Effects of Pectin and Guar Gum on Food Protein Functionality

Ph. D. Thesis

in

Food Engineering

University of Gaziantep

Supervisor

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by

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December, 2006

ABSTRACT

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Ph. D. in Food Engineering

Supervisor: Assoc. Prof. Dr. Esra İBANOĞLU

December, 2006, 143 pages

In this study, the effect of hydrocolloids (pectin, guar gum, *iota*-carrageenan) on functional properties of whey protein isolate (WPI) and egg proteins (egg white, egg yolk plasma, egg yolk granule) were investigated. In protein-polysaccharide mixtures, phase separation behavior and thermal properties of these proteins were determined. In addition, the influence of hydrocolloids on emulsifying properties, foaming properties and rheological properties was investigated. And also the effect of heat treatment on foaming properties of egg white and emulsifying properties of egg yolk proteins and WPI was also studied. The presence of hydrocolloids did not affect the thermal stability of egg white but increased thermal stability of WPI and egg yolk plasma. WPI-pectin and WPI-guar gum systems exhibited phase separation but no phase separation was observed in WPI-i-carr systems. Except for plasma-pectin mixtures, all other egg protein-hydrocolloid mixtures occupied a two-phase system; the top phase was defined as protein-rich phase and the bottom phase was defined as polysaccharide-rich phase. Therefore, emulsions were prepared below the phase separation threshold and the emulsifying activity and emulsion stability were determined spectrophotometrically by measuring time-dependent changes in turbidity. Emulsion activity of egg yolk granule stabilized emulsions without the addition of hydrocolloids was determined as 2.61 m^2/mg. While the addition of pectin slightly caused a slight change in emulsion activity

of these emulsions, addition of guar gum (0.5%) increased it to 3.41 m^2/mg. Emulsion stability was also observed to be changing with the concentration of hydrocolloids and more stable emulsions were obtained at higher hydrocolloid concentrations. Only transient foaming (foam volume was 19.2 cm^3 and collapsed within 9-10 sec) was observed with egg yolk plasma and granule. But quite stable foams were produced with WPI (80 s) and egg white (64 s) with a foam volume of 119.3 cm^3 and 120.7 cm^3, respectively, in the absence of hydrocolloids. Addition of hydrocolloids did not change foam volume but increased foam stability. Emulsifying and foaming properties were also studied by using heat-treated (65-80°C) protein or protein-hydrocolloid mixtures. Foam volume of heat-treated egg white decreased as compared to that of non-heated egg white. Foam stability of egg white increased as heating temperature was increased from 65°C to 75°C. However, it reduced when the protein solution was treated at 80°C. As the temperature was increased emulsifying properties of both egg yolk proteins and WPI also decreased. Finally, rheological behavior of these proteins was determined in emulsion systems. Elastic modulus and viscous modulus increased with increasing frequency and they were regarded as frequency dependent. Emulsions prepared in the absence of hydrocolloids showed Newtonian behavior ($n \cong 1.0$). Generally addition of pectin increased viscosity slightly but did not change flow type. Addition of higher amounts of guar gum (0.5%) changed rheological properties of these emulsions and they showed pseudoplastic behavior ($n < 1.0$).

Key words: Whey protein isolate, Egg proteins, Hydrocolloids, Emulsion, Foam, DSC, Heat treatment, Rheology

ÖZET

PEKTİN VE GUAR ZAMKININ GIDA PROTEİNLERİNİN FONKSİYONEL ÖZELLİKLERİNE ETKİSİ

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Aralık, 2006, 143 sayfa

Bu çalışmada, hidrokolloidlerin (pektin, guar zamkı, *iyota*-karragenan) peynir altı suyu protein izolatı (PAP) ve yumurta proteinlerinin (yumurta beyazı, yumurta sarısı plazması, yumurta sarısı granülü) fonksiyonel özelliklerine etkisi araştırılmıştır. Protein-polisakkarit karışımları kullanılarak bu proteinlerin faz ayrım özelliği ve ısısal özellikleri belirlenmiştir. Bunun yanında, hidrokolloidlerin emülsiyon oluşturma, köpürme ve reolojik özelliklere etkisi incelenmiştir. Ayrıca ısıl işlemin yumurta beyazının köpük oluşturma, yumurta sarısı proteinleri ve PAP'ın emülsiyon oluşturma özelliğine etkisi çalışılmıştır. Hidrokolloidlerin yumurta beyazının ısısal stabilitesini etkilemediği, ancak PAP ve yumurta sarısı plazmasının ısısal stabilitesini arttırdığı görülmüştür. PAP-pektin ve PAP-guar zamkı sistemlerinde faz ayrımı gözlenmiş, ancak PAP-iyota karragenan sistemlerinde faz ayrımı görülmemiştir. Plazma-pektin karışımları dışında diğer bütün yumurta proteini-hidrokolloid karışımları iki fazlı bir sistem oluşturmuştur; üst faz proteince zengin alt faz polisakkaritce zengin olarak tanımlanmıştır. Bu yüzden, emülsiyonlar faz ayrımının gerçekleştiği en küçük konsantrasyonun altındaki konsantrasyonlarda hazırlanmış; emülsiyon aktivitesi ve emülsiyon stabilitesi spektrofotometrik metotla bulanıklığın zamanla değişimi olarak ölçülmüştür. Hidrokolloid eklenmeden yumurta sarısı granülü ile stabilize edilmiş emülsiyonların

emülsiyon aktivitesi 2.61 m^2/mg olarak belirlenmiştir. Pektin eklenmesi bu emülsiyonların emülsiyon aktivitesinde küçük bir değişime neden olurken, guar zamkı (%0.5) eklenmesi ile bu değer 3.41m^2/mg'a çıkmıştır. Stabilitenin de hidrokolloid konsantrasyonuyla değiştiği gözlenmiş ve yüksek hidrokolloid konsantrasyonunda daha stabil emülsiyonlar elde edilmiştir. Yumurta sarısı plazması ve granülü ile ancak geçici bir köpürme (19.2 cm^3'lük köpük 9-10 s içinde söndü) elde edilmiştir. Hidrokolloid eklenmeden PAP ve yumurta beyazı ile köpük hacmi sırasıyla 119.3 cm^3 ve 120.7 cm^3 olan oldukça stabil köpükler (PAP için 80 s, yumurta beyazı için 64 s) üretilmiştir. Hidrokolloid eklenmesi köpük hacmini değiştirmdi ancak köpük stabilitesini arttırmıştır. Emülsiyon oluşturma ve köpürme özellikleri ısıl işlem görmüş (65-80°C) protein veya protein-hidrokolloid karışımları kullanılarakta çalışılmıştır. Isıl işlem görmüş yumurta beyazı ile hazırlanan köpüklerin hacmi ısıl işlem görmemiş olana kıyasla azalmış fakat köpük stabilitesi sıcaklık 65°C'den 75°C'ye yükseldikçe artmıştır. Ancak 80°C'de ısıtılmış protein çözeltisi ile hazırlanan köpüklerin stabilitesi azalmıştır. Sıcaklık arttıkça hem yumurta sarısı proteinlerinin hem de PAP'ın emülsiyon özellikleri azalmıştır. Son olarak, bu proteinlerin reolojik özellikleri emülsiyon sistemlerinde belirlenmiştir. Elastik modülü ve viskoz modülü frekanstaki artışla arttı ve bu mödüller frekansa bağımlı olarak nitelendirilmiştir. Hidrokolloid eklenmeden hazırlanan emülsiyonlar Newtonian davranış ($n \cong 1.0$) göstermiştir. Genellikle pektin eklenmesi viskoziteyi çok az değiştirirken akış çeşidini değiştirmemiştir. Yüksek miktarlarda (%0.5) eklenen guar zamkı bu emülsiyonların reolojik özelliğini değiştirmiş ve sistemler pseudoplastik davranış ($n < 1.0$) göstermiştir.

Anahtar Kelimeler: Peynir altı suyu protein izolatı, Yumurta proteinleri, Hidrokolloidler, Emülsiyon, Köpük, DSTK, Isıl İşlem, Reoloji

ACKNOWLEDGEMENTS

I wish to express my gratitude to my supervisor Doç.Dr. Esra İbanoğlu for her encouragement and invaluable suggestions throughout this study. I am grateful for her support and guidance.

I would like to thank Prof. Dr. Ahmet Kaya for his interest and helps.

I would like to thank to my committee members, Prof.Dr. Fahrettin Göğüş and Yrd. Doç. Dr. Bülent Belibağlı for their enlightening suggestions throughout this study.

I wish to thank my family for their support.

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LIST OF ABBREVIATIONS

WPI	Whey protein isolate
EYG	Egg yolk granule
EYP	Egg yolk plasma
EW	Egg white
i-car	*iota*-carrageenan
SLS	Sodium lauryl sulfate
HDL	High density lipoproteins
LDL	Low density lipoproteins
DSC	Differential scanning calorimetry
T_d	Denaturation temperature
ΔH	Denaturation enthalpy
EAI	Emulsion activity index
ESI	Emulsion stability index
FV	Foam volume
n	Flow behavior index
k	Consistency index
G'	Elastic (storage) modulus
G"	Viscous (loss) modulus
δ	Phase angle
γ	Shear rate
σ	Shear stress
η	Viscosity

CHAPTER 1

1. INTRODUCTION

In food applications, proteins in addition to providing essential amino acids should ideally possess several desirable characteristics referred to as functional properties. The functional properties, rather than the nutritional value, of protein in protein-containing products largely determine their acceptability as ingredients in prepared foods. Generally, proteins are present together with polysaccharides in many kinds of food systems. The overall texture and stability of food products depend not only on the properties of proteins and polysaccharides, but also the nature and strength of protein/polysaccharide interactions. Proteins and polysaccharides may be thermodynamically incompatible and therefore phase separation may take place in a protein-polysaccharide mixture. This is usually unwanted in consumer products since the phase separation is important in food applications from the point of long-term stability, rheology, transport processes and energy consumption. In literature, relatively limited studies have been performed on phase separation behavior of proteins and role of protein-polysaccharide interactions in food stability. Therefore, in first part of this study, we aimed to determine the phase separation behavior of some food proteins in the presence of polysaccharides to obtain more detailed information which allows the manipulation of sensory properties, stability and rheology of food by adjusting the interaction in a desirable way.

In our model protein-polysaccharide systems, whey protein isolate and egg proteins were chosen since they both are widely used functional ingredients in many food products and both are protein mixtures. Recently, a series of investigations dealing with whey protein-polysaccharide mixtures have been made but there is not enough study to compare the effect of neutral (guar gum) and anionic polysaccharide (pectin, *i*-carrageenan) on phase separation behavior and functional properties of whey proteins. Egg proteins are another widely used protein ingredients in food products.

Egg proteins generally used in the form of egg yolk or egg white. Besides their high nutritional value, egg yolk is used as an emulsifier and egg white is used as a foaming agent. Although egg proteins are key ingredients of many food systems, its functional properties have not received the attention they deserve. Yolk is still used empirically and physical properties of yolk emulsions are not entirely controlled. Because yolk is a complex mixture of several lipoproteins and proteins and the effects of interactions with other components and phase separation behavior are not well understood. Therefore, egg yolk and egg white powder were used in our model systems. This study also shows the functionality of egg and whey proteins side by side.

Food dispersions often take the form of emulsions and foams. The foaming and emulsifying characteristics are important attributes during the production stage of such dispersions. In addition, stability is an important property of food dispersions, since the consumer perception of quality is influenced by appearance. The rheological properties of food emulsions are of great importance for many industrial applications influenced by the flow behavior such as mixing units, pipelines and pumps. In addition, the long-term storage stability and the texture as related to mouth feel are influenced by the rheological behavior of the product and are of great importance for consumer perception. Therefore, by knowing the product rheology one can predict the requirements for the design and processing and also the product quality. For these reasons, in addition to phase separation behavior emulsifying, foaming and rheological properties of whey protein isolate and egg proteins were also investigated.

During manufacture of many food products heat treatment is applied to ensure microbial safety and to obtain desirable organoleptic attributes. Heat treatment cause changes in protein structure. This can be desirable or undesirable. Therefore, the effect of heat treatment on foaming and emulsifying properties of food proteins were investigated by using heat-treated protein solutions or protein-polysaccharide mixtures. Thermal properties of protein-polysaccharide mixtures were determined by using Differential Scanning Calorimetry (DSC).

CHAPTER 2

2. LITERATURE SUMMARY

2.1. Egg Proteins

2.1.1. Egg Yolk

Hen's egg yolk can be found in a number of food products such as mayonnaise and salad dressings, in general, bakery products and cakes, custard etc., being an ingredient of high nutritional value with unique sensory characteristics and excellent functionality (Kiosseoglou, 2003). Thus, the preparation and stability of yolk-based products depend on yolk constituent ability, to a large extend to adsorb at oil-water interfaces and form a strong and cohesive film around oil droplets. During the production of certain foods, e.g. cakes, yolk constituents may have to function as emulsifiers and foaming agents as well as network structure formers. But despite the fact that egg yolk has long been empirically recognized as a reference-emulsifying ingredient, its emulsifying properties still remain fairly poorly understood, presumably because of its remarkably complex chemical composition (Anton et al., 2003; Kiosseoglou, 2003; le Denmat et al., 2000). Therefore, fractionation of yolk constituents can help to screen those, which effectively contribute to the emulsifying properties, provided that these constituents are recovered undenatured.

Egg yolk (EY) contains approximately 50% water, 35% lipids and 15% proteins (Mel'nikov, 2002). It is a complex system containing a large number of polydispersed particles, granules, which are dispersed in clear yellow fluid, plasma. Plasma (supernatant) and granule (precipitate) can be separated by centrifugation (Anton et al., 2000; Anton et al., 2003; le Denmat et al., 2000). The granule mainly consists mainly of 70 % high-density lipoproteins (HDL), which are also called lipovitellins, 12% low-density lipoproteins (LDL), and 16% a highly phosphorylated protein called phosvitin (Aluko and Mine, 1998; Causeret et al., 1991).

Plasma is composed of 85% LDL and 15% livetin, a globular protein (Le Denmat et al., 2000; Mel'nikov, 2002). Plasma constituents are remarkably soluble in the

common pH range of food emulsions and whatever the NaCl concentration (le Denmat et al., 2000). The soluble plasma constituents can adsorb efficiently at the oil water interface and LDL displaces competitively livetins from the interface. Contrary to plasma, specific conditions are required to have granule proteins to be soluble, namely high NaCl concentration. Granule aggregates have poor surface activity and they do not adsorb efficiently when they compete with soluble proteins. But when the physicochemical conditions do allow granule solubilisation, solubilised HDL could provide additional emulsifiers and favor wider interface area coverage. At low ionic strength (< 0.17 M NaCl), native granules form non-soluble HDL-phosvitin aggregates (Anton et al., 2000) and at high ionic strength (> 0.5 M NaCl), HDL and phosvitin are solubilised. Acidification and alkalinisation cause also the disruption of granules and the solubilisation of these constituents. At acidic pH, the increase in the number of positive charges (NH_3^+) induces electrostatic repulsions and the results in disruption of granule constituents. At basic pH, the repulsion of negative charges (COO^-) is involved (le Denmat et al., 2000).

Most studies devoted to the adsorption behavior of yolk constituents have been performed with individual components: LDL (Anton et al., 2003), phosvitin (Chung and Ferrier, 1992) and livetin, or with granules and plasma (Kiosseoglou, 2003; Mel'nikov, 2002). It has been demonstrated that HDL, LDL, livetin and phosvitin have a strong propensity to absorb at the oil-water interface, whereas the protein contribution to the emulsifying capacity of EY is believed to be greater than the phospholipid contribution. Following the adsorption, EY proteins and lipoproteins form relatively strong film at the oil-water interface, which is largely responsible for the stability of emulsions against coalescence. Mine (1998) reported that the apoproteins have a higher adsorption capacity, compared with the globular proteins, due to their flexible molecular structure and a greater surface hydrophobicity. It has been also hypothesized that lipoproteins disintegrate at the interface, followed by the coalescence of neutral lipids with oil droplets and adsorption of apoproteins at the oil-water interface.

Several studies have compared emulsifying properties of granules constituents after disruption of granular structure by NaCl (Aluko and Mine, 1997; Aluko and Mine, 1998). They observed that stability of emulsions prepared with granules was higher

at pH 7.0 and 9.0 than at pH 4.0. Further, they reported that HDL is more surface active than phosvitin. In the recent years, several authors have compared the emulsifying properties of yolk with those of plasma and granules (Anton and Gandemer, 1997; le Denmat et al., 2000). Granules provide better emulsion stability than yolk and plasma, and could be potential stabilizers in food emulsions (Kiosseoglou, 2003). However, Tsutsui, (1988) reported that LDL's emulsifying capacity is higher than that of HDL, and the diameter of the LDL emulsion is smaller than that of the HDL emulsion.

The emulsifying properties of plasma constituents, i.e. LDL and livetins, have been evaluated in comparison with other food emulsifiers, particularly proteins. Mizutani and Nakamura (1984) showed that LDL are better emulsifiers than bovine serum albumin. Aluko et al. (1998) showed that LDL are more surface active than whey proteins and were also more surface active than caseins. Concurrently, livetins have been revealed to have poor adsorption capacity at the oil-water interface when they are emulsified together with milk caseins or yolk lipoproteins.

Despite numerous studies on the individual properties of EY constituents (e. g. separate protein or phospholipid fractions), little is known on the behavior of EY as a whole. In recent years, several attempts were made in order to understand dependence of the intrinsic properties of EY on the environmental factors, such as pH, low-molecular weight salt concentration, temperature, presence of water-soluble polymers etc (Mel'nikov, 2002). However, often the data from different studies were in contradiction and, therefore, it was difficult to apply them for the optimization of the EY application as an emulsifier. Consequently, to optimize the use of egg yolk in food emulsions, it was necessary to have more detailed information about the adsorption of yolk constituents at the oil-water interface, an their ability to prepare and stabilize oil-in-water emulsions.

2.1.2. Egg White

Egg white remains an interesting ingredient in the food industry due to its multifunctional properties (Nagano and Nishinari, 2001; der Plancken et al., 2005). Egg white proteins are well-known for their gelling, foaming and emulsifying characteristics, in addition to their high nutritional quality. Because of gelling ability,

a large amount of high-gelling type of egg white is used in meat and surumi products, particularly in Japan. The major proteins present in egg whites are ovalbumin (54%), conalbumin (13%), ovomucoid (11%), ovomucin (2%), lysozyme (3.5%) and ovoglobulins (4%) (Perez and Pilosof, 2004; Sagis et al., 2001). Of the major proteins in egg white, conalbumin is the most sensitive to heat (Perez and Pilosof, 2004). Of these proteins conalbumin, ovomucin and the ovoglobulins are thought to be the main proteins responsible for the stabilization of the foam (Sagis et al., 2001). Ovalbumin is extensively utilised by the food industry because of its ability to form foam and to form gels upon heating (Galazka et al., 1999). It has molecular weight of 44 kDa, with four free sulphydryl groups and one disulphide bond. Ovalbumin also appears to be involved in stabilization of the foam, but to a lesser extent (Sagis et al., 2001). Aggregation at the interface is thought to be responsible for the stabilization of the foam. When the denaturation and aggregation processes are very fast the foam will have poor stability. The proteins must have some resistance to surface denaturation to produce stable long-lived foam.

Although they have the poorest foaming ability, lysozyme was used in some researches. Lysozyme is a compact globular protein with a well-defined tertiary structure maintained by four disulphide bonds as well as by non-covalent interactions (Mckenzie and White, 1991). Lysozyme consists of 129 residues and has a molecular weight of 14.5 kD. Its isoelectric point is 10.5. It possesses relatively low total hydrophobicity (Song et al., 2002). Because of its compact and rigid structure, it unfolds at the interface very slowly. However, the films formed exhibit high surface viscosity, the resistance to shear depending on the degree of cross-linking and the intermolecular interactions (Scaman et al., 2006). This imparts stability. Heat treatment improves the surface properties of lysozyme. The controlled dry-heating of lysozyme in the presence of polysaccharides has been shown to be very effective at improving its emulsifying and foaming properties (Scaman et al., 2006). It was also reported that lysozyme-galactomannan conjugate showed excellent emulsifying activity and enhanced bactericidal effect to Gram-negative bacteria (Song et al., 2002).

2.2. Whey Proteins

Whey proteins possess excellent physicochemical, gelation and binding properties; and therefore, are widely used as functional ingredients in many formulated bakery, dairy and sausage products (Xiong et al., 1993). They are highly nutritive and stable in mildly acidic conditions (Ibrahim et al., 1993). However, commercial whey protein sources vary considerably in functional properties because of differences in salt content and composition in the whey products (Xiong et al., 1993). Furthermore, a number of environmental factors (e.g., pH, ionic strength, protein concentration, and time and temperature of heating) influence whey protein gelation behavior and other functional characteristics.

β-lactoglobulin (β-lg) is the major component of whey, constituting about 50% of the proteins in whey protein concentrate and is largely responsible for whey protein functionality (Xiong et al., 1993). It is a globular protein of 162 amino acid residues with a molecular mass of 18,400 Da and pI of 5.1 (Relkin, 1998). The protein possesses two disulfide bonds, a free thiol group and two tryptophan residues (Hoffmann et al., 1997).

α-lactalbumin (α-lac) is the second major protein of whey representing approximately 20% of the whey protein (Boye and Alli, 2000). α-lactalbumin has a highly ordered secondary structure, and a compact, spherical tertiary structure. It has a molecular weight of 14,000 Da with 123 residues. α-lac is a calcium-binding protein containing four intermolecular disulfides and no free thiol group. Thermal denaturation and pH <4.0 results in the release of bound calcium. The removal of bound calcium greatly decreases the thermal stability of α-lactalbumin. In food applications, α-lac has poor surface properties and no thermal gelation properties (due to the absence of free sulfhydryl group) at neutral pH (Relkin, 1994).

Bovine serum albumin (BSA) is a third protein of interest in the whey of milk. Bovine serum albumin is a large globular protein (66,000 Da) with a good essential amino acid profile. Its isoelectric point is 4.7. BSA has 17 disulfide bonds and one free thiol group. This protein constitutes only 5% of the protein in whey but possesses excellent gelling characteristics. It is reported to be partially unfold between 40 and

50°C, exposing non-polar residues on the surface and facilitating reversible protein-protein interactions (Hayakawa et al., 1992).

In the food industry, whey proteins are frequently used in the form of concentrates or isolates which contain a mixture of all five different proteins found in whey. More recently, there has been growing interest in the use of the individual whey proteins, particularly α-lactalbumin, in the development of specialty gels. Recent studies have shown that mixing the individual whey proteins in specific proportions (e.g. β-lactoglobulin and α-lactalbumin or BSA and α-lactalbumin) can also give gels with structural properties and strengths which vary from those of traditional whey protein concentrates and isolates (Boye et al., 2000).

Whey proteins are widely used in foods as emulsifiers because of their ability to facilitate the formation and stability of oil-in-water emulsions. Emulsions made with whey proteins have been shown to be stable at pH 7.0 an adsorption of the two major protein components is proportional to their initial concentration in the isolate (Sliwinski et al., 2003).

Whey proteins are very sensitive to heat (Mishra et al., 2001). The extent of heat treatment given during the manufacture of whey protein concentrate (WPC) is very critical because it may cause the denaturation of the proteins which alternatively affects the solubility and other functional properties of the proteins and due to the alteration of these functional properties, there is limited use of these WPCs in various food products. Therefore, in order to increase the applicability of WPCs, researchers investigated improvement of functional properties by the formation of complexes of WPC with polysaccharide (Akhtar and Dickinson, 2003; Ibrahim et al., 1993; Mishra et al., 2001).

2.3. Polysaccharides

Food hydrocolloids are high-molecular weight hydrophilic biopolymers used as functional ingredients in the food industry for the control of microstructure, texture, flavor and shelf-life. The term hydrocolloid embraces all polysaccharides that are extracted from plants, seaweeds and microbial sources, as well as gums derived from

plant exudates, and modified biopolymers made by enzymatic treatment of starch or cellulose (Dickinson, 2003).

2.3.1. Pectin

The hydrocolloid pectin is widely used in the food industry for gelling, thickening and stabilizing properties. Commercially, it is extracted mostly from citrus peel (lemon, lime and grapefruit) and apple pomace. Under certain well-defined conditions, pectin can form viscoelastic solutions and structured networks, and is widely exploited in jams, jellies and marmalades. Pectin can also be used to stabilize clouding in beverages, with effectiveness of stabilization dependent on the nature and amount of pectin present (Akhtar et al., 2002).

Pectin is defined as a mixture of heteropolysaccharides consisting predominantly methoxylated galacturonic acid residues. The functional properties are sensitive to the degree of esterification, DE, which in turn dependent on the type of plant tissue from which the pectin is extracted. High methoxyl pectins (≥ 50% DE) form gels under acidic conditions (pH ≤ 3.5) in the presence of a cosolute (typically > 50 wt% sucrose), whereas low methoxyl pectins (< 50% DE) form gels by different mechanism in the presence of calcium ions (Akhtar et al., 2002).

Like most polysaccharides, pectins are generally not considered to be emulsifying agents. But, pectins from citrus fruits and apples can exhibit good surface activity and emulsion stabilizing characteristics if the average molecular weight is reduced to <80 kDa by severe acid hydrolysis (Dickinson, 2003).

2.3.2. Guar gum

Galactomannans are seed polysaccharides, most widely used in the food industry as thickening agents. Guar gum is the most widely used galactomannans. The polymer is relatively large with a molecular weight of about 220,000 Da (Fennema, 1985). It hydrates rapidly in cold water to give a highly viscous, thixotropic solution. It is usually used at a concentration of 1.0 % or less in foods. Since the gum is neutral its solution viscosity is little affected by changes in pH. Salts have little effect on its solution viscosity. It can be used in ice cream, pastry icings, and dressings and sauces.

Purified guar gum does not exhibit any surface activity. Nevertheless, there are some statements in the literature that this polymer has the capacity to emulsify oils and stabilize fairly coarse emulsions at a moderately low gum/oil ratio. It has been suggested that, based on light microscopy observations of strong birefringency at the oil-water interface, this gum stabilize emulsions by forming liquid crystalline layers around the droplets (Dickinson, 2003).

2.3.3. Carrageenans

Carrageenans are anionic polysaccharides extracted from red seaweed. It is a high molecular weight linear polysaccharide, comprising repeating galactose units (Zhang and Foegeding, 2003). They exist in three main forms like kappa, iota or lambda (Hemar et al., 2002). They contain sulfate groups. The structures of the three main forms of carrageenan differ only in the number of sulphate groups per disaccharide: kappa has one, iota has two and lambda has three (Langendorff et al., 2000). Iota- and kappa-carrageenan in aqueous solution undergo a temperature-dependent coil (disordered state) to helix (ordered) transition. The helix-formation is closely associated with gelation, although the exact relationship between the two events is still not understood. Lambda-carrageenan adopts a coil conformation whatever the ionic and temperature conditions and is unable to form gels.

Carrageenan, as a stable colloidal dispersion, is added to chocolate milk to prevent chocolate precipitation. It is used in puddings as stabilizing powder. In cheese products it acts as an emulsion stabilizer. It is an inhibitor of ice crystallization in frozen foods. It increases the loaf volume, improves appearance and crumb texture in cakes (Fennema, 1985).

2.4. Protein-polysaccharide interactions

Protein-polysaccharide mixtures are widely used in the food industry as they play an essential role in the structure, texture, stability and rheology of many foodstuffs. Proteins contribute to emulsion and foam formation, whereas polysaccharides are present as thickening and water-holding agents. The overall texture and stability of food products depends not only on the properties of proteins and polysaccharides but also on the nature and the strength of protein/polysaccharide interactions. Therefore, knowledge

of the mechanisms occurring in protein-polysaccharide systems is important to develop desirable properties in food products (Hemar et al., 2001).

In aqueous solution, a binary mixture of protein + hydrocolloid can exhibit one of three different equilibrium situations: (a) miscibility, (b) thermodynamic incompatibility, and (c) complex coacervation (or complexation) (de Kruif and Tuinier, 2001; Martinez et al., 2005).

Miscibility occurs commonly at low biopolymer concentrations, and either incompatibility or coacervation (or complexation) at high concentrations, depending on whether the protein polysaccharide interaction is net repulsive or net attractive, respectively (Dickinson, 2003).

Thermodynamic incompatibility implies the separation into two distinct aqueous phases, one rich in protein and the other rich in hydrocolloid (Dickinson, 2003). Above the isoelectric point of the protein, thermodynamic incompatibility between the protein and polysaccharide generally occurs because of the repulsive electrostatic interactions and different affinities towards the solvent. Therefore, protein and polysaccharide may co-exist in a single phase (miscibility) in domains in which they mutually exclude one another or, above a critical concentration, segregate into different phases (Martinez et al., 2005).

Complex coacervation mainly occurs below the protein isoelectric point as a result of net electrostatic interactions between the polymers carrying opposite charges and implies the separation of two distinct phases, one phase is rich in the two biopolymers and the other phase is depleted in both (Dickinson, 2003; Martinez et al., 2005). Unless protein and hydrocolloid carry opposite net charges, complex coacervation does not take place (Dickinson, 2003). The phenomenon tends to be suppressed also at low biopolymer charge densities. Precipitation and/or gelation may occur at high charge densities. The maximum coacervation yield occurs for the case of an equal ratio mixture (by weight) of biopolymers at the pH where they carry equal and opposite charges. The extent to which a particular biopolymer is involved in Coulombic interactions depends on how far its isoelectric point pI differs from the solution pH. Most food proteins ($pI_p \sim 5$) form complex coacervates with anionic

hydrocolloids (pI_h ~ 3) in the intermediate region of pH where the two macromolecules carry opposite net charges (pI_p < pH < pI_h). The strength of complexation between protein and polysaccharide depends on the distribution of ionisable groups on the surface of the protein, the ease of unfolding of the protein's native structure and the backbone flexibility and charge distribution on the polysaccharides. The extent of reversibility of complexation depends on the aqueous environment and on mixing conditions. The tendency towards the non-equilibrium formation of an insoluble coacervate is enhanced at low salt concentrations and at pH values significantly below pI_p (Dickinson, 2003). When both biopolymers carry a net negative charge, soluble complexes may be produced. In this case, any electrostatic interaction involves the anionic polyelectrolyte interacting with positively charged local patches on the protein. Increasing the net negative charge on the protein has two effects: (a) it enhances protein-protein electrostatic repulsion and (b) it reduces protein-hydrocolloid attraction by screening the interactions of positively charged groups. The positively charged groups on a protein ($-NH_3^+$) interact more strongly with $–OSO_3^-$ groups than with $–CO_2^-$ groups. This means that, in terms of the relative contributions of effects (a) and (b), a useful distinction can be made between a sulfated hydrocolloid like carrageenan and a carboxylated hydrocolloid like pectin. At low ionic strength, sulfated hydrocolloids of relatively high charged density form fairly strong reversible complexes with proteins, even at neutral or alkaline pH (i.e. well above pI_p). In contrast, at pH > pI_p , any interaction of a protein with a carboxylated hydrocolloid interaction is quite weak or non-existent- or the system may even exhibit thermodynamic incompatibility (Dickinson, 2003).

Previous studies (Bourriot et al., 1999; Hemar et al., 2001; Hemar et al., 2002) have shown that mixtures of biopolymers, such as milk proteins and polysaccharides, can separate into two phases, which is the result of either thermodynamic incompatibility or depletion flocculation phenomena. Phase separation in biopolymers due to thermodynamic incompatibility is related to the entropy of the system and arises from the fact that the free energy of mixing is minimized. In the case of depletion flocculation, the aggregation of particles is caused by the exclusion of polymer molecules from the space between the particles, resulting in a difference in polymer concentration between the interparticle region and the outside region surrounding the particles. The osmotic pressure of the polymer solution being higher than the osmotic

pressure in the polymer-free interparticle region causes an additional attractive force between particles. This attractive force depends on the size, shape and concentration of the polymer molecules and the particles. Recent studies (Bourriot et al., 1999; Hemar et al., 2001; Maroziene, A. and de Kruif, 2000) on casein micelles and polysaccharide mixed systems have suggested depletion flocculation mechanisms to be responsible for phase separation. These systems included guar gum, carrageenan, pectin and exocellular polysaccharides, produced by lactic acid bacteria.

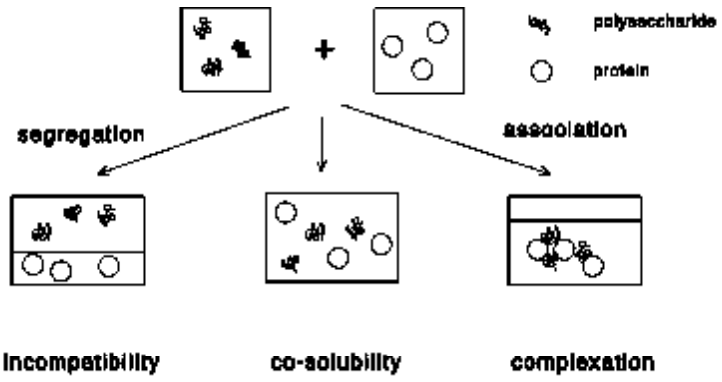


Figure 2. 1. Main trends in the behaviors of protein/polysaccharide mixtures (de Kruif and Tuinier, 2001)

In consumer products, phase separation is usually unwanted. However, many aspects related to interactions between biopolymers can be used for improving the products. Firstly, a fundamental understanding of the interactions leads to predictions of the phase line and interpretation of the measured phase behavior. The unwanted effect of phase separation can then be suppressed by using only biopolymer concentrations in the stable regions. The theory for depletion interaction allows the phase lines to be predicted from the sizes and molar masses of the particles involved. In the stable region the system does not behave ideally, and the equilibrium and transport properties of a solution of biopolymer 1 changes when biopolymer 2 is added. The transport properties, such as diffusion and especially the viscosity, affect aspects such as mouthfeel. An understanding of the biopolymer interactions may thus also lead to a directed manipulation of the properties of food dispersions. In separation technology the concepts of phase separation can be used to lower the energy consumption of the process. For example, concentration of caseins can be achieved

easily by adding the right polysaccharide dose. The depletion mechanism can also be exploited to separate large and small biocolloids, thereby replacing the procedure of centrifugal centrifugation (de Kruif and Tuinier, 2001).

When one of the components is much larger than the other a gel phase can form due to depletion interaction when the attraction is large and the volume fraction of the particles is sufficiently high. This is an arrested phase separation that behaves macroscopically as a stable highly viscous/gel-like mixture. In products such as salad dressings this mechanism gives the product its characteristic consistency (de Kruif and Tuinier, 2001).

Phase separation behavior of κ-carrageenan with milk proteins, especially caseins, was investigated (Thaiudom and Goff, 2003) and it was suggested that κ-carrageenan prevents phase separation (even at very low concentration) by adsorbing on casein micelles thus forming a filament gel-network that leads to prevention of the sedimentation of caseins.

Polysaccharide gums are often added to emulsions to enhance viscosity and alter textural characteristics. The presence of non-adsorbing polysaccharide in the aqueous phase of a protein-stabilised emulsion causes an increase in the attractive force between the droplets due to an osmotic effect associated with the exclusion of the biopolymers from a narrow region surrounding each droplet. This attractive force increases as the concentration of biopolymers increases, until eventually it may become large enough to overcome the repulsive interactions between the droplets and cause them to flocculate. This type of droplet aggregation is usually referred to as depletion flocculation (McClements, 2004). If the amount of polysaccharide is not large enough to completely cover the protein, a polysaccharide may adsorb onto more than one protein surface, thereby bridging two or more protein particles. Such a process is called as bridging flocculation (de Kruif and Tuinier, 2001). Within oil-in-water emulsions, complexation arising from attractive interactions between biopolymers may have either positive or negative influence over stability depending on the proportion of polysaccharide present (Dickinson and James, 2000). At high protein-polysaccharide ratios, bridging flocculation may result from the development of macromolecular linkages between protein-coated oil droplets. In contrast,

enhanced emulsion stability may occur at low protein-polysaccharide ratios, since biopolymer complexes (or conjugates) may coat the entire oil droplet surface (cooperative adsorption) forming a thick macromolecular multilayer, which provides both electrostatic and steric contributions to stability. For instance, interactions between β-lactoglobulin and various polysaccharides can result in the formation of complexes with substantially improved emulsifying properties (Dickinson and James, 2000).

Previous studies have identified significant electrostatic interactions between globular bovine serum albumin (BSA) and anionic sulfated polysaccharides as the cause of bridging flocculation in these emulsions. Moreover, a correlation between the density of charged sulfate groups on the polymer and the strength of the protein-polysaccharide complex has been identified. As determined by levels of induced oil droplet flocculation, protein interactions with κ-carrageenan are weaker than those with ι-carrageenan owing to a higher proportion of anionic sulfate groups in the latter molecular form. The strength of biopolymer interaction is governed by the aqueous solution conditions. At neutral pH, for instance, carrageenans are unreactive toward BSA, whereas complexation is induced under acidic conditions (Dickinson and James, 2000).

2.5. Functional Properties of Proteins

The functional properties of proteins are those physicochemical properties that enable proteins to contribute to the desirable characteristics of food. The conformation of individual proteins in solution is dependent on interactions with water. Most foods are hydrated solid systems and the physicochemical, rheological behavior of proteins is strongly influenced by the presence of water (Fennema, 1985). Many functional properties of a protein preparation are related to this progressive hydration: water absorption, swelling, cohesion, adhesion, dispersibility, solubility and viscosity etc. Also gelation implies the formation of a well-hydrated insoluble mass, but specific protein-protein interactions are also required. Finally, surface properties, such as emulsification and foaming, necessitate a high degree of protein hydration and dispersion (Voutsinas et al., 1983).

2.5.1. Solubility

High protein solubility is necessary for good foaming or emulsion capacity and stability. The degree of insolubility is probably the most practical measure of protein denaturation + aggregation, and because proteins that initially exist in a denatured, partially aggregated state often exhibit impaired ability to participate effectively in gelation, emulsification and foaming (Fennema, 1985).

2.5.2. Viscosity

The viscosity and consistency of proteins are important functional properties in fluid foods such as beverages, soups, sauces and creams. Knowledge of flow properties of protein dispersions is of practical significance in optimizing operations, such as pumping, mixing, heating, cooling and spray drying that involve mass and/or heat transfers (Gladwell et al., 1985). Solutions, dispersions, emulsions, pastes, or gels of most hydrophilic macromolecules, including proteins, do not behave like Newtonian fluids, as their viscosity decreases when the flow rate increases. This behavior is called pseudoplastic or shear thinning. When protein-protein interactions are numerous enough, as in pastes or gels, a plastic viscoelastic behavior is displayed, and fluid flow occurs only above a yield stress value necessary for the rupture of some of the interactions. The viscosity of most proteins increases exponentially with protein concentration, because of protein-protein interactions. Soluble proteins with high water absorption develop a high viscosity (Fennema, 1985).

Two aspects of rheology are important in relation to the stability of food colloids; i) modifying the rheological properties of aqueous continuous phase, ii) modifying the surface rheology of adsorbed macromolecular films at the air-water, oil-water interfaces. In the former, viscous and gel-like bulk phase reduces the rate of creaming and the rate of film rupture and drainage. In the latter, viscoelasticity of adsorbed protein film is important in determining the coalescence stability of oil droplets and gas bubbles. These aspects can be achieved by adding high molecular weight polysaccharides in the former and by forming complexes between polysaccharides and proteins in the latter. However, favorable rheological features of the polysaccharides from the colloidal stability point of view are dependent on the optimum polysaccharide concentration and the extent of interaction between proteins and polysaccharides (Saldamlı, 1998).

2.5.3. Gelation

Gelation is the association of long polymer chains to form a three dimensional continuous network that is resistant to flow under pressure. Protein gelation is utilized not only for the formation of solid viscoelastic gels, but also for improved water absorption, thickening, particle binding (adhesion) and emulsion or foam-stabilizing effects (Boye et al., 2000).

2.5.4. Emulsifying Properties

A great number of processed foods exist as emulsions, such as infant formulae, clinical nutrition products and beverages. The production of stable emulsions is a key issue in the food industry and requires a good understanding of the complex macromolecular interactions arising during technological processes (Reiffers-Magnani et al., 2000).

Food emulsions are formed by mixing oil and water together to form droplets, usually in the presence of an emulsifier (Chung and Ferrier, 1991). Emulsifying properties are commonly discussed in terms of emulsifying activity and emulsion stability. Emulsifying activity is a measure of turbidity of a diluted emulsion, and directly related to the interfacial area. Emulsion stability can be defined as the change in absorbance with time (Pearce and Kinsella, 1978).

