utilisant les gélatines (types ‘A’, et ‘B’), l'amidon modifié et la gomme acacia modifié seule et avec les modificateur de viscosité (gomme de xanthane et alginate de propylène glycol). Des propriétés mécaniques et physiques des émulsions préparées ont été évaluées. Les émulsions démontré la stabilité raisonnable, ont été choisies et incorporées aux boissons simulées de jus et de lait. Écrémant et la stabilité des boissons simulées, contenant l'émulsion de 2%, ont été évaluées pendant le stockage de 2 mois. Les propriétés visqueuses et élastiques des émulsions concentrées aussi bien que leur opacité ont augmenté avec une augmentation de concentration hydrocolloïde. Le type de gélatine ‘A’ au pH neutre et le type ‘B’ à pH 3.4 étaient moins stables probablement à cause de l'agrégation de protéine (près de leurs points isoélectriques) et perte de force répulsive. L'amidon modifié a eu plus petite taille de particule et une stabilité appropriée possédée aux deux niveaux de pH. La gomme acacia modifiée était plus stable au pH neutre. En boissons simulées, ceux contenant l'amidon modifié, gomme acacia modifiée, gélatine type ‘A’, conjugues de d'amidon modifiés ont demontré une illustrées stabilité raisonnable et sans des signes de l'écrémage. Les résultats obtenus fournissent des informations utiles pour la préparation des émulsions o/w stables (émulsions de boisson) sans addition des agents de poids réglées (e.g. huile végétale bromée, résine estérifiée, isobutyrate d'acétate de sucrose).

ACKNOWLEDGMENTS

I would like to extend my sincere gratitude to my supervisor, Dr. Hosahalli S. Ramaswamy (Professor, Food Science Department), for his financial support, valuable time, ceaseless encouragement, professional advices, and endless help throughout by masters. I am privileged to get an opportunity to be one of his students.

I would like to express my sincere thanks to Dr. Ali R. Taherian (Food Rheologist, Food Research and Development Center (FRDC), St-Hyacinthe, Quebec) for his support to streamline this project, precious guidance, expertise and valuable time throughout my work. I am thankful to Food Research and Development Center, Quebec for providing me some resources to carry out a part of the analysis.

I would also like to thank Dr. S. Kermasha (Chair, Food Science Department), Dr V. Yalayan (Professor, Food Science Department), for their help and understanding, my committee member, Dr. W.D. Marshall for his time and consideration, Ms. Leslie Ann LaDuke, Ms. Diane Chan-Hum, and Debra Martin, Food Science Department secretaries, for all their administrative and secretarial help.

I wish to convey my thankfulness to members of the food processing group for their friendly advices and encouragement in my thesis work: Ms. Elham Azarpazhooh, Dr. Pedro Alvarez, Dr. Yousef Karmini, Dr. Kevin Shao, Mr. Ajaypal Singh, Mr. Hussein Hassan, Mr. Liang Yu, Dr. Mritunjay Dwivedi, and Mr. Sibtain Saqib.

This thesis is dedicated to my respected parents, to my brother and sister-in-law for their blessings and love.

CONTRIBUTION OF AUTHORS

Part of the thesis research has been presented at two scientific conferences and selected portions are being prepared for publication. Three authors have been involved at different levels in the thesis research and preparation of the thesis.

Jaideep Kaur Arora is the M.Sc. candidate who participated actively in the planning of the experiments, and actually carried out all experiments, gathered data, analyzed the results and prepared the first draft of the thesis manuscript. Dr. H.S. Ramaswamy is the thesis supervisor, under whose guidance the thesis was planned and carried out and who critically reviewed and edited the thesis to its final form. Dr. Ali R. Taherian provided the technical supervision to set up the experiments, train the candidate and analyze the results.

LIST OF PUBLICATIONS AND PRESENTATIONS

Selected part of this thesis has been presented at two scientific conferences:

1. Arora JK, Ramaswamy, HS, Taherian, AR. 2009. Rheology and stability of oil-in-water beverage emulsions. Agricultural and Biosystems Engineering Technical Conference, March 25, 2009, Food Research and Development Center, Saint-Hyacinthe, QC, CANADA.
2. Arora JK, Ramaswamy, HS, Taherian, AR. 2009. Effect of formulations and pH on rheological and physical properties, and stability of prepared oil-in-water emulsions. IFT Annual Meeting & Food Expo, June 6-9, 2009, Anaheim, CA, USA.

Part of this thesis is being prepared for publications:

1. Arora JK, Ramaswamy, HS, Taherian, AR, 2009. Effect of formulation and pH on the rheological and physical properties on concentrated oil-in-water beverage emulsions.
2. Arora JK, Ramaswamy, HS, Taherian, AR, 2009. Effect of storage on rheological properties, droplet size distribution and stability of concentrated oil-in-water emulsions.
3. Arora JK, Ramaswamy, HS, Taherian, AR, 2009. Stability of thermally and high pressure pasteurized simulated juice and dairy beverages.

TABLE OF CONTENTS

NOMENCLATURE

γ	Interfacial tension, dyne/cm
ΔS	Increase in surface area, μm^2
V	Volume of the emulsified liquid, m^3
d	droplet diameter, µm
ø	Dispersed phase volume fraction
V_D	Volume of Emulsion Droplets
V_E	Total volume of the emulsion
U_{stoke}	Velocity of Creaming or sedimentation, ms^{-1}
g	gravity
r	Droplet Radius, µm
ρ_{oil}	Oil phase density, g/cm^3
ρ_w	Water phase density, g/cm3
η_w	Velocity of water phase, Pa.s
σ	Shear stress, Pa
δl	Change in length, cm or m
$\dot{\gamma}$	Shear rate, s^{-1}
m	Consistency coefficient, mPa
n	Flow behavior index
G*	Complex modulus, G'+ iG". Pa
G'	Storage modulus, Pa
G"	loss modulus, Pa
$n_{W. ph.}$	Flow behavior index of water phase
$n_{O. ph.}$	Flow behavior index of oil phase

n_{Em}	Flow behavior index of total emulsion
$m_{\mathrm{W.\ ph.}}$	Consistency coefficient of water phase, Pas
$m_{\mathrm{O.\ ph}}$	Consistency coefficient of oil phase, Pas
m_{Em}	Consistency coefficient of emulsion phase, Pas
$\eta_{\mathrm{app.\ W.\ ph}}$	Viscosity of the water phase, Pas
$\eta_{\mathrm{app.\ O.\ ph.}}$	Viscosity of the oil phase, Pas
$\eta_{\mathrm{app.\ Em}}$	Viscosity of the water phase, Pas
H_C	Height of the cream, cm
H_B	Total height of the beverage, cm
t	Time, s
λ	Time constant, s

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Consumers demand food that taste good, appear great and have excellent functionality (high nutrient value) and this demand has ever been increasing with growing awareness. Keeping up with this growing perception has always been challenging for the food industry. Besides the nutritional importance, sensory qualities with desirable texture and rheological properties, is the ultimate test for the acceptability of the food. Beverages represent a major secondary processed food group and form an essential component of a nation's agribusiness system. Market demand for new low-cost, health beneficial products has been driving the industry to explore different beverage alternatives. Novel and beneficial ways are being researched to deliver functional ingredients through beverages that have tangible advantages to health such as essential oils and vitamins. Bottled water and beverages with functional ingredients, such as vitamin waters, energy drinks and sports beverages, are getting increasingly popular. Beverage emulsions are important for successful marketing of these products.

Beverage emulsions have similar composition, preparation procedures, and physical properties to other typical food emulsions. Beverage emulsions can be divided into two categories, flavor emulsions and cloud emulsions. Beverage flavor emulsions provide the beverage with flavor, color and cloudiness, whereas beverage cloud emulsions are implicated only with the cloud. Both types contain an oil phase and a water phase and fall under the category of oil-in-water emulsions. The oil phase consists of vegetable or flavor oils and weighing agents. Oil is responsible for giving opacity to the beverage. Flavor oils are generally composed of essential oils or citrus oils. Pure or deodorized vegetable oils are also commonly used along with terpenes in cloud emulsions. Weighting agents are added to oil to adjust the oil-phase density. Weighing agents are lipophilic compounds soluble in oil and have density higher than the oil and are added to the oils in order to elevate the oil phase density to the aqueous phase levels. However, the use of weighting agents is becoming too restrictive because of carcinogenic concerns. In addition to vegetable oils, edible waxes are also added to the beverage cloud emulsions (Friberg, 2004). In most beverage emulsions the water content is about 60-70%, and in certain formulations it can be as high as 80%. The aqueous phase typically consists of water, emulsifier, acids, sugar, and preservatives.

Beverage emulsions are an important component of the food beverage industry. Beverages containing emulsions are very common in the market, they are incorporated in all dairy based drinks and many of the citrus drinks. Beverage emulsions are a unique class of oil in water emulsions as they are prepared in concentrated form but consumed in highly diluted form. The emulsion is stored and transported in the concentrated form to reduce costs associated with large quantities of water present (McClements, 2004a). Emulsion stability in these beverages which influences product quality, is the most desirable feature for the consumer. Therefore, the emulsion stability is a major issue in the beverage industry.

The stability of emulsion in diluted form is more difficult to achieve than in the concentrated form. This is due to the extreme low viscosity of the diluted beverage which contains very small amount of concentrated emulsion (Taherian, 2006). From a physicochemical point of view, emulsions are thermodynamically unstable systems. They separate into the two immiscible phases based on the stability kinetics. Oil and water do not coexist comfortably because of the surface energy of the oil-water interface. Physical destabilization mechanisms of emulsions include particle migration phenomena like sedimentation and creaming and oil droplets size variation processes such as flocculation and coalescence (Comas et al., 2006). *Creaming and sedimentation* are based on gravitational separation. Creaming describes the upward movement of droplets due to lower density of the oil than the surrounding liquid, whereas sedimentation is the downward movement of the droplets. In beverage industry, the common term for creaming in bottled drinks is known as 'ringing', as the oil carrying emulsion separates and move to the top forming a whitish ring or oily ring at the neck of the bottle (which is referred to as oiling-off). *Flocculation and coalescence* are both forms of droplet aggregation. Flocculation occurs when two or more droplets come together to form an aggregate retaining their individual integrity, whereas in coalescence two or more droplets merge as a bigger droplet (McClements, 2004a). This leads to decreased number of droplets, enhances creaming and eventually causes emulsion breakdown. *Ostwald ripening* is defined as growth of the larger droplets at the expense of the smaller ones, due to mass transport of soluble dispersed phase through the continuous medium. Ostwald ripening is negligible unless the dispersed phase is at least sparingly soluble in the continuous phase. Since essential oils are somewhat soluble in water, beverage emulsions are prone to Ostwald ripening (Buffo and Reineccius, 2001; McClements, 2004a; Friberg, 2004). The rate of creaming can be enhanced by other factors such as oil droplet viscosity, polydispersity, electrical charge and zeta potential, particle size characterization, surface activity, and emulsion rheology (Taherian, 2006).

The knowledge of the rheological properties of food emulsions is important for a variety of reasons. The shelf-life of many food emulsions depends on the rheological characteristics of component phases, for example, creaming of oil droplets is strongly dependent on the viscosity differential between the oil and the aqueous phase. The efficiency of droplet disruption in a homogenizer depends on the viscosity of the separate components, as well as on the overall rheology of the product (McClements, 2004b). In addition, the rheological properties of emulsions are known to be affected by the contribution of attractive and repulsive interactions between the oil droplets, e.g. van der Waals, electrostatic, steric, depletion, and hydrophobic. When the attractive interactions outweigh the repulsive interactions, the droplets tend to aggregate (e.g. flocculation and creaming), which increases their effective size and volume fraction. While under the influence of repulsive forces, the effective volume fraction and size of the droplets is also increased, but they are not able to approach as closely together as hard spheres. When the distance of closest approach is significant compared to the particle radius, these systems behave as though they have a particle concentration that is much greater than the actual droplet concentration. As a result they may be much more viscous than expected or even exhibit elastic behavior (Valdez et al., 2006). Some of the parameters that control the properties of emulsions are particle size and shape distribution, inter-particle interaction forces and the volume fraction of the dispersed phase. These parameters control the flow rheology of these systems. Control of flow or viscoelastic properties of the suspension is crucial during preparation, behavior on standing (e.g., its settling characteristics) and application of the emulsion (Tadros, 1996).

Substantial work has been focused on study of emulsions; however, stabilizing beverage emulsions without the use of weighting agents (which have now a limited use because of apparent health risks and other disadvantages) hasn’t been well studied yet. In this study, the stability of concentrated and dilute emulsions was studied by including different types of biopolymer emulsifiers: gelatin, modified starch, modified gum Arabic, and other stabilizers such as Xanthan gum and propylene glycol alginate without the addition of weighting agents.

The main objective of this research was to characterize the factors affecting the stability of beverage emulsions without the use of weighting agents in both concentrated and diluted forms.

The specific objectives were:

I. To evaluate the rheological properties of concentrated oil-in-water emulsions as affected by concentration and pH of selected proteins (gelatin type ‘A’ and type ‘B’) and polysaccharides (modified starch and modified gum Arabic) and studying the effect of conjugation of proteins with polysaccharides (modified starch, modified gum Arabic, Xanthan gum and propylene glycol alginate) on viscosity.
II. To study the changes in the rheological properties, particle size distribution of the concentrated oil-in-water emulsions during storage over a period of 2 weeks.
III. To analyze the effect of thermal and non-thermal (high pressure) pasteurization on the diluted oil-in-water emulsions in simulated juices and dairy beverages.

CHAPTER 2

LITERATURE REVIEW

2.1 Emulsion science

Emulsion science combines aspects of physics, chemistry, biology and engineering. In the beginning, the fundamental principles of emulsion science were largely derived from the disciplines of polymer science, colloidal science, interfacial chemistry, and fluid mechanics; nevertheless, it did not take much time for researchers to relate emulsion science to food. As a result, a substantial number of natural and processed foods consist either partially or wholly of emulsions (McClements, 2004a).

2.2 Food emulsions

2.2.1 Definition

Food products are complex microstructures composed of water, proteins, carbohydrates, fats, lipids and minor components. One category of liquid food products is emulsions. An emulsion in general consists of two immiscible liquids (usually oil and water), with one of the liquids dispersed as small spherical droplets in the other. The diameters of the droplets dispersed usually lie between 0.1 and 100 micrometers, considering major part of foods.

Food emulsions are known to impart desirable mouthfeel, textural characteristics, appearance and flavor to the food. Although food emulsion products have different tastes and appearances (such as milk, creams, cheese, salad dressings, mayonnaise, coffee, dips, deserts, soups, sauces, fruit and milk beverages etc), dispersion of droplets of one liquid in another, is inherent in their basic formulation.

2.2.2 Types of food emulsions

There are two main types of simple emulsions for food applications: oil-in-water and water-in-oil emulsion, classified according to the relative spatial distribution of the oil and aqueous phases. A system that consists of oil droplets dispersed in an aqueous solution is called oil-in-water or simply O/W emulsions. They are the most versatile of the emulsions and exist in many forms such as mayonnaise, milk, cream liquor, creamer, ice-cream mix, dressing, beverage, soup and sauce, to name a few. The second type is called water-in-oil (W/O) emulsions, which are characterized by butter, margarine, and low fat spreads in general. The concentration of droplets in an emulsion is usually described in terms of the disperse phase volume fraction, 'ø' (Benichou and Garti, 2002; McClements, 2004a; Friberg et al., 2004).

In addition to the conventional emulsions, other complex systems are also feasible. These are called as multiple emulsions. Multiple emulsions (or double emulsions) are very complex dispersion systems which are characterized by a low thermodynamic stability. They are "emulsions of emulsions", e.g. water-in-oil-in-water (W/O/W) or oil-in-water-in-oil (O/W/O). Potential applications of multiple emulsions have been demonstrated in medicine, pharmaceutics, cosmetics, and industrial applications. These structured emulsions were also envisaged in the food industry owing to their capability to entrap, in the internal compartments, water-soluble substances, e.g., NaCl and vitamins with the possible use of bovine serum albumin (BSA), casein, gelatin and other natural occurring macromolecules together with monomeric–amphiphilic molecules (Garti, 1997; Benichou and Garti, 2004; Fechner et al., 2007). Bonnet et al. (2009) indicated a possible use of W/O/W emulsions loaded with magnesium ions in food applications. However, commercially, the use of multiple food emulsions is still low as these double emulsions are susceptible to breakdown (to become simple emulsions) during processing. They have a more complicated formation, since they require additional homogenization steps for complete emulsification (McClements, 2004b; Muschiolik, 2007; Charcosset, 2009).

2.2.3 Purpose of emulsification

Emulsions and colloidal systems are thermodynamically unstable systems and tend to destabilize due to an excess surface free energy. This destabilization can occur due to the following 2 reasons:

1) The contact between oil and water molecules is energetically unfavorable and so there is a tendency for the system to reduce the contact area between oil and water.

2) Liquid food oils normally have a lower density than aqueous solutions and so oil tends to move upwards (McClements, 2000).

Therefore, the main objective of emulsification is addition of energy to the system to create interfaces between the two media while the system tends to return to its thermodynamically stable state. Conventionally, oil-in-water (O/W) emulsions are created by homogenizing an oil phase and an aqueous phase together in the presence of one or more emulsifiers.

Theoretically, the energy necessary to create an interface was given by Friberg (1976):

$$E = \gamma * \Delta S \text{ and } \Delta S = 6V/ d \qquad \text{(Eq 2.1)}$$

Where 'γ' is the interfacial tension, 'ΔS' is increase in surface area, 'V' is volume of the emulsified liquid and 'd' is the droplet diameter (Taherian, 2006). The energy 'E' is inversely proportional to the diameter of the droplets. However, it is recognized that the energy that must be provided for the emulsion is significantly higher than that indicated by the formula since other factors such as type of mechanical agitation, the difference in density between the two immiscible liquids and the viscosity of the continuous phase has a significant impact on the energy required (McClements, 2004b; Taherian, 2006).

2.2.4 Emulsifiers

To help stabilize pure emulsions, it is normally necessary to add an additional third component, an emulsifying agent. Emulsifiers are surface active ingredients which increase the short and long-term kinetic stability of food emulsion systems by adsorption at the interface. They reduce the interfacial tension between the oil and water by absorbing at the liquid-liquid interface, facilitating the production of small droplets during homogenization. Emulsifiers also improve the stability of emulsions to droplet aggregation by generating repulsive forces between the droplets and/or by forming interfacial membranes around the droplets that are resistant to rupture (Surh et al., 2005). Emulsifying agents include low molecular weight surfactants, such as ionic and non-ionic molecules, and macromolecules, such as proteins, carbohydrates and other biopolymers (Taherian, 2006). Proteins cause stability largely through electric and steric repulsion, controlled by the extent of unfolding or denaturing (Hunter, 2008).

2.3 Emulsion properties:

There are many properties of emulsion useful for the formulation of the final product. Some of the main properties have been described by McClements (2004a) and Akoh (2008):

2.3.1 Dispersed phase volume fraction (DPVF): Dispersed phase volume fraction ($\o$) is equal to the volume of emulsion droplets (V_D) divided by the total volume of the emulsion (V_E):

$$\o = V_D/ V_E \qquad \text{(Eq 2.2)}$$

DPVF determines the relative proportion of oil and water in a product as well as influencing many of the physiochemical and sensory properties of the emulsions such as appearance, rheology, taste and stability. For instance, emulsion tends to become thicker and to have a higher viscosity when the concentration of the droplets is increased. Vanapalli et al. (2002), also observed that high dispersed phase volume fractions lead to partial destabilization, resulting in the formation of a flocculated gel (Vanapalli et al., 2002; McClements, 2004a).

2.3.2 Particle size distribution: Many of the most important properties of emulsion-based food products are determined by the size of the droplets that they contain. If all the droplets in the emulsion are of the same size, it is referred to as mono-disperse emulsion, but if there is a range of droplet sizes present it is a poly-disperse emulsion. However, real food emulsions never contain the same droplet size. Therefore, the average size of the droplets is characterized for quality comparisons. The distribution is usually represented by a plot of droplet frequency (number or volume) versus droplet size (radius or diameter).

2.3.3 Interfacial properties: The interfacial region only makes up a tiny fraction of the total volume of the emulsion. Even then, it plays a significant role in determining many of the most important physiochemical and organoleptic properties of food emulsions. The most important properties of the interface are the concentration of emulsifier molecule, thickness, viscoelasticity, electrical charge and (interfacial) tension of the interface.

The thickness and rheological properties of the interfacial region may influence physiochemical stability mechanisms such as gravitational separation, coalescence, flocculation, Ostwald ripening, compositional ripening, and flavor release and also the overall rheology of the emulsion.

2.3.4 Droplet charge: The charge on a droplet is important as it helps to determine the nature of its interactions with other charged species. Emulsifiers have hydrophilic head groups that may be neutral, positively charged, or negatively charged. Proteins may also be positive, negative or neutral depending on the pH of the solution compared to their isoelectric point. Surface active polysaccharides may also have an electrical charge depending on the type of functional groups along their backbone.

2.3.5 Physical state: The transition of the components in a food emulsion from one physical state to other often has a pronounced influence on its overall properties. In oil-in-water emulsions, phase transitions of emulsified fat or oil, and in water-in-oil emulsions, phase transition of emulsified water is considered. However, in general, the primary concern is the crystallization and melting of emulsified fats.

2.3.6 Droplet interactions: Van der Waals, electrostatic, steric, depletion and hydrophobic interactions are the main interactions occurring in food emulsions. These interactions vary in their direction (attractive or repulsive), strength (strong or weak), and range (short or long). Dominance in the attractive forces may result in close approach of droplets, but if the repulsive forces dominate, the droplets tend to remain as individual entities. All these interactions (attractive or repulsive) can largely affect stability, rheology, appearance and flavor of the food emulsions and so it is crucial to understand their nature and characteristics (McClements, 2004a; Akoh, 2008).

2.4 Emulsion stability

A stable emulsion is one with no apparent change in the size distribution of the droplets, or their state of aggregation, or their spatial arrangement within the test container, over the time-scale of observation. This time scale may vary from hours to months depending on many factors (Dickinson, 2003). Emulsion stability is the measure of the rate at which an emulsion creams, flocculates or coalesces. The rate of these changes can be measured by determining the size distribution of the particles and the viscosity of the phases in the emulsion (Huang et al., 2001).

For a polymer to be effective as an emulsifying agent, it must be surface active. To retain small droplets during emulsification, the time between droplet collisions should be long compared to the time for emulsifier to adsorb at the new oil-water interface and to create a stabilizing layer which can cover the droplet temporarily. It clearly must have amphiphilic character. Hence, an ideal emulsifying agent capable of making small droplets is typically composed of species of relatively low molecular mass with good solubility in the aqueous continuous phase. The limited emulsifying capacity of some biopolymers can be ascribed to poor solubility and/or insufficient amphiphilic character to produce a rapid and considerable reduction in the interfacial tension during droplet disintegration.

To stabilize dispersed droplets, a polymer should have following four characteristics:

1. ***Strong Adsorption:*** This implies that the amphiphilic polymer has a substantial degree of hydrophobic character (e.g. non-polar side chains or a peptide/protein moiety) in addition to being hydrophilic to keep it permanently stuck to the interface.

2. ***Complete surface coverage:*** This implies sufficient polymer should be present to fully saturate the surface.

3. ***Formation of a thick steric stabilizing layer:*** This states that the polymer is predominately hydrophilic and of high molecular weight (10^4-10^6 Da) within an aqueous medium with good solvent properties.

4. ***Formation of a charged stabilizing layer:*** This suggests the presence of charged groups on the polymer that contribute to the net repulsive electrostatic interaction between droplet surfaces, especially at low ionic strength (Dickinson, 2003).

Hence, the selection of the competent emulsifier is not an easy task since many additional parameters such as: surface viscosity, surface pressure, dynamic surface tension, rate of migration to the interface, relaxation time on the interface, and competitive adsorption with other surfactants must be considered (Benichou and Garti, 2002).

2.5 Mechanisms of emulsion instability

As mentioned previously, emulsions are thermodynamically unstable systems that tend to revert back to the separate oil and water phases with time. The most important mechanisms of physical instability are creaming, flocculation, coalescence, Ostwald ripening and phase separation (Figure 2.1). In practice, these mechanisms act in concert and can influence each other (Akoh, 2008).

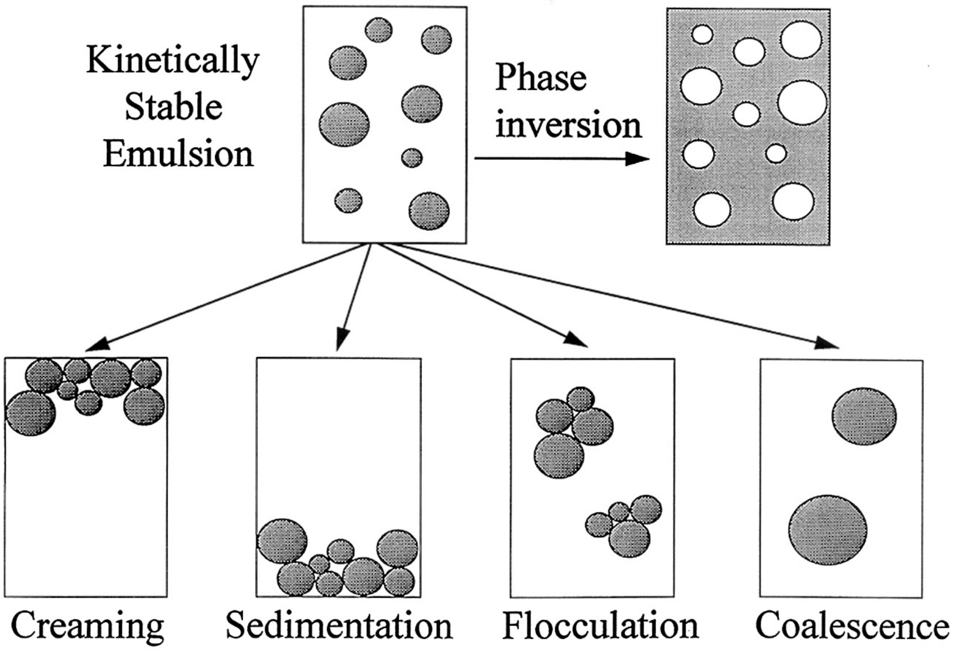


FIGURE 2.1: Food emulsions may become unstable through a variety of physical mechanisms, including creaming, sedimentation, flocculation, coalescence, and phase inversion (McClements, 2004).

2.5.2 Creaming: Creaming occurs because of the density difference between the dispersed phase and the continuous phase and leads to a bulk separation under gravity. The oil forms a dense layer at the top of the emulsion on storage, leaving behind the thin water like layer at the bottom. Conversely, if they have higher density they tend to move down resulting in what is referred to as sedimentation. Most liquid oils have density lower than that of water, so creaming is more frequent than sedimentation. There is no change in the droplet size of the emulsion. Stokes' law gives the rate of creaming of a single isolated spherical droplet as :

$$U_{stoke} = \frac{2\,g\,r^2\,(\rho_{oil} - \rho_w)}{9\eta_w} \qquad \text{(Eq 2.3)}$$

Where 'U_{stoke}' is the rate of creaming or sedimentation, 'g' is the acceleration of gravity, 'r' is the oil droplet radius, 'ρ_{oil}' is the density of oil phase, 'ρ_w' is the density of the water phase and 'η_w' is the viscosity of the water phase. The sign of U_{stoke} determines whether the particle moves upward (-) or downward (+). Stokes law also demonstrates that the more uniform the droplets are, the slower the creaming process is. According to stokes equation, the velocity of the droplet is also indirectly related to the viscosity of the continuous phase. In the initial stages of creaming, the droplets move upward and the droplet depleted layer is observed at the bottom of the container (Taherian, 2006; Akoh and Min, 2008).

In dilute beverage bottles, the emulsion separates and forms a whitish creamy ring at the neck of the bottle (Dickinson, 1992). Creaming kinetics may be determined from the visual observations of the cream or serum layer thickness, from oil volume fraction profiles detected ultrasonically, spectroscopically or from any other technique which measures the local oil/water ratio in emulsions (Dickinson et al., 1995).

2.5.2 Flocculation: Flocculation refers to the aggregation of droplets in three-dimensional clumps, under the influence of inter-particle colloidal forces (which are net attractive) retaining their integrity (Taherian, 2006). Droplets on colliding may either move apart or remain aggregated, depending upon relative magnitude of the attractive and repulsive forces between them. If the net force acting is strongly attractive they bunch, but if it is strongly repulsive they remain distinct. Flocculation is potentially a reversible process. Under the influence of shear flow or Brownian motion, or by manipulation of the inter-particle forces, a (partially) flocculated emulsion may be converted into partially dispersed emulsion (Dickinson, 1992; Dickinson et al., 1995; Akoh, 2008). In concentrated emulsions, a perceptible increase in viscosity occurs with flocculation. In dilute beverage systems, the droplet concentration is so low that flocculation is often reversible (McClements, 2004b).

Flocculation may be induced by polymers bridging between droplets (bridging flocculation) as well as by non-adsorbing polymers (depletion flocculation). Bridging flocculation occurs when a polyelectrolyte adsorbs to the surface of more than one droplet and links them together. Bridging flocculation by strongly adsorbing polymers may be effectively irreversible. Such aggregation occurs during the formation of emulsions when there is insufficient presence of polymer to completely envelop the surface. Strong bridging flocculation may also result from competitive adsorption during emulsification in systems containing a mixture of protein + polysaccharide (Dickinson, 1992). On the other hand, depletion flocculation occurs when the free unabsorbed polymer concentration in the continuous phase generates an attractive osmotic force that is strong enough to overcome the various repulsive forces (Guzey and McClements, 2006). Attraction arises when droplet surfaces are at sufficient close separation that polymer molecules are depleted from the region of aqueous phase between them. The resulting osmotic pressure gradient between the pure solvent in the space and the bulk polymer solution tends to push the droplets together and leads to spontaneous flocculation. It has been suggested that even trace amounts of free polymer can start the formation of weak flocs (Dickinson, 1992). This phenomenon had been also stated for emulsions stabilized by surfactants, due to micelle depletion, when the surfactant concentration exceeds a critical limit (Velez et al., 2003).

2.5.3 Coalescence: Possibility of flocculated droplets to merge together is called coalescence. It is a physical phenomenon whereby two or more droplets combine together to form a single large droplet. It is due to the tendency of the emulsion to achieve a thermodynamically more stable state by a decrease in contact area between oil and water phase. It is desired during manufacture of butter, where churning of cream leads to the separation of butter granules due to phase inversion process that involves coalescence of milk fat globules. However, coalescence is usually undesired (Aken, 2004). In oil in water emulsions (concentrate or dilute), coalescence eventually leads to the formation of a layer of oil on top of the emulsion, which is referred to as oiling off (McClements, 2004a). This latter process of coalescence is irreversible.

In food emulsions, coalescence is not caused by a single step but instead by a sequence of several processes prior the final step of coalescence. Two main consecutive steps are involved: (1) encounter of droplet surface and formation of a thin film and (2) initiation of thin film rupture. The first step corresponds to the flocculation and aggregation of the droplets. The second step corresponds to the actual irretrievable process of coalescence. This may depend not only on the properties of the adsorbed layer but also on other factors such as droplet size distribution, the volume fraction of the droplets, the hydrodynamics of liquid flow and colloidal forces acting across the film (Aken, 2004). In protein emulsions, the kinetics of rupture depends on degree of the local mechanical and thermal fluctuations, and on the viscoelastic properties of the adsorbed protein layers that bound the thin liquid lamella (Dickinson, 1992).

2.5.4 Ostwald ripening: Larger droplets grow at the expense of the smaller ones. This happens because of the transport of dispersed phase molecules from the smaller to the larger droplets through the dominant continuous phase. The mechanism of emulsion destabilization with time in these systems is attributed to Ostwald ripening. This, along with other instability mechanisms (e.g. creaming, flocculation, coalescence) cause changes in the spatial distribution and size of the droplets, which may cause significant alterations in emulsion rheology (Valdez et al., 2006). Ostwald ripening is negligible in many foods because triglycerides have very low water solubilities. Nevertheless, in emulsions (e.g. beverage flavor emulsions) that contain more water soluble lipids, such as flavor oils, Ostwald ripening may play a significant role.

2.5.5 Phase inversion: In phase inversion, a system changes from oil-in-water to water-in-oil emulsion or vice versa. This process usually occurs as a result of some transformation in the system's composition or environmental conditions (Akoh, 2008).

2.6 Factors affecting emulsion stability:

Several factors affect the formation and stabilization of emulsions. Dickinson and Stainsby (1988) described the numerous factors affecting emulsion stability:

1. The physical nature of the interfacial film
2. The steric or electric barrier
3. Viscosity and rheological behavior
4. Oil droplet viscosity
5. Particle size distribution and polydispersity
6. Electrical charge and zeta potential
7. Phase volume ratio of the dispersed phase
8. Temperature
9. Emulsifying agents (surfactants or surface-active agents stabilizers)

2.7 Emulsion ingredients

2.7.1 Oil

Oil is responsible for providing cloudiness and opacity to food emulsions as the light passing through the emulsion is scattered by the oil droplets. Edible fats and oils are a major source of energy and essential nutrients and they influence the nutritional, organoleptic, and physicochemical properties of food emulsions in many ways. Oils used in emulsions can be obtained from a variety of different sources including plants, seeds, nuts, animals, and fish. Commercial vegetable oils contain different lipids. The fact that there are many different types of fatty acid molecules, and that these fatty acids can be located at different positions on the glycerol molecule, means that there is a huge number of possible triacylglycerol molecules present (McClements, 2004a). Most food fats have triglycerides as their major hydrophobic component (~90%). These non-polar lipids are the evident carriers of lipophilic bioactives in emulsion systems. The chemical and physical properties of oils govern the micro-structural characteristics, the colloidal stability, rheological properties and moisture barrier properties of food emulsions. Polar lipids (e.g. monoglycerides, phospholipids, glycolipids), because of their amphiphilic properties, are surface-active and can be used to stabilize emulsions containing active food ingredients (Augustin and Hemar, 2009).

Phospholipids (3-9%) form an outer shell of the polar material separating the triglycerides from the aqueous environment. This layer is mainly responsible for oil-surfactant-emulsifier interactions as it is located at oil-water interface. The presence of phospholipids and sterols at the interface will constitute films whose nature depends on the arrangement of the molecules relative to each other. The stability of an emulsion will therefore depend greatly on the structure of the interfacial film (liquid crystalline interfaces) and also on the concentration of phospholipids and sterols, which varies in different oils and therefore, on the hydrophobicity of the oil (Taherian, 2006).

2.7.2 Hydrocolloids

A wide variety of different hydrocolloid emulsifiers are utilized in food manufacturing. Each type of emulsifiers vary in their efficiency to produce small oil droplets during homogenization, and their ability to prevent droplet aggregation under different environmental stresses, such as pH, ionic strength, thermal and non-thermal processing etc. There is no single emulsifying agent that is ideal for use in every type of food emulsion (Aoki et al., 2005). Various emulsion based food products principally contain proteins and polysaccharides which contribute to their stability and texture. Amongst all the emulsifiers, the most common used in the emulsion preparations are amphiphilic proteins, polysaccharides, phospholipids and small molecule surfactants (Guzey and McClements, 2006).

2.7.2.1 Proteins as emulsifiers

Food emulsions of the oil-in-water type are largely stabilized by proteins. Proteins have been known for their wide availability, natural origin, non-toxicity; these features make them an attractive emulsifier. They have the ability to facilitate the formation, improve the stability, and offer desirable physicochemical properties in oil-in-water (O/W) emulsions; e.g. soy, whey, casein, fish, meat and plant proteins extracted from a variety of natural sources.

Emulsion stabilization is partly due to:

1) their ability to absorb to the oil-water interface and
2) their ability to increase the aqueous phase viscosity or to form a gel in the aqueous phase.

