EFFECTS OF EMULSIFIERS ON MIXING PROPERTIES, VISCOELASTIC
PROPERTIES AND STABILITY OF ZEIN-STARCH DOUGHS

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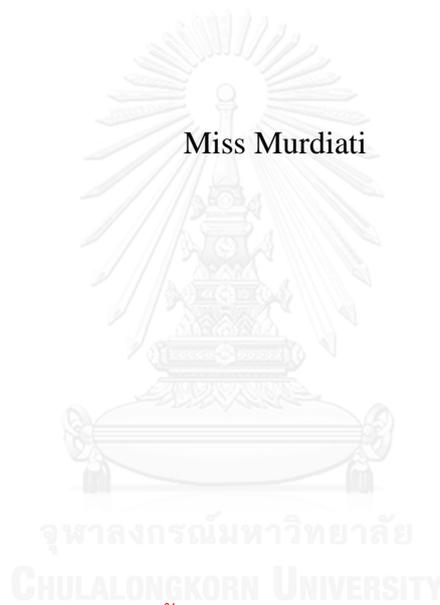
A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Food Science and Technology

Department of Food Technology

Faculty of Science

Chulalongkorn University

Academic Year 2015

ผลของสารอิมัลซิไฟเออร์ต่อสมบัติการนวดผสม สมบัติหยุ่นหนืด และความคงตัวของโดสตาร์ช
ผสมเซอีน

นางสาวมูร์ดาทิ

Thesis Title	EFFECTS OF EMULSIFIERS ON MIXING PROPERTIES, VISCOELASTIC PROPERTIES AND STABILITY OF ZEIN-STARCH DOUGHS
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มูร์ดาทิ : ผลของสารอิมัลซิไฟเออร์ต่อสมบัติการนวดผสม สมบัติหยุ่นหนืด และความคงตัวของโดสตาร์ช ผสมเซอีน (EFFECTS OF EMULSIFIERS ON MIXING PROPERTIES, VISCOELASTIC PROPERTIES AND STABILITY OF ZEIN-STARCH DOUGHS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: คริษ กวักเพชูรย์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: น้ำฝน ลำดับวงศ์, หน้า.

เซอีนซึ่งเป็นโปรตีนของข้าวโพดสามารถเกิดโดที่มีสมบัติหยุ่นหนืดแต่จะต้องนวดผสมที่อุณหภูมิสูงกว่า อุณหภูมิห้อง (35°C) และโดที่ได้ยังมีสมบัติที่ด้อยกว่าโดฟลาวร์ข้าวสาลี วัตถุประสงค์ของงานวิจัยนี้คือการศึกษาอิทธิพล ของสารอิมัลซิไฟเออร์ต่อสมบัติต่างๆของโดสตาร์ชผสมเซอีน ได้แก่ สมบัติการนวดผสม สมบัติหยุ่นหนืด และความคง ตัวของโด โดยนำสตาร์ชข้าวโพด (90% โดยน้ำหนักฟลาวร์) เซอีน (10% โดยน้ำหนักฟลาวร์) สารอิมัลซิไฟเออร์ (PATCO-3^{TM} 4-6% หรือ sodium stearoyl lactilates (SSL) 2-4% โดยน้ำหนักฟลาวร์) และน้ำ (ปริมาณที่เหมาะสม % โดยน้ำหนักฟลาวร์) มานวดผสมเป็นโดที่อุณหภูมิ 35°C และ 28°C จากการวิเคราะห์สมบัติการนวดผสมโดด้วยเครื่อง farinograph พบว่าโดที่เติมสารอิมัลซิไฟเออร์จะใช้เวลาการขึ้นโดที่นานกว่าและใช้น้ำในปริมาณที่ต่ำกว่าโดสูตรควบคุมที่ ไม่มีการเติมสารอิมัลซิไฟเออร์ โดยโดที่เติม SSL จะใช้เวลาในการขึ้นโดที่เร็วกว่าและใช้น้ำในปริมาณที่ต่ำกว่าโดที่เติม PATCO-3^{TM} โดที่เติมสารอิมัลซิไฟเออร์จะมีอุณหภูมิการเปลี่ยนแปลงค่า G' ที่ต่ำกว่าโดสูตรควบคุม โดยการเติม SSL จะ ทำให้อุณหภูมิการเปลี่ยนแปลงค่า G' ของโดลดลงได้มากกว่าการเติม PATCO-3^{TM} และ อุณหภูมิการเปลี่ยนแปลงค่า G' ของโดจะลดลงมากขึ้นตามปริมาณสารอิมัลซิไฟเออร์ที่เติมในโด เมื่อนำโดมาทดสอบการเปลี่ยนรูปร่างระดับต่ำด้วยเครื่อง rheometer พบว่าค่า δ ของโดจะลดลงเมื่อมีการเติมสารอิมัลซิไฟเออร์ซึ่งแสดงถึงโดมีความยืดหยุ่นที่เพิ่มขึ้น นอกจากนั้น ค่า G*, G', และ G' ของโดที่เติมสารอิมัลซิไฟเออร์ยังมีค่าสูงขึ้นซึ่งแสดงถึงโดมีความแข็งแรงเพิ่มขึ้น โดยการ เปลี่ยนแปลงนี้จะพบในโดที่เติม SSL มากกว่า โดที่เติม PATCO-3^{TM} สมบัติหยุ่นหนืดของโดเหล่านี้สามารถพบได้ทั้งใน โดที่ผ่านการนวดผสมที่อุณหภูมิ 35°C และ 28°C ซึ่งแสดงว่าเซอีนสามารถแสดงสมบัติเชิงหน้าที่ได้ที่อุณหภูมิห้องด้วย กลไกการลดอุณหภูมิการเปลี่ยนแปลงค่า G' ของโดด้วยสารอิมัลซิไฟเออร์ ผลจากการวิเคราะห์ด้วย FTIR พบว่าโดที่เติม PATCO-3^{TM} มีความคงตัวที่ดีกว่าโดที่เติม SSL โดยสังเกตุจากค่าที่ค่อนข้างคงที่และปริมาณที่สูงกว่าในโครงสร้างทุติย ภูมิแบบ β-sheet ของเซอีน ระหว่างการเก็บเป็นเวลา 15 นาที ที่อุณหภูมิห้อง (25-28°C) อิทธิพลของสารอิมัลซิไฟเออร์ต่อ ความคงตัวของโดนี้มีผลที่ชัดเจนกับโดที่ผ่านการนวดผสมที่อุณหภูมิ 35°C มากกว่าที่อุณหภูมิ 28°C อย่างไรก็ตามไม่ สามารถนำผลความคงตัวของโดสูตรควบคุมมาเปรียบเทียบกับโดเติมสารอิมัลซิไฟเออร์ได้เนื่องจากเกิดการแยกวัฏภาค ภายในโดสูตรควบคุมระหว่างการเก็บ ผลที่ได้จากการศึกษานี้สนับสนุนว่าสารอิมัลซิไฟเออร์ (PATCO-3^{TM} และ SSL) สามารถช่วยกระบวนการนวดผสมที่อุณหภูมิห้องให้ได้โดสตาร์ชผสมเซอีนที่มีสมบัติหยุ่นหนืด โดยการเติม PATCO- 3^{TM} มีผลที่ดีต่อการลดลงของอุณหภูมิการเปลี่ยนแปลงค่า G' สมบัติหยุ่นหนืด และความคงตัวของโดสตาร์ชผสมเซอีน ถึงแม้ว่าโดที่เติม SSL จะมีอุณหภูมิการเปลี่ยนแปลงค่า G' ที่ลดลงได้มากกว่าและมีสมบัติหยุ่นหนืดที่ดีกว่าแต่ไม่สามารถ รักษาความคงตัวไว้ได้ระหว่างการเก็บ ดังนั้นอาจจะกล่าวได้ว่าการพัฒนาโครงสร้างร่างแหของเซอีนไม่ได้ขึ้นอยู่กับการ ลดอุณหภูมิการเปลี่ยนแปลงค่า G' เท่านั้นแต่อาจยังขึ้นอยู่กับอันตรกิริยาระหว่างเซอีนและสารอิมัลซิไฟเออร์ ความ สมดุลย์ที่เหมาะสมระหว่างความชอบน้ำและความไม่ชอบน้ำของสารอิมัลซิไฟเออร์น่าจะเป็นปัจจัยที่ต้องนำมาพิจารณา เพื่อให้ได้สภาวะที่เหมาะสมต่อการลดอุณหภูมิการเปลี่ยนแปลงค่า G' สมบัติหยุ่นหนืดและความคงตัวของโดสตาร์ชผสม เซอีน

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5672606123 : MAJOR FOOD SCIENCE AND TECHNOLOGY

KEYWORDS: ZEIN / DOUGH / VISCOELASTICITY / SECONDARY STRUCTURE / DOUGH STABILITY / STORAGE MODULUS TRANSITION / MIXING PROPERTIES

MURDIATI: EFFECTS OF EMULSIFIERS ON MIXING PROPERTIES, VISCOELASTIC PROPERTIES AND STABILITY OF ZEIN-STARCH DOUGHS. ADVISOR: DARIS KUAKPETOON, Ph.D., CO-ADVISOR: ASST. PROF. NAMFONE LUMDUBWONG, Ph.D., pp.