Emulsions are thermodynamically unstable systems, which ultimately tend to separate into two immiscible fat and aqueous phases, after creaming and coalescence of the oil droplets. Breakdown processes of emulsions may be distinguished: (i) Creaming or sedimentation (caused by gravity). (ii) Flocculation caused by van der Waals attraction when there is not sufficient repulsion between the droplets. (iii) Ostwald ripening caused by the difference in solubility between the small and large droplets. (iv) Coalescence induced by thinning and disruption of the liquid film between the droplets. (v) Phase inversion the dispersed phase and medium interchange (Tadros, 2004).

Settling under gravity may occur when the density of the oil is higher than that of the medium. This may also occur with water-in-oil emulsions when the density of the aqueous droplets is higher than the of the oil phase. In most cases, creaming rather

than sedimentation occurs, since most oils have densities lower than the continuous aqueous phase (Tadros, 2004). The creaming kinetics can be described by the Stokes law, which involves the dependence of the creaming rate on the fat globule diameter, the viscosity of the continuous phase and the density difference between continuous and dispersed phases (Reiffers-Magnani et al., 2000). However, this law often fails to describe the creaming processes arising in many food emulsions, especially those resulting from flocculation of the oil droplets. Flocculation also referred to as reversible aggregation arises when several droplets associate with each other, due to unbalanced repulsive and attractive forces (when the van der Waals attractive energy exceeds the repulsive energy). For example the presence of polysaccharides in the emulsion bulk phase can promote depletion flocculation of the oil droplets by inducing an osmotic pressure gradient within the continuous phase surrounding the globules and their following aggregation. Ostwald ripening (disproportionation) may occur when the oil solubility is significant (the small droplets which have higher solubility than the larger ones, tend to dissolve on storage and become deposited on the larger ones) (Tadros, 2004). Coalescence results from the thinning and disruption of the liquid film between the droplets, with the ultimate joining of these droplets and finally some oil separation can be observed. In phase inversion the dispersed medium forms the droplets and the dispersed droplets form the continuous phase (e.g. an O/W emulsion reverting to a W/O emulsion and vice versa). A schematic representation of the above break down processes is given in Figure 2.2.

In a food emulsion of the oil-in-water type, the primary emulsifying and stabilizing agent that protects droplets against coalescence is typically a multicomponent mixture of adsorbed proteins. Hydrocolloid gums are mostly hydrophilic polymers, and do not exhibit surface activity. However, as a stabilizer in food emulsions, some gums migrate slowly to the air-water and oil-water interfaces and exhibits significant surface and interfacial activities. Various polysaccharides may also be present as thickening and gelling agents. Segregative or associative interactions between these polysaccharides and adsorbed proteins may have additional secondary implications for aggregation and creaming behavior.

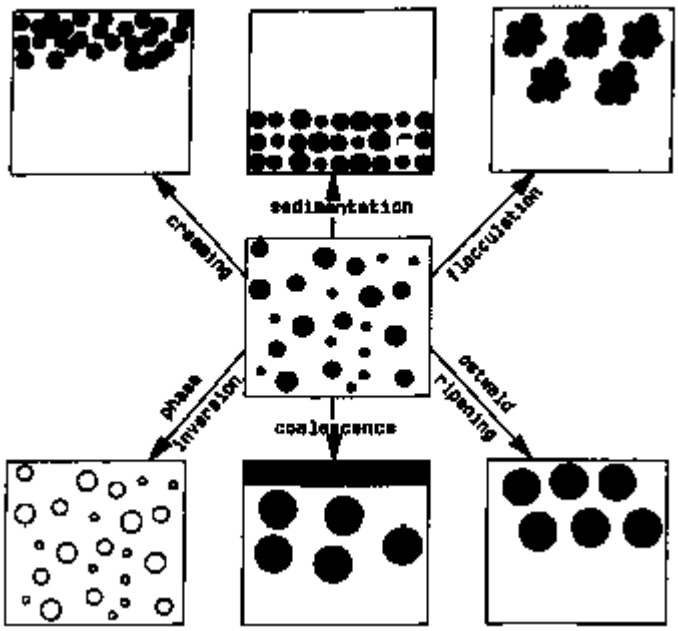


Figure 2.2. Schematic representation of the break-down processes in emulsions (Tadros, 2004)

2.5.5. Foaming Properties

A foam is a high-volume-fraction dispersion of gas in liquid. Bubbles are separated by thin films. Three adjacent thin films intersect in a Plateau border and the continuous phase is interconnected through a network of Plateau borders. Foams are usually formed by incorporating a high volume fraction of gas in a surfactant solution, which stabilizes the bubbles (Wang and Narsimhan, 2004). Incorporation of air cells influence the texture of a food product and they can impart body and smoothness, can ensure desirable structure and rheological properties, and can facilitate flavour dispersion. Product appearance and shelf-life are determined by the amount of air incorporated and the sizes and physical stability of the bubbles. Examples of food products where incorporated air creates or enhances desirable characteristics include bread, cakes, ice-cream, confectionery, and beer. In each case, it is essential for the manufacturer to be able to control the size distribution of air bubbles and the spatial distribution of the gaseous phase in order to maintain the quality of the product (Ka Lau and Dickinson, 2005). Protein stabilized foams are extensively employed in many food formulations. Proteins, being surface active, tend to adsorb at the gas–liquid interface, thus stabilizing the foam. Foamability and foam

stability are two important functional properties of proteins. Foamability is the ability of protein to form large volume of foam and is governed by the ability of protein to adsorb rapidly at the gas–liquid interface leading to substantial reduction in surface tension. Foam stability is governed by the ability of protein to stabilize the thin film separating the gas bubbles thus preventing bubble coalescence (Ka Lau and Dickinson, 2005) .

The stability of an aerated system is affected by three basic mechanisms: gravitational drainage, coalescence, and disproportionation (Ka Lau and Dickinson, 2005). In a spherical bubble foam, upward movement of large bubbles due to buoyancy results in a vertical phase separation (creaming) of the dispersed air. In a polyhedric foam, gravity acts directly on the draining film liquid. Because of the radius of curvature of a Plateau border, the pressure inside the Plateau border is less than that in thin film by capillary pressure (Wang and Narsimhan, 2004). This difference, known as Plateau border suction, also leads to drainage of liquid from thin films to the neighboring Plateau border. This is counteracted by disjoining pressure caused by van der Waals, electrostatic and steric interactions between two approaching faces of a draining film. The draining thin film may reach an equilibrium at which the Plateau border suction is counterbalanced by the disjoining pressure. The film may also rupture due to the growth of imposed thermal and mechanical perturbations, thus leading to coalescence of neighboring gas bubbles. Coalescence of adjacent bubbles by rupture of inter-bubble lamellae, and disproportionation (diffusion from small to large bubbles by differences in the Laplace pressure) due to polydispersity in the bubble-size distribution, combine to produce a gradual coarsening of the gaseous dispersion (Ka Lau and Dickinson, 2005). These structural changes also serve to accelerate the gravity-driven separation processes. All these instability mechanisms are acting together, gradually resulting in non-uniform microstructure and reduced product quality, which is obviously undesirable.

Addition of xanthan gum was found to result in enhanced foam stability of egg white foams over a wide range of ionic strength and pH (Wang and Narsimhan, 2004).

Martinez et al (2005) have been previously studied the effect of polysaccharides on foaming properties of intact food proteins and shown that foam stability is strongly increased. For more low-viscosity foams such as those containing the carrageenans and galactomannans, the surface and rheological properties should become important and stability would be determined by both bulk and interfacial properties. Polysaccharides alongside increasing the viscosity of the aqueous phase may influence the viscoelastic character and thickness of the adsorbed macromolecular layer reducing the thinning rate of lamella and hence increasing the stability of the foam. This behavior is quite different from that observed for oil-in-water emulsions where small amounts of polysaccharides (i.e. xanthan) have been shown to reduce the stability with respect to creaming by promoting droplet flocculation through a depletion mechanism (Martinez et al., 2005).

2.6. Improvement in Functional Properties

Improvements of functional properties may be achieved by modifying the protein structure by chemical, enzymatic methods or physical treatments (Dickinson, 1992). Physical modification is preferable since it does not add any chemicals (Tedford and Schaschke, 2000). Limited heat treatment improves foaming and emulsifying properties of some proteins (Pittia et al., 1996).

2.6.1. Heat Treatment

Heat treatment is often required to ensure microbial safety or to obtain desirable organoleptic attributes of food products (der Plancken et al., 2005). To ensure that egg products are free from pathogenic bacteria and particularly *Salmonella* whole egg, egg yolk and egg white need to be pasteurized under certain conditions. Severe heat treatments can further ensure microbial safety and increase shelf-life of egg products but can have detrimental effects on the functional properties of egg proteins resulting in commercially undesirable finished products. Therefore several attempts have been made by researchers to identify methods that would allow eggs to withstand severe heat treatments without altering or at least damaging their physical and functional properties (Campbell et al., 2005).

Heat treatments are also of great importance in the production, concentration and processing of food proteins. Changes in protein structures have desirable or

undesirable effects on their functional properties. The thermal denaturation process may be reversible or irreversible and more or less cooperative as a function of the sequence of the amino-acid residues and of the chemical environment, such as pH, concentration, ionic strength, etc. The heating of globular proteins is accompanied by the destruction of some of the forces which stabilize the tertiary and/or secondary structures, e.g. hydrogen interactions between the polar groups and interactions of non-polar groups (hydrophobic interactions) through the surrounding water molecules which form cages around them (Relkin, 1994). The disruption of electrostatic and van der Waals interactions are observed to a lesser extent.

Voutsinas et al. (1983) showed that protein solubility decreased as heating time increased due to the progressive denaturation of the protein. As protein denaturation progressed, protein solubility decreased and the hydrophobicity usually increased. This is due to the gradual exposure of hydrophobic amino acid residues of native proteins, which are usually buried in the interior of the molecules as a result of the protein unfolding. This effect might be eliminated with the addition of polysaccharides. Because hydrocolloid molecules may behave as steric spacers between the protein molecules, protecting the globular protein against aggregation by blocking the hydrophobic binding sites on the surface (İbanoğlu, 2005). Laneuville et al (2000) also reported that in heat-treated protein-hydrocolloid systems, adding a polysaccharide could prevent an excessive protein aggregation by minimizing protein-protein interactions, either by shielding active charged groups or by decreasing the collision rate between molecules through an increase in viscosity of the solution.

It was reported that the size distribution of whey protein-stabilised emulsions was not affected by heating at 70°C. However, heating at higher temperatures in the range 75-80°C, led to significant droplet aggregation; an effect that decreased with increasing heating temperature. The authors explained this trend by the predominance inter-droplet interactions leading to aggregation of emulsion droplets in the range of 75-80°C, while at higher temperatures intra-droplet protein-protein interactions are favored (Sliwinski et al., 2003). Other researchers confirmed the temperature dependence of the aggregation of whey protein-stabilised emulsions. They proposed that in the temperature range of 65-80°C due to partial unfolding at

the oil-water interface not all-hydrophobic side chains are oriented towards the oil phase leading to a relatively high hydrophobicity of the surface of the adsorbed layer and thereby making it susceptible to aggregation. On the contrary, at higher heating temperatures intra-droplet protein-protein interactions are promoted, because hydrophobic groups due to complete unfolding of whey proteins are able to rearrange themselves to be in contact with the oil phase thereby reducing the droplet surface hydrophobicity (Sliwinski et al., 2003). Recently, it was shown that aggregation of emulsions was more extensive and proceeded more rapidly as the concentration of whey protein in the emulsion increased. Removal of non-adsorbed protein from the emulsion decreased the rate of the aggregation reaction by several times.

In whey protein-stabilised emulsions, heating above 65°C causes the adsorbed protein to partially unfold and expose some reactive amino acids, which were inaccessible in folded proteins. The sequence of amino acids can participate in the formation of cross-links between proteins of the interfacial film and/or between proteins of the aqueous phase. These cross-links consist of both chemical bonds (disulfide linkages) and physical interactions (hydrophobic, electrostatic, hydrogen bonding). Firstly, such cross-links could change the viscoelastic properties of the interfacial film, thus modifying their resistance towards coalescence. Secondly, the cross-links, involving proteins of the aqueous phase, can lead to a three-dimensional solid-like network, which contributes to the development of textured emulsions. Consequently, thermal treatment of emulsions significantly alters their stability towards coalescence and their flowing properties (Anton et al., 2001).

Emulsifying properties of egg yolk granules and plasma have been investigated and it was demonstrated that granule solutions withstand more severe heat treatment than yolk and plasma solutions. The structure of native granules, in which HDL and phosvitin are aggregated through phosphocalcic bridges, cannot prevent protein denaturation, but can avoid aggregation of LDL and HDL from different granules (Anton et al., 2001).

Thermal denaturation of globular proteins has been extensively studied and thermodynamic parameters have been determined (Boye and Alli, 2000). Differential scanning calorimetry (DSC) has been established as a sensitive technique for

studying thermal denaturation and conformational transitions of proteins providing qualitative and quantitative information as to the thermodynamic properties of proteins (İbanoğlu, 2005).

2.6.1.1. Differential Scanning Calorimetry

The most commonly used method in studies of the protein denaturation is differential scanning calorimetry (DSC). DSC provides detailed information about both the physical and energetic properties of a substance. Differential scanning calorimetry is used to study what happens to polymers when they are heated. It represents the amount of heat absorbed by the polymer during thermal denaturation and gives the heat flow plot against temperature. The heat of transition or enthalpy is calculated by measuring the area under the peak. The peak temperature is defined as the temperature of denaturation, T_d (Delben and Stefancich, 1998; Relkin, 1994). This instrument provides quantitative information about exothermic, endothermic and heat capacity changes as function of temperature and time (such as melting, purity and glass transition temperature). This technique consists of a two-pan configuration (sample and reference). DSC measures energy differences between the sample and reference pan (Clas et al., 1999).

A second heating of the sample solution, which should show a similar peak transition to that observed in the first heating curve, generally checks the reversibility of the denaturation process (Hoffmann et al., 1997).

2.7. Rheological Properties

2.7.1. Definition of Rheology

The science of rheology has many applications in the fields of food acceptability, food processing, and food handling. Foods, however, are complex materials structurally and rheologically and, in many cases, they consist of mixtures of solids as well as fluid structural components (Tabilo-Munizaga and Barbosa-Ca´novas, 2005).

Rheology concerns the flow and deformation of substances and, in particular, to their behavior in the transient area between solids and fluids. Moreover, rheology attempts to define a relationship between the stress acting on a given material and the

resulting deformation and/or flow that takes place (Tabilo-Munizaga and Barbosa-Ca´novas, 2005).

Rheology is concerned with how all materials respond to applied forces and deformations. Basic concepts of stress (force per area) and strain (deformation per length) are key to all rheological evaluations. Stress (σ) is always a measurement of force per unit of surface area and is expressed in units of Pascals (Pa). The direction of the force with respect to the impacted surface area determines the type of stress. Normal stress occurs when the force is directly perpendicular to a surface and can be achieved during tension or compression. Shear stress occurs when the forces act in parallel to a surface. On the other hand, strain represents a dimensionless quantity of relative deformation of a material. The direction of the applied stress with respect to the material surface will determine the type of strain. Normal strain (ε) occurs when the stress is normal to a sample surface. Foods show normal strain when compressed (compressive stress) or pulled apart (tensile stress) (Tabilo-Munizaga and Barbosa-Ca´novas, 2005.

2.7.2. Rheological Measurement Equipment

The rheometer, or viscometer, which measures the rheological properties of fluids by the resistance to flow under a known force or the produced stress by a known amount of flow, is an essential tool in food rheological studies. The two common approaches used in rheometers are controlled rate and controlled stress. In the controlled rate approach, the material being studied is placed between two plates. One of the plates is rotated at a fixed speed and the torsional force produced at the other plate is measured. In the controlled stress approach, the situation is reversed. A torque (stress) is applied to one plate and the displacement or rotational speed (strain rate) of that same plate is measured (Tabilo-Munizaga and Barbosa-Ca´novas, 2005).

2.7.3. Rheological Measurement Techniques

Generally, steady state flow measurements and dynamic (oscillatory) measurements have been employed for the characterization of emulsions (Kontogiorgos et al., 2004).

2.7.3.1. Steady State Flow Measurements

This technique determines the relationship between shear rate (γ) and shear stress (σ). For a Newtonian system σ is related to γ by the equation,

$$\sigma = \eta\gamma \qquad (2.1)$$

where η is the Newtonain viscosity (that is independent of the applied shear rate) (Tadros, 2004). The majority of the foods do not show Newtonian flow behavior. This means that the plot of σ vs. γ is not linear and/or the plot does not begin at the origin. For non-Newtonian liquid the viscosity is a function of the rate of shear. The measured shear stress viscosity may increase or decrease with time even through the shear rate is maintained constant. In such as case the fluid is called a time dependent fluid. Time independent deviation from ideal Newtonian behavior will cause the relationship between shear stress and shear rate to be nonlinear. When the material exhibit a diminish of viscosity as shear rate increases it is called shear thinning or pseudoplastic, however if the viscosity increase with shear rate and is called shear thickening or dilatent. Pseudoplasticity and dilatency are time independent properties (Tabilo-Munizaga and Barbosa-Ca´novas, 2005).

2.7.3.2. Dynamic (oscillatory) Measurements

These are the most commonly used methods to obtain information on the viscoelastic and gel characteristics of foods. A strain is applied in a sinusoidal manner, with an amplitude and a frequency. From dynamic rheological tests in the linear viscoelastic range, the storage modulus (elastic modulus), G′, and the loss modulus (viscous modulus), G″, and tan δ = G″/G′, the loss factor, can be obtained. G′ value is a measure of the deformation energy stored in the sample during the shear process, representing the elastic behavior of a sample. In contrary, G″ value is a measure of the deformation energy used up in the sample during the shear and lost to the sample afterwards, representing the viscous behavior of a sample. If G′ is much greater than the G″, the material will behave more like a solid; that is, the deformations will be essentially elastic or recoverable. However, if G″ is much greater than G′, the energy used to deform material is dissipated viscously and the materail's behavior is liquid-like. On the other hand, the loss factor (or dampling factor) reveals the ratio of the viscous to the elastic portion of the deformation behavior. A phase angle δ = 0° or tan δ = 0° correponds to an elastic response and

$\delta = 90°$ or $\tan \delta = \infty$ is a viscous response. If the phase angle is within the limits of $0° < \delta < 90°$, the material is called viscoelastic (Tabilo-Munizaga and Barbosa-Ca´novas, 2005).

Three types of dynamic tests can be conducted: (1) frequency sweep studies in which G′ and G″ are determined as a function of frequency at fixed temperatures, (2) temperature sweep in which G′ and G″ are determined as function of temperature at fixed frequency, (3) time sweep in which G′ and G″ are determined as a function of time at fixed frequency and temperature (Tabilo-Munizaga and Barbosa-Ca´novas, 2005). The frequency sweep test is the most commonly used method because it shows how the viscous and elastic behavior of the material changes with the rate of application of strain stress.

2.7.4. Rheology of Emulsions

The rheology of emulsions is focusing a great interest mainly due to its relationship with emulsion stability, the most important factor to be considered in emulsion technology. Rheological properties of food emulsions should be carefully taken into account for plant and process designing and modeling, consumer acceptability and long-term stability purposes. The huge number of structural parameters that influence emulsion stability also affect rheology. Thus, during the last years, a large number of researchers have addressed a wide range of topics related to emulsion rheology (Diftis et al., 2005; Kontogiorgos et al., 2003; Mandala et al., 2004). Concerning emulsion rheological behavior, it ranges between Newtonian and pseudoplastic. Emulsion formed of milk protein and soya oil showed Newtonian behavior, but kappa carrageenan addition resulted in viscosity increase and clear pseudoplastic behavior, indicating reversible flocculation of oil droplets. Protein emulsions containing xanthan and gum arabic showed pseudoplastic behavior (Hennock et al., 1984), which approached Newtonian character as the concentration of gum increased (İbanoğlu, 2002). Generally, gums and stabilizers have non-Newtonian rheology and they impart non-Newtonian character to the emulsion even when the amount of the dispersed phase is low (Mandala et al., 2004). Tavares and Silva (2003) reported that the presence of galactomannan had a negative effect on the network development of

galactomannan-WPI systems, increasing the temperature of gelation and the viscous character of the system, and decreasing the storage modulus (G′). The viscoelastic behavior of egg yolk stabilised emulsions after the addition of polysaccharide was futher increased (Kontogiorgos et al.,2004). The flow became more pseudoplastic with increased values of viscosity and the storage modulus was also increased.

CHAPTER 3

3. MATERIALS AND METHODS

3.1. Materials

Powdered whey protein isolate (98%) (WPI, BIOPRO, Lot No. JE 030-3-420) was obtained from Davisco Foods International (Le Sueur, MN, USA). Hen egg albumen powder (sample no.1392) (egg white) and hen egg yolk powder (sample no.1393) were kindly supplied from NIVE (Nederlandse Industrie van Eiproducten, Holland). Low-methoxyl pectin (P-9135), guar gum (G-4129), *i*-carrageenan (C-1138), bovine serum albumin (A-7906) and glucose (D-9559) were purchased from Sigma Company. Buffer salts, potassium dihydrogen orthophosphate, disodium hydrogen orthophosphate and sodium lauryl sulfate (SLS) were purchased from Analar analytical reagent; BDH Chemical Ltd. Sodium chloride (JT-Baker), sodium hydroxide (Riedel-de-Haen), sulfuric acid (Carla Erba), phenol (Riedel-de-Haen) were used. Copper sulfate, sodium potassium tartrate, potassium iodide, hydrocholoric acid were obtained from Merck Company. All chemicals used were analytical grade.

3.2. Preparation of Stock Protein Solutions

Phosphate buffer was prepared by dissolving potassium dihydrogen orthophosphate (3.76 gr) and disodium hydrogen orthophosphate (3.44 gr) in 2 lt distilled water and pH was adjusted to 7.0 by using 0.1 M NaOH and 0.1 M HCl. Whey protein isolate (WPI) was dissolved in phosphate buffer (ionic strength 0.05 M and pH 7.0) at room temperature while stirring with magnetic stirrer for 1 h.

Egg yolk powder was dissolved in phosphate buffer containing 0.17 M NaCl, pH 7.0, by stirring for 1 h. This solution was then centrifuged at 2100x g for 45 min and the supernatant (plasma) was separated from the precipitate (granules) by decantation. Plasma was again centrifuged under the same conditions for a complete removal of granules. Then granules were dissolved in phosphate buffer containing 0.5 M NaCl

(Reiffers-Magnani et al.,2000; Akthar et al., 2002). The protein content of plasma and granule preparation was determined by Biuret method. Egg white powder was prepared by dissolving powder in phosphate buffer containing 0.17 M NaCl solution.

3.3. Preparation of Stock Polysaccharide Solutions

ι-carrageenan, guar gum and pectin (low methoxyl) stock solutions were prepared by dispersing the powders in phosphate buffer and vigorously stirring for 30 min at room temperature, followed by heating at 50°C until the solution became clear (Turgeon and Beaulieu, 2001; Zhang and Foegeding, 2003; Thaiudom and Goff,2003).

3.4. Determination of Phase Diagrams

Stock solutions of proteins and polysaccharides were mixed at different ratios at pH 7.0 and throughly stirred at room temperature in glass test tubes. Protein concentration in these solutions was changed from 0.0% to 12.0% (w/w) and polysaccharide concentration was changed from 0.0% to 1.4% (w/w). All mixtures were mixed until a homogeneous mixture was obtained. Then, the solutions were kept at refrigerator for 24 h to ensure complete hydration. After aging, the mixtures were warmed to 25°C for 30 min in a controlled temperature water bath and then centrifuged at 850x g for 15 min. The speed of centrifugation were determined by means of a series of preliminary experiments and chosen due to observation of distinct phase separation. Phase separation boundary was then detected visually after a clear separation of two phases was obtained. Protein content and polysaccharide concentration in each phase were determined. The position of the phase separation boundary line was plotted following the various concentrations of both protein and polysaccharide that showed phase separation (Thaiudom and Goff, 2003).

3.4.1. Determination of Protein Content

Protein content of each phase after phase separation was determined by Biuret method. For the preparation of Biuret reagent, 1.5 g copper sulfate ($CuSO_4.5H_2O$) and 6.0 g sodium potassium tartrate ($NaKC_4H_4O_6.4H_2O$) were dissolved in sufficient distilled water separately and then they were mixed and 300 ml 10% sodium hydroxide (NaOH) was added to this mixture with constant swirling.And the 1.0 g potassium iodide (KI) was added into the mixture and the solution was diluted to 1.0 lt with distilled water.

Standard protein solutions (0.0-1.4 mg/ml) were prepared by using bovine serum albumin as a standard protein with a final volume of 1.0 ml. 4.0 ml of Biuret reagent was added to each solution and mixed thoroughly. They were waited at room temperature for 30 min and the absorbance was read in spectrophotometer (Pharmacia Biotech, Novaspec II, UK) at 540 nm against the blank (water). Standard curve was prepared from the data as plotting absorbance versus concentration (Figure A.1).

After phase separation, 1.0 ml of sample was taken from each phase and protein content was determined by following the above procedure.

3.4.2. Determination of Polysaccharide Content

Polysaccharide content of each phase after phase separation was determined by phenol/sulfuric acid method (Dubois et al., 1956). 80% (w/w) phenol was prepared with distilled water. Standard polysaccharide solutions (0.0-0.05 mg/ml) were prepared by using glucose with a final volume of 2.0 ml. Then 0.05 ml of 80% phenol was added onto each solution and then 5.0 ml of concentrated sulfuric acid (H_2SO_4) was added rapidly and mixed thoroughly. The tubes were allowed to stand 10 min in a water bath at 25°C and after 10 min the absorbance was read in spectrophotometer (Pharmacia Biotech, Novaspec II, UK) at 490 nm and standard curve was prepared by plotting the absorbance versus concentration (Figure A.2).

After phase separation, 2.0 ml of sample was taken from each phase and the above procedure was followed and polysaccharide content of each phase was determined by using standard curve.

3.5. Differential Scanning Calorimetry

Stock whey protein isolate (40.0% w/v), egg proteins (20.0% w/v) and polysaccharide (2.0% w/v) solutions were prepared. For each protein, 25.0 μl of protein and 25.0 μl polysaccharide solution were placed into aluminum DSC pans and the pans were sealed. The samples were placed in the DSC (Perkin-Elmer DSC 6 equipped with a Pyris software, Perkin-Elmer Inc., Wellesley USA) and scanned from 15 to 110°C at a heating rate of 5°C/min using an empty pan as a reference. The

enthalpy of denaturation (ΔH) and the denaturation temperature (Td) were determined from each curve (Relkin et al., 1999; Relkin and Sourdet, 2005)

3.6. Emulsifying Properties

3.6.1. Preparation of Emulsions

The emulsifying properties of whey protein isolate, egg yolk plasma, egg yolk granule and egg white were determined by the modified Pearce and Kinsella (1978) method. To prepare emulsions, 25.0% (v/v) soybean oil and 75.0% (v/v) of protein solution were homogenized using lab scale homogenizer (Art-Miccra D-8, Germany) at 33,000 rpm for 5 min to give a final protein concentration of 0.50% (w/v) in whey protein isolate stabilised emulsions and of 1.0%(w/v) in egg proteins stabilised emulsions. Emulsions were also prepared in the presence of pectin/guar gum (0.0-0.50% w/v) to observe the effects of hydrocolloid gums.

To observe the influence of heat treatment on emulsifying properties, 6.0 ml of protein solutions (egg yolk granule, egg yolk plasma, whey protein isolate) both in the absence and presence of pectin/guar gum (0.1% (w/v)) were heated at 65, 70 and 75°C for 2 min and immediately cooled in the ice-bath and then emulsions were prepared as above and emulsifying properties were determined. Emulsions were prepared in duplicate.

3.6.2. Emulsifying Activity and Emulsion Stability

A 50 μL aliquot of the emulsion was taken from the bottom of the container at different time intervals and diluted with 5 ml of 0.1% SLS solution. The absorbance of the diluted emulsion was then determined at 500 nm by spectrophotometer (Pharmacia Biotech, Novaspec II, UK). The emulsifying activity was determined from the absorbance measured immediately after emulsion formation and the emulsion stability, which was described as emulsion stability index (ESI), was estimated by following the time-dependent change in the absorbance readings. The result was expressed as surface area (m^2) per unit weight of protein used in the emulsions. Emulsion experiments were replicated two times.

3.6.3. Creaming Analysis

The creaming stability of emulsions was assessed by transferring 10 ml of emulsion into a test tube and storing at ambient temperature and changes in the serum volume was observed regularly during 10 h. During this time interval the volume of emulsion creamed at the top and the volume of sedimented water phase were both noticed and the percentage of creaming was calculated as follows:

$$\%\text{creaming} = 100 - [(V_c/V_t) \times 100] \quad (3.1)$$

where V_c is the volume of the creamed phase and V_t is the volume of the creamed phase + volume of the aqueous phase (Anton et al., 2000).

3.7. Foam Formation and Stability

Foaming properties of whey protein isolate, egg yolk proteins and egg white were investigated. Stock protein solutions were diluted to 0.01% (w/v). The samples were foamed in a glass-sintered column of 250 mm in length and 35 mm in internal diameter. Air was fed into the column by an air pump (EYELA, Tokyo, Japan) at a constant flow rate of 0.5 nl/min and 5 ml of sample was sparged for 10 sec. Immediately after turning off the gas, the foam height was read from the calibrated markings on the column and the foam volume (FV) was calculated. The time for the collapse of the foam to half of its initial value is measured and expressed as foam stability. The foaming experiments were also done in the presence of pectin/guar gum (0.01-0.50% w/v) to observe the effects of polysaccharides on foaming behavior of proteins (İbanoglu and Karataş, 2001).

To investigate the effect of heat treatment on foaming properties of proteins, egg white solutions (0.01% w/v) were heated at 65, 70, 75 and 80°C for 2 min and then immediately cooled in an ice-bath to prevent further aggregation (Campbell et al., 2005) and then foamed as above. The effects of hydrocolloid gums on the foaming behavior of heated and unheated egg white were investigated in the presence of 0.01, 0.1 and 0.5% (w/v) pectin/guar gum. Heat treatment was applied to either protein solution (1.0% w/v) which was diluted to the desired concentration and mixed with biopolymer hydrocolloids or the protein-hydrocolloid mixture at the desired concentration. Foaming experiments were replicated three times.

3. 8. Rheological Measurements

Dynamic rheological measurements were performed at 25 ± 0.01°C, with a RheoStress RS-1 controlled stress rheometer (HAAKE, Karlsruhe, Germany), using a cone and plate geometry (cone diameter 35 mm, angle 2°). For each measurement, 2.0 ml of emulsion were carefully deposited over the plateau of the rheometer. After the plateau has been contact with the cone, the exposed surface of sample was covered with a thin layer of silicone oil to prevent evaporation during the measurement. Stress sweep tests (1 Hz at 25°C) were made to determine the linear viscoelastic region of all samples; a stress value of 1 Pa were chosen for all the frequency tests. All the dynamic frequency sweep tests were done inside the linear viscoelastic region in a frequency range of 0.001 to 10 Hz.and the elastic (G′) and viscous (G″) moduli were recorded versus frequency. Data analysis software (Reowin Pro Data Manager Version 2.64) was used to obtain the experimental data (elastic, viscous modulus, phase angle etc.).

Steady flow measurements were carried out in the range of 0-200 s^{-1} during 300 sec and rheological parameters (shear stress, shear rate, apparent viscosity) were obtained from the software. Flow curves were fitted to power law model ($\sigma = k\gamma^n$) using Sigma Plot 6.0 software. The k and n parameters which are consistency and flow index, respectively, were calculated.

3.9. Statistical Analysis

All statistical analysis were completed using the SPSS 8.0 system software. Experimental results were subjected to one-way and two way analysis of variance (ANOVA) according to the General Linear Model (GLM) procedure with least-square means effects.

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1. Phase Separation Behavior of Protein-Polysaccharide Mixtures

4.1.1. Phase Separation Behavior of Whey Protein Isolate

After aging and centrifugation, phase separation was observed with a clear boundary between the lower layer and the upper layer in WPI–pectin or WPI–guar gum mixtures. Figure 4.1 illustrates the phase diagram of WPI–guar gum and WPI–pectin mixtures. The binodal curve (solid line) separates the one-phase region from the two-phase region and was built by direct observation of the phase separation in the test tubes (Bourriot et al., 1999). The mixtures were stable at compositions below the curves (one-phase region) and were unstable at compositions above this curve (two-phase region). At low concentrations of WPI and guar gum, homogeneous protein-polysaccharide mixtures were formed (stable region) which indicates the compatibility of WPI and guar gum. As the concentration of WPI increased, the phase separation was induced even at a low (0.20% w/w) guar gum concentration. It was visually observed that a clear upper layer and a relatively opaque and white lower layer were formed after separation. When the samples showing phase separation were moderately shaken by hand and remixing of two phases was observed. Therefore, we suggested that the phase separation in WPI–gum systems was a reversible process and relatively weak interactions were present between protein and either pectin or guar gum.

The region under binodal curve gives information about the extent of incompatibility (Turgeon and Beaulieu, 2001). Zhang and Foegeding (2003) suggested that when binodal curve is close to both axes, compatibility decreases. Therefore, the extent of incompatibility was observed to be greater in samples containing guar gum as compared to those containing pectin. As observed from the region of incompatibility (one-phase region in Figure 4.1A) on the graph, the phase separation occurs over a wide range of biopolymer concentrations. While a small amount of guar gum (0.15%

w/w) could induce phase separation in the mixture of WPI (4.0% w/w) with hydrocolloids, a 1.20% (w/w) pectin was needed for the same amount of protein. Bourriot et al. (1999) reported that a low molecular weight hydrocolloid occupies a smaller volume in the medium than a high molecular weight hydrocolloid and the exclusion of the small polymer occurs to a lesser extent. Therefore, the binodal line shifts towards the high polysaccharide concentration as in the case of WPI–pectin system in our work. This reveals the difference between two hydrocolloids in terms of phase separation with WPI.

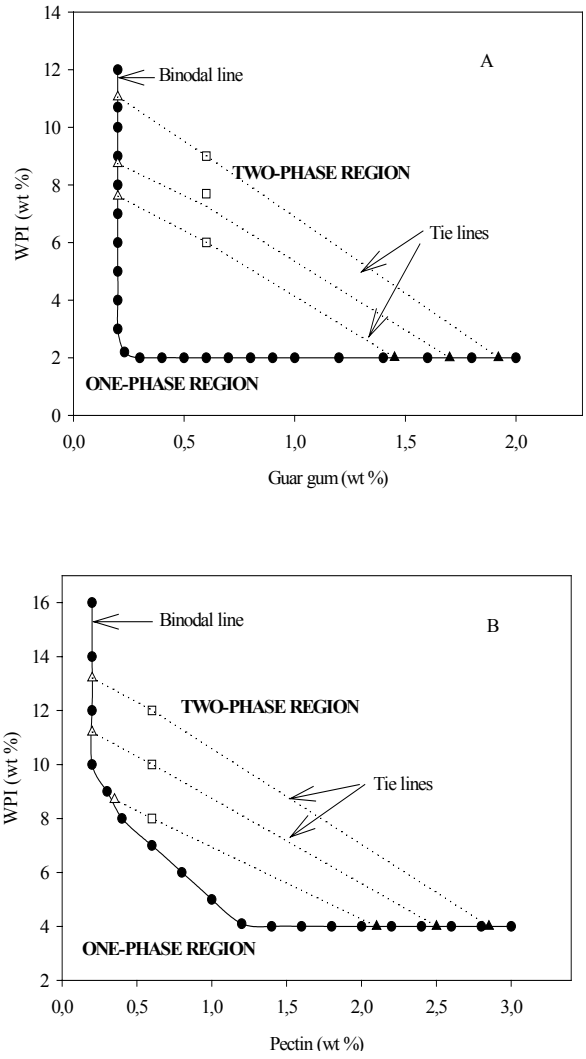


Figure 4.1. Phase diagrams of WPI–guar gum (A) and WPI–pectin (B) mixtures (in potassium phosphate buffer, pH 7.0). Binodal (solid curve, points ●); tie lines (dotted lines); initial mixtures (□); upper phase (△); lower phase (▲).

The mixture of WPI (8.0% w/w) and gum (0.60% w/w pectin or guar gum) exhibited two distinct layers after centrifugation and each phase was analysed for protein and

hydrocolloid gums. Table 4.1 represents the composition of top and bottom phases. Chemical analysis showed that the top phase was high in protein (78.8% of initial protein) and low in pectin (17.0% of initial amount), so-called protein-rich phase, while the bottom phase was polysaccharide-rich phase which had 21.20% of initial protein content and 83.0% of initial pectin content. Distributions of components within two phases were similar with guar gum and pectin. However, protein contents were slightly high in the upper phase (80.5%) and low in the lower phase (19.5%) with guar gum. Phase separation has been shown with pectin–heat denatured whey protein (Kim et al., 2005) and pectin–skim milk (Marazione and de Kruif, 2000). Hemar et al. (2001) have reported that depletion flocculation and thermodynamic incompatibility are the two main mechanisms, which could lead to phase separation in milk protein–polysaccharide mixtures. It has also been reported that guar gum–milk mixtures showed phase separation, which is induced by depletion flocculation due to neutral guar gum, which does not adsorb on milk proteins (Langerdorff et al., 2000). In the case of depletion flocculation, the aggregation of particles is caused by the exclusion of polymer molecules from the space between the particles, resulting in a difference in polymer concentration between the interparticle region and the outside region (Hemar et al., 2001).

Table 4.1. Volume and composition of separated phases resulting from phase separation of solutions containing 8.00 % (w/w) whey protein isolate and 0.60 % (w/w) polysaccharide

	Top phase			Bottom phase		
	Protein (%)[a]	Polysaccharide (%)[b]	Volume (%)	Protein (%)[a]	Polysaccharide (%)[b]	Volume (%)
Pectin	78.8	17.0	72.1	21.2	83.0	27.9
Guar gum	80.5	9.6	74.6	19.5	90.4	25.4

[a] As a weight percent of the total protein fraction in the system before separation
[b] As a weight percent of the total polysaccharide fraction in the system before separation

The phase separation behavior of WPI–*i*-carrageenan mixture was also investigated. A visually observed separation of distinct phases was not detected. The mixtures of WPI and hydrocolloid gum at various concentrations exhibited a homogeneous appearance. A gel formation was observed visually in all mixtures. The gelation of WPI–carrageenan mixtures might be the reason for inhibition of phase separation. A previous study (Hemar et al., 2002) reported a phase separation in the skim milk

powder-κ-carrageenan and milk protein concentrate-κ-carrageenan mixtures at the microscopic level while homogeneous samples at the macroscopic level without phase separation have been detected. They have also reported that mixtures containing whey protein isolate and κ-carrageenan does not show phase separation even at the microscopic level. They have suggested that phase separation may arise from depletion flocculation in these systems.

4.1.2. Phase Separation Behavior of Egg Yolk Granule

Phase separation behavior of egg yolk granule was investigated by mixing protein solution (1.0-4.0% w/w) and pectin (0.01-1.0% w/w) solution and by centrifugating the mixtures after 24 h of storage. In all mixtures, phase separation was observed. Even at very low concentrations of protein and pectin, homogeneous solutions were not detected. At the bottom of the test tube, a small amount of yellow precipitate was observed and the amount of this precipitate increased with increasing protein concentration. An opaque, light yellow homogeneous solution was formed at the top of the precipitate. At a high protein concentration (4.0% w/w), a gel formation was observed in mixtures. The reason may be the increasing of protein concentration, which may lead to protein-protein interactions and may enhance gel formation (Fitzsimons et al., 2006). Although the emulsifying properties of egg yolk proteins have been studied by several authors (Aluko et al., 1997; Anton et al., 2000; Anton et al., 2001; Anton ve Gandemer, 1997), there are limited numbers of studies reporting the phase separation behavior of either egg yolk or its components in the presence of polysaccharides. The granules are made of 70.0% high-density lipoproteins (HDL), 16.0% phosvitin and 12.0% low-density lipoproteins (LDL) (Le Denmat et al., 2000). The isoelectric region of apo-LDL isoelectric region is between pH 6.5 and 7.3, and the isoelectric point (pI) of phosvitin is around 4.0, while the isoelectric point of apo-HDL is expected to be in an enlarged neutral region (Le Denmat et al., 2000). At pH < pI, the protein molecules carry net positive charges and at pH > pI, they carry net negative charges (Dickinson and James, 2000). At the isoelectric point, proteins have minimum net charge and solubility. Therefore, at neutral pH (pH 7.0) HDL is expected to be sparingly soluble and can be observed as precipitate at the bottom of test tube. Similarly, some LDL fractions carrying net positive charges may form complexes with pectin and may precipitate at the bottom of the tube. Phosvitin carries a net negative charge at pH 7.0 and an electrostatic repulsion is expected to

occur between pectin and phosvitin. Phosvitin as well as negatively charged LDL fractions may take place in the homogeneous solution above the precipitate.