Conformation changes on adsorption

After adsorption to an oil-water interface, a protein may undergo substantial changes in the structure in order to maximize the number of favorable interactions and minimize the number of unfavorable interactions in their new environment. Protein initially surrounded by water molecules is covered by oil molecule (hydrophobic) on one side and water (hydrophilic) on the other upon adsorption, which increases the binding energy and decreases the interfacial tension. Partial unfolding of the native structure on adsorption also contributes to the formation of the interfacial layer (Dickinson, 1992; McClements, 2004b; Hunter, 2008). This conformational change is largely dependent on the hydrophobic interactions between the surface and the amino acid side chains in the protein. Ideally, hydrophobic side chains will be close to the oil surface and hydrophilic residues will favor the aqueous phase, but this will be restricted by the distribution of the amino acid residues in the protein. The conformation that is adopted also depends on the surface area that the protein is required to cover (Dalgleish, 1997). Protein emulsifiers differ in the rate at which they adsorb to droplet surfaces during homogenization, in the minimum amount that is required to saturate the droplet surface, and in their ability to protect droplet against coalescence under different environmental conditions (Dickinson, 1992).

Globular proteins also tend to unfold when the temperature exceeds the thermal denaturation temperature (T_m) exposing reactive nonpolar and sulfhydryl groups (Harnsilawat et al., 2007). After adsorption to the oil-water interface, proteins slowly change their conformation (unfold), adapting their molecular structure to the changed environment at the interface. Globular protein molecules have been found to largely retain their secondary structure of α- helices, β-sheets but their tertiary structure changes. This reorganization leads to an exposure of hydrophobic moieties and sulphydryl groups at the aqueous side of the adsorbed protein layer (Aken, 2004).

Factors affecting protein stabilized emulsions

Temperature, pH, ionic strength, protein concentration and protein/oil ratio, oil volume fraction and molecular interactions are the major factors that affect the physical properties of protein stabilized emulsion (Khalloufi et al., 2009). The ability of proteins to generate repulsive interactions (e.g., steric and electrostatic) between oil droplets and to form an interfacial membrane that is resistant to the rupture plays a considerable role against flocculation and coalescence during long-term storage (McClements, 2004b).

The major mechanism preventing droplet flocculation in protein stabilized emulsions is strong electrostatic repulsion between the charged droplets as the interfacial membrane formed is fairly thin and has some electric charge.

Sensitivity to environmental conditions

Proteins are charged polymers. Protein-stabilized emulsions are particularly sensitive to pH and ionic strength effects. Aqueous phase solvent conditions (especially pH) may have a substantial influence on the adsorption and stability behavior. Emulsions produced tend to flocculate at pH values close to the isoelectric point (pI) of the adsorbed proteins and when the ionic strength exceeds a critical level. At a pH below their isoelectric point, the proteins adsorbed on the oil droplet will be positively charged. Along with other factors, proteins are sensitive to temperature as well, as they tend to unfold when the temperature exceeds a critical value exposing reactive non-polar sulfyhydral groups. These reactive groups increase the attractive interaction between droplets, which may lead to droplet flocculation.

The sensitivity of protein-stabilized emulsions to environmental stresses (such as pH, ionic strength, and temperature) limits their application in many types of commercial product. More stable emulsions can be formed if the droplets and polymer are mixed at a pH where they have the same sign charge, and then the pH is adjusted to a value where they have opposite charges. Results from study done by Harnsilawat et al., (2007) indicate that most of the anionic polysaccharides (e.g., carrageenan, alginate and pectin) adsorb to the surfaces of protein-coated droplets (e.g., β-Lg) at a pH where both the protein and polysaccharide have similar net negative charges and that this adsorption prevents droplet flocculation around the isoelectric point of the adsorbed proteins (Harnsilawat et al., 2007).

A number of methods have being tried to improve the emulsifying properties of protein ingredients, including limited hydrolysis to form peptides, modification of structure of protein physically, chemically, genetically or by use of enzymes, and blending of the proteins with other ingredients. However, these methods haven't yet received legal acceptance (McClements, 2007a). For example, covalent attachment of hydrophobic groups to a protein molecule, may lead to a significant improvement in the surface activity of the protein (Toledano and Magdaddi, 1998).

The structure and composition of the adsorbed layer in a protein-stabilized food emulsion is also affected by various kinds of competition at the interface: protein/protein, protein/surfactant and protein/polysaccharide. Therefore, in order to make quantitative prediction of stability of the protein stabilized emulsions, we should know the thickness and structure of the adsorbed layers, effect of presence of other components such as surfactants and polymers (polysaccharides) and theories relating the characteristics of the protein layers to the inter-particle interactions (Dickinson, 1992).

2.7.2.2 Polysaccharides

The term 'hydrocolloid' embraces the very many polysaccharides that are extracted from plants, seaweeds, and microbial sources, and modified biopolymers made by the chemical or enzymatic treatment of starch or cellulose. As proteins are known for their emulsifying and foaming properties, polysaccharides are identified for their water-holding and thickening properties. These make up an important group of materials in food, cosmetic, biomedical or pharmaceutical applications. Polysaccharides play an important role as hydrating, thickening, emulsifying, and suspending polymers. From a general view point, they are principally important in the category of water-soluble polymers (Dumitriu, 2004).

Polysaccharide gums are mostly hydrophilic polymers and do not exhibit significant surface activity (Dickinson and Stainsby, 1988). They are not considered to be strong surface active agents or emulsifiers. However, as a stabilizer in food emulsions and foams, some gums are found to migrate slowly to the air–water and oil–water interfaces and exhibit some surface and interfacial activities. Researchers have further investigated that hydrocolloid gums, although water-soluble, rigid and very hydrophilic, can precipitate/adsorb onto oil droplets and sterically stabilize emulsions against flocculation and coalescence (Huang et al., 2001; Rinaudo, 2008).

Viscosity modification upon adsorption

The main stabilizing action of food polysaccharides is via viscosity modification or gelation in the aqueous continuous phase. The incorporation of polysaccharide into oil in water emulsions retards the upward droplet creaming by enhancing the viscosity of the continuous phase, which produces desirable textural characteristics.

At sufficiently high concentrations, polysaccharides form a three-dimensional network of interacting or entangled molecules that traps the droplets and effectively inhibits their movement. At this concentration, creaming is retarded because even though the droplets might have aggregated they are incapable of moving owing to the high viscosity or the gel-network formed by the polysaccharides. The influence of polysaccharides on the creaming stability of emulsions is not straightforward and depends on the characteristics of the system. For instance, polysaccharides are also capable of promoting droplet flocculation in emulsions through a depletion mechanism. Over an intermediate polysaccharide concentration, droplet flocculation may cause creaming instability because the increase in effective size of the particles which promotes creaming more than compensates for the increase in continuous phase viscosity which hinders creaming (McClements and Chanamai, 2000; Velez et al., 2003).

Sensitivity to environmental conditions

Emulsions stabilized by polysaccharides (for e.g. gum Arabic and modified starch), are often more resistant to pH changes, high ionic strength, and elevated temperatures than those stabilized by proteins. This has been attributed to the fact that polysaccharide stabilized droplets are surrounded by a relatively thick permeable hydrocolloid layer, which increases the steric repulsion and decreases the van der Waals attraction between oil droplets (Harnsilawat et al., 2007).

Protein in polysaccharide

Many polysaccharides have a good emulsion stabilizing properties because their predominant hydrophilicity and network-forming behavior assists in the generation of a desirable polymeric barrier in the aqueous medium between dispersed oil droplets. However, it is possible that small amounts of protein may be present in some of the commercial gums either as a contaminant or as an intrinsic part of the molecular structure which may be partially responsible for the emulsification properties, acting as a strong anchor point at the oil-water interface, providing the protective layer that confers effective steric stabilization during extended storage. Covalent protein-polysaccharide complexes occur widely in nature e.g. in the block copolymers of mucus glycoproteins or in the hydrocolloid emulsifier gum Arabic. Consequently, an ideal biopolymer would perhaps combine the favorable surface-active character of protein with the steric stabilizing properties of the polysaccharide. Such a hybrid biopolymer can also be produced by direct covalent linkage of protein and polysaccharide (Dickinson, 1992; Dickinson, 1993).

2.8 Food rheology

The rheology has been generally applied to products like inks, paints, plastics, rubber and other industrial materials. Likewise, this science has many applications in the field of food acceptability, food processing, and food handling. The sensory acceptability factors of foods are extremely important as people enjoy eating their food with great pleasure. Hence, from a practical point of view, the rheological characterization of foods is very essential, particularly in relation to structure, processing design and stability (Bourne, 2002). Food rheology is the study of deformation and flow of the food under well-defined conditions (McKenna, 2003).

2.8.1 Basic concepts of food rheology

Food rheology is concerned with the description of the mechanical properties of food materials under various deformation conditions. Under external force, food materials exhibit the ability to flow, or accumulate deformations which can be partially or fully recoverable, or both.

Since rheology is the study of deformation, basic concepts of stress and strain are necessary keys for all rheological evaluations. Stress and strain are two relevant terms when force is applied on a material.

Strain: Strain refers to the change in shape or size of a material when subjected to a stress. It is a dimensionless quantity. However, it may be expressed in dimensions of meter, to remind that there is a change in length.

$$\text{Strain} = \gamma = \delta l / l$$

Where l is the length of the solid.

Shear stress: Shear stress is defined as the force per unit area. It is a stress component applied tangentially. It can be tensile, compressive and shear. It is a force vector that possesses both magnitude and direction. The SI unit for shear stress is the Pascal (Pa) with units of Newton meter^{-2} (Nm^{-2}). It is denoted by σ.

Shear rate: Shear rate is the gradient of velocity established in a fluid as a result of an applied shear stress. It is expressed in units of reciprocal seconds (s^{-1}) or inverse seconds. It is denoted by $\dot{\gamma}$ (Bourne, 2002).

Rheological properties are determined by measuring force and deformation as a function of time. There are two kinds of rheological measurements; fundamental and empirical: fundamental methods have an advantage over empirical of being of known equations and concepts, whereas empirical methods are always used when sample composition or geometry is too complex to account for forces and deformation (Bourne, 2002).

The science of rheology has many applications in the emulsion industry as well as food industry as a whole (Welti-Chanes and Oguilera, 2002):

1. Plant design: Pump and pipe sizing and selection, heat and mass transfer calculations, and other processing engineering calculations including mixers, extruders, coaters and homogenizers, heat exchangers, filler designs.
2. Quality control: Both raw material and finished product at different stages of the process by drawing wealth of correlations between rheological and other data.
3. Evaluation of sensory attributes: Rheological and textural properties are a major factor in evaluation of food quality by the sense of touch, as texture is sensed mainly by the feeling of touch, usually in mouth.
4. Assessment of food structure and conformation of molecular constituents.
5. Shelf life testing

2.8.2 Rheology of food emulsions

In particular, the rheological properties of food emulsions are among the most important physical attributes. It is important to obtain a good quality product with respect to texture, stability and creaminess, all of which are governed by the rheology of the system.

The rheology of emulsions is a subject of considerable importance from both fundamental and applied points of view. The knowledge of the rheological properties of food emulsions is important for a number of reasons: (1) the efficiency of droplet distribution in a homogenizer depends on the viscosity of the individual components, as well as on the overall rheology of the emulsion product, (2) rheological techniques can be used to investigate various processes that occur in emulsion system such as creaming and sedimentation, flocculation, coalescence of droplets, Ostwald ripening and phase inversion, (3) the shelf life of many food emulsions depends on the rheological characteristics of the component phases and lastly, (4) the properties of the interfacial film (surfactant, polymer or mixtures of these) can be studied from the rheological analysis i.e. viscosity and elasticity of the interfacial film (Tadros, 1994).

Liquid food emulsions range from low viscosity emulsion such as milk to high viscosity such as mayonnaise and salad dressings. The basic concepts of food rheology can be classified into viscous flow, elastic deformation and viscoelasticity (Barbosa-Canovas et al., 1996).

2.8.2.1 Rheological classifications

1. ***Newtonian ideal liquid emulsions:*** These have true viscous flow, which means the shear stress is directly proportional to shear rate and the viscosity is independent of the shear rate within the laminar flow range and the plot begins at the origin. Examples of the Newtonian emulsions are tea, coffee, sugar syrups, beer, carbonated beverages, filtered fruit juices, edible oils, and milk. Dynamic viscosity and coefficient of viscosity are synonyms of the term ‘viscosity’ when referring to Newtonian emulsions. They exhibit simplest types of flow properties. All the other types of food are non-Newtonian. Here

$$Viscosity = \frac{Shear\ Stress}{(shear\ Rate)}\ or\ \eta = \frac{(\sigma)}{(\dot{\gamma})} \qquad \text{(Eq 2.4)}$$

2. ***Non-Newtonian Emulsions*****:** The viscosity of the emulsions depend upon applied shear rate (time independent) and/or time over which shear stress is applied (time dependent):

a) ***Time independent non-Newtonian Fluids*****:**
   - **i.** ***Plastic (Bingham) fluid emulsions:*** Such a behavior is displayed by fluids which behave like a solid until a finite shear stress known as yield stress (σ_0) is applied to begin the flow. Elastic deformation is exhibited at stress below the yield value while the plastic flow is observed at high shear stresses. Some examples of this kind of fluid emulsion are mayonnaise, whipped cream, and margarine.

   - **ii.** ***Pseudo-plastic or shear thinning fluids:*** Here the apparent viscosity of the emulsion decreases as the shear rate is increased. The curve begins from the origin but concaves upward. Shear-thinning may be thought of being due to breakdown of structural units in a food due to the hydrodynamic forces generated during shear. Most non-Newtonian food emulsions exhibit shear-thinning behavior, including many salad dressings and some concentrated beverages (Rao, 1999).

   - **iii.** ***Dilatant or shear thickening fluid emulsions:*** The shear stress-shear rate plot of this type of a flow begins at the origin but equal increments in the shear stress gives less than proportional increase in the shear rate. There is an apparent increase in viscosity with increasing shear rate. e.g. high solids, raw starch suspensions, chocolate syrups. Although, dilatant flow is rarely seen in food industry and finished food products.

In all the above three cases, viscosity at any given shear rate can be calculated by (eq 2.5):

$$\eta = \sigma_0 + m\dot{\gamma}^{n-1} \qquad \text{(Eq 2.5)}$$

All the above flow behaviors can be represented by this equation as represented in Figure 2.2. Newtonian flow is represented by a straight line starting from the origin, dilatant flow starts from the origin and concaves downward whereas Pseudoplastic flow starts at the origin and concaves upward. Plastic or Bingham flow is linear and does not begin from the origin, has some yield stress before the liquid flows. For Herschel-Bulkley, the flow behavior above the yield value of a plastic fluid is not linear.

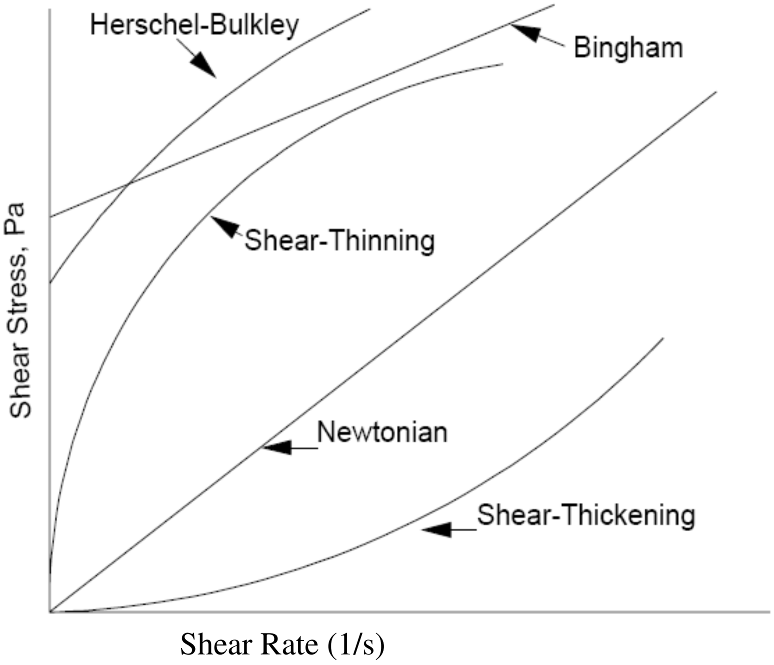


FIGURE 2.2. Basic shear diagram of shear rate versus shear stress for the classification of time-independent flow behavior of fluid foods (Bourne, 2002).

b) ***Time dependent fluid emulsions:*** These exhibit shear rate as a function of both magnitude and duration of shear, and time lapse between two successive shearing actions (Figure 2.3).

   i. ***Thixotropic flow:*** The apparent viscosity decreases with shearing time but the change is reversible. This phenomenon is explained by the breakdown of forces that exist between particles of a system which return back to original state on standing, e.g. starch gel pastes, starch-thickened baby food or yogurt.

   ii. ***Rheopectic flow:*** The apparent viscosity increases with time of shearing and the change is irreversible, e.g. egg white foam and whipped cream. When egg white is beaten, its viscosity increases until it becomes stiff, in other words it thickens.

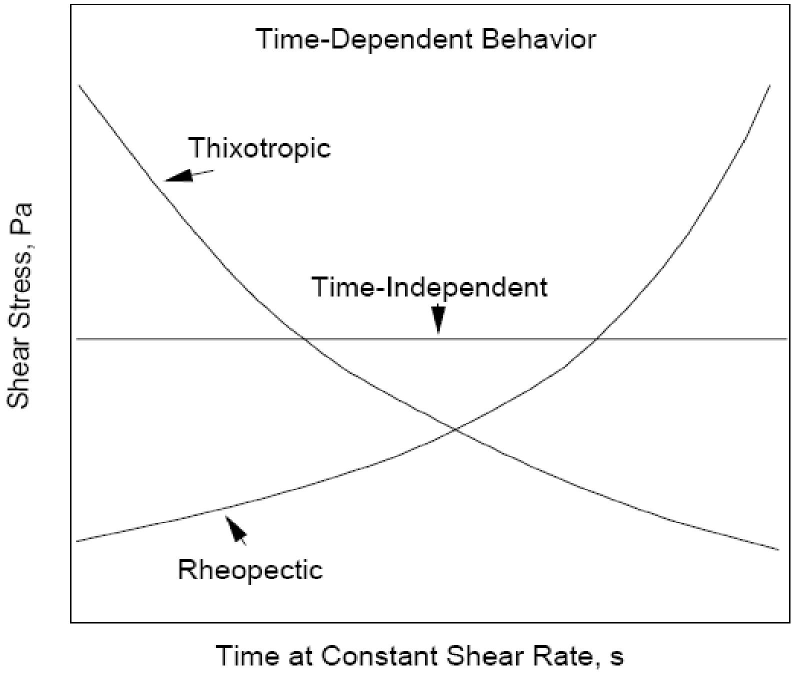


FIGURE 2.3: Time dependent non-Newtonian fluids (Bourne, 2002)

3. Viscoelastic emulsions: The emulsions which exhibit both the viscous and elastic properties simultaneously are viscoelastic (Bourne, 2002). When a deforming force is applied on a viscoelastic emulsion, it continuous to deform as long as the force is pressing against it. When the force is removed there is some recovery or the original shape, due to elastic component, also calculated as storage (or elastic) modulus. But the recovery is not complete because of the viscous component. Due to this partial recovery, some energy is lost which is defined as the loss (or viscous) modulus. The rheological properties of viscoelastic materials are therefore characterized as

$$G^* = G' + iG'' \qquad \text{(Eq 2.6)}$$

Where G’ is known as the storage modulus and G” as the loss modulus.

2.8.1.2 Factors affecting emulsion rheology:

1. ***Dispersed phase volume fraction:*** One of the most important factors which affect the emulsion rheology is the oil phase volume fraction. The viscosity of an emulsion increases linearly with oil phase volume fraction.

2. ***Viscosity of the dispersed droplets:*** Another factor that may affect the rheology of the emulsions is the viscosity of the dispersed droplets. The viscosity of an emulsion increases linearly with dispersed phase volume fraction.

3. ***Droplet size distribution:*** The third factor that affects emulsion rheology is the droplet size distribution and polydispersity. The effect of both droplet size and droplet size distribution on the rheology of an emulsion depends upon oil phase volume fraction and the nature of the colloidal interactions. At a critical distance of separation between the droplets, which depends on droplet size, the viscosity shows a rapid increase.

4. ***Continuous phase:*** Three main properties of the continuous phase must be considered. The first and the most important is the viscosity of the medium. The viscosity of the emulsion is directly proportional to the viscosity of the water phase. Addition of additives such as excess emulsifiers and thickeners (e.g. polysaccharides) may also affect the viscosity. The second property of the medium is the chemical composition such as polarity and pH which affect the charge on the droplets and hence their repulsion. The viscosity of the emulsion is directly related to the magnitude of the repulsive forces. The latter are also affected by the nature and concentration of electrolyte in the system, which represents the third important attribute of the aqueous phase.

5. ***Interfacial film:*** The rheology of emulsion may also be influenced by the interfacial rheology of the emulsifier film surrounding the droplets. When the shear is applied to an interfacial film, its constituent molecules as well as the molecules of the oil and water phases in its immediate vicinity are displaced from their equilibrium positions. This will have an effect on the interfacial viscosity of the film, consequently on the bulk rheology of the emulsion, if the latter is formed from large deformable droplets. The nature and concentration of the emulsifier, in particular, its solubility and distribution in both phases, also have a large effect on the rheology of the system (Tadros, 1994; McClements, 2004b; Friberg et al., 2004).

Hence, rheological characterization of emulsions provides important information to improve and optimize production and manufacturing processes of emulsions. Parameters from rheological tests are interwoven, complement each other, and describe properties of formulations during processing, application and storage of emulsions. Therefore, reliable research and a thorough understanding of rheological properties of emulsions is necessary.

2.9 Beverage emulsions:

Beverage emulsions are an exclusive class of oil-in-water emulsions. This is because first they are prepared in a concentrated form as an emulsion concentrate and later diluted in an acidified sugar solution or milk beverage to get the final beverage known as beverage emulsion. When the emulsion concentrate is diluted, it usually undergoes a dilution of several hundred times. The concentration of oil droplets in dilute beverage is less than 0.2% which is responsible for their slightly opaque or turbid appearance. Beverage emulsions can be categorized into beverage flavor emulsions (to provide a combination of flavor, cloudiness and color to finished products) and beverage cloud emulsions (which specifically provides just the cloudy appearance to the beverage without any flavor). These oil-in-water emulsions contain a dispersed oily phase comprised of essential oil, vegetable oil and weighting agents and continuous phase containing water, emulsifiers (proteins or polysaccharides), citric acid, preservatives and color. The emulsions in both concentrate and diluted forms must have a high degree of stability. Consequently, the stability of cloud or flavor emulsions for a desired period of time becomes a common concern in the beverage industry and ringing or creaming has been a frequent problem in beverages. The main origin and nature of the instability mechanisms are gravitational separation (creaming), flocculation, and coalescence. The rate of the creaming could be characterized through assessment of particle size distribution, electrical charge and zeta potential, polymer surface activity, oil droplet viscosity, and water phase rheology (Taherian et al., 2008). This stability is easy to achieve in concentrated form. The reason is the higher viscosity of the concentrate due to the high concentration of hydrocolloids which act as stabilizer. Stability decreases when the emulsion concentrate is dispensed in a second water phase. Hydrocolloids serve as emulsifiers and stabilizers in beverage emulsions. Hydrocolloids stabilize these emulsions through viscosity effects, steric hindrance and electrostatic interactions (Buffo et al., 2001; Tan, 2004).

2.9.1 Phase component of beverage emulsions:

Beverage cloud emulsions are composed of two immiscible phases namely oil phase (dispersed phase) and water phase (continuous phase).

2.9.1.1 Oil phase

Oil component is one of the chief ingredients of beverage emulsions. It is responsible for providing cloudy appearance to the beverage. The clouding agent must contribute to the opacity without affecting the stability of the cloud (creaming, ringing or separation), color, taste or odor of the finished beverage (Garti et al., 1991). The oil phase in beverage emulsions normally contains a mixture of non-polar carrier oils (e.g., terpenes), flavor oils, and weighting agents (Harnsilawat et al., 2006). Pure and deodorized vegetable oils are also commonly used with terpenes in the cloud emulsion. In addition to vegetable oils, edible waxes can also be used. Weighting agents are a group of substances added to the oil to increase its density and prevent creaming. A large variety of materials are being used and investigated in producing cloud beverages such as brominated vegetable oil (BVO), ester gum (EG), dammar gum (DG), and sucrose acetate isobutyrate (SAIB). BVO is made by bromination of vegetable oil. Ester gum is made by esterification of wood rosin with glycerol. Damar gum is a natural exudate obtained from the shrubs of the *Caesalpinaceae* and *Dipterocarpaceae* families. SAIB is made by the esterification of sucrose with acetic and isobutyric anhydrides. BVO, SAIB and ester gum are predominantly used in beverage emulsions. Certain limitations of these materials have been approved individually by many countries, however, none has been approved universally (McClements, 2000; Tan, 2004; Given, 2009).

2.9.1.2 Water phase:

Water phase is a major component of beverage emulsions which consists of 60-70% of the formulation and can reach to 80% for some rare compositions. The water phase components are:

Hydrocolloids: Hydrocolloids stabilize beverage emulsions through viscosity effect, film formation, steric hindrance, and electrostatic interactions (Buffo et al., 2001; Tan, 2004). There are many hydrocolloids used in beverage emulsions: gum Arabic, modified food starch, whey protein, gelatin, pectin, whey protein, iotacarrageenan, chitosan, propylene glycol alginate, Xanthan gum, gellan gum, guar gum etc. Hydrocolloids have been studied and their work has been summarized in many publications (Ray et al., 1995; Tadros, 1996; Chanamai and McClements, 2002; Anonymous, 2005;

Surh et al., 2005; Aoki, 2005; Taherian, 2006; Taherian et al., 2006; Harnsilawat et al., 2007; Sosa-Herrera et al., 2008).

To perform as an effective stabilizer for beverage emulsion, the hydrocolloid must have the following characteristics (Friberg et al., 2004):

1) It should have high solubility in cold water and must solubilize readily.
2) It should rapidly reduce the interfacial tension at the freshly formed oil–water interface
3) It should have high emulsifying property.
4) It should not thicken or gel on ageing.

Surface-active hydrocolloids:

1. Proteins

- Casein & Whey protein: The most commonly used proteins in food applications are the caseins and whey protein derived from bovine milk. Caseins are a group of proline-rich proteins with relatively small secondary structure and low solubility at pH 4.6 and are much more stable to heat. Whey protein on the other hand, are a group of globular proteins which have a compact and ordered molecular structure, organized in tight conformations, such as α- helices, β-sheets, and stabilized by bridges, are heat-sensitive (Aken, 2004; Dickinson and Parinson, 2006).

- Gelatin: Gelatin, one of the most popular biopolymers, is widely used in food, pharmaceutical, cosmetic, and photographic applications because of its unique functional and technological properties. In the food industry, gelatin is utilized in confections, low-fat spreads, dairy, baked goods to provide emulsification, gelling and stabilization, and also in meat products. Gelatin is a relatively high molecular weight protein derived from animal collagen, e.g. pig, cow or fish. It is prepared by hydrolyzing collagen by boiling in the presence of either acid (Type ‘A’ gelatin) or alkali (Type ‘B’ gelatin). The structure is broken down to such an extent that warm water soluble collagen i.e. gelatin is formed (Karim and Bhat, 2009).

The iso-electric point of gelatin type ‘A’ (~7–9) is higher than that of gelatin type ‘B’ (~5). Some previous studies have shown that gelatin is surface-active and that it is capable of acting as an emulsifier in oil-in-water emulsions. Yet, it is important to verify whether gelatin can be used to prepare emulsions that are physically stable as well (Surh et al., 2006).

2. Polysaccharides

- Gum Arabic: Gum Arabic is the most commonly used hydrocolloid in beverage emulsions. It is a dried exudate from the stems and branches of trees of the genus Acacia. The best commercial grades used for emulsions give clear solutions when dissolved in water, are viscous, colorless, tasteless and odorless.

- Modified starch: Modified starch is being most widely accepted as an alternative to gum Arabic for use as a stabilizer. They are a group of specially designed starch derivatives with balanced lipophilic and hydrophilic groups on the starch molecules (Friberg et al., 2004; Tan, 2004).

  The level of surface activity of gum and modified starch is low in comparison with typical food protein emulsifiers. To compensate for this in generating stabilized droplets, it is necessary to add a high gum-to-oil weight ratio, approximately, 1:1, as compared with 1:10 for equivalent protein-stabilized emulsions (Dickinson, 2003).

3. Hydrocolloid thickeners

- Xanthan Gum: Xanthan gum is produced by fermentation of bacterium *Xanthomonas campestris*. Aqueous solutions of Xanthan exhibit strong pseudoplasticity. This pseudoplasticity makes it advantageous for use in beverages. It is used in fruit beverages to suspend fruit juices, prevent oil ringing and by providing viscosity (even at low concentrations) without masking natural flavor of the fruits. It gives enhanced mouthfeel with less viscous taste and good flavor release to the fruit beverage. Xanthan gum is found to be stable at wide range of pH (Imeson, 1997; Angelo, 2006).

- Propylene glycol alginate (PGA): Alginate is a natural hydrocolloid usually extracted from certain species of brown seaweed. The alginic acid from this seaweed is reacted with propylene oxide to produce propylene glycol alginate. This PGA is widely used as a stabilizer and thickening agent in fruit and milk beverages (McClements, 2004a).

Acids: Acids in the drinks are to provide taste and control pH. In a beverage emulsion, acid also provides protection against microbial growth. Citric acid is the most commonly used acid in beverages.

Preservatives: Benzoic acid or sodium benzoate is added to beverage emulsion as a preservative. However, the preservation of beverages by benzoic acid is most effective at pH less than 4.5 (Tan, 2004).

CONNECTING STATEMENT TO CHAPTER 3

Flavor or cloud emulsions are added to fruit beverages to give similar opaque appearances as juices. Many studies have focused on preparation of beverage cloud emulsions using different hydrocolloids by addition of weighting agents and evaluating their rheological measurements. Only few studies have focused on the rheological properties of cloud emulsions in the absence of weighting agents. Chapter 3 focuses on the rheology of concentrated oil-in-water emulsions prepared with different emulsifiers without using weighting agents. Protein and polysaccharide emulsions at different concentrations were prepared at two pH levels and their steady shear and dynamic rheological characteristics were determined. This study also focused on conjugated emulsions formed by combining different emulsifiers with xanthan gum and propylene glycol alginate.

Part of the results of this study has been presented at the following conference:

Arora JK, Ramaswamy, HS, Taherian, AR. 2009. Rheology and stability of oil-in-water beverage emulsions. Agricultural and Biosystems Engineering Technical Conference, March 25, 2009, Food Research and Development Center, Saint-Hyacinthe, QC, CANADA.

A manuscript is also being prepared for publication based on the studies highlighted in this chapter:

Arora JK, Ramaswamy, HS, Taherian, AR, 2009. Effect of formulation and pH on the rheological and physical properties of concentrated oil-in-water beverage emulsions.

All experiment work and data analysis were carried out by the candidate under the overall supervision of Dr. H.S. Ramaswamy. Dr. Taherian provided the technical supervision to set up the experiments, train the candidate and analyze the results.

CHAPTER 3

EFFECT OF FORMULATION AND PH ON THE RHEOLOGICAL AND PHYSICAL PROPERTIES ON CONCENTRATED OIL-IN-WATER BEVERAGE EMULSIONS

3.1 ABSTRACT

Steady and dynamic shear rheological properties of concentrated oil-in-water beverage emulsions prepared with different hydrocolloids were investigated both individually and in combination formulations. Emulsions were prepared with addition of 10% canola oil into the hydrated solutions of gelatin type ‘A’ and type ‘B’, modified starch and modified gum Arabic, Xanthan gum and propylene glycol alginate (PGA) in a buffer solution at pH of 3.4 and deionized water (pH 7.0). The concentration effects of different hydrocolloids on the rheological properties were studied at the two pH levels. The results showed that an increase in concentration of hydrocolloid was associated with an enhancement in viscous and elastic properties of concentrated emulsions. pH had a significant effect on the apparent viscosity on protein stabilized emulsions. With the exception of emulsion prepared with gelatin type ‘B’ in combination with Xanthan gum and PGA, the flow behavior index (n) for concentrated emulsions varied from 0.88 to 0.97. The emulsion containing gelatin type ‘B’, Xanthan gum and PGA indicated significantly higher viscosity with an “n” value of 0.33.

3.2 INTRODUCTION

Oil-in-water (O/W) emulsions are prepared by homogenizing the oil phase into an aqueous phase in the presence of an emulsifier which can help to enhance the kinetic stability of the emulsion (Buffo and Reineccius, 2002; McClements, 2004a). Beverage emulsions are a class of oil-in-water emulsions. The oil phase is the oil blended with weighting agents and, water or the aqueous phase which is the key component in beverage emulsions which holds various hydrocolloids, acid, preservatives and/or colors together (Tan, 1998). These emulsions are different from other food emulsions in that they are to be consumed in a highly diluted form, rather than in their original concentrate form. They are first prepared as an emulsion concentrate and later diluted into the beverage in order to produce the finished product. In soft drinks, the beverage emulsion may provide flavor, color and cloudy appearance for the beverage, or just the cloudiness.

The emulsions in both the concentrate and the dilute form must have a high degree of stability. This stability is difficult to achieve as oil droplets in the dispersed form have lower specific gravity than the water phase. Therefore, weighting agents (for example brominated oil) are used to balance the density difference between oil and the continuous aqueous phase. They have density higher than the flavoring oil and contain lipophilic compounds soluble in oil (Tan, 2004). Beverage emulsions are thermodynamically unstable systems that tend to break down during storage. The most common sign of beverage emulsion deterioration is “ringing” or “oiling-off”. Ringing is the formation of a whitish “ring” around the neck of the container, whereas oiling-off is the formation of a shiny oil slick on top of the product. These defects are the result of certain physicochemical mechanisms that occur within the dilute beverage, including gravitational separation (resulting in creaming), flocculation, and coalescence (Chanamai and McClements, 2000).

Creaming occurs due to the imbalance between oil and water phase density because of which oil droplets tend to move upwards. Flocculation occurs when oil droplets of the dispersed phase form, stick together retaining their individual integrity, which accelerates the rate of creaming. In emulsion concentrates, a perceptible increase in viscosity is observable when flocculation occurs (Taherian et al., 2008). Coalescence implies localized disruption of neighboring droplets in aggregates, so that oil droplets merge together to form large ones. This leads to a decrease in the number of droplets, enhances creaming and can eventually cause emulsion breakdown. Ostwald ripening is defined as the growth of larger droplets at the expense of smaller ones, due to mass transport of soluble dispersed phase through the continuous medium. Ostwald ripening is negligible unless the dispersed phase is at least sparingly soluble in the continuous phase. Since essential oils are somewhat soluble in water, beverage emulsions are prone to Ostwald ripening (Buffo and Reineccius, 2001; Comas et al., 2006; Taherian et al., 2006; Mirhosseini et al., 2007). The rate of creaming is governed by Stokes law (Eq 3.1);

$$U_{stoke} = \frac{2gr^2 (\rho_{oil} - \rho_w)}{9\eta_w} \qquad \text{(Eq 3.1)}$$

Where 'U_{stoke}' is the rate of creaming or sedimentation, 'g' is the acceleration due to gravity, 'r' is the oil droplet radius, 'ρ_{oil}' is the density of oil phase, 'ρ_w' is the density of the water phase and 'η_w' is the viscosity of the water phase. The sign of U_{stoke} determines whether the particle move upward (-) or downward (+). Therefore, reducing the diameter of suspended globules and increasing the water phase viscosity will reduce the settling or creaming rate of the cloud emulsion in a beverage by a factor of r^2.

Rheology of beverage emulsions is a subject of high importance for industrial applications. Primarily, the rheology of emulsions is a direct manifestation of the various interactions that occur within an emulsion system (Tadros, 1994). Understanding the rheological behavior of emulsions has been of great interest for its strong relationship to many properties of emulsions that are vital for various applications in food industry. Creaming of oil droplets in beverage emulsions is strongly dependent on the viscosity of the component phases. Oil phase volume fraction, droplet size distribution, colloidal interactions, chemical composition (polarity and pH which affect the charge on the droplets and hence their attraction or repulsion) are other major contributors to the emulsion rheology. In dilute systems, where the dispersed phase volume fraction is less than 0.05 (beverage emulsions), the emulsion is a fluid with a relatively low viscosity that is dominated by the viscosity of the continuous phase. In addition to flow rheology, examination of dynamic properties is also essential in the characterization of concentrated beverage emulsions.