Zein, maize protein, has ability to form viscoelastic dough but requires mixing above room temperature (35°C) and has inferior dough properties compared to wheat dough. The objective of this research was to study effect of emulsifiers on zein-starch dough's properties, including the mixing properties, viscoelastic properties, and dough stability. Corn starch (90%, w/w of flour), zein (10%, w/w of flour), emulsifier (4-6% PATCO-3™ or 2-4% sodium stearoyl lactilates (SSL), w/w of flour) and water (optimum %) were mixed at 35°C and 28°C into dough. Farinograph results revealed that emulsifier addition decreased optimum water amount and prolonged mixing time. SSL dough exhibited lower water amount and less mixing time than PATCO-3™ dough. Dough with emulsifier showed lower storage modulus G' transition temperature (T of G' transition) compared to the control. More pronounced depression in T of G' transition was shown by addition of SSL than PATCO-3™ and the higher the emulsifier concentration, the greater the decrease obtained. Small deformation test showed that the phase angle value decreased with emulsifier incorporation, indicating enhancement of its elastic characteristic. Complex modulus, storage modulus, and viscous modulus values also increased, recognized as higher dough strength. Those were more obvious in SSL dough than PATCO-3™ dough. Those trends also were shown for dough mixed at 28°C and 35°C, indicating zein could be functionalized at below room temperature through decreasing in T of G' transition. FTIR results exhibited that PATCO-3™ dough had better stability than SSL dough, as shown by relatively higher and constant of β-sheet secondary structure amount during storage for 15 min at room temperature (25-28°C). More apparent effect was seen in dough mixed at 35°C than at 28°C. Comparison with the control was not possible due to severe phase separation. The results of this study suggested that PATCO-3™ and SSL might help to form viscoelastic zein-starch dough at room temperature. PATCO-3™ had more positive effect on G' transition temperature, viscoelastic properties and dough stability. SSL addition even though resulted in lower G' transition temperature and more improved viscoelastic nature, the dough was unstable during storage. Thus, structural improvement in zein cannot be attributed only by the effect of G' transition but also the interaction between zein and emulsifier. The proper balance between hydrophobic and hydrophilic parts of emulsifier might be required to get optimum decreased T of G' transition, viscoelastic properties, and stability of zein-starch dough.

Department:	Food Technology	Student's Signature	....
Field of Study:	Food Science and Technology	Advisor's Signature	....
Academic Year:	2015	Co-Advisor's Signature	....

ACKNOWLEDGEMENTS

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CHAPTER I

INTRODUCTION

1.1   Introduction

Celiac disease is a lifetime intolerance to the gliadin of wheat which causes immunologically inflammatory damage to the small intestine mucosa (Murray 1999). At present, a lifelong avoidance of gluten products is the only treatment for the patients (Cureton and Fasano 2009). On the other hand, economics concern in the countries where wheat cannot be produced resulting in high economic dependence is also another consideration. Taking Indonesia as an example, as the net importer of wheat, total Indonesian wheat consumption in 2014 was 7.4 million metric ton, while corn as the second top grain that locally produced only covered 4.4 million metric ton (Wright 2015). That corn as the second highest local crop production in Indonesia suggests that the consumption of this commodity or other cereals that locally produced in Indonesia are supposed to be high. Thereby, looking at those above-mentioned perspectives, finding alternative cereal source as gluten substitute is highly necessary.

Wheat gluten by far is acknowledged as the only cereal protein could form viscoelastic dough (Wieser 2007; Arendt et al. 2008). However, zein, corn prolamin, could also participate in viscoelastic dough formation with properties similar to wheat dough but it requires mixing at 35°C, which is above the glass transition temperature of zein (28°C) (Lawton 1992). Moreover, the dough is attributed by poor dough properties compared to wheat dough and also not stable when the mixing is stopped and during resting time (Lawton 1992; Mejia et al. 2007; Andersson et al. 2011).

In zein plasticization, the good plasticizer for zein is defined as a substance that has proper balance between polar and nonpolar functional group, such as amphiphilic plasticizer (Di Gioia et al. 1999; Lawton 2002, 2004). Moreover, addition of compatible plasticizer generally might result in a lowering of processing temperature (Di Gioia and Guilbert 1999; Sothornvit and Krochta 2005). Lawton (1992) found that when dibutyl tartrate incorporation as amphiphlic plasticizer besides water is added, 28°C was the minimum temperature for dough formation in a farinograph. However, in this case, it is necessary to use more commonly used additives in food applications, thus the application is legal and permitted in various countries.

Emulsifier is one of food additives which possesses both hydrophilic and lipophilic properties. It is also widely used in breadmaking process as dough strengthener (Stauffer 2005). In this study, addition of two types of emulsifier to zein-starch dough was proposed, including stearoyl sodium lactylate (SSL) and PATCO-3™. The hypotheses of this study were that SSL and PATCO-3™ could lower down the mixing temperature and affect mixing properties, viscoelastic properties, and stability of zein-starch dough.

1.2   Objectives

The objectives of this present study were to investigate the influence of emulsifiers on 1) mixing properties of zein-starch dough, 2) viscoelastic properties of zein-starch dough, and 3) stability of zein-starch dough.

CHAPTER II

LITERATURE REVIEW

2.1 Dough formation

All amorphous polymers exhibit a glass transition phenomenon, including protein polymers. Glass transition is a reversible transition from glassy to rubbery state in which can be stimulated by addition of heat and plasticizer. The temperature when the transition in the amorphous polymer between glassy and rubbery occurs is known as the glass transition temperature (T_g). Below its T_g, a polymer is rigid and in glassy state, while above its T_g, it becomes rubbery and flexible (Delcour and Hoseney 2010). Above the T_g, protein polymers experience in increasing mobility and free volume, leading to enhancement of polymer reactivity. Delcour and Hoseney (2010) showed that gluten with moisture content of 16% or higher was rubbery at below room temperature (16-18°C). In turns, the gluten chains will become more mobile and could interact with other gluten chains to form gluten network (Pouplin et al. 1999).

Wheat flour components (dry basis) generally includes six groups, such as starch, storage proteins (gluten), non-starch polysaccharides (pentosans), lipids; water-soluble proteins, and inorganic compounds (ash) (Stauffer 1998). However, in this recent study, the flour model system is proposed, thus the dough system only involves three major groups of flour components, including starch, water, and protein. In dough formation, starch is relatively inert, but plays a role as a 'filler' that is responsible to increased dough viscoelasticity (Stauffer 1998). Gluten proteins are composed of two protein fractions: monomeric gliadins and polymeric glutenins

(Stauffer 1998). The latter are subdivided into low and high molecular species. It has been hypothesized that high molecular weight (HMW) glutenin subunits contribute to gluten viscoelasticity via β-sheet structure formation. Gluten is the main factor determines how well these requirements are met.

During dough formation, when adequate amount of water is introduced to dry flour the two proteins undergo change from "frozen state" to be flexible and more mobile. The process of wetting the proteins is known as hydration. Water is responsible in hydrating the protein fibrils. This is a prerequisite for operations as water is required as a medium within which all flour components become mobile and through which the many complex interactions for dough formation may take place (Millar and Tucker 2012). Optimum amount of water level is required to form cohesive, viscoelastic dough (Zaidel et al. 2010). Excessive water addition to the flour will result in slurry while too little water results in low cohesive dough. The optimum water level varies among types of flour. The strong flours, indicated by high content of protein, require mainly higher water level than weak flours as result of the higher protein content and dense particles in the strong flours (Zaidel et al. 2010).

Hydration alone is not sufficient to form dough. Applying mechanical energy (mixing) is necessarily required to develop the dough. During mixing, the mixture of flour and water transforms from thick, viscous slurry to a smooth viscoelastic mass. The final result is expected to be a dry, silky appearance, and able to form continuous membrane upon extension (Pyler and Gorton 1988). Upon hydration and mixing, the hydrated proteins are introduced to each other and start to interact. These interactions literally will form chemical bonds so that the protein chains will stick to each other. This stage is called as cross-linked of protein chains (Stauffer 1998). Letang et al.

(1999) proposed a molecular models explaining gluten development during mixing, as shown in Fig. 2.1. In these models, interaction among glutenin proteins in the loop by disulphide bonds is a key of gluten development. At the early stage of mixing, the gluten fibrils are in contact with the mixer blade, the sides of the bowl and other flour particles. The hydrated gluten fibrils and starch granules are continuously dispersed throughout. Glutenins, which are the long polymeric proteins, are folded and the chains are in random. As mixing proceeds, more protein becomes hydrated and the glutenins tend to align because of the shear and stretching forces imposed. At this stage, gluten networks are more developed by the cross-linking of protein with disulphide bonds.
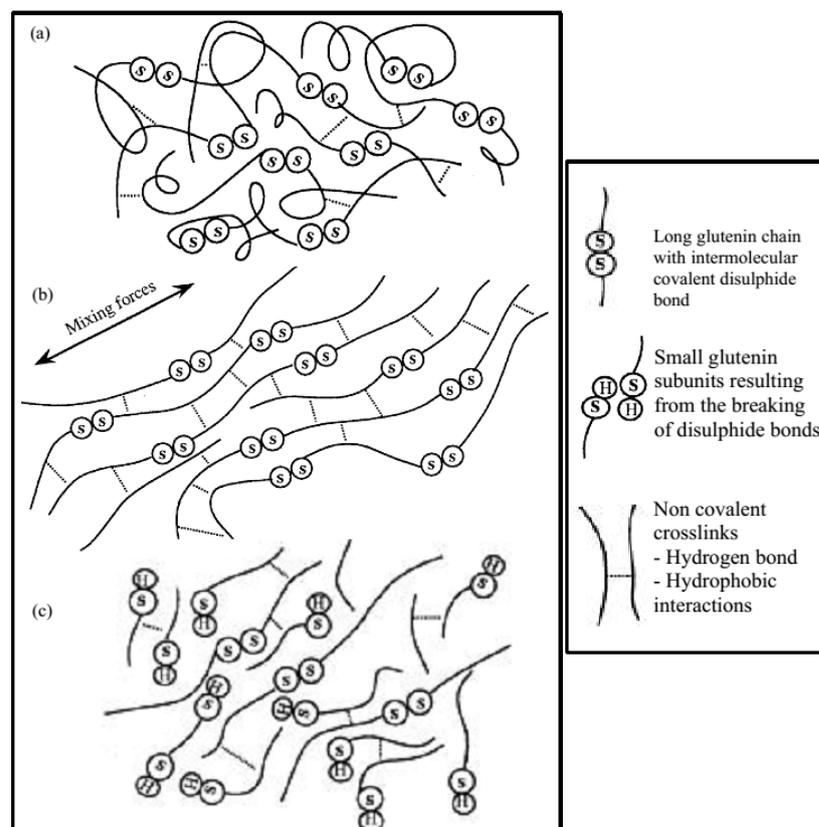
Figure 2.1 Molecular interpretation of gluten development (a) beginning of mixing, (b) optimum development and (c) overmixing (Letang et al. 1999).