In granule proteins–pectin mixtures, protein and polysaccharide contents of the top phase were determined after centrifugation and the results were given in Table 4.2. It was observed that most of the initially present protein and polysaccharide collected at the top phase. As the polysaccharide concentration increased protein content of the top phase decreased except for the mixtures initially containing 4.00% (w/w) protein and 0.20% (w/w) polysaccharide.

Table 4.2. Protein and polysaccharide contents of top phase after phase separation in egg yolk granule proteins–pectin systems

Initial protein concentration (%)	Initial pectin concentration (%)	Protein (%)[a]	Pectin (%)[b]
1.0	0.10	79.0	61.0
1.0	0.20	51.0	64.0
1.0	0.40	51.0	70.0
1.0	0.60	51.0	71.0
1.0	0.80	43.0	78.0
1.0	1.00	35.0	84.0
2.0	0.20	71.0	68.0
2.0	0.40	55.0	73.0
2.0	0.60	38.0	74.0
2.0	0.80	34.0	82.0
4.0	0.10	67.0	63.0
4.0	0.20	75.0	65.0

[a] As a weight percent of the total protein fraction in the system before separation
[b] As a weight percent of the total polysaccharide fraction in the system before separation

When the mixtures of egg yolk granule proteins and guar gum system were examined, a phase separation was observed. The top phase was opaque and light yellow, while the bottom phase was opaque and white. A small amount of precipitate was also observed at the bottom of the tube. It was visually detected that the volume of the bottom phase increased (Table 4.3) and it became highly viscous with increasing guar gum concentration. Since guar gum is a non-ionic hydrocolloid gum, an electrostatic interaction with egg yolk granule proteins at pH 7.0 (where granule components carry net negative charges) may not occur. However, incompatibility in egg yolk granule proteins–guar gum system may appear. A study on protein-polysaccharide interactions involving canola protein isolates showed (Uruakpa and

Arntfield, 2005) that neutral polysaccharides such as guar gum tend to be incompatible with canola proteins, which could lead to improved gelation properties. With increasing protein and gum concentration highly viscous solutions eventually leading to gel formation were observed e.g. in mixtures containing 4.0% (w/w) protein. This could be resulted from a high degree of protein-protein interactions at high concentrations and the development of network structure (Kioesseoglou, 2003). When the protein and polysaccharide contents of separated phases were determined, the top phase was identified as protein-rich phase and the bottom phase was obtained as polysaccharide-rich phase. The protein content at the top phase was high at low gum concentration whereas it decreased as the gum concentration was increased. In samples containing originally 1.0 (w/w) protein, the top phase exhibited 72.6% protein with 0.10% (w/w) guar gum concentration, while it contained 95.0% protein with 0.01% (w/w) guar gum concentration. This result can be explained by incompatibility and may suggest that granule proteins interact with guar gum although it is non-ionic. Thermodynamic incompatibility between the protein and polysaccharide generally occurs because of the repulsive electrostatic interactions (Martinez et al., 2005) but it was also reported that thermodynamic incompatibility can arise in conditions when the protein is in the presence of a neutral nonadsorbed polysaccharide or of an anionic polysaccharide bearing a charge of the same sign as the protein (close to neutrality) (Doublier et al., 2000). Increasing of gum concentration decreased the volume fraction of the top phase. At low gum concentrations, the phase volume occupied by guar gum was low. The volume occupied by guar gum increased with increase in hydrocolloid concentration. It has been reported that this phenomenon resulted from increase in osmotic pressure of hydrocolloid, which induced flocculation of proteins (Marozione and de Kruif 2000).

4.1.3. Phase Separation Behavior of Egg Yolk Plasma

Egg yolk plasma–pectin mixtures were prepared at varying the protein contents (from 1.0 to 10.0% w/w) and polysaccharide contents (from 0.0 to 1.0% w/w). After centrifugation, homogeneous and light yellow solutions were obtained without any sign of phase separation. Egg yolk plasma is composed of 85.0% low-density lipoproteins (LDL) and 15.0% livetins (Le Denmat et al., 2000). Apo-LDL isoelectric region is between pH 6.5 and 7.3, the isoelectric point of livetins varies between pH 4.3 and 5.5. Above these defined pH values both LDL and livetins carry

net negative charges. In this case two types of interactions may take place. Firstly, an electrostatic repulsion may occur between two biopolymers, pectin and protein. Secondly, when both biopolymers carry a net negative charge an electrostatic interaction occurs between anionic polysaccharide and positively charged local patches on the protein (Dickinson, 2003). Even in the presence of these interactions, protein and polysaccharide could homogeneously distribute in the solution (Dickinson, 2003) and therefore, no phase separation was observed. However, a phase separation was observed in egg yolk plasma proteins-guar gum mixtures (Figure 4.2). The phase diagram showed two distinct regions. At relatively low plasma (0.50% w/w) and guar gum concentrations (0.01% w/w) the solutions formed a single homogeneous mixture of protein and polysaccharide molecules. Above the phase boundary, the solutions separated into two distinct phases. The top phase was opaque and light yellow while the bottom phase was opaque and white.

Table 4.3. Volume and composition of top phase resulting from phase separation of egg yolk granule proteins–guar gum systems

Initial protein conc. (%)	Initial guar gum conc. (%)	Protein (%)[a]	Guar gum (%)[b]	Volume (%)
1.0	0.01	95.0	6.10	88.5
1.0	0.02	88.2	6.10	87.0
1.0	0.04	77.0	6.30	86.0
1.0	0.06	73.0	6.50	84.5
1.0	0.08	80.0	6.50	84.0
1.0	0.10	72.6	6.80	82.0
2.0	0.01	99.0	6.80	85.0
2.0	0.02	99.0	7.00	85.0
2.0	0.04	96.0	7.10	83.0
2.0	0.06	94.4	6.90	81.0
2.0	0.08	92.0	7.10	80.0
2.0	0.10	88.4	7.10	80.8
4.0	0.01	99.0	9.20	73.0
4.0	0.02	82.3	9.10	73.6
4.0	0.04	75.0	9.20	72.0
4.0	0.06	71.5	9.40	71.0
4.0	0.08	67.0	9.50	70.2
4.0	0.10	67.2	9.50	70.0

[a] As a weight percent of the total protein fraction in the system before separation
[b] As a weight percent of the total polysaccharide fraction in the system before separation

After phase separation protein and guar gum contents of each phase were analyzed and the results were given in Table 4. 4. In mixtures containing initially 2.0% (w/w)

protein, the top phase exhibited 88.8% protein with 0.06% (w/w) guar gum, while it contained 85.0% protein with 0.10% (w/w) guar gum. Both protein content and volume fraction of top phase decreased with increasing gum concentration. Analysis of the composition of these two phases indicated that the top phase was rich in protein and the bottom phase was rich in guar gum. The volume occupied by the top phase was higher than that of bottom phase. The volume of bottom phase increased with increasing gum concentration.

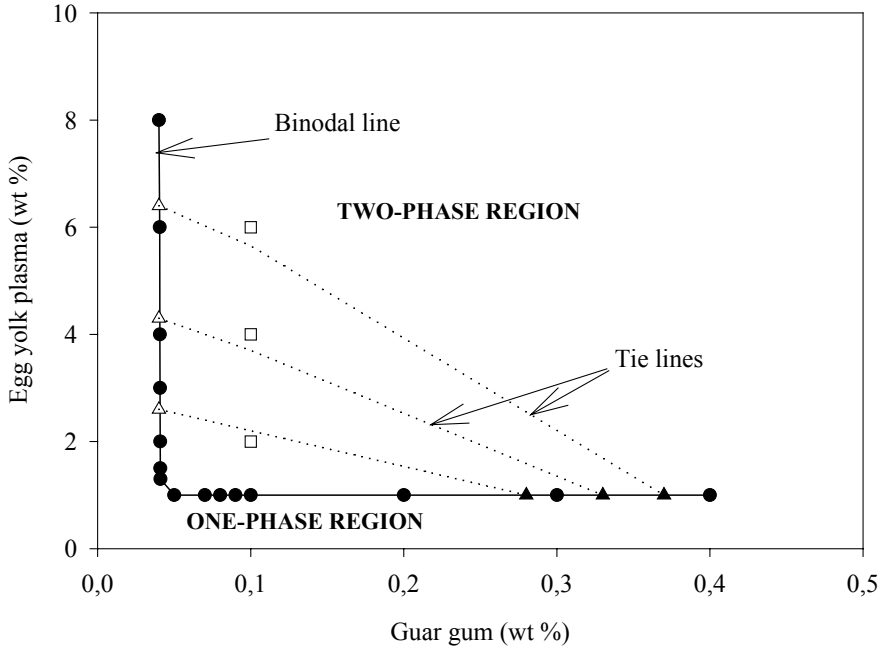


Figure 4.2. Phase diagram of egg yolk plasma proteins–guar gum mixtures (in potassium phosphate buffer, pH 7.0). Binodal (solid curve, points ●); tie lines (dotted lines); initial mixtures (□); upper phase (△); lower phase (▲).

Table 4.4. Volume and composition of separated phases resulting from phase separation of egg yolk plasma proteins–guar gum systems

Protein (%)	Guar gum (%)	Top phase			Bottom phase		
		Protein (%)[a]	Guar gum (%)[b]	Volume (%)	Protein (%)[a]	Guar gum (%)[b]	Volume (%)
2.0	0.06	88.8	29.5	78.0	11.2	70.5	22.0
2.0	0.08	87.0	29.5	78.2	13.0	70.5	21.8
2.0	0.10	85.0	29.8	76.5	15.0	70.2	23.5
4.0	0.06	69.0	9.7	75.0	31.0	90.3	25.0
4.0	0.08	68.8	9.7	74.0	31.2	90.3	26.0
4.0	0.10	67.5	9.8	72.2	32.5	90.2	27.8

[a] As a weight percent of the total protein fraction in the system before separation
[b] As a weight percent of the total polysaccharide fraction in the system before separation

4.1.4. Phase Separation Behavior of Egg White

In all of egg white proteins–pectin mixtures, a trace amount of white precipitate was observed at the bottom of the tube and a white homogeneous solution was observed above this precipitate. Amount of this precipitate increased with increasing pectin concentration. As the concentration of biopolymers increased, the top phase became a highly viscous solution. This could be resulted from gelling ability of egg white (Van der Plancken et al., 2005) and thickening ability of pectin (Dickinson and James, 2000). Egg white constituents other than lysozyme have an isoelectric point (pI) of below 6.0. But lysozyme has a pI of 10.5 and at pH 7.0 lysozyme carries net positive charges while other constituents carry net negative charges and an electrostatic attraction is expected between lysozyme and pectin. Therefore, the precipitation observed at the bottom might be the result of lysozyme–pectin interaction and above this precipitate a homogeneous solution was observed due to the repulsions that take place between other egg white constituents and pectin. Table 4.5 shows the protein and polysaccharide content of the top phase after separation of samples containing different amounts of pectin and egg white proteins. The top phase was characterized by high protein and polysaccharide contents in all mixtures. A reduction was observed in the amount of polysaccharide detected in the top phase when initial pectin concentration increased.

A two-phase system was observed after centrifugation in egg white–guar gum mixtures. The top phase was white and transparent, while the bottom phase was white and opaque. Figure 4.3 illustrates the phase diagram of egg white–guar gum mixtures. The binodal curve (solid line) separates the one-phase region from the two-phase region. The mixtures were stable at compositions below this curve and are unstable at compositions above the curve. Therefore, at low concentrations of egg white and guar gum, homogeneous protein–polysaccharide mixtures were formed. Analysis of the composition of these two phases indicated that the top phase was rich in protein and the bottom phase was rich in guar gum (Table 4.6). In originally 1.0% (w/w) protein containing samples, more than 90.0% of the initially present protein was detected at the top phase after phase separation. The volume occupied by the top phase was higher than that occupied by the bottom phase.

Table 4.5. Protein and polysaccharide contents of top phase after phase separation in egg white proteins–pectin systems

Initial protein conc. (%)	Initial pectin conc. (%)	Protein (%)[a]	Pectin (%)[b]
1.0	0.01	97.0	91.0
1.0	0.02	96.0	91.0
1.0	0.04	94.0	93.5
1.0	0.06	93.0	92.0
1.0	0.08	94.0	95.0
1.0	0.10	91.0	96.0
2.0	0.01	98.0	92.0
2.0	0.02	97.0	91.5
2.0	0.04	98.0	94.0
2.0	0.06	86.0	95.0
2.0	0.08	95.0	97.0
2.0	0.10	91.0	98.0
4.0	0.01	94.0	88.5
4.0	0.02	93.0	90.0
4.0	0.04	91.0	90.0
4.0	0.06	91.0	91.0
4.0	0.08	89.0	92.0
4.0	0.10	87.0	93.6

[a] As a weight percent of the total protein fraction in the system before separation
[b] As a weight percent of the total polysaccharide fraction in the system before separation

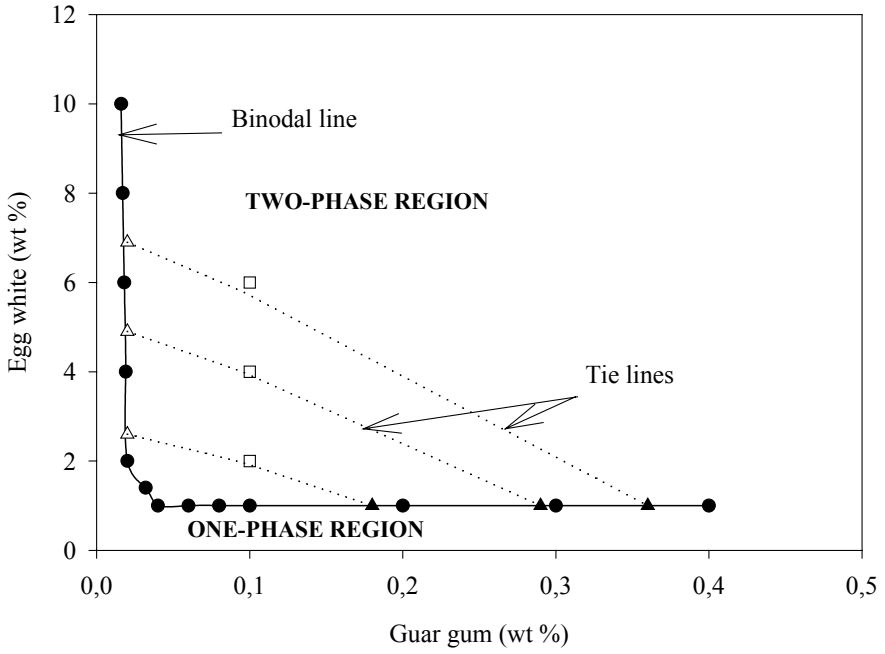


Figure 4.3. Phase diagram of egg white proteins–guar gum mixtures (in potassium phosphate buffer, pH 7.0). Binodal (solid curve, points ●); tie lines (dotted lines); initial mixtures (□); upper phase (△); lower phase (▲).

Table 4.6. Volume and composition of separated phases resulting from phase separation of egg white proteins–guar gum systems

Protein (%)	Guar gum (%)	Top phase			Bottom phase		
		Protein (%)[a]	Guar gum (%)[b]	Volume (%)	Protein (%)[a]	Guar gum (%)[b]	Volume (%)
2.0	0.0.6	93.2	10.6	69.0	6.8	89.4	31.0
2.0	0.08	91.0	11.0	68.0	9.0	89.0	32.0
2.0	0.10	85.8	11.8	66.0	14.2	88.2	34.0
4.0	0.06	82.0	10.2	69.5	18.0	89.8	30.5
4.0	0.08	81.8	10.7	67.0	18.2	89.3	33.0
4.0	0.10	77.1	10.8	63.0	22.9	89.2	37.0
6.0	0.06	75.0	11.2	59.0	25.0	88.8	41.0
6.0	0.08	72.0	11.0	54.0	28.0	89.0	46.0
6.0	0.10	65.6	11.5	57.0	34.4	88.50	43.0

[a] As a weight percent of the total protein fraction in the system before separation
[b] As a weight percent of the total polysaccharide fraction in the system before separation

4. 2. DSC Measurements

Denaturation thermograms of the WPI, egg white, egg yolk granule and egg yolk plasma were obtained by DSC (Table 4.7). The enthalpy of transition (ΔH) was calculated from the area under the peak. Denaturation enthalpies correspond to the loss of favorable intramolecular interactions within the protein molecule. Denaturation temperature (Td) was defined as the temperature at which a local maximum occurs in the excess heat capacity (Welzel, 2002).

The thermograms obtained for the pure WPI solution display one major endothermic peak of 16.09 J/g (ΔH) and a T_d of 77.62 °C. A single peak for the transition of whey protein concentrate has been reported by Boye and Alli (2000). The addition of carrageenan, guar gum and pectin to the WPI solutions caused a shift of peak temperature to 81.51, 80.10 and 83.05 °C, respectively. We concluded that the presence of hydrocolloids increased the thermal stability of WPI. ANOVA results revealed that the addition of hydrocolloids had a significant effect ($p < 0.05$) on denaturation temperature of WPI (Table A.1). The highest thermal stability was observed in the presence of pectin (83.05 °C), whereas the lowest transition temperature was observed in the presence of guar gum (80.10 °C). A significant reduction ($p < 0.05$) was observed in denaturation enthalpy of WPI after addition of hydrocolloids (Table A.2). The lower values suggest that less energy is required for the denaturation. This may arise either from the partial denaturation of WPI prior to

heat treatment due to high charge density of biopolymers or from the aggregation of protein as a result of phase separation (İbanoğlu, 2005).

Table 4.7. The peak temperatures (T_d) and the enthalpy changes (ΔH) for heat denaturation of pure proteins and protein–hydrocolloid mixtures

	Pure Protein		**Pectin**		**Guar gum**		**Carrageenan**	
	T_d (°C)	ΔH (J/g)	T_d (°C)	ΔH (J/g)	T_d (°C)	ΔH (J/g)	T_d (°C)	ΔH(J/g)
WPI	77.62	16.09	83.05	7.89	80.10	7.47	81.51	7.28
EW	84.10	23.30	84.70	23.60	84.60	23.60	84.30	17.80
EYG	85.90	18.50	86.70	17.20	85.40	9.10	86.10	18.70
EYP	86.10	16.60	85.80	11.09	86.40	11.40	86.30	17.20

Egg white was observed to exhibit two main thermograms at temperatures 68.50°C and 84.10°C. While the dominant latter is due to ovalbumin, conalbumin and lysozyme appear as a single peak in the former as concealed by each other (Donovan et al., 1975). The thermograms and the denaturation enthalpies of egg white correlate well with the results obtained by Perez and Pilosof (2004). The transition temperatures of egg white were not significantly ($p > 0.05$) influenced from the presence of pectin and guar gum (Table A.3). However, the presence of *i*-carrageenan reduced the former transition temperature by 5.0°C. The reason why we have examined the effect of *i*-carrageenan was related with the relative effects of carboxylated hydrocolloids (e.g. pectin) and sulfated hydrocolloids (e.g. carrageenan) in protein–polysaccharide systems. It has been stated (Dickinson, 2003) that the positively charged groups on a protein ($-NH_3^+$) attract $-OSO_3^-$ groups more strongly compared with $-CO_2^-$ groups. Therefore, the protein is capable of forming fairly strong complexes with sulfated hydrocolloids, even at neutral or alkaline pH. The decrease in the former transition temperature may result from the reduction in the thermal stability of proteins as a result of complexation with *i*-carrageenan. A lower denaturation enthalpy, that was obtained for egg white–*i*-carrageenan mixture as compared with native egg white and egg white–pectin/guar gum, may give emphasis to this altered heat stability. The residual enthalpy is actually a net value from a combination of endothermic reactions such as the disruption of hydrogen bonds, and exothermic processes, including the break-up of hydrophobic interactions and protein aggregation (van der Plancken et al, 2005). Therefore, an enhanced

aggregation of denatured proteins may cause a reduction in the denaturation enthalpy.

A single peak was observed for each of egg yolk plasma and egg yolk granule. Although granule proteins (HDL and phosvitin) has been demonstrated as being more resistant to heat denaturation and subsequent aggregation (Le Denmat et al. 1999) than plasma proteins in which LDL proteins has been described as heat sensitive (Anton et al., 2001), transition temperatures of both granule and plasma proteins were obtained very close to each other (at temperatures 85.90 and 86.10, respectively). The presence of hydrocolloid gums did not significantly ($p > 0.05$) modify the peak temperatures, however, the residual denaturation enthalpies were influenced (Tables A.5-A.8). Both granule and plasma proteins gave low denaturation enthalpies with guar gum. It has to be remembered that thermal analysis were performed on high concentrations of egg protein–hydrocolloid gums. Preliminary studies showed that there was a phase separation in the mixtures of both biopolymers indicating the availability of thermodynamic incompatibility at the concentrations studied, especially with guar gum. Therefore, the solution concentration of each biopolymer in each phase would be twice the initial concentration. A high concentration and heat effect may result in an exothermic contribution due to aggregation to the overall transition heat.

4.3. Emulsifying Properties

4.3.1. Emulsifying Properties of Whey Protein Isolate

The ability of a protein to form an emulsion is related to its ability to adsorb at the oil-water interface and to stabilize the film (Pearce and Kinsella, 1978). Emulsifying activity of WPI–pectin, WPI–guar gum systems were given in terms of emulsion activity index (EAI) in Figure 4.4. The emulsions were prepared at WPI and hydrocolloid concentrations, which were below the phase separation threshold concentration. The addition of pectin increased EAI of WPI. EAI was measured as 1.90 m^2/mg in the absence of hydrocolloids while it was detected as 1.95 m^2/mg and 2.38 m^2/mg after adding 0.01% (w/v) and 0.50% (w/v) pectin, respectively. Similarly, EAI of emulsions prepared with WPI–guar gum increased as compared to EAI of WPI alone. Statistical analysis showed that there was a significant difference

($p < 0.05$) among the EAI of emulsions stabilized with 0.01, 0.05, 0.10, 0.20 and 0.50% (w/v) hydrocolloids (Tables A.9-A.10). The change in EAI values with an increase in hydrocolloid concentration was much higher in emulsions containing guar gum than those containing pectin.

Figures 4.5 and 4.6 illustrate the emulsion stability index of WPI in the absence and presence of pectin (Figure 4.5) and guar gum (Figure 4.6). Emulsion stability index (ESI) decreased rapidly for the first 30 minutes and then it was slowed down. Reduction was retarded at high polysaccharide concentrations (≥0.10% w/v). This was more obvious in the emulsions containing guar gum and stable emulsions were prepared at high concentrations (Figure 4.6). As the concentration of hydrocolloid gums increased, ESI increased and the highest value was measured in emulsions containing 0.50% (w/v) hydrocolloid. It was determined that ESI at each hydrocolloid concentration was significantly different ($p < 0.05$) (Table A.11). Mishra et al., (2001) described that in emulsion systems containing both protein and polysaccharide, proteins typically form an adsorbed layer at the oil-water interface, whereas, hydrophilic polysaccharides possibly form a secondary thick layer which enhances the steric stabilizing properties on the outside of protein coated droplets. Also, a high hydrocolloid concentration increases the viscosity and restricts the movement of oil droplets, and thus, increases stability (Dickinson et al., 1998; Huang et al.; Cui, 2001). It has been detected (Uruakpa and Arntfield 2005) that dispersed systems such as whey protein–hydrocolloid-stabilized emulsions could be more stable by improving the surface film via incompatibility and complexing of the surface active protein with polysaccharides. The incompatibility in commercial canola protein-guar gum systems, which occurs at pH>pI, has been responsible for the enhancement in emulsifying activity and stability as guar gum level increases, whereas in canola protein-pectin systems, the enhancement of emulsifying properties has arisen from complexing. Tolstoguzov (1991) has observed that the emulsion stability of legumin increased with pectin under conditions of complexing at pH=4.2 and it increased via incompatibility at pH=7.6.

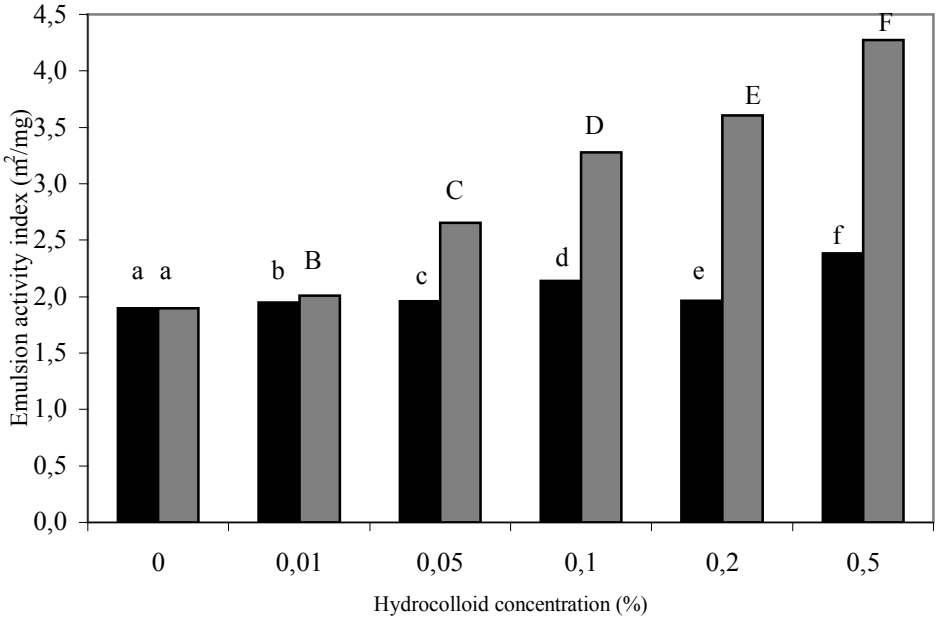


Figure 4.4. Effect of pectin and guar gum on emulsion activities of whey protein stabilised emulsions (*solid bars:* pectin, *dotted bars*: guar gum)
*Different letters (small letters for pectin, capital letters for guar gum) indicate statistical difference at α=0.05 level between hydrocolloid concentration

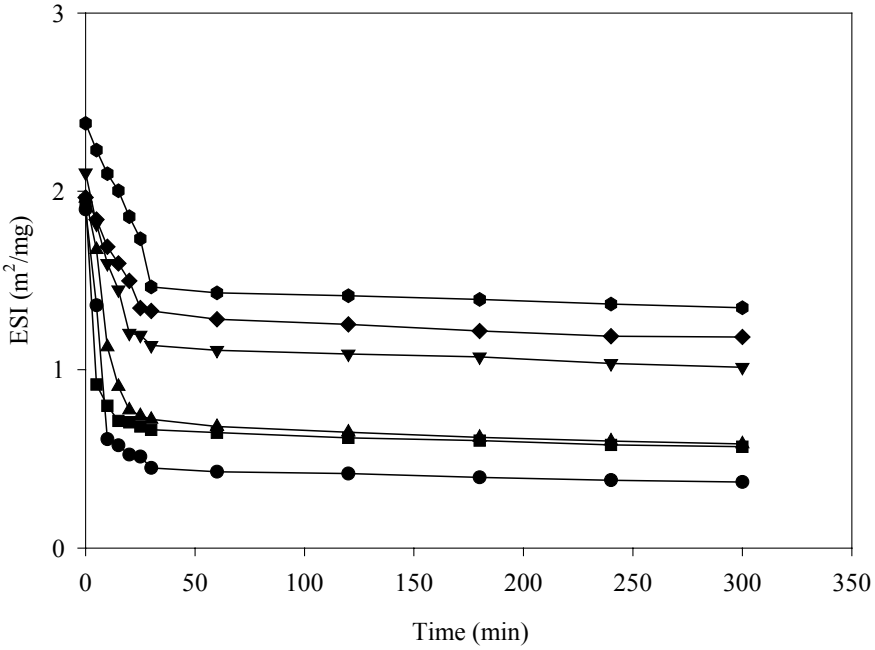


Figure 4.5. Emulsion stability of whey protein isolate in the presence of pectin (● 0.00%, ■ 0.01%, ▲ 0.05%, ▼ 0.10%, ◆ 0.20%, ⬢ 0.50% (w/v))

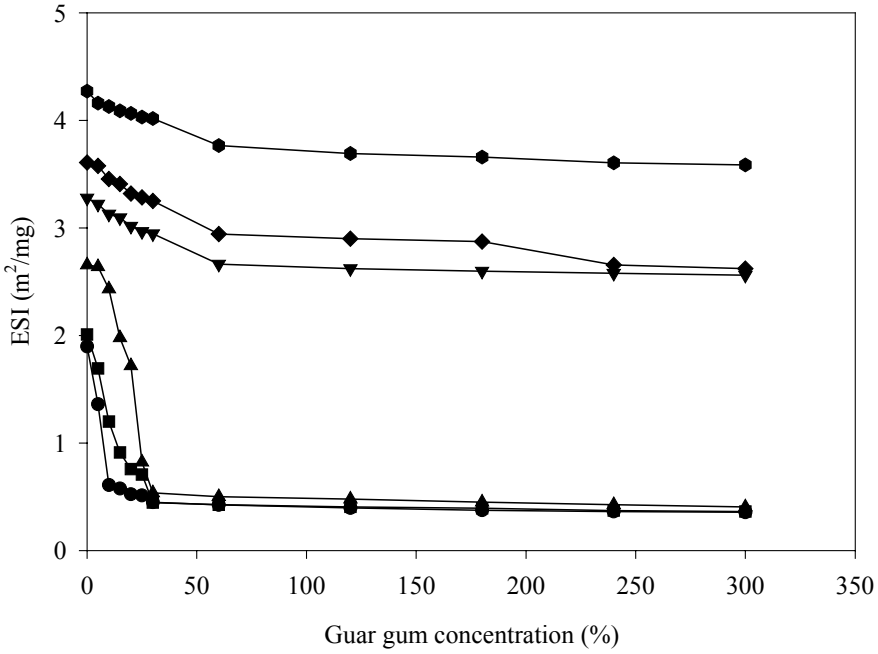


Figure 4.6. Emulsion stability of whey protein isolate in the presence of guar gum (● 0.00%, ■0.01%, ▲ 0.05%, ▼ 0.10%, ◆ 0.20%, ⬢ 0.50% (w/v))

The term creaming denotes the gravitational process in which dispersed particles, being less dense than the suspending fluid, tend to rise to the top of the sample, forming a more concentrated region (the cream). Creaming is a sign of emulsion instability and subsequent break down (Pinfield et al., 1997). Creaming behaviour of whey protein isolate in the absence and presence of pectin or guar gum was given in Figures 4.7 and 4.8. Cream separation proceeded fast for the first 2 h and then gradually slowed down and reached at the plateu value for the emulsion stabilized by WPI alone. A similar trend was obtained in emulsions stabilized by WPI–pectin or WPI–guar gum at low gum concentrations. (≤0.10% w/v pectin and ≤0.01% w/v guar gum). However the creaming rate was retarded when the gum concentration was increased. While 60.0% creaming was observed after 5 h standing of WPI-stabilized emulsion, creaming was measured as 45.0%, 36.0% and 12.0% for emulsions containing 0.10%, 0.20% and 0.50% (w/v) pectin, respectively.

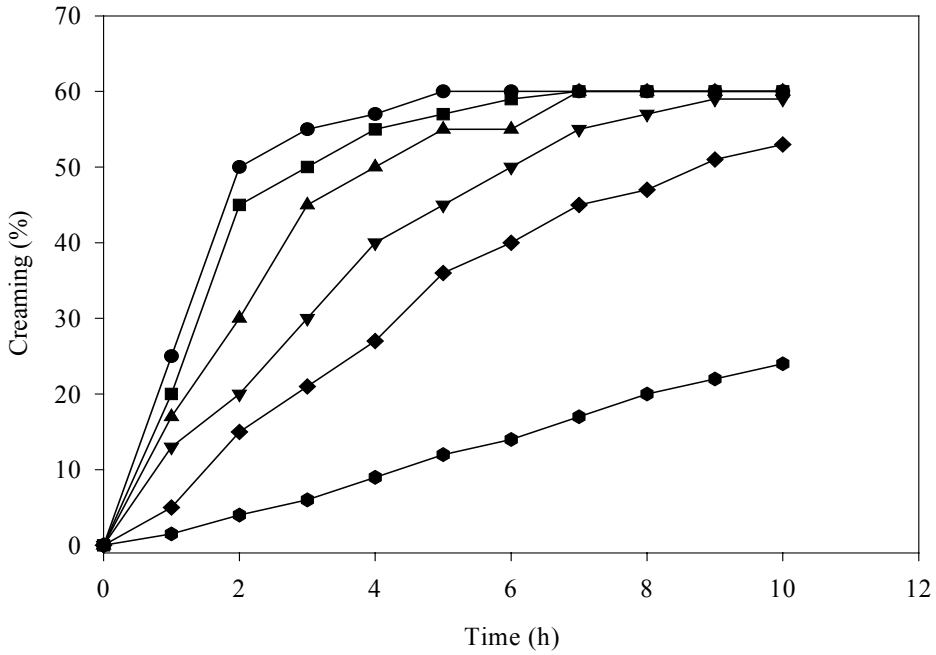


Figure 4.7. Creaming behavior of whey protein isolate in the presence of pectin (● 0.00%, ■0.01%, ▲ 0.05%, ▼ 0.10%, ◆ 0.20%, ⬢ 0.50% (w/v))

A dramatic change was observed in the creaming behaviour when the guar gum concentration was increased from 0.01 to 0.05% (w/v). The change of concentration from 0.01 to 0.10% (w/v) reduced the creaming approximately by 60.0% (percent creaming from 60.0% to 22.0%). The percentage of creaming was reduced to 3.5% at 0.2% (w/v) pectin concentration. The creaming was not detected in the presence of 0.50% (w/v) guar gum within the experimental time scale (after 24 h). This may result from the gel formation in the protein–gum system at high gum concentrations. Gelation may retard serum separation and increased emulsion stability against creaming. The stability of emulsions can be increased by using high-molecular weight polysaccharides, which keep the droplets apart after formation and thus, protect them against creaming, flocculation and coalescence (Akthar and Dickinson 2003).

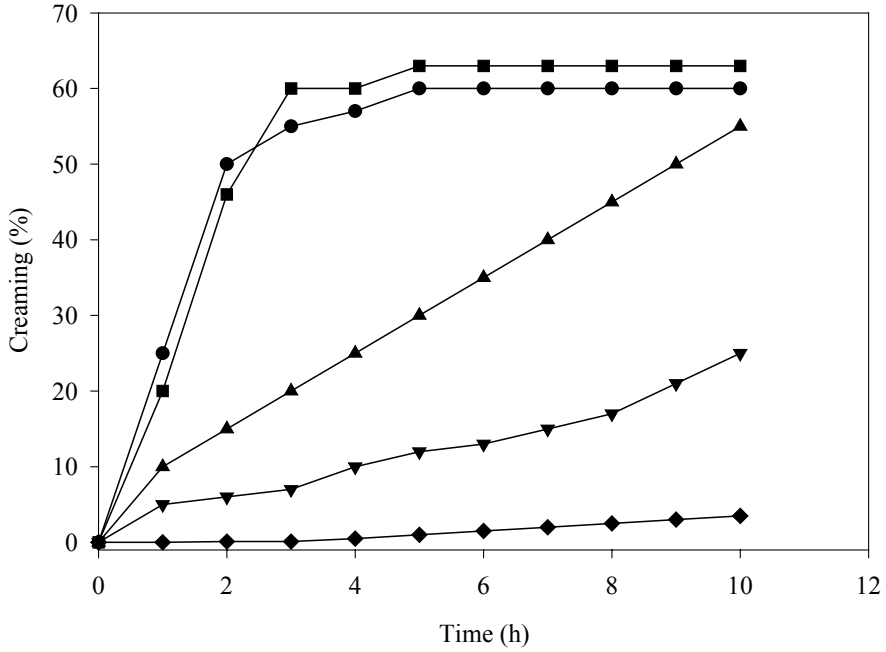


Figure 4.8. Creaming behavior of whey protein isolate in the presence of guar gum (●0.00%, ■0.01%, ▲ 0.05%, ▼ 0.10%, ◆ 0.20% (w/v))

4.3.2. Emulsifying Properties of Egg Yolk Granule

Figure 4.9. illustrates the effects of pectin and guar gum on emulsifying activity index (EAI) of egg yolk granule. EAI of egg yolk granule without the addition of hydrocolloids was determined 2.61 m^2/ mg. Addition of pectin cause a change in the emulsion activity. Whereas, addition of 0.01% (w/v) guar gum had very little influence on emulsion activity, increasing concentration of guar gum gradually increased EAI. The highest EAI value was recorded in the presence of 0.50% (w/v)guar gum. There was a statistically significant difference ($p < 0.05$) between different hydrocolloid concentration in terms of EAI both in the presence of pectin and guar gum (Table A.12-13). The effects of pectin and guar gum on emulsion stability of egg yolk granule were shown in Figures 4.10 and 4.11, respectively. In the absence of hydrocolloids emulsion stability rapidly reduced within first 10 minutes and this reduction was continued up to 30^{th} min and after this point only a very small reduction was observed in stability. Emulsions prepared with pectin showed similar trend. Addition of low amounts of pectin (0.01% w/v) caused a very slight change on emulsion stability. However, an improvement was observed in emulsion stability in the presence of a high amount of pectin (0.05-0.50% (w/v)). Stability of emulsions containing 0.01-0.05% (w/v) guar gum rapidly decreased as

shown from Figure 4.11. Increasing gum concentration retarded this reduction and especially when guar gum concentration increased to 0.20 and 0.50% (w/v) more stable emulsions were obtained. This high polysaccharide concentration retarded serum separation and increased stability. This may be due to an increase in viscosity of the continuous phase surrounding the oil droplets restricting their movement and/or to the adsorption/precipitation of the polysaccharide at the oil-water interface causing a reduction in interfacial tension (Huang et al., 2001). ESI of granule stabilised emulsions with 0.01, 0.05, 0.10, 0.20and 0.50% (w/v) pectin/guar gum was significantly different ($p < 0.05$) (Table A.14). The effect was more noticeable for guar gum and stable emulsions were obtained in the presence of guar gum as compared with pectin. This difference might be resulted from higher viscosity of guar gum.