Emulsion stabilization is usually achieved by adding a small amount of surfactant molecules and/or proteins, and/or thickening agents to the water phase. These hydrocolloids in water phase promote the emulsion formation and short term stabilization by interfacial action. The term hydrocolloid includes many proteins, polysaccharides that are extracted from plants, seaweeds as well as gum derived from plant exudates, modified biopolymers made by the chemical and enzymatic treatment of starch or cellulose. The main stabilization of food polysaccharides is via viscosity modification or gelation in the aqueous continuous phase (Garti, 1999; Dickinson, 2003; Mirhosseini et al., 2008a). For a polymer to be effective as an emulsifying agent it must be surface active, it should have the capacity to lower the tension of the oil-water interface. It should be amphiphilic with a substantial degree of hydrophobic character. It should also be in sufficient quantity to cover the droplet surface and form a thick steric stabilizing layer around the droplet. To retain small droplets, time between droplet-collisions should be long compared with the time of emulsifier to adsorb at the new oil-water interface and to create the transient stabilizing layer (Dickinson, 2003; Taherian, 2006).

Modification of rheological properties of water phase has been a promising alternative to weighting agents in beverage emulsions. Addition of biopolymers such as gum Arabic, modified starch, pectin etc can modify emulsion rheology by raising the viscosity of the continuous phase or by causing adhesion between droplets without coalescence (Mason, 1999). Polysaccharides form three-dimensional network of interacting or entangled molecules that traps the droplets and effectively inhibits their movement. They provide specific rheological properties for achieving stability of the emulsion (McClements, 2000). Gum Arabic is one of the most common emulsifiers currently used to stabilize beverage emulsions. Modified gum Arabic (MGA) used in this study is a cold water soluble hydrocolloid. MGA is produced by reacting natural acacia with l-octenyl succinic anhydride (OSA) in a production process that is similar to that used to produce modified food starch-OSA (Sklar, 2008). It has been enriched with a protein rich high molecular weight component (the arabino-galacto-protein complex) which provides emulsifying ability to the gum Arabic (Garti, 1999). Modified starches are also widely used in the beverage industry. One of the most commonly used modified starches (Purity Gum BE) is an octenyl succinate derivative of waxy-maize which is also used in this work. It consists primarily of amylopectin that has been chemically modified to contain a side group that is anionic and non-polar. Modified starch is known to be stable over a wide range of pH (Chanamai and McClements, 2002; McClements, 2004b).

Many proteins are surface-active ingredients as well that can be used as emulsifiers because of their ability to facilitate the formation, improve the stability and produce desirable physiochemical properties in beverage emulsions (McClements, 2004a). Proteins used in this research are gelatin type

‘A’ and type ‘B’. Gelatin, one of the most popular biopolymers, is widely used in food, pharmaceutical, cosmetic, and photographic applications because of its unique functional and technological properties. In the food industry, gelatin is utilized in confections, low-fat spreads, dairy emulsions, baked goods, beverages, soups, sauces etc as thickening and gelling agents, and also to provide emulsification. Gelatin is a relatively high molecular weight protein derived from animal collagen, e.g. pig, cow or fish. It is prepared by hydrolyzing native collagen by boiling in the presence of either acid (type ‘A’) or salt (type ‘B’), resulting in partial cleavage of the cross links (Karim and Bhat, 2009). The iso-electric point of gelatin type ‘A’ (~7–9) is higher than that of gelatin type ‘B’ (~5). The relatively high iso-electric point of type ‘A’ means that oil-in-water emulsions can be created that have a positive charge over a wider range of pH values than other regularly used protein emulsifiers, such as soy, casein or whey proteins. Consequently, gelatin type ‘A’ may be suitable for creating oil-in-water food emulsions with high oxidative stability. Some previous studies have shown that gelatin is surface-active and that it is capable of acting as an emulsifier in oil-in-water emulsions (Surh et al., 2006). Yet, it is important to verify whether gelatin can be used to prepare beverage emulsions that are physically stable as well. It is postulated that the interactions between gelatin and surfactants may lead to formation of various surfactant-protein complexes, which also have surface activity, and stabilize emulsions (Vinetsky and Magdassi, 1997).

Protein-polysaccharide mixtures are commonly found in the food industry. In most applications, these biopolymers are used to formulate emulsion-based food systems. Mixing polysaccharides and proteins in aqueous systems may result in miscible solutions or co-solubility and improve protection against environmental stresses in the dilute beverage emulsions (Aoki et al., 2005). Relatively thick and highly charged interfaces can be produced using these protein-polysaccharide complexes, which means that steric and electrostatic repulsion between the droplets can be increased, thereby improving droplet stability to aggregation (Harnsilawat et al., 2006). With this regard, the stabilization effect of various gums has been the subject of many studies. There are reports on using hydrocolloid materials such as Xanthan gum, propylene glycol alginate, pectin, gellan gum, gum tragcanth, ghatty gum and carboxymethylcellulose in addition to surface active biopolymers to enhance the stability of flavor and cloud emulsions (Tan, 2004). Xanthan gum (XG) is an anionic microbial polysaccharide produced by the bacterium, *Xanthomonas campestris*. It was developed at Northern Regional Research Laboratory in Peoria, Illinois and approved by the FDA for use in food (Coia and Stauffer, 1987). The most important properties of XG are high low-shear viscosity and strong shear-thinning character exhibited even at low concentrations. This high viscosity behavior gives the product advantage of easy pourability, mixing or pumping, provides good suspension properties and lends stability to colloidal suspensions (Imeson, 1997; Sun et al., 2007).

Alginates are also important hydrocolloids used in the food industry. Alginic acid, the free acid form of alginate, is the intermediate product in the commercial manufacture of alginates. Propylene glycol alginate (PGA) is a derivative of alginic acid formed by reacting alginic acid with propylene oxide. It is a high molecular weight compound with molecular weight ranging from 30,000 to 200,000 Daltons. Many of the food emulsions are also stabilized by PGA. In contrast to XG, PGA displays a low degree of pseudo-plasticity in solution and promotes creaminess without significant rheological changes (Pettitt et al., 1995; Imeson, 1997).

There is a limited amount of published information on rheological characteristics of beverage emulsions stabilized with gelatin, and their comparison with other widely used hydrocolloids. Hence, the objective of this study was to evaluate rheological properties as affected by: (a) concentration & pH of selected proteins (gelatin type ‘A’ & type ‘B’) & polysaccharides (modified starch, modified gum Arabic) (b) conjugation of proteins (gelatin type ‘A’ & type ‘B’) with polysaccharides (modified starch, modified gum Arabic, Xanthan gum & propylene glycol alginate). This study would help to optimize the quality of oil-in-water beverage formulation without using the regulated weighting agents.

3.3 MATERIALS AND METHODS

3.3.1 Materials

Commercially available refined vegetable canola oil was obtained from the local market. Modified starch (Purity Gum^{TM} Be) was obtained from National Starch (Bridgewater, NJ), gelatin type ‘A’ and type ‘B’ from Rousselot (a VION Company, Dubuque, IA). Modified gum Arabic, viscosity builders: Ticaxan Xanthan 200 (Xanthan gum) and, Propylene glycol alginate (PGA/LV Powder) were obtained from TIC GUMS (Belcamp, MD). Food grade citric acid and dibasic sodium phosphate were used to prepare buffer solutions and were bought from Fisher Scientific (Montreal, QC). Deionized water was used to prepare solutions and emulsions.

3.3.2 Emulsion preparation

Hydrocolloids were introduced into the buffer solution at pH 3.4 or deionized water at pH 7.0. The water phases were prepared using four different hydrocolloids at two different levels: gelatin type 'A' (0.75 & 1% w/w) & gelatin type 'B' (0.75 & 1% w/w), modified starch (6 & 12% w/w) & modified gum Arabic (6 & 12% w/w). Hydrocolloids were added to water phase and stirred using a high speed glass mixer (Waring Commercial Blender, Montreal, QC). The mixtures were kept overnight to allow complete hydration. Then the oil was added to the water phase at a fixed level of 10% w/w with continuous mixing. The mixing was first done using the glass mixer for 5 min followed by high speed homogenization using an ultrasonic homogenizer for 90s (at intervals to prevent heating) at 75% amplitude for final homogenization. Sodium azide (0.02% w/w) was added to the emulsions to inhibit any microbial growth. For conjugate emulsions, gelatin and polysaccharide powders were mixed and hydrated. Thickening agents (Xanthan gum and PGA) were dissolved separately in aqueous solutions and stirred in the emulsion containing hydrocolloids.

3.3.3 Evaluation of specific gravity

Specific gravity of each water phase and emulsion was evaluated at the given concentrations using a 50 mL specific gravity bottle (Fisherbrand, Montreal, QC).

3.3.4 Flow and dynamic rheological measurements:

For rheological measurements AR2000 Rheometer (TA instruments, New Castle, DE) was used. The sample was placed in 60mm cone geometry of 2° and solvent trap at room temperature. Flow curves were determined at increasing shear rate (0.1-100 s^{-1}) within 8 minutes. Viscosity was calculated as a function of shear rate. Experimental flow curves were compared to Power's Law Model:

$$\eta = m\dot{\gamma}^{(n-1)} \qquad \text{(Eq 3.2)}$$

Where n = 1 for a Newtonian fluid, n < 1 for a shear thinning fluid and n > 1 for a shear thickening fluid. Variation in consistency coefficient (m) and flow behavior index (n) values were determined and apparent viscosity (at a particular stress rate of 1 s^{-1}) were determined at different levels of concentration and 2 different pH.

Emulsion rheology was measured for the four hydrocolloids i.e. proteins (gelatin type 'A' and gelatin type 'B') and polysaccharides (modified starch and modified gum Arabic) at two different concentrations 0.75 & 1% w/w and 6 & 12% w/w, respectively. Measurements were done immediately after preparation. The emulsions were prepared at two pH levels, i.e. buffer at pH 3.4 and deionized water corresponding pH 7.0. High values of coefficient of determination ($R^2 > 0.98$) indicated a good fit for all the flow measurements. Standard deviation and coefficient of variance were also computed for all samples.

For dynamic or oscillatory measurements, a frequency sweep from 1 up to 50 radians per sec was given at fixed oscillation stress (1 Pascal) and G' (elastic modulus) and G" (viscous modulus) were obtained. Measurements were done in triplicate per each emulsion.

Dynamic measurements were conducted to describe the rheological properties of emulsions more clearly. The storage modulus (G'), loss modulus (G") and phase angle (δ) are among the parameters that characterize a system in a dynamic rheological study. The region of linear viscoelasticity was determined prior to each frequency sweep performing stress sweeps to verify the linear relationship between stress and strain. G' is a measure of the energy stored in a cycle of oscillation whereas G" is the measure of energy lost as viscous flow in a cycle of oscillation. The focus was to study the loss modulus (G") and storage modulus (G'). For a purely viscous system, $G' = 0$ and $G'' = G^*$, where G^* is the complex modulus. Conversely, $G' = G^*$ and $G'' = 0$, if the system is purely elastic.

3.3.5 Statistical analysis

The effect of hydrocolloid concentration and pH on the rheology of water phase and emulsions were statistically tested using ANOVA and the means were compared at a significant level of 5%. Statistical analysis was done using Microsoft excel and triplicate readings were taken.

3.4 RESULTS AND DISCUSSION

Steady shear flow properties

Table 3.1 & 3.2 show the flow properties of concentrated protein and polysaccharide stabilized emulsions. An empirical power law equation was used to calculate apparent viscosity. Apparent viscosity at a particular shear rate of 1 s^{-1} was used for quality comparison. In general, increase in emulsifier concentration contributed to change the flow behavior (n) of water phase from Newtonian to slightly shear thinning and resulted in higher consistency coefficient (m) and apparent viscosity of water phase and emulsion at low shear rate of 1 s^{-1} which was steady during the whole shear rate as shown in Figure 3.1 & Figure 3.2 plots of apparent viscosity as a function of shear rate

TABLE 3.1(a): Flow properties of water phases alone and together in the form of emulsions as a function of gelatin concentration and pH.

Properties	Concentration of proteins (gelatin type ‘A’ and type ‘B’) stabilized emulsions in deionized water (pH 7)			
	0.75% Gelatin type ‘A’	1% Gelatin type ‘A’	0.75% Gelatin type ‘B’	1% Gelatin type ‘B’
$n_{\text{W. ph.}}$	0.93 ± 0.06	0.94 ± 0.00	0.98 ± 0.00	0.99 ± 0.00
$n_{\text{O. ph.}}$	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01
$n_{\text{Em.}}$	0.86 ± 0.01	0.85 ± 0.01	0.90 ± 0.00	0.99 ± 0.00
$m_{\text{W. ph.}}$ (mPa)	4.22 ± 0.03	9.99 ± 0.33	3.18 ± 0.05	3.32 ± 0.01
$m_{\text{O. ph.}}$ (mPa)	76.56 ± 1.25	76.56 ± 1.25	76.56 ± 1.25	76.56 ± 1.25
$m_{\text{Em.}}$ (mPa)	4.50 ± 0.12	5.45 ± 0.19	2.81 ± 0.04	4.01 ± 0.08
$\eta_{\text{app. W. ph}}$ 1 s^{-1} (mPa s)	4.22 ± 0.30	10.00 ± 0.33	3.18 ± 0.05	3.32 ± 0.01
$\eta_{\text{app. O. ph.}}$ 1 s^{-1} (mPa s)	76.56 ± 1.24	76.56 ± 1.24	76.56 ± 1.24	76.56 ± 1.24
$\eta_{\text{app. Em.}}$ 1 s^{-1} (mPa s)	4.50 ± 0.12	5.45 ± 0.44	2.81 ± 0.00	4.01 ± 0.08

TABLE 3.1(b): Flow properties of water phases alone and together in the form of emulsions as a function of gelatin concentration and pH.

Properties	Concentration of proteins (gelatin type ‘A’ and type ‘B’) stabilized emulsions in buffer system (pH 3.4)			
	0.75% Gelatin type ‘A’	1% Gelatin type ‘A’	0.75% Gelatin type ‘B’	1% Gelatin type ‘B’
$n_{\text{W. ph.}}$	0.94 ± 0.00	0.91 ± 0.02	0.98 ± 0.01	0.98 ± 0.00
$n_{\text{O. ph.}}$	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01
$n_{\text{Em.}}$	0.96 ± 0.01	0.97 ± 0.00	0.80 ± 0.01	0.84 ± 0.00
$m_{\text{W. ph.}}$ (mPa)	4.26 ± 0.04	7.30 ± 0.67	2.62 ± 0.06	13.56 ± 0.15
$m_{\text{O. ph.}}$ (mPa)	76.56 ± 1.25	76.56 ± 1.25	76.56 ± 1.25	76.56 ± 1.25
$m_{\text{Em.}}$ (mPa)	2.99 ± 0.06	3.37 ± 0.02	2.64 ± 0.08	3.04 ± 0.04
$\eta_{\text{app. W. ph.}}$ 1 s^{-1} (mPa s)	4.26 ± 0.04	6.94 ± 0.29	2.62 ± 0.06	13.56 ± 0.15
$\eta_{\text{app. O. ph.}}$ 1 s^{-1} (mPa s)	76.56 ± 1.24	76.56 ± 1.24	76.56 ± 1.24	76.56 ± 1.24
$\eta_{\text{app. Em.}}$ 1 s^{-1} (mPa s)	3.00 ± 0.06	3.37 ± 0.02	2.64 ± 0.08	3.04 ± 0.04

TABLE 3.2(a): Flow properties of water phases alone and together in the form of emulsion as a function of polysaccharide concentration and pH.

Properties	Concentration of polysaccharide (modified starch and modified gum arabic) stabilized emulsions in deionized water (pH 7)			
	6% Modified Starch	12% Modified Starch	6% Modified gum Arabic	12% Modified gum Arabic
$n_{\text{W. ph.}}$	0.97 ± 0.04	0.97 ± 0.00	0.99 ± 0.02	0.98 ± 0.00
$n_{\text{O. ph.}}$	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01
$n_{\text{Em.}}$	0.97 ± 0.00	0.93 ± 0.00	0.98 ± 0.00	0.98 ± 0.00
$m_{\text{W. ph.}}$ (mPa)	5.54 ± 0.19	20.98 ± 0.52	4.13 ± 0.06	11.56 ± 0.78
$m_{\text{O. ph.}}$ (mPa)	76.56 ± 1.25	76.56 ± 1.25	76.56 ± 1.25	76.56 ± 1.25
$m_{\text{Em.}}$ (mPa)	9.06 ± 0.25	67.10 ± 2.94	7.27 ± 0.04	26.41 ± 0.19
$\eta_{\text{app. W. ph}}$ 1 s^{-1} (mPa s)	5.54 ± 0.19	20.98 ± 0.52	4.13 ± 0.06	11.56 ± 0.78
$\eta_{\text{app. O. ph.}}$ 1 s^{-1} (mPa s)	76.56 ± 1.24	76.56 ± 1.24	76.56 ± 1.24	76.56 ± 1.24
$\eta_{\text{app. Em.}}$ 1 s^{-1} (mPa s)	9.06 ± 0.25	67.11± 2.95	7.27 ± 0.41	26.41 ± 0.19

TABLE 3.2(b): Flow properties of water phases alone and together in the form of emulsion as a function of polysaccharide concentration and pH.

Properties	Concentration of polysaccharide (modified starch and modified gum arabic) stabilized emulsions in buffer system (pH 3.4)			
	6% Modified Starch	12% Modified Starch	6% Modified gum Arabic	12% Modified gum Arabic
$n_{\text{W. ph.}}$	0.98 ± 0.00	0.97 ± 0.00	0.99 ± 0.00	0.98 ± 0.00
$n_{\text{O. ph.}}$	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01
$n_{\text{Em.}}$	0.97 ± 0.00	0.92 ± 0.00	0.98 ± 0.00	0.98 ± 0.00
$m_{\text{W. ph.}}$ (mPa)	5.36 ± 0.19	20.97 ± 0.14	3.83 ± 0.12	12.38 ± 0.08
$m_{\text{O. ph.}}$ (mPa)	76.56 ± 1.25	76.56 ± 1.25	76.56 ± 1.25	76.56 ± 1.25
$m_{\text{Em.}}$ (mPa)	9.45 ± 0.08	65.33 ± 1.89	6.75 ± 0.02	31.84 ± 0.46
$\eta_{\text{app. W. ph}}$ 1 s^{-1} (mPa s)	5.36 ± 0.02	20.97 ± 0.14	3.83 ± 0.12	12.38 ± 0.81
$\eta_{\text{app. O. ph.}}$ 1 s^{-1} (mPa s)	76.56 ± 1.24	76.56 ± 1.24	76.56 ± 1.24	76.56 ± 1.24
$\eta_{\text{app. Em.}}$ 1 s^{-1} (mPa s)	9.44 ± 0.08	65.33± 1.90	6.75 ± 0.02	31.84 ± 0.46

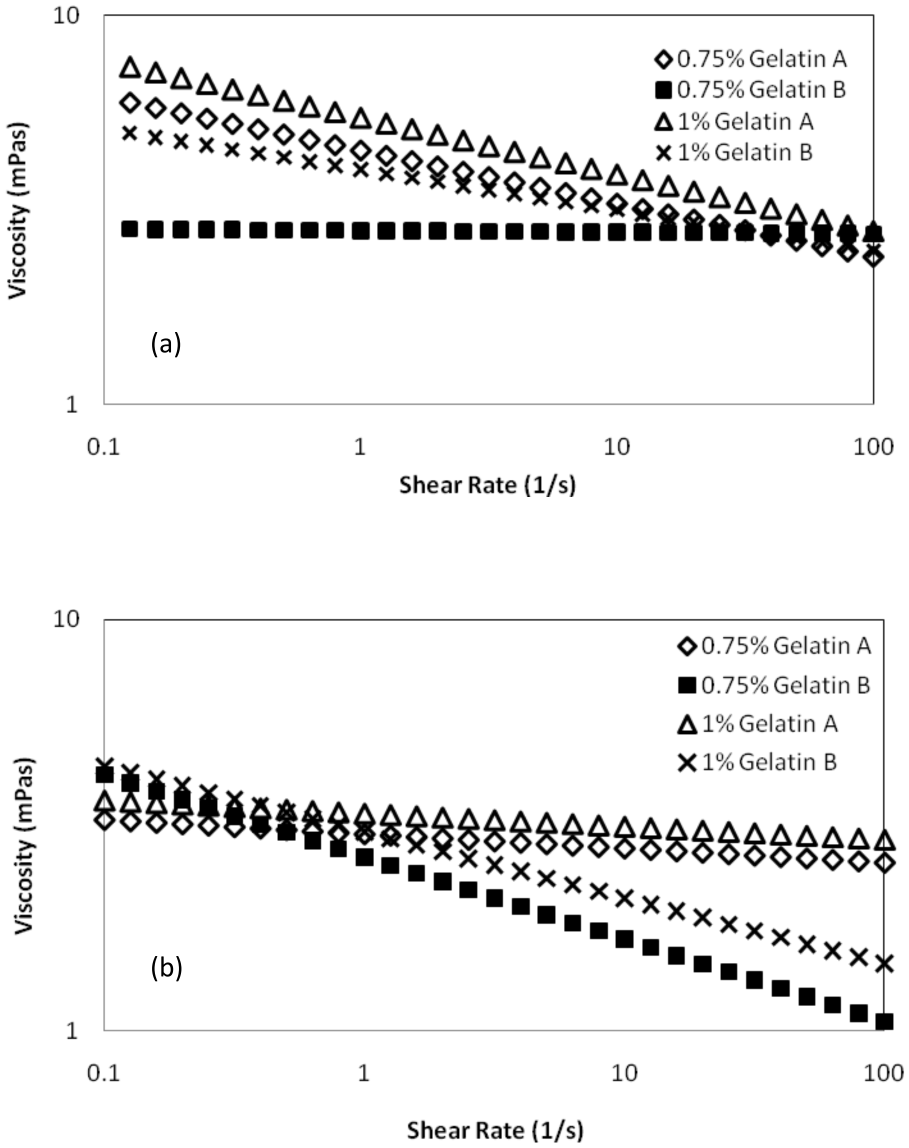


FIGURE 3.1. Apparent viscosity of gelatin stabilized emulsions at selected concentrations in deionized water at (a) pH 7.0 and in acidic buffer at (b) pH 3.4 respectively.

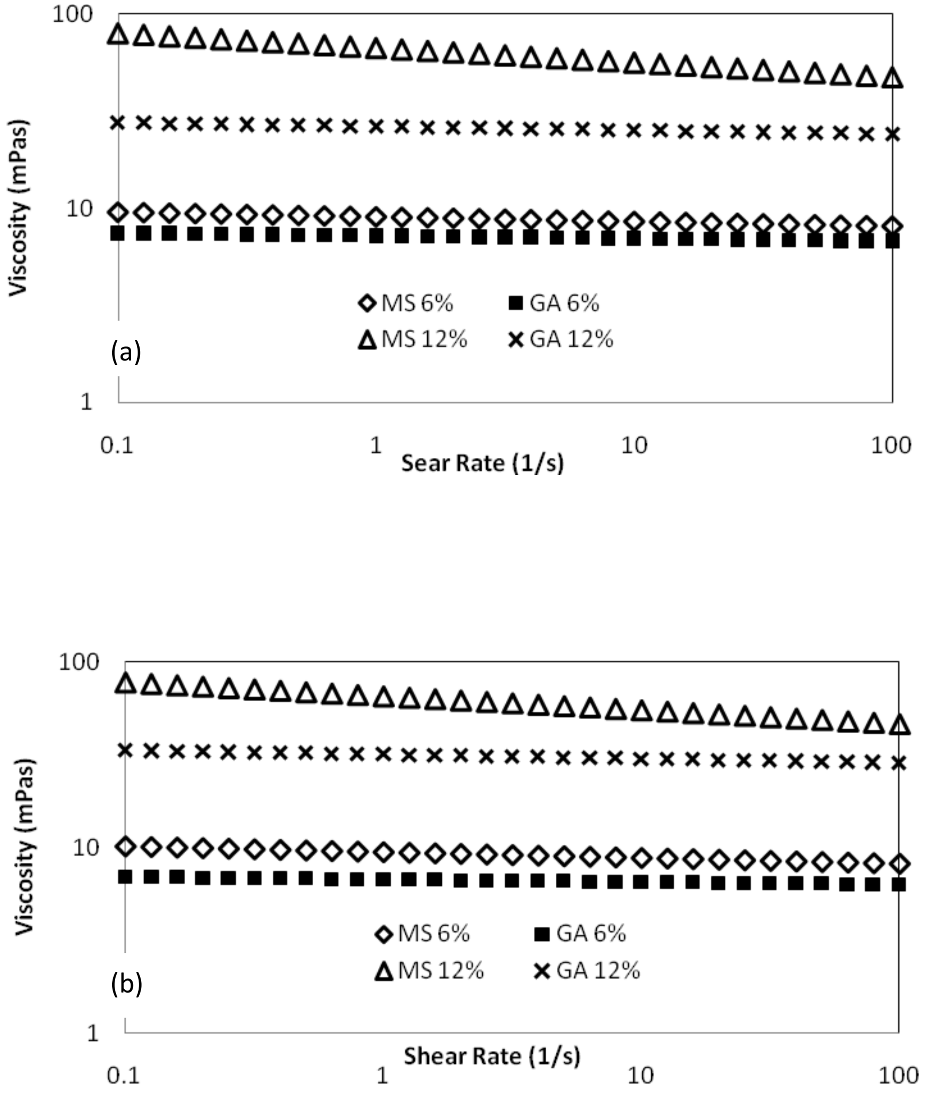


FIGURE 3.2. Apparent viscosity of polysaccharide (modified starch (MS) & modified gum Arabic (GA)) stabilized emulsions at selected concentrations in deionized water at (a) pH 7.0 and in acidic buffer at (b) pH 3.4 respectively.

(0.1 to 100 s^{-1}). The rheological properties of oil-in-water emulsions showed that the rheological behavior ranged between Newtonian and pseudoplastic.

3.4.1 Effect of concentration

The effect of emulsifier concentration on apparent viscosity and shear-thinning was investigated for two levels (0.75 & 1% w/w) for gelatin (both type 'A' and type 'B' gelatin) and two levels (6 & 12% w/w) for polysaccharide (modified starch and modified gum Arabic). For all the emulsions, concentration had a considerable effect on the viscosity. The consistency index is an indicator of the viscous nature of the system and was observed to be increasing with the increase in hydrocolloid concentration. Therefore, the apparent viscosity of the water phase as well as the fresh emulsions was observed to be increasing with the raise in concentration. Figures 3.3(a) & (b) represent the change in apparent viscosity of protein and polysaccharide stabilized emulsion as a function of concentration at shear rate of 1 $s^{-1.}$ The results indicate that higher concentrations yield higher viscosity solutions. Further, the apparent viscosity of stabilized emulsions only increased slightly with protein concentration as compared to polysaccharide emulsions which is also obvious from the flow curves. This was due to the higher concentration of polysaccharides (6 and 12% w/w) than gelatins (0.75 and 1% w/w).

Among the polysaccharides, the apparent viscosity for modified starch was above modified gum Arabic, indicating modified starch to be more effective in increasing apparent viscosity of the emulsions (thicker emulsions). Apparent viscosity value for modified starch was 65.3 mPas & 67.1 mPas and for gum Arabic it was 31.8 mPas & 26.4 mPas at 12% concentration in pH 3.4 emulsion and at neutral pH, respectively. This can be related to the increase in the layer thickness due to high concentration. Slight and sharp increase in consistency coefficients of water phase and emulsions ($m_{W.\,ph}$ and $m_{Em.}$) were related to the replacement of water with oil in emulsion formulations. Higher 'n' values almost reaching 1 were observed for all the polysaccharide emulsions; however 'n' for modified starch decreased slightly when emulsion was formed at higher concentrations. The reason for increase in consistency coefficient and apparent viscosity with concentration of emulsifying agent might relate to apparent increase in effective volume concentration of the emulsion (Suzuki et al., 1991).

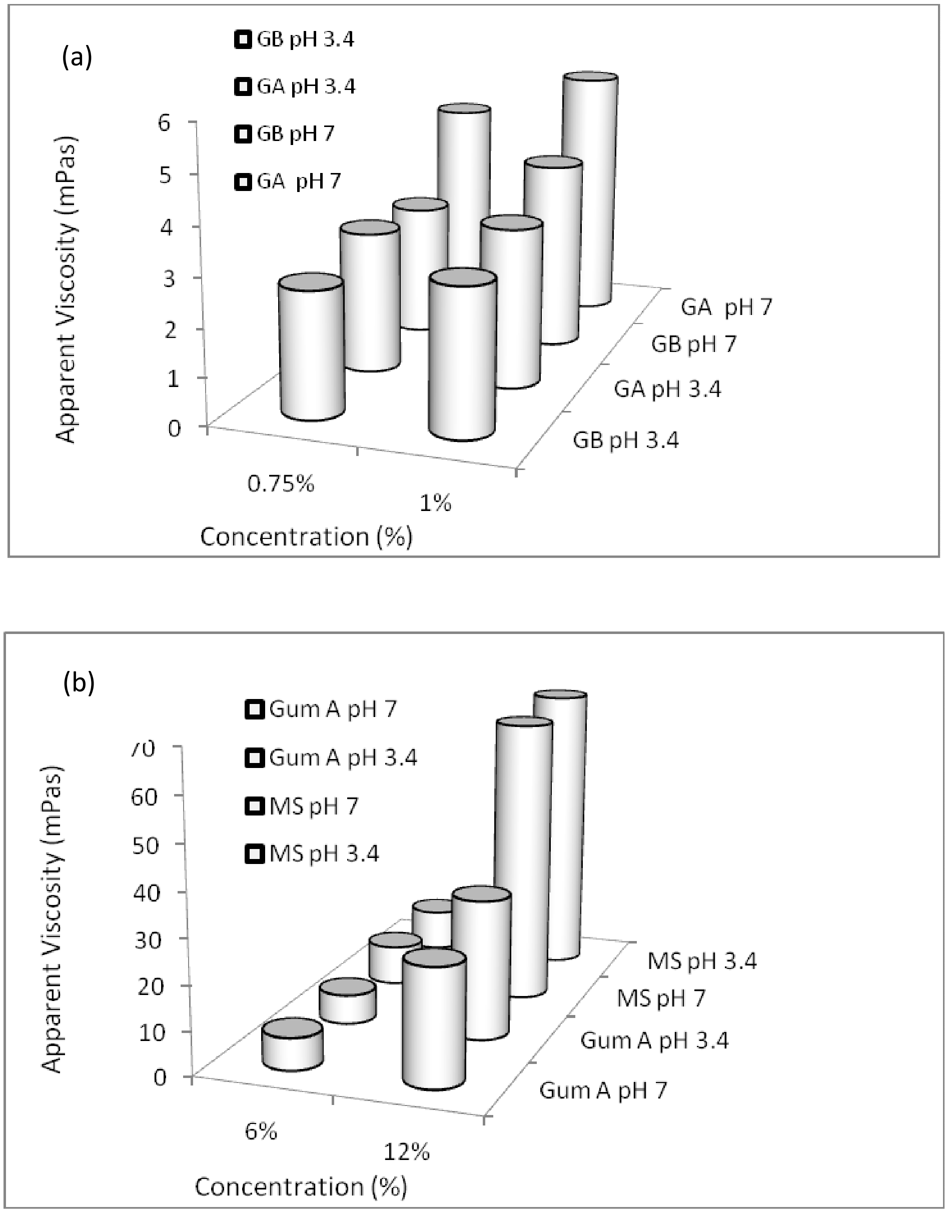


FIGURE 3.3. Apparent Viscosity at shear rate of 1/s for (a) protein stabilized emulsion at 0.75 and 1% w/w concentration and (b) polysaccharide stabilized emulsions as a function of concentration.

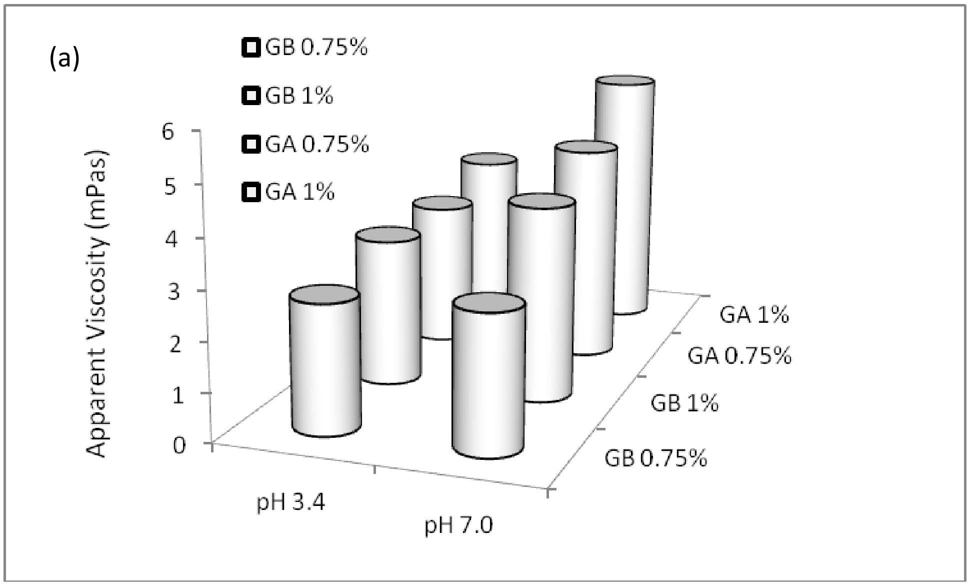
3.4.2 Effect of pH

Figure 3.4 (a) and (b) compares the apparent viscosity of protein (gelatin type 'A' and type 'B') and polysaccharide stabilized emulsions, respectively. For protein stabilized emulsions, the apparent viscosity of both gelatin type 'A' and type 'B' is higher at neutral pH than in acidic buffer at pH 3.4.

Table 3.1(a) compares the flow properties of gelatin stabilized emulsions prepared in deionized water at pH 7.0. At pH 7.0, the flow behavior indices of type 'A' emulsions (n = 0.86 for 0.75% & n = 0.85 for 1%), were lower than the flow behavior index of associated water phase (n = 0.93 for 0.75% & n = 0.94 for 1%), for both concentrations showing thinning behavior, indicating onset of flocculation or instant instability of the emulsions. Shear-thinning is the result of progressive deformation and disruption of flocs in the shear field as the shear stress is increased. This phenomenon can be due to closeness of isoelectric point of gelatin type 'A' (~7-9) to the pH of the medium which could result in formation of cold set gel and loss of repulsive force (McClements, 2004a). However, the flow for gelatin type 'B' was Newtonian with n = 0.99 at the 1% concentration, indicating the absence of cold set gel and presence of repulsive forces. The isoelectric point of this gelatin was close to 5.0 and far from isoelectric point of medium. From Table 3.1(b) which gives the flow properties for protein stabilized emulsions in pH 3.4, it was indicative that for gelatin type 'A', emulsion formation contributed to build up of Newtonian viscosity; however there was a converse effect on gelatin type 'B' emulsion resulting in aggregation of protein at pH 3.4 which was close to its iso-electric point.

Figure 3.1 makes the results clearer. At small shear rates, flocs did not separate; however at high shear rates, sudden reduction in viscosity was observed which was related to the disruption of flocs with greater shear.