At optimum dough development, the interactions between the polymers cross-links become stronger which leads to an increase in dough strength, maximum resistance to extension and restoring force after deformation. When the dough is mixed longer past its optimum development, the cross-links begin to break due to the breaking of disulphide bonds. The glutenins become depolymerised and the dough is overmixed. The presence of smaller chains in the dough makes the dough stickier. The monomeric proteins, gliadins form a matrix within the long polymer networks and contribute to resistance to extension by forming viscous behavior. Increasing the interactions between protein polymers increases gluten viscous resistance and resistance to extension. It was said that gliadins acted like a plasticizer, promoting viscous behavior and extensibility of gluten (Letang et al. 1999).

2.2   Gluten network formation

Gluten protein is typically divided into two fractions, which are gliadins and glutenins. Gliadins are monomeric proteins that has major role on viscosity and extensibility of dough, while glutenins are polymeric protein responsible for the dough strength and elasticity (Delcour and Hoseney 2010). Glutenins are divided into two forms, including high molecular weight glutenin subunits (HMW-GS) (range of molecular weights within 80,000-120,000) and low molecular weight glutenin subunits (LMW-GS) (range of molecular weight within 40,000-55,000) (Stauffer 1998). HMW-GS are chiefly responsible for gluten elasticity, even though the content in wheat flour is low, covering 12% of wheat flour proteins or 1-1.7% of flour dry weight (Shewry et al. 2000). It is believed that formation of β-sheet secondary

structures by HMW-GS is the key factor to the elastic behavior of gluten in wheat dough (Belton 1999).

Viscoelastic wheat dough only could be resulted from interactions between gliadin and glutenin. Either gliadin or glutenin alone could not form a cohesive dough alone. Its amino acid composition, structure, and its polymeric nature are the factors that determine the unique behavior of gluten (Lasztity 1995; Shewry et al. 2000). Gluten amino acid composition contains high glutamine residues, relatively high amount of proline, and low contents of basic amino acids (lysine, arginine, and histidine). Amino acids of gluten shows high amount of hydrophobic side chains (leucine, isoleucine, and proline). Thiol groups and disulfide bonds from cysteine/cystine contribute an important role in determining gluten and dough properties (Shewry et al. 2000). Sulfhydryl groups promote disulfide-sulfhydryl interchange that involves cleavage and reformation of disulfide bonds, which has contribution in dough stability and strength. Hydrophobic interactions from the hydrophobic side chains, together with the hydrogen bonding from high amide content as well as low charge density, also contribute key roles in stabilizing gluten structure and in creation of the rheological and leavening properties of wheat dough.

The importance of HMW-GS on dough elasticity was related with two factors (Shewry et al. 1995): 1) The cysteine residues involving in both intra- and inter-molecular disulfide bond formations. The elastic property of material is affected by the degree of disulfide bond. High degree of disulfide linkages will result in rubber-like elasticity, whereas low degree of disulfide linkages will give more extensible materials; 2) The β-spiral structure. Deformation and reformation of this domain play an important role in the viscoelasticity of wheat gluten. As a result of water addition

to wheat flour during mixing, HMW-GS could develop β-sheets structures through physical entanglements of aligned peptide chains (uncoiled protein structures) and through formation of end-to-end disulphide bonds and intra and intermolecular hydrogen bonds (Belton 1999).

Belton (1999) proposed the loop-and-train model (Fig. 2.2) to explain the mechanism of gluten viscoelasticity in which HMW-GS are stabilized by intermolecular hydrogen bonds, forming aligned β-sheet structures. At low hydration levels, proteins is in the form of dense mass (the train formation), resulting from protein-protein interactions through inter-molecular disulfide bonds. Upon hydration (intermediate hydration), interaction between water and protein will increase to form hydrogen bonds. Thereby, protein-protein and protein-water interactions will be in balance and loop formation will start to happen. At high hydration, more protein-water hydrogen bonds and cleavage of protein-protein inter-chain linkages will occur, resulting in a high loop to train ratio. The train regions represent β-sheet structure, whereas loop regions are associated with extended hydrated β-turn structures. Thus, at first, β-sheet content increases upon hydration. However, further hydration (excess) will lead to transition from β-sheet to β-turn structure. When extension or stretching out is applied to the system, such as during mixing, the loops will disappear and the trains are formed. If the extension force is removed and the polymer relaxes, then loops may be re-formed. For this reason, such polymers are believed to have high resistance to extension and stable viscoelastic polymers during dough mixing and proofing (Mejia et al. 2007).
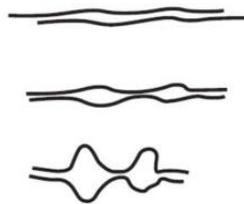
Figure 2.2 Effect of hydration on interactions between protein and water in
loop-and-train model.

2.3 Zein structure

Zein is the main protein component of the corn kernel (Lasztity 1995). It is a
prolamin protein which is located in encapsulated protein bodies (Argos et al. 1982;
Shukla and Cheryan 2001; Lawton 2002). The amino acid composition of zein shows
high hydrophobicity due to rich in hydrophobic amino acid residues such as leucine,
proline, alanine, and phenylalanine (Argos et al. 1982); whereas it lacks of basic and
acidic amino acid and has substantial amounts of glutamic acid (21-26%), leucine
(20%), proline (10%) and alanine (10%) (Shukla and Cheryan 2001). Zein is formed
from a heterogeneous mixture of aggregates linked by disulfide bonds (Shukla and
Cheryan 2001). Isolated zeins are available in commercial and the application is
mainly used as coating for food products (Lawton 2002). Based on the solubility, zein
was subdivided into four fractions: α-, β-, γ- and δ-zein (Lawton 1992).

The α-zein contributes 75 to 80% of the total maize prolamins in maize and
this type is the major fraction found in commercial zein (Lawton 2002). Its molecular
weight is in the range of 19,000 and 22,000 (Shewry and Tatham 1990). The α-zein
consists of only one or two cysteine residues in its N terminal domain. Due to limited
number of cysteine residues, this fraction is present as monomers or oligomers
(Shewry and Halford 2002). When dissolved in aqueous alcohol, α-zein displays high

contents of α-helix (≈45-60%) and low contents of β-sheet secondary structures (Shewry and Tatham 1990). Furthermore, a high constituent of inter-molecular hydrogen bonded β-sheet structures is observed upon drying of zein and lowering its water content, indicating the presence of protein aggregates whereas and increase in water activity will decrease β-sheet and increase α-helix and β-turn structures (Mizutani et al. 2003). Conversely, Duodu et al. (2001) showed that wet cooking will lead to more antiparallel intermolecular β-sheet secondary structures that might happen possibly at the expense of some α-helical conformation.

The first structural model for α-zein mixture in methanol was proposed by Argos et al. (1982). The structure of zein was concluded to have roughly 50% α-helices character. The model was composed of nine antiparallel and adjacent helices formed from repeat units of central repetitive domain and they are clustered within a cylinder (Fig. 2.3; Fig. 2.4). At the top and bottom of these helical cylinders, the glutamine residues were accumulated. This structure ensured spanning of the helices. Furthermore, polar residues were distributed along the helical surfaces, favoring intra- and inter-molecular linkages between adjacent helices. This model later was improved by Matsushima et al. (1997). It was reported that the helices arrangement was in linear instead of cylindrical arrangement (Fig. 2.5).
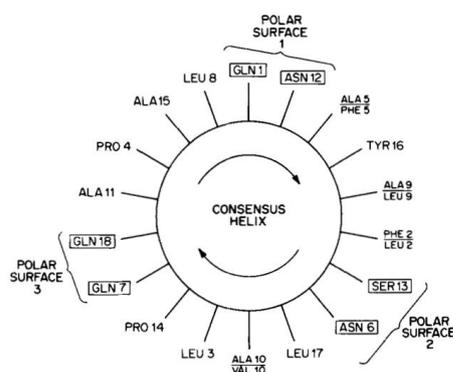
Figure 2.3 Helical wheel for the repeat units in α-zein (Argos et al. 1982).
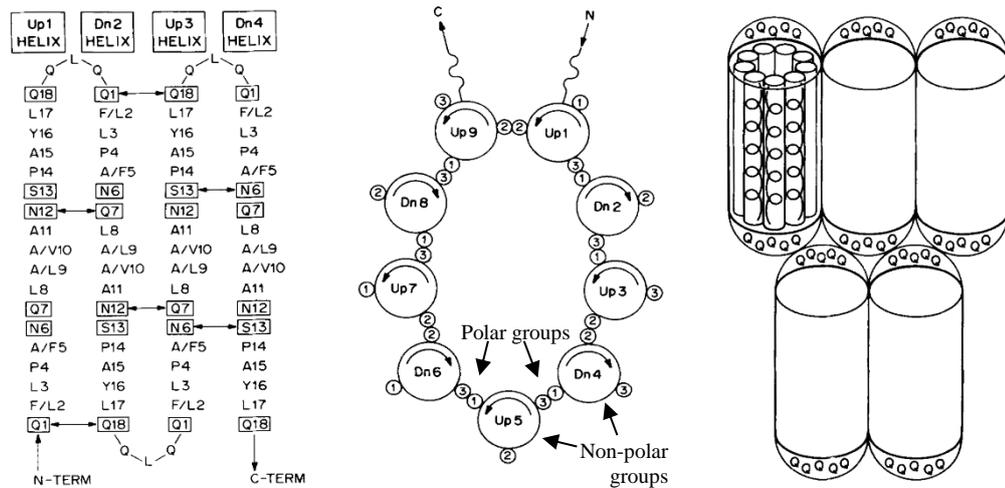
Figure 2.4   (left) A possible hydrogen bonding (↔) between polar groups in consensus of α-zein; (center) A possible nine-helical zein protein structural model; (right) A possible model for the arrangement of zein proteins within a plane and a stacking formation (Argos et al. 1982).
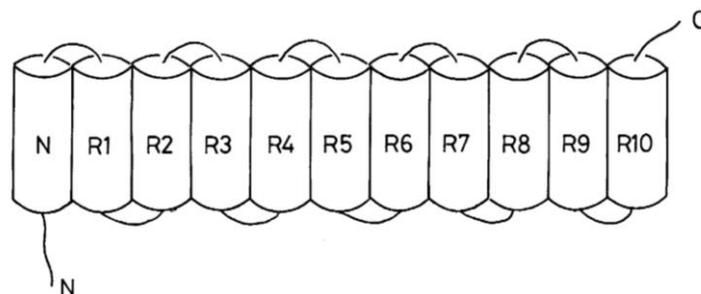
Figure 2.5 Linear arrangement of consensus helices (Matsushima et al. 1997).