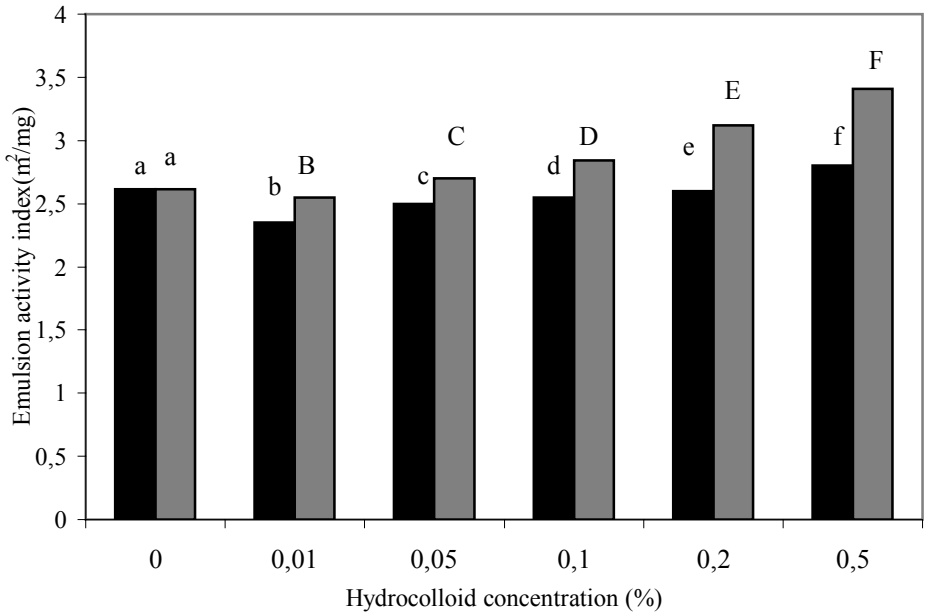


Figure 4.9. Effect of pectin and guar gum on emulsion activities of egg yolk granule stabilized emulsions (*solid bars:* pectin, *dotted bars*: guar gum)
*Different letters (small letters for pectin, capital letters for guar gum) indicate statistical difference at α=0.05 level between hydrocolloid concentration

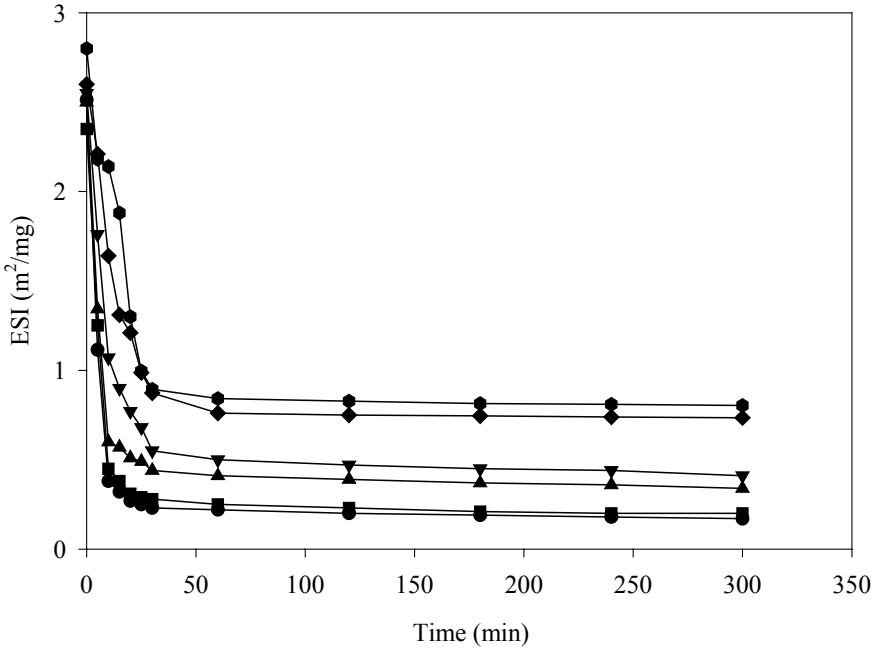


Figure 4.10. Emulsion stability of egg yolk granule in the presence of pectin (● 0.00%, ■ 0.01%, ▲ 0.05%, ▼ 0.10%, ◆ 0.20%, ⬢ 0.50% (w/v))

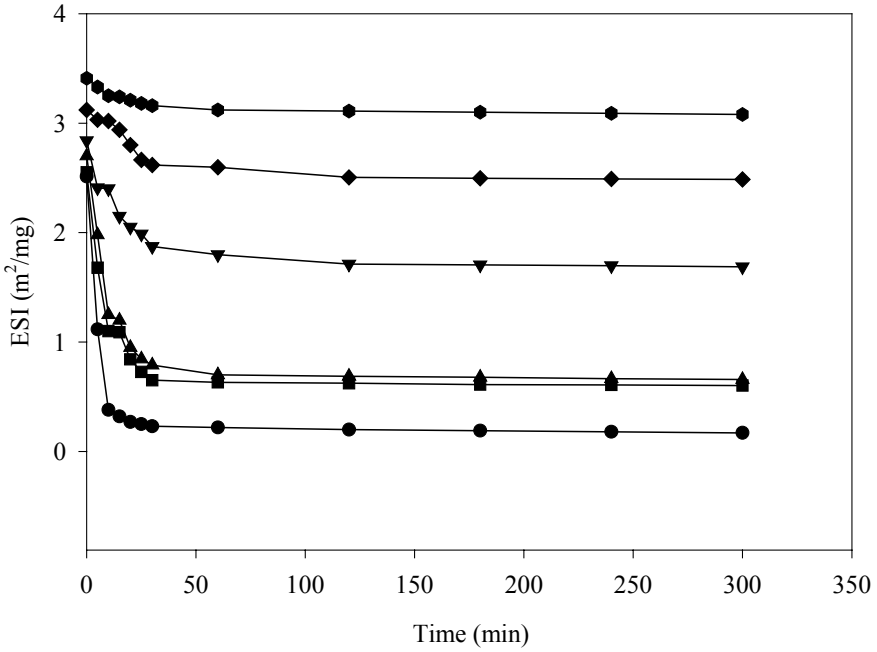


Figure 4.11. Emulsion stability of egg yolk granule in the presence of guar gum (● 0.00%, ■ 0.01%, ▲ 0.05%, ▼ 0.10%, ◆ 0.20%, ⬢ 0.50% (w/v))

Creaming behavior of egg yolk granule with and without hydrocolloids was also determined and the results were given on Figures 4.12 and 4.13. Granule stabilized

emulsions in the absence of hydrocolloids creamed readily within 4 h and creaming percentage was determined as 50.0% and did not change up to 10 h. Low levels of pectin caused a slight change on creaming, but the increase in the amount of pectin gradually decreased creaming. In granule-stabilised emulsions creaming was decreased from 50.0 to 45.0% with the addition of 0.20% (w/v) pectin. This effect was more pronounced in the presence of 0.50% (w/v) pectin. Addition of guar gum slowed down creaming and at a high gum concentration (0.05-0.20% (w/v)) more stable emulsions against creaming were produced. Emulsions containing 0.20% (w/v) guar gum creamed only 10% after 10 h. No creaming was observed when gum concentration increased to 0.50% (w/v) even after 24 h. In the presence of high molecular weight polysaccharides, the nonadsorbed hydrocolloid molecules could be expected to form liquid crystalline lamellae in the continuous water phase and stabilize the emulsion by physically trapping the emulsion droplets in the microgel matrix (Hennock et al., 1984). ANOVA results indicated that there was a significant difference between different hydrocolloid concentrations in terms of creaming percentage of egg yolk granule stabilized emulsions (Table A.15).

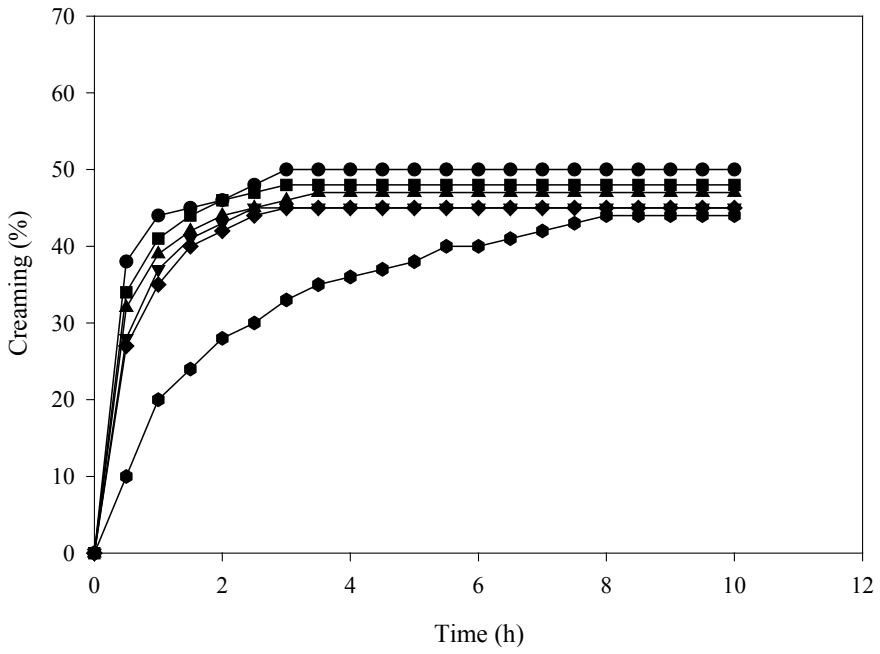


Figure 4.12. Creaming behavior of egg yolk granule in the presence of pectin (● 0.00%, ■0.01%, ▲ 0.05%, ▼ 0.10%, ◆ 0.20%, ⬢ 0.50% (w/v))

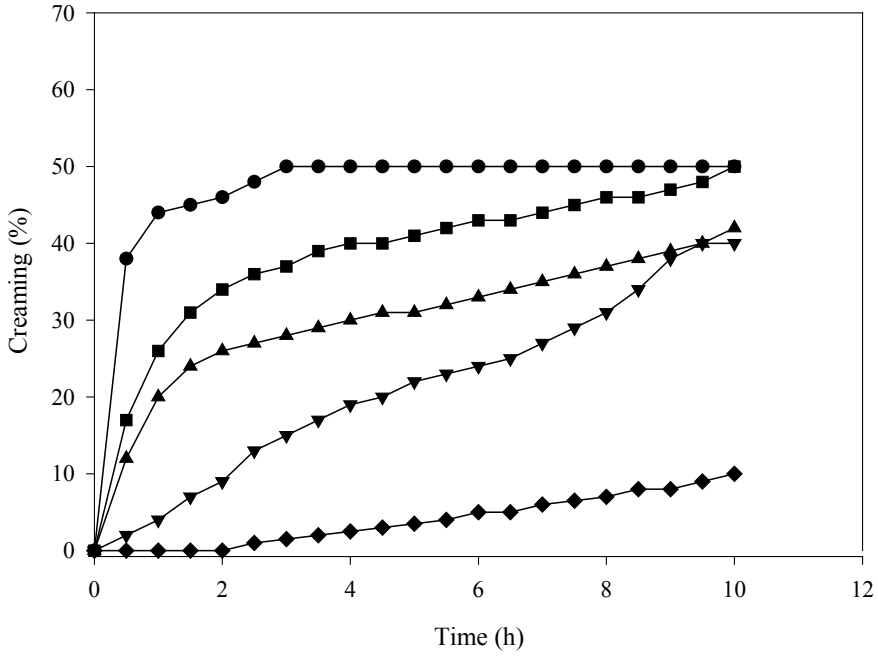


Figure 4.13. Creaming behavior of egg yolk granule in the presence of guar gum (● 0.00%, ■0.01%, ▲ 0.05%, ▼ 0.10%, ◆ 0.20% (w/v))

4.3.3. Emulsifying Properties of Egg Yolk Plasma

The effect of hydrocolloids, pectin and guar gum, on emulsion activity index of egg yolk plasma was given in Figure 4.14. EAI of only plasma-stabilized emulsions was found, as 1.34 m^2/mg. Addition of 0.01% (w/v) of pectin or guar gum caused a very little change on EAI. But it was observed that the increase in the hydrocolloid concentration increased EAI of plasma proteins. With the addition of 0.50% (w/v) pectin and guar gum, EAI values were measured as 1.80 and 2.31 m^2/mg, respectively. EAI of egg yolk plasma stabilized emulsions were observed to be significantly different ($p < 0.05$) at different hydrocolloid concentrations (Table A.16-A.17). Figures 4.15 and 4.16 show the changes in emulsion stability of plasma-stabilized emulsions with and without pectin and guar gum, respectively. Emulsions prepared without hydrocolloids readily broke up and a serum separation was observed at the bottom of container. This explains the rapid reduction in Figures 4.15-4.16 within initial 30 min. Addition of 0.01-0.10% (w/v) pectin did not prevent this phenomenon. When the pectin concentration increased to 0.20% (w/v) serum separation was retarded and stable emulsions were produced. But the most stable emulsion was obtained in the presence of 0.50% (w/v) pectin. On the other hand, guar gum increased the emulsion stability even at very low level (0.05% (w/v)).

Increasing hydrocolloid concentration increases the stability due to restriction of movement of oil droplets with increased viscosity of the continuous phase (Huang et al., 2001). A high hydrocolloid (pectin/guar gum) concentration was observed to cause a significant increase ($p < 0.05$) in ESI (Table A.18). In the present work, when the emulsifying properties of egg yolk granule and plasma were compared, it was observed that egg yolk granule had better emulsifying activity and stability than plasma. A better emulsion stability of granules has been reported by Le Denmat et al. (2000). Granule proteins have been considered to be globular-like proteins and HDL has been indicated as the main constituents, which adsorb at the oil-water interface (Mine, 1998). Thus, they are better in stabilizing emulsions than plasma proteins.

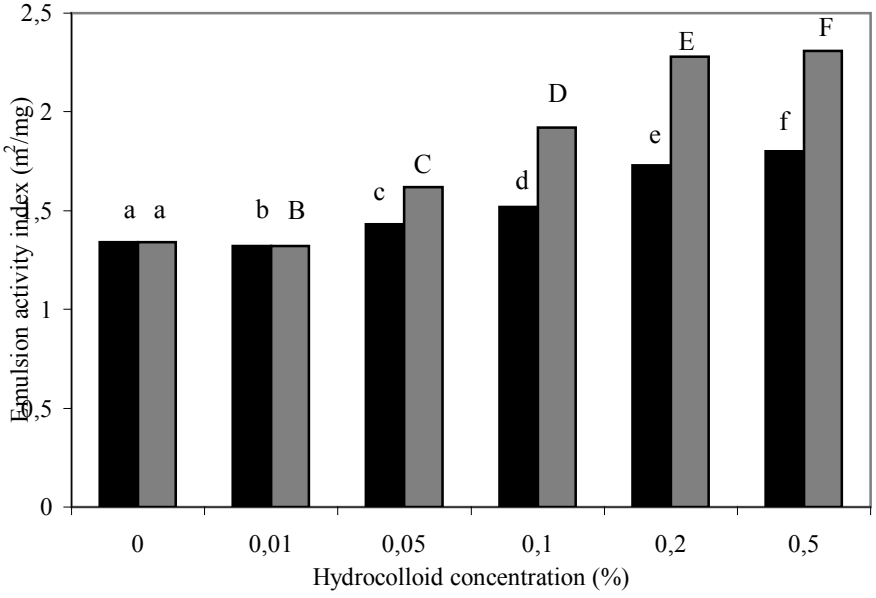


Figure 4.14. Effect of pectin and guar gum on emulsion activities of egg yolk plasma stabilised emulsions (*solid bars:* pectin, *dotted bars*: guar gum)
*Different letters (small letters for pectin, capital letters for guar gum) indicate statistical difference at α=0.05 level between hydrocolloid concentration

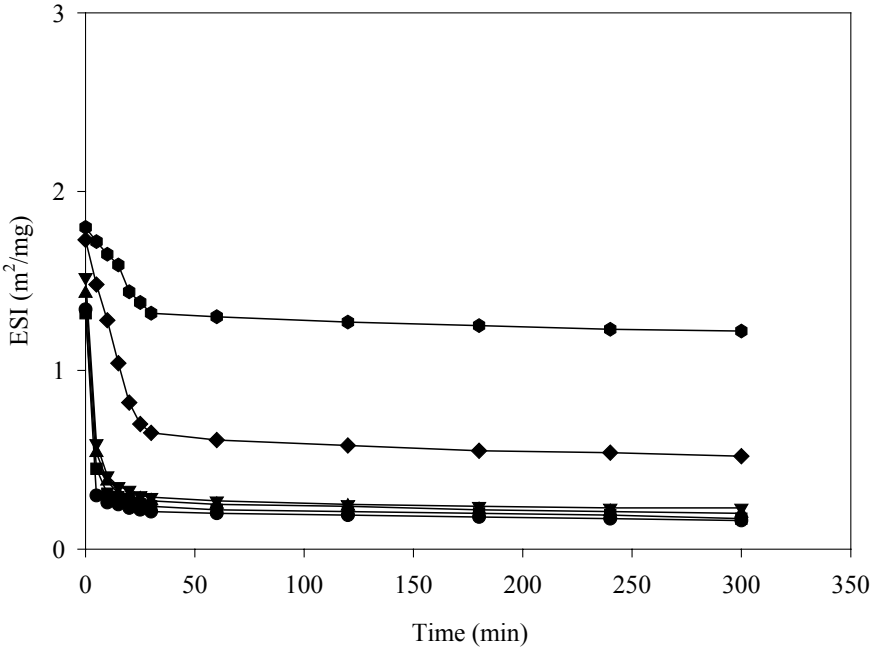


Figure 4.15. Emulsion stability of egg yolk plasma in the presence of pectin (● 0.00%, ■ 0.01%, ▲ 0.05%, ▼ 0.10%, ◆ 0.20%, ⬢ 0.50% (w/v))

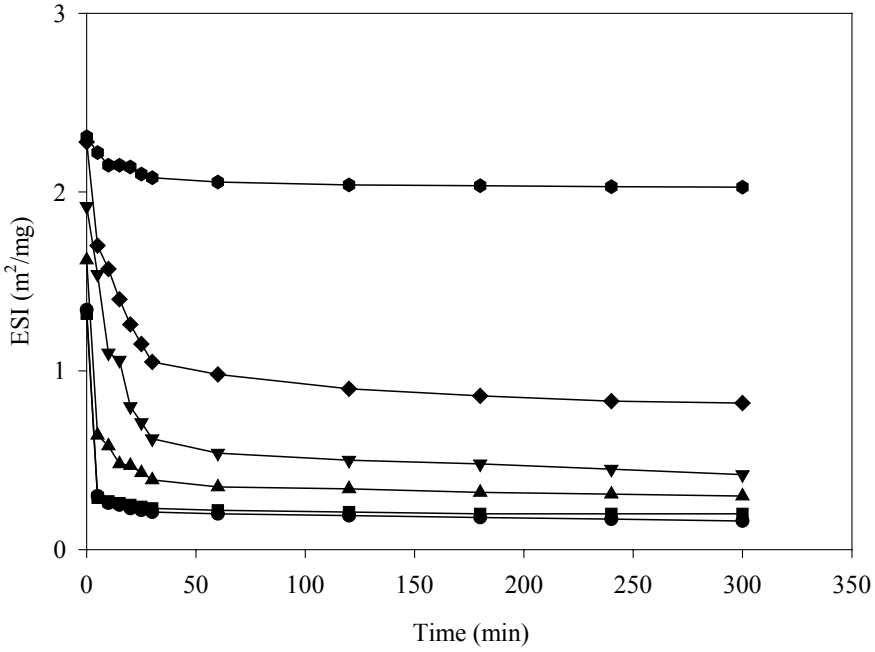


Figure 4.16. Emulsion stability of egg yolk plasma in the presence of guar gum (● 0.00%, ■ 0.01%, ▲ 0.05%, ▼ 0.10%, ◆ 0.20%, ⬢ 0.50% (w/v))

Creaming behavior of egg yolk plasma in the presence of pectin and guar gum was shown in Figure 17 and Figure 18, respectively. In the absence of hydrocolloids, plasma-stabilized emulsions dramatically creamed in 2 h (approximately 50.0%) and

then creaming rate slowed down and after 10 h creaming was measured as 61.0%. Addition of 0.01 to 0.10% (w/v) pectin slightly decreased creaming percentage. But creaming decreased from 61.0 to 54.0 with the addition of 0.20% (w/v) pectin and no creaming was detected with the addition of 0.50% (w/v) pectin. Although addition of guar gum at low levels (0.01-0.05% (w/v)) reduced creaming slightly, higher amounts of guar gum caused a pronounced decrease in creaming percentage. Creaming decreased from 61.0 to 57.0% with the addition of 0.05% (w/v) guar gum. This value was reduced to 39.0% with the addition of 0.20% (w/v) guar gum. In the presence of 0.50% (w/v) guar gum no creaming was detected. It was reported that stability of emulsions could be increased by using high-molecular weight polysaccharides that keep droplets apart after their formation and thus protect them against creaming, flocculation and coalescence (Akthar and Dickinson, 2003). ANOVA results revealed that there was a statistically significant difference ($p < 0.05$) between different hydrocolloid concentrations in terms of the degree of creaming (Table A.19).

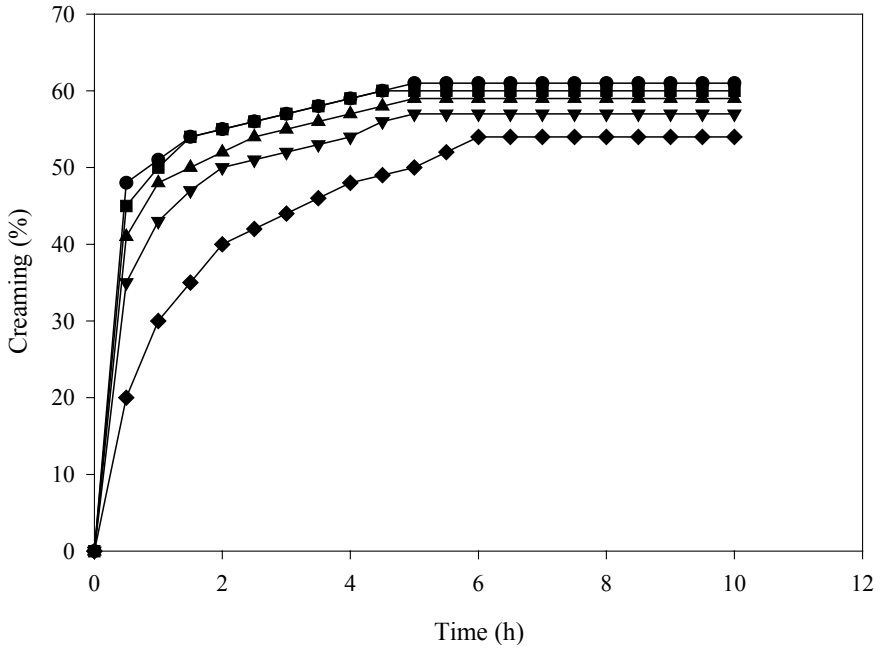


Figure 4.17. Creaming behavior of egg yolk plasma in the presence of pectin (● 0.00%, ■0.01%, ▲ 0.05%, ▼ 0.10%, ◆ 0.20% (w/v))

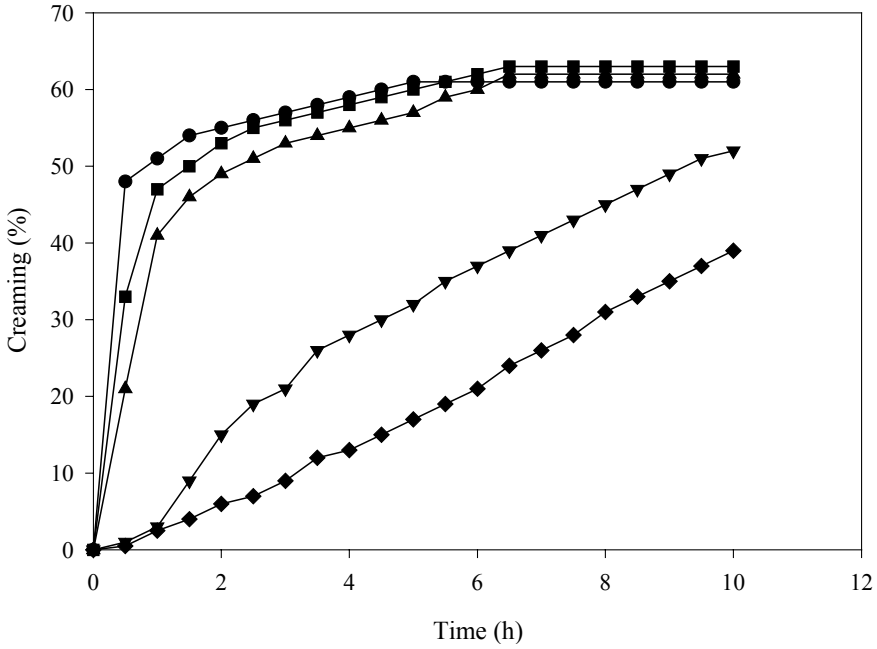


Figure 4.18. Creaming behavior of egg yolk plasma in the presence of guar gum (● 0%, ■0.01%, ▲ 0.05%, ▼ 0.1%, ◆ 0.2% (w/v))

When creaming behaviors of egg yolk granule and egg yolk plasma were compared, egg yolk granule showed higher stability of emulsion against creaming than egg yolk plasma in the absence of polysaccharides. Some researchers have reported that at neutral pH, emulsions made with granules exhibite the same droplet size as those made with plasma (Le Denmat et al., 2000). Concurrently, they show better stability to creaming. It appears that the native structure of granule is disrupted partially as depended on NaCl concentration and the soluble granule constituents become efficient emulsion stabilizers.

4.3.4. Emulsifying Properties of Egg White

Emulsion activity index of egg white in the absence and presence of hydrocolloids was determined spectrophotometrically and the results were shown on Figure 4.19. In the absence of hydrocolloids EAI of egg white was determined as 0.72 m^2/mg. This value increased to 0.79 and 0.87 m^2/mg with the addition of 0.01% (w/v) pectin and guar gum, respectively. Increasing hydrocolloid concentration improved EAI significantly ($p < 0.05$) (Tables A.20-A.21). Maximum EAI was observed in the presence of 0.50% (w/v) pectin/guargum.

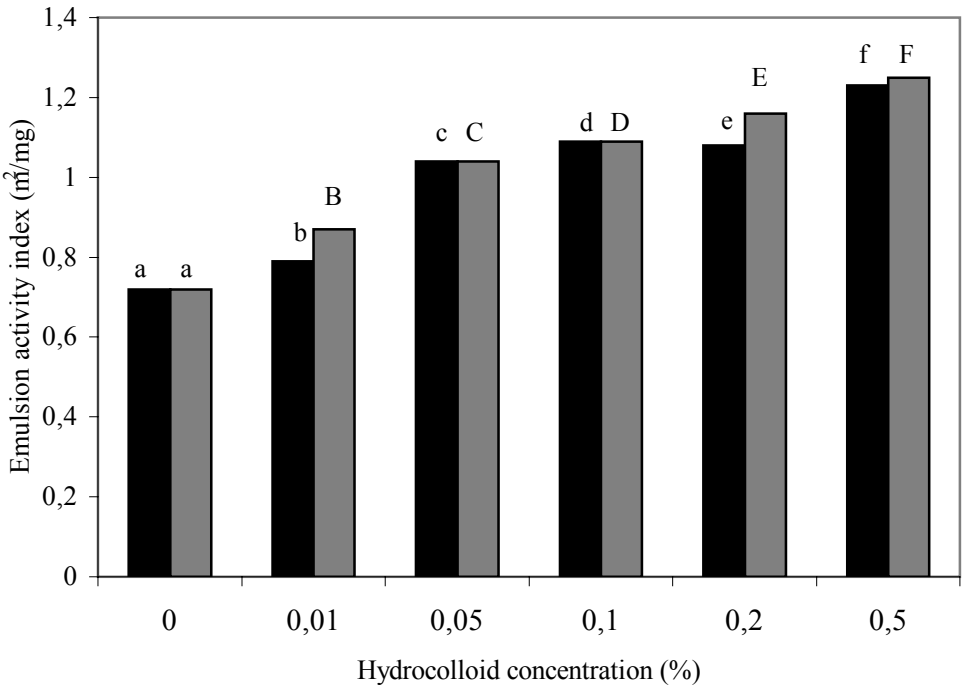


Figure 4.19. Effect of pectin and guar gum on emulsion activities of egg white stabilised emulsions (*solid bars:* pectin, *dotted bars*: guar gum)
*Different letters (small letters for pectin, capital letters for guar gum) indicate statistical difference at α=0.05 level between hydrocolloid concentration

Figures 4.20 and 4.21 illustrate the effect of pectin and guar gum on emulsion stability of egg white stabilized emulsions, respectively. Both in the presence of low amounts (0.01 (w/v)) of pectin and guar gum, a rapid decrease was observed in emulsion stability within first 35 min. During this period serum separation was observed at the bottom of container and emulsions immediately broke up. Serum separation prevented with the increase in the amount of hydrocolloids and emulsion stability increased. In both pectin and guar gum stabilized emulsions, increasing of polysaccharide concentration increased the emulsion stability. It has been reported that this could be resulted from the hydrophobic residues of protein denatured at the oil-water interface may be anchored to the surface of oil droplets in the emulsion and the hydrophilic parts of polysaccharide may be oriented to the water phase, thereby inhibiting the coalescence of oil droplets or water drainage (Huang et al., 2001; Song et al., 2002).

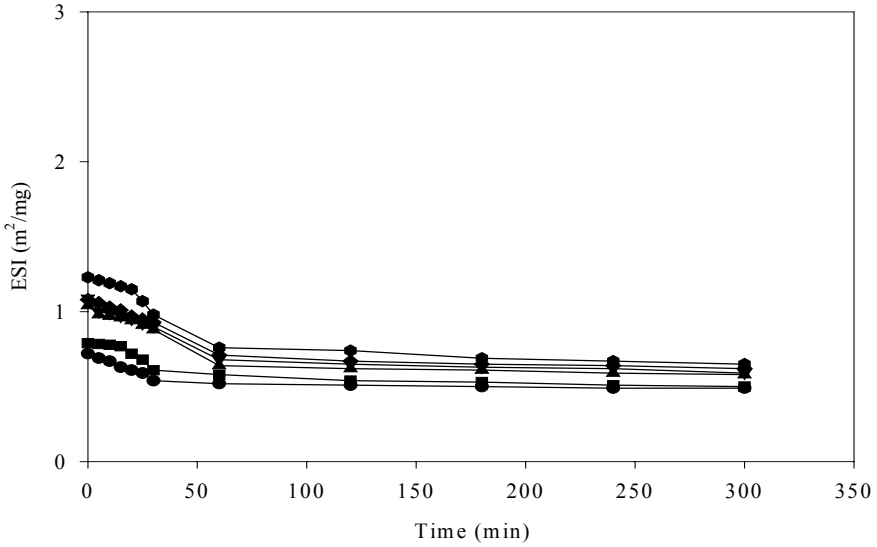


Figure 4.20. Emulsion stability of egg white in the presence of pectin (● 0.00%, ■ 0.01%, ▲ 0.05%, ▼ 0.10%, ◆ 0.20%, ⬢ 0.50% (w/v))

Creaming behavior of egg white in the presence of pectin and guar gum was shown on Figures 4.22 and 4.23, respectively. Creaming was measured as 58.0% after 10 h without pectin and as 57.0 and 54.0% with the addition of 0.01 and 0.05% (w/v) pectin, respectively. A slight change in the degree of creaming was observed at low levels of pectin. On the other hand, when the pectin concentration was increased to 0.50% (w/v), creaming was measured as 33.0%. High levels of pectin increased the stability of emulsion and decreased the serum separation and also creaming. After addition of 0.20% (w/v) guar gum, 3.0% creaming was observed. Increasing of gum concentration decreased the creaming due to restriction of movement of emulsion droplets. In 0.50% (w/v) guar gum containing emulsion, no serum separation was observed. The gel formation resulted from both gelling ability of egg white and guar gum prevented this separation. Statistical analysis showed that increasing hydrocolloid concentration caused a significant difference ($p < 0.05$) in terms of ESI and the creaming percentage (Tables A.22-A.23).

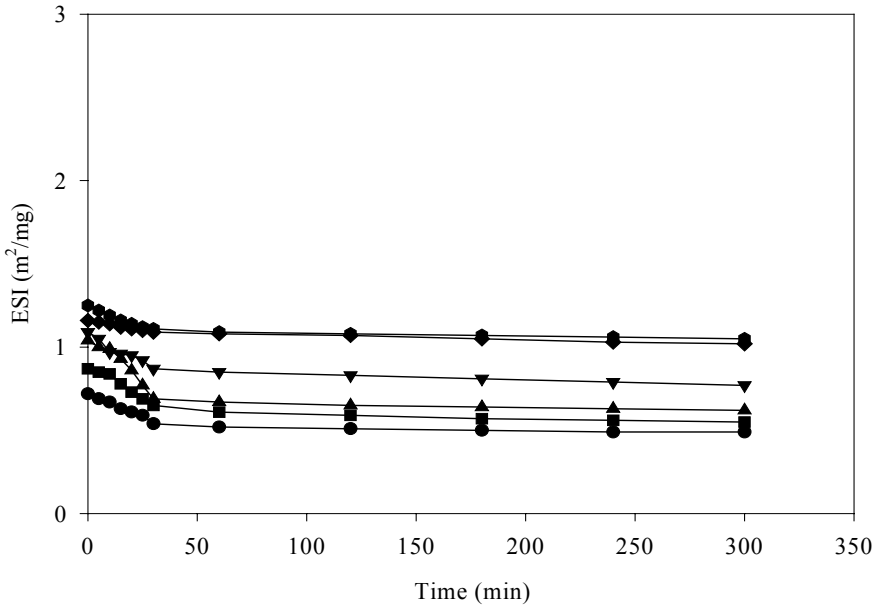


Figure 4. 21. Emulsion stability of egg white in the presence of guar gum (● 0.00%, ■ 0.01%, ▲ 0.05%, ▼ 0.10%, ◆ 0.20%, ⬢ 0.50% (w/v))

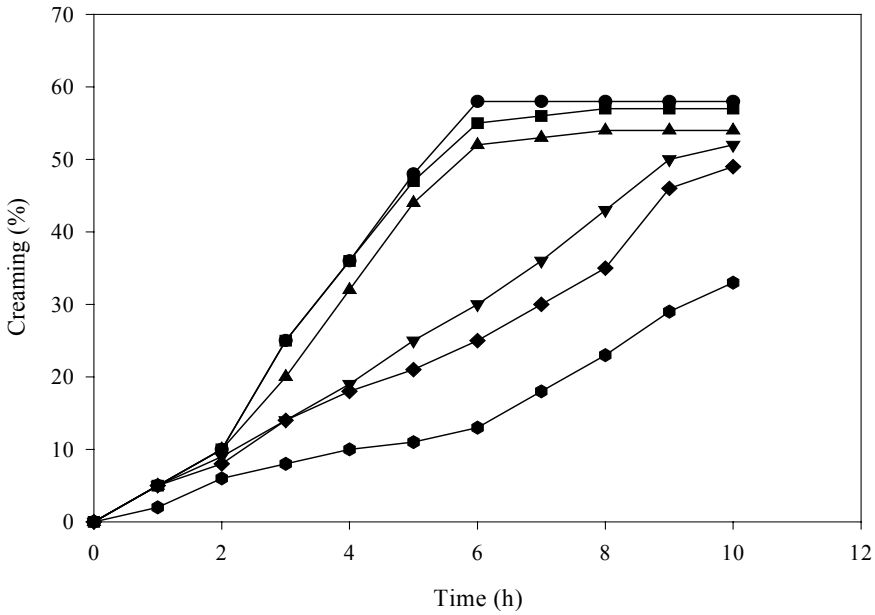


Figure 4.22. Creaming behavior of egg white in the presence of pectin (● 0.00%, ■0.01%, ▲ 0.05%, ▼ 0.10%, ◆ 0.20%, ⬢ 0.50% (w/v))

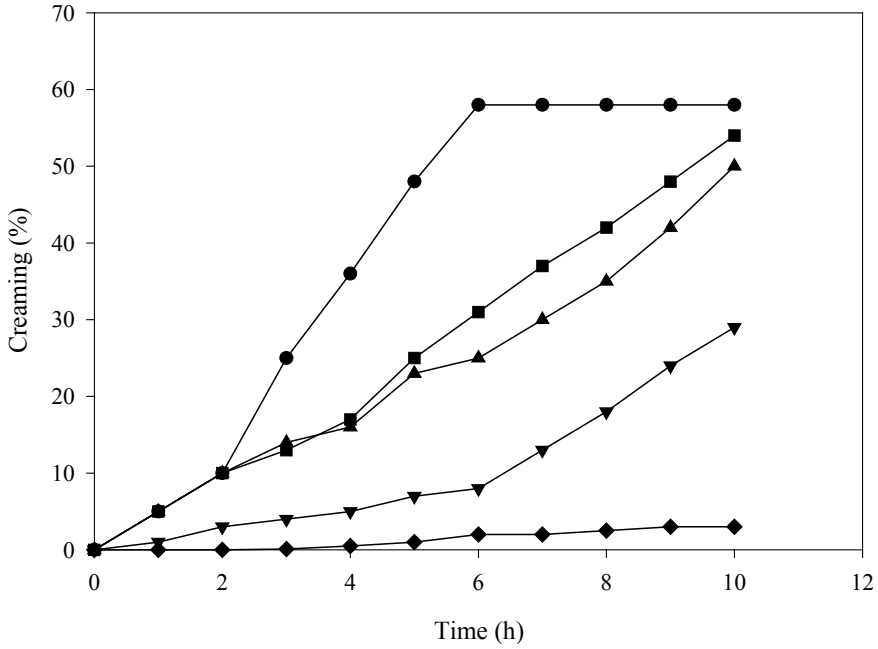


Figure 4.23. Creaming behavior of egg white in the presence of guar gum (● 0.00%, ■0.01%, ▲ 0.05%, ▼ 0.10%, ◆ 0.20% (w/v))

4.4. Foaming Properties

Foams were made by sparging air into protein solutions for a certain time and foam volume and half-life time of foam collapse as foam stability were determined and the results were given in Figures 4.24- 4.27. When foams were initially formed, the air bubbles were spherical and small and as time passed air bubbles became polyhedral and larger bubbles were observed.

As shown from figures, foam volume of egg white in the absence of hydrocolloid was measured as 120.7 cm^3. After addition 0.01% pectin or guar gum, foam volume was determined as 121.7 cm^3. Addition of pectin or guar gum and changes in the hydrocolloid concentration had no significant ($p > 0.05$) effect on foam volume (Tables A.24-A.26). Foam stability of egg white was measured as 64.0 s in the absence of hydrocolloids. Stability increased to 70.0 s and 96.0 s after addition of 0.01% (w/v) pectin and guar gum, respectively. When pectin and guar gum concentration were increased to 0.50%, stability increased to 234.0 and 170.0 s, respectively. ANOVA results indicated that increasing hydrocolloid concentration caused a significant ($p < 0.05$) change on foam stability both for pectin and guar

gum but there was no significant difference between the effect of pectin and guar gum in terms of foam stability (Tables A.27-A.29).

In foams prepared with whey protein isolate, 119.3 cm^3 foam volume was measured. Addition of pectin or guar gum and changing hydrocolloid concentration had no significant ($p > 0.05$) effect on foam volume (Tables A.30-A.32). Foam stability of whey protein isolate was detected as 80.0 s in the absence of hydrocolloids, but this value was recorded as 101.0 s with 0.01% (w/v) pectin addition and increased with increasing of pectin concentration. An increase in the foam stability was also observed with the addition of guar gum. But a low foam stability was obtained with guar gum as compared with pectin. It was determined that there was a significant difference ($p < 0.05$) between guar gum and pectin for 0.00, 0.01, 0.10 and 0.50% (w/v) hydrocolloid concentration in terms of foam stability (Tables A.33-A.35). Previous studies on the effect of polysaccharides on foaming properties of proteins have shown that foam stability is enhanced in the presence of non-surface active polysaccharides (Mishra et al., 2001). Polysaccharides alongside increasing the viscosity of the aqueous phase may influence the viscoelastic behavior and thickness of the adsorbed macromolecular layer reducing the thinning rate of lamella and hence increasing the stability of the foam. Therefore, the increased stability of foams in the presence of polysaccharides would be related to the increased bulk viscosity and surface viscosity (Martinez et al.,2005).

To explain the influence of protein concentration on foaming properties, foams were also prepared at higher protein concentration (0.05% (w/v)). When protein concentration increased, both foam volume and foam stability increased. The increase in the foam volume and stability can be explained by a high rate of diffusion of molecules to the interface at high protein concentration. The foam capacity (foam volume) becomes high, because the number of molecules at the interface is high (Sanchez et al., 2005). At high protein concentration, denser foams were observed. Due to the foam stiffness, some problems were encountered during measurements. More consistent results were obtained in foams containing 0.01% (w/v) protein. For that reason, foaming experiments were performed at low protein concentration (0.01% (w/v)).