Tables 3.2(a) and 3.2(b) compare the flow properties of emulsions and related phases at selected concentration and pH of modified starch and modified gum Arabic. The non-flocculated polysaccharide emulsions in this case exhibited more similar behavior at both the pH. Both polysaccharides showed less variability at different pH levels. These results suggest that emulsions prepared with modified starch and modified gum Arabic as hydrocolloid were predominantly stabilized by steric interactions, as



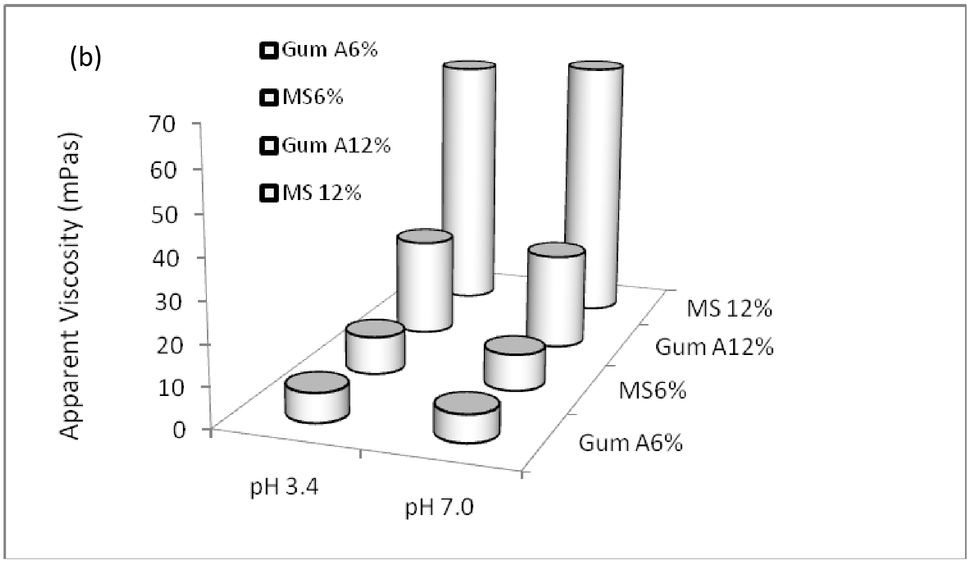


FIGURE 3.4. Apparent viscosity at shear rate of 1/s for (a) protein stabilized emulsion and (b) polysaccharide stabilized emulsions as a function of pH.

changes in electrostatic interactions (pH) did not have a significant impact on droplet aggregation (Chanamai and McClements, 2002).

Figure 3.2 compares the shear dependency of viscosity at selected shear rate range for polysaccharide added emulsions at both the pHs. The apparent viscosity decreased very slightly as the shear rate increased, suggesting less flocculation and greater stabilization characteristics in polysaccharide emulsions compared to gelatin emulsions.

3.4.3 Dynamic (visco-elastic) flow properties

Figure 3.5 shows the effect of gelatin type, concentration and pH on the frequency dependence of elastic modulus (G’) and loss modulus (G”). For all the cases, the modulus (G’ and G”) increased with increasing frequency. However, G’ increased more than G”. This increase in elastic modulus as a result of progress in frequency has been related to the closer packing of micro-gelled colloidal particles which leads to greater frictional forces between droplets subjected to shear (Valdez et al., 2006). Results also indicate that G” was greater than G’ for all the emulsions and with increase in concentration, there was an increase in viscoelastic properties of emulsions. This was clear with plotting G’ and G” at a single frequency of 10 radians per second (Figure 3.6). In Figure 3.6(a) which is for gelatin stabilized emulsions in deionized water (pH = 7.0), frequency development for gelatin type ‘A’ emulsions were much greater than gelatin type ‘B’ at same pH. However, in acidic buffer at pH 3.4 (Figure 3.6(b)), the trends were reversed. This suggests there is more solid like behavior when the protein is closer to its isoelectric point which could be due to the aggregation of protein at pH close to its iso-electric point. The dynamic properties of gelatin type ‘B’ were observed to be more stable in deionized water than in pH 3.4 buffers. Thus dynamic results are in agreement with previously stated flow rheological results.

Figure 3.7(a) & (b) represent the dynamic behavior of modified starch and modified gum Arabic stabilized emulsions in pH 7.0 and pH 3.4, respectively. The results indicated again that with increase in concentration, there was an increase in the viscoelastic properties of polysaccharide stabilized emulsions. In all the cases G” i.e. the viscous

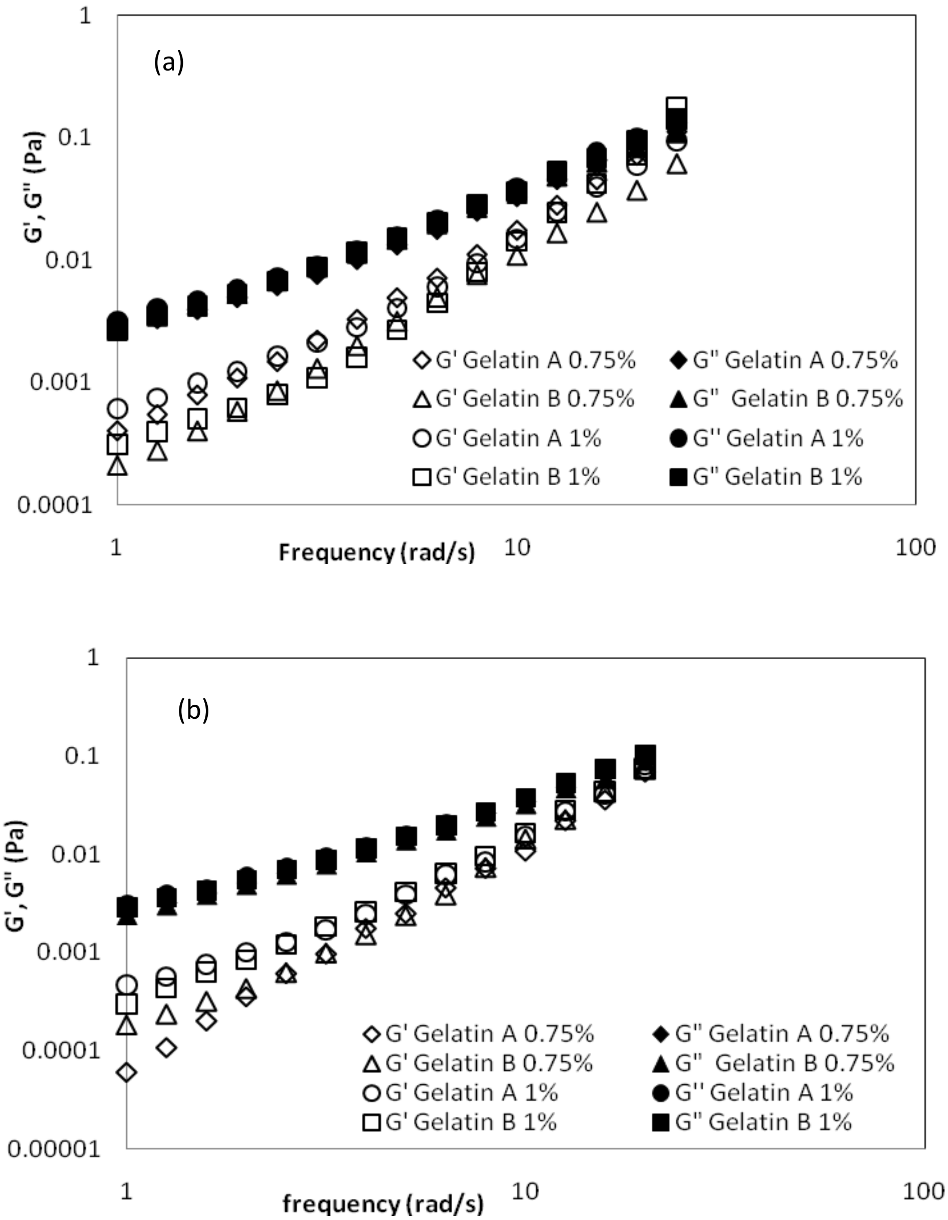
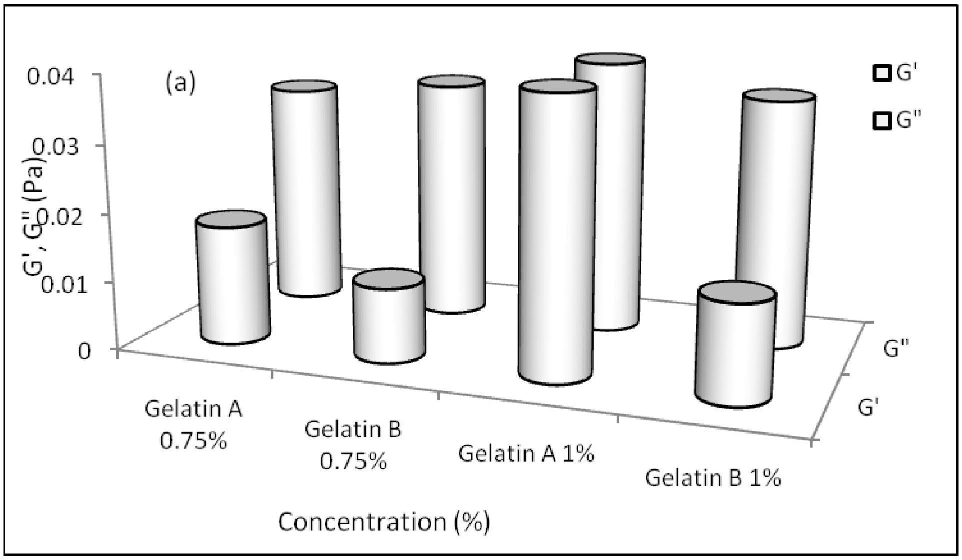


FIGURE 3.5. Frequency dependence of elastic modulus (G') and loss modulus (G'') for gelatin stabilized emulsions at (a) pH 7.0 and (b) pH 3.4.



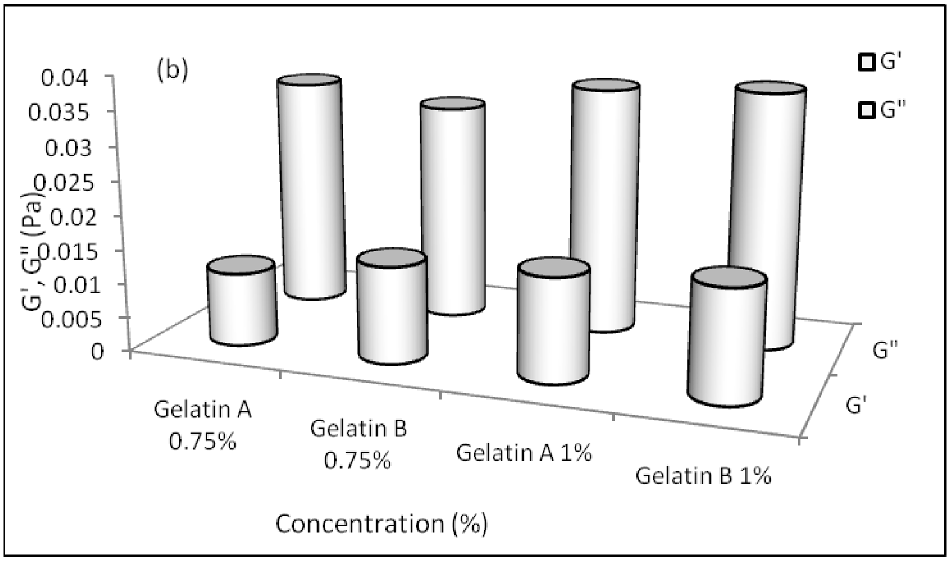


FIGURE 3.6. G'(elastic modulus), G"(loss modulus) at 10 radians per second for gelatin stabilized emulsions at selected concentrations at (a) pH 7.0 and in acidic buffer at (b) pH 3.4 respectively.

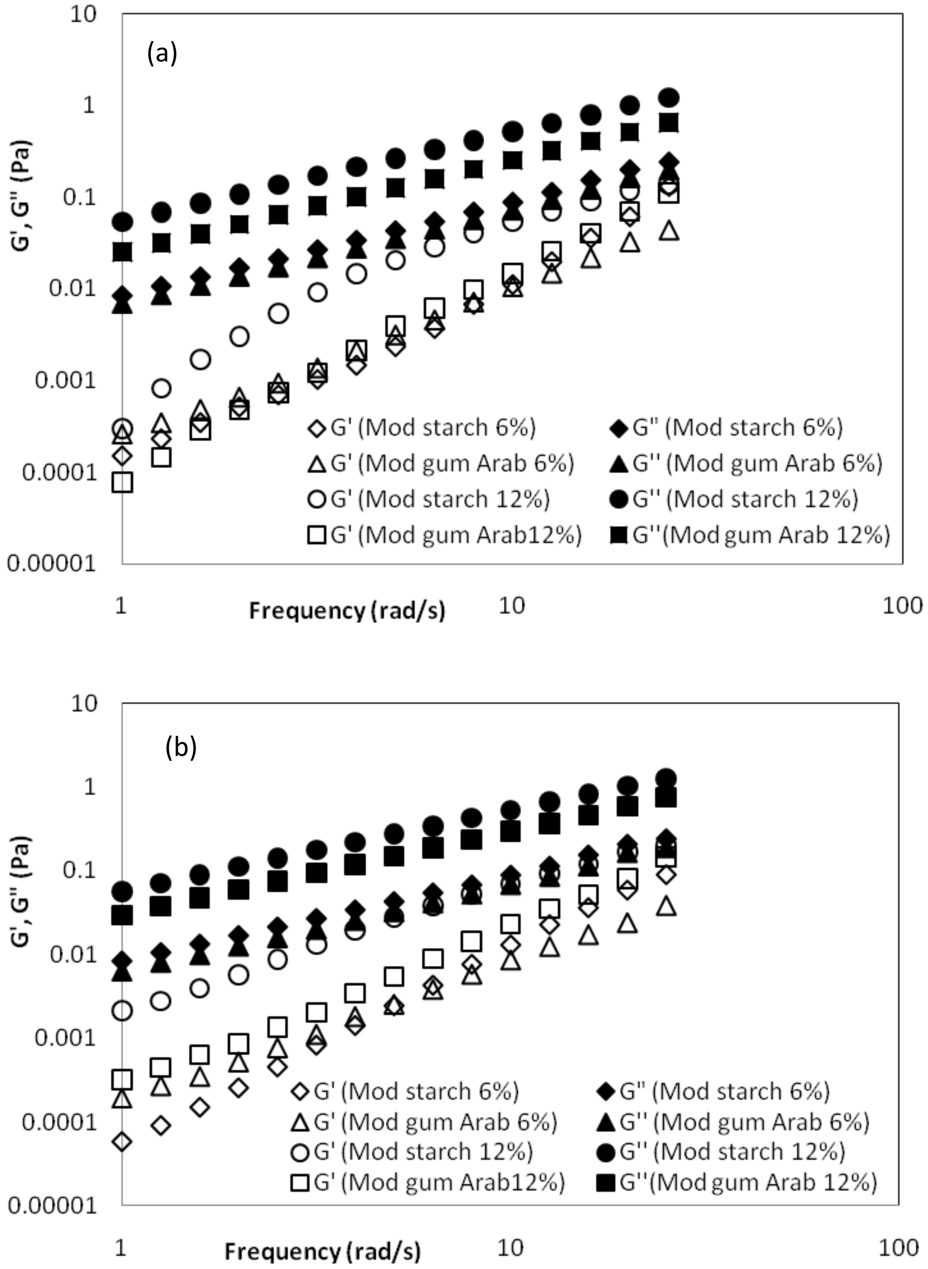


FIGURE 3.7. Frequency dependence of elastic modulus (G’) and loss modulus (G”) for polysacchairde stabilized emulsions at (a) pH 7.0 and (b) pH 3.4

modulus was found to be more dominant than G' i.e. the elastic modulus, indicating the predominance of viscous liquid like behavior over solid elastic like behavior. Also, there was negligible variation in properties upon change in pH for polysaccharides. This could be due to the formation of a thick film layer around the droplet that enables the emulsion to be sterically stabilized in concentrated as well as diluted form. Also, G' and G" were plotted at frequency of 10 radians per second for quality comparisons (Figure 3.8). These results match with the previous rheological flow measurements.

3.4.4 Effect of conjugation

In the previous sections, the rheological properties of proteins and polysaccharide emulsions were discussed individually. It was found that gelatin type 'A' had less shear thinning in low pH system and gelatin type 'B' in high pH system. And modified starch and modified gum Arabic were able to provide similar rheological properties in both pH systems. Based on these rheological properties following conjugates were formed. Also conjugates of gelatin with viscosity builders (Xanthan gum and PGA) were prepared:

Conjugates in deionized water (pH 7.0): (i) gelatin (type B, 1% w/w) and modified starch (12% w/w), (ii) gelatin (type B, 1% w/w) and modified gum Arabic (12% w/w), (iii) gelatin (type B, 1% w/w) with Xanthan gum (0.3% w/w) and PGA (0.3% w/w). Conjugates in acidic buffer at pH 3.4: (i) gelatin (type A, 1% w/w) and modified starch (12% w/w), (ii) gelatin (type A, 1% w/w) and modified gum Arabic (12% w/w), (iii) gelatin (type A, 1% w/w) with Xanthan gum (0.3% w/w) and PGA (0.3% w/w).

Table 3.3 gives the flow properties of water phases and emulsions for conjugate systems. Combining biopolymers resulted in much higher apparent viscosity of emulsions than gelatin or polysaccharide emulsions prepared individually. This may be interpreted by the reason that large number of particles increased the resistance to the flow. Hence this phenomenon led to an increase in apparent viscosity. For all the conjugates, except gelatin type 'B' with Xanthan and PGA, the flow behavior index of emulsion decreased when oil was added to the water phase, indicating possibility of flocculation.

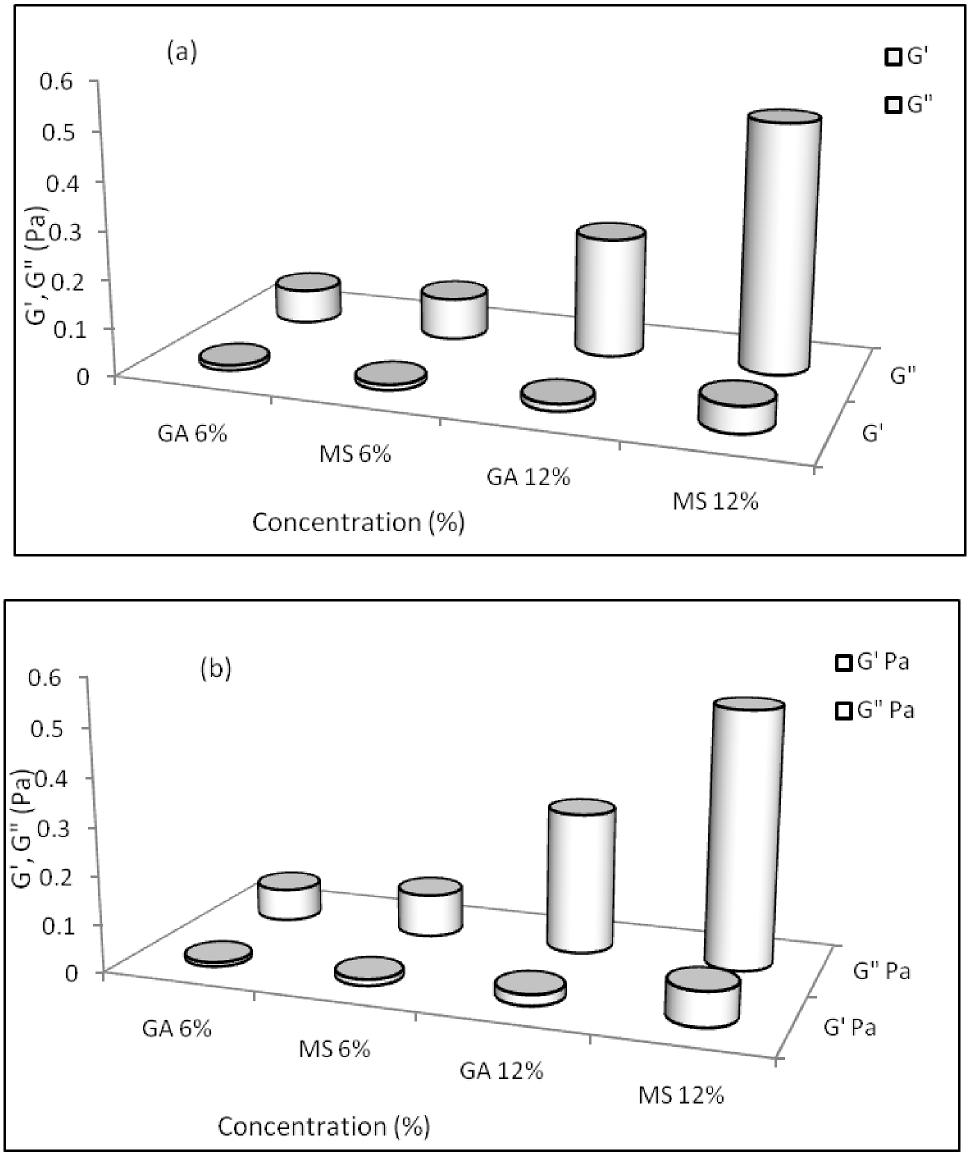


FIGURE 3.8. G'(elastic modulus), G"(loss modulus) at 10 radians per second for polysaccharide (modified starch (MS) & modified gum Arabic (GA)) stabilized emulsions at selected concentrations at (a) pH 7.0 and in acidic buffer at (b) pH 3.4 respectively.

Conjugate of gelatin type ‘B’ and modified starch had $n_{w.ph}$ (flow behavior index of water phase) = 0.91 which decreased to n_E (flow behavior index of emulsion) = 0.63 with emulsion formation. However, flow behavior of gelatin type ‘A’ and modified starch combination decreased from $n_{w.ph}$ = 0.88 to n_E = 0.84. The conjugate of gelatin type ‘A’ and modified starch was formed at pH 3.4 which is quite far from the IEP of gelatin type ‘A’. This might have favored the repulsive interactions between charged biopolymers (Dickinson, 1993). The consistency coefficients of the water phase also shifted up with mixing of the polymers together.

A major decrease in the flow behavior index for water phase ($n_{w.ph}$ = 0.22) after addition of Xanthan gum and PGA to gelatin type ‘B’ in deionized water was observed. This emulsion had the highest consistency coefficient and hence maximum apparent viscosity (3455 mPas) amid all the conjugated systems. The emulsion was highly pseudoplastic, with apparent viscosity 10 times larger than the emulsion formed in acidic solution. Significant decrease in flow behavior index and increased consistency coefficient with addition of Xanthan gum has also been previously observed by Coia and Stauffer (1987) who studied and evaluated various hydrocolloids for self stability which included Xanthan gum and PGA. Xanthan gums showed a strong resistance to serum separation but were susceptible to oiling off during storage. They were not surface active and therefore oiling off occurred more readily than with other surface active gum systems (Coia and Stauffer, 1987).

The conjugates of gelatin type ‘A’ and type ‘B’ with modified gum Arabic were unstable. It was found that these conjugated emulsions prepared in either deionized water or acidic buffer separated within hours of preparation. However, this was not predictable from flow properties of water phase. Lifting of the oil containing hydrocolloid was observed leaving the water phase behind. Interactions between these two polymers seemed to be segregated. This might be due to incompatibility between the two polymers. This incompatibility or coacervation can occur at high concentrations depending on whether the protein polysaccharide interaction is net repulsive or net attractive, respectively (Dickinson, 2003).

The shear rate dependence of viscosities of resultant emulsions from conjugates is shown in Figure 3.9. The apparent viscosities of all the emulsions decreased with increasing shear rate indicating shear thinning behavior revealing a pseudo-plastic behavior with a pronounced shear thinning phenomenon under shear. The zero shear-rate limiting viscosity was greatest for gelatin type ‘B’ emulsion stabilized in conjugation with viscosity builders (Xanthan gum and PGA). This increase in zero-shear limiting viscosity can be partially due to higher degree of flocculation in emulsion and more stress was needed to break the flocs. Buffo and Reineccius (2002) and McClements (2004a) have also indicated that at lower shear rates the hydrodynamic forces are not large enough to disrupt the flocs which then act like particles with fixed size and shape, resulting in constant viscosity. But as the shear rate increased, the values of stress exceed the linear range, and the hydrodynamic forces become large enough to deform & disrupt the flocs, which align with the shear field & result in rapid decline in viscosity (Buffo and Reineccius, 2002). This has also been explained in study done by Pettitt et al., in 1995, which demonstrated that under low shear conditions Xanthan gum displayed high solution viscosity; however sharp reductions in viscosity occurred under high shear. They found around 3- and 5-fold increase in the limiting viscosity of salad dressing with addition of 0.33% w/w and 0.55% w/w PGA to Xanthan gum, respectively (Pettitt et al., 1995). Yilmazer et al., 1991 also indicated that PGA and Xanthan gum used together affected the rheological stability of oil-in-water emulsions and the degree of stabilization was a function of concentration and ratio used. Little change for viscosity of Xanthan and PGA added emulsion was found over a period of study (Yilmazer, 1991).

TABLE 3.3: Flow properties of water phases alone and together in the form of emulsions of conjugate emulsion systems

Properties	Conjugates					
	a) Gel B-MS	b) Gel B-MGA	c) Gel A-MS	d) Gel A-MGA	e) Gel B-X-PGA	f) Gel A-X-PGA
$n_{w.ph}$	0.91 ± 0.00	0.87 ± 0.00	0.88 ± 0.00	0.99 ± 0.00	0.22 ± 0.01	0.90 ± 0.01
$n_{O.ph}$	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
$n_{Em.}$	0.63 ± 0.00	0.58 ± 0.01	0.84 ± 0.00	0.70 ± 0.01	0.34 ± 0.00	0.55 ± 0.00
$m_{W.\ ph.}$ (mPa)	54.26 ± 0.62	37.19 ± 0.73	62.83 ± 0.00	13.43 ± 0.44	17021.99 ± 166.28	8.27 ± 0.26
$m_{O.\ ph.}$ (mPa)	76.56 ± 1.25	76.56 ± 1.25	76.56 ± 1.25	76.56 ± 1.25	76.56 ± 1.25	76.56 ± 1.25
$m_{Em.}$ (mPa)	421.05 ± 23.05	379.32 ± 1.23	168.26 ± 11.22	172.61 ± 1.32	3455.42 ± 5.63	200.48 ± 14.67
$\eta_{app.\ W.\ ph.} 1\ s^{-1}$ (mPa s)	54.26 ± 0.62	37.20 ± 1.03	62.83 ± 0.00	13.29 ± 0.22	17021.99 ± 166.28	8.27 ± 0.17
$\eta_{app.\ O.\ ph.} 1\ s^{-1}$ (mPa s)	76.56 ± 1.24	76.56 ± 1.24	76.56 ± 1.24	76.56 ± 1.24	76.56 ± 1.24	76.56 ± 1.24
$\eta_{app.\ Em.} 1\ s^{-1}$ (mPa s)	421.05 ± 23.05	379.32 ± 1.23	168.26 ± 11.22	172.61 ± 1.32	3455.42 ± 5.63	200.48 ± 14.67

a) 1% Gelatin ‘B’ & 12% Modified Starch in deionized water, b) 1% Gelatin ‘B’ & 12% Gum Arabic in deionized water, c) 1% Gelatin ‘A’ & 12% Modified Starch in pH 3.4 buffer, d) 1% Gelatin ‘B’ & 12% gum Arabic in pH 3.4 buffer, e) 1% Gelatin ‘B’ - 0.3% Xanthan gum - 0.3% PGA, f) 1% gelatin ‘A’ - 0.3% Xanthan gum - 0.3% PGA.

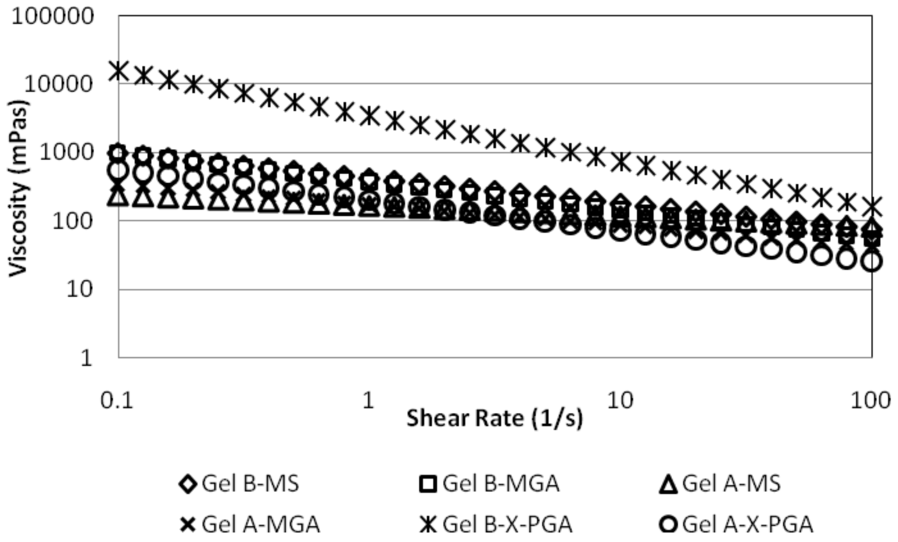


FIGURE 3.9. Apparent viscosity of conjugate stabilized emulsions as a function of shear rate.

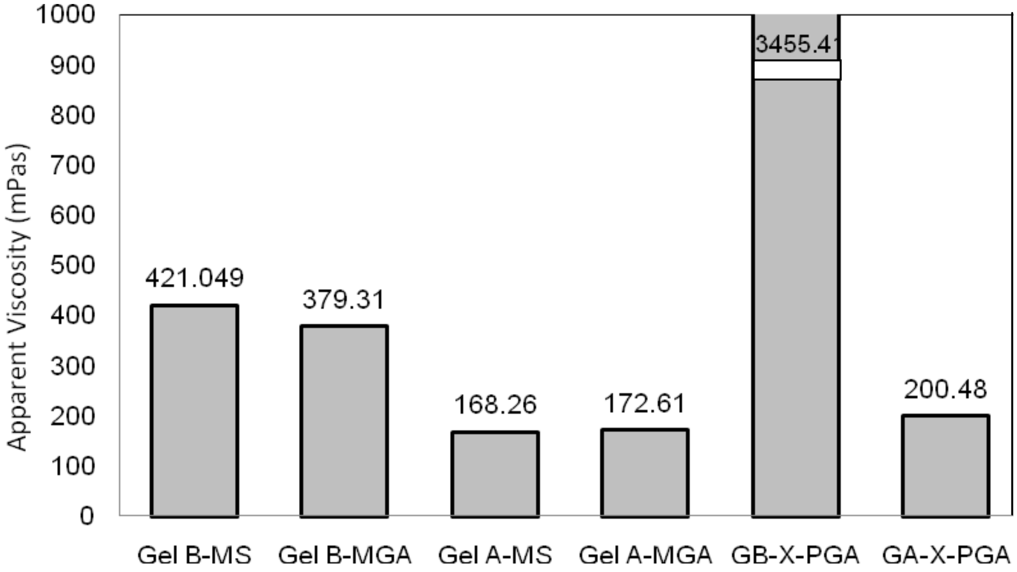


FIGURE 3.10. Apparent viscosity of conjugates at shear rate of 1 s^{-1}.

3.5 CONCLUSIONS

Rheological examinations revealed that with an increase in concentration and pH there was increase in the apparent viscosity. G" was greater than G' suggesting emulsions had a more viscous than elastic behavior. The pH plays an important role in binding degree of gelatin. Gelatin stabilized emulsions were more susceptible to pH than modified starch and modified gum Arabic stabilized emulsions. Gelatin type 'A' possessed greater shear thinning at high pH and showed Newtonian behavior at acidic pH. On the other hand, gelatin type 'B' showed thinning at low pH. This instability of gelatin type 'B' in acidic emulsion and gelatin type 'A' in deionized water (neutral buffer) can be due to aggregation of proteins at pH close to their respective iso-electric points. Apparent viscosity increased significantly on conjugation of hydrocolloids with different magnitudes for each and viscosity decreased with increasing shear rate indicating shear thinning behavior.

CONNECTING STATEMENT TO CHAPTER 4

In Chapter 3, the rheological properties of freshly prepared emulsion concentrates with different protein and polysaccharide emulsifiers were investigated. In order to assess the emulsion stability, these properties need to be evaluated and monitored over a period of storage. Hence in Chapter 4, the rheological properties and particle size distribution of these emulsions were evaluated over a period of time to examine their storage stability. Emulsifiers found relatively stable were also conjugated and evaluated to determine their influence on stability over a period of 2 weeks. The aim was to select out stable concentrated emulsions which can be later diluted in order to provide desirable stability to cloud beverages.

Part of the results of this study has been presented at the following conference:

Arora JK, Ramaswamy, HS, Taherian, AR. 2009. Effect of formulations and pH on rheological and physical properties, and stability of prepared oil-in-water emulsions. IFT Annual Meeting & Food Expo, June 6-9, 2009, Anaheim, CA, USA.

A manuscript also is also being prepared for publication based on the studies highlighted in this chapter:

Arora JK, Ramaswamy, HS, Taherian, AR, 2009. Effect on storage on rheological properties, droplet size distribution and stability of concentrated oil-in-water emulsions.

All experiment work and data analysis were carried out by the candidate under the overall supervision of Dr. H.S. Ramaswamy. Dr. Taherian provided the technical supervision to set up the experiments, train the candidate and analyze the results.

CHAPTER 4

EFFECT OF STORAGE ON RHEOLOGICAL PROPERTIES, DROPLET SIZE DISTRIBUTION AND STABILITY OF CONCENTRATED OIL-IN-WATER EMULSIONS

4.1 ABSTRACT

In this work, effect of storage on rheological, particle size and optical properties of concentrated oil-in-water beverage emulsions were studied. Emulsions were prepared individually using gelatin type ‘A’ and type ‘B’, modified starch and modified gum Arabic with canola oil. Dispersions were adjusted to two pH conditions, namely pH 3.4 and 7.0. Emulsions were optically characterized for droplet size distribution using ZetaSizer and rate of creaming was determined using Quick Scan. Gelatin was conjugated with polysaccharides and viscosity builders (Xanthan and PGA). Concentrated emulsions were stored for a period of 2 weeks and the rate of increase in droplet size and transmission of light were evaluated.

Oil-phase concentration had a significant effect on increasing the opacity of emulsion. Increase in apparent viscosity was also observed with storage. This raise in viscosity was more pronounced for gelatin type ‘A’ emulsions at pH 7.0 and for gelatin type ‘B’ emulsions at pH 3.4. Emulsions showed shear thinning behavior with different magnitude for each which was associated with droplet flocculation. Increase in slope of particle size distribution size was more for protein (gelatin) stabilized emulsions than polysaccharide stabilized emulsions. Emulsions stable at particular pH were conjugated. Gelatin type ‘A’-modified starch was observed to have smaller particle size and greater stability in concentrated form at pH 3.4, followed by gelatin type ‘B’-modified starch and gelatin type ‘B’-Xanthan-PGA both at pH 7.0. Gelatin -modified gum Arabic underwent rapid separation upon preparation. Stable concentrated forms were selected for dilution.

4.2 INTRODUCTION

Shelf-life of emulsions depends on their stability. This stability is an important quality criterion in dairy and citrus beverages. Stability of cloud emulsions for a desired period of time is a common issue in the beverage industry. Oil-in-water (O/W) emulsions are prepared by homogenizing an oil phase into an aqueous phase in the presence of an emulsifier which can help to enhance the kinetic stability of the emulsion (Buffo and Reineccius, 2002; McClements, 2004a). Beverage emulsions are primarily formulated to provide opacity to clear juices (Taherian et al., 2006). Over time, the emulsions tend to break due to their thermodynamic instability resulting in creaming (due to gravitational separation), flocculation and coalescence (Tan, 2004). In creaming, oil droplets tend to move upwards; sticking of the oil droplets is referred to as flocculation, whereas in coalescence, the oil droplets merge together to form bigger droplets. This consequently decreases the number of droplets, enhances cr eaming and eventually leads to emulsion breakdown (Buffo and Reineccius, 2001; Comas et al., 2006; Taherian et al., 2006; Mirhosseini et al., 2007). It has been stated by McClements (2004b) that Stoke's law is applicable to beverage emulsions. Therefore, the rheological parameters, droplet size and distribution are highly responsible for emulsion stability. Hence, reducing the diameter of suspended globules and increasing the water phase viscosity will help reduce creaming rate of the cloud emulsion.