β-zein accounts for 10-15% of total zein, contributing the second highest amount of zein fraction. The molecular weight is in the range of 14,000 and 16,000 (Shewry and Tatham 1990). The composition includes high amounts of methionine and cysteine. The secondary structure of purified β-zein is composed by little α-helix with the remaining being β-sheet and aperiodic (β-turn and random coil) structure (Shewry and Tatham 1990).

The γ-zein contributes 5-10% of total zein and has a molecular weight of 28,000. The extraction of γ-zein requires reducing agent. The reduced form of γ-zein is readily soluble in water (Shewry and Tatham 1990). The least fraction of total zein is δ-zein that has a molecular weight of 10,000. Similar to γ-zein, the addition of a reducing agent is needed for the extraction of this fraction (Shewry and Tatham 1990).

2.4 Sodium stearoyl-2-lactylate and calcium stearoyl-2-lactylate

Emulsifiers are surface-active agents with hydrophilic and lipophilic properties. The ratio of hydrophilic domain to lipophilic domain mainly determines the emulsifying potential of the surfactant. This ratio is called hydrophilic lipophilic balance (HLB) and is scaled from 0 to 20. Emulsifiers are further classified according to their ionization potential; ionic and nonionic. The ionic emulsifiers, namely cationic (not used in foods) and anionic emulsifiers, are used for different purposes during baking. Anionic emulsifier interact with most proteins and in turn will generally lead to unfolding of the protein structure (Nylander et al. 2008). The binding of anionic emulsifier opens up the polymer molecules, due to the increased electrostatic repulsion, and unveils new hydrophobic domains, which can bind additional surfactants (Nylander et al. 2008), as shown in Fig. 2.6. While interactions between nonionic emulsifiers and proteins are weaker and seldom affect the structure of proteins (Nylander et al. 2008).

Although the mechanisms of emulsifiers in dough strengthening are not fully understood, theories suggests that effective emulsifiers form a thin interfacial layer in between the gluten and starch granules that improved the integrity of the dough

during baking (Stampfli and Nersten 1995). Numerous studies have been carried out to determine the role of emulsifiers in bread making. Emulsifiers have been suggested to form complexes with gluten proteins that increase the strength of gluten matrix resulting in increased dough height during proofing (Gómez et al. 2004).
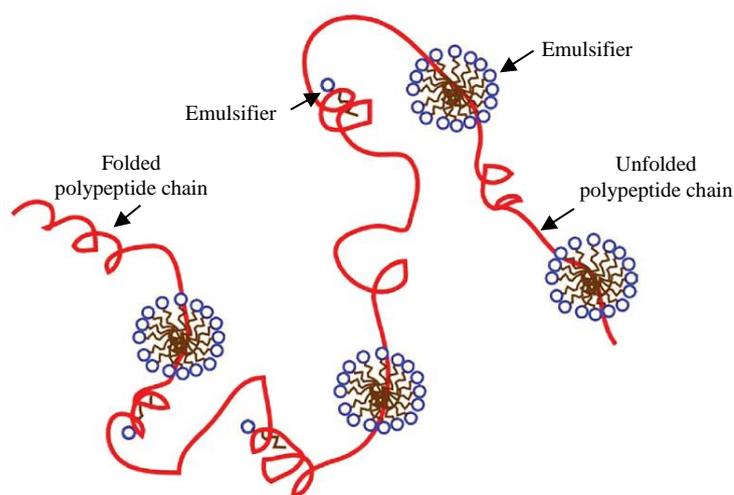
Figure 2.6   Schematic representation of the so-called necklace model for the interaction between anionic emulsifier and proteins (Nylander et al. 2008).

Among commonly used emulsifiers in bakery industry are sodium stearoyl-2-lactylate (SSL) and calcium stearoyl-2-lactylate (CSL). These emulsifiers are excellent dough strengtheners and anionic in nature (Stampfli and Nersten 1995). The HLB of CSL (E481) and SSL (E482) are 8-10 and 22, respectively. The molecular structures of SSL and HLB are given in Fig. 2.7. SSL and CSL are lactic acid esters of fatty acids. They are made by esterification of lactic acid with 1:1 blends of palmitic and stearic acids in the presence of sodium or calcium hydroxides. Thus, the sodium or calcium salts of stearyol lactylates, fatty acids salts and free fatty acids are produced (Young 2014). Lactic acid esters easily polymerise to form lactoyllactic or

polylactic acids, giving rise to a variety of lactylated compounds. SSL is a versatile, anionic, water-dispersible emulsifier, which is more frequently used than the less water-dispersible but oil-soluble CSL (Young 2014).

SSL and CSL are permitted for use as food additives in essentially all countries around the world. The maximum legal limit of SSL in the United States and Europe is 0.5% based on flour. However, in relatively mild processing conditions, it is often not more than 0.375% (Boutte and Skogerson 2004). Based on No-Observed-Adverse-Effect-Level (NOAEL) of 2200 mg/kg bw/day derived from the one-year toxicity study in rats and an uncertainty factor of 100, an Acceptance Daily Intake (ADI) is 22 mg/kg bw/day for SSL and CSL, either in single or in combination usage (EFSA 2013).

(a)

(b)
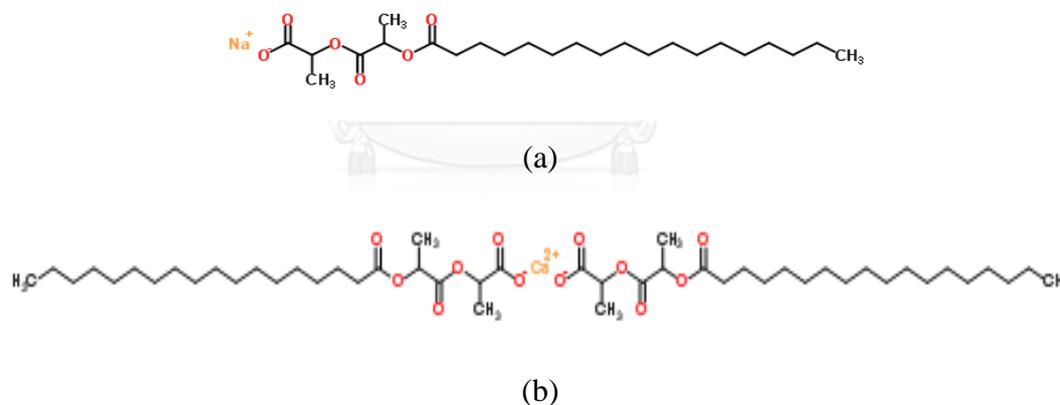
Figure 2.7 The general molecular structure of (a) SSL and (b) CSL (US-NLM 2015).

2.5 Viscoelasticity of zein-starch dough

A study by Lawton (Lawton 1992) had initiated the idea of application of zein protein in dough system and recently successive intensive studies on the viscoelastic properties of zein dough follow (Mejia et al. 2007); (Schober et al. 2008); (Fevzioglu et al. 2012); (Mejia et al. 2012). Lawton (2002) found that maize zein was unable to

form viscoelastic fibrils at room temperature like what gluten. However, α-zein was reported to form a viscoelastic protein network when the protein was held and mixed at 35°C, which is above its glass transition temperature of approximately 28°C at >20% moisture content.

Furthermore, viscoelastic properties of zein dough was affected by temperature (Lawton 1992). As dough rests at room temperature, it caused a loss of viscoelasticity. It was possibly because of the T_g of zein. Another finding of Lawton (1992) showed that the addition of dibutyl tatrate affected the extensibility of zein dough. When extended, the behavior of a zein-starch dough containing no dibutyl tartrate differs from that of doughs containing dibutyl tartrate. The former dough has little extensibility immediately after mixing and loses most of its extensibility after resting, whether kept warm or not. Obviously, dibutyl tartrate affects dough extensibility.

Moreover, zein could not form the cross-linked matrix, resulting lower resistance to extension. To test whether a relative lack of covalent intermolecular crosslinks decreases extensibility and stability of zein-starch doughs, zein was treated to be cross-linked with formaldehyde (Lawton 1992). Addition of formaldehyde could result in greater resistance to extension of dough and its dough resistance did not decrease as rapidly as dough was extended.

Study of Mejia et al. (2007) showed that relaxation of zein dough was observed to happen within seconds in the absence of stress and rapid loss of elastic properties was determined. It will not be stable for a long period of time and will collapse during baking unless modifiers are added to the gluten-free dough. They also studied on the effect of temperature and shear to content of β-sheet structures. It was

observed that zein dough exhibited similar β-structure content to that found in gluten polymers. However, upon removal of shear at 35°C, zein underwent a rapid change in its secondary structure favoring unordered structures. In contrast, gluten, under the same conditions, formed a polymeric network with stable β-sheet structures following the removal of shear. Furthermore, when the temperature was cooled down into 25°C the β-sheet structure of zein also decreased drastically, while gluten dough was maintained in its β-sheet structure. This suggested that temperature and shear are necessary not only to maintain the viscoelastic nature of the zein polymer, as found by Lawton (1992), but also to maintain the polymeric β-sheet structure.

A possible reason to explain the rapid loss of viscoelastic properties was the lack of analogous high molecular weight (HMW) subunits in maize zein that might provide stability to the β-sheet alignments (Mejia et al. 2007). Zein lacks of HMW prolamins, thus, the chains of glutenin polymers are not able to form hydrogen bonding and to contribute to the alignment of the molecules (Mejia et al. 2007). Therefore, when proofing and baking, the structure breaks, and the secondary structure collapses and returns to random coil.