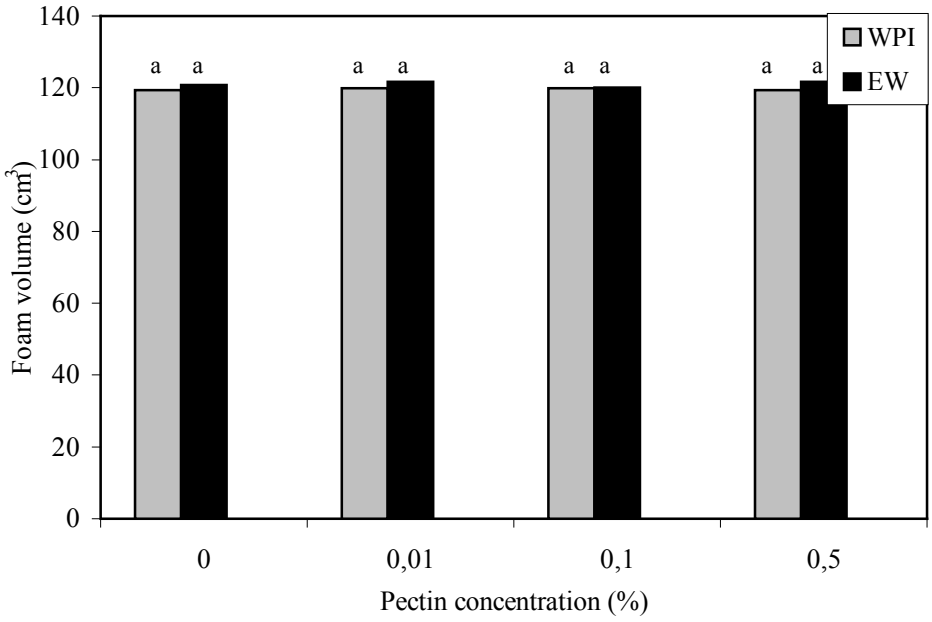


Figure 4. 24. Foam volume of proteins in the absence and presence of pectin
*Small letters indicate no statistical difference at α=0.05 level between hydrocolloid concentration

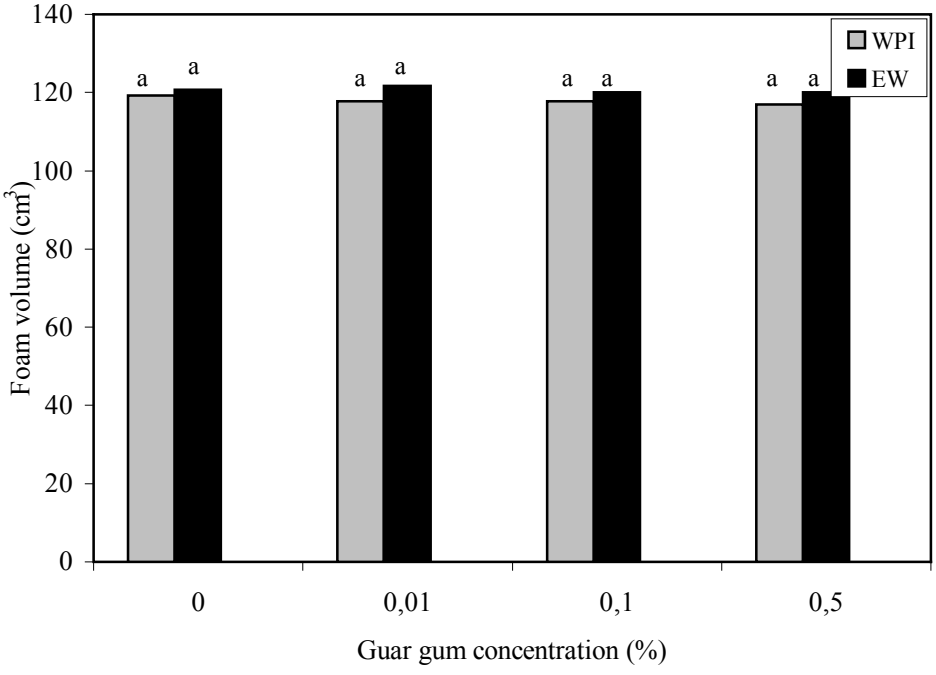


Figure 4.25. Foam volume of proteins in the absence and presence of guar gum
*Small letters indicate no statistical difference at α=0.05 level between hydrocolloid concentration

In additon to whey protein isolate and egg white, foaming properties of both egg yolk plasma and egg yolk granule were also investigated. Transient foams were obtained

with 0.01% (w/v) egg yolk plasma. Foams collapse immediately after turning off the air flow. When protein concentration was increased to 0.05% (w/v), foam volume was recorded as 77.0 cm^3, but it collapsed within a few seconds (9.0 s). Addition of guar gum increased this time to 18.0 s while pectin addition did not affect the stability.

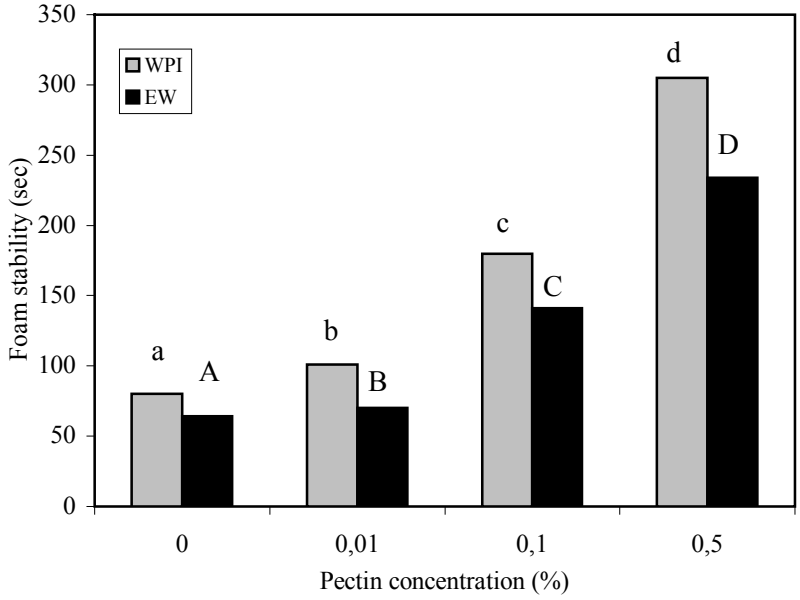


Figure 4.26. Foam stabilities of proteins in the absence and presence of pectin
*Different letters (small letters for WPI, capital letters for EW) indicate statistical difference at α=0.05 level between hydrocolloid concentration

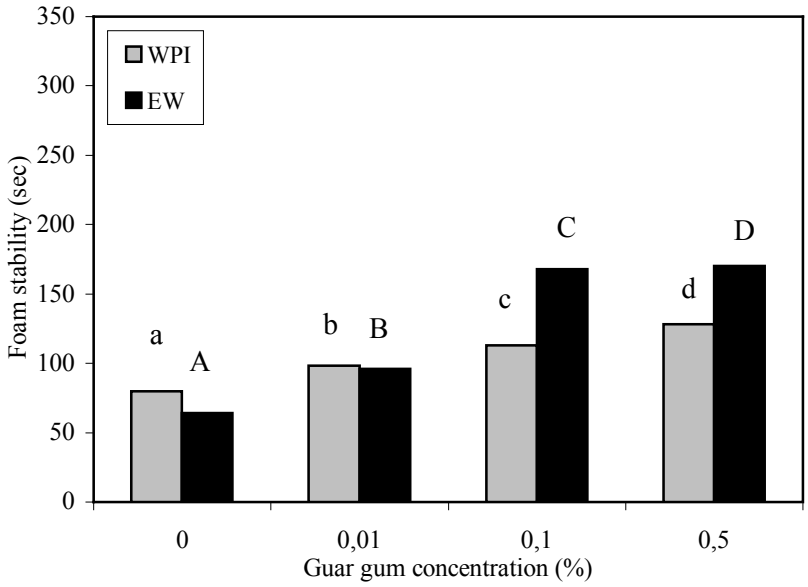


Figure 4.27. Foam stabilities of proteins in the absence and presence of guar gum
*Different letters (small letters for WPI, capital letters for EW) indicate statistical difference at α=0.05 level between hydrocolloid concentration

Foam volume was measured as 19.2 and 96.0 cm^3 in foams prepared from 0.01 and 0.05% (w/v) egg yolk granule, respectively. Similar to egg yolk plasma, granule foams collapsed rapidly. Foam stability of granule (0.01% (w/v)) was detected as 10.0 s. This value increased to 14.0 s and 20.0 s in the presence of 0.10% (w/v) pectin and guar gum, respectively. As a result we concluded that although egg yolk proteins were good emulsifying agent, they have poor foaming ability.

4.5. Heat Treatment

In the present study, investigation of foaming behavior after heat treatment was performed by using egg white due to its high foaming capacity. However, egg yolk proteins (granule and plasma) and whey protein isolate were usually regarded as good emulsifying agents. Therefore, the influence of heat treatment on emulsifying properties was investigated by using egg yolk proteins and whey protein isolate. Heat treatment was applied at low (40-60 °C) and high temperatures (65-80 °C) for a certain time (0.5-3.0 mins). The preliminary experiments showed that the effect of heat treatment was more pronounced at high temperatures for 2.0 mins.

4.5.1. Effects of Heat Treatment on Foaming Properties of Egg White

Foam volumes of heat-treated and nonheat-treated egg white were given in Figure 4.28. Heat treatment was applied in two different ways. Firstly, the stock protein solution was heat-treated and then diluted to the desired concentration to be foamed (A). Secondly, the protein solution was directly exposed to heat treatment at the concentration being foamed (B, C). The foam volume decreased when a stock protein solution (%1.0 (w/v)) was heat-treated and then diluted to the concentration (0.01 % (w/v)) to be foamed (A). As shown from Figure 4.28 relatively high foam volume was observed in the case of B and C after heat. The lowest foam volume was obtained in samples heated at 80°C in all cases. Foam volume was observed to be slightly improved when the concentration was increased to 0.05% (w/v) (C) probably due to large number of molecules in the solution. This shows the importance of the protein concentration during heat treatment. The rate of protein denaturation and aggregation in solution was shown to be strongly dependent on concentration (Le Bon et al., 1999; de la Fuente et al., 2002). This may result in reduction in foam volume in the former treatment due to aggregation.

Figure 4.29 illustrates the effects of pectin and guar gum on the foam volume of heat-treated and untreated egg white. In these experiments the heat treatment was applied to the mixture of protein and polysaccharide at the final concentration being foamed. Exposure to high temperature reduced the foam volume of the egg white-hydrocolloid mixture solution. Heat treatment and hydrocolloid concentration had a significant effect ($p < 0.05$) on foam volume of egg white (Table A.36).

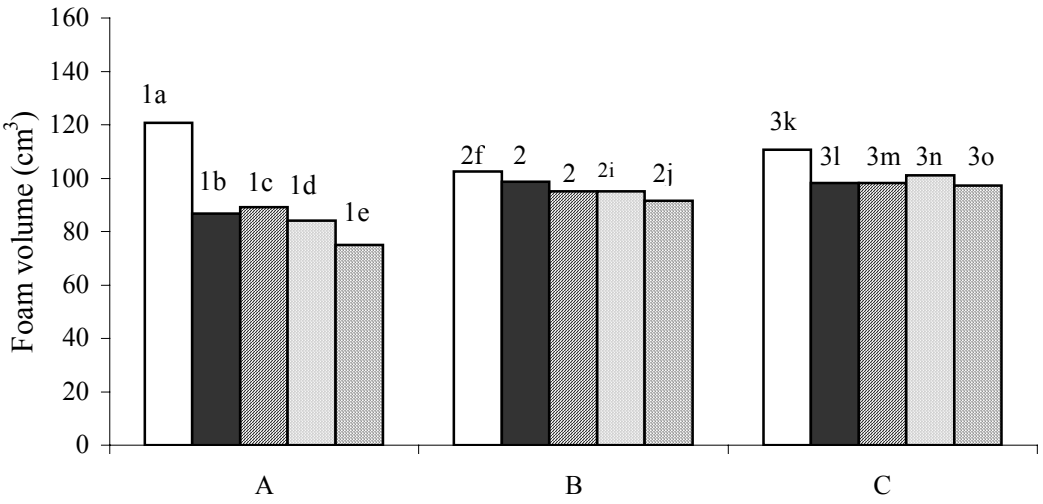


Figure 4.28. Foam volume of egg white, *open bars*: no heat treatment, *solid bars*: 65°C, *dashed bars*: 70°C, *dotted bars*: 75°C, *crossed bars*: 80°C. A. 1.0% egg white solution was heated and diluted to 0.01% (w/v), B. 0.01% (w/v) egg white solution was prepared and heat treatment was applied, C. 0.05% (w/v) egg white solution was prepared and heat treatment was applied
*Different numbers indicate statistical difference at α=0.05 level between treatments
*Different small letters indicate statistical difference at α=0.05 level between temperatures

The influence of heat treatment on foam volume and stability of egg white both in the absence and presence of hydrocolloids were given in Figures 4.30- 4.33. As shown from the figures, foam stability of egg white was observed to be improved with the heat treatment. Foam stability increased as the heating temperature was increased from 65°C to 75°C. However, the foam stability reduced when the protein solution was treated at 80°C. The foam stability of heat-treated samples was also enhanced in the presence of pectin and guar gum. It was observed that the stability of foams increased as the hydrocolloid concentration increased. So, it was concluded that foam stability seemed to be dependent on the hydrocolloid concentration. ANOVA results also indicated that foam stability of egg white was significantly

different ($p < 0.05$) at 65, 70, 75 and 80°C at all hydrocolloid concentration (Table A.37). In the presence of pectin, stable foams were prepared as compared with guar gum. Both bulk and surface rheological properties have been shown to impact foam destabilization (Kloek et al., 2001). Polysaccharides increase the viscosity of aqueous phase and may influence the viscoelastic character and thickness of the adsorbed macromolecular layer reducing the thinning rate of lamella and hence increasing the stability of foam (Dickinson and İzgi, 1996). Therefore, the increased stability of egg white foams in the presence of hydrocolloids would be related to the increased bulk viscosity and formation of strong elastic film as a consequence of protein-polysaccharide interaction at the interface (Martinez et al, 2005). The enhancement of egg white-pectin foam stability with heat treatment may be attributed to increased aggregation of the interfacial film, which has been related to enhanced resistance to foam collapse (Carp et al., 2001).

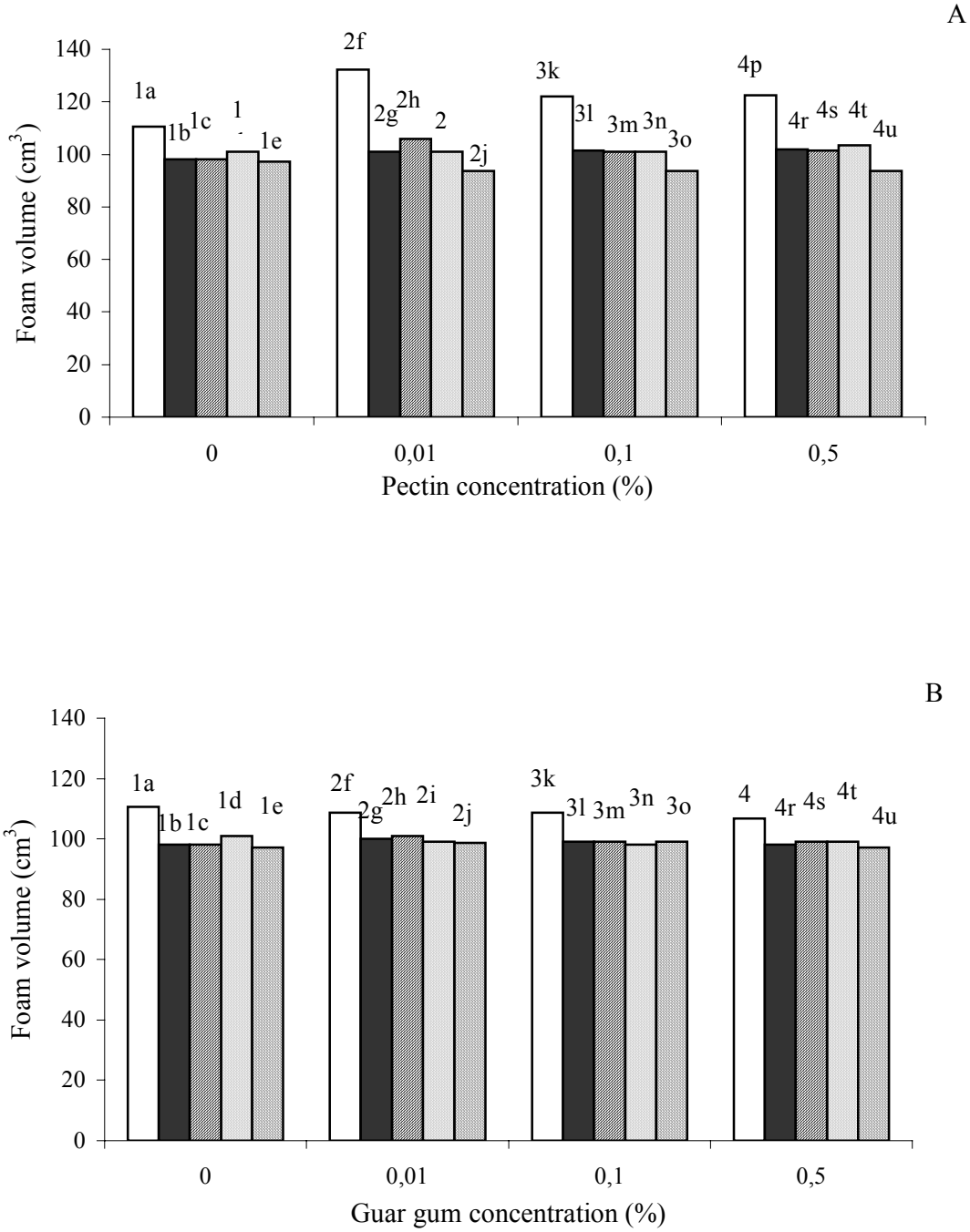


Figure 4.29. Foam volume of non-heated and heat-treated egg white solution (0.05%w/v)) with hydrocolloids, A. pectin, B. guar gum, open *bars*: no heat treatment, *solid bars*: 65°C, *dashed bars*: 70°C, *dotted bars*: 75°C, *crossed bars*: 80°C

*Different numbers indicate statistical difference at α=0.05 level between hydrocolloid concentration

*Different letters indicate statistical difference at α=0.05 level between temperatures

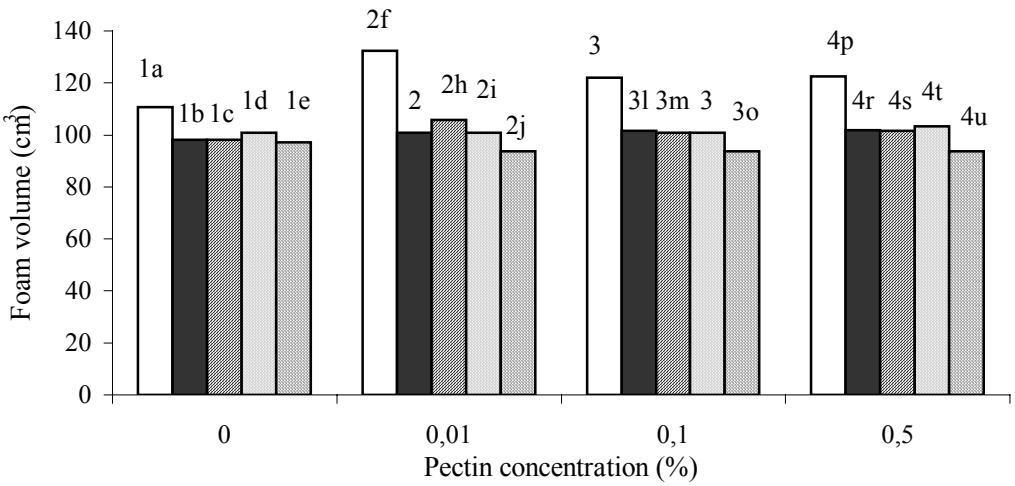


Figure 4.30. Foam volume of 1.0% (w/v) heat treated and then diluted to 0.05% (w/v) egg white solution with pectin, open *bars*: no heat treatment, *solid bars*: 65°C, dashed *bars*: 70°C, *dotted bars*: 75°C, *crossed bars*: 80°C.

*Different numbers indicate statistical difference at α=0.05 level between hydrocolloid concentration

*Different letters indicate statistical difference at α=0.05 level between temperatures

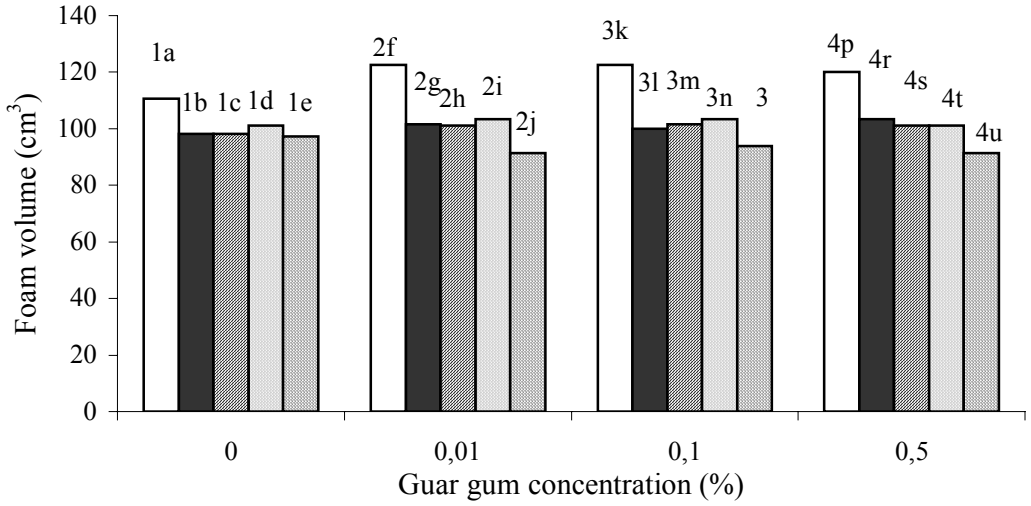


Figure 4.31. Foam volume of 1.0% (w/v) heat treated and then diluted to 0.05% (w/v) egg white solution, with guar gum, open *bars*: no heat treatment, *solid bars*: 65°C, dashed *bars*: 70°C, *dotted bars*: 75°C, *crossed bars*: 80°C

*Different numbers indicate statistical difference at α=0.05 level between hydrocolloid concentration

*Different letters indicate statistical difference at α=0.05 level between temperatures

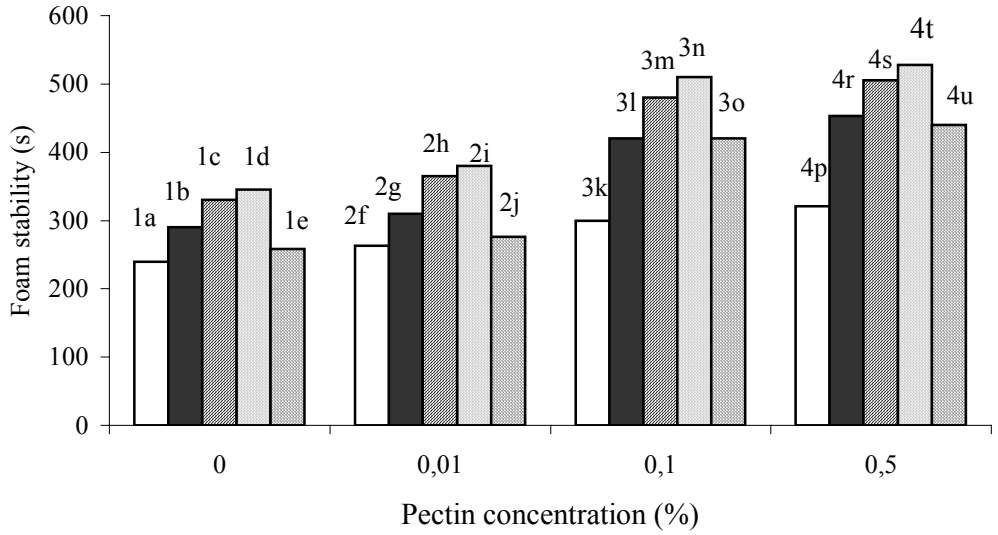


Figure 4.32. Foam stability of non-heated and heat-treated egg white solution (0.05% (w/v)) with pectin, *open bars*: no heat treatment, *solid bars*: 65°C, *dashed bars*: 70°C, *dotted bars*: 75°C, *crossed bars*: 80°C

*Different numbers indicate statistical difference at α=0.05 level between hydrocolloid concentration

*Different letters indicate statistical difference at α=0.05 level between temperatures

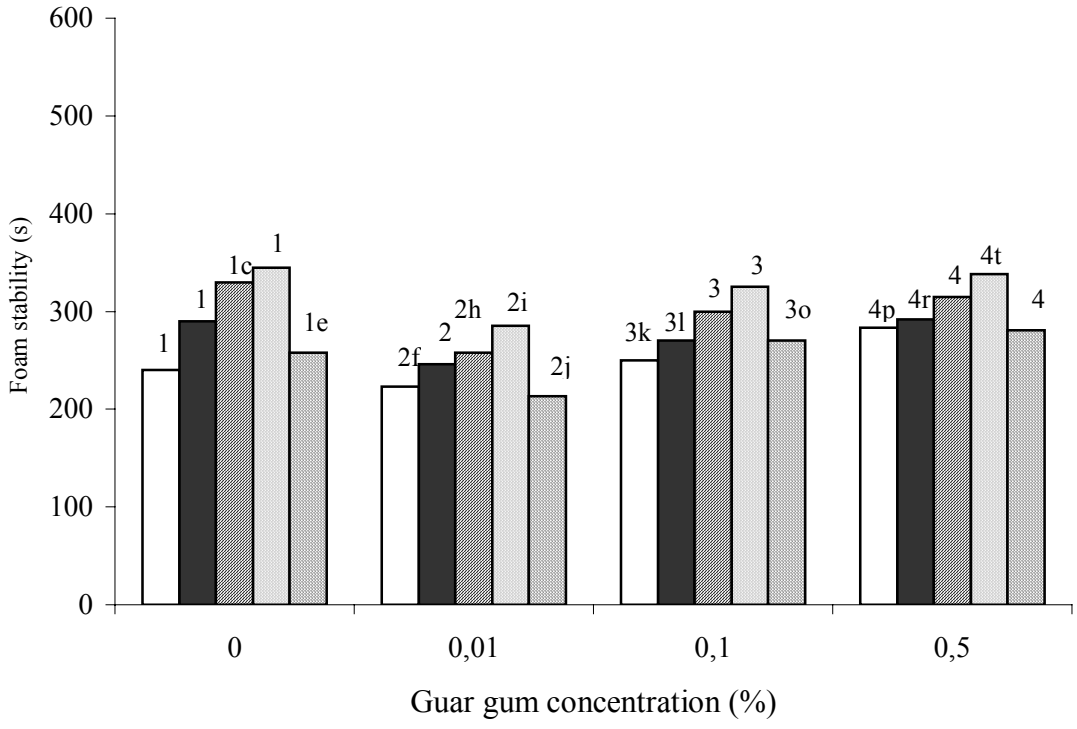


Figure 4.33. Foam stability of non-heated and heat-treated egg white solution (0.05% (w/v)) with guar gum, *open bars*: no heat treatment, *solid bars*: 65°C, *dashed bars*: 70°C, *dotted bars*: 75°C, *crossed bars*: 80°C

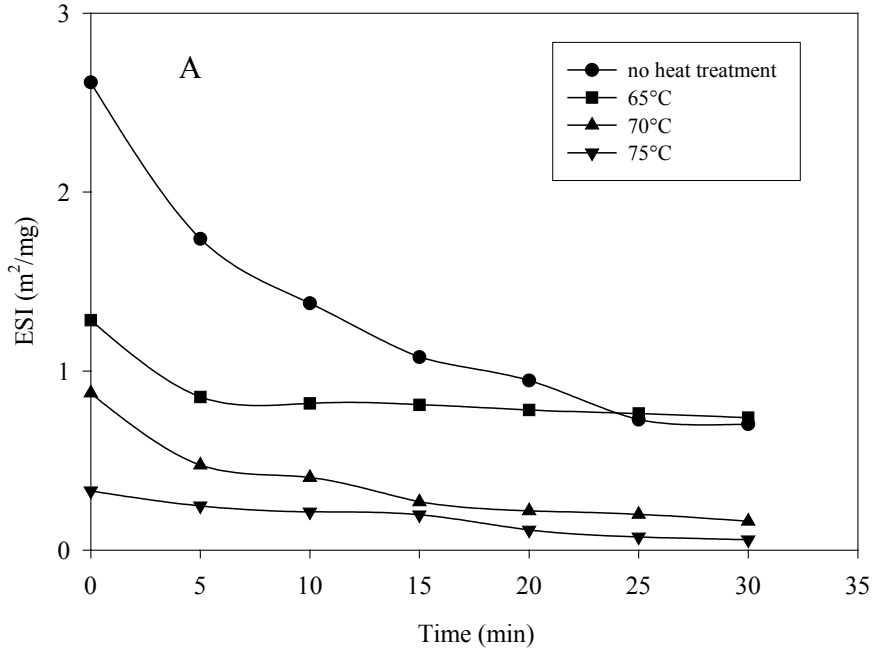
*Different numbers indicate statistical difference at α=0.05 level between hydrocolloid concentration

*Different letters indicate statistical difference at α=0.05 level between temperatures

4.5.2. Effects of Heat Treatment on Emulsifying Properties of Egg Yolk

Egg yolk is generally used as emulsifying agent in food systems. Therefore, in this part of our study the effect of heat treatment on emulsifying properties of egg yolk constituents has been investigated. Heat treatment was applied to protein–hydrocolloid mixture before the emulsion was formed. Emulsion stability index (ESI) of native and heat-treated egg yolk granule (A) and egg yolk plasma (B) were given in Figure 4.34. ESI at time zero has been defined as emulsifying activity and the emulsion stability was examined by measuring the change in ESI with time. Unheated granule had better emulsifying activity than plasma (ESI of 2.61m^2/mg, and 1.34 m^2/mg for granule and plasma, respectively). The emulsion stability of the granule was also observed to be better than that of plasma. Heat treatment significantly ($p < 0.05$) reduced the emulsifying activities and emulsion stabilities of plasma and granule (Tables A.38-A.40). As the temperature was increased emulsifying properties decreased and it was more pronounced with granule proteins than with plasma proteins.

The creaming behavior of non-heated and heat-treated egg yolk plasma and granule was shown in Figures 4.35 and 4.36. After 10 h, higher percentage of creaming was measured for plasma proteins than granule proteins. There was a significant difference ($p < 0.05$) between creaming percentages of emulsions stabilized with granule/plasma proteins heat-treated at 65-75°C (Tables A.41-A.42). Plasma proteins exhibited a low rate and extent of creaming than granule proteins after thermal treatment. This might be resulted from the gelation of egg yolk plasma. The heat sensitivity of it has been reported to be mainly due to LDL which has undergone denaturation at 70°C and formed gel at 75°C in a pH range of 4-9 (Anton et al. 2001). Le Denmat et al. (1999) reported that the heat treatment have not influenced the emulsifying activity and emulsion stability of granules even at 76°C. Thus, the granule proteins have been demonstrated to be heat resistant. A better emulsion stability of granules compared to plasma has been revealed by Le Denmat et al. (2000). Granule proteins have been considered to be globular-like proteins and HDL has been indicated as the main constituents that adsorb at the oil-water interface (Mine, 1998). Thus, they have been regarded as good in stabilizing emulsions than plasma proteins.



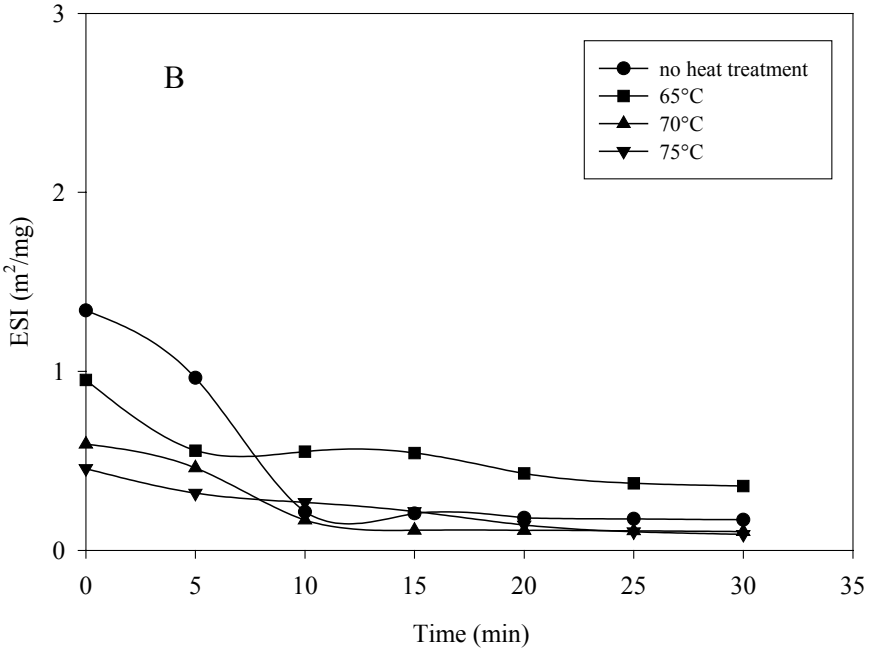


Figure 4.34. Emulsion stability index of non-heated and heat-treated egg yolk granule (A) and plasma (B) without hydrocolloids

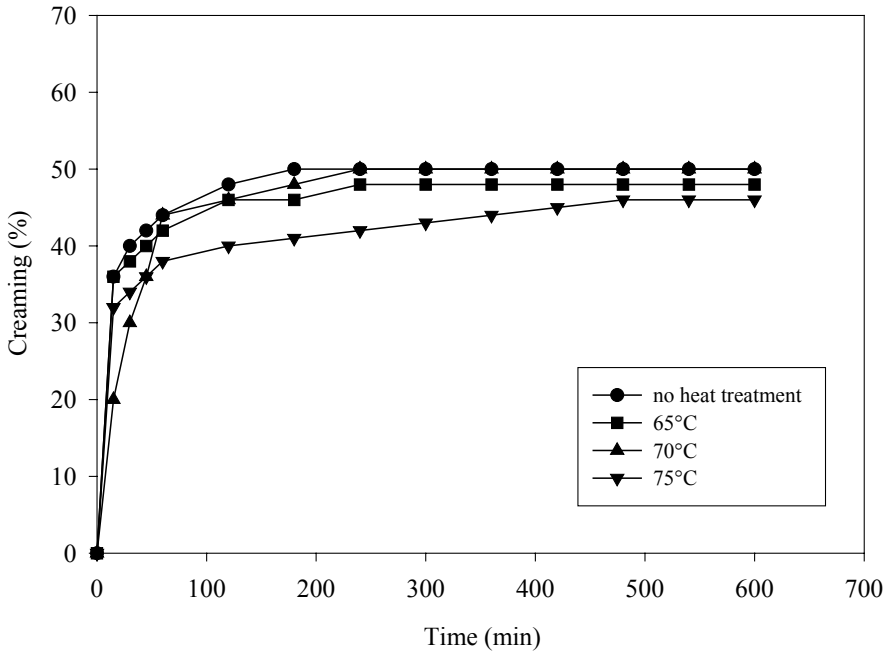


Figure 4.35. Creaming behaviors of non-heated and heat-treated egg yolk granule without hydrocolloids

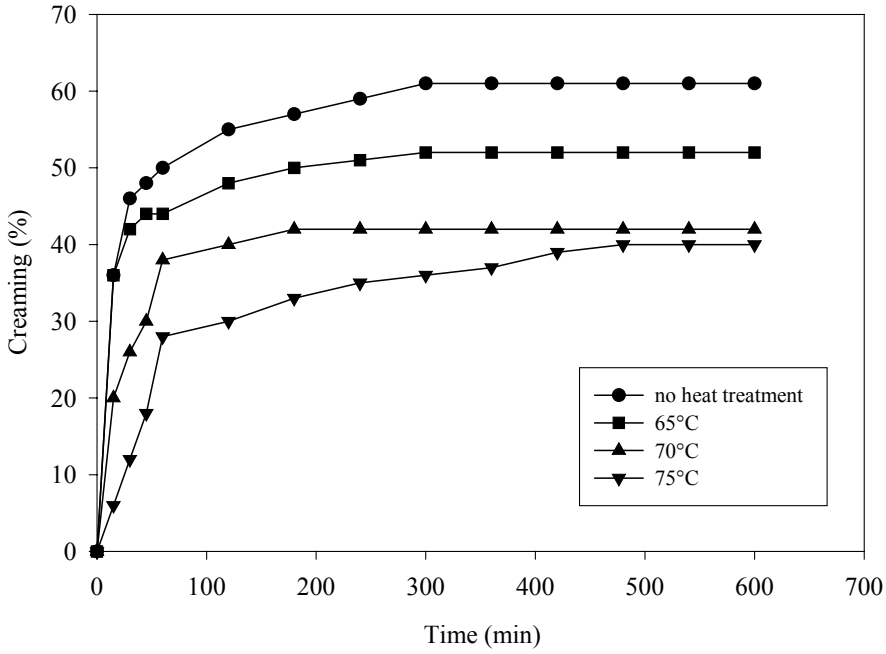


Figure 4.36. Creaming behaviors of non-heated and heat-treated egg yolk plasma without hydrocolloids

Figure 4.37 represents the emulsifying activity and emulsion stability of non-heated and heat-treated egg yolk granule and plasma in the presence of 0.10 % (w/v) pectin. It was resulted that emulsion activity and stability of egg yolk proteins heated

together with pectin were not improved. However, guar gum increased the emulsion activity and stability of heated and unheated yolk plasma and granule (Figures 4.38 and 4.34). The increase in the stability may arise from the change in the bulk rheology, which retards the coalescence of droplets. A very slight change was observed on the rate and extent of creaming of plasma/granule-stabilized emulsions in the presence of pectin (Figures 4.39 and 4.35) after heat treatment. However, the creaming rate was dramatically reduced by heated plasma proteins and granule proteins in the presence of guar gum (Figures 4.40 and 4.35). The percentage creaming was reduced in a great extent with heat-treated plasma proteins at 70°C and 75°C probably due to protein gelation (Anton et al. 2000).