Polysaccharides are usually added to oil-in-water food emulsions to stabilize emulsions via viscosity modification or gelation in the aqueous continuous phase (Garti, 1999; Dickinson, 2003; Mirhosseini et al., 2008a) thereby enhancing textural characteristics and retardation of creaming of oil droplets (McClements, 2000; Buffo et al., 2001; Chanamai and McClements, 2001; Aoki et al., 2007). Over a range of intermediate polysaccharide concentrations droplet flocculation causes creaming instability because the increase in effective size of the particles (which promotes creaming) more than compensates for the increase in continuous phase viscosity (which retards creaming). At higher polysaccharide concentrations, creaming is retarded because even though the droplets are aggregated they are incapable of moving, owing to the high viscosity or the gel-network formed by the polysaccharides. The influence of polysaccharides on the creaming stability of emulsions is therefore not straightforward and depends on the system characteristics (McClements, 2000).

Gum Arabic is one of the most common emulsifiers currently being used in beverage emulsions. Modified gum arabic (MGA) used in this study is a cold water soluble hydrocolloid. MGA is produced by reacting natural acacia gum with l-octenyl succinic anhydride (OSA) in a production process that is similar to that used to produce modified food starch-OSA. It has been enriched with a protein rich high molecular weight component (the arabinogalacto-protein complex) which provides emulsifying ability to the gum Arabic (Garti, 1999). Another polysaccharide, modified starch has also been widely studied (Chanamai and McClements, 2002; Prochaska et al., 2007). One of the most commonly used modified starch (Purity Gum BE) also used in this work is an octenyl succinate derivative of waxy-maize. It consists primarily of amylopectin that has been chemically modified to contain a side group that is anionic and non-polar (Taherian, 2006). Modified starch is known to be stable over a wide range of pH (Chanamai and McClements, 2002; McClements, 2004a). Proteins also have been known to have greater binding affinities, improving the stability and producing desirable physiochemical properties in emulsions (Dickinson, 2001; Akhtar and Dickinson, 2003; McClements, 2004b; Harnsilawat, et al., 2006). Nevertheless, not much attention has been given to emulsifying properties of gelatin which can also prove to be an excellent surface active protein. In the food industry, gelatin is utilized as thickening and gelling agents, and also to provide emulsification in confections, baked goods, soups, sauces, etc. Gelatin is a relatively high molecular weight protein derived from animal collagen, e.g. pig, cow or fish. It is prepared by hydrolyzing native collagen by boiling in the presence of either acid (gelatin type 'A') or salt (gelatin type 'B'), resulting in partial cleavage of the cross links (Karim and Bhat, 2009). The iso-electric point of gelatin type 'A' (~7–9) is higher than that of gelatin type 'B' (~5). Some previous studies have shown that gelatin is surface-active and that it is capable of acting as an emulsifier in oil-in-water emulsions (Surh et al., 2006). Yet, it is important to verify whether gelatin can be used to prepare emulsions that are visibly stable in diluted beverage forms.

Along with single hydrocolloids, protein-polysaccharide mixtures are commonly found in the food industry. Polysaccharides and proteins mixtures have known to result in miscible solutions and improve against environmental stresses in emulsion systems (Akhtar and Dickinson, 2003; Aoki et al., 2005; Akhtar and Dickinson, 2007). There is ample literature on using hydrocolloid materials such as Xanthan gum, pectin, gellan gum, gum tragcanth, ghatty gum etc in addition to surface active biopolymers to enhance the stability of flavor and cloud emulsions (Tan, 2004).

Several studies in the past two decades have characterized rheological properties and particle size distribution of food emulsions. It is normally important that emulsion droplets are made as small as possible in order to minimize gravity creaming effects. Suzuki et al. (1991) and Klinkesorn et al. (2004)

studied the rheology and stability of various oil-in-water emulsions stabilized by different surface-active hydrocolloids and justified the apparent viscosity of emulsions as affected by volume concentration of the dispersed phase, particle size, nature of emulsifying agent and emulsifying conditions (Suzuki, 1991; Klinkesorn et al., 2004; Ye et al., 2004; Taherian et al., 2006). However, not much has been studied on rheological properties, particle size distribution and stability of gelatin and gelatin-polysaccharide stabilized emulsions. There is a need for further investigation of these emulsifying agents and factors affecting emulsion stability in comparison to traditionally used polysaccharides such as modified starch and gum Arabic.

Therefore, the objective of this study was to evaluate the storage stability of concentrated oil-in-water emulsions using gelatin (gelatin type ‘A’ and ‘B’) and polysaccharides (modified starch, modified gum arabic, Xanthan and PGA) and their conjugates based on their rheological properties, particle size distribution and optical properties over a period of 14 days at two pH levels (3.4 & neutral). The outcomes were then related to the stability of emulsions prior to and after dispersion in simulated juice and dairy beverages.

4.3 MATERIAL AND METHODS

4.3.1 Materials

Commercially available refined vegetable canola oil was obtained from the local market. Modified starch (Purity Gum^{TM} Be) was obtained from National Starch (Bridgewater, NJ). Gelatin type ‘A’ and type ‘B’ were obtained from Rousselot (a VION Company, Dubuque, IA). Modified gum Arabic, viscosity builders: Ticaxan Xanthan 200 (Xanthan gum) and Propylene glycol alginate (PGA/LV Powder) were obtained from TIC GUMS (Belcamp, MD). Food grade citric acid and dibasic sodium phosphate were used to prepare buffer solutions and were bought from Fisher Scientific (Montreal, QC). Deionized water was used to prepare solutions and emulsions.

4.3.2 Emulsion Preparation

Hydrocolloids were introduced into the buffer solution at pH 3.4 and deionized water at pH 7.0. The water phase was prepared using four different hydrocolloids at two different levels: gelatin type ‘A’ (0.75 & 1% w/w) & type ‘B’ (0.75 & 1% w/w), modified starch (6 & 12% w/w) & modified gum Arabic (6 & 12% w/w). Hydrocolloids were added to water phase and stirred using a high speed commercial blender (Waring, Montreal, QC). The mixtures were kept overnight to allow complete hydration. Then the oil was added to the water phase at a fixed level of 10% w/w with continuous mixing. The mixing was first done using the blender for 5 min followed by high speed homogenizer (ultrasonic homogenizer) for 90s (at intervals to prevent heating) at 75% amplitude for final homogenization. 0.02% w/w sodium azide was added to the emulsions to inhibit any microbial growth. For conjugate emulsions, dry gelatin and polysaccharide powders were mixed and hydrated. Thickening agents (Xanthan gum and PGA) were dissolved separately in aqueous solutions and stirred in the emulsion containing gelatin. All the emulsion samples were stored for a period of 14 days for stability evaluation.

4.3.3 Evaluation of specific gravity

Specific gravity of each water phase and emulsion was evaluated at the given concentrations using a 50 mL specific gravity bottle (Fisherbrand, Montreal, QC, Canada).

4.3.4 Flow and dynamic rheological measurements

For rheological measurements AR2000 Rheometer (TA instruments, New Castle, DE, U.S.A.) was used. The sample was placed in 60mm cone geometry of 2° and solvent trap at room temperature. Flow curves were determined at increasing shear rate (0.1-100 s^{-1}) in 8 min. Apparent viscosity was calculated as a function of shear rate. Experimental flow curves were compared to Power's law model (Eq 4.1):

$$\eta = m\dot{\gamma}^{(n-1)} \tag{4.1}$$

Where n = 1 for a Newtonian fluid, n < 1 for a shear thinning fluid and n > 1 for a shear thickening fluid. Variation in consistency coefficient (m) and flow behavior index (n) values were determined and apparent viscosity (at a particular stress rate of 1 s^{-1}) were determined at different levels of concentration and two different pH.

Emulsion rheology was measured for the four hydrocolloids i.e. proteins (gelatin type 'A' and gelatin type 'B') and polysaccharides (modified starch and modified gum Arabic) at two different concentration levels 0.75% & 1% and 6% & 12%, respectively. Measurements were done on day 1, 7 & 14. The emulsions were prepared at two pH levels, i.e. deionized water corresponding pH 7.0 and buffer at pH 3.4. Apparent viscosity at a particular shear rate of 1 s^{-1} was employed for quality comparison.

In order to determine the linear viscoelastic region, the rheological parameters (elastic modulus i.e. G’, and loss modulus i.e. G”) were first measured by conducting a stress sweep test at a fixed frequency. G’ is a measure of the energy stored in a cycle of oscillation whereas G” is the measure of energy lost as viscous flow in a cycle of oscillation. The focus was to study the loss modulus (G”) and storage modulus (G’). For a purely viscous system, G’ = 0 and G” = G*, where G* is the complex modulus. Conversely, G’ = G* and G” = 0, if the system is purely elastic. For dynamic or oscillatory measurements, a frequency sweep from 1 up to 50 radians per sec was given at fixed oscillation stress (1 Pascal) and G’ (elastic modulus) and G” (viscous modulus) were obtained. Measurements were done in triplicate per each emulsion.

4.3.5 Particle size distribution

Particle size distribution of concentrated emulsions was determined by the integral light scattering technique using a ZetaSizer 4 (Malvern Instruments Ltd., Malvern, UK). Emulsions were analyzed immediately after preparation in duplicate. The instrument uses the method of photon correlation spectroscopy (PCS) to measure particle size in constant random thermal, or Brownian motion. Photon Correlation Spectroscopy (PCS) is based on dynamic light scattering. The time decay of the near-order of the droplets caused by the Brownian motion is used to evaluate their size (via the Stokes-Einstein relation). As a consequence, PCS requires highly diluted suspensions in order to avoid multiple scattering. Due to the Brownian motion, intensity of light scattered from the particles vary with time. The frequency of these fluctuations depend on the speed at which the droplets move and hence on their size. Large particles move slower than the smaller ones, so that the rate of fluctuation of the light scattered from them is also slower. The particle diameter range and number of photon counts per second were evaluated at room temperature. To avoid multiple scattering, concentrated emulsions were diluted (1:1000) with deionized water prior to analysis. The droplet size distribution of each emulsion was measured twice and the mean droplet diameter was reported as the average.

4.3.6 Stability of emulsions

For instrumental stability evaluation, 6 ml of sample was poured into a flat-bottom cylindrical glass tube (100mm height, 16mm internal diameter) and subjected to an optical scanning screening (Quick Scan, Coulter Crop., Maimi, FL). The transmission of monochromatic light ($\lambda = 850$ nm) from the sample was measured as a function of their height in order to quantify the creaming rate. Creaming rate was then calculated from the height of the interfaces between the opaque droplet rich layer and transparent droplet depleted layer as a function of time for 12 days. Creaming rate was expressed as slope of absolute thickness of layers per unit time (Taherian et al., 2006).

4.4 RESULTS AND DISCUSSION

4.4.1 Storage effect on flow rheology of protein stabilized emulsions

The emulsion flow curves for protein stabilized emulsions at pH 7.0 and pH 3.4 are shown in Figure 4.1(a, b, c and d) and Figure 4.2 (a, b, c and d), respectively. High values of coefficient of determination ($R^2 > 0.98$) indicated a good fit for all the steady flow measurements. Standard deviation and coefficient of variance were also computed for all samples. Figures represent a shear thinning behavior. The apparent viscosity increased for all the emulsions after two weeks at different magnitudes. It is likely that the increase in viscosity partially corresponds to higher degree of flocculation, thus greater stress is required to break down the clumps resulting in higher viscosity.

Tables 4.1 & 4.2 give the power law model's flow behavior index as a measure of non-Newtonian pseudoplasticity in pH 7.0 and in system at pH 3.4, respectively. The low values of the flow behavior index of gelatin stabilized emulsions revealed non-Newtonian character with a typically pseudoplastic behavior throughout the storage time. Apparent viscosity at a shear rate of $1s^{-1}$ was also plotted to better compare the emulsions from stability point of view (in this case the shear rate can be chosen at any appropriate level). Change in apparent viscosity with time for protein stabilized emulsions at shear rate of 1/s in pH 7.0 is shown in Figure 4.3(a). Gelatin type 'A' (1%) in neutral pH exhibited a prominent change in apparent viscosity (358 mPas) during storage with a corresponding decrease in the flow behavior index (n = 0.47) indicating high degree of shear thinning behavior due to disruption of flocculated droplets. However at low pH (Figure 4.3(b)), the value of apparent viscosity was smaller (159 mPas) with a relatively higher n of 0.54 indicating less flocculation than former. This phenomenon can be due to closeness of isoelectric point of gelatin type 'A' (~7-9) to the pH of the medium (i.e. pH 7.0) which could result in formation of cold set gel and high flocculation resulting in loss of repulsive force. Probably, at pH values close to the isoelectric point (as in this case IEP of gelatin type 'A' is ~7-9), two major competing effects occur, that is (1) flocculation of emulsion droplets and

(2) their incorporation into a gel formed with the protein in solution. The flocculation might have increased effective size of the dispersed

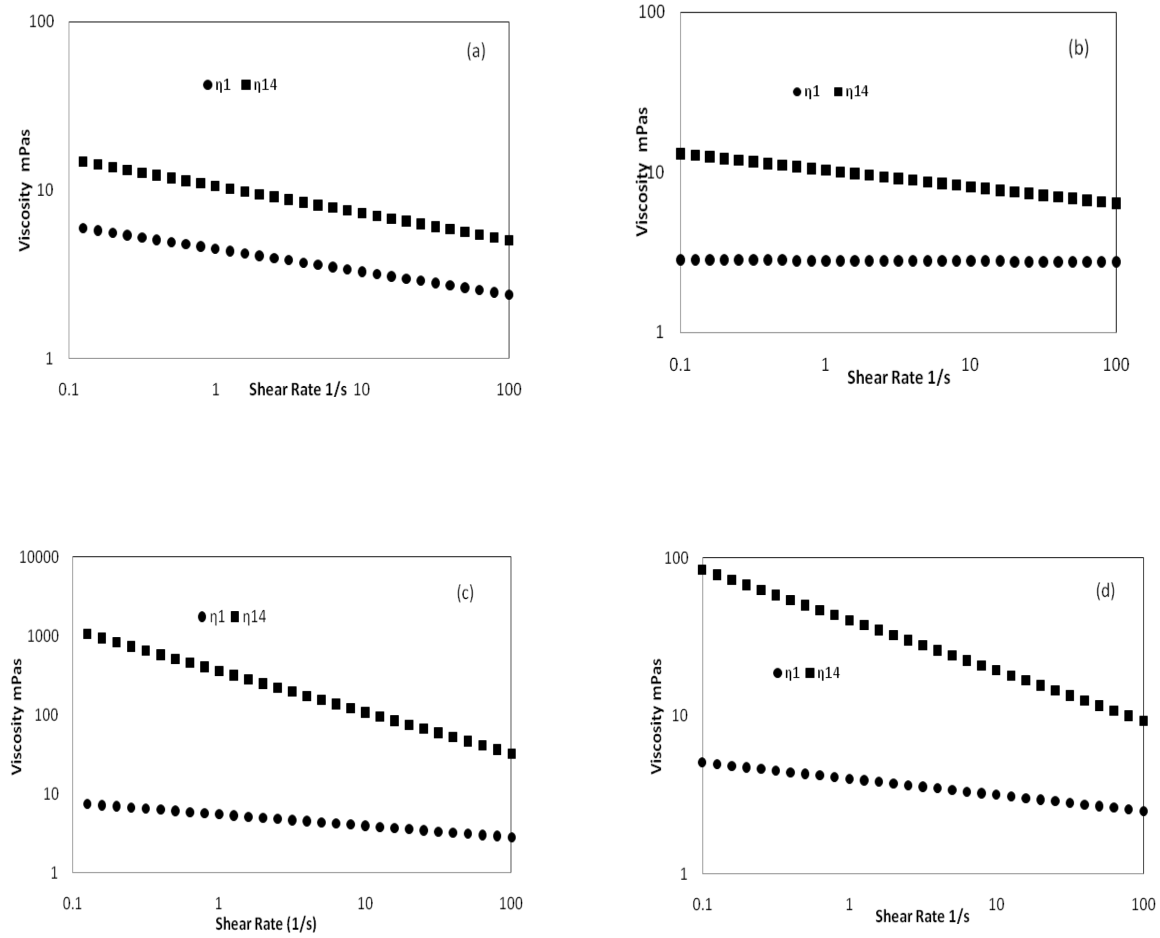


FIGURE 4.1. Shear state flow curves for (a) 0.75% gelatin type 'A', (b) 0.75% gelatin type 'B', (c) 1% gelatin type 'A', (d) 1% gelatin type 'B' at day 1 (η1) and day 14 (η14) in pH 7.0.

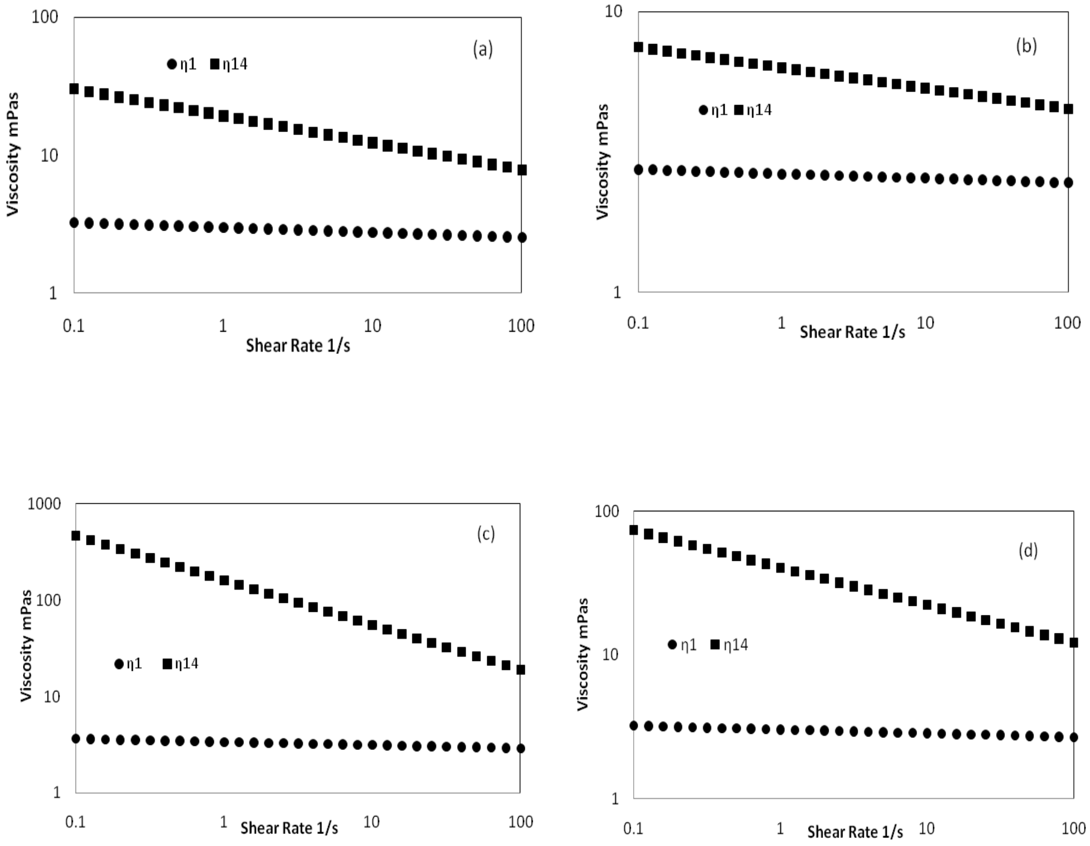


FIGURE 4.2. Shear state flow curves for (a) 0.75% gelatin type 'A', (b) 0.75% gelatin type 'B', (c) 1% gelatin type 'A', (d) 1% gelatin type 'B' at day 1 (η1) and day 14 (η14) in pH 3.4.

phase and altered its incorporation into the gel matrix and, therefore, contributing to the changing appearance of the gels and hence increasing the viscosity (Hunt and Dalgleish, 1995). Gelatin type ‘B’ emulsions, however, had a lower flow behavior index at 0.72 & 0.63 at 0.75% and 1% concentration in pH 3.4 compared to that in pH 7.0 at 0.90 & 0.68 at 0.75% & 1% concentration, respectively.

4.4.2 Storage effect on flow rheology of polysaccharide stabilized emulsions

The emulsion flow curves for polysaccharide stabilized emulsions at pH 7.0 and pH 3.4 are shown in Figure 4.4 and Figure 4.5, respectively. Both figures suggest slight shear thinning behavior. Flow curves were almost identical for the three measurements and time period of two weeks. Flow behavior index (n) for these samples indicated a slight decrease and correspondingly there was minor increase in the consistency coefficient (m) for these samples after two weeks storage time (Table 4.2). This could partially correspond to a slight degree of flocculation. Due to its high molar mass and branched polymer structure, modified starch that is adsorbed at the interface may give rise to steric stabilization in emulsions and other dispersed systems (Nilsson and Bergenstahl, 2006).

Apparent viscosity at a shear rate of $1 s^{-1}$ was plotted to better compare the emulsions from a stability point of view (Figure 4.6). There was a small increase in apparent viscosity of modified starch stabilized emulsions with time. However this increase was nothing compared to the viscosity change in gelatin stabilized emulsions. Also, insignificant change in apparent viscosity was observed in modified gum Arabic emulsions studied during the 14 days that the experiment lasted (at both the concentration levels). This may imply that there was not much variation in the mean droplet diameter during this period of time. Findings by Chanamai and McClements, 2002 have also suggested that modified starch and gum Arabic stabilized emulsions are not sensitive to pH and temperature changes.

Table 4.1(a): Consistency coefficient (mPa) and flow behavior index of protein stabilized emulsions as a function of storage time at pH 7.0.

Emulsion	m (day 1)	m (day 7)	m (day 14)	n (day 1)	n (day 7)	n (day 14)
Gelatin A 0.75 %	4.50 ± 0.12	10.61 ± 0.50	40.30 ± 1.23	0.86 ± 0.01	0.84 ± 0.01	0.52 ± 1.01
Gelatin B 0.75 %	2.81 ± 0.01	8.94 ± 1.22	10.30 ± 0.11	0.99 ± 0.00	0.91 ± 0.01	0.90 ± 0.00
Gelatin A 1.0 %	5.45 ± 0.19	215.03 ± 7.46	358.27 ± 11.78	0.85 ± 0.01	0.53 ± 0.00	0.47 ± 0.00
Gelatin B 1.0 %	4.01 ± 0.01	31.41 ± 2.30	40.27 ± 3.02	0.90 ± 0.01	0.70 ± 0.00	0.68± 0.03

Table 4.1(b): Consistency coefficient (mPa) and flow behavior index of protein stabilized emulsions as a function of storage time at pH 3.4.

Emulsion	m (day 1)	m (day 7)	m (day 14)	n (day 1)	n (day 7)	n (day 14)
Gelatin A 0.75 %	3.01 ± 0.06	9.90 ± 0.09	19.26 ± 0.27	0.96 ± 0.00	0.88 ± 0.00	0.80 ± 0.00
Gelatin B 0.75 %	2.64 ± 0.07	4.98 ± 0.03	6.30 ± 0.07	0.80 ± 0.01	0.75 ± 0.00	0.72 ± 0.00
Gelatin A 1.0 %	3.37 ± 0.02	74.25 ± 1.58	159.16 ± 5.70	0.97 ± 0.00	0.64 ± 0.00	0.54 ± 0.00
Gelatin B 1.0 %	3.04 ± 0.04	29.47 ± 0.33	40.64 ± 2.73	0.84 ± 0.01	0.72 ± 0.00	0.63 ± 0.00

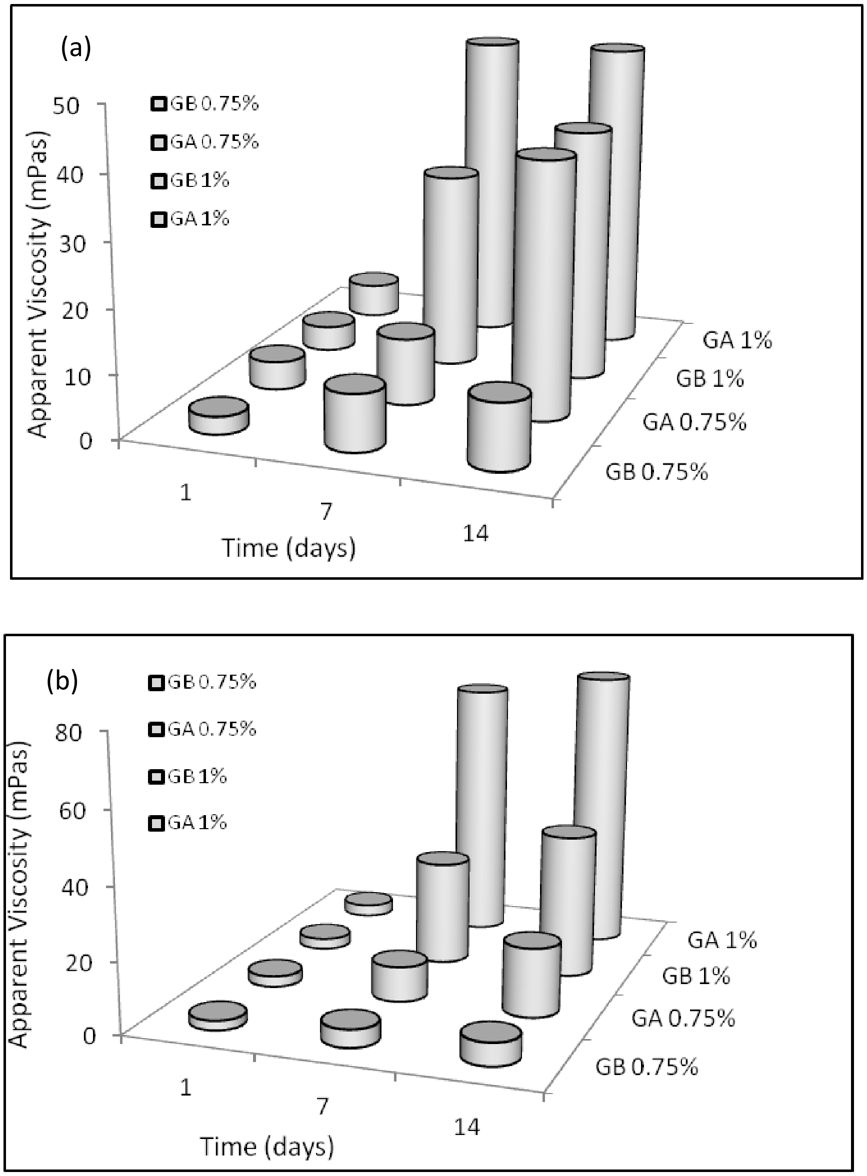


FIGURE 4.3. Change in apparent viscosity with time for protein stabilized emulsions at shear rate of 1/s in (a) pH 7 and (b) pH 3.4 systems.

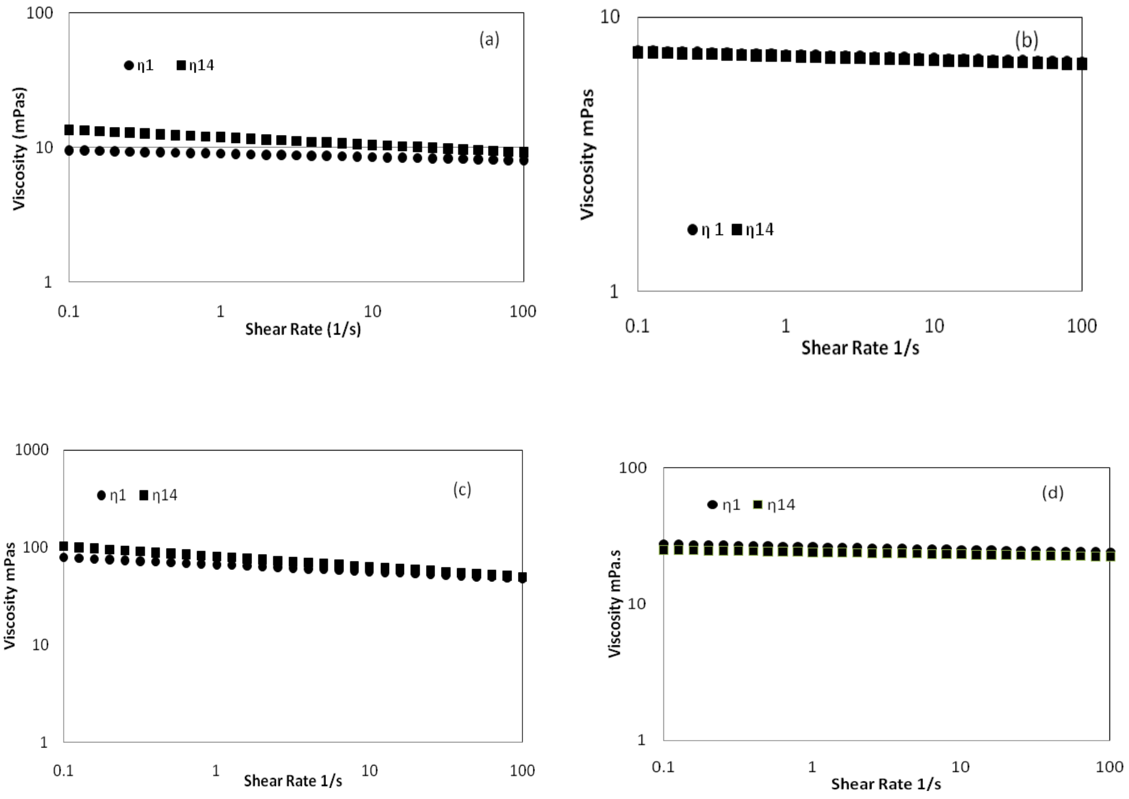


FIGURE 4.4. Shear state flow curves for (a) 6% modified starch, (b) 6% modified gum Arabic, (c) 12% modified starch, (d) 12% modified gum Arabic in pH 7.0 at day 1 (η1) and day 14 (η14).

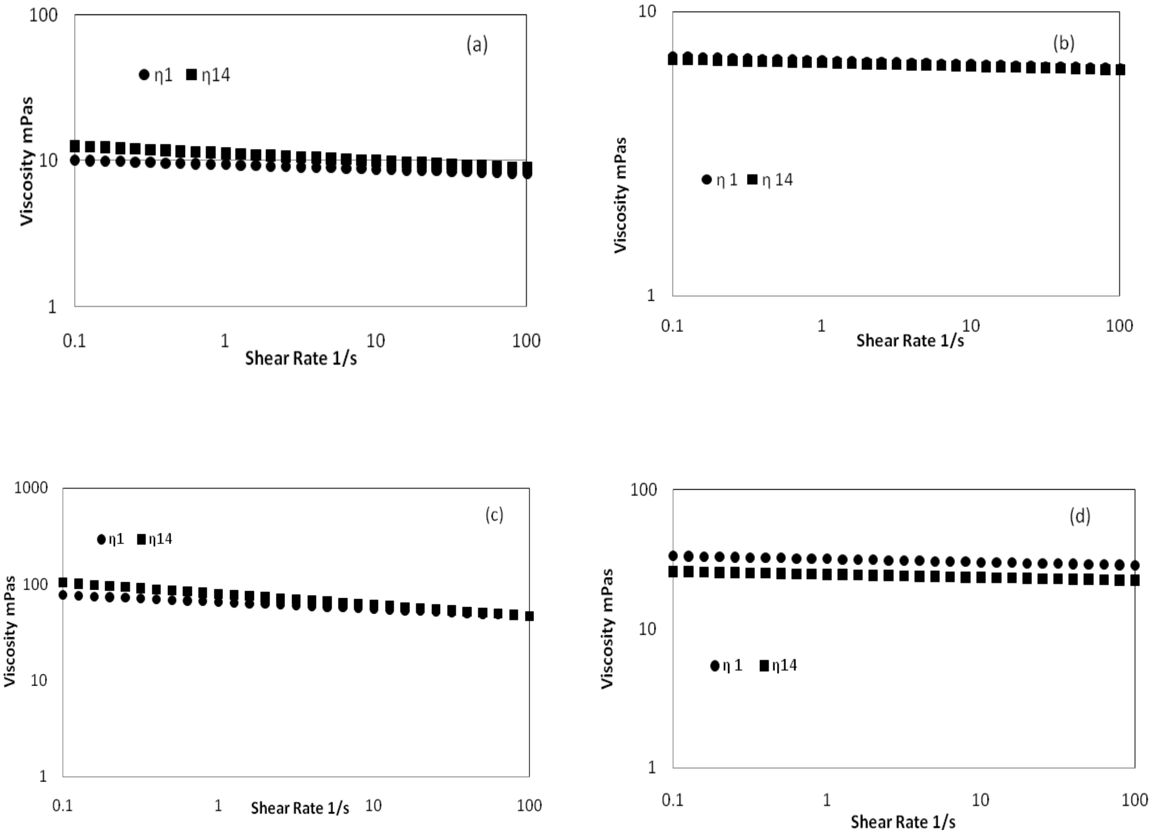


Figure 4.5. Shear state flow curves for (a) 6% modified starch, (b) 6% modified gum Arabic, (c) 12% modified starch, (d) 12% modified gum Arabic in pH 3.4 at day 1 (η1) and day 14 (η14).

TABLE 4.2(a): Consistency coefficient (mPa) and flow behavior index of polysaccharide stabilized emulsions as a function of storage time at pH 7.0.

Emulsion	m (day 1)	m (day 7)	m (day 14)	n (day 1)	n (day 7)	n (day 14)
Modified Starch 6%	9.06 ± 0.25	11.69 ± 0.11	11.93 ± 0.11	0.97 ± 0.00	0.95 ± 0.00	0.94 ± 0.00
Modified G. Arabic 6%	7.27 ± 0.04	7.28 ± 0.08	7.17 ± 0.06	0.98 ± 0.00	0.98 ± 0.00	0.98 ± 0.00
Modified Starch 12 %	67.11 ± 2.95	79.31 ± 0.76	80.72 ± 0.49	0.93 ± 0.00	0.89 ± 0.00	0.89 ± 0.00
Modified G. Arabic 12 %	26.41 ± 0.19	24.17 ± 0.19	24.00 ± 0.24	0.98 ± 0.00	0.98 ± 0.00	0.98± 0.00

TABLE 4.2(b): Consistency coefficient (mPa) and flow behavior index of polysaccharide stabilized emulsions as a function of storage time at pH 3.4.