2.6 Rheological measurements of dough

Rheology is defined as the science of the deformation and flow of matter (Hui 2006). The relationship between applied stress and the resulting deformation is studied by rheological measurements (Hui 2006). Stress is defined as the amount of force applied per unit area and strain is the relative deformation observed in the material structure. Response of materials to applied stress is various and it determines their rheological behavior (Zaidel et al. 2010). Ideally rheological behavior of

materials is categorized into two groups, including solid (elastic) and fluid (viscous) behavior. However, many food materials possess a rheological behavior that is categorized between the liquid and solid states. It means that they show viscous and elastic behaviors, which is defined as viscoelasticity (Zaidel et al. 2010).

The rheological property is one of dough properties that chiefly affect the quality of its final product. Thereby, many instruments have been developed to provide a description on the mechanical properties of dough to help in predicting its baking performance. Of the instruments are farinograph, mixograph, alveograph, and extensigraph (Zaidel et al. 2010). Those instruments are categorized as empirical rheological techniques in which the results could not be used to evaluate the material properties. On the other hand, fundamental rheological testing provides examination of physical properties of dough. These techniques are commonly conducted to 1) acquire quantitative information on mechanical properties of the materials, 2) explain the molecular structure and composition of the material and, 3) characterize the material behavior during processing (Dobraszczyk and Morgenstern 2003). Dynamic or oscillatory test is of the most common applied analysis. This testing is generally performed at small deformations. Thus, it is also known as 'small amplitude oscillatory shear' (Steffe 1996).

Dynamic oscillatory technique is ideal and the most sensitive testing to characterize the structural properties of viscoelastic materials (Morrison 2001). During the testing, the sample is subjected to harmonically sinusoidal that could be either stress or strain as the input. As the output, either strain or stress will be measured as a response, depending on the input used. This technique also could provide very sensitive results on chemical composition and physical structure of the

material. It is important to note that the analysis should be conducted in the linear region to avoid the strain or stress dependency (Steffe 1996). Typical parameters obtained from this testing are shear storage modulus (G') and shear loss modulus (G"). G' indicates the component of stress in phase with the strain. It is called as storage modulus since it informs the energy storage of materials. This information is useful to explain the elastic nature of material. On the other hand, G" is related to the viscous nature of the material. It provides information about the energy dissipation due to permanent molecular displacements (Rao 2007). Thus, it is termed as the loss modulus and is defined as the component of stress that is 90° out of phase with the strain (Steffe 1996). Mathematical explanations of G' and G" are shown below (Equations 2.61; 2.6.2; 2.6.3). The response of viscoelastic materials such as dough could be 1) viscous deformation as described by G", 2) elastic deformation as described by G', and 3) the ratio of loss modulus to storage modulus as defined by phase angle (δ) or tangent of phase angle (tan δ). The lower phase angle values, the higher elasticity obtained (Steffe 1996).

$$G'(\omega) = \frac{\sigma 0}{\gamma 0} \cos(\delta) \qquad (2.6.1)$$

$$G''(\omega) = \frac{\sigma 0}{\gamma 0} \sin(\delta) \qquad (2.6.2)$$

$$tan\,(\delta) = \frac{G''}{G'} \qquad (2.6.3)$$

where,

γ_0 Amplitude of the strain equal to $\frac{L \sin(\omega t)}{h}$

ω Frequency of oscillation in rad/s

σo Amplitude of shear stress. Peak force/unit area received by the fixed plate

δ Phase lag, phase shift, mechanical loss angle relative to the strain

G' Storage modulus (solid or elastic character)

G'' Loss modulus (liquid or viscous character)

$tan(\delta)$ Tangent of the phase lag or phase angle

$G*$ Complex Modulus (strength of material)

2.7   Glass transition

When the material undergoes glass transition, the material will transform from glassy state into rubbery state. A range of temperature in which the transition occurs is known as the glass transition temperature (T_g) (Roos 1992). At temperatures below the T_g, amorphous polymers are in a metastable glassy and stiff state whereas above the T_g, they soften and become rubbery (Pouplin et al. 1999). During this transition, a large change in the thermo-mechanical properties of the material at particular temperature or temperature range will be observed (Abiad et al. 2009). The material will experience smooth changes in temperature derivative, heat capacity, and expansion coefficient that are considered as a kinetic (physical change) rather than a thermodynamic phenomenon (change of phase).

Glass transition of material is affected by several factors, including water content, water activity, time, temperature, molecular weight, cross-linking degree, and concentration of plasticizer addition (Abiad et al. 2009). Particularly, plasticizers obviously affect the T_g of the material as they cause change in polymer chains structure. The T_g generally is depressed with increasing in plasticizer molar content and this is defined as a measure of plasticizer efficiency (Pommet et al. 2005). Moreover, addition of plasticizer can modify the water vapor barrier and mechanical properties of materials through increasing in extensibility, decreasing in mechanical

resistance, and may also change permeability to water vapor, which depends on the hydrophilicity of the material. In the breadmaking procedure, water is the most common plasticizer to form a dough. For example, water as plasticizer will affect T_g of gluten, resulting in change from glassy state at room temperature to be rubbery once water content (plasticizer) increased.

There are many theories explaining the mechanisms of plasticization that can lower the processing temperature (Di Gioia and Guilbert 1999; Sothornvit and Krochta 2005). The "lubricity theory" assumes that the plasticizer performs as a lubricant that enable macromolecules' movements over each other, while the "gel theory" states that plasticizer participates in the disruption of polymer-polymer interactions (hydrogen bonds and van der Waals or ionic forces) (Di Gioia and Guilbert 1999; Sothornvit and Krochta 2005). On the other hand, the "free volume theory" explains plasticization effect is related with the ability of plasticizer to increase free volume (Sothornvit and Krochta 2005). Regardless of which theory is the most suitable, the action of plasticizer is to interpose itself between the polymer chains that lead to alteration in the forces holding the chains together (Entwistle and Rowe 1978). Since hydrogen bonds (H-bonds) and nonpolar interactions act as the bonds to stabilize the protein conformation, necessarily the plasticizers must be polar but is not totally good solvents for the protein. Entwistle and Rowe (1978) also observed that the degree of plasticizer efficiency increases with decreasing degree of plasticizer-polymer interactions. The higher plasticizing effect of small molecules, such as glycerol, has been attributed to ease of insertion and positioning within the three-dimensional protein network (Kalichevsky et al. 1992). The interactions between protein chains and plasticizer are not fully understood and could be a

function of molecular weight, size, ,and hydroxyl groups number of the plasticizer (Guéguen et al. 1998). Moreover, the uniformity of plasticizer molecules distribution among the proteins is still questioned (Di Gioia and Guilbert 1999).

Commonly measurement of physical properties (thermal properties, elasticity, specific volume, conductivity deformation) of amorphous food materials as a function of temperature is used to determine the glass transition. Observation in change of viscoelastic properties is one of the method to identify the T_g (Abiad et al. 2009). This principal is applied in several techniques, such as Dynamic Mechanical Analysis (DMA), Broad Band Frequency Squeezing Flow/ Oscillatory Squeezing Flow (OSF), and rheometer. These techniques can provide information about the mechanical and thermomechanical properties of a given material by applying a sinusoidally oscillating either stress or strain to the sample causing a sinusoidal response (Abiad et al. 2009). The output will be either the strain or the stress respectively, depending on the applied input. The glass transition temperature then could be reported as the onset temperature where the first changes in the monitored properties are observed, or as the inflection point, midpoint of the steepest slope connecting the onset and offset horizontals (Abiad et al. 2009). The relationship between the stress and the strain of the sample allows the calculation of the sample mechanical modulus often known as "stiffness". The time shift between the stress and the strain is a measure of the friction generated on polymer molecules when it is deformed. The time shift is used to calculate the viscoelastic properties of the material such as the loss modulus and storage modulus. Those techniques also facilitate various testing modes such as sweep across temperature or frequency within the linear viscoelastic region (Abiad et al. 2009).

The technical challenge in T_g measurement of dough is that the sample preparation causes a change in the sample material, from the fresh dough into other state of material, such as strip, pressed dough, or powder. In the case of rheometer, Schober et al. (2008) estimates the T_g of the zein resin and zein dough by using the peak-like maximum of the tangent of the phase angle during temperature gradients. This technique was suitable for zein resin, indicating by smooth and obvious peak. However, in contrast, the curves measured with zein dough appeared very noisy. On the other hand, DMA and OSF are more commonly used testing for T_g of dough. However, the sample preparation for DMA testing requires the dough to be loaded and pressed under very high pressure (5000 lbs or more). By this procedure, the question remains if there is any effect of high pressure that might change the sample material (Abiad et al. 2009). Other sample preparation could be in the powdery material for testing using powder cell fixture. However, as the material is in a powdery form, the calculation of moduli might not represent fundamental measurements of the elastic and viscous nature of the sample. The same limitation also occurs for OSF testing that also requires the sample in the powdery state.

2.8   Fourier Transform Infrared (FT-IR) spectroscopy

Of the methods to study secondary structure of proteins is Fourier Transform Infrared (FT-IR) spectroscopy. FT-IR spectroscopy is advanced development of conventional infrared spectroscopy (Hui 2006). Infrared (IR) spectroscopy in fact is one of the earliest methods for identifying secondary structure estimation of polypeptides and proteins. The use of IR spectroscopy to determine the secondary structure of proteins was first established in 1950's. However, low sensitivity and

interfering absorptions from surrounding media or solvents limited its applications (Surewicz and Mantsch 1988).

The basis of FT-IR spectroscopy is the invention of the interferometer and conversion of the interference pattern into a spectrum using Fourier transformation (Hui 2006). The interferometer generates particular signal as results of encoding of all infrared frequencies (Hui 2006). Interferometer principally employs beamsplitter that divides each incoming infrared beam into two optical beams. One beam reflects onto a fixed flat mirror, whereas another beam reflects onto a moving flat mirror. These two beams then reflect onto their respective mirrors and will meet back at the beamsplitter to be combined into one beam. Since there are different two paths, which are a fixed length of path and constantly changing path due to moving mirror, the existing signal in the interferometer is the result from two beams interfere each other. This resulting signal is known as an interferogram (Hui 2006). The measured interferogram later will be decoded into a frequency spectrum by using mathemical technique of Fourier transformation. The typical spectrum is a plot of the intensity versus each individual frequency. The spectrum then will be used to make an identification for analysis (Hui 2006).