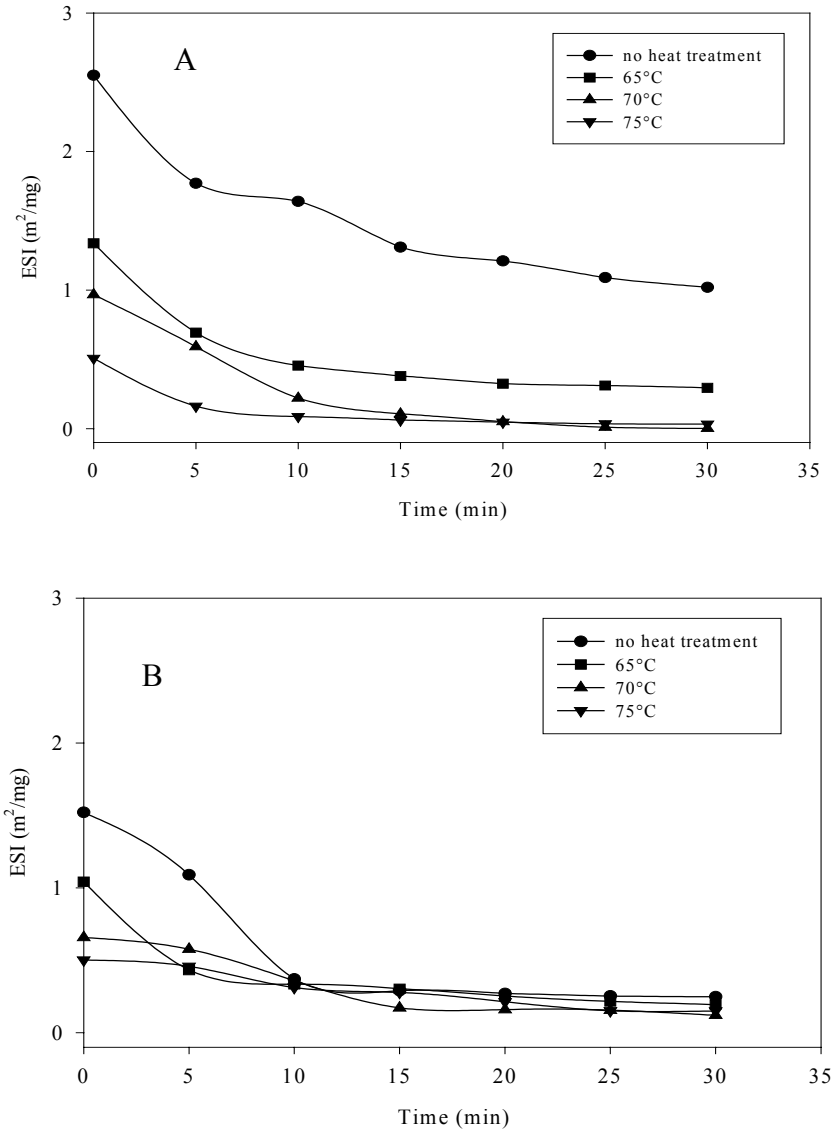
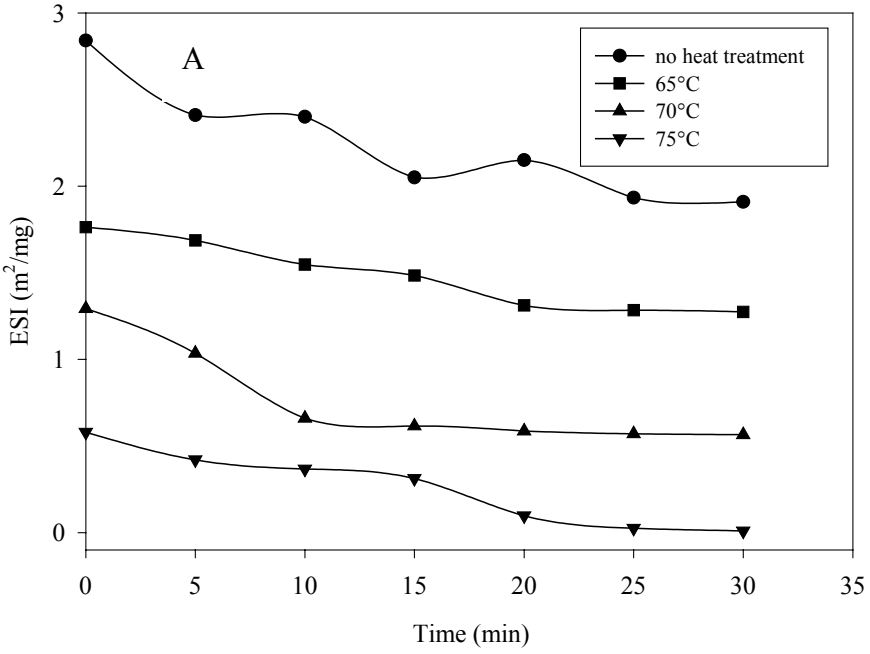


Figure 4.37. Emulsion stability index of non-heated and heat treated egg yolk granule (A) and plasma (B) in the presence of pectin



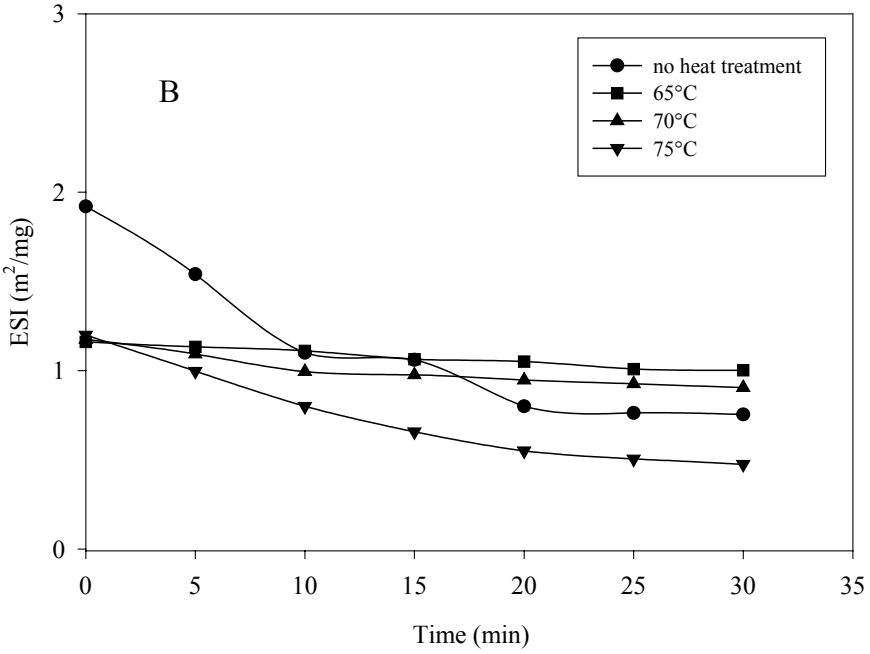


Figure 4.38. Emulsion stability index of non-heated and heat treated egg yolk granule (A) and plasma (B) in the presence of guar gum

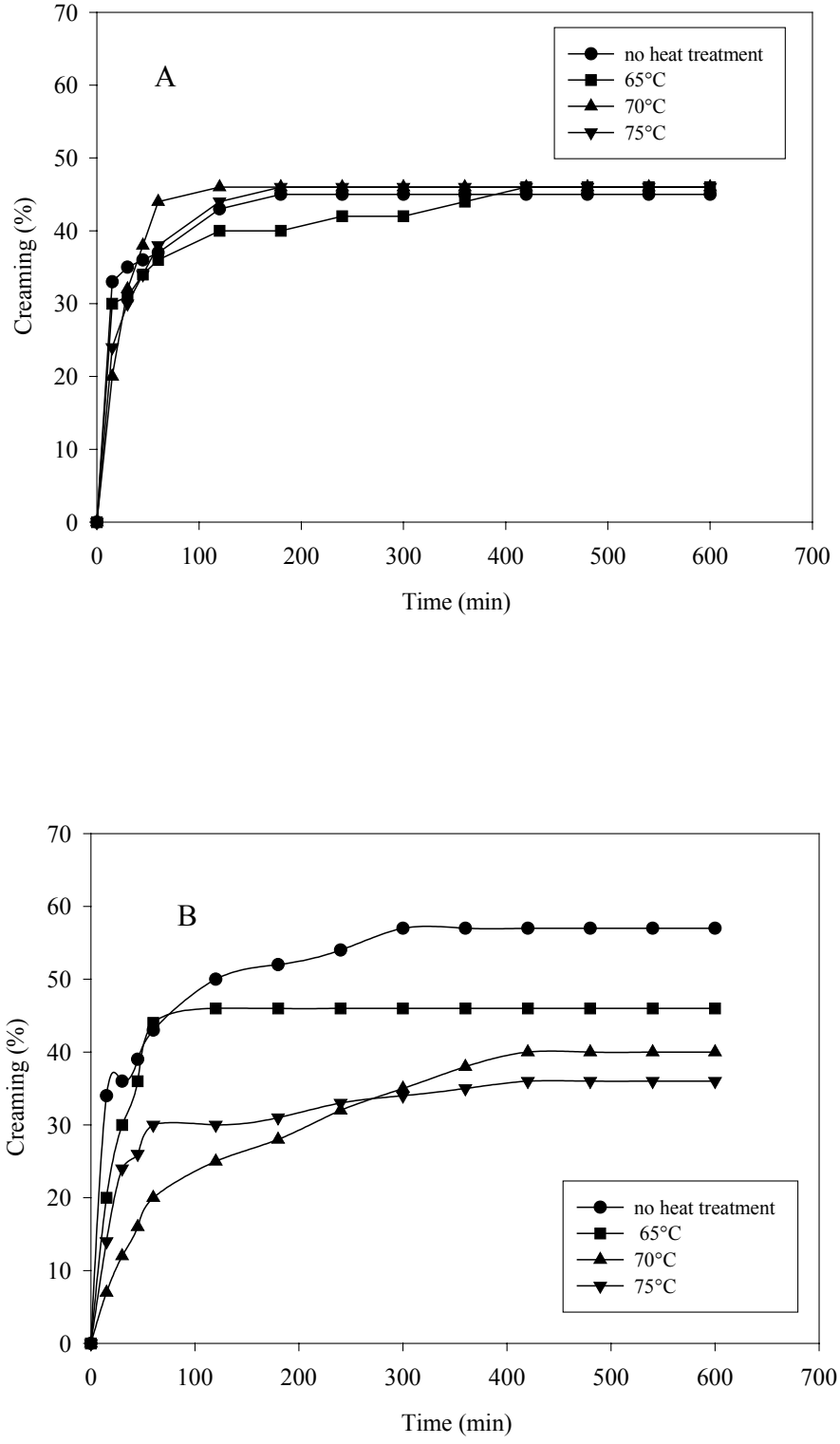
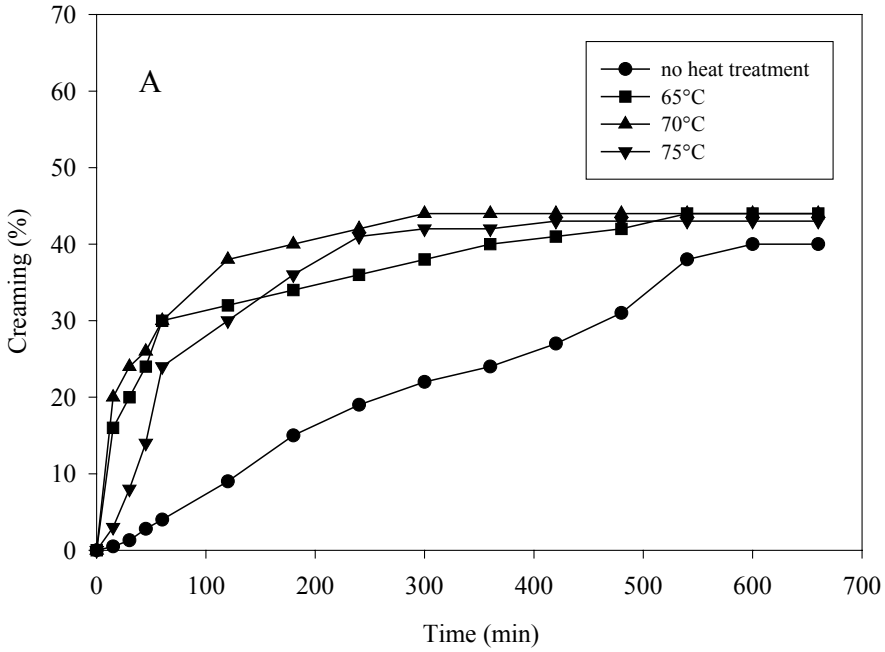


Figure 4.39. Creaming behaviors of non-heated and heat treated egg yolk granule (A) and plasma (B) in the presence of pectin



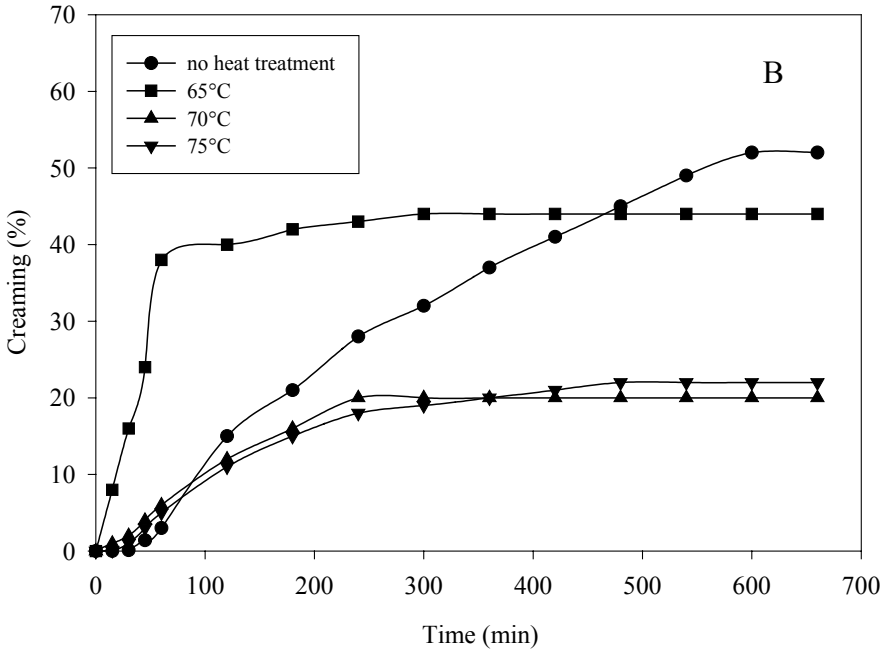


Figure 4.40. Creaming behaviors of non-heated and heat treated egg yolk granule (A) and plasma (B) in the presence of guar gum

4.5.3. Effects of Heat Treatment on Emulsifying Properties of Whey Protein Isolate

The effects of heat treatment on emulsifying properties of whey protein isolate (WPI) were investigated. Protein solutions and protein–hydrocolloid mixtures were heated at 65°C-75 °C for 2.0 minutes and then immediately cooled and emulsions were prepared (0.50 % (w/v) protein, pH 7.0) with and without hydrocolloids. Emulsion stability index (ESI) and % creaming were determined and the results were given in Figures 4.41-4.46. Emulsifying activity (ESI at time zero) was measured as 1.90 and 1.88 m^2/mg for non-heated and heat-treated (at 65°C) WPI, respectively (Figure 4.41). As the temperature was increased, only an incremental decrease was observed in EAI. ESI rapidly decreased within first 10 min; after 10^{th} min a slow reduction was observed. ESI gradually reduced with increasing temperature and the lowest stability was obtained with WPI heated at 75°C. EAI and ESI of WPI at each temperature were significantly different ($p < 0.05$) (Tables A.43-A.44). Ye and Singh (2006) reported that upon heat treatment above 70°C whey proteins unfold and aggregate. In whey protein stabilised emulsions, this may lead to aggregation of the emulsion droplets during heat treatment. It was known that this aggregation induced a reduction in emulsion stability.

The creaming behavior of non-heated and heat-treated WPI was shown on Figure 4.42. At the end of 10 h, creaming was measured as 60.0% for non-heated WPI. It was detected as 59.0, 58.0 and 57.0% for WPI heat-treated at 65, 70 and 75°C, respectively. Heat treatment may cause gelation of proteins and this gelation may retard serum separation and cause a decrease in creaming percentage. Heat treatment had a significant ($p < 0.05$) influence on the creaming behavior of WPI (Table A.45). Hunt and Dalgleish (1995) reported that emulsions made with whey protein were stable against heating at low and neutral pH at low ionic strength. But Sliwinski et al. (2003) reported that the size distribution of whey protein stabilized emulsions was not affected by heating at 70°C. However, heating at higher temperatures in the range of 75-80°C led to significant droplet aggregation, which was decreased with increasing temperature.

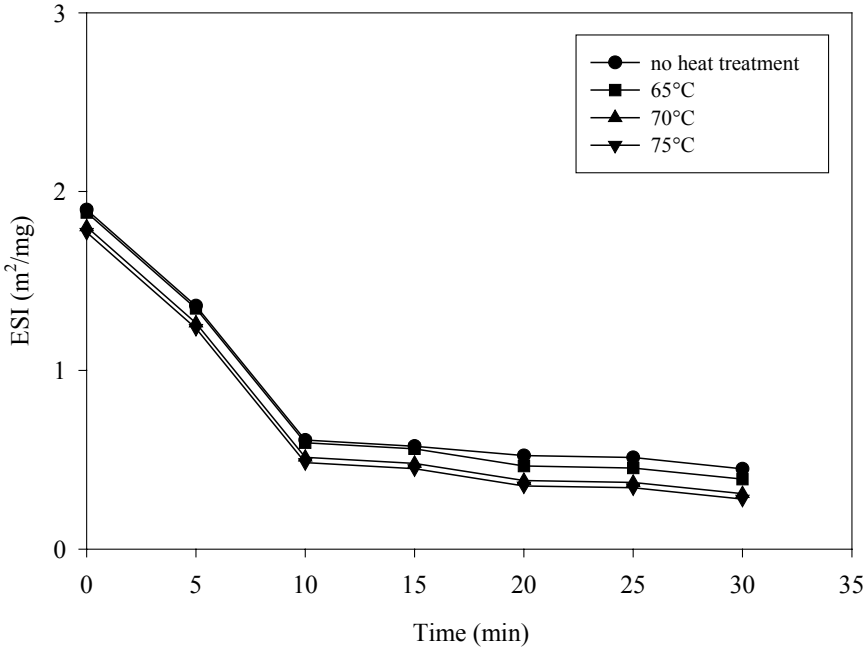


Figure 4.41. Emulsion stability index of non-heated and heat-treated whey protein isolate without hydrocolloids

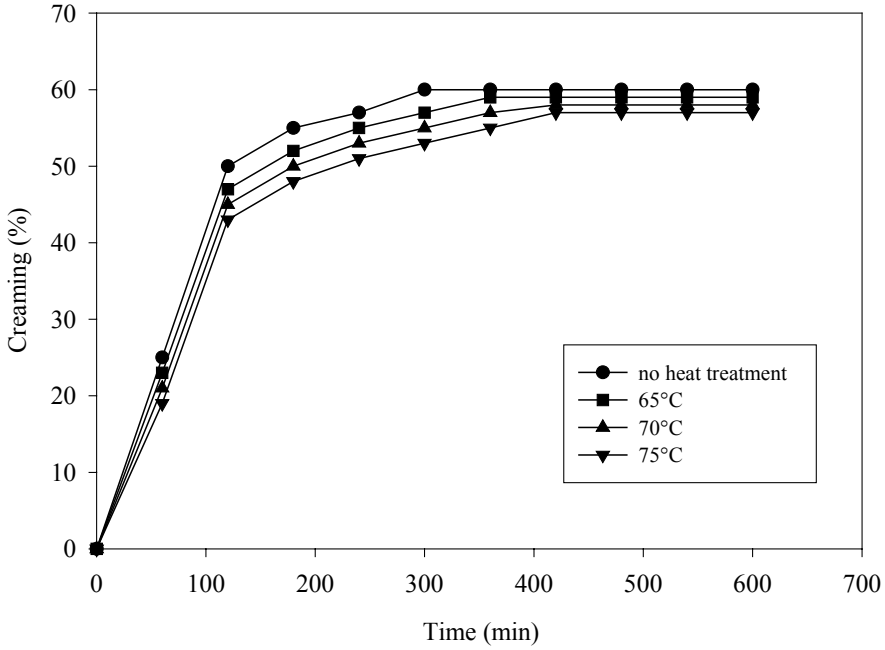


Figure 4.42. Creaming behaviors of non-heated and heat-treated whey protein isolate without hydrocolloids

Emulsion activity and emulsion stability index of non-heated and heat-treated whey protein isolate in the presence of 0.10% (w/v) pectin and guar gum were given in Figures 4.43 and 4.44, respectively. As shown from figures heat treatment did not improve the emulsion activity and emulsion stability index of whey protein isolate even in the presence of hydrocolloids.

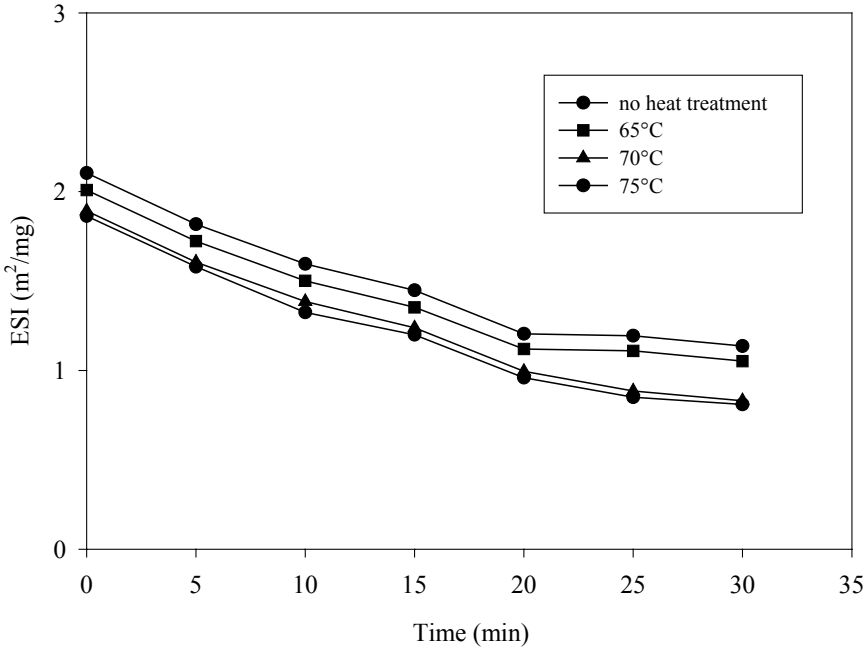


Figure 4.43. Emulsion stability index of non-heated and heat-treated whey protein isolate in the presence of pectin

Figures 4.45 and 4.46 illustrate the creaming behavior of non-heated and heat-treated WPI in the presence of pectin and guar gum, respectively. The creaming stability of both unheated and heated emulsions increased after adding hydrocolloids. Creaming percentage of non-heated WPI–pectin stabilised emulsion was measured as 59.0%. This value decreased to 57.0, 55.0 and 53.0% after application of heat treatment at 65, 70 and 75°C, respectively. In non-heated WPI–guar gum stabilised emulsions, creaming was measured as 25.0% at the end of 10 h. After heat treatment (at 75°C) was applied it was detected as 20.0%. We concluded that heat treatment decreased the creaming behavior of WPI in the absence (Figure 4.42) and presence of hydrocolloids (Figures 4. 45 and 4.46). The stabilisation against creaming in these emulsions can be attributed to the formation of network structure in the emulsions and the high viscosity of aqueous phase (Ye and Singh, 2006). They reported that the viscosity of the continuous phase increases with the addition of polysaccharide to the

emulsions, which results in reduced mobility of the droplets and increased creaming stability.

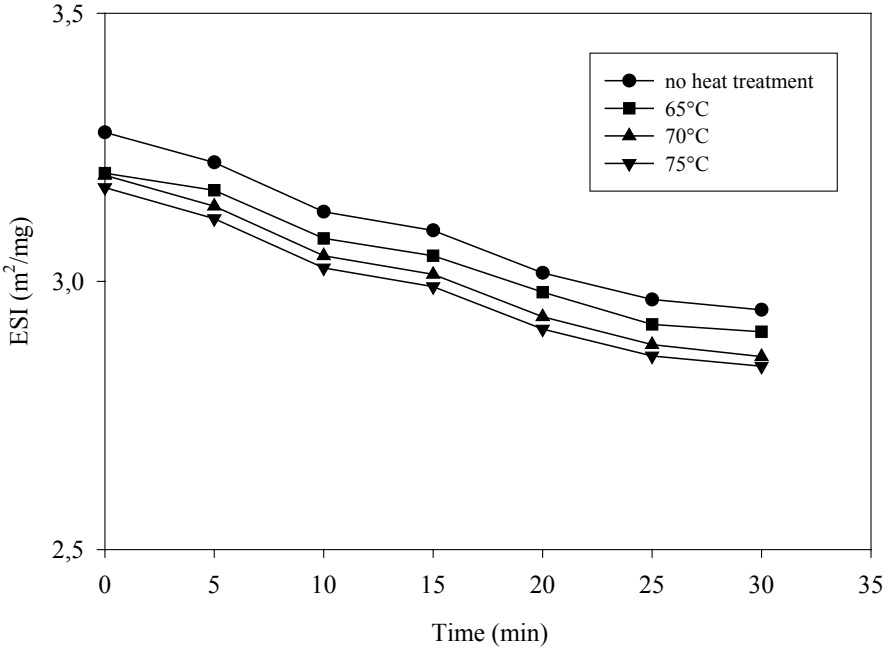


Figure 4.44. Emulsion stability index of non-heated and heat-treated whey protein isolate in the presence of guar gum

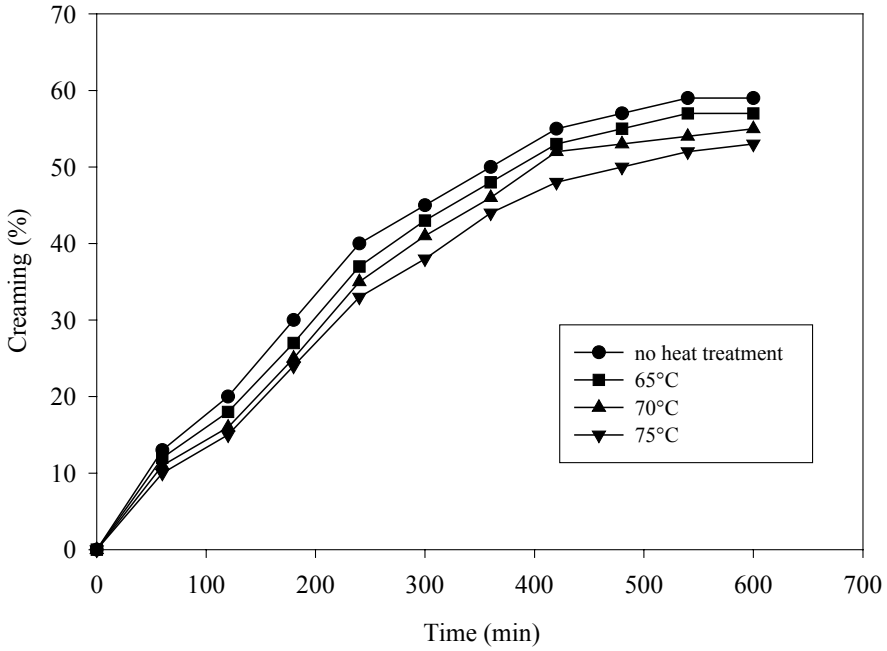


Figure 4.45. Creaming behaviors of non-heated and heat-treated whey protein isolate in the presence of pectin

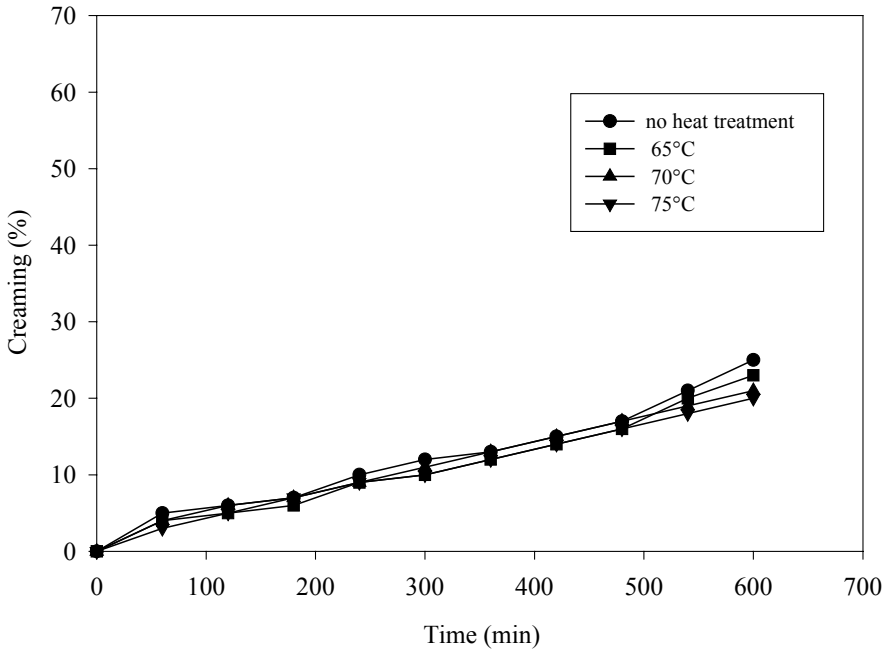


Figure 4.46. Creaming behaviors of non-heated and heat-treated whey protein isolate in the presence of guar gum

4.6. Rheological Properties

4.6.1. Rheological Properties of Whey Protein Isolate-Stabilised Emulsions

Dynamic frequency sweep tests were performed in the linear viscoelastic range to determine the frequency dependence of the elastic and viscous moduli. Figure 4.47 illustrates the mechanical spectra describing the viscoelastic behavior of whey protein isolate stabilized emulsions in the absence and presence of pectin/guar gum. Magnitudes of both G′ and G″ increased with frequency. While G′ was relatively independent of pectin concentration, G″ seemed to decrease at high pectin concentration (0.50% (w/v)). All pectin concentrations studied exhibited a fluid-like behavior at low frequencies where loss modulus (viscous modulus) (G″) was higher than storage modulus (elastic modulus) (G′). This behavior corresponds to the characteristics of viscoelastic fluids (Steffe, 1996). It has been stated that G′ and G″ curves intersect in the frequency range, showing a clear tendency for more solid-like behavior at frequencies higher than the crossover frequency. At low frequencies, when G″ is much higher than G′, the energy used to deform the material is dissipated viscously and the material's behavior is liquid-like (Rao, 1999). Both G′ and G″ were not greatly changed when 0.10% guar gum was included in the emulsion, but addition of 0.50% (w/v) guar gum greatly increased both elastic and viscous

modulus. At lower frequencies WPI-guar system showed viscous behavior (since G″ > G′). However, at high frequencies G′ and G″ cross-over and G′ became greater than G″ and these systems behaved like an elastic system (Salvador et al., 2005). Tavares and Silva (2003) have studied the rheological behavior of whey protein–galactomannan mixtures in aqueous solutions under gelling conditions at neutral pH and reported that the presence of the neutral polysaccharide had significant effect on the viscoelastic behavior.

Figure 4.48 shows the shear stress versus shear rate plot of WPI-stabilised emulsions in the absence and presence of different concentrations of pectin/guar gum. The flow behavior of WPI-alone stabilized emulsions was Newtonian; the shear stress showed a practically linear dependence on shear rate (Lizarraga et al., 2006). For all pectin concentrations the shear stress increased with pectin concentration. But the addition of pectin did not significantly change the flow type. Emulsions prepared with WPI–guar gum (0.10% (w/v)) behaves like a Newtonian fluid. When guar gum concentration was increased to 0.50% (w/v), these emulsions showed shear-thinning behaviour and therefore, identified as non-Newtonian. It was reported that after addition of gums apparent viscosity decreased with increasing shear rate, therefore they exhibit a shear-thinning behavior (Kayacier and Doğan, 2006). This result was also correlated with the results of Ye and Singh (2006).

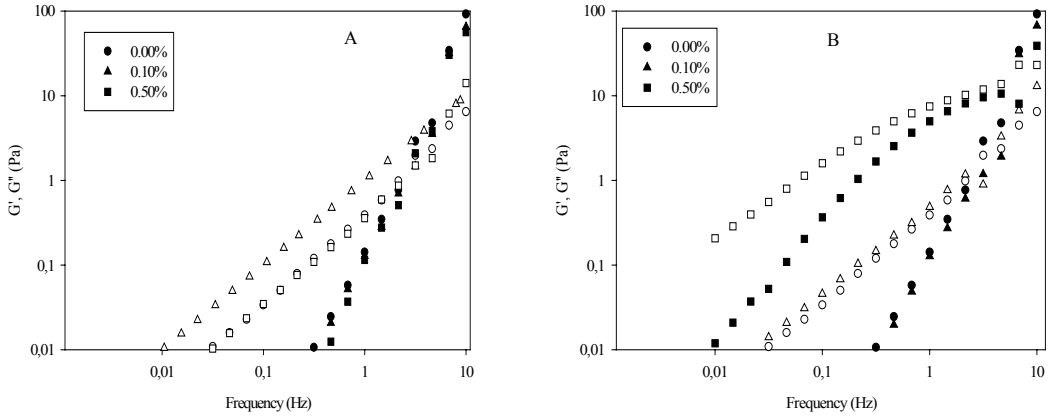


Figure 4.47. Viscoelastic moduli (G′- filled symbols, G″- open symbols) as a function of frequency for WPI–pectin (A) and WPI–guar gum (B) systems

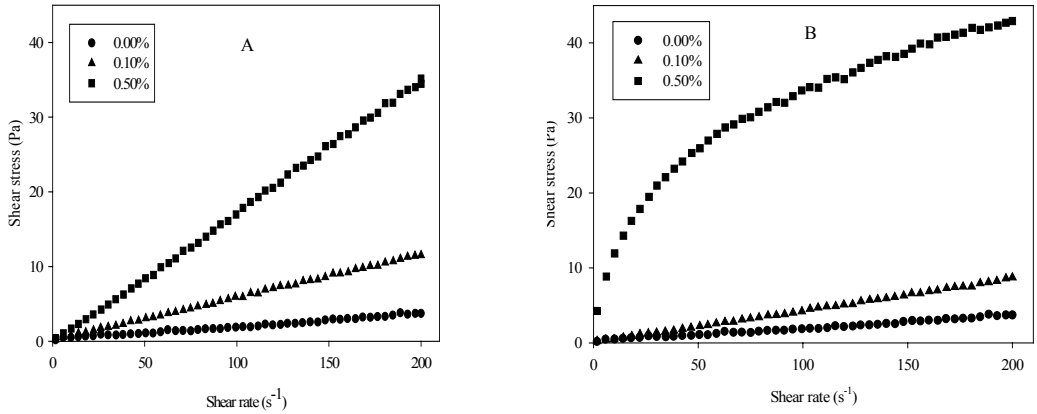


Figure 4.48. Flow behavior of WPI–pectin (A) and WPI–guar gum systems(B)

Flow curves were modelled with the power law ($\sigma = k\gamma^n$) and the parameters were given in Table 4.8. In equation, k is the consistency index, n is the flow behavior index. n <1.0 corresponds to shear thinning behavior, n >1.0 corresponds to shear thickening behavior and n = 1.0 corresponds to Newtonian behavior (Anton et al., 2001a). In WPI stabilised emulsions, consistency index (k) decreased with the addition of pectin (0.10 and 0.50% (w/v)) and 0.10% (w/v) guar gum and increased when 0.50% (w/v) guar gum was added. The consistency index (k) is an indicator of the viscous behavior and was observed to be increased when the hydrocolloid concentration increased from 0.10 to 0.50% (w/v) and the results were in agreement with the results of Lizarraga et al. (2006) and Kayacıer and Doğan (2006). In emulsions stabilised with WPI–pectin (0.10-0.50% (w/v)) and WPI–guar gum (0.10% (w/v)), the flow behaviour index (n) was practically 0.90 which corresponds to Newtonian behavior. Flow behaviour index of WPI-stabilised emulsions decreased from 0.876 to 0.396 after adding 0.50% (w/v) guar gum and it was indicating the shear thinning behavior. This is usually the case with weakly flocculated emulsions or those to which a thickener is added. The lower the value of the n the more shear thinning behavior the emulsion exhibits (Tadros, 2004). Increasing hydrocolloid concentration induced a significant difference ($p < 0.05$) on flow behavior index (n) and consistency index (k) (Table A.46).

Table 4.8. Rheological parameters of emulsions prepared with different proteins and hydrocolloids

Protein	Hydrocolloid	Hydrocolloid concentration (%)	n	k	R^2
WPI	Pectin	0.00	0.876	0.352	0.980
		0.10	0.973	0.067	0.999
		0.50	1.000	0.147	0.999
	Guar gum	0.10	0.984	0.047	0.998
		0.50	0.396	5.382	0.994
EYP	Pectin	0.00	0.904	0.047	0.991
		0.10	1.000	0.035	0.997
		0.50	0.915	0.062	0.998
	Guar gum	0.10	0.994	0.076	0.997
		0.50	0.446	3.373	0.995
EYG	Pectin	0.00	0.828	0.042	0.982
		0.10	0.830	0.041	0.987
		0.50	1.000	0.107	0.999
	Guar gum	0.10	0.978	0.073	0.998
		0.50	0.627	0.973	0.998
EW	Pectin	0.00	0.777	0.154	0.993
		0.10	0.775	0.082	0.997
		0.50	0.775	0.102	0.995
	Guar gum	0.10	0.782	0.112	0.992
		0.50	0.369	8.252	0.996

WPI: whey protein isolate, EYP: egg yolk plasma. EYG: egg yolk granule, EW: egg white

4.6.2. Rheological Properties of Egg Yolk Plasma-Stabilised Emulsions

Dynamic and steady shear measurements of egg yolk plasma stabilized emulsions in the absence and presence of hydrocolloids were performed. Plasma proteins-stabilised emulsions with and without pectin showed Newtonian flow and liquid-like behavior with G″ being greater than G′ at frequencies lower than approximately 7.0 Hz and solid-like behavior with G′ being greater than G″ at frequencies above 7.0 Hz (Figure 4.49). The addition of pectin did not affect elastic behavior (Figure 4.49(A)) , but the viscous modulus (Figure 4.49(A)) and the apparent viscosity (Figure 4.49(A′)) were slightly increased in the presence of 0.50% (w/v) pectin. It was observed that addition of guar gum did not effect G′. (Figure 4.49(B)) Whereas addition of 0.10% (w/v) guar gum did not change viscous modulus (Figure 4.49(B)), 0.50% (w/v) guar gum increased viscous modulus and these emulsions behaves as more liquid-like than the emulsion containing 0.10% (w/v) guar gum. Plasma proteins–guar gum stabilized emulsions showed Newtonian behavior at low guar

gum concentration (0.10% w/v) and shear thinning behavior at high gum concentration (0.50% w/v) (Table 4.8). Generally, gums have non-Newtonian rheology and they impart non-Newtonian character to the emulsion even when the amount of the dispersed phase is low (Mandala et al., 2004). A dramatic change was observed in the apparent viscosity of emulsions containing 0.50% (w/v) guar gum. This high viscosity restricts the movement of emulsion droplets as reported by Huang et al (2001) and provides better stability and this correlates with our previous results.

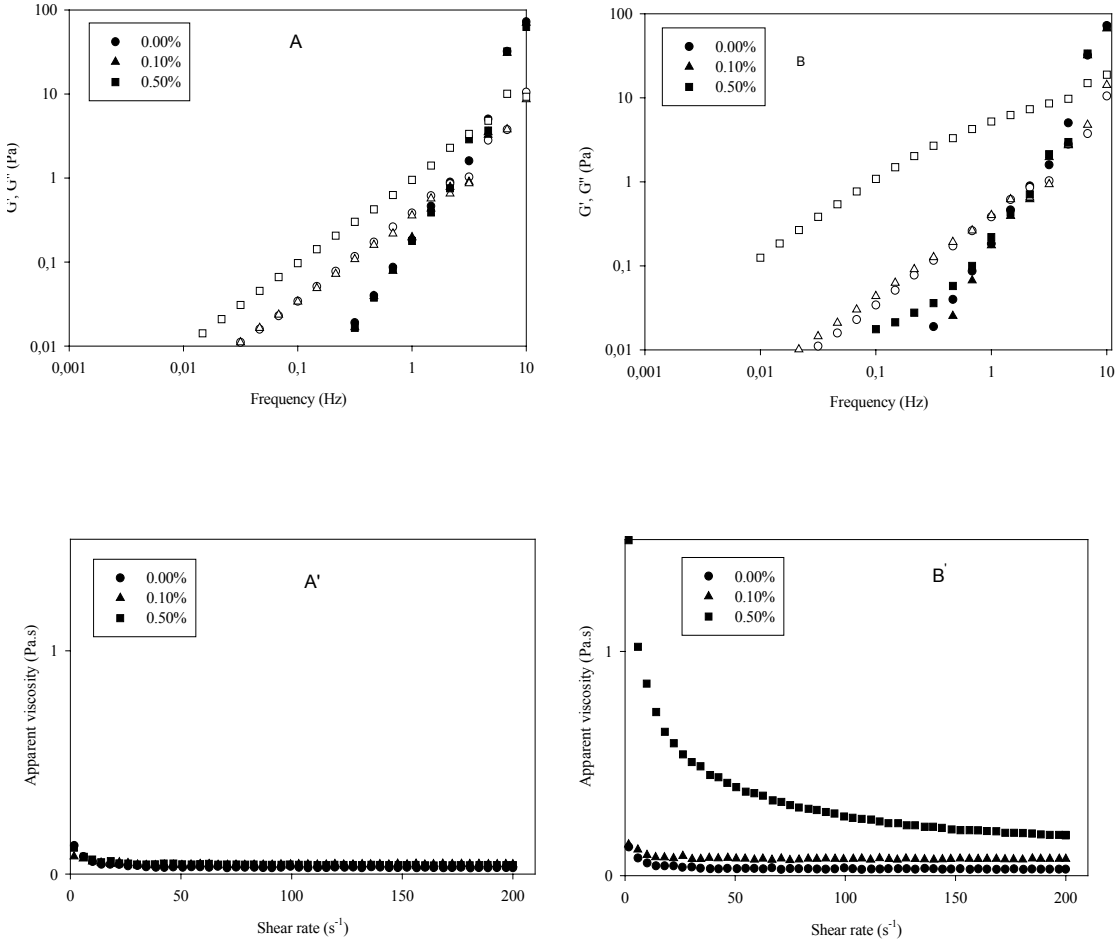


Figure 4.49. Viscoelastic moduli (G′- filled symbols, G’’- open symbols) and apparent viscosities of plasma stabilized emulsions containing different concentrations of pectin (A, A′) and guar gum (B, B′)

To examine the effect of protein concentration on viscoelastic properties, an emulsion containing 2.0% (w/v) protein and 0.50% (w/v) guar gum was prepared and the results were compared to emulsions containing 1.0 (w/v) % plasma and 0.50%

(w/v) guar gum and it was observed that G′, G″ increased when the protein concentration was increased (Figure 4.50). These system again showed viscous (liquid-like) behavior with being G″ greater than G′ at low frequencies and showed elastic behavior at high frequency (10 Hz). Apparent viscosity of the emulsion of 2.0% (w/v) plasma proteins was higher than that of containing the 1.0% (w/v) plasma proteins (Figure 4.50). Anton et al. (2001a) studied the rheology of hen egg yolk stabilized emulsions and found that viscosity of emulsions prepared at pH 7.0 increased with increasing protein concentration and this could be attributed to a flocculation between oil droplets.