Emulsion	m (day 1)	m (day 7)	m (day 14)	n (day 1)	n (day 7)	n (day 14)
Modified Starch 6%	9.45 ± 0.08	10.86 ± 0.02	11.27 ± 0.05	0.97 ± 0.00	0.96 ± 0.00	0.95 ± 0.00
Modified G. Arabic 6%	6.75 ± 0.02	6.49 ± 0.05	6.60 ± 0.10	0.98 ± 0.00	0.99 ± 0.00	0.98 ± 0.00
Modified Starch 12 %	65.33 ± 1.89	68.94 ± 1.97	79.81 ± 1.87	0.92 ± 0.00	0.91 ± 0.00	0.89 ± 0.00
Modified G. Arabic 12 %	31.84 ± 0.46	26.20 ± 0.24	24.47 ± 0.28	0.98 ± 0.00	0.98 ± 0.00	0.98± 0.00

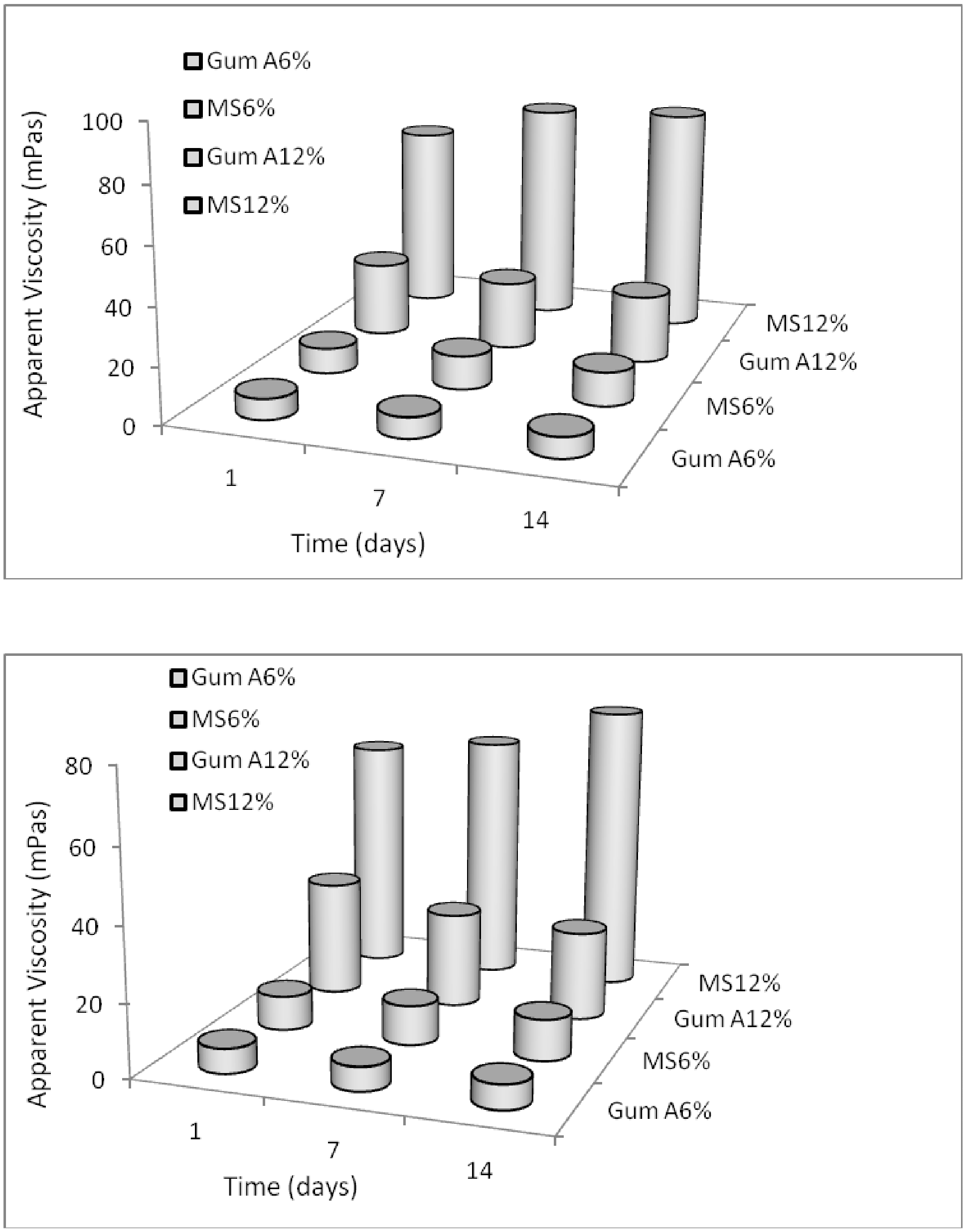


FIGURE 4.6. Change in apparent viscosity with time for polysaccharide stabilized emulsions at shear rate of 1/s in (a) pH 7 and (b) pH 3.4 systems.

4.4.3 Storage effect on dynamic properties of protein and polysaccharide stabilized emulsions

The frequency development of the viscoelasticity of typical protein and polysaccharide emulsions on day 1 and day 14 are shown in Figure 4.7. The elastic modulus (G') and loss modulus (G") for emulsion prepared with gelatin (Figure 4.7(a)) increased to a greater extent while polysaccharide systems had negligible variation during storage (Figure 4.7(b)).

Figure 4.8 shows the frequency dependence of G' and G" at a single frequency of 10 radians per second as a function of time. The figures indicate that G" is greater than G' for all the emulsion at day 1. However, for gelatin stabilized emulsions (Figure 4.3 (a)) there was much greater increase in the storage modulus (G') with time which suggested that the droplets might have flocculated or coalesced and behaved as rigid solid particles resulting in high values of G'. On the other hand, for polysaccharide emulsions (Figure 4.3(b)), G" i.e. the loss modulus value was higher than G' indicating the polysaccharide emulsions were viscous throughout without much accumulation. This suggests that gelatin stabilized emulsions were more sensitive to pH than polysaccharide stabilized emulsions. These results match with the previous flow rheological measurements.

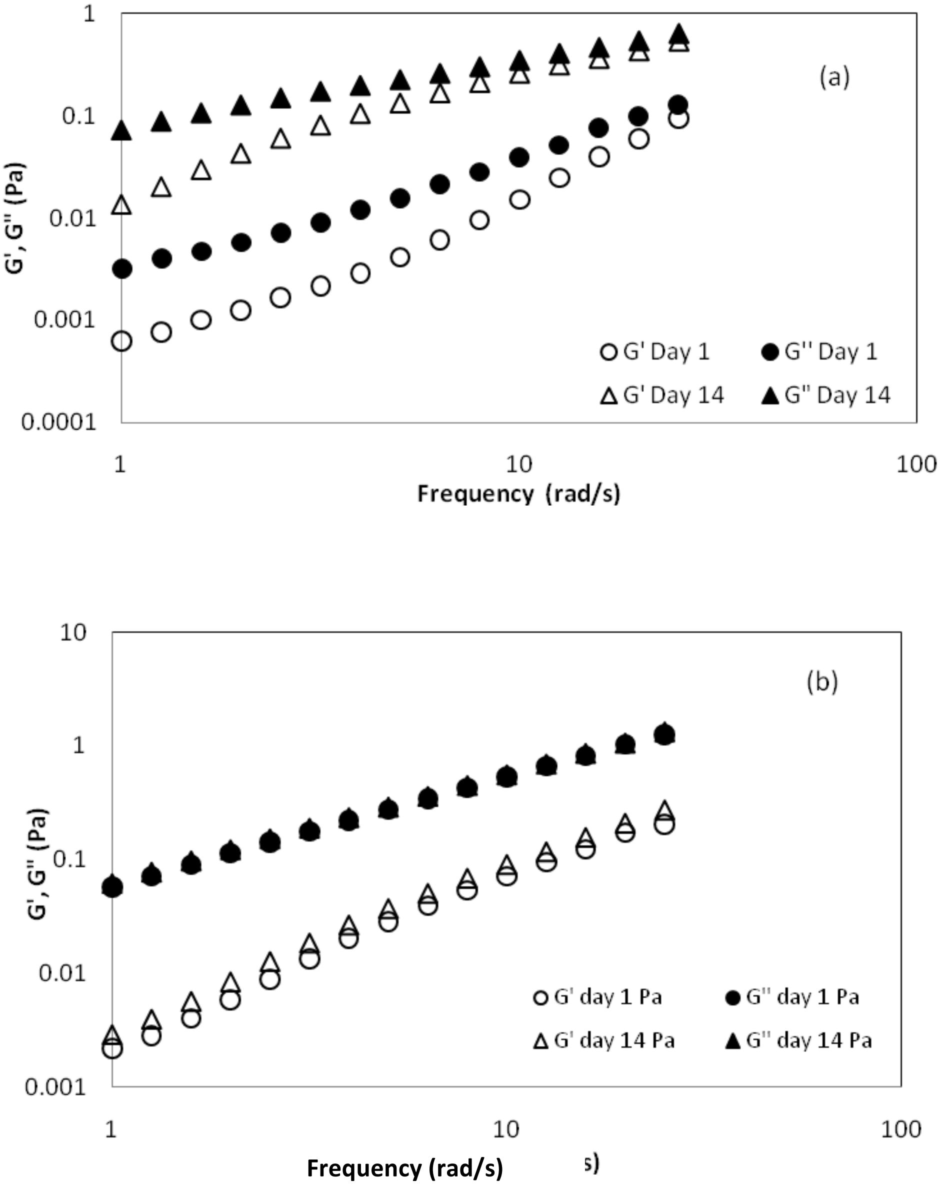


FIGURE 4.7. Frequency dependence of elastic modulus (G') and loss modulus (G'') for (a) gelatin stabilized emulsion, (b) polysaccharide stabilized emulsion.

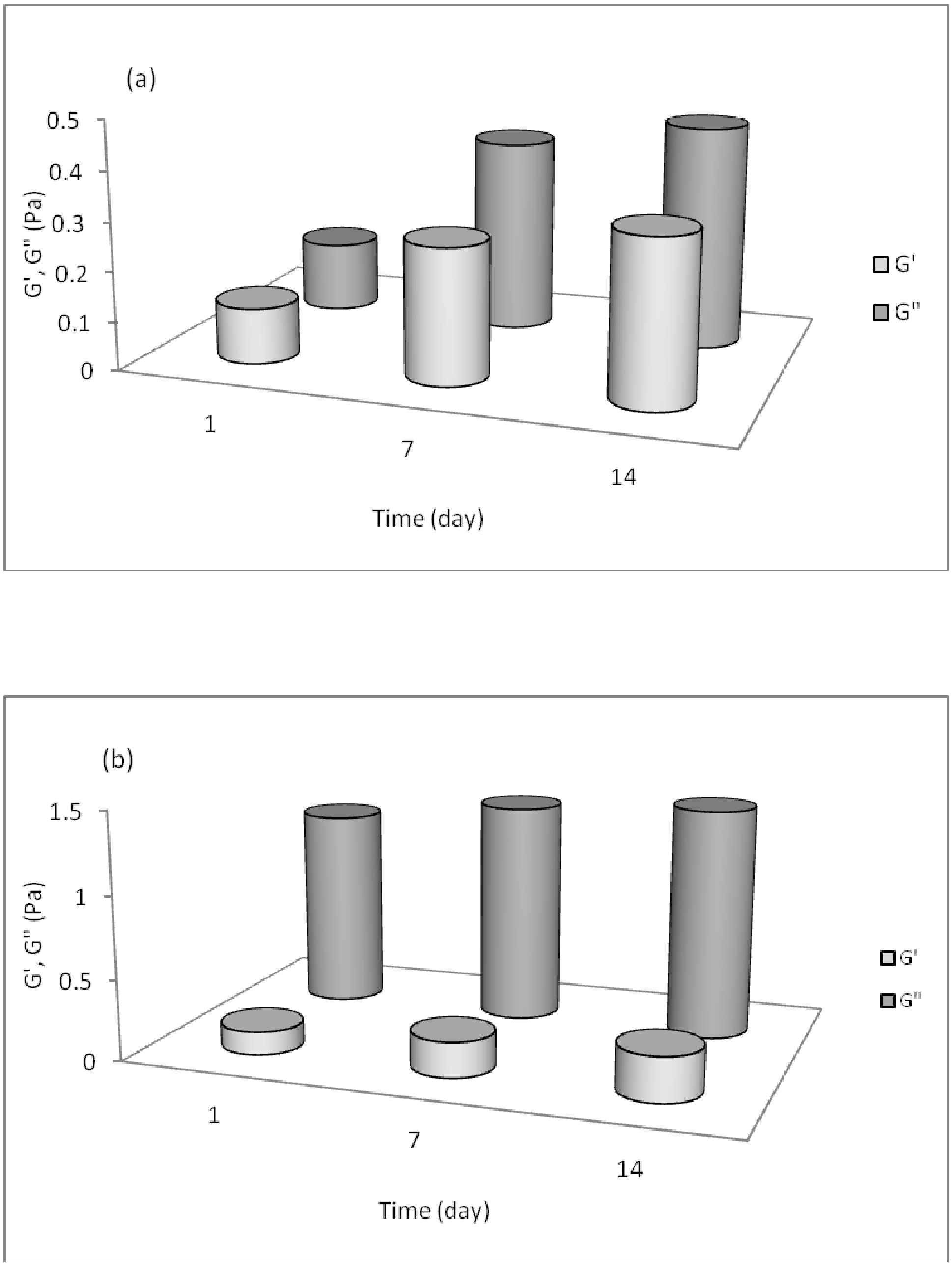


FIGURE 4.8. G' and G" at a single frequency of 10 radians per second as a function of time for a (a) gelatin stabilized emulsion and (b) polysaccharide stabilized emulsion.

4.4.4 Storage effect on rheology of conjugated emulsions

The apparent viscosity of emulsions increased when proteins were conjugated with polysaccharides. A combination of high content of all main emulsion components result in the highest viscosity value, in agreement with the conclusions by Mirhosseini et al. (2007). Table 4.3 describes the apparent viscosity at shear rate of 1/s for conjugate emulsions at pH 7 and pH 3.4 for day 1, 7 and 14. For conjugates stabilized in pH 7.0, freshly prepared 1% gelatin type 'B'-0.3% Xanthan -0.3% PGA conjugate (gel B-X-PGA) had much higher apparent viscosity (3455 mPas) than gelatin type 'A' conjugate (200 mPas). Both the emulsions illustrated shear thinning behavior as the apparent viscosity increased and flow behavior index decreased on storage. Yilmazer et al. (1991) also indicated that PGA and Xanthan gum used together affected the rheological stability of oil-in-water emulsions and the degree of stabilization was a function of concentration and ratio used. Due to the high viscosity, at low shear rates the hydrodynamic forces are large enough to disrupt the flocs which then act like particles with fixed size and shape (Yilmazer et al., 1991). However, gelatin type 'A' destabilized within few days of storage and the viscosity of destabilized emulsion increased abruptly (200 mPas on day 1, 2615 mPas on day 7, 3743 mPas on day 14).

Gelatin type 'A' (1%) and modified starch (12%) conjugate had apparent viscosity (471 mPas) with greater 'n' value (0.84) than gelatin type 'B' and modified starch combination (168.26 mPas) with lower n = 0.63. Also with time, the flow behavior index for gelatin type 'B'-modified starch conjugate decreased more (n = 0.42) suggesting greater flocculation than gelatin type 'A'-modified starch (n = 0.51). This may be interpreted by the reason that the presence of large particles (flocs) in gelatin type 'B'-modified starch which increased the resistance to the flow. We know the flocculated emulsions exhibit strong shear thinning behavior (Demetriades et al., 1997). Hence, this phenomenon led to an increase in the shear thinning index (Mirhosseini et al., 2007).

For gelatin and modified gum Arabic emulsions, apparent viscosity was measured by mixing the separated emulsion. The apparent viscosity showed higher values as there was excessive flocculation.

TABLE 4.3: Consistency coefficient (mPa) and flow behavior index of conjugate emulsions

Emulsion	m (day 1)	m (day 7)	m (day 14)	n (day 1)	n (day 7)	n (day 14)
Gel B-MS	471.05 ± 23.30	1818.06 ± 171.33	1918.99 ± 49.98	0.63± 0.00	00.47 ± 0.01	0.42 ± 0.01
Gel B-MGA	251.64 ± 1.23	408.32 ± 73.28	1119.44 ± 345.76	0.65 ± 0.01	0.60 ± 0.02	0.49 ± 0.01
Gel B-X-PGA	3455.42 ± 5.62	9857.12 ± 4.81	9494.1 ± 113.06	0.34 ± 0.00	0.28 ± 0.00	0.30 ± 0.00
Gel A-MS	168.26 ± 11.22	3076.17± 30.05	3092.74 ± 25.02	0.84 ± 0.00	0.51 ± 0.00	0.51 ± 0.00
Gel A-MGA	172.61 ± 1.32	1750.25 ± 25.37	1923.09 ± 14.84	0.70 ± 0.01	0.43 ± 0.06	0.38 ± 0.21
Gel A-X-PGA	200.48 ± 14.67	2615.92 ± 416.90	3743.95 ± 101.79	0.55 ± 0.00	0.30 ± 0.02	0.28 ± 0.00

4.4.5 Particle properties

The droplet size distribution of emulsions containing different hydrocolloids and conjugated emulsions was measured. Typical particle size distribution at day 1, day 7 and day 14 for emulsions containing 1% gelatin type 'A' and 1% gelatin type 'B', 12% modified starch and 12% modified gum Arabic with 10% oil at two pH are shown in Figure 4.9 and Figure 4.10, respectively. Noticeably, the droplets were not of the same size, i.e. the emulsions were polydisperse. Since the droplet size varied, an average droplet size was considered for comparison. It can be clearly seen that for all the emulsions, with increase in storage time, the size of the droplets increased and the distribution shifted to larger sizes but the magnitude of the shift varied for each. Chanamai and McClements (2001) observed increase in size of the flocs when the emulsions were left on the microscope slide, the floc size increased over time which was probably caused by droplet-droplet, droplet-floc, and floc-floc collisions generated by Brownian motion.

Table 4.4 compares the average droplet size and slope of average particle size growth in micrometers (µm) of protein and polysaccharide stabilized emulsions. We can observe a clear increase in size of the droplets with storage time. The influence of pH on mean droplet size for the different systems was monitored. In case of gelatin type 'A' emulsion, the average droplet size on day 1 for the freshly prepared emulsion in deionized water was smaller (0.076µm) than the emulsion prepared in pH 3.4 buffer (0.604µm). However, with time, the average size for gelatin type 'A' in deionized water sharply increased around 10 times higher than original (0.076µm at day 1 to 0.746µm at day 7) within 7 days of storage and rose to 1.485µm on day 14. The observed increase in particle size might have been caused by droplet flocculation or coalescence. This was likely because the pH of neutral water was close to the isoelectric point of gelatin type 'A' (~7-9), because of which the droplets of the gelatin type 'A' stabilized emulsions might have formed aggregates in deionized water as mentioned earlier. The droplet distribution and the shift from small to large droplet size is a clear indication of aggregation or bridging flocculation. An increase in mean particle size due to flocculation and coalescence usually leads to an increase in the instability of the droplets to gravitational separation (McClements, 2007b). In comparison to this, the increase in size for type 'A' emulsion prepared in acidic buffer at pH 3.4 was minor from day 1 to day 14 (0.604µm to 0.756µm).

Gelatin type 'B' had a sharp increase in the mean diameter after two weeks of storage (0.37 µm & 2.44 µm on day 1 and day 14, respectively) in acidic buffer system at pH 3.4 and the distribution shifted to larger extent than the original emulsion indicating extensive droplet aggregation. However, in deionized water emulsion droplet size was bigger initially, but with a small increase thereafter (1.18 µm and 1.38 µm at day 1 and day 14, respectively).

For modified starch stabilized emulsions, there was no significant difference in droplet size with respect to pH and storage time, as both systems displayed more or less similar size (0.43 µm) with two weeks storage (Table 4.4). These results are in agreement with Chanamai et al. (2002) who reported there was no extensive difference in mean droplet size of emulsions stabilized with modified starch in pH range from 3 to 9. The constancy in the mean droplet diameter, with time, indicates the absence of coalescence between droplets. No coalescence is an indication of better stability (Nunez et al., 2000). For modified gum Arabic, the size of the droplets was significantly smaller in pH 7.0 (0.13 µm) than pH 3.4 buffer system (1.52 µm). However, the droplet size did not change considerably during the observation time in both pH systems.

Figure 4.11 and Table 4.5 shows the comparative particle size distribution for conjugate emulsions at pH 3.4 and pH 7.0 at different systems studied. Figure 4.11(a) and 4.11(d) compare the particle size and growth for conjugate of gelatin type 'B'- modified starch (gel B-MS) in pH 7 and conjugate of gelatin type 'A'- modified starch (gel A-MS) in pH 3.4 buffer respectively. The sizes of droplets for gel B-MS conjugate are smaller (1.52 µm on day 1) than gel A-MS conjugate (2.37 µm). However on storage, there was not a significant difference in the size distribution for gel A-MS stabilized emulsion with a similar average particle size ranging from 2.37 µm to 2.42 µm. Gelatin and modified gum Arabic conjugates at both pH which separated within few hours of preparation presented a poor behavior as emulsifier; this was signified by enormous increase in size of the particles kept for 14 days (Figures 4.11(b) and 4.11(e)). Figures 4.11(c) and 4.11(f) demonstrate the effect on particle size for gelatin combination with viscosity builders,

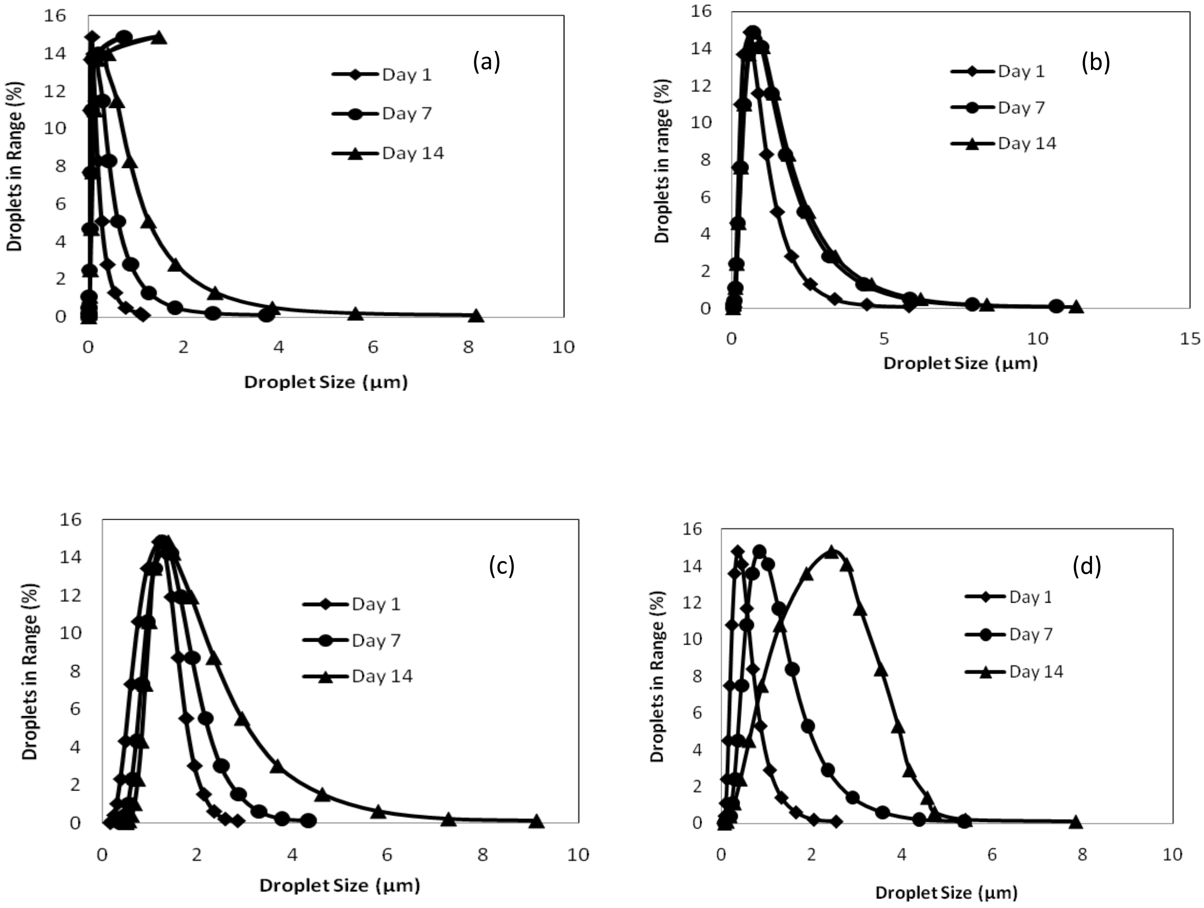


FIGURE 4.9. Particle size and distribution for (a) 1wt% gelatin type 'A' in pH 7, (b) 1% gelatin type 'A' in pH 3.4, (c) 1% gelatin type 'B' in pH 7.0 and (d) 1% gelatin type 'B' in pH 3.4 measured at day 1, 7, and 14.

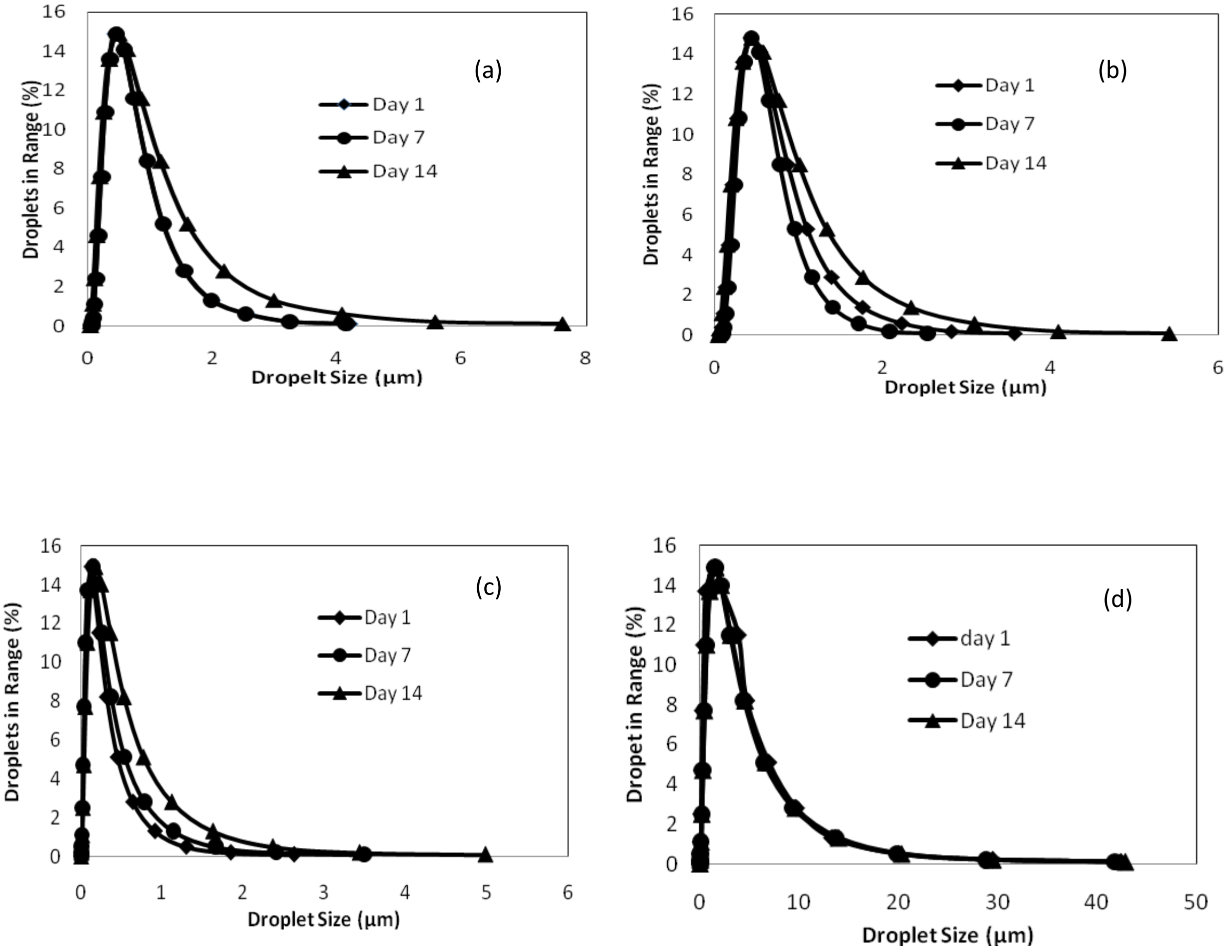


FIGURE 4.10. Particle size and distribution for (a) 12% modified starch in pH 7.0, (b) 12% modified starch in pH 3.4, (c) 12% modified gum Arabic in pH 7.0 and (d) 12% modified gum Arabic in pH 3.4 measured at day 1, 7, and 14.

TABLE 4.4: Average Particle size of protein and polysaccharide stabilized emulsions

Emulsion	Average particle size (μm)			Particle size growth
	Day 1	Day 7	Day 14	Slope
Gel ‘A’ in pH 7	0.07	0.75	1.45	0.11
Gel ‘A’ in pH 3.4	0.60	0.69	0.75	0.01
Gel ‘B’ in pH 7	1.18	1.24	1.38	0.02
Gel ‘B’ in pH 3.4	0.36	0.84	2.44	0.16
Mod. starch in pH 7	0.43	0.45	0.48	0.004
Mod. starch in pH 3.4	0.43	0.43	0.43	0.006
Mod. gum Arabic in pH 7	1.50	1.56	1.63	0.01
Mod. gum Arabic in pH 3.4	0.13	0.15	0.18	0.004

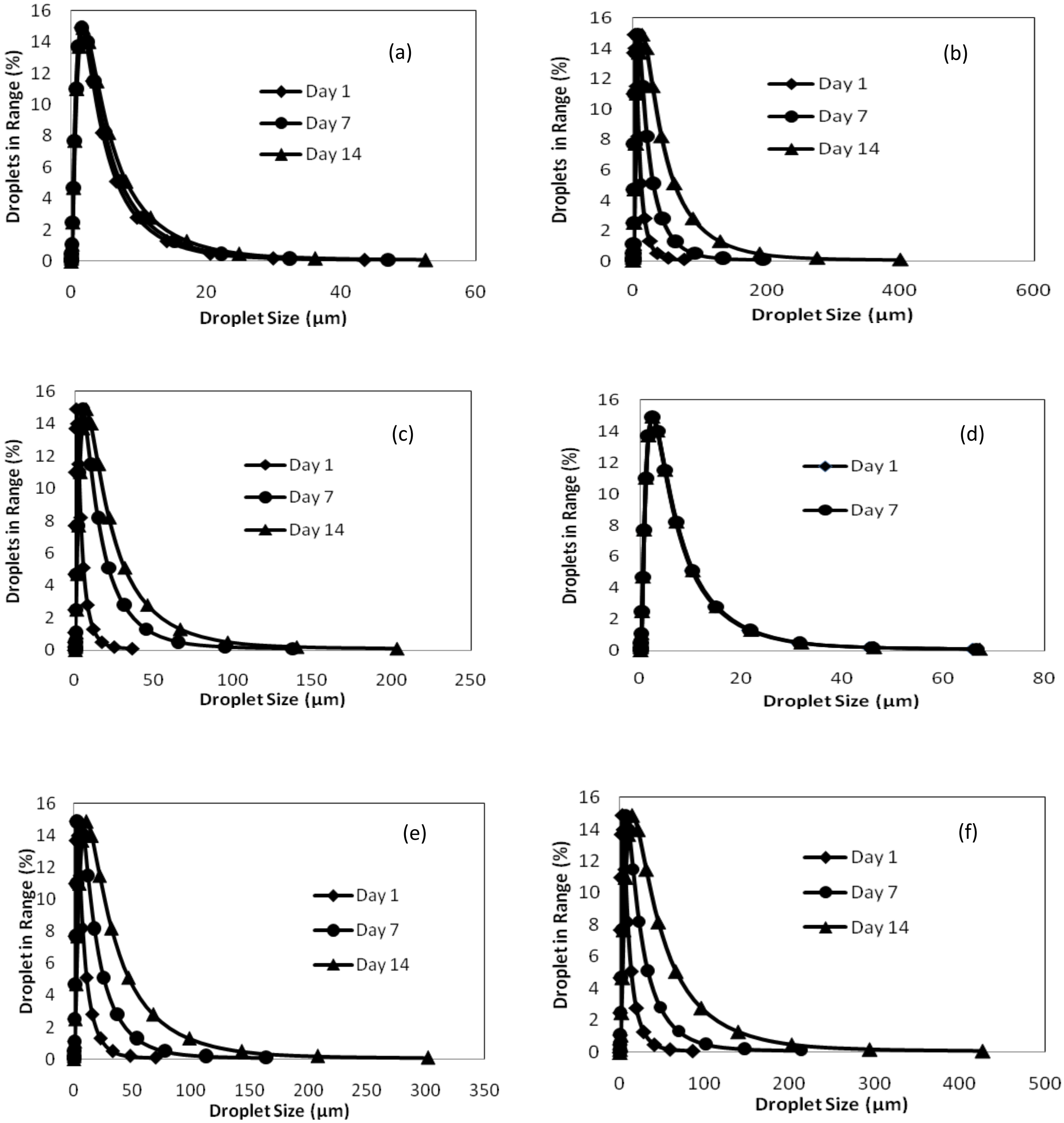


FIGURE 4.11. Particle size and distribution for (a) 1% gelatin 'B'-12% modified starch in pH 7.0, (b) 1% gelatin (type A), modified gum Arabic in pH 7.0, (c) 1% gelatin 'B'-Xanthan-PGA in pH 7.0, (d) 1% gelatin (type A)-modified starch in pH 3.4, (e) 1% w/w gel 'A'-12% modified gum Arabic, (f) Gelatin (type A) A-0.3% Xanthan -0.3% PGA measured at day 1, 7, and 14.

TABLE 4.5: Average particle size of conjugate emulsions

Conjugate emulsion	Average particle size (µm)			Particle size growth
	Day 1	Day 7	Day 14	Slope
Gel B-MS-pH 7	1.52	1.64	1.83	0.02
Gel B-MGA-pH 7	2.66	6.77	13.95	0.87
Gel B-X-PGA-pH 7	1.27	4.81	7.11	0.44
Gel A-MS-pH 3.4	2.37	2.40	2.42	0.004
Gel A-MGA-pH 3.4	1.67	2.86	10.85	0.72
Gel A-X-PGA-pH 3.4	2.98	7.45	14.90	0.92

Xanthan and PGA at pH 7 and pH 3.4 respectively. Gelatin type ‘A’ hybrid displayed significantly bigger particle size (14.90 µm) than gelatin type ‘B’ conjugate (7.11 µm) on day 14. This implies much higher flocculation in gelatin ‘A’. This suggests that gelatin B-Xanthan-PGA having smaller size may exhibit better emulsifying behavior in comparison to those prepared with gelatin ‘A’.

4.4.6 Stability of emulsions

The optical characterization of the concentrated emulsions using a Quick Scan was carried out. The transmission of light from the sample was calculated as a function of their height. The rate of creaming (aggregation) or creaming velocity for gelatin type ‘A’, gelatin type ‘B’, modified starch and modified gum Arabic stabilized emulsions are illustrated in figures 4.12 (a), (b), (c) and (d), respectively. For all the emulsions, the creaming velocity decreased as the biopolymer concentration increased (Taherian, 2006). The rate of creaming was high for gelatin type ‘A’ emulsions stabilized in deionized water and low in acidic buffer. However for gelatin type ‘B’ it was reverse. As mentioned earlier lower values of average particle size were observed relative to gelatin type ‘B’ at high pH (far from the isoelectric point) which correlated to the lower transmission. For modified starch, transmission was small for all the formulations however 12% concentration showed even smaller percentage transmission (i.e. greater stability) than 6% concentration. At higher polysaccharide concentrations creaming was retarded, because even though the droplets were aggregated they are incapable of moving, owing to the high viscosity or the gel-network formed by the polysaccharides (McClements, 2000). Modified gum Arabic at elevated concentration in deionized water indicated the lower aggregation rate than acidic buffer and hence was considered more stable at neutral pH. Thus the creaming behavior of non-flocculated and flocculated emulsions was clearly different. Creaming was much more rapid in the flocculated emulsion than in the non-flocculated emulsion, as would be expected because of the increase in the size of the particles in the system (Chanamai and McClements, 2001). All these results were in excellent agreement with rheology and particle size observations.