When IR radiation goes through the sample, some of the infrared radiation will be absorbed by the sample while some of it is just transmitted (passing through). Absorption of IR by a molecule results in transition from ground state to a specific excited state (Hollas 2002), corresponding to changes in vibrational motions of molecules and a change in the dipole moment of the molecule (Stuart 2005). Some of the chemical bonds' vibrations due to a radiation are stretching and twisting (Haris and Severcan 1999). In protein, application of IR radiation produces vibrations of

different bonds in peptide moieties (C=O, C-N and N-H groups). Those vibrations happen at discrete frequencies for specific secondary structural motifs as a result of differences in hydrogen bonding patterns and geometric orientation (Singh 2000). Vibrations of the peptide bond are categorized into different nine characteristic bands, knowing as amide bands (Table 2.1) (Singh 2000). Amide bands of protein includes Amide A, Amide B, and Amide I-VII with decreasing order of wavenumber (Stuart 2005).

Table 2.1 Characteristic amide bands of proteins with corresponding to peptide linkage vibrational motions

Designation	Wavenumber (cm^{-1})	Description
Amide A	3300	N-H stretching
Amide B	3100	N-H stretching
Amide I	1700-1600	80% C=O stretching
		10% C-N stretching
		10% N-H bending
Amide II	1575-1480	60% N-H bending
		40% C-N stretching
Amide III	1350-1200	30% C-N stretching
		30% N-H bending
		10% C=O stretching
		10% O=C-N bending
		20% other
Amide IV	770-625	40% O=C-N bending
		60% other
Amide V	800-640	N-H bending
Amide VI	610-535	C=O bending
Amide VII	200	C-N torsion

Amide I (1700-1600 cm-1) is commonly used for quantification of proteins secondary structure by FT-IR spectroscopy (Stuart 2005). This amide band is based on stretching vibrations of peptide carbonyl (C=O) groups generated by particular secondary structural forms (Stuart 2005). The nature of hydrogen bonds such as the C=O and N-H moieties is used to determine the exact frequencies of these bands (Surewicz and Mantsch 1988).

CHAPTER III

MATERIALS AND METHODS

3.1   Materials

All materials used in this study were as follows:

1. Commercial zein was purchased from Flo Chemical Corporation (Ashburnham, MA, USA) (Appendix A.1)

2. Native maize starch was donated by Friendship Corn Starch Co., Ltd. (Thailand) (Appendix A.2).

3. Sodium stearoyl lactylate (SSL) was provided by Danisco Co., Ltd. (Thailand) (Appendix A.3).

4. PATCO-3™ was purchased from UFM Food Centre Co. Ltd. (Thailand) (Appendix A.4)

5. Distilled water was used for all experiments.

3.2   Methods

3.2.1   Dough mixing properties

Dough mixing properties for all treatments were investigated by using a 300-g Brabender farinograph (Brabender® GmbH and Co. KG, Duisburg, Germany). As shown in Table 3.1, the control was made from blend of zein-starch composite flour; while incorporation of PATCO-3™ or SSL into zein dough was employed in three different concentration based on dry flour weight (w/w): PATCO-3™ at 4, 5 and 6% (P-4, P-5, P-6 respectively) and SSL at 2, 3 and 4% (S-2, S-3, S-4 respectively). Those concentrations were selected based on the emulsifier

concentration required to produce standard dough consistency according to American Association of Cereal Chemist (AACC). All dried ingredients were pre-mixed in a glass jar with a spatula and incubated at $35^{\circ}C$ for 24 hr before dough formation. Composite flour and distilled water were individually mixed into dough for 16 min at $35^{\circ}C$. Mixing properties were evaluated in terms of water absorption and dough development time. Mixing in farinograph was conducted according to AACC standard method 54-21 (AACC 1995b) and was based on dough consistency at the 475-500 FU (Farinograph Unit) line. The standard dough consistency values were resulted from several mixing trials.

Table 3.1 Composition of zein composite flour for measurements using farinograph

	Maize starch (g)	Zein (g)	PATCO-3™ (g)	SSL (g)
Control	270	30	-	-
P-4	270	30	12	-
P-5	270	30	15	-
P-6	270	30	18	-
S-2	270	30	-	6
S-3	270	30	-	9
S-4	270	30	-	12

3.2.2 Dough preparation

This dough preparation was used to prepare the sample for the rest of analysis in this study, including determination of storage modulus transition temperature, small oscillatory test, and secondary structural analysis. A modified hand mixer (Kenwood HM320, from Kenwood Limited, Havant, UK) was used to prepare all dough samples (Appendix A.5). The mixer was modified into a single kneader

mixer with a flat-bottom cylinder glassware as the mixing bowl (dimension were 3 cm diameter and 10 cm height). The mixing speed was set at 28 rpm by using a power inverter. The 12-g composite flour (Table 3.2) was pre-mixed inside glassware and incubated at $35^{o}C$ for 24 hr before dough formation. The control was blend of zein-starch added with water. Each emulsifier was employed in three levels of addition based on dry flour weight (w/w); PATCO-3™ at 4, 5 and 6% (P-4, P-5, P-6 respectively) and SSL at 2, 3 and 4% (S-2, S-3, S-4 respectively).

Distilled water ($35^{o}C$) was gradually added to the pre-mixed flour in the glassware sitting in water bath set at the temperature of $35^{o}C$. The amount water added were based on water absorption, while the optimum mixing time was adopted from the dough development time from farinogram (results of farinograph) through several trials based on the result of windowpane test (Appendix B.1). The windowpane test was conducted by stretching, pulling and turning a small piece of dough to see whether it will form a paper-thin and translucent membrane that indicates the protein network properly developed (Reinhart 2001).

Table 3.2 Composition of zein composite flour for dough preparation

	Maize starch (g)	Zein (g)	PATCO-3™ (g)	SSL (g)	Water amount (%, w/w dry flour)
Control	10.80	1.20	-	-	75.0
P-4	10.80	1.20	0.48	-	64.5
P-5	10.80	1.20	0.60	-	63.2
P-6	10.80	1.20	0.72	-	60.3
S-2	10.80	1.20	-	0.36	56.3
S-3	10.80	1.20	-	0.24	54.1
S-4	10.80	1.20	-	0.48	51.9

3.2.3 Determination of storage modulus transition temperature

Small amplitude oscillatory testing (Schober et al. 2008) was performed to determine the storage modulus transition temperature of zein doughs. Measurements were made in a rheometer (Bohlin C-VOR rheometer, from Bohlin Instruments Ltd., Gloucester, UK). Triplicate samples were analyzed with parallel plate geometry (20 mm diameter plate) at 35°C. After mixing at 35°C, dough samples were transferred to the plate with the gap of 3 mm. Sample trimming using spatula was done to excessive sample and the exposed edges were covered with grease to reduce water loss from the sample. In addition to coating, a lid was used to cover sample and plate.

The tests were performed in two modes: (a) stress sweep test and (b) temperature sweep test (Appendices B.2.1, B2.2). Stress sweep tests were conducted at a constant frequency of 1 Hz and stress range between 1 and 1×10^{3} Pa to determine the linear viscoelastic region (LVR). Within the LVR, the complex modulus, storage modulus and loss modulus were constant, indicating the structure of sample was undisturbed by the oscillatory motion; while beyond the LVR, the structure of the material was disturbed and started to break down. Therefore, any subsequent test was done within the LVR. The result was shown by plotting the complex modulus against the strain. Subsequently the temperature sweep tests were conducted to investigate changes in the sample as function of glass transition. Based on the stress sweep test results, temperature sweep tests for all samples were performed at strain of 3×10^{-3} at 1 Hz. The measurements were started at 35°C and decrease to 10°C at the cooling rate of 1°C/min (Appendix B.2.3).

The storage modulus (G') transition temperature was determined by interpretation adapted from Laaksonen and Labuza (2001). The result was expressed by plotting the increasing in storage modulus against a decreasing in temperature. The storage modulus transition (T of G' transition) was reported as the onset temperature where the slope of a linear regression line of the increasing in G' just began to increase significantly. The results of T of G' transition were used to determine two different mixing temperatures for small deformation oscillatory testing and secondary structural analysis.

The moisture contents of doughs were also determined according to AACC Method 44-15A (at 130°C for 60 min) (AACC 1995a). One-way ANOVA with post-hoc LSD test using SPSS Statistics 22.0 (IBM Corp., New York, USA) was used to form statistical grouping (α=0.01).

3.2.4 Viscoelastic properties

To investigate viscoelastic properties of zein dough samples, small amplitude oscillatory testing (Fevzioglu et al. 2012) was carried out using a rheometer (Bohlin C-VOR rheometer, from Bohlin Instruments Ltd., Gloucester, UK) with parallel plate geometry (20 mm diameter plate) at two different temperatures which were 28°C and 35°C. After mixing at 28°C or 35°C, dough sample was placed on the pre-heated bottom plate (28°C or 35°C). Then, the upper plate was lowered until the gap with the bottom plate was 3 mm. The excessive sample then was trimmed using spatula. To reduce water loss from the sample during measurement, grease was used to cover the exposed edges. In addition to coating, sample and plate are covered by a lid.

Viscoelasticity measurements were conducted using frequency sweep tests. Prior to frequency sweep test, the stress sweep test to determine the linear viscoelastic region was done as explained above (sub-chapter 3.2.3) (Appendices B.3.1, B.3.2). Based on the stress sweep , the frequency sweep tests for all samples were carried out at the strain of 3 x 10^{-3} over frequency range of 0.01-100 rad/s. Complex modulus (G*), G' (storage modulus), G" (viscous modulus) and phase angle (δ) were recorded as the results. Triplicate measurements were made.

3.2.5 Secondary structural analysis

Fourier Transform Infrared (FT-IR) spectroscopy was performed using a Nicolet Nexus 870 spectrometer (Thermo Electron Corp., Madison, WI), equipped with a diamond attenuated total reflectance (ATR) accessory and a mercury-cadmium-telluride (MCT/A) detector cooled by liquid nitrogen (Thermo Electron Corp.). According to Mejia et al. (2007), spectra were collected at a resolution of 4 cm-1 within the frequency range of 4000-650 cm^{-1} at room temperature. Interferograms from 256 scans were coadded and Fourier-transformed using the Happ/Ganzel apodization function. The empty crystal was used as background. Each dough sample was transferred to the crystal and pressed firmly to achieve a better contact after collecting the background spectra. Prior to analysis, protein spectra were obtained by subtraction of starch-emulsifier-water paste from dough spectra (Dong et al. 1990) by using OMNIC software program (Thermo Electron Corp., Madison, WI).