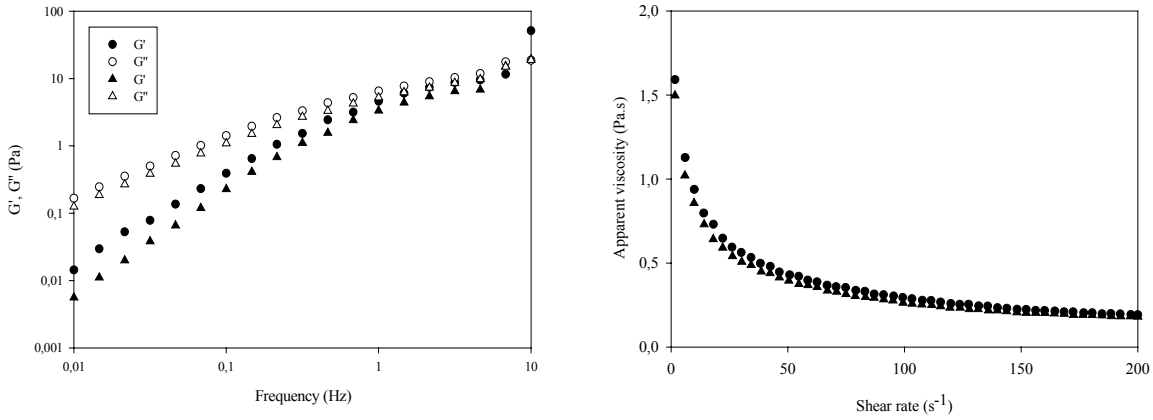


Figure 4.50. Viscoelastic moduli (G′- filled symbols, G″- open symbols) and apparent viscosities of 1.0% (w/v) plasma–0.50% (w/v) guar gum (▲) and 2.0% (w/v) plasma–0.50% (w/v) guar gum (●) containing emulsions

4.6.3. Rheological Properties of Egg Yolk Granule-Stabilised Emulsions

In Figure 4.51, the elastic modulus (G′) and viscous modulus (G″) values were plotted, against frequency of oscillation for egg yolk granule proteins (EYG) stabilised emulsions in the absence and presence of pectin or guar gum. Elastic modulus and viscous modulus increased with increasing frequency and they were regarded as frequency dependent. In the case of emulsions prepared with pectin, no changes were observed in elastic behavior whatever the pectin concentration was. For emulsions prepared with EYG –0.1% (w/v) pectin, approximately no change was observed in the viscous modulus. However, we observed a decrease in the viscous modulus for emulsions prepared with EYG–0.50% (w/v) pectin . The presence of 0.10% (w/v) guar gum had a very slight effect on both elastic and viscous moduli, while 0.50% (w/v) guar gum increased both moduli. The changes in viscoelastic profile mean that the molecular mobility was decreased due to the

presence of the neutral polysaccharide (Tavares et al., 2003). At low frequencies G″ values were greater than G′ values, showing lower elastic characteristics in the frequency range studied. This was a typical behavior of weakly flocculated emulsions (Riscardo et al., 2005). At higher frequencies, a cross-over between G′ and G″ was noticed and G′ becames greater than G″ which indicates the emulsion system behaves like an elastic solid.

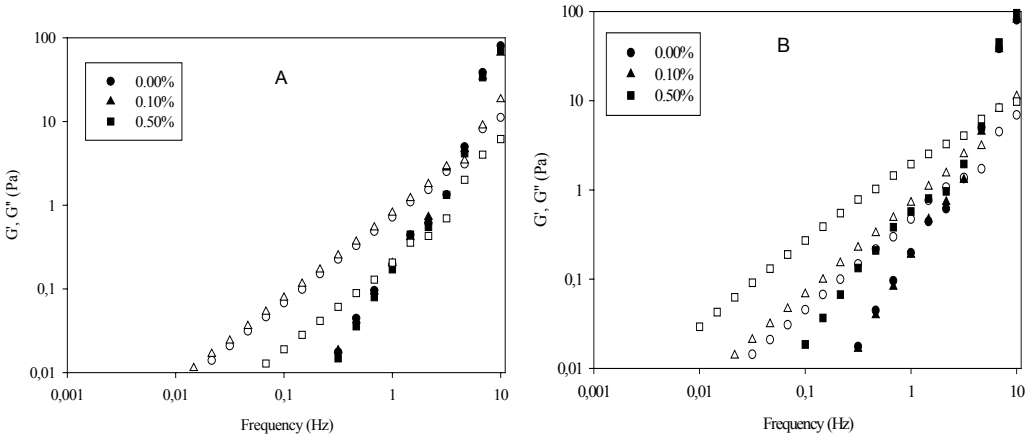


Figure 4.51. Viscoelastic moduli (G′- filled symbols, G″- open symbols) of egg yolk granule stabilized emulsions containing different concentrations of pectin (A) and guar gum (B)

Figure 4.52 shows the flow curves EYG–pectin and EYG–guar gum emulsion systems. As may be observed, viscosity of EYG–pectin emulsions did not change with increasing shear rate and showed Newtonian behavior. At high shear rates no difference was observed between the viscosity of emulsions containing no pectin and 0.10% (w/v) pectin. This may be due to the orientation of macromolecules in the direction of shear and to the structure distruption (Mandala et al., 2004). In emulsions prepared with EYG and guar gum, increasing of gum concentration increased the viscosity and the viscosity decreases with increasing shear rate. Emulsions containing 0.10% (w/v) guar gum showed slightly pseudoplastic behavior. When the gum concentration was increased to 0.50% (w/v), the system showed more pseudoplastic behavior. Kontogiorgos et al. (2004) have been studied the rheological behavior of egg yolk stabilized concentrated emulsions containing cereal polysaccharides and found that the viscoelastic behavior of emulsions after the addition of polysaccharide was further enhanced and the flow became more

pseudoplastic with increased values of viscosity and the storage (elastic) modulus was also increased.

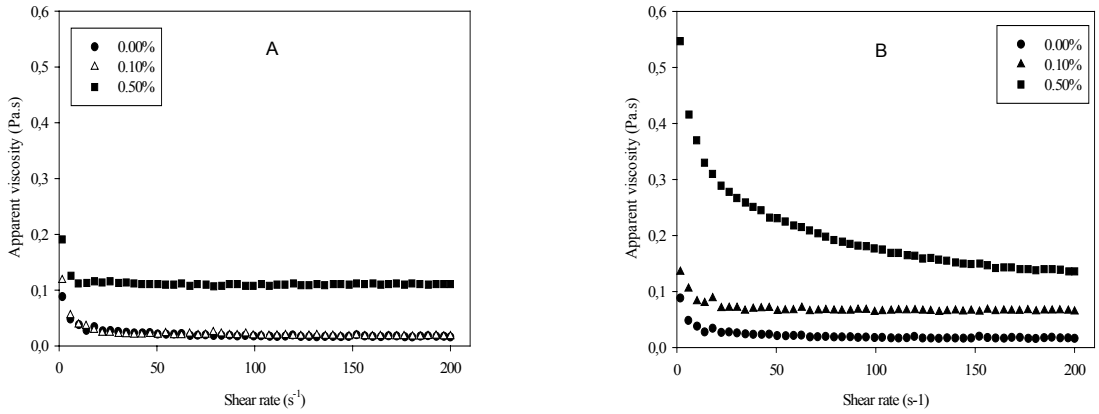


Figure 4.52. Flow behavior of EYG-pectin (A) and EYG-guar gum systems(B)

EYP and EYG stabilised emulsions showed Newtonian behavior in the presence of pectin and low concentration of guar gum (Table 4.8). Higher amounts (0.50% (w/v)) of guar gum containing emulsions had lower n values and shear thinning behavior was observed. Consistency indexes of these emulsions decreased with the addition of 0.10% (w/v) pectin and a high consistency was observed in the presence of 0.50% (w/v) pectin and guar gum (0.10-0.50% (w/v)) and consistency index increased with increasing hydrocolloid concentration (Kayacıer and Doğan, 2006). Statistical analysis revealed that there was a significant difference ($p < 0.05$) among different hydrocolloid concentrations in terms of flow behavior index of egg yolk proteins (granule and plasma) and consistency index (Tables A.47-A.48).

4.6.4. Rheological Properties of Egg White-Stabilised Emulsions

Dynamic rheological tests of egg white-stabilised emulsions with and without hydrocolloids showed liquid-like character at lower frequencies and the moduli crossing each other at high frequencies (Figure 4.53). These two moduli gradually increased with frequency and crossed-over especially above a frequency of 1.0 Hz. The two moduli of high hydrocolloid containing emulsions crossed-over at lower frequency values. This is the indicative of a stronger structure in the presence of higher amounts of hydrocolloids. This might be resulted from high gelling ability of egg white. Ngarize et al.(2004) reported that egg white protein solutions at high protein concentration cross-linked at 20°C and showed higher G′ values than G″.

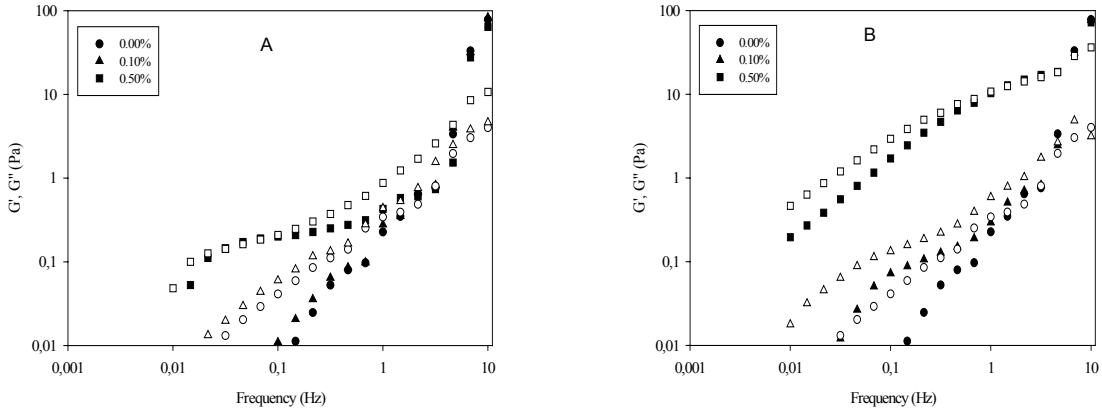


Figure 4.53. Viscoelastic moduli (G′- filled symbols, G″- open symbols) of egg white stabilized emulsions containing different concentrations of pectin (A) and guar gum (B)

Figure 4.54 illustrates the flow behavior of egg white stabilised emulsions. Emulsions prepared without hydrocolloids had low viscosity (0.28 Pa.s) and showed Newtonian flow. Viscosity was measured as 0.31 and 0.43 Pa.s after the addition of 0.10% (w/v) pectin and guar gum, respectively. We observed that the addition of low amounts of hydrocolloids increased viscosity but did not change flow type. But the additon of higher amounts of hydrocolloids, especially guar gum, increased viscosity greatly (up to 4.70 Pa.s) and the system showed pseudoplastic behavior. Concerning emulsion rheological behavior, it ranges between Newtonian and pseudoplastic (Mandala et al., 2004). Emulsion (o/w) formed of milk protein and soya oil showed Newtonian behavior, but kappa-carrageenan addition resulted in viscosity increase and clear pseudoplastic behavior, indicating reversible flocculation of oil droplets.

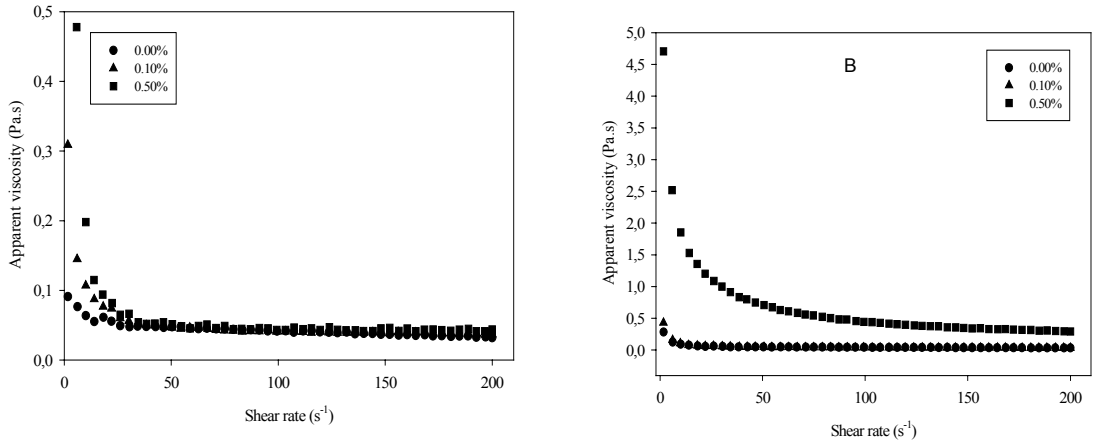


Figure 4.54. Flow behavior of egg white-pectin (A) and egg white-guar gum systems(B)

Emulsions prepared with EW-alone has a consistency index of 0.15 Pa s^n (Table 4.8). This value decreased to 0.08 and 0.10 Pa s^n with the addition of 0.10 and 0.50% (w/v) pectin, respectively. While the addition of 0.10% guar gum decreased k value, addition of 0.50% (w/v) guar gum increased consistency. The increase in k with respect to hydrocolloid concentration was more pronounced for guar gum compared to pectin. Flow behavior index of EW–pectin stabilised emulsions was approximately 0.80 and can be regarded as Newtonian fluid. But EW–guar gum (0.50% (w/v)) stabilized emulsions showed shear thinning behavior with n value of 0.37. It was statistically confirmed that hydrocolloid concentration had a significant effect on flow behavior index (Table A.49).

CHAPTER 5

CONCLUSIONS

Phase separation was observed at low biopolymer concentrations in protein-polysaccharide mixtures as a result of either thermodynamic incompatibility or depletion flocculation. In the case of thermodynamic incompatibility, a two-phase system was observed, one of the phases was rich in protein and the other was rich in polysaccharide. In the case of depletion flocculation, on phase rich in both of two biopolymers and the other was depleted in both.

The thermograms obtained for whey protein isolate, egg yolk granule proteins and egg yolk plasma proteins solutions displayed one major endothermic peak, but for egg white proteins two endothermic peak. The presence of hydrocolloids influenced thermal stability of proteins and reduced denaturation enthalpies due to protein aggregation and less energy is required for denaturation.

Stability of many food systems in the form of emulsion can be controlled with the addition of hydrocolloid gums. Pectin and especially guar gum increase the viscosity of food emulsion and thereby stabilize the emulsion by decreasing droplets movement and increase long-term storage stability.

Addition of pectin and guar gum decreased creaming of emulsions by increasing viscosity of aqueous phase and also they keep droplets apart and protect them against creaming. The effect of guar gum was more pronounced than that of pectin, because guar gum has higher viscosity.

Addition of pectin or gum and changing hydrocolloid concentration had no significant effect on foam volume. On contrary, hydrocolloids enhanced foam stability by influencing the viscoelastic behavior of aqueous phase and reducing the thinning rate of lamella.

Heat treatment especially at 80°C caused a reduction in foam volume of egg white due to protein aggregation. The effect of heat treatment was more pronounced at high protein concentration. Therefore, it was concluded that the rate of protein denaturation and aggregation in solution were dependent on protein concentration. Foam stability increased as the heating temperature was increased from 65°C to 75°C, however, it reduced at higher temperatures as a result of protein denaturation.

Heat treatment induced a reduction in emulsion activity and stability of egg yolk proteins and whey protein isolate, but increased stability against creaming. Both native and heat-treated egg yolk granule proteins were better emulsifier than egg yolk plasma proteins.

Emulsions stabilized without hydrocolloids showed Newtonian behavior except for egg white stabilized ones, and the viscosity of these systems did not change with shear rate. Pectin did not affect flow type. Guar gum was changed flow type from Newtonian to pseudoplastic depending on added concentration. High consistency indexes were observed in 0.5% guar gum containing emulsions. Elastic modulus and viscous modulus increased with increasing frequency and they were regarded as frequency dependent. At low frequencies emulsions behaved like a liquid and at higher frequencies they showed a solid or elastic behavior.

Whey protein isolate can be used in new food products as a good emulsifying and foaming agent in the presence of pectin and guar gum. Egg white can be used as a good foaming agent while egg yolk as a good emulsifying agent.

Functional properties of proteins are affected from environmental conditions. Therefore, to obtain more detailed information, these properties should be investigated at different environmental conditions such as pH, ionic strength etc. Also the effect of different hydrocolloids on functionality of these proteins should be investigated under different environmental conditions.

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APPENDICES

Table A.1. ANOVA and Multiple comparisons (LSD test) table for the effect of different hydrocolloids on the denaturation temperature of whey protein isolate

ANOVA table

	Sum of squares	df	Mean square	F	Significance
Between groups	27.211	3	9.070	32.785	0.0003
Within groups	1.107	4	0.277		
Total	28.317	7			

Multiple comparisons (LSD test)

Hydrocolloid	Hydrocolloid	Mean Difference	Std. Error	Significance
No hydrocolloid	Pectin	-4.7150*	0.526	0.001
	Guar gum	-1.7900*	0.526	0.027
	Carrageenan	-3.9100*	0.526	0.002
Pectin	No hydrocolloid	4.7150*	0.526	0.001
	Guar gum	2.9250*	0.526	0.005
	Carrageenan	0.8050	0.526	0.201
Guar gum	No hydrocolloid	1.7900*	0.526	0.27
	Pectin	-2.9250*	0.526	0.005
	Carrageen	-2.2120*	0.526	0.016
Carrageenan	No hydrocolloid	3.9100*	0.526	0.002
	Pectin	-0.8050	0.526	0.201
	Guar gum	2.1200*	0.526	0.016

*The mean difference is significant at the 0.05 level

Table A.2. ANOVA and Multiple comparisons (LSD test) table for the effect of different hydrocolloids on the transition enthalpy of whey protein isolate

ANOVA table

	Sum of squares	df	Mean square	F	Significance
Between groups	109.873	3	36.624	6260.544	0.000
Within groups	$2.340*10^{-3}$	4	$5.85*10^{-3}$		
Total	109.896	7			

Multiple comparisons (LSD test)

Hydrocolloid	Hydrocolloid	Mean Difference	Std. Error	Significance
No hydrocolloid	Pectin	8.2000*	0.076	0.000
	Guar gum	8.6200*	0.076	0.000
	Carrageenan	8.8100*	0.076	0.000
Pectin	No hydrocolloid	-8.2000*	0.076	0.000
	Guar gum	0.4200*	0.076	0.005
	Carrageenan	0.6100*	0.076	0.001
Guar gum	No hydrocolloid	-8.6200*	0.076	0.000
	Pectin	-0.4200*	0.076	0.005
	Carrageen	0.1900	0.076	0.068
Carrageenan	No hydrocolloid	-8.8100*	0.076	0.000
	Pectin	-0.6100*	0.076	0.001
	Guar gum	-0.1900	0.076	0.068

*The mean difference is significant at the 0.05 level

Table A.3. ANOVA and Multiple comparisons (LSD test) table for the effect of different hydrocolloids on the denaturation temperature of egg white

ANOVA table

	Sum of squares	df	Mean square	F	Significance
Between groups	0.250	3	$8.333*10^{-2}$	3.030	0.156
Within groups	0.110	4	$2.750*10^{-2}$		
Total	0.360	7			

Multiple comparisons (LSD test)

Hydrocolloid	Hydrocolloid	Mean Difference	Std. Error	Significance
No hydrocolloid	Pectin	-0.3500	0.166	0.102
	Guar gum	-0.4000	0.166	0.073
	Carrageenan	$-5.00*10^{-2}$	0.166	0.778
Pectin	No hydrocolloid	0.3500	0.166	0.102
	Guar gum	$-5.00*10^{-2}$	0.166	0.778
	Carrageenan	0.3000	0.166	0.145
Guar gum	No hydrocolloid	0.4000	0.166	0.073
	Pectin	$5.00*10^{-2}$	0.166	0.778
	Carrageen	0.3500	0.166	0.102
Carrageenan	No hydrocolloid	$5.00*10^{-2}$	0.166	0.778
	Pectin	-0.3000	0.166	0.145
	Guar gum	-0.3500	0.166	0.102

*The mean difference is significant at the 0.05 level

Table A.4. ANOVA and Multiple comparisons (LSD test) table for the effect of different hydrocolloids on the transition enthalpy of egg white

ANOVA table

	Sum of squares	df	Mean square	F	Significance
Between groups	48.855	3	16.285	47.203	0.001
Within groups	1.380	4	0.345		
Total	50.235	7			

Multiple comparisons (LSD test)

Hydrocolloid	Hydrocolloid	Mean Difference	Std. Error	Significance
No hydrocolloid	Pectin	-0.0300	0.587	0.636
	Guar gum	-0.0300	0.587	0.636
	Carrageenan	-5.5000*	0.587	0.001
Pectin	No hydrocolloid	0.0300	0.587	0.636
	Guar gum	0.0000	0.587	1.000
	Carrageenan	5.8000*	0.587	0.001
Guar gum	No hydrocolloid	0.0300	0.587	0.636
	Pectin	0.0000	0.587	1.000
	Carrageen	5.8000*	0.587	0.001
Carrageenan	No hydrocolloid	-5.5000*	0.587	0.001
	Pectin	-.5.8000*	0.587	0.001
	Guar gum	-.5.8000*	0.587	0.001

*The mean difference is significant at the 0.05 level

Table A.5. ANOVA and Multiple comparisons (LSD test) table for the effect of different hydrocolloids on the denaturation temperature of egg yolk granule

ANOVA table

	Sum of squares	df	Mean square	F	Significance
Between groups	3.294	3	1.098	6.506	0.051
Within groups	0.675	4	0.169		
Total	3.969	7			

Multiple comparisons (LSD test)

Hydrocolloid	Hydrocolloid	Mean Difference	Std. Error	Significance
No hydrocolloid	Pectin	-0.0850	0.411	0.107
	Guar gum	0.9500	0.411	0.082
	Carrageenan	-0.1500	0.411	0.733
Pectin	No hydrocolloid	0.8500	0.411	0.107
	Guar gum	1.8000*	0.411	0.012
	Carrageenan	0.7000	0.411	0.164
Guar gum	No hydrocolloid	-0.9500	0.411	0.082
	Pectin	-1.8000*	0.411	0.012
	Carrageen	-1.1000	0.411	0.055
Carrageenan	No hydrocolloid	0.1500	0.411	0.733
	Pectin	-.0.7000	0.411	0.164
	Guar gum	1.1000	0.411	0.055

*The mean difference is significant at the 0.05 level

Table A.6. ANOVA and Multiple comparisons (LSD test) table for the effect of different hydrocolloids on the transition enthalpy of egg yolk granule

ANOVA table

	Sum of squares	df	Mean square	F	Significance
Between groups	125.055	3	41.685	362.478	0.000
Within groups	0.460	4	0.115		
Total	125.515	7			

Multiple comparisons (LSD test)

Hydrocolloid	Hydrocolloid	Mean Difference	Std. Error	Significance
No hydrocolloid	Pectin	1.3000*	0.339	0.019
	Guar gum	9.4000*	0.339	0.000
	Carrageenan	-0.2000	0.339	0.587
Pectin	No hydrocolloid	-1.3000*	0.339	0.019
	Guar gum	8.1000	0.339	0.000
	Carrageenan	-1.5000*	0.339	0.011
Guar gum	No hydrocolloid	-9.4000*	0.339	0.000
	Pectin	-8.1000*	0.339	0.000
	Carrageen	-9.6000*	0.339	0.000
Carrageenan	No hydrocolloid	0.2000	0.339	0.587
	Pectin	1.5000*	0.339	0.011
	Guar gum	9.6000*	0.339	0.000

*The mean difference is significant at the 0.05 level

Table A.7. ANOVA and Multiple comparisons (LSD test) table for the effect of different hydrocolloids on the denaturation temperature of egg yolk plasma

ANOVA table

	Sum of squares	df	Mean square	F	Significance
Between groups	1.524	3	0.508	1.113	0.442
Within groups	1.825	4	0.456		
Total	3.349	7			

Multiple comparisons (LSD test)

Hydrocolloid	Hydrocolloid	Mean Difference	Std. Error	Significance
No hydrocolloid	Pectin	1.1000	0.675	0.179
	Guar gum	0.7500	0.675	0.329
	Carrageenan	0.2000	0.675	0.782
Pectin	No hydrocolloid	-1.1000	0.675	0.179
	Guar gum	-0.3500	0.675	0.632
	Carrageenan	-0.9000	0.675	0.254
Guar gum	No hydrocolloid	-0.7500	0.675	0.329
	Pectin	0.3500	0.675	0.632
	Carrageen	-0.5500	0.675	0.461
Carrageenan	No hydrocolloid	-0.2000	0.675	0.782
	Pectin	0.9000	0.675	0.254
	Guar gum	0.5500	0.675	0.461

*The mean difference is significant at the 0.05 level

Table A.8. ANOVA and Multiple comparisons (LSD test) table for the effect of different hydrocolloids on the transition enthalpy of egg yolk plasma

ANOVA table

	Sum of squares	df	Mean square	F	Significance
Between groups	63.880	3	21.293	462.775	0.000
Within groups	0.184	4	$4.601*10^{-2}$		
Total	64.064	7			

Multiple comparisons (LSD test)

Hydrocolloid	Hydrocolloid	Mean Difference	Std. Error	Significance
No hydrocolloid	Pectin	5.4650*	0.215	0.000
	Guar gum	5.2000*	0.215	0.000
	Carrageenan	-0.6000*	0.215	0.049
Pectin	No hydrocolloid	-5.4650*	0.215	0.000
	Guar gum	-0.2650*	0.215	0.284
	Carrageenan	-6.0650*	0.215	0.000
Guar gum	No hydrocolloid	-5.2000*	0.215	0.000
	Pectin	0.2650	0.215	0.284
	Carrageen	-5.8000*	0.215	0.000
Carrageenan	No hydrocolloid	0.6000*	0.215	0.049
	Pectin	6.0650*	0.215	0.000
	Guar gum	5.8000*	0.215	0.000

*The mean difference is significant at the 0.05 level

Table A.9. ANOVA table for the effect of different pectin concentrations on the emulsion activity index (EAI) of whey protein isolate

	Sum of squares	df	Mean square	F	Significance
Between groups	0.332	5	$6.646*10^{-2}$	113.925	0.000
Within groups	$3.5*10^{-3}$	6	$5.833*10^{-4}$		
Total	0.336	11			

Table A.10. ANOVA table for the effect of different guar gum concentrations on the emulsion activity index (EAI) of whey protein isolate

	Sum of squares	df	Mean square	F	Significance
Between groups	8.734	5	1.747	28325.056	0.000
Within groups	$3.7*10^{-4}$	6	$6.167*10^{-5}$		
Total	8.734	11			

Table A.11. ANOVA and Multiple comparisons (LSD test) table for the effect of different hydrocolloid concentrations on the emulsion stability index (ESI) of whey protein isolate

ANOVA table

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	ESIPECTIN	113.470	17	6.675	339.395	0.000
	ESIGUAR	449.558	17	26.445	267.186	0.000
TIME	ESIPECTIN	102.888	12	8.574	435.972	0.000
	ESIGUAR	335.039	12	27.920	282.092	0.000
CONC	ESIPECTIN	10.582	5	2.116	107.611	0.000
	ESIGUAR	114.519	5	22.904	231.412	0.000
Error	ESIPECTIN	1.082	55	$1.967*10^{-2}$		
	ESIGUAR	5.444	55	$9.897*10^{-2}$		
Total	ESIPECTIN	114.551	72			
	ESIGUAR	455.002	72			

ESIPECTIN: ESI of emulsions in the presence of pectin
ESIGUAR: ESI of emulsions in the presence of guar gum

Multiple comparisons (LSD test)

Dependent Variable	(I) CONC	(J) CONC	Mean Difference (I-J)	Std. Error	Sig.
ESIPECTIN	0.00	0.01	-0.132833(*)	0.057	0.024
		0.05	-0.266083(*)	0.057	0.000
		0.10	-0.664500(*)	0.057	0.000
		0.20	-0.795417(*)	0.057	0.000
		0.50	-1.073333(*)	0.057	0.000
	0.01	0.00	0.132833(*)	0.057	0.024
		0.05	-0.133250(*)	0.057	0.024
		0.10	-0531667(*)	0.057	0.000
		0.20	-0.662583(*)	0.057	0.000
		0.50	-0.940500(*)	0.057	0.000
	0.05	0.00	0.266083(*)	0.057	0.000
		0.01	0.133250(*)	0.057	0.024
		0.10	-0.398417(*)	0.057	0.000
		0.20	-0.529333(*)	0.057	0.000
		0.50	-0.807250(*)	0.057	0.000
	0.10	0.00	0.664500(*)	0.057	0.000
		0.01	0.531667(*)	0.057	0.000
		0.05	0.398417(*)	0.057	0.000
		0.20	-0.130917(*)	0.057	0.026
		0.50	-0.408833(*)	0.057	0.000
	0.20	0.00	0.795417(*)	0.057	0.000
		0.01	0.662583(*)	0.057	0.000
		0.05	0.529333(*)	0.057	0.000
		0.10	0.130917(*)	0.057	0.026
		0.50	-0.277917(*)	0.057	0.000
	0.50	0.00	1.073333(*)	0.057	0.000
		0.01	0.940500(*)	0.057	0.000
		0.05	0.807250(*)	0.057	0.000
		0.10	0.408833(*)	0.057	0.000
		0.20	0.277917(*)	0.057	0.000

ESIGUAR	0.00	0.01	-0.153667	0.128	0.237
		0.05	-0.600333(*)	0.128	0.000
		0.10	-2.236250(*)	0.128	0.000
		0.20	-2.504417(*)	0.128	0.000
		0.50	-3.269083(*)	0.128	0.000
	0.01	0.00	0.153667	0.128	0.237
		0.05	-0.446667(*)	0.128	0.001
		0.10	-2.082583(*)	0.128	0.000
		0.20	-2.350750(*)	0.128	0.000
		0.05	-3.115417(*)	0.128	0.000
	0.05	0.00	0.600333(*)	0.128	0.000
		0.01	0.446667(*)	0.128	0.001
		0.10	-1.635917(*)	0.128	0.000
		0.20	-1.904083(*)	0.128	0.000
		0.50	-2.668750(*)	0.128	0.000
	0.10	0.00	2.236250(*)	0.128	0.000
		0.01	2.082583(*)	0.128	0.000
		0.05	1.635917(*)	0.128	0.000
		0.20	-0.268167(*)	0.128	0.041
		0.50	-1.032833(*)	0.128	0.000
	0.20	0.00	2.504417(*)	0.128	0.000
		0.01	2.350750(*)	0.128	0.000
		0.05	1.904083(*)	0.128	0.000
		0.10	0.268167(*)	0.128	0.041
		0.50	-0.764667(*)	0.128	0.000
	0.50	0.00	3.269083(*)	0.128	0.000
		0.01	3.115417(*)	0.128	0.000
		0.05	2.668750(*)	0.128	0.000
		0.10	1.032833(*)	0.128	0.000
		0.20	0.764667(*)	0.128	0.000

* The mean difference is significant at the 0.05 level
ESIPECTIN: ESI of emulsions in the presence of pectin
ESIGUAR: ESI of emulsions in the presence of guar gum

Table A.12. ANOVA table for the effect of different pectin concentrations on the emulsion activity index (EAI) of egg yolk granule

	Sum of squares	df	Mean square	F	Significance
Between groups	0.268	5	$5.35*10^{-2}$	4.587	0.045
Within groups	$7.0*10^{-2}$	6	$1.167*10^{-2}$		
Total	0.338	11			

Table A.13. ANOVA table for the effect of different guar gum concentrations on the emulsion activity index (EAI) of egg yolk granule

	Sum of squares	df	Mean square	F	Significance
Between groups	1.105	5	0.221	21.946	0.010
Within groups	$6.04*10^{-2}$	6	$1.007*10^{-2}$		
Total	1.165	11			

Table A.14. ANOVA and Multiple comparisons (LSD test) table for the effect of different hydrocolloid concentrations on the emulsion stability index (ESI) of egg yolk granule

ANOVA table

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	ESIPECTIN	88.054	17	5.180	165.017	0.000
	ESIGUAR	301.401	17	17.729	253.274	0.000
CONC	ESIPECTIN	61.277	6	10.213	325.370	0.000
	ESIGUAR	289.616	6	48.269	689.550	0.000
TIME	ESIPECTIN	26.776	11	2.434	77.551	0.000
	ESIGUAR	11.785	11	1.071	15.305	0.000
Error	ESIPECTIN	1.726	55	$3.139*10^{-2}$		
	ESIGUAR	3.850	55	$7.0*10^{-2}$		
Total	ESIPECTIN	89.780	72			
	ESIGUAR	305.251	72			

ESIPECTIN: ESI of emulsions in the presence of pectin
ESIGUAR: ESI of emulsions in the presence of guar gum

Multiple comparisons (LSD test)

Dependent Variable	(I) CONC	(J) CONC	Mean Difference (I-J)	Std. Error	Sig.
ESIPECTIN	0.00	0.01	$-3.033*10^{-2}$	0.072	0.677
		0.05	-0.1900(*)	0.072	0.011
		0.10	-0.0376(*)	0.072	0.000
		0.20	-0.071017(*)	0.072	0.000
		050	-0.854167(*)	0.072	0.000
	0.01	0.00	$3.033*10^{-2}$	0.072	0.677
		0.05	-0.160167(*)	0.072	0.031
		0.10	-0.345667(*)	0.072	0.000
		0.20	-0.679833(*)	0.072	0.000
		0.50	-0.823833(*)	0.072	0.000
	0.05	0.00	0.190500(*)	0.072	0.011
		0.01	0.160167(*)	0.072	0.031
		0.10	-0.185500(*)	0.072	0.013
		0.20	-0.519667(*)	0.072	0.000
		0.50	-0.663667(*)	0.072	0.000
	0.10	0.00	0.376000(*)	0.072	0.000
		0.01	0.345667(*)	0.072	0.000
		0.05	0.185500(*)	0.072	0.013
		0.20	-0.334167(*)	0.072	0.000
		0.50	-0.478167(*)	0.072	0.000
	0.20	0.00	0.710167(*)	0.072	0.000
		0.01	0.679833(*)	0.072	0.000
		0.05	0.519667(*)	0.072	0.000
		0.10	0.334167(*)	0.072	0.000
		0.50	-0.144000	0.072	0.051
	0.50	0.00	0.854167(*)	0.072	0.000
		0.01	0.823833(*)	0.072	0.000
		0.05	0.663667(*)	0.072	0.000
		0.10	0.478167(*)	0.072	0.000

		0.20	0.144000	0.072	0.051
ESIGUAR	0.00	0.01	-0.473583(*)	0.108	0.000
		0.05	-0.588750(*)	0.108	0.000
		0.10	-1.522750(*)	0.108	0.000
		0.20	-2.227167(*)	0.108	0.000
		0.50	-2.686833(*)	0.108	0.000
	0.01	0.00	0.473583(*)	0.108	0.000
		0.05	-0.115167	0.108	0.291
		0.10	-1.049167(*)	0.108	0.000
		0.20	-1.753583(*)	0.108	0.000
		0.50	-2.213250(*)	0.108	0.000
	0.05	0.00	0.588750(*)	0.108	0.000
		0.01	0.115167	0.108	0.291
		0.10	-0.934000(*)	0.108	0.000
		0.20	-1.638417(*)	0.108	0.000
		0.50	-2.098083(*)	0.108	0.000
	0.10	0.00	1.522750(*)	0.108	0.000
		0.01	1.049167(*)	0.108	0.000
		0.05	0.934000(*)	0.108	0.000
		0.20	-0.704417(*)	0.108	0.000
		0.50	-1.164083(*)	0.108	0.000
	0.20	0.00	2.227167(*)	0.108	0.000
		0.01	1.753583(*)	0.108	0.000
		0.05	1.638417(*)	0.108	0.000
		0.10	0.704417(*)	0.108	0.000
		0.50	-0.459667(*)	0.108	0.000
	0.50	0.00	2.686833(*)	0.108	0.000
		0.01	2.213250(*)	0.108	0.000
		0.05	2.098083(*)	0.108	0.000
		0.10	1.164083(*)	0.108	0.000
		0.20	0.459667(*)	0.108	0.000

* The mean difference is significant at the 0.05 level
ESIPECTIN: ESI of emulsions in the presence of pectin
ESIGUAR: ESI of emulsions in the presence of guar gum

Table A.15. ANOVA and Multiple comparisons (LSD test) table for the effect of different hydrocolloid concentrations on the creaming behavior of egg yolk granule

ANOVA table

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	CREAMPECTIN	230438.825	26	8863.032	179.954	0.000
	CREAMGUAR	109627.206	26	4216.431	115.494	0.000
TIME	CREAMPECTIN	228602.667	21	10885.841	221.024	0.000
	CREAMGUAR	73847.833	21	3516.563	96.323	0.000
CONC	CREAMPECTIN	1836.159	5	367.232	7.456	0.000
	CREAMGUAR	35779.373	5	7155.875	196.009	0.000
Error	CREAMPECTIN	4925.175	100	49.252		
	CREAMGUAR	3650.794	100	36.508		
Total	CREAMPECTIN	235364.000	126			
	CREAMGUAR	113278.000	126			

CREAMPECTIN: Creaming percentage of emulsions in the presence of pectin
CREAMGUAR: Creaming percentage of emulsions in the presence of guar gum

Multiple comparisons (LSD test)

Dependent Variable	(I) CONC	(J) CONC	Mean Difference (I-J)	Std. Error	Sig.
CREAMPECTIN	0.00	0.01	1.857143	2.166	0.393
		0.05	3.095238	2.166	0.156
		0.10	4.857143(*)	2.166	0.027
		0.20	5.142857(*)	2.166	0.019
		0.50	12.095238(*)	2.166	0.000
	0.01	0.00	-1.857143	2.166	0.393
		0.05	1.238095	2.166	0.569
		0.10	3.000000	2.166	0.169
		0.20	3.285714	2.166	0.132
		0.50	10.238095(*)	2.166	0.000
	0.05	0.00	-3.095238	2.166	0.156
		0.01	-1.238095	2.166	0.569
		0.10	1.761905	2.166	0.418
		0.20	2.047619	2.166	0.347
		0.50	9.000000(*)	2.166	0.000
	0.10	0.00	-4.857143(*)	2.166	0.027
		0.01	-3.000000	2.166	0.169
		0.05	-1.761905	2.166	0.418
		0.20	.285714	2.166	0.895
		0.50	7.238095(*)	2.166	0.001
	0.20	0.00	-5.142857(*)	2.166	0.019
		0.01	-3.285714	2.166	0.132
		0.05	-2.047619	2.166	0.347
		0.10	-0.285714	2.166	0.895
		0.50	6.952381(*)	2.166	0.002
	0.50	0.00	-12.095238(*)	2.166	0.000
		0.01	-10.238095(*)	2.166	0.000
		0.05	-9.000000(*)	2.166	0.000
		0.10	-7.238095(*)	2.166	0.001
		0.20	-6.952381(*)	2.166	0.002

CREAMGUAR	0.00	0.01	8.380952(*)	1.865	0.000
		0.05	16.523810(*)	1.865	0.000
		0.10	25.333333(*)	1.865	0.000
		0.20	42.333333(*)	1.865	0.000
		0.50	46.238095(*)	1.865	0.000
	0.01	0.00	-8.380952(*)	1.865	0.000
		0.05	8.142857(*)	1.865	0.000
		0.10	16.952381(*)	1.865	0.000
		0.20	33.952381(*)	1.865	0.000
		0.50	37.857143(*)	1.865	0.000
	0.05	0.00	-16.523810(*)	1.865	0.000
		0.01	-8.142857(*)	1.865	0.000
		0.10	8.809524(*)	1.865	0.000
		0.20	25.809524(*)	1.865	0.000
		0.50	29.714286(*)	1.865	0.000
	0.10	0.00	-25.333333(*)	1.865	0.000
		0.01	-16.952381(*)	1.865	0.000
		0.05	-8.809524(*)	1.865	0.000
		0.20	17.000000(*)	1.865	0.000
		0.50	20.904762(*)	1.865	0.000
	0.20	0.00	-42.333333(*)	1.865	0.000
		0.01	-33.952381(*)	1.865	0.000
		0.05	-25.809524(*)	1.865	0.000
		0.10	-17.000000(*)	1.865	0.000
		0.50	3.904762(*)	1.865	0.039
	0.50	0.00	-46.238095(*)	1.865	0.000
		0.01	-37.857143(*)	1.865	0.000
		0.05	-29.714286(*)	1.865	0.000
		0.10	-20.904762(*)	1.865	0.000
		0.20	-3.904762(*)	1.865	0.039

* The mean difference is significant at the 0.05 level
CREAMPECTIN: Creaming percentage of emulsions in the presence of pectin
CREAMGUAR: Creaming percentage of emulsions in the presence of guar gum