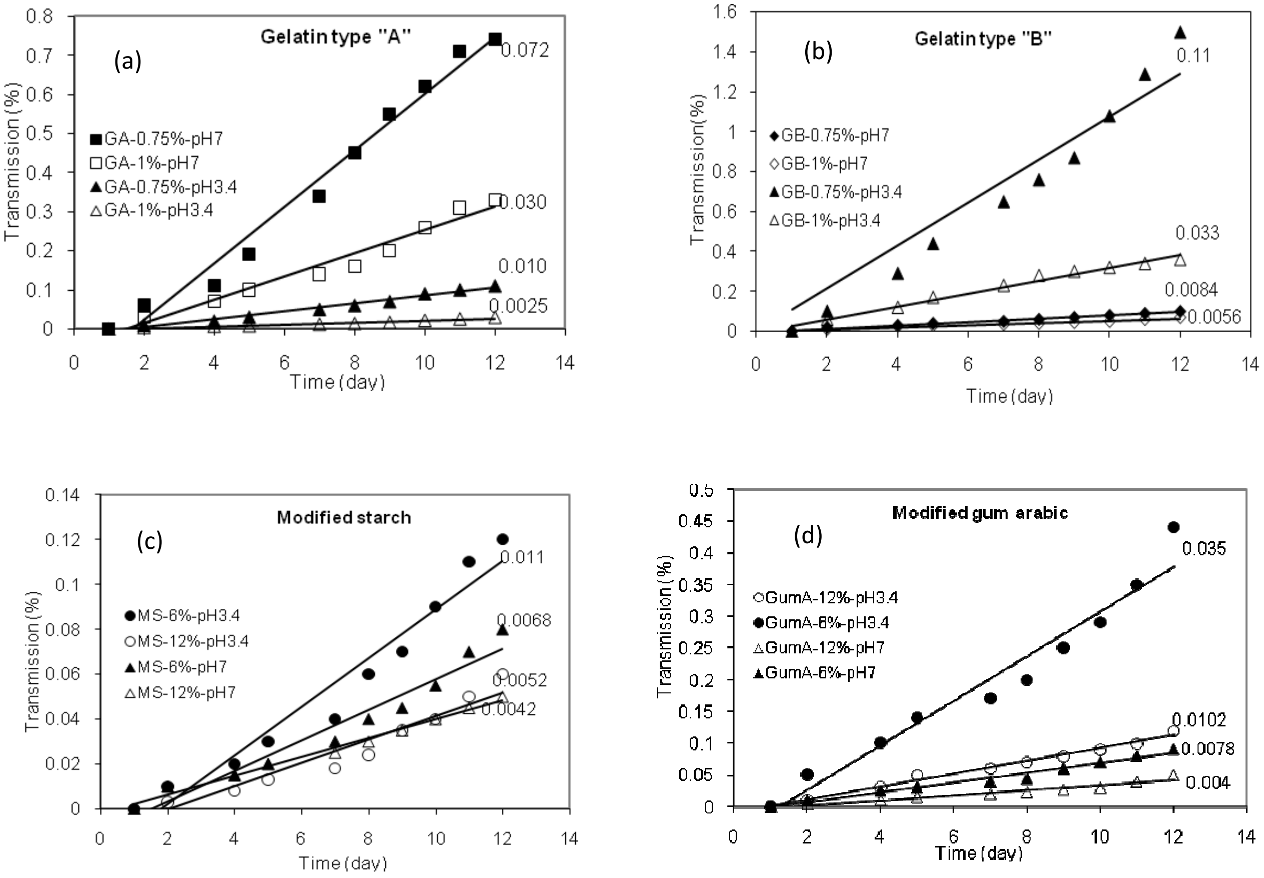
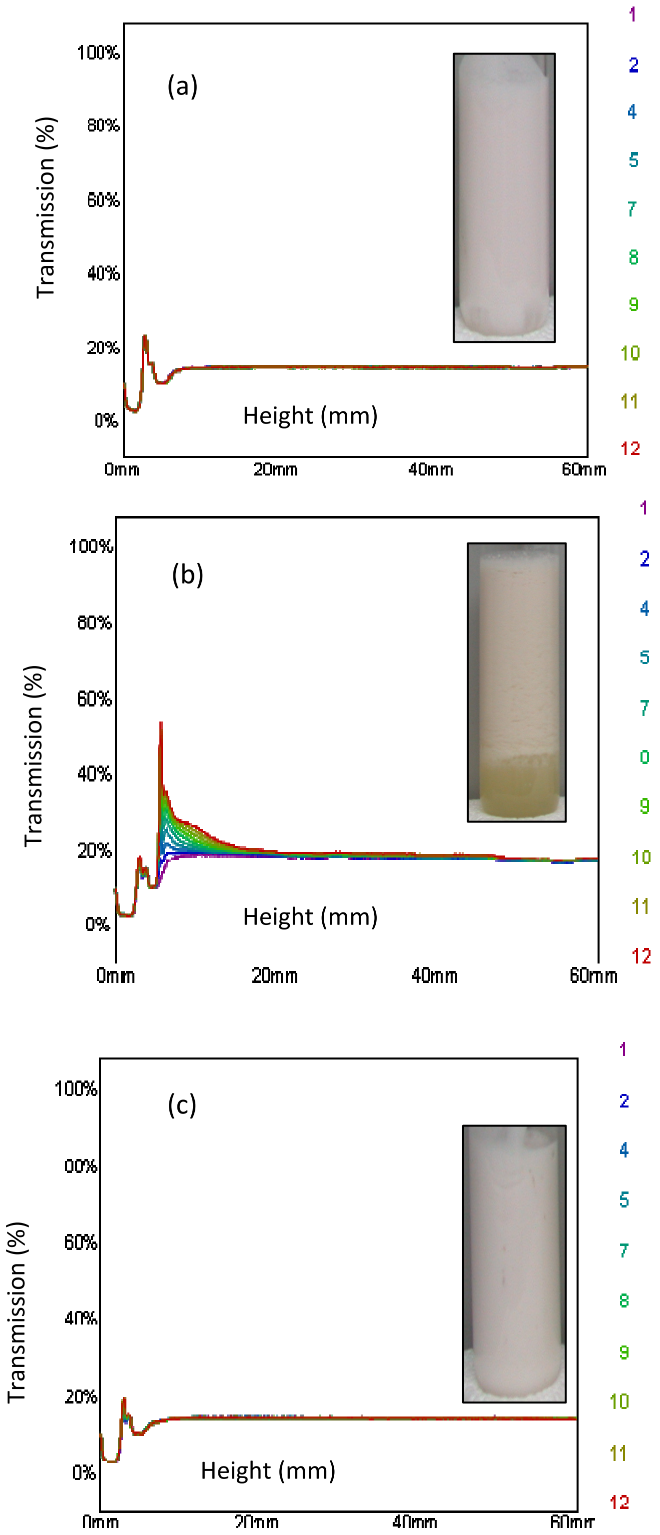


FIGURE 4.12. Time evolution of transmission for (a) gelatin type 'A' (GA), (b) gelatin type 'B' (GB), (c) modified starch (MS), and (d) modified gum Arabic (Gum A) stabilized emulsions as a function of storage time.

Figure 4.13 shows the creaming destabilization kinetics (transmission profiles) of the conjugate emulsions measured by % transmission as a function of time. Transmission increases as the size of droplets increases with storage. Initially, the transmission was fairly constant along the entire height of the emulsion because there was an even distribution of droplets throughout the system. With time, as the droplets moved upward, there was an increase in transmission at the bottom of unstable emulsions (because the droplet concentration decreased). From this condition it was easy to observe that for an unstable conjugate, the creaming process was faster than the others. As creaming proceeded a clear interface formed between the droplet-depleted layer at the bottom and the droplet-rich layer at the top for unstable emulsions (for example, Figure 4.13(b)). For a stable emulsion however, transmission was comparatively constant (for example, as shown in Figure 4.13(d)).

Figure 4.14 displays the time evolution of transmission for conjugated emulsions. It was seen that the slope of transmission (rate of aggregation) is highest for gelatin type ‘A’-Xanthan-PGA followed by gelatin and modified gum Arabic conjugates. These results are in agreement with the particle size distribution previously analyzed. Gelatin type ‘A’-Modified starch indicated lowest aggregation rate and hence, was the most stable conjugate followed by gelatin type ‘B’-Modified starch and gelatin-Xanthan-PGA.


(a)
Transmission (%)
100%
80%
60%
40%
20%
0%
Height (mm)
0mm
20mm
40mm
60mm
1
2
4
5
7
8
9
10
11
12
(b)
Transmission (%)
100%
80%
60%
40%
20%
0%
Height (mm)
0mm
20mm
40mm
60mm
1
2
4
5
7
0
9
10
11
12
(c)
Transmission (%)
100%
00%
60%
40%
20%
0%
Height (mm)
0mm
20mm
40mm
60mm
1
2
4
5
7
8
9
10
11
12

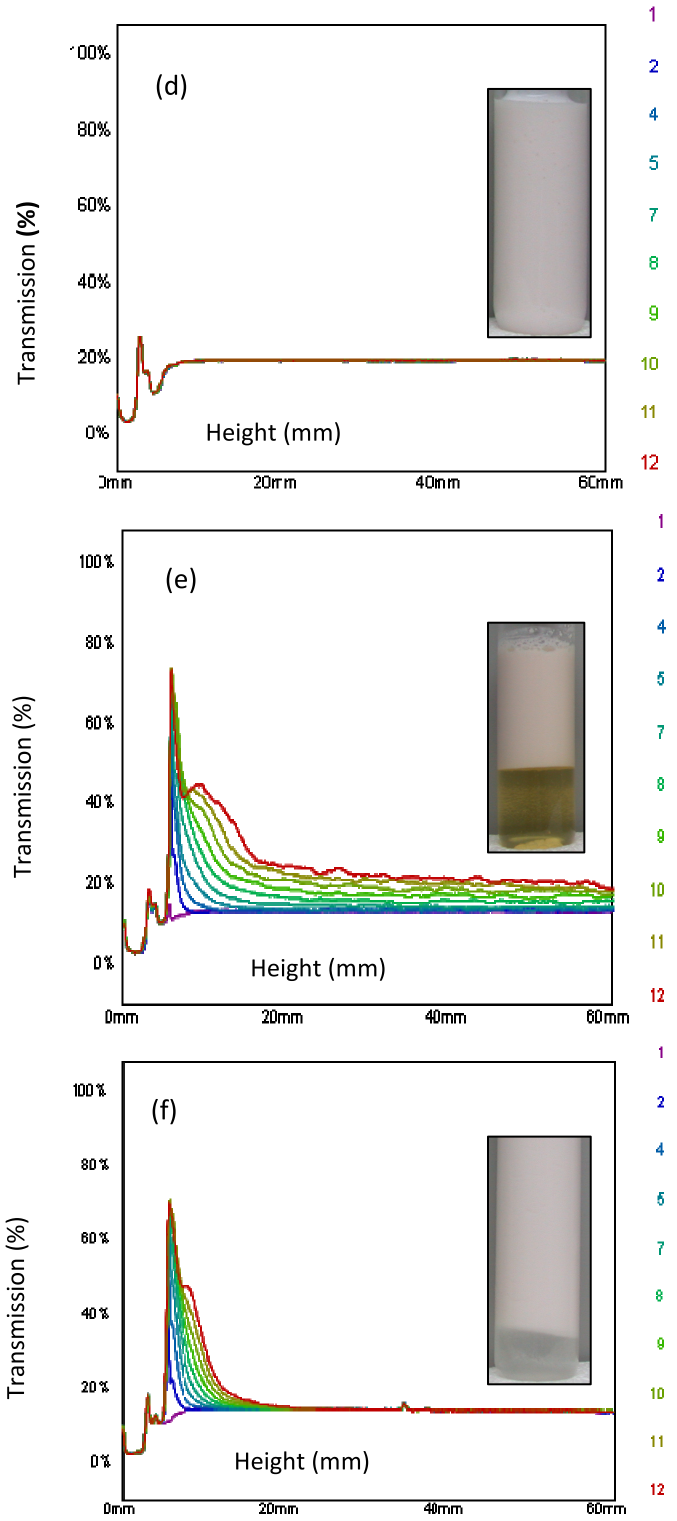


FIGURE 4.13. Transmission profiles for conjugate emulsions (a) gelatin type 'B'-modified starch, (b) gelatin type 'B'-modified gum Arabic, (c) gelatin type 'B'-Xanthan gum-PGA, (d) Gelatin type 'A'-modified starch, (e) gelatin type 'A'-modified gum Arabic, (f) gelatin type 'A'-Xanthan gum-PGA.

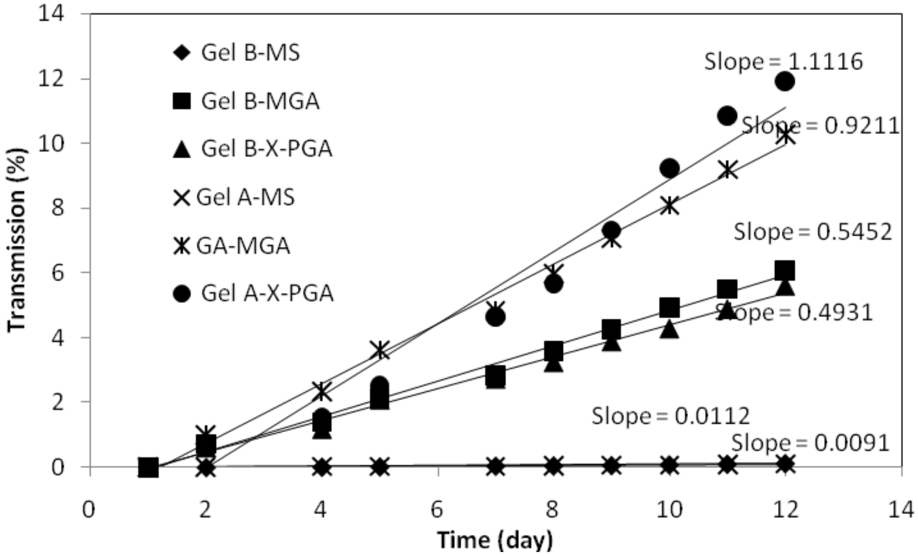


FIGURE 4.14. Time evolution of transmission for conjugated emulsions.

4.5 CONCLUSIONS

Higher concentrations showed less transmission (i.e. greater stability) for all the biopolymers. Near the isoelectric point of the gelatin, flocculation occurred in the emulsion which lead to considerable increase in the apparent viscosity and greater instability due to creaming. Gelatin emulsions were stable at pH far from their specific isoelectric points. In order to produce a stable gelatin emulsion, it is important to ensure that the pH is significantly far from the isoelectric point of the protein. Modified starch was stable in both the pH conditions as expected. Modified gum Arabic showed smaller particle size and slighter rate of aggregation at neutral pH. Gelatin type ‘A’-modified starch was observed to be the most stable conjugate in concentrated form at pH 3.4 followed by gelatin type ‘B’- modified starch and gelatin type ‘B’-Xanthan-PGA both at pH 7.0. Gelatin and modified gum Arabic hybrids were not stable and destabilized within hours of preparation. This study demonstrated that oil-in-water emulsions stable in concentrated form can be diluted into fruit juices and dairy beverages to form highly stabilized beverage cloud and flavor emulsions without the use of restrictive weighting agents.

CONNECTING STATEMENT TO CHAPTER 5

In this chapter, reformulation of the emulsions was done based on results obtained in the previous chapter. The individual emulsions and conjugates which were stable in concentrated form were diluted in a simulated juice and mimicked milk beverage and their shelf stability was investigated. We aimed to prepare beverage emulsions with natural emulsifiers without the restricted weighting agents. The results of this work will be useful for industrial practices to develop quality emulsions and enriched milk and fruit drinks

A manuscript is also being prepared for publication based on the studies highlighted in this chapter:

Arora JK, Ramaswamy, HS, Taherian, AR, 2009. Stability of thermally and high pressure pasteurized simulated juice and dairy beverages.

All experiment work and data analysis were carried out by the candidate under the overall supervision of Dr. H.S. Ramaswamy. Dr. Taherian provided the technical supervision to set up the experiments, train the candidate and analyze the results.

CHAPTER 5

STABILITY OF THERMALLY AND HIGH PRESSURE PASTEURIZED SIMULATED JUICE AND DAIRY BEVERAGES

5.1 ABSTRACT

In our previous study, concentrated emulsions were prepared using different hydrocolloids such as gelatin type ‘A’, type ‘B’, modified starch, modified gum Arabic individually and in conjugation. In these studies, a constant amount of canola oil was added to each solution followed by addition of viscosity builders such as Xanthan gum and PGA as stabilizers. In order to test the emulsion stability in the dilute form in final preparations, the emulsions that were considered stable in concentrated form were diluted in a simulated juice (pH 3.0) and mimicked dairy beverage (pH 6.8). These were pasteurized by heat treatment and high pressure. Stability tests were carried out with the formulated beverage solutions. Modified starch stabilized emulsions produced stability in both simulated juice and dairy beverage. Modified gum Arabic was stable in the dairy beverage with no sign of ringing. Gelatin type ‘A’ and modified starch conjugate resulted in greater stability compared to the other conjugated emulsions. However, gelatin alone stabilized systems were not stable. Ringing was characteristically associated with emulsions formed with gelatin alone. Neither thermal processing nor high pressure treatment resulted in destabilization of the emulsion.

5.2 INTRODUCTION

Citrus and dairy beverages have been a rapidly growing segment of the beverage world, having shown tremendous growth in the market for past few years. Global imports of beverages have increased significantly from a value of \$17.2 million in 1996 to \$179.5 million in 2007 (Statistics Canada). Among all the beverages such as ready to serve juice and milk drinks (RTD), frozen fruit juice concentrates, carbonated beverages, beverage emulsions cannot be overlooked.

Beverage emulsions are the sole class of oil-in-water emulsions which are prepared in concentrated form and diluted into juice or dairy drinks to provide cloudy appearance and/or desirable fragrance and color in the finished product. This cloudy appearance is one of the most important quality attributes of beverage emulsions. Suspended particles (usually oil globules) dispersed in the medium scatter light which determines the turbidity, cloudiness, opacity or lightness of the dilute beverage emulsion. The intensity of the cloud in the beverage will depend mainly on the concentration and size of the oil globules. The size of globules in cloud beverages generally ranges from 0.5-5 microns (Tan, 2004; Taherian et al., 2008).

The major components of beverage emulsions are a water phase consisting of 60-70% water, amphiphilic polysaccharides to reduce the surface tension and allow steric stabilization, stabilizer gums to prolong stability and control rheological properties, citric acid to control pH and preservatives to prevent spoilage (Taherian, 2006), while the oil phase contains vegetable or flavor oils and weighting agents. Weighting agents (like brominated vegetable oils, also known as density adjusting agents), usually added to the oil, largely eliminate the gravitational separation (Tan, 2004) of the emulsion components. However, many countries have withdrawn or have permitted limited use of weighting agents, and therefore producing cloud emulsions that achieve stability with density matching and prevent creaming has become a challenging task for the beverage industry (Taherian, 2006). The most common sign of beverage emulsion deterioration is “ringing” or “oiling-off”. Ringing is the formation of a whitish “ring” around the neck of the bottle, whereas oiling-off is the formation of a shiny oil slick on top of the product. Ringing and oiling-off are the result of certain physicochemical mechanisms that occur within the dilute beverage, including gravitational separation (resulting in creaming), flocculation, and coalescence (Chanamai and McClements, 2000). These mechanisms promote creaming and may cause breakdown of the emulsion into top oil rich phase and bottom water phase. A stable beverage emulsion is therefore portrayed by long term stability in diluted form with absence of ringing resulting from raised flocculated or coalesced droplets around the neck of the bottle.

Emulsions for beverages are generally prepared by dispersing the oil phase in water, using emulsifiers (such as hydrocolloids) and subjecting the mixture to homogenization (Chanamai and McClements, 2001). Hydrocolloids serve as emulsifiers and stabilizers in beverage emulsions. Three factors: viscosity effects, steric hindrance and electrostatic interactions, and govern hydrocolloid stability. As detailed in previous chapters, modified gum Arabic (MGA) is used to prepare the emulsions in this study. MGA is produced by reacting natural acacia with l-octenyl succinic anhydride (OSA) (TIC Gums, Belcamp, MD). High enriched molecular mass arabinogalactan–protein complex fraction (AGP) in gum Arabic containing most of the total protein is mostly responsible for the emulsifying properties (Buffo et al., 2001; Chanamai and McClements, 2002). It has been postulated that gum Arabic adsorbs at the oil–water interface in the manner where the arabinogalactan (AG) groups protrude into the solution, while the polypeptide backbone rests at the interface (Acedo-Carrillo et al., 2006). Modified starches are a group of specially designed starch derivatives with balanced lipophilic and hydrophilic groups on the starch molecules. Purity Gum$^{\text{TM}}$ Be (octenyl-succinate starch, OSA starch) used in this study is made by esterification of starch and anhydrous octenyl succinic acid under alkaline condition. Purity gum is believed to provide surface activity as high as gum Arabic (Taherian et al., 2006).

Yet, polysaccharides have less surface activity compared to proteins. This is associated to their low flexibility, pronounced hydrophilicity, and monotonic repetition of the monomer units in the backbone (Acedo-Carrillo et al., 2006) and therefore a large excess must be added to ensure that all the droplet surfaces are sufficiently coated. One advantage of protein as an emulsifier is that it is used at relatively low concentrations compared to other polysaccharides. Therefore, many proteins are also surface active ingredients that can be used as emulsifiers because of their ability to facilitate the formation of a protective membrane by adsorbing to the surface of freshly formed oil droplets which prevents droplets from coalescing, improve stability and produce desirable physicochemical properties in beverage emulsions. The appearance, rheology and stability of protein stabilized emulsions depend largely on the molecular structure, interfacial interactions and environmental conditions such as temperature, pH and ionic strength of the continuous phase and the emulsion (Chanamai and

McClements, 2000). Gelatin has been widely utilized for foods, photographic applications, cosmetic and other medical materials, and microorganism culture materials etc. Recently, its use is expanding to new applications such as health foods because of its physical functionality. Gelatin type ‘A’ and type ‘B’ are used in this work to stabilize oil-in-water beverage emulsions. Gelatin is manufactured from collagen obtained from mammalian resources using a controlled acid (resulting in gelatin type ‘A’) or alkaline (resulting in gelatin type ‘B’) hydrolysis (Cho et al., 2006). Some previous studies have shown that gelatin is surface-active and that it is capable of acting as an emulsifier in oil-in-water emulsions. But it hasn’t been confirmed if we can use gelatin to prepare stable emulsions in beverage form. Therefore, based on our previous results, gelatin stable at specific pH solutions will be incorporated into dilute simulated beverages. Also, in addition to utilizing gelatin alone, conjugates of gelatin with polysaccharides (modified starch, Xanthan gum and PGA) stable in concentrate form will be prepared into dilute solutions and their stability will be examined.

Conventionally, thermal processing has been used to pasteurize beverage emulsions. Thermal pasteurization treatments extend the shelf life of food products by inactivating the pathogens and also reducing the spoilage bacteria but also affect the product quality. Use of non-thermal techniques as an alternative to heat processing is acquiring relevance in recent times. One of these techniques is high hydrostatic pressure technology which produces high quality foods that are microbiologically safe with an extended shelf life and provides similar characteristics to the food with minimal effect on the sensory qualities associated with ‘fresh-like’ attributes such as texture, color and flavor. Increasing consumer demand for high-quality, minimally processed, additive-free and microbiologically secure foods has ensured worldwide acceptability of the potential to high pressure (HP) technology in food processing. The HP technology is also known to have several advantages over conventional thermal processing, such as low-temperature, uniform pressure, short treatment time, minimum quality loss, and waste free (Zhu et al., 2008). High pressure ranges of approximately 300–700 MPa for periods from approximately 30 seconds to a few minutes are required to destroy pathogenic bacteria such as *Listeria, Escherichia coli, Salmonella* and *Vibrio*, as well as yeasts and moulds that cause food spoilage (Bull et al., 2004).

Only limited amount of data is available about the effect of thermal processing and HP treatment on the stability of beverage emulsions. The objective was to determine whether model beverage emulsions can be created with gelatin type ‘A’ & type ‘B’, modified starch, modified gum Arabic, Xanthan gum and PGA followed by pasteurization either achieved thermally and non thermally (using high pressure processing) and then to evaluate their stability during storage. The use of natural occurring biopolymers will help resolve health restrictions warranted by the need for weighting agents in the formulation of beverages.

5.3 MATERIAL AND METHODS

5.3.1 Materials

Commercially available refined vegetable canola oil was obtained from the local market. Modified starch (Purity Gum^{TM} Be) was obtained from National Starch (Bridgewater, NJ), Gelatin type 'A' and type 'B' from Rousselot (a VION Company, Dubuque, IA,). Modified Gum Arabic, Viscosity builders: Ticaxan Xanthan 200 and, Propylene glycol alginate (PGA/LV Powder) were obtained from TIC GUMS (Belcamp, MD,). Food grade citric acid and dibasic sodium phosphate were used to prepare buffer solutions and were bought from Fisher Scientific (Montreal, QC). Deionized water was used to prepare solutions and emulsions.

5.3.2 Preparation of emulsions

Selected emulsions as detailed below (which were stable in concentrated form) were again reformulated with same procedures as described in the previous chapter:

Emulsions at pH 3.4	**Emulsions at pH 7.0**
1. Gelatin (Type A, 1% w/w) **2.** Modified starch (12% w/w) **3.** Gelatin (Type A, 1% w/w)-Modified starch (12% w/w)	**1.** Gelatin (Type B, 1% w/w) **2.** Modified starch (12% w/w) **3.** Modified gum Arabic (12% w/w) **4.** Gelatin (Type B, 1% w/w)-Modified starch (12% w/w) **5.** Gelatin (Type B, 1% w/w)- Xanthan (0.3% w/w)-PGA (0.3% w/w)

5.3.3 Preparation of simulated beverages

Sugar solutions (11°Brix) were prepared by adding calculated amount of sugar to deionized water and pH was adjusted using anhydrous citric acid to get pH 3 (similar to a orange juice) and mixed with 2% w/w of emulsions prepared at pH 3.4. Acidified sugar solution without the addition of emulsion was used as blank. For simulating the dairy beverage, a buffer having a pH 6.8 (similar to that of milk) was made and mixed with 2% w/w of emulsions prepared at pH 7.0.

All the formulated beverages were homogenized before being filled into previously sterilized 300 mL glass bottles (for thermal pasteurization) and plastic bottles (for high pressure pasteurization). Beverages were prepared in duplicates.

5.3.4 Thermal processing

Glass bottles filled with simulated juice beverages were thermally pasteurized in a temperature controlled glycerin bath (Julabo 26, Fisher Scientific, Montreal, QC,) with a programmable temperature controller. Sample temperature was gathered using a thin thermocouple located at the cold spot of the bottle sample using a HP data logger (Model 34970A, 20 channels multiplexer, Hewlett Packard, Austin, TX). The bottles were treated at 90°C for 12 min, cooled and then stored at room temperature (22°C) (Taherian et al., 2007). Whereas, bottles of simulated milk beverages were given a heat treatment at 71.7°C for 15s in a temperature controlled bath and refrigerated at 4°C (to mimic milk pasteurization) (Ramesh, 2007).

5.3.5 High pressure pasteurization

High pressure treatments were given in a HP unit capable of operation at pressures up to 650 MPa (model ACIP 6500-5-12VB, ACB, Nantes, France) with a chamber volume of 5 L. Water was used as the pressure-transfer medium.

Plastic bottles were filled with the beverage drinks. Simulated juice beverage samples were subjected to a pressure of 550 MPa for 1 min at room temperature. This condition was chosen based on a previous study done in our lab, which resulted in excellent mango juice quality that was comparable to fresh juice for up to 60 days (Hiremath, 2005).

For milk beverages, 600 MPa pressure was applied with 10 min holding time. Similar pressure-time combination was applied by Harper (2005). The treatment had resulted in reduction of the psychrotrophic bacteria counts by 5 logs for skim and chocolate milk and by 2 logs for 2% milk.

5.3.6 Stability

The emulsion added simulated drinks were stored at ambient temperature (22^{o}C) and milk beverages were refrigerated (4^{o}C), for a period of up to two months. Gravity creaming of emulsion samples after dilution were monitored visually every two weeks. At the end of the storage period, samples with the presence of severe whitish ring on the top of the bottle were graded as '+ +' and others showing no signs of creaming were considered stable with negative sign '-'. Monitoring of samples was performed in duplicate.

The emulsion stability was also expressed with respect to the maintenance of the homogeneous structure of the system. The shelf stability of the beverages was rated on the bases of uniformity of the cloud present throughout the height of the bottle determined every 2 weeks until the end of storage (8 weeks). The results were expressed as percentage of the cream segment height in relation to the total height of the beverage in the bottle (H_B): Creaming 'C' = $100 \times H_C/H_B$. H_C is the height of the cream (Klinkesorn et al., 2004). The emulsions were also graded from '1' to '9', '1' indicating no lifting and '9' being maximum lift of the oil droplets to the top. The emulsions provided opacity to all the beverages. This was evident comparing the blank (acidified sugar solution without emulsion) with simulated juice carrying emulsions.

5.4 RESULTS AND DISCUSSION

Thermal treatment and high pressure processing were carried out to pasteurize the emulsion added beverages. There was no differences in visual stability of emulsions pasteurized either by thermal or high pressure. The beverages stable with thermal processing also indicated stability with high pressure treatment. All stability parameters studied gave identical value over the two month storage period. However, additional research needs to be done to determine whether high pressure pasteurization can represent a better alternative to thermal pasteurization for processed beverage emulsions by providing similar stability characteristics with minimal effect on their sensory qualities such as texture, color and flavor while ensuring pasteurization.

The representative results concerning the stability of the emulsion added simulated juice beverages are presented in Table 5.1. The table shows the percentage rate of creaming at intervals of 2 weeks over a 2 month storage period. For gelatin type ‘A’ added emulsion beverage a thin layer of creaming (C = 1.25%) was observed after 2 weeks which increased within storage time (C = 4.90% after 8 weeks) with severe ringing at the neck of the bottle. However for modified starch added emulsion beverage the creaming rate was slight for the 2 month storage period (C = 0.40%). The conjugate of gelatin type ‘A’ and modified starch beverage were the most stable with no visible creaming (C = 0%). ‘Lifting’ refers to rising of the flocculated oil droplets to the top leaving behind the water phase. The greater the lift, the lower will be the stability of the beverage. Grading on the basis of lifting is also shown in Table 5.1. Lifting was much higher for gelatin type ‘A’ beverage indicating lower stability. Systems showing greater shelf stability illustrated low lifting (modified starch and gelatin A-modified starch conjugate systems).

Table 5.1. Visual quality rating for the emulsion added simulated juice beverages.

Emulsion: Gelatin type ‘A’			
Week	**[1]Lifting**	**[2]Creaming (C%)**	**[3]Ringing in bottle**
2	4	1.25	+
4	5	3.23	+ +
6	6	4.56	+ +
8	7	4.90	+ +
Emulsion: Modified starch			
2	1	0.00	-
4	2	0.16	±
6	2	0.25	±
8	2	0.40	±
Emulsion: Gelatin type ‘A’-Modified starch			
2	1	0.00	-
4	1	0.00	-
6	1	0.00	-
8	1	0.00	-

[1] Lifting: ‘1’ = no lifting, ‘9’ = maximum lifting of emulsion

[2] Creaming ‘C’ = 100 × H_C/H_B

[3] Ringing in bottle: ‘-’ = no ringing (excellent), ‘±’ = negligible, ‘+’ = moderate, ‘+ +’ = severe (non-acceptable).

Shelf stability of the emulsions in simulated juice drinks after thermal and high pressure pasteurization upon standing for 2 months is visually demonstrated in Figure 5.1 and Figure 5.2, respectively. In the beverage containing gelatin type ‘A’ emulsion, it was observed that during first 2 weeks the opacity of beverage greatly decreased and a whitish ring was formed at the neck of the bottle. The thickness of the creamy ring kept on increasing with storage time. As illustrated, gelatin type ‘A’ indicated instability with high amount of creaming after 2 months. This creaming was considered non-acceptable from a stability viewpoint. The beverage prepared with the addition of the modified starch emulsion however showed good stability. After 2 weeks, there was no creaming observed and with subsequent 2 months storage, very slight creaming was observed at the surface of the bottle. It was interesting to note that the gelatin type ‘A’-modified starch conjugate which was the most stable conjugate in concentrated form indicated excellent stability of all formulations with no signs of ringing even after 2 months of storage.

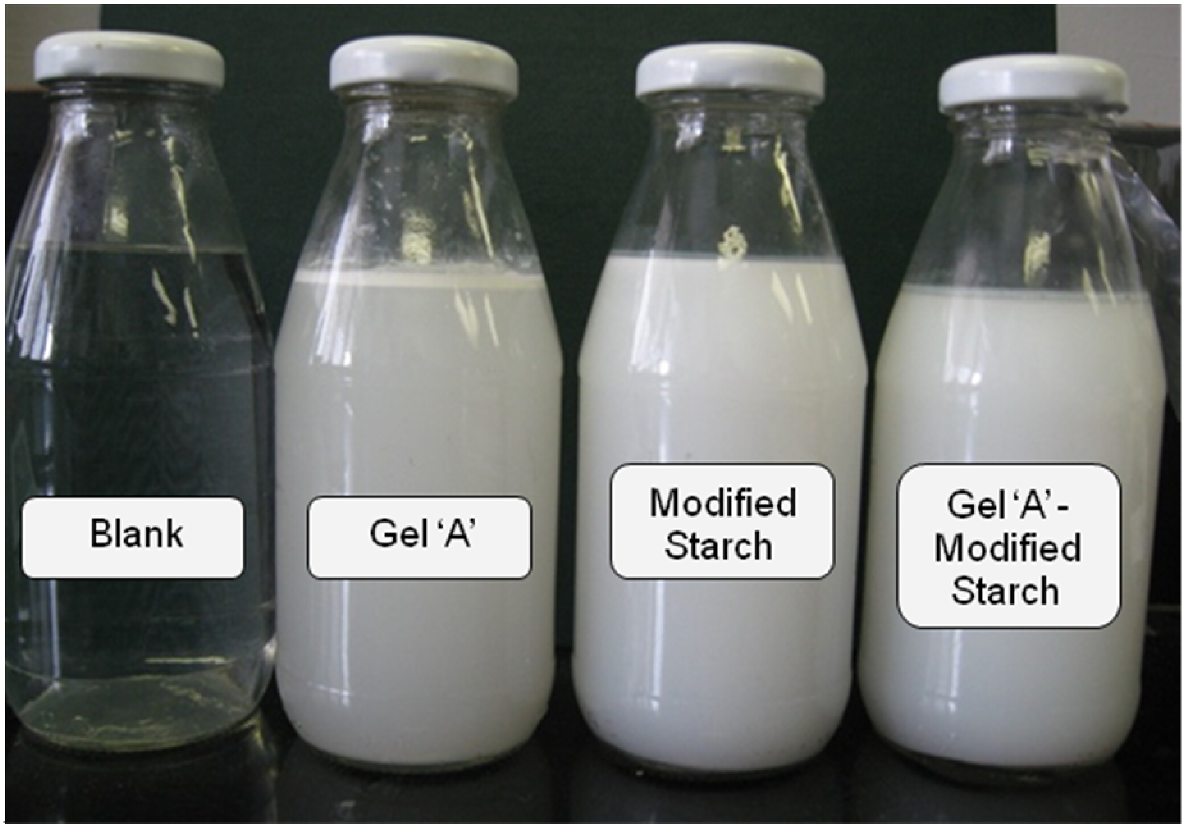


FIGURE 5.1. Shelf stability of thermally pasteurized simulated juice drinks containing 2% concentrated emulsion after 2 months of storage.