The dough samples were mixed for each optimum mixing time at two different mixing temperatures (35°C and 28°C). Each dough sample was monitored in change of its secondary structure during the fifteen minutes of sample relaxation at

room temperature. The zero minute was accounted after collecting the background spectra immediately. FT-IR spectra of the samples were taken after 0, 5, 10 and 15 min of storage (relaxation time) subsequently without removing the sample from the crystal.

Quantitative information on protein secondary structure was obtained by decomposition of Amide I (1600-1700 cm^{-1}) band into their components (Appendix B.4). Curve deconvolution, fitting, and peak assignment were done with OMNIC software (Thermo Electron Corp.) to quantify protein secondary structures, including 1) β-sheet (lower frequency) at 1615-1640 cm^{-1}, 2) α-helix at 1652−1657 cm^{-1}, 3) β-sheet and β-turn at 1660-1690, 4) β-sheet (higher frequency) at 1690-1700 cm^{-1} from the resolved spectra (Popineau et al. 1994). A Gaussian shape was assumed for resolved components. Full-width at half-maxima was 6.75 and kept constant for all peaks during deconvolution. Samples were analyzed in duplicate. Integrated areas were calculated for the assigned peaks that correspond to the structural elements of the protein. The fraction percentages of secondary structure were obtained by the area of the component being measured is divided by the total area of all other peaks (Equation 3.2.5.1, Appendix B.5). The amount of β-sheet secondary structures was the sum of amount of β-sheet (lower frequency), β-sheet and β-turn, and β-sheet (higher frequency) secondary structures.

$$\text{percent component peak } C = \frac{area\ of\ C}{total\ of\ peak\ areas} \times 100 \qquad (3.2.5.1)$$

where,

C = peak area of each component

CHAPTER IV

RESULTS AND DISCUSSION

4.1   Dough mixing properties

The optimum water amount is the amount of water required to reach dough consistency of 500 Farinograph Unit (FU) while the development time is indicated by peak time which is time required for dough to reach maximum consistency (Sahin and Sumnu 2006). When mixed at $35^{\circ}C$ by using a farinograph, addition of emulsifier as expected affected the mixing properties of zein-starch doughs (Table 4.1). Farinogram of all treatments are shown on Appendix C.1. Generally water absorption decreased and development time increased for all dough with emulsifier and the change was greater with higher level of emulsifier addition. While the water absorption value for control dough was found to be 75.0%, PATCO-3™ samples were observed to absorb more water (60.3-64.5%) than SSL samples (51.9-56.3%). Previous finding in wheat dough also observed the same evident of decreasing in water absorption due to addition of SSL (Gómez et al. 2004).

Table 4.1 Effect of emulsifier on mixing properties

	Water absorption (%, w/w dry flour)	Development time (minute)
Control	75.0	2.7
P-4	64.5	4.0
P-5	63.2	4.7
P-6	60.3	4.7
S-2	56.3	3.7
S-3	54.1	3.7
S-4	51.9	4.9

The time needed for dough development was prolonged by SSL and PATCO-3™, and the effect was more noticeable with incorporation of SSL than that of with PATCO-3™. Those results agree with previous findings showing SSL increased the mixing time of wheat dough (Gómez et al. 2004). However, there was no difference in dough development time at 5% and 6% added PATCO-3™ and also at 2% and 3% added SSL.

The resistance to mixing was also recorded and showed by the consistency of dough in farinogram (Fig. 4.1). At 75.0% water amount, the consistency of control did not yield at 500 FU (Fig. 4.1A) as required by AACC standard method and higher water amount addition (78.0%) could not reduce the consistency (data is not shown); while doughs with emulsifier were able to reach 500 FU (Fig. 4.1B and 4.1C).

The control reached a maximum peak within 2.7 min and all doughs with emulsifier showed a slower increase in consistency with a peak within 4.9 min of mixing (Table 4.1). Moreover, it exhibited phase separation between protein and starch-water, as shown by a slight amount of starchy liquid surrounding the dough after mixing (Appendix C.2). Similar observations were also found by Andersson et al. (2011) and Oom et al. (2008). On the other hand, zein-starch doughs with either SSL or PATCO-3™ mixed evenly and did not result in phase separation. However, the consistency of doughs containing emulsifier showed a very rapid breakdown as indicated by a rapid drop in consistency (Fig. 4.1B and 4C) while the consistency of the control was more prolonged and constant (Fig. 4.1A).

Hydration step in dough formation principally is the plasticization phenomenon by water. Water during hydration acts as main plasticizer and softener of
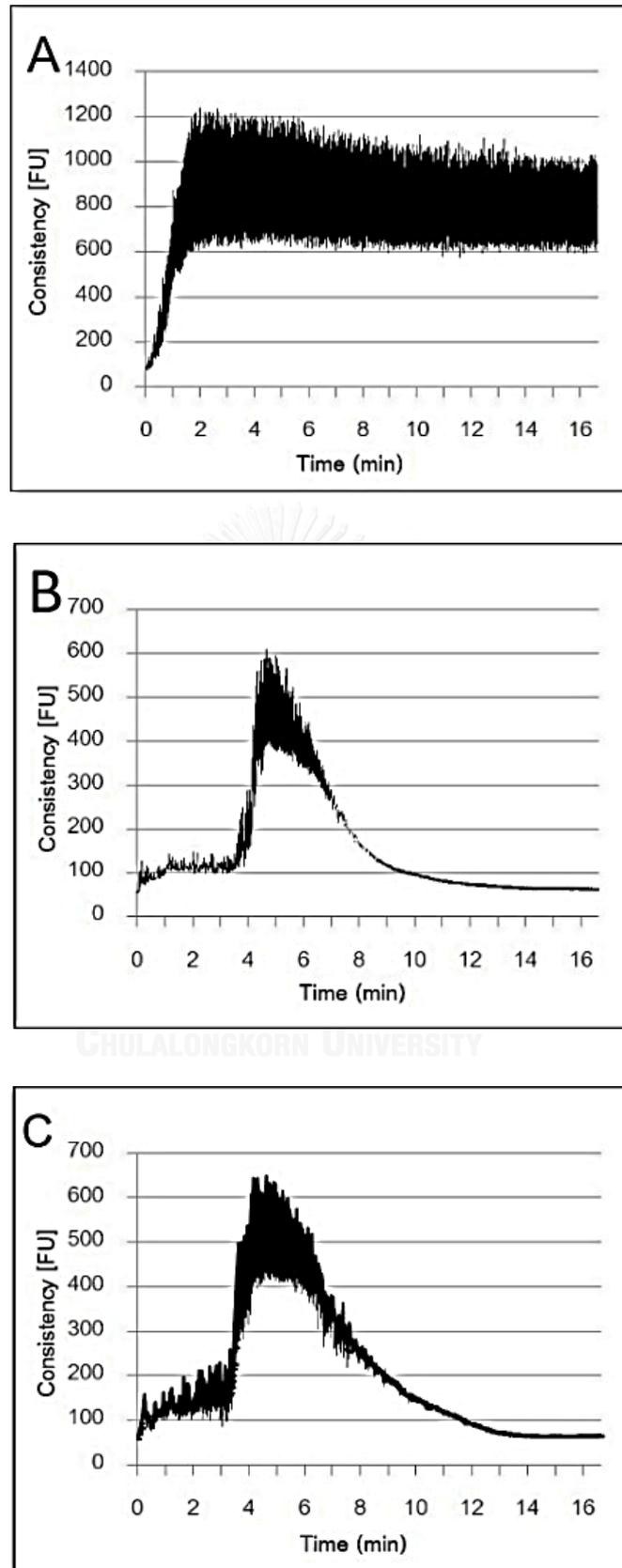
Figure 4.1 Farinogram profiles of zein-starch doughs. A: Control, B: P-6, C: S-4.

major hydrogen bonding of proteins (Slade and Levine 1991; Roos et al. 1996). It leads to breakage of hydrogen bonds resulting in conformational changes of protein structures from tertiary structures into α-helix secondary structures (coiled structures) and further hydration and mixing action facilitate a change from coiled structures into uncoiled primary structures (Griffin and Lynch 1973; Amemiya and Menjivar 1992). It in turn lets the formation of β-sheet structures as the main component determining dough viscoelasticity (Belton et al. 1995).

Particularly on a molecular basis, Argos et al. (1982) proposed a model of zein's tertiary structure based on nine α-helices secondary structures rich in hydrophobic residues joining by intra- and intermolecular hydrogen bonds and those nine α-helices form nine adjacent repeats arranged in an antiparallel ring connecting by glutamine-rich loops. The highly hydrophobicity of of α-helices secondary structures' surface confirms that hydration by water alone in zein dough system might be difficult to take place. Moreover, the fact that the high consistency of the control dough at mixing temperature of 35$^\text{o}$C also suggests that even at temperature above the zein's T_g, zein is still difficult to hydrate. For this reason, it is thought that the consistency of the control dough might be mostly as a result of frictions of flour components instead of protein network formation. The friction had more contribution to the dough consistency than the protein network and it was generated almost immediately after mixing started and it occurred faster than protein network formation. Thereby, the control dough obtained much shorter development time. It is also an indication that water alone might be not able to hydrate the composite flour, thus short time of mixing was sufficient. Furthermore, to reduce the consistency due to frictions, water might have a role as a lubricant. This is why the control dough

required higher amount of water. However, the water was added beyond the ability of flour to hold the water due to low hydrophilicity of zein. As a result, phase separation occurred. On the other hand, less water amount but resulting in good dough consistency for dough with emulsifier could give insight that better plasticization might occur in those dough samples. It also might mean that total plasticizer amount is sufficiently available for dough formation. Thus, it is believed that the good consistency of the dough samples with emulsifier might be resulted mainly from protein network.