Table A.16. ANOVA table for the effect of different pectin concentrations on the emulsion activity index (EAI) of egg yolk plasma

	Sum of squares	df	Mean square	F	Significance
Between groups	0.406	5	$8.117*10^{-2}$	23.192	0.001
Within groups	$2.1*10^{-2}$	6	$3.5*10^{-3}$		
Total	0.427	11			

Table A.17. ANOVA table for the effect of different guar gum concentrations on the emulsion activity index (EAI) of egg yolk plasma

	Sum of squares	df	Mean square	F	Significance
Between groups	1.959	5	0.392	1958.567	0.000
Within groups	$1.2*10^{-3}$	6	$2.0*10^{-4}$		
Total	1.960	11			

Table A.18. ANOVA and Multiple comparisons (LSD test) table for the effect of different hydrocolloid concentrations on the emulsion stability index (ESI) of egg yolk plasma

ANOVA table

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	ESIPECTIN	88.054	17	5.180	165.017	0.000
	ESIGUAR	301.401	17	17.729	253.274	0.000
CONC	ESIPECTIN	61.277	6	10.213	325.370	0.000
	ESIGUAR	289.616	6	48.269	689.550	0.000
TIME	ESIPECTIN	26.776	11	2.434	77.551	0.000
	ESIGUAR	11.785	11	1.071	15.305	0.000
Error	ESIPECTIN	1.726	55	$3.139*10^{-2}$		
	ESIGUAR	3.850	55	$7.0*10^{-2}$		
Total	ESIPECTIN	89.780	72			
	ESIGUAR	305.251	72			

ESIPECTIN: ESI of emulsions in the presence of pectin
ESIGUAR: ESI of emulsions in the presence of guar gum

Multiple comparisons (LSD test)

Dependent Variable	(I) CONC	(J) CONC	Mean Difference (I-J)	Std. Error	Sig.
ESIPECTIN	0.00	0.01	$-3.033*10^{-2}$	0.072	0.677
		0.05	-0.190500(*)	0.072	0.011
		0.10	-0.376000(*)	0.072	0.000
		0.20	-0.710167(*)	0.072	0.000
		0.50	-0.854167(*)	0.072	0.000
	0.01	0.00	$3.033*10^{-2}$	0.072	0.677
		0.05	-0.160167(*)	0.072	0.031
		0.10	-0.345667(*)	0.072	0.000
		0.20	-0.679833(*)	0.072	0.000
		0.50	-0.823833(*)	0.072	0.000
	0.05	0.00	0.190500(*)	0.072	0.011
		0.01	0.160167(*)	0.072	0.031
		0.10	-0.185500(*)	0.072	0.013
		0.20	-0.519667(*)	0.072	0.000
		0.50	-0.663667(*)	0.072	0.000
	0.10	0.00	0.376000(*)	0.072	0.000
		0.01	0.345667(*)	0.072	0.000
		0.05	0.185500(*)	0.072	0.013
		0.20	-0.334167(*)	0.072	0.000
		0.50	-0.478167(*)	0.072	0.000
	0.20	0.00	0.710167(*)	0.072	0.000
		0.01	0.679833(*)	0.072	0.000
		0.05	0.519667(*)	0.072	0.000
		0.10	0.334167(*)	0.072	0.000
		0.50	-0.144000	0.072	0.051
	0.50	0.00	0.854167(*)	0.072	0.000
		0.01	0.823833(*)	0.072	0.000
		0.05	0.663667(*)	0.072	0.000
		0.10	0.478167(*)	0.072	0.000
		0.20	0.144000	0.072	0.051

ESIGUAR	0.00	0.01	-0.473583(*)	0.108	0.000
		0.05	-0.588750(*)	0.108	0.000
		0.10	-1.522750(*)	0.108	0.000
		0.20	-2.227167(*)	0.108	0.000
		0.50	-2.686833(*)	0.108	0.000
	0.01	0.00	0.473583(*)	0.108	0.000
		0.05	-0.115167	0.108	0.291
		0.10	-1.049167(*)	0.108	0.000
		0.20	-1.753583(*)	0.108	0.000
		0.50	-2.213250(*)	0.108	0.000
	0.05	0.00	0.588750(*)	0.108	0.000
		0.01	0.115167	0.108	0.291
		0.10	-0.934000(*)	0.108	0.000
		0.20	-1.638417(*)	0.108	0.000
		0.50	-2.098083(*)	0.108	0.000
	0.10	0.00	1.522750(*)	0.108	0.000
		0.01	1.049167(*)	0.108	0.000
		0.05	0.934000(*)	0.108	0.000
		0.20	-0.704417(*)	0.108	0.000
		0.50	-1.164083(*)	0.108	0.000
	0.20	0.00	2.227167(*)	0.108	0.000
		0.01	1.753583(*)	0.108	0.000
		0.05	1.638417(*)	0.108	0.000
		0.10	0.704417(*)	0.108	0.000
		0.50	-0.459667(*)	0.108	0.000
	0.50	0.00	2.686833(*)	0.108	0.000
		0.01	2.213250(*)	0.108	0.000
		0.05	2.098083(*)	0.108	0.000
		0.10	1.164083(*)	0.108	0.000
		0.20	0.459667(*)	0.108	0.000

* The mean difference is significant at the 0.05 level
ESIPECTIN: ESI of emulsions in the presence of pectin
ESIGUAR: ESI of emulsions in the presence of guar gum

Table A.19. ANOVA and Multiple comparisons (LSD test) table for the effect of different hydrocolloid concentrations on the creaming behavior of egg yolk plasma

ANOVA table

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	CREAMGUAR	226203.623	26	8700.139	133.933	0.000
	CREAMPECTIN	299082.016	26	11503.154	341.038	0.000
TIME	CREAMGUAR	170203.750	21	8104.940	124.771	0.000
	CREAMPECTIN	250282.167	21	11918.198	353.343	0.000
CONC	CREAMGUAR	55999.873	5	11199.975	172.417	0.000
	CREAMPECTIN	48799.849	5	9759.970	289.357	0.000
Error	CREAMGUAR	6495.877	100	64.959		
	CREAMPECTIN	3372.984	100	33.730		
Total	CREAMGUAR	232699.500	126			
	CREAMPEC	302455.000	126			

CREAMPECTIN: Creaming percentage of emulsions in the presence of pectin
CREAMGUAR: Creaming percentage of emulsions in the presence of guar gum

Multiple comparisons (LSD test)

Dependent Variable	(I) CONC	(J) CONC	Mean Difference (I-J)	Std. Error	Sig.
CREAMGUAR	0.00	0.01	0.666667	2.487	0.789
		0.05	3.380952	2.487	0.177
		0.10	26.000000(*)	2.487	0.000
		0.20	37.619048(*)	2.487	0.000
		0.50	55.666667(*)	2.487	0.000
	0.01	0.00	-0.666667	2.487	0.789
		0.05	2.714286	2.487	0.278
		0.10	25.333333(*)	2.487	0.000
		0.20	36.952381(*)	2.487	0.000
		0.50	55.000000(*)	2.487	0.000
	0.05	0.00	-3.380952	2.487	0.177
		0.01	-2.714286	2.487	0.278
		0.10	22.619048(*)	2.487	0.000
		0.20	34.238095(*)	2.487	0.000
		0.50	52.285714(*)	2.487	0.000
	0.10	0.00	-26.000000(*)	2.487	0.000
		0.01	-25.333333(*)	2.487	0.000
		0.05	-22.619048(*)	2.487	0.000
		0.20	11.619048(*)	2.487	0.000
		0.50	29.666667(*)	2.487	0.000
	0.20	0.00	-37.619048(*)	2.487	0.000
		0.01	-36.952381(*)	2.487	0.000
		0.05	-34.238095(*)	2.487	0.000
		0.10	-11.619048(*)	2.487	0.000
		0.50	18.047619(*)	2.487	0.000
	0.50	0.00	-55.666667(*)	2.487	0.000
		0.01	-55.000000(*)	2.487	0.000
		0.05	-52.285714(*)	2.487	0.000
		0.10	-29.666667(*)	2.487	0.000
		0.20	-18.047619(*)	2.487	0.000

CREAMPECTIN	0.00	0.01	0.714286	1.792	0.691
		0.05	2.333333	1.792	0.196
		0.10	4.809524(*)	1.792	0.009
		0.20	10.809524(*)	1.792	0.000
		0.50	55.666667(*)	1.792	0.000
	0.01	0.00	-0.714286	1.792	0.691
		0.05	1.619048	1.792	0.369
		0.10	4.095238(*)	1.792	0.024
		0.20	10.095238(*)	1.792	0.000
		0.50	54.952381(*)	1.792	0.000
	0.05	0.00	-2.333333	1.792	0.196
		0.01	-1.619048	1.792	0.369
		0.10	2.476190	1.792	0.170
		0.20	8.476190(*)	1.792	0.000
		0.50	53.333333(*)	1.792	0.000
	0.10	0.00	-4.809524(*)	1.792	0.009
		0.01	-4.095238(*)	1.792	0.024
		0.05	-2.476190	1.792	0.170
		0.20	6.000000(*)	1.792	0.001
		0.50	50.857143(*)	1.792	0.000
	0.20	0.00	-10.809524(*)	1.792	0.000
		0.01	-10.095238(*)	1.792	0.000
		0.05	-8.476190(*)	1.792	0.000
		0.10	-6.000000(*)	1.792	0.001
		0.50	44.857143(*)	1.792	0.000
	0.50	0.00	-55.666667(*)	1.792	0.000
		0.01	-54.952381(*)	1.792	0.000
		0.05	-53.333333(*)	1.792	0.000
		0.10	-50.857143(*)	1.792	0.000
		0.20	-44.857143(*)	1.792	0.000

* The mean difference is significant at the 0.05 level

CREAMPECTIN: Creaming percentage of emulsions in the presence of pectin

CREAMGUAR: Creaming percentage of emulsions in the presence of guar gum

Table A.20. ANOVA table for the effect of different pectin concentrations on the emulsion activity index (EAI) of egg white

	Sum of squares	df	Mean square	F	Significance
Between groups	0.381	5	$7.617*10^{-2}$	118.704	0.000
Within groups	$3.85*10^{-3}$	6	$6.417*10^{-4}$		
Total	0.385	11			

Table A.21. ANOVA table for the effect of different guar gum concentrations on the emulsion activity index (EAI) of egg white

	Sum of squares	df	Mean square	F	Significance
Between groups	0.381	5	$7.611*10^{-2}$	27.846	0.000
Within groups	$1.64*10^{-2}$	6	$2.733*10^{-3}$		
Total	0.397	11			

Table A.22. ANOVA and Multiple comparisons (LSD test) table for the effect of different hydrocolloid concentrations on the emulsion stability index (ESI) of egg white

ANOVA table

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	ESIPECTIN	46.960	17	2.762	756.354	0.000
	ESIGUAR	57.324	17	3.372	1721.641	0.000
CONC	ESIPECTIN	45.205	6	7.534	2062.920	0.000
	ESIGUAR	56.719	6	9.453	4826.551	0.000
TIME	ESIPECTIN	1.755	11	.160	43.681	0.000
	ESIGUAR	.604	11	$5.494*10^{-2}$	28.053	0.000
Error	ESIPECTIN	.201	55	$3.652*10^{-3}$		
	ESIGUAR	.108	55	$1.959*10^{-3}$		
Total	ESIPECTIN	47.161	72			
	ESIGUAR	57.431	72			

ESIPECTIN: ESI of emulsions in the presence of pectin
ESIGUAR: ESI of emulsions in the presence of guar gum

Multiple comparisons

Dependent Variable	(I) CONC	(J) CONC	Mean Difference (I-J)	Std. Error	Sig.
ESIPECTIN	0.00	0.01	$-6.9583*10^{-2}$(*)	0.025	0.007
		0.05	-0.230000(*)	0.025	0.000
		0.10	-0.252500(*)	0.025	0.000
		0.20	-0.280000(*)	0.025	0.000
		0.50	-0.379167(*)	0.025	0.000
	0.01	0.00	$6.9583*10^{-2}$	0.025	0.007
		0.05	-0.160417(*)	0.025	0.000
		0.10	-0.182917(*)	0.025	0.000
		0.20	-0.210417(*)	0.025	0.000
		0.50	-0.309583(*)	0.025	0.000
	0.05	0.00	0.230000(*)	0.025	0.000
		0.01	0.160417(*)	0.025	0.000
		0.10	$-2.250*10^{-2}$	0.025	0.366
		0.20	$-5.00*10^{-2}$ (*)	0.025	0.048
		0.50	-0.149167(*)	0.025	0.000
	0.10	0.00	0.252500(*)	0.025	0.000
		0.01	0.182917(*)	0.025	0.000
		0.05	$2.2500*10^{-2}$	0.025	0.366
		0.20	$-2.750*10^{-2}$	0.025	0.270
		0.50	-0.126667(*)	0.025	0.000
	0.20	0.00	0.280000(*)	0.025	0.000
		0.01	0.210417(*)	0.025	0.000
		0.05	$5.00*10^{-2}$ (*)	0.025	0.048
		0.10	$2.750*10^{-2}$	0.025	0.270
		0.50	$-9.9166*10^{-2}$ (*)	0.025	0.000
	0.50	0.00	0.379167(*)	0.025	0.000
		0.01	0.309583(*)	0.025	0.000
		0.05	0.149167(*)	0.025	0.000
		0.10	0.126667(*)	0.025	0.000
		0.20	$9.916*10^{-2}$ (*)	0.025	0.000

ESIGUAR	0.00	0.01	-0.110833(*)	0.018	0.000
		0.05	-0.210833(*)	0.018	0.000
		0.10	-0.325000(*)	0.018	0.000
		0.20	-0.513333(*)	0.018	0.000
		0.50	-0.548333(*)	0.018	0.000
	0.01	0.00	0.110833(*)	0.018	0.000
		0.05	$-1.000*10^{-1}$ (*)	0.018	0.000
		0.10	-0.214167(*)	0.018	0.000
		0.20	-0.402500(*)	0.018	0.000
		0.50	-0.437500(*)	0.018	0.000
	0.05	0.00	0.210833(*)	0.018	0.000
		0.01	$1.000*10^{-1}$(*)	0.018	0.000
		0.10	-0.114167(*)	0.018	0.000
		0.20	-0.302500(*)	0.018	0.000
		0.50	-0.337500(*)	0.018	0.000
	0.10	0.00	0.325000(*)	0.018	0.000
		0.01	0.214167(*)	0.018	0.000
		0.05	0.114167(*)	0.018	0.000
		0.20	-0.188333(*)	0.018	0.000
		0.50	-0.223333(*)	0.018	0.000
	0.20	0.00	0.513333(*)	0.018	0.000
		0.01	0.402500(*)	0.018	0.000
		0.05	0.302500(*)	0.018	0.000
		0.10	0.188333(*)	0.018	0.000
		0.50	$-3.500*10^{-2}$	0.018	0.058
	0.50	0.00	0.548333(*)	0.018	0.000
		0.01	0.437500(*)	0.018	0.000
		0.05	0.337500(*)	0.018	0.000
		0.10	0.223333(*)	0.018	0.000
		0.20	$3.500*10^{-2}$	0.018	0.058

* The mean difference is significant at the 0.05 level
ESIPECTIN: ESI of emulsions in the presence of pectin
ESIGUAR: ESI of emulsions in the presence of guar gum

Table A.23. ANOVA and Multiple comparisons (LSD test) table for the effect of different hydrocolloid concentrations on the creaming behavior of egg white

ANOVA table

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	CREAMGUAR	36948.655	16	2309.291	23.228	0.000
	CREAMPECTIN	78909.909	16	4931.869	107.210	0.000
TIME	CREAMGUAR	24712.618	11	2246.602	22.598	0.000
	CREAMPECTIN	74071.000	11	6733.727	146.380	0.000
CONC	CREAMGUAR	12236.037	5	2447.207	24.616	0.000
	CREAMPECTIN	4838.909	5	967.782	21.038	0.000
Error	CREAMGUAR	4970.855	50	99.417		
	CREAMPECTIN	2300.091	50	46.002		
Total	CREAMGUAR	41919.510	66			
	CREAMPECTIN	81210.000	66			

CREAMPECTIN: Creaming percentage of emulsions in the presence of pectin
CREAMGUAR: Creaming percentage of emulsions in the presence of guar gum

Multiple comparisons

Dependent Variable	(I) CONC	(J) CONC	Mean Difference (I-J)	Std. Error	Sig.
CREAMGUAR	0.00	0.01	12.000000(*)	4.252	0.007
		0.05	14.909091(*)	4.252	0.001
		0.10	27.454545(*)	4.252	0.000
		0.20	36.354545(*)	4.252	0.000
		0.50	37.636364(*)	4.252	0.000
	0.01	0.00	-12.000000(*)	4.252	0.007
		0.05	2.909091	4.252	0.497
		0.10	15.454545(*)	4.252	0.001
		0.20	24.354545(*)	4.252	0.000
		0.50	25.636364(*)	4.252	0.000
	0.05	0.00	-14.909091(*)	4.252	0.001
		0.01	-2.909091	4.252	0.497
		0.10	12.545455(*)	4.252	0.005
		0.20	21.445455(*)	4.252	0.000
		0.50	22.727273(*)	4.252	0.000
	0.10	0.00	-27.454545(*)	4.252	0.000
		0.01	-15.454545(*)	4.252	0.001
		0.05	-12.545455(*)	4.252	0.005
		0.20	8.900000(*)	4.252	0.041
		0.50	10.181818(*)	4.252	0.020
	0.20	0.00	-36.354545(*)	4.252	0.000
		0.01	-24.354545(*)	4.252	0.000
		0.05	-21.445455(*)	4.252	0.000
		0.10	-8.900000(*)	4.252	0.041
		0.50	1.281818	4.252	0.764
	0.50	0.00	-37.636364(*)	4.252	0.000
		0.01	-25.636364(*)	4.252	0.000
		0.05	-22.727273(*)	4.252	0.000
		0.10	-10.181818(*)	4.252	0.020
		0.20	-1.281818	4.252	0.764

CREAMPECTIN	0.00	0.01	0.818182	2.892	0.778
		0.05	3.272727	2.892	0.263
		0.10	11.909091(*)	2.892	0.000
		0.20	14.818182(*)	2.892	0.000
		0.50	23.727273(*)	2.892	0.000
	0.01	0.00	-0.818182	2.892	0.778
		0.05	2.454545	2.892	0.400
		0.10	11.090909(*)	2.892	0.000
		0.20	14.000000(*)	2.892	0.000
		0.50	22.909091(*)	2.892	0.000
	0.05	0.00	-3.272727	2.892	0.263
		0.01	-2.454545	2.892	0.400
		0.10	8.636364(*)	2.892	0.004
		0.20	11.545455(*)	2.892	0.000
		0.50	20.454545(*)	2.892	0.000
	0.10	0.00	-11.909091(*)	2.892	0.000
		0.01	-11.090909(*)	2.892	0.000
		0.05	-8.636364(*)	2.892	0.004
		0.20	2.909091	2.892	0.319
		0.50	11.818182(*)	2.892	0.000
	0.20	0.00	-14.818182(*)	2.892	0.000
		0.01	-14.000000(*)	2.892	0.000
		0.05	-11.545455(*)	2.892	0.000
		0.10	-2.909091	2.892	0.319
		0.50	8.909091(*)	2.892	0.003
	0.50	0.00	-23.727273(*)	2.892	0.000
		0.01	-22.909091(*)	2.892	0.000
		0.05	-20.454545(*)	2.892	0.000
		0.10	-11.818182(*)	2.892	0.000
		0.20	-8.909091(*)	2.892	0.003

* The mean difference is significant at the 0.05 level
CREAMPECTIN: Creaming percentage of emulsions in the presence of pectin
CREAMGUAR: Creaming percentage of emulsions in the presence of guar gum

Table A.24. ANOVA table for the effect of different pectin concentrations on the foam volume (FV) of egg white

	Sum of squares	df	Mean square	F	Significance
Between groups	3644.834	3	1214.945	0.974	0.480
Within groups	4990.515	4	1247.629		
Total	8635.349	7			

Table A.25. ANOVA table for the effect of different guar gum concentrations on the foam volume (FV) of egg white

	Sum of squares	df	Mean square	F	Significance
Between groups	2.014	3	0.671	2.672	0.183
Within groups	1.005	4	0.251		
Total	3.019	7			

Table A.26. ANOVA table for the effect of different hydrocolloids on the foam volume (FV) of egg white

	Sum of squares	df	Mean square	F	Significance
Between groups	0.661	1	0.661	1.403	0.281
Within groups	2.828	6	0.471		
Total	3.489	7			

Table A.27. ANOVA table for the effect of different pectin concentrations on the foam stability (FS) of egg white

	Sum of squares	df	Mean square	F	Significance
Between groups	37627.00	3	12542.333	10033.867	0.000
Within groups	5.000	4	1.250		
Total	37632.00	7			

Table A.28. ANOVA table for the effect of different guar gum concentrations on the foam stability (FS) of egg white

	Sum of squares	df	Mean square	F	Significance
Between groups	16297.375	3	5642.458	5015.519	0.000
Within groups	4.500	4	1.125		
Total	16931.875	7			

Table A.29. ANOVA table for the effect of different hydrocolloids on the foam stability (FS) of egg white

	Sum of squares	df	Mean square	F	Significance
Between groups	666.125	1	666.125	0.467	0.520
Within groups	8564.750	6	1427.458		
Total	9230.875	7			

Table A.30. ANOVA table for the effect of different pectin concentrations on the foam volume (FV) of whey protein isolate

	Sum of squares	df	Mean square	F	Significance
Between groups	0.495	3	0.165	2.538	0.195
Within groups	0.260	4	$6.5*10^{-2}$		
Total	0.755	7			

Table A.31. ANOVA table for the effect of different guar gum concentrations on the foam volume (FV) of whey protein isolate

	Sum of squares	df	Mean square	F	Significance
Between groups	2.150	3	0.717	2.783	0.174
Within groups	1.030	4	0.258		
Total	3.180	7			

Table A.32. ANOVA table for the effect of different hydrocolloids on the foam volume (FV) of whey protein isolate

	Sum of squares	df	Mean square	F	Significance
Between groups	1.125	1	1.125	2.199	0.189
Within groups	3.070	6	0.512		
Total	4.195	7			

Table A.33. ANOVA table for the effect of different pectin concentrations on the foam stability (FS) of whey protein isolate

	Sum of squares	df	Mean square	F	Significance
Between groups	61653.375	3	20551.125	6576.360	0.000
Within groups	12.500	4	3.125		
Total	61665.875	7			

Table A.34. ANOVA table for the effect of different guar gum concentrations on the foam stability (FS) of whey protein isolate

	Sum of squares	df	Mean square	F	Significance
Between groups	2374.500	3	791.500	633.200	0.000
Within groups	5.000	4	1.250		
Total	2379.500	7			

Table A.35. ANOVA table for the effect of different hydrocolloids on the foam stability (FS) of whey protein isolate

	Sum of squares	df	Mean square	F	Significance
Between groups	29890.125	1	29890.125	11.398	0.015
Within groups	15734.750	6	2622.458		
Total	45624.875	7			

Table A.36. ANOVA tables for the effects of heat treatment and hydrocolloid concentration on foam volume (FV) of heat-treated egg white

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	FVPECT	218416.285	8	27302.036	1615.386	0.000
	FVGUAR	214310.888	8	26788.861	2780.081	0.000
CONCENTRATION	FVPECTIN	216696.988	4	54174.247	3205.340	0.000
	FVGUAR	212872.740	4	53218.185	5522.850	0.000
HEAT TREATMENT	FVPECTIN	1719.297	4	429.824	25.432	0.000
	FVGUAR	1438.148	4	359.537	37.312	0.000
Error	FVPECTIN	202.815	12	16.901		
	FVGUAR	115.632	12	9.636		
Total	FVPECTIN	218619.100	20			
	FVGUAR	214426.520	20			

FVPECTIN: Foam volume in the presence of pectin
FVGUAR: Foam volume in the presence of guar gum

Table A.37. ANOVA tables for the effects of heat treatment and hydrocolloid concentration on foam stability (FS) of heat-treated egg white

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	FSPECTIN	2916932.500	8	364616.562	600.563	0.000
	FSGUAR	1599623.300	8	199952.913	1431.046	0.000
HEAT TREATMENT	FSPECTIN	2826727.500	5	565345.500	931.185	0.000
	FSGUAR	1590290.500	5	318058.100	2276.315	0.000
CONCENTRATION	FSPECTIN	90205.000	3	30068.333	49.526	0.000
	FSGUAR	9332.800	3	3110.933	22.265	0.000
Error	FSPECTIN	7285.500	12	607.125		
	FSGUAR	1676.700	12	139.725		
Total	FSPECTIN	2924218.000	20			
	FSGUAR	1601300.000	20			

FSPECTIN: Foam stability in the presence of pectin
FSGUAR: Foam stability in the presence of guar gum

Table A.38. ANOVA tables for the effects of heat treatment on emulsion activity index (EAI) of heat-treated egg yolk plasma

		Sum of Squares	df	Mean Square	F	Sig.
EAINOPOL	Between Groups	0.918	3	0.306	5815.154	0.000
	Within Groups	$2.105*10^{-4}$	4	$5.263*10^{-5}$		
	Total	0.918	7			
EAIPECTIN	Between Groups	1.013	3	0.338	22317.826	0.000
	Within Groups	$6.050*10^{-5}$	4	$1.513*10^{-5}$		
	Total	1.013	7			
EAIGUAR	Between Groups	0.216	3	$7.213*10^{-2}$	1.095	0.048
	Within Groups	0.264	4	$6.588*10^{-2}$		
	Total	0.480	7			

EAINOPOL: EAI of emulsions without hydrocolloids
EAIPECTIN: EAI of emulsions in the presence of pectin
EAIGUAR: EAI of emulsions in the presence of guar gum

Table A.39. ANOVA tables for the effects of heat treatment on emulsion stability index (ESI) of heat-treated egg yolk plasma

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	ESINOPOL	5.766	10	0.577	19.689	0.000
	ESIPECTIN	6.663	10	0.666	20.357	0.000
	ESIGUAR	29.109	10	2.911	91.296	0.000
HEAT TREATMENT	ESINOPOL	4.305	4	1.076	36.749	0.000
	ESIPECTIN	4.729	4	1.182	36.117	0.000
	ESIGUAR	27.971	4	6.993	219.321	0.000
Error	ESINOPOL	0.527	18	$2.929*10^{-2}$		
	ESIPECTIN	0.589	18	$3.273*10^{-2}$		
	ESIGUAR	0.574	18	$3.188*10^{-2}$		
Total	ESINOPOL	6.294	28			
	ESIPECTIN	7.253	28			
	ESIGUAR	29.683	28			

ESINOPOL: ESI of emulsions without hydrocolloids
ESIPECTIN: ESI of emulsions in the presence of pectin
ESIGUAR: ESI of emulsions in the presence of guar gum

Table A.40. ANOVA tables for the effects of heat treatment on emulsion stability index (ESI) of heat-treated egg yolk granule

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	ESINOPOL	20.660	10	2.066	29.447	0.000
	ESIPECTIN	21.824	10	2.182	83.964	0.000
	ESIGUAR	56.495	10	5.649	551.705	0.000
HEAT TREATMENT	ESINOPOL	18.498	4	4.625	65.914	0.000
	ESIPECTIN	18.747	4	4.687	180.308	0.000
	ESIGUAR	54.998	4	13.749	1342.714	0.000
Error	ESINOPOL	1.263	18	$7.016*10^{-2}$		
	ESIPECTIN	0.468	18	$2.599*10^{-2}$		
	ESIGUAR	0.184	18	$1.024*10^{-2}$		
Total	ESINOPOL	21.923	28			
	ESIPECTIN	22.292	28			
	ESIGUAR	56.679	28			

ESINOPOL: ESI of emulsions without hydrocolloids
ESIPECTIN: ESI of emulsions in the presence of pectin
ESIGUAR: ESI of emulsions in the presence of guar gum

Table A.41. ANOVA tables for the effects of heat treatment on creaming behavior of heat-treated egg yolk plasma

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	CREAMNOPOL	102836.500(a)	17	6049.206	367.762	0.000
	CREAMGUAR	36550.382(b)	17	2150.022	44.141	0.000
	CREAMPECTIN	81550.786(c)	17	4797.105	277.078	0.000
HEAT TREATMENT	CREAMNOPOL	93039.571	4	23259.893	1414.085	0.000
	CREAMGUAR	28268.790	4	7067.197	145.094	0.000
	CREAMPECTIN	72740.429	4	18185.107	1050.362	0.000
Error	CREAMNOPOL	641.500	39	16.449		
	CREAMGUAR	1899.598	39	48.708		
	CREAMPECTIN	675.214	39	17.313		
Total	CREAMNOPOL	103478.000	56			
	CREAMGUAR	38449.980	56			
	CREAMPECTIN	82226.000	56			

CREAMNOPOL: Creaming percentage of emulsions without hydrocolloids
CREAMPECTIN: Creaming percentage of emulsions in the presence of pectin
CREAMGUAR: Creaming percentage of emulsions in the presence of guar gum

Table A.42. ANOVA tables for the effects of heat treatment on creaming behavior of heat-treated egg yolk granule

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	CREAMNOPOL	102836.500	17	6049.206	367.762	0.000
	CREAMPECTIN	81550.786	17	4797.105	277.078	0.000
	CREAMGUAR	36550.382	17	2150.022	44.141	0.000
HEAT TREATMENT	CREAMNOPOL	93039.571	4	23259.893	1414.085	0.000
	CREAMPECTIN	72740.429	4	18185.107	1050.362	0.000
	CREAMGUAR	28268.790	4	7067.197	145.094	0.000
Error	CREAMNOPOL	641.500	39	16.449		
	CREAMPECTIN	675.214	39	17.313		
	CREAMGUAR	1899.598	39	48.708		
Total	CREAMNOPOL	103478.000	56			
	CREAMPECTIN	82226.000	56			
	CREAMGUAR	38449.980	56			

CREAMNOPOL: Creaming percentage of emulsions without hydrocolloids
CREAMPECTIN: Creaming percentage of emulsions in the presence of pectin
CREAMGUAR: Creaming percentage of emulsions in the presence of guar gum

Table A.43. ANOVA tables for the effects of heat treatment on emulsion activity index (EAI) of heat-treated whey protein isolate

		Sum of Squares	df	Mean Square	F	Sig.
EAINOPOL	Between Groups	$2.07*10^{-2}$	3	$6.904*10^{-3}$	505.753	0.000
	Within Groups	$5.460*10^{-5}$	4	$1.365*10^{-5}$		
	Total	$2.077*10^{-2}$	7			
EAIPECT	Between Groups	$6.474*10^{-2}$	3	$2.158*10^{-2}$	15.615	0.011
	Within Groups	$5.528*10^{-3}$	4	$1.382*10^{-3}$		
	Total	$7.027*10^{-2}$	7			
EAIGUAR	Between Groups	$1.038*10^{-2}$	3	$3.459*10^{-3}$	43.163	0.002
	Within Groups	$3.205*10^{-4}$	4	$8.013*10^{-5}$		
	Total	$1.070*10^{-2}$	7			

EAINOPOL: EAI of emulsions without hydrocolloids
EAIPECTIN: EAI of emulsions in the presence of pectin
EAIGUAR: EAI of emulsions in the presence of guar gum

Table A.44. ANOVA tables for the effects of heat treatment on emulsion stability index (ESI) of heat-treated whey protein isolate

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	ESINOPOL	24.643	10	2.464	18658.775	0.000
	ESIPECTIN	54.821	10	5.482	7170.889	0.000
	ESIGUAR	258.181	10	25.818	556341.056	0.000
HEAT TREATMENT	ESINOPOL	16.867	4	4.217	31927.846	0.000
	ESIPECTIN	51.394	4	12.849	16806.671	0.000
	ESIGUAR	257.811	4	64.453	1388858.603	0.000
Error	ESINOPOL	$2.377*10^{-3}$	18	$1.321*10^{-4}$		
	ESIPECTIN	$1.376*10^{-2}$	18	$7.645*10^{-4}$		
	ESIGUAR	$8.353*10^{-4}$	18	$4.641*10^{-5}$		
Total	ESINOPOL	24.645	28			
	ESIPECTIN	54.835	28			
	ESIGUAR	258.182	28			

ESINOPOL: ESI of emulsions without hydrocolloids
ESIPECTIN: ESI of emulsions in the presence of pectin
ESIGUAR: ESI of emulsions in the presence of guar gum

Table A.45. ANOVA tables for the effects of heat treatment on creaming behavior of heat-treated whey protein isolate

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	CREAMNOPOL	113540.364	14	8110.026	8209.535	0.000
	CREAMPECTIN	73015.545	14	5215.396	5137.554	0.000
	CREAMGUAR	7241.909	14	517.279	964.419	0.000
HEAT TREATMENT	CREAMNOPOL	99020.727	4	24755.182	25058.926	0.000
	CREAMPECTIN	57642.364	4	14410.591	14195.507	0.000
	CREAMGUAR	5382.000	4	1345.500	2508.559	0.000
Error	CREAMNOPOL	29.636	30	0.988		
	CREAMPECTIN	30.455	30	1.015		
	CREAMGUAR	16.091	30	0.536		
Total	CREAMNOPOL	113570.000	44			
	CREAMPECTIN	73046.000	44			
	CREAMGUAR	7258.000	44			

CREAMNOPOL: Creaming percentage of emulsions without hydrocolloids
CREAMPECTIN: Creaming percentage of emulsions in the presence of pectin
CREAMGUAR: Creaming percentage of emulsions in the presence of guar gum

Table A.46. ANOVA table for the effect of different hydrocolloid and hydrocolloid concentrations on rheological parameters (n, k) of whey protein isolate stabilized emulsions

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	n	4.533	4	1.133	16.464	0.058
	k	4.375	4	1.093	12.780	0.053
CONCENTRATION	n	4.468	3	1.489	21.635	0.045
	k	4.542	3	1.514	17.707	0.041
HYDROCOLLOID TYPE	n	$6.54*10^{-2}$	1	$6.54*10^{-2}$	0.950	0.433
	k	$6.53*10^{-2}$	1	$6.53*10^{-2}$	0.763	0.425
Error	n	0.138	2	$6.884*10^{-2}$		
	k	0.171	2	$8.550*10^{-2}$		
Total	n	4.671	6			
	k	4.546	6			

Table A.47. ANOVA table for the effect of different hydrocolloid and hydrocolloid concentrations on rheological parameters (n, k) of egg yolk plasma stabilized emulsions

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	n	4.680	4	1.170	35.189	0.028
	k	4.783	4	1.195	35.250	0.033
CONCENTRATION	n	4.635	3	1.545	46.471	0.021
	k	4.810	3	1.603	47.286	0.024
HYDROCOLLOID TYPE	n	$4.467*10^{-2}$	1	$4.467*10^{-2}$	1.343	0.366
	k	$4.87*10^{-2}$	1	$4.87*10^{-2}$	1.437	0.416
Error	n	$6.65*10^{-2}$	2	$3.325*10^{-2}$		
	k	$6.78*10^{-2}$	2	$3.39*10^{-2}$		
Total	n	4.747	6			
	k	4.851	6			

Table A.48. ANOVA table for the effect of different hydrocolloid and hydrocolloid concentrations on rheological parameters (n, k) of egg yolk granule stabilized emulsions

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	n	4.349	4	1.087	29.359	0.033
	k	4.528	4	1.132	28.089	0.036
CONCENTRATION	n	4.340	3	1.447	39.065	0.025
	k	5.594	3	1.864	46.253	0.034
HYDROCOLLOID TYPE	n	$8.994*10^{-3}$	1	$8.994*10^{-3}$	0.243	0.671
	k	$9.04*10^{-3}$	1	$9.04*10^{-3}$	0.224	0.402
Error	n	$7.406*10^{-2}$	2	$3.703*10^{-2}$		
	k	$8.06*10^{-2}$	2	$4.03*10^{-2}$		
Total	n	4.423	6			
	k	4.610	6			

Table A.49. ANOVA table for the effect of different hydrocolloid and hydrocolloid concentrations on rheological parameters (n, k) of egg white stabilized emulsions

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Model	n	3.101	4	0.775	27.689	0.035
	k	4.113	4	1.028	34.381	0.045
CONCENTRATION	n	3.075	3	1.025	36.603	0.027
	k	3.962	3	1.320	44.147	0.032
HYDROCOLLOID TYPE	n	$2.656*10^{-2}$	1	$2.656*10^{-2}$	0.948	0.433
	k	$2.851*10^{-2}$	1	$2.851*10^{-2}$	0.953	0.421
Error	n	$5.60*10^{-2}$	2	$2.80*10^{-2}$		
	k	$5.99*10^{-2}$	2	$2.99*10^{-2}$		
Total	n	3.157	6			
	k	4.173	6			

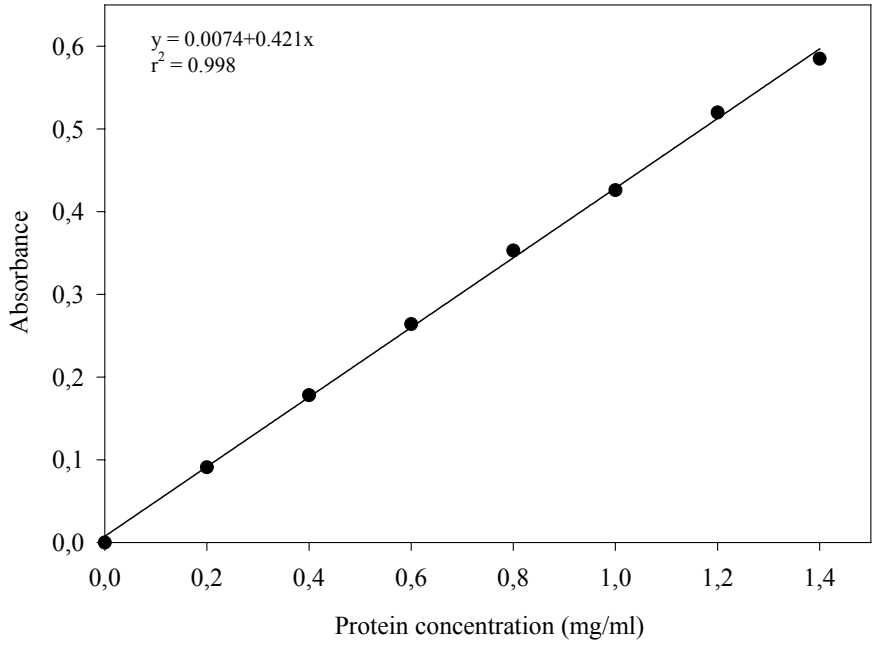

Figure A.1. Standard curve for protein determination by Biuret method

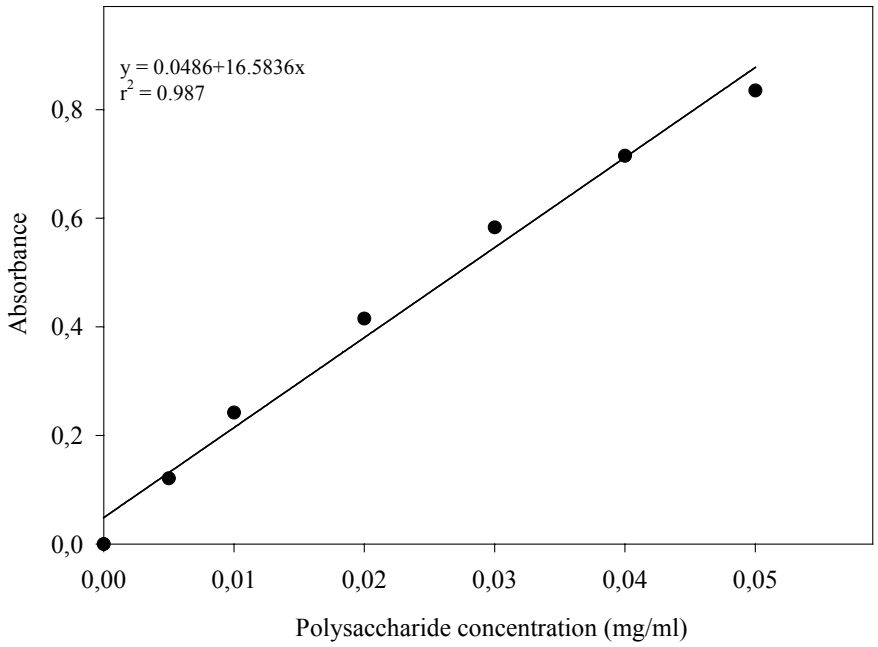

Figure A.2. Standard curve for polysaccharide determination by phenol/sulfuric acid method

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