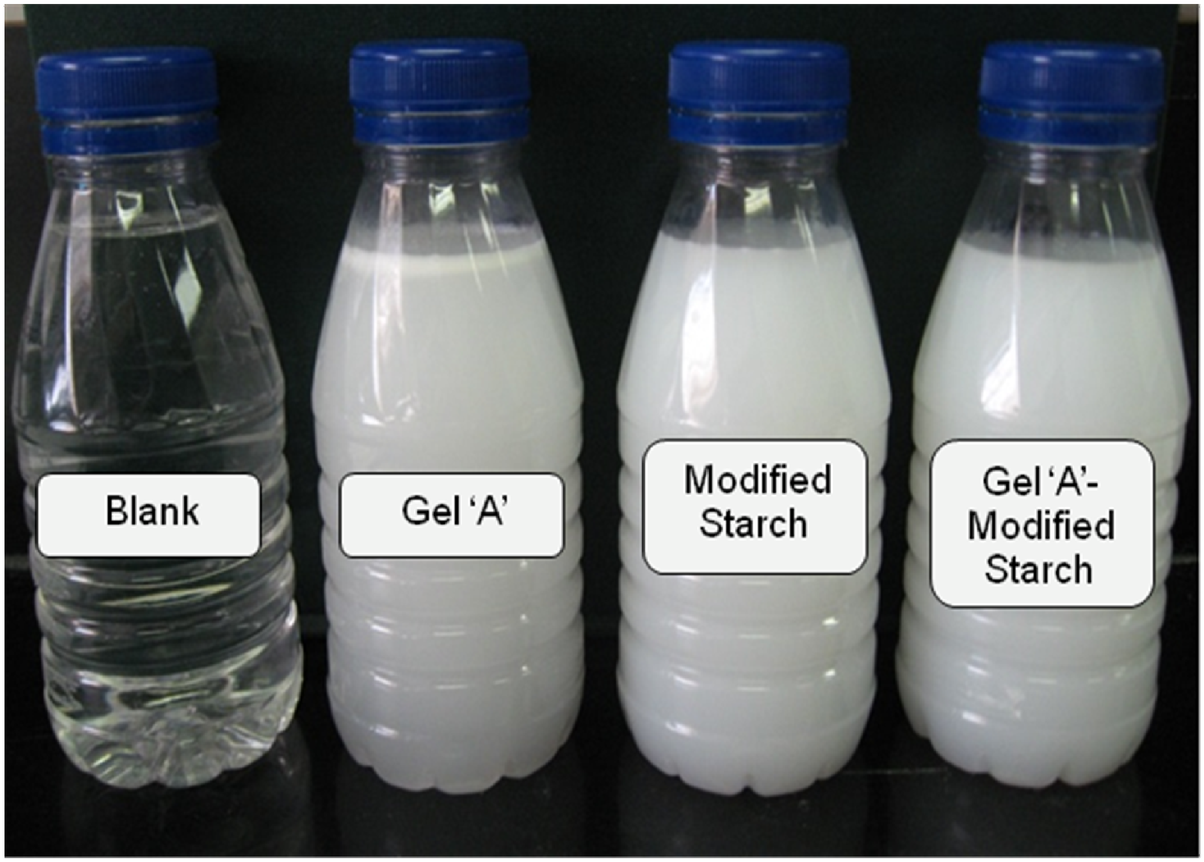


FIGURE 5.2. Shelf stability of high pressure pasteurized simulated juice drinks containing 2% concentrated emulsion after 2 months of storage.

Table 5.2 gives the stability rating for simulated milk beverages at pH 6.8. Compared to creaming in gelatin type ‘A’ beverage (C = 4.90% in 8 weeks), gelatin type ‘B’ had elevated creaming rate within storage time (C = 8.30% in 8 weeks). Also, greater rates of creaming were associated with gelatin conjugates; gelatin type ‘B’- modified starch (C = 9.33%) and gelatin type ‘B’ -Xanthan -PGA (C = 11.40%). These conjugates demonstrated highest lifting and visibly severe ringing. However, modified starch which exhibited negligible creaming (C = 0.40% in 8 weeks) in simulated juice exhibited zero creaming in mimicked milk beverage without lifting. Modified gum Arabic also had insignificant creaming rate (C = 0.31% after 8 weeks storage).

TABLE 5.2. Visual quality rating for the emulsion added simulated milk beverages.

Emulsion: Gelatin type ‘B’			
Week	**[1]Lifting**	**[2]Creaming (%)**	**[3]Ringing in bottle**
2	5	3.67	+
4	6	4.44	+ +
6	7	6.96	+ +
8	7	8.30	+ +
Emulsion: Modified starch			
2	1	0.00	-
4	1	0.00	-
6	1	0.00	-
8	1	0.00	-
Emulsion: Modified gum Arabic			
2	1	0.21	-
4	1	0.23	±
6	1	0.28	±
8	2	0.31	±
Emulsion: Gelatin type ‘B’-Modified starch			
2	3	2.86	±
4	7	6.90	+
6	7	7.87	+ +
8	8	9.33	+ +
Emulsion: Gelatin type ‘B’-Xanthan –PGA			
2	8	10.82	+ +
4	8	11.33	+ +
6	8	11.40	+ +
8	8	11.40	+ +

[1] Lifting: ‘1’ = no lifting, ‘9’ = maximum lifting of emulsion

[2] Creaming ‘C’ = 100 × H_C/H_B

[3] Ringing in bottle: ‘-’ = no ringing (excellent), ‘±’ = negligible, ‘+’ = moderate, ‘+’ ‘+’ = severe (non-acceptable).

Figures 5.3 and 5.4 show shelf stability of pasteurized milk beverages refrigerated at 4^0C for a storage period of 2 months. The turbidity decreased relatively fast and ringing and aggregation of droplets was associated with beverages containing gelatin emulsions. Samples containing a conjugate of gelatin type 'B'and Xanthan -PGA started showing separation into two visibly distinct layers after only a day. The solution started getting clear from the bottom and oil droplets accumulated and lifted to the top within 2 weeks. There was a major cloudy layer on the top and a small portion of a somewhat clearer layer on the bottom of the sample bottles. Towards the 4th week of the observation time, the rate of change decreased and the results after that did not show a significant difference. This system was considered the least stable conjugate. In two weeks, the turbidity also decreased for gelatin type 'B' emulsion and gelatin type 'B'-modified starch conjugate. There was formation of a thick ring at the neck of the bottles. These emulsions did not show favorable shelf stability on standing. The beverage containing modified starch showed the highest turbidity therefore indicating highest shelf stability among milk simulations. The beverage with modified gum Arabic also showed good stability with very thin ring after 2 months storage.

The formation of a thick adsorbed layer around the oil droplets in polysaccharide stabilized emulsions keeps them apart far enough by steric interaction, which also minimizes attractive Van der Waals forces. Jayme (1999) concluded, therefore, that the mechanism by which gum Arabic imparts stability to emulsions is via an electro-steric mechanism. The steric stabilization results from the macromolecules adsorbed to the droplet surface and as two such droplets approach together, the osmotic pressure between them increases due to the spatial captivity of the adsorbed polysaccharide molecule and are manifested as a repulsive interaction. The electrostatic stabilization on the other hand, arises from the electrostatic repulsion between droplets. However, the steric contribution is the dominant in case of polysaccharides (Taherian, 2006). Also the protein content of modified gum Arabic samples may be partially or fully responsible for the emulsifying activity (Randall et al., 1988). The interfacial membranes formed by proteins are usually relatively thin and electrically charged, hence, the major mechanism preventing droplet flocculation in protein (gelatin)-stabilized emulsions is electrostatic repulsion, rather than steric repulsion. However, protein emulsions are found to be very sensitive to pH, ionic strength effects and thermal processing (McClements, 2004b). This could be the reason for the instability of gelatin stabilized beverages. Also, the lower density of oil droplets might have resulted in higher number of thermodynamically favorable collisions among droplets causing highly flocculated droplets giving rise to ringing. Hence, advance study should be done to investigate the influence of solution conditions, ingredient interactions and environmental stresses on gelatin stabilized emulsions.

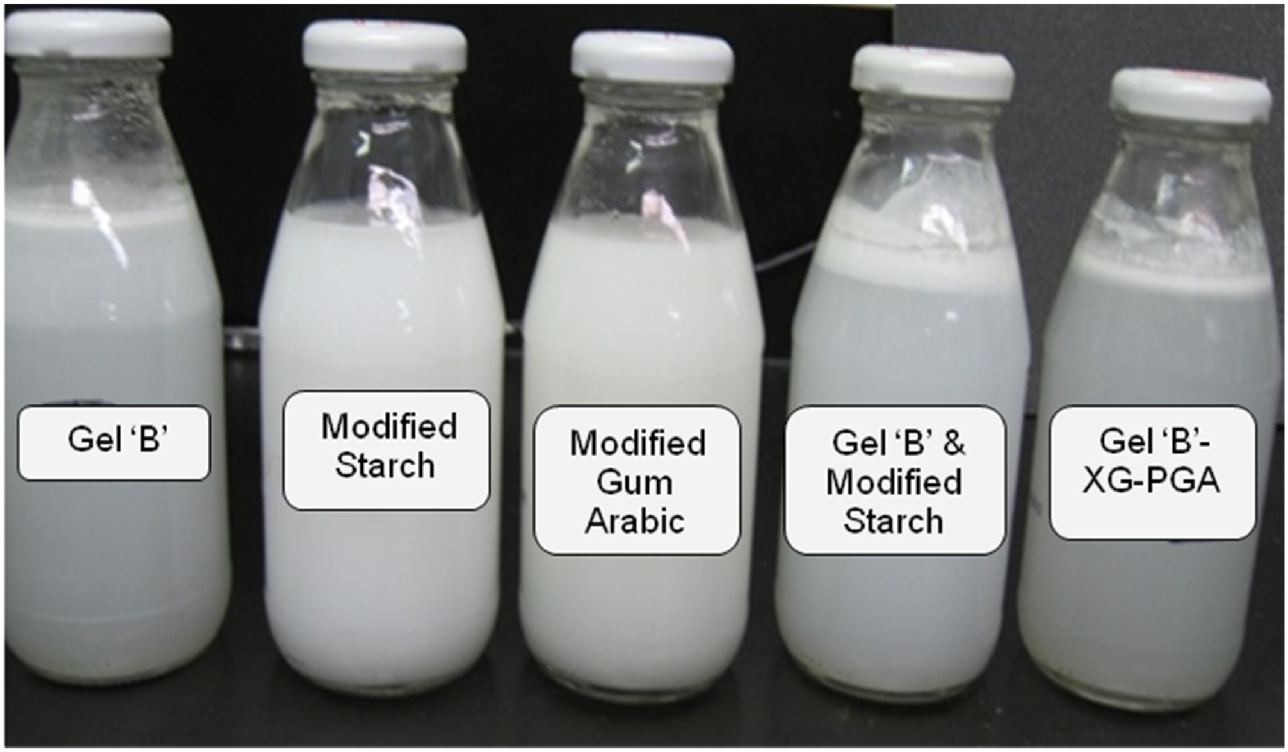


FIGURE 5.3. Shelf stability of thermally pasteurized simulated dairy drinks containing 2% concentrated emulsion after 2 months of storage.

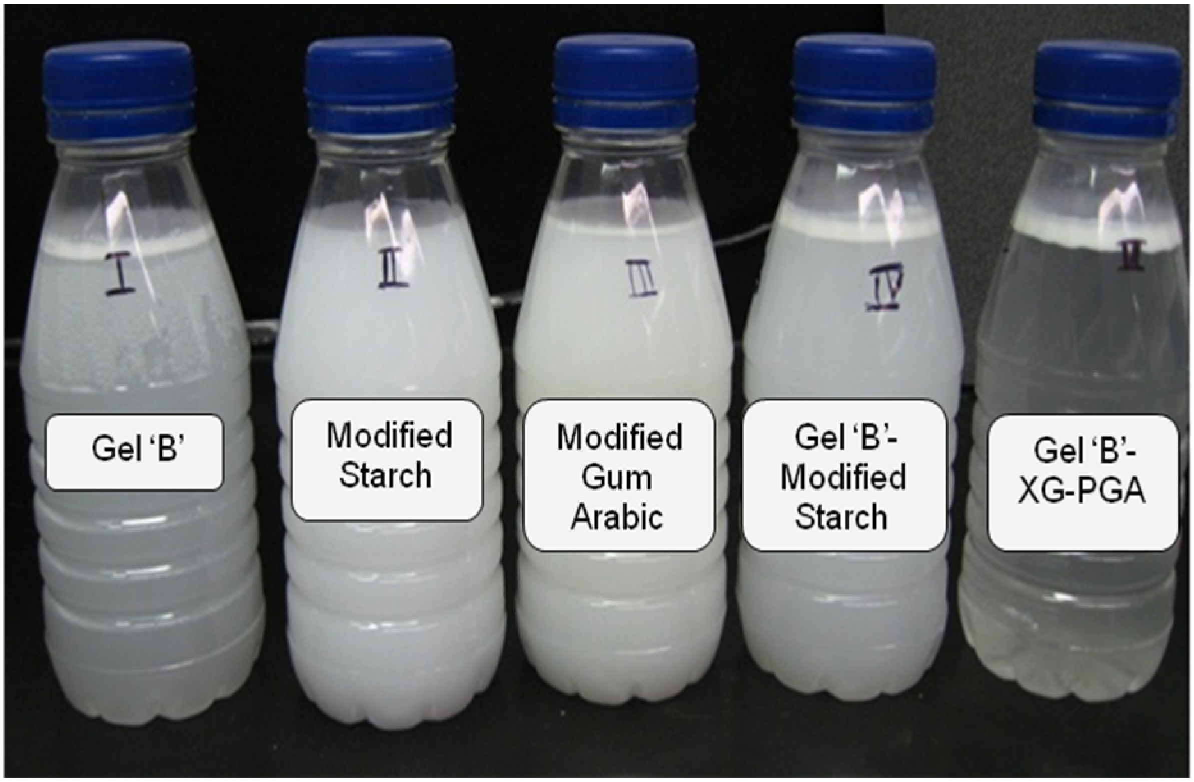


FIGURE 5.4. Shelf stability of high pressure processed simulated dairy drinks containing 2% concentrated emulsion after 2 months of storage.

5.5 CONCLUSIONS

Addition of emulsion concentrates to beverages affected the opacity and showed that oil content is readily responsible for the cloudiness in the beverage. The study confirmed that model beverage emulsions can be formulated even without the use of restricting weighting agents. Modified starch stabilized emulsions produced stability in both simulated juice and dairy beverage. Modified gum Arabic was stable in mimicked milk beverage. The most stable conjugate in concentrated form i.e. gelatin type ‘A’ and modified starch combination indicated shelf stability in dispersed form as well. However, gelatin stabilized systems did not provide stability may be due to their sensitivity to environmental factors. This work can be very useful for manufacture of beverage drinks without the use of weighting agents.

CHAPTER 6

GENERAL CONCLUSIONS

Beverage emulsions are oil-in-water emulsions with the prime purpose to give opacity to clear beverages. They are prepared in a concentrated form and then diluted several hundred times in sugar/acid solution in order to provide the opaque appearance to the drink. However, beverage emulsions are thermodynamically unstable and tend to destabilize over time. This instability occurs due to various breakdown processes which include gravitational separation (i.e. creaming), flocculation, and coalescence. Oil droplets, which have lower density than the continuous medium, cream to the top, and result in ringing.

For beverage emulsions, the most critical norm is its stability in the finished drink. Stability in the concentrated form is easier to achieve because the viscosity is much higher due to the high concentration of hydrocolloid.

In this study different type of natural proteins (gelatin type 'A' and type 'B') and polysaccharides (modified starch and modified gum Arabic) were used to prepare concentrated emulsions at two percentage concentrations and two different pH levels. The steady flow and dynamic rheological properties of the water phase and emulsions were first examined. It was found that with the increase in concentration there was increase in the apparent viscosity of all the emulsions. This means that high concentrations provide thicker layer in order to cover the oil droplet surface completely. The protein stabilized emulsions indicated higher viscosity at neutral pH. However, pH variation did not have a major effect on apparent viscosity of polysaccharide stabilized emulsions.

In the second step, we studied how the storage times affected the rheological properties of concentrated emulsions. It was observed that with storage, gelatin type 'A' emulsions showed greater shear thinning behavior at high pH conditions and gelatin type 'B' had lower flow behavior index at low pH. This could be due to the fact that emulsion droplets stabilized by gelatin were highly unstable to aggregation near the isoelectric point of the proteins because of the relatively low electrostatic repulsion between the droplets. In contrast, for polysaccharide stabilized emulsions, there was a slight increase in apparent viscosity with time. G" was greater than G' demonstrating the emulsions had more liquid like characteristics. With time however, G’ increased in case of protein stabilized emulsions. Particle size and instrumental stability of emulsions was also characterized.

Depending on the rheological, particle constancy of gelatin types at particular pH conditions, conjugates were prepared and their physical properties were observed for a two weeks storage period. The individual and conjugate emulsions stable in concentrated form were diluted into juice and milk simulated beverages. The results demonstrated that concentrates were successful in providing opacity to the beverages. Modified starch and modified gum Arabic showed good stability in milk simulation, and modified starch and gelatin ‘A’-modified starch conjugate was stable in dilute juice simulation. However, gelatin stabilized emulsions did not show appropriate stability upon dilution. Since the stability attributes were different for different hydrocolloids, it can be concluded that the hydrocolloid type, concentration and conditions are responsible for physiochemical properties of the emulsions, such as rheological properties, particles properties and their overall stability.

These results are an excellent alternative to replace existing weighting agents which have a restricted level of use due to the health disadvantages.

Recommendations for future work

1. Investigations should be done to determine commercial acceptance of beverage emulsions without the addition of weighting agents.
2. Use of other hydrocolloids (such as Pectin, casein, Tara gum) should be explored individually or in combinations to stabilize cloud beverage emulsions, thereby evaluating the compatibility of surface active biopolymer and gum.
3. In this study, visual quality comparison between thermal pasteurization and high pressure treatment was carried, therefore, there is need to investigate other quality characteristics obtained with high pressure processing.

REFERENCES

Acedo-Carrillo, J.I., Rosas-Durazo, A., Herrera-Urbina, R., Rinaudo, M., Goycoolea, F. M., and Valdez, M. A. (2006). "Zeta potential and drop growth of oil in water emulsions stabilized with mesquite gum." Carbohydrate Polymers 65(3): 327-336.

Aken, G.A.V. (2004). Coalescence Mechanisms in Protein-Stabilized Emulsions. Food Emulsions. K. L. Stig E. Friberg, Johan Sjoblom, Marcel Dekker, Inc: 299-325.

Akhtar, M. and Dickinson, E. (2003). "Emulsifying properties of whey protein-dextran conjugates at low pH and different salt concentrations." Colloids and Surfaces B: Biointerfaces 31(1-4): 125-132.

Akhtar, M. and Dickinson, E. (2007). "Whey protein-maltodextrin conjugates as emulsifying agents: An alternative to gum Arabic." Food Hydrocolloids 21(4): 607-616.

Akoh, C.C. and Min, D.B. (2008). Food Lipids: chemistry, nutrition and biotechnology, CRC Press.

Angelo, L.L.D. (2006). Application of Hydrocolloids in the Beverage Industry. Gums and Stabilizers for the Food Industry 13. Atlanta, RSC Publishing: 421-430.

Anonymous (2005). "Acacia gum stabilizers for flavor and beverage emulsions." Agro Food Industry Hi-Tech 16(3): 48-48.

Aoki, T., Decker, E.A., McClements, D.J. (2005). "Influence of environmental stresses on stability of O/W emulsions containing droplets stabilized by multilayered membranes produced by a layer-by-layer electrostatic deposition technique." Food Hydrocolloids 19: 209–220.

Aoki, H., Katayama, T., Ogasawara, T., Sasaki, Y., Al-Assaf, S., and Phillips, G.O. (2007). "Characterization and properties of Acacia senegal (L.) Willd. var. Senegal with enhanced properties (Acacia (sen) SUPER GUM (TM)): Part 5. Factors affecting the emulsification of Acacia senegal and Acacia (sen) SUPER GUM (TM)." Food Hydrocolloids 21(3): 353-358.

Augustin, M.A. and Hemar, Y. (2009). "Nano- and micro-structured assemblies for encapsulation of food ingredients." Chemical Society Reviews 38(4): 902-912.

Barbosa-Canovas, G.V., Kokini, J.L., MA, L. and Ibarz, A. (1996.). ‘The Rheology of Semiliquid Foods’. Edited by Charlotte Brabants, Academic Press

Benichou, A. and Garti, N. (2002). "Protein-Polysaccharide Interactions for Stabilization of Food Emulsions." J. Dispersion Science and Technology 23(1-3): 93-123.

Benichou, A.A. and Garti, N. (2004). "Double emulsions stabilized with hybrids of natural polymers for entrapment and slow release of active matters." Advances in Colloid and Interface Science 108-109: 29-41.

Bonnet, M., Cansell, M., Berkaouia, A., Ropersb, M.H., Anton, M., and Leal-Calderon, F. (2009). "Release rate profiles of magnesium from multiple W/O/W emulsions." Food Hydrocolloids 23(1): 92-101.

Bourne, M.C. (2002). Food Texture and Viscosity:Concept and Measurement, Academic Press.

Buffo, R.A. and Reineccius, G.A. (2001). "Shelf-life and mechanisms of destabilization in dilute beverage emulsions." Flavour and Fragrance Journal 16(1): 7-12.

Buffo, R.A. and Reineccius, G.A. (2002). "Modeling the rheology of concentrated beverage emulsions." Journal of Food Engineering 51(4): 267-272.

Buffo, R.A., Reineccius, G.A., and Oehlert,G.W. (2001). "Factors affecting the emulsifying and rheological properties of gum acacia in beverage emulsions." Food Hydrocolloids 15(1): 53-66.

Bull, M.K., Zerdin, K., Howe, E., Goicoechea, D., Paramanandhan, P., Stockman, R., Sellahewa, J., Szabo, E.A., Johnson, R.L., and Stewart, C.M.(2004). "The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice." Innovative Food Science & Emerging Technologies 5(2): 135:149.

Chanamai, R. and McClements, D.J. (2000). "Impact of Weighting Agents and Sucrose on Gravitational Separation of Beverage Emulsions." Journal of Agricultural and Food Chemistry 48(11): 5561-5565.

Chanamai, R. and McClements, D.J. (2001). "Depletion flocculation of beverage emulsions by gum Arabic and modified starch." Journal of Food Science 66(3): 457-463.

Chanamai, R. and McClements, D.J. (2001). "Prediction of emulsion color from droplet characteristics: dilute monodisperse oil-in-water emulsions." Food Hydrocolloids 15(1): 83-91.

Chanamai, R. and McClements D.J. (2002). "Comparison of gum Arabic, modified starch, and whey protein isolate as emulsifiers: Influence of pH, CaCl2 and temperature." Journal of Food Science 67(1): 120-125.

Charcosset, C. (2009). "Preparation of emulsions and particles by membrane emulsification for the food processing industry." Journal of Food Engineering 92(3): 241-249.

Cho, S.H., Jahncke, M.L., Chin, K.B., and Eun, J.B. (2006). "The effect of processing conditions on the properties of gelatin from skate (Raja Kenojei) skins." Food Hydrocolloids 20(6): 810-816.

Coia, K.A., Stauffer, K.R. (1987). "Shelf Life Study of Oil/Water Emulsions using Various Commercial Hydrocolloids." Journal of Food Science 52(1): 166-172.

Comas, D.I., Wagner, J.R., Tomas, M.C. (2006). "Creaming stability of oil in water (O/W) emulsions: Influence of pH on soybean protein-lecithin interaction." Food Hydrocolloids 20(7): 990-996.

Dalgleish, D.G. (1997). "Adsorption of protein and the stability of emulsions." Trends in Food Science & Technology 8(1): 1-6.

Demetriades, K., Coupland, J.N. and McClements, D.J. (1997). "Physical Properties of Whey Protein Stabilized Emulsions as Related to pH and NaCl." Journal of Food Science 62(2): 342-347.

Dickinson, E. (1992). "Structure and Composition of Adsorbed Protein Layers and the Relationship to Emulsion Stability." J. Chem Soc. Faraday Trans 88(20): 2973-2983.

Dickinson, E. (1993). "Protein–polysaccharide interactions in food colloids." Food colloids and polymers: stability and mechanical properties: 77–93.

Dikinson, E., Gollar, M.I., Wedlock, D.J. (1995). "Osmotic Pressure, Creaming and Rheology of Emulsions containing Nonionic Polysaccharide." Journal of Colloid and Interface Science 172: 192-202.

Dickinson, E. (2001). "Milk protein interfacial layers and the relationship to emulsion stability and rheology." Colloids and Surfaces B: Biointerfaces 20(3): 197-210.

Dickinson, E. (2003). "Hydrocolloids at interfaces and the influence on the properties of dispersed systems." Food Hydrocolloids 17(1): 25-39.

Dickinson, E., Stainsby, G. (1988). Advances in Food Emulsion and Foams. Emulsion stability Elsevier Applied Science: (pp. 1–44).

Dickinson, E. Parinson., E. (2006). Understanding the stabilizing properteis of casein in heated milk protein emulsions. Gums and stability for the Food Industry 13. Leeds, RSCPublishing: 308.

Dumitriu, S. (2004). Polysaccharides: Structural diversity and functional versatility, CRC Press.

Fechner, A, Knoth, A., Scherze, I. and Muschiolik, G. (2007). "Stability and release properties of double-emulsions stabilised by caseinate–dextran conjugates." Food Hydrocolloids 21: 943-952.

Friberg, S.E. (2004). Food Emulsions, Marcel Dekker, Inc.

Garti, N. Aserin, A. and Azaria, D. (1991). "A clouding agent based on modified soy protein." Internation Journal of Food Science and Technology 26: 259-270.

Garti, N. (1997). "Progress in Stabilization and Transport Phenomena of Double Emulsions in Food Applications." Lebensm.-Wiss. u.-Technol. 30: 222-235.

Garti, N. (1999). "Hydrocolloids as emulsifying agents for iol in water emulsions." J. Dispersion Science and Technology 20(1&2): 327-355.

Given Jr, P.S. (2009). "Encapsulation of Flavors in Emulsions for Beverages." Current Opinion in Colloid & Interface Science 14(1): 43-47.

Guzey, D., McClements, D.J., (2006). "Formation, stability and properties of multilayer emulsions for application in the food industry." Advances in Colloid and Interface Science 128-130: 227-248.

Harnsilawat, T., Pongsawatmanit, R., and McClements, D.J. (2006). "Stabilization of model beverage cloud emulsions using protein-polysaccharide electrostatic complexes formed at the oil-water interface." Journal of Agricultural and Food Chemistry 54(15): 5540-5547.

Harnsilawat, T., Pongsawatmanit, R., and McClements, D.J. (2007). "Influence of pH and ionic strength on formation and stability of emulsions containing oil droplets coated by β-lactoglobulin Alginate interfaces." Biomacromolecules 7: 2052-2058.

Harper, J. (2005). "Use high pressure to extend shelf life of pasteurized fluid milk products." Microbial Update International.

Hiremath, N.D. (2005). Studies on high pressure processing and preservation of mango juice : pressure destruction kinetics, process verification and quality changes during storage. Food Science and Agricultural Chemistry. Montreal, McGill University. M.Sc.

Huang, X., Kakuda, Y., and Cui, W. (2001). "Hydrocolloids in emulsions: particle size distribution and interfacial activity." Food Hydrocolloids 15(4-6): 533-542.

Hunt, J.A., Dalgleish, D.G. (1995). "Heat Stability of Oil-in-Water Emulsions Containing Milk Proteins: Effect of Ionic Strength and pH." Journal of Food Science 60(2): 1120-1123.

Hunter, T.N., Pugh, R.J., Franks, G.V., and Jameson, G.J. (2008). "The role of particles in stabilising foams and emulsions." advances in colloid and interface science 137(2): 57-81.

Imeson, A. (1997). Thickening and Gelling Agents for Food, Blakie Academic & Professional.

Jayme M.L., Dunstan D.E., and Gee, M.L. (1999). Zeta potential of gum Arabic stabilized emulsions. Food Hydrocolloids, 13:459-465.

Karim, A.A and Bhat, R. (2009). "Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins." Food Hydrocolloids 23: 563-576.

Keowmaneechai, E. and McClements, D.J. (2002). "Effect of CaCl2 and KCl on physiochemical properties of model nutritional beverages based on whey protein stabilized oil-in-water emulsions." Journal of Food Science 67(2): 665-671.

Khalloufi, S., Corredig, M., Goff, H.D., and Alexander, M. (2009). "Flaxseed gums and their adsorption on whey protein-stabilized oil-in-water emulsions." Food Hydrocolloids 23(3): 611-618.

Klinkesorn, U., Sophanodora, P., Chinachoti, P. and McClements, D.J. (2004). "Stability and rheology of corn oil-in-water emulsions containing maltodextrin." Food Research International 37(9): 851-859.

Mason, T. G. (1999). "New fundamental concepts in emulsion rheology." Current Opinion in Colloid & Interface Science 4(3): 231-238.

McClements, D.J., Chanamai R. (2000). "Impact of Weighting Agents and Sucrose on Gravitational Separation of Beverage Emulsions." J. Agric. Food Chem. 48(11).

McClements, D.J. (2000). "Comments on viscosity enhancement and depletion flocculation by polysaccharides." Food Hydrocolloids 14: 173–177.

McClements, D.J. (2004a). Food Emulsions Principles, Practices, and Techniques, CRC Press.

McClements, D.J. (2004b). "Protein-stabilized emulsions." Current Opinion in Colloid & Interface Science 9: 305-313.

McClements, D.J. (2007a). "Critical Review of Techniques and Methodologies for Characterization of Emulsion Stability." Critical Reviews in Food Science and Nutrition 47(7): 611 - 649.

McClements, D.J. (2007b). Emulsification and Encapsulation. Handbook of Industrial Water Soluble Polymers. A. W. Peter: 98-133.

McKenna, B. (2003). Texture in Food: Volume 1: Semi-Solid Foods, CRC Press.

Mirhosseini, H., Tan, C.P., Hamid, N.S.A. and Yusof, S. (2007). "Modeling the relationship between the main emulsion components and stability, viscosity, fluid behavior, zeta-potential, and electrophoretic mobility of orange beverage emulsion using response surface methodology." Journal of Agricultural and Food Chemistry 55(19): 7659-7666.

Mirhosseini, H., Tan, C.P., Hamid, N.S.A. and Yusof, S. (2008a). "Effect of Arabic gum, Xanthan gum and orange oil contents on [zeta]-potential, conductivity, stability, size index and pH of orange beverage emulsion." Colloids and Surfaces A: Physicochemical and Engineering Aspects 315(1-3): 47-56.

Mirhosseini, H., Tan, C.P., and Taherian, A.R. (2008b). "Effect of glycerol and vegetable oil on physicochemical properties of Arabic gum-based beverage emulsion." European Food Research and Technology 228(1): 19-28.

Muschiolik, G. (2007). "Multiple emulsions for food use." Current Opinion in Colloid & Interface Science 12(4-5): 213-220.

Nilsson, L. and Bergenstahl, B. (2006). "Adsorption of Hydrophobically Modified Starch at Oil/Water Interfaces during Emulsification." Langmuir 22(21): 8770-8776.

Nunez, G.A., Sanchez, G., Gutierrez, X., Silva, F., Dalas, C., and Rivas, H. (2000). "Rheological Behavior of Concentrated Bitumen in Water Emulsions." Langmuir 16(16): 6497-6502.

Pettitt, D.J., Wayne, J.E.B., Nantz, J.J.R., Shoemaker, C.F. (1995). "Rheological Properties of Solutions and Emulsions Stabilized with Xanthan Gum and Propylene Glycol Alginate." Journal of Food Science 60(3): 528-531.

Prochaska, K., Kedziora, P., Le Thanh, J., and Lewandowicz, G.,(2007). "Surface activity of commercial food grade modified starches." Colloids and Surfaces B: Biointerfaces 60(2): 187-194.

Ramesh, M.N. (2007). Pasteurization and Food Preservation. Handbook of Food Preservation. M. S. Rahman, CRC Press: 571-582.

Randall, R.C., Phillips, G.O., and Williams, P.A. (1988). "The role of the proteinaceous component on the emulsifying properties of gum arabic." Food ydrocolloids 2(2):131-140.

Rao, M.A. (1999). Rheology of Fluid and Semisolid Foods Principles and Applications, An Aspen Publishers, Inc.

Ray, A.K., Bird, P.B., Iacobucci, G.A., and Clark, B.C..(1995). "Functionality Of Gum-Arabic - Fractionation, Characterization And Evaluation OF Gum Fractions In Citrus Oil-Emulsions And Model Beverages." Food Hydrocolloids 9(2): 123-131.

Rinaudo, M. (2008). "Main properties and current applications of some polysaccharides as biomaterials." Polymer International 57(3): 397-430.

Sosa-Herrera, M.G., Berli, C.L.A., and Martínez-Padilla, L.P. (2008). "Physicochemical and rheological properties of oil-in-water emulsions prepared with sodium caseinate/gellan gum mixtures." Food Hydrocolloids 22(5): 934-942.

Sun, C., Gunasekaran, S. and Richards, M.P. (2007). "Effect of Xanthan gum on physicochemical properties of whey protein isolate stabilized oil-in-water emulsions." Food Hydrocolloids 21(4): 555-564.

Surh, J., Decker, E.A., and McClements, D.J. (2006). "Properties and stability of oil-in-water emulsions stabilized by fish gelatin." Food Hydrocolloids 20(5): 596-606.

Surh, J., Gu, Y.S., Decker, E.A., and McClements, D. J. (2005). "Influence of environmental stresses on stability of OM emulsions containing cationic droplets stabilized by SDS-fish gelatin membranes." Journal of Agricultural and Food Chemistry 53(10): 4236-4244.

Suzuki. K, Matsouka, T.M.K., and Kubota, K. (1991). "Effect of constituent of rheological properties of corn oil-in-water emulsions." Journal of Food Science 56(3): 796-798.

Tadros, T.F. (1994). "Fundamental principles of emulsion rheology and their application." Colloids and Surfaces A: Physicochemical and Engineering Aspects 91(1994): 39-5539.

Tadros, T.F. (1996). "Correlation of viscoelastic properties of stable and flocculated suspensions with their interparticle interactions." Advances in colloid and interface science 68: 97-200.

Taherian, A.R. (2006). Rheology and Stability of Beverage Emulsions in Concentrated and Diluted Forms. Department of Food Science and Agricultural Chemistry. Montreal, McGill University. PhD.

Taherian, A.R., Fustier, P. Britten, M. and Ramaswamy, H.S. (2008). "Rheology and stability of beverage emulsions in the presence and absence of weighting agents: A review." Food Biophysics 3(3): 279-286.

Taherian, A.R., Fustier, P., and Ramaswamy, H.S. (2006). "Effect of added oil and modified starch on rheological properties, droplet size distribution, opacity and stability of beverage cloud emulsions." Journal of Food Engineering 77(3): 687-696.

Taherian, A.R., Fustier, P. and Ramaswamy, H.S. (2007). "Effects of added weighting agent and Xanthan gum on stability and rheological properties of beverage cloud emulsions formulated using modified starch." Journal of Food Process Engineering 30(2): 204-224.

Tan, C-T., Contis, E.T., Ho, C.T., Mussinan, C.J. Parliament, T.H., Shahidi, F., and Spanier, A.M. (1998). Beverage flavor emulsion--A form of emulsion liquid membrane microencapsulation. Developments in Food Science, Elsevier. Volume 40: 29-42.

Tan, C.P. (2004). Beverage Emulsions. Food Emulsions, Marcel Dekker, Inc.

Toledano, O. and Magdaddi, S. (1998). "Emulsification and Foaming Properties of Hydrophobically Modified Gelatin." Journal of Colloid and Interface Science 200: 235-240.

Valdez, M.A., Acedo-Carrillo, J.I., Rosas-Durazo, A., Lizardi, J., Rinaudo, M., and Goycoolea, F.M. (2006). "Small-deformation rheology of mesquite gum stabilized oil in water emulsions." Carbohydrate Polymers 64(2): 205-211.

Vanapalli, S.A., Palanuwech, J., and Coupland, J.N. (2002). "Stability of emulsions to dispersed phase crystallization: effect of oil type, dispersed phase volume fraction, and cooling rate." Colloids and Surfaces A: Physicochemical and Engineering Aspects 204(1-3): 227-237.

Velez, G., Munoz, J., Williams, P.A., and English, R.J. (2003). "Role of Hydrocolloids in the Creaming of Oil in Water Emulsions." Journal of Agricultural and Food Chemistry 51: 265-269.

Vinetsky, Y., and Magdassi, S. (1997). "Formation and surface properties of microcapsules based on gelatin-sodium dodecyl sulphate interactions." Colloids and Surfaces A: Physicochemical and Engineering Aspects 122: 227-235.

Welti-Chanes, J., and Aguilera J.M. (2002). Engineering and Food for the 21st Century, CRC Press.

Ye, A., Hemar, Y., and Singh, H. (2004). "Enhancement of coalescence by Xanthan addition to oil-in-water emulsions formed with extensively hydrolysed whey proteins." Food Hydrocolloids 18(5): 737-746.

Yilmazer , G., Carrillo A. and Kokini, J.F. (1991). "Effect of Propylene Glycol Alginate and Xanthan Gum on Stability of O/W Emulsions." Journal of Food Science 56(2): 513-517.

Zhu, S., Naim, Marcotte, F., M., Ramaswamy, H.S., and Shao, Y. (2008). "High-pressure destruction kinetics of Clostridium sporogenes spores in ground beef at elevated temperatures." International Journal of Food Microbiology 126(1-2):86-92.