The lower optimum water amount due to added emulsifier might be related with the role of emulsifier as plasticizer to improve plasticization prior to dough mixing. The gel theory states that the mechanism of plasticization is initiated by attachment of plasticizer molecules along the polymer chains (Sothornvit and Krochta 2005). This will replace polymer–polymer attachments at places and hinder the forces holding polymer chains together. In turn, this reduces the rigidity of the gel structure, resulting in increased gel flexibility. Furthermore, plasticizer molecules that are not attached to polymer form aggregated plasticizer domains, acting like a lubricant to facilitate the movement of polymer molecules. From the point of view of its molecular structure, amphiphilic structure owned by emulsifier might help plasticization upon addition of water to zein composite flour. Its amphiphilic nature of emulsifier might provide an ease for emulsifier to insert into the tertiary structures of zein. According to the plasticization theory mentioned above, the polar side of emulsifier might interrupt hydrogen bonding along the zein's tertiary structures, leading to conformational changes into its secondary structures. As a result, the subsequent steps will follow. Further hydration together with mixing action will

promote a change from coiled structures into uncoiled primary structures with subsequent formation of β-sheet structures.

During hydration, the total water required for dough mixing is divided into two types of water, which are 1) bound water which is firmly attached to the protein structure and has ability to penetrate the conformational protein structures. This water is lost both in its mobility and its solvent properties; 2) free water that exists as the least firmly held water, still retaining much of its mobility and solvent action (Pyler and Gorton 1988). For this reason, that the lower optimum water amount either for doughs with SSL or doughs with PATCO-3™ is considered as a result of the role of emulsifier as a second plasticizers for zein dough besides water. In this case, some amount of emulsifier might replace a function of bound water, thus it compensates the required total amount of 'water', resulting lower optimum water amount in zein doughs with emulsifier.

The molecular structure of SSL is a linear hydrophobic chain attached by one molecule of Na^+ as the hydrophilic side and its molecular weight is 450.58 g/mol (EFSA 2013; US-NLM 2015). On the other hand, CSL in PATCO-3™ has bigger structure as shown by Ca^+ as its polar side attached between two hydrocarbon chains and its molecular weight is 895.26 g/mol (EFSA 2013; US-NLM 2015). By that linear structure and smaller molecular weight, SSL is easier to attach to the polymer chain sites of zein to hinder the forces holding zein polymers together. In contrast, CSL might be more difficult to occupy the polymer chain sites of zein due to more complicated chemical structure. Moreover, that polar side (Na^+) of SSL adjacent to only one hydrocarbon chain provides more accessible interaction for water as surrounding environment and it is also able to act like plasticizer molecules that are

not attached to polymer, facilitating the movement of polymer molecules; whereas CSL with its polar side attached between two hydrocarbon chains is more difficult to interact with water for acting as lubricant. For that reason, SSL is considered to be more compatible plasticizer to water than PATCO-3™. Hence, compared to PATCO-3™ and water, SSL together with water is considered as better medium in making the zein protein chains more mobile. This is why lower amount of water and lower level of added SSL was enough for zein-starch dough formation. On the other hand, higher water amount was needed for PATCO-3™ doughs due to the less compatibility of CSL in PATCO-3™ to water. Moreover, higher level of PATCO-3™ incorporation is required to compensate the effect of CSL.

The mixing time or dough development time is related with the transition from dry flour and water to the viscoelastic dough, indicating by the initial part of the mixing curve showing an increase in resistance until reach the peak or maximum consistency when all the flour particles are hydrated (Letang et al. 1999; Millar and Tucker 2012). Doughs with stronger or more elastic property generally need longer times to reach the peak resistance (Stauffer 1998). Hence, the higher degree of plasticization in zein dough with emulsifier might be a reason why longer mixing time was required to develop the dough. Doughs with PATCO-3™ had slightly longer mixing time than doughs with SSL, which might mean the protein network developed slower in PATCO-3™ doughs than SSL doughs. It might be partly due to the less ability of PATCO-3™ to help hydration compared to SSL. The similar trend is also observed by Andersson et al. (2011). They found that hydrocolloid which could help dough hydration better than β-glucan required shorter mixing time to produce zein dough.

4.2 Determination of storage modulus transition temperature

Principally, mixing temperature in breadmaking process is strongly related to the glass transition concept and its effects on protein system. However, due to technical challenge in determination of glass transition temperature in dough system, this experimental study was focused on finding the minimum mixing temperature. The proposed method was through observation of the rheological change in dough. The used parameter for observation was storage modulus (G') since it confers the elastic properties of dough that highly related with the protein network of dough.

Storage modulus transition profiles and moisture content of doughs were obtained for each sample to study the influence of emulsifier on mixing temperature of zein-starch dough (Fig. 4.2 and Table 4.2). Figure 4.2 shows typical changes in the dynamic-mechanical properties of the zein-starch dough, as a function of temperature measured by rheometer at Hz. As the temperature cooled down from 35°C, the storage modulus (G') of all dough samples increased (Appendix C.4). There was a temperature in which the rate of increasing G' significantly changed. This temperature could be identified by looking at the first temperature making the G' failed out of the regression line of the initial modulus. This temperature could be considered as a storage modulus transition temperature (T of G' transition). This interpretation was adapted from glass transition concept in which at temperature below glass transition temperature a material becomes hardened as a result of limited interaction among the component (Matveev et al. 1997; Abiad et al. 2009). Moreover, its ability to store energy is partially increased resulting in higher G' at lower temperature (Nikolaidis and Labuza 1996; Laaksonen and Labuza 2001; Abiad et al. 2009). The determined T
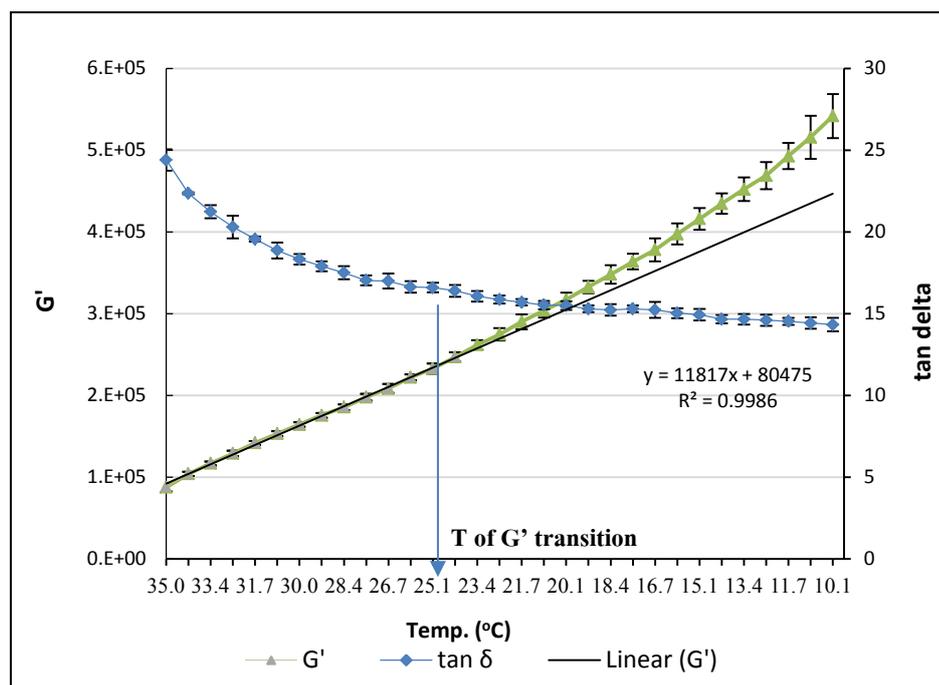
Figure 4.2   Example of a dynamic-mechanical properties profile for zein-starch dough containing emulsifier (P-6).

Table 4.2   G' transition temperature of zein-starch doughs with different moisture content

	% Dough moisture (wb)	T of G' transition (^{o}C)
Control	46.35 ± 1.12^{a}	25.03 ± 0.85^{a}
P-4	43.62 ± 0.18^{b}	26.17 ± 0.92^{b}
P-5	42.90 ± 0.22^{b}	25.33 ± 0.98^{b}
P-6	41.13 ± 0.49^{c}	24.50 ± 0.52^{c}
S-2	40.88 ± 0.30^{c}	22.53 ± 0.85^{c}
S-3	38.90 ± 0.21^{d}	18.67 ± 1.32^{d}
S-4	37.34 ± 0.07^{e}	13.93 ± 0.92^{e}

Means followed by different letters within the same column were significant differences ($p<0.01$).

of G' transition are shown in Appendix C.3 and the analysis of variance is shown in Appendices D.1 and D.2.

Both PATCO-3™ and SSL clearly showed the plasticizing effect of emulsifier, as shown by lower T of G' transition (Table 4.2), and the greater effect was more obvious with higher level of emulsifier. The T of G' transition of doughs with SSL had more pronounced T of G' transition depression compared to doughs with PATCO-3™. Those results support previous works explaining that good plasticizer for zein should have amphiphilic property (Lawton 2002, 2004). Furthermore, the T of G' transition of the control was 25.03°C at 46.35% of moisture content. The current result confirms previous findings that development of viscoelastic zein dough required mixing above 25°C (Lawton 1992; Mejia et al. 2007; Schober et al. 2008; Andersson et al. 2011).

The moisture content of the doughs were between 37.34-46.35% (Table 4.2). Doughs with added emulsifier had significantly lower moisture content than the control ($p<0.01$) and doughs with added SSL had lower moisture content than doughs with added PATCO-3™ counterparts. The lower moisture contents were due to lower optimum water amount required for dough formation. Furthermore, with a significant decrease ($p<0.01$) in moisture content, all dough samples with PATCO-3™ showed the T of G' transition at close temperature with that of the control dough, while the T of G' transition of all dough samples with SSL were observed at significantly lower temperature ($p<0.01$).

In glass transition concept, it is well known that principally when the water content decreases, the glass transition temperature increases. Referring to this concept, the results of the present study did not follow the theory. Even though

doughs with PATCO-3™ resulted in similar T of G' transition to the control dough, by their lower water content values the results were still considered to demonstrate a T of G' transition depression. Furthermore, the lower T of G' transition of doughs with emulsifier but with lower dough water content values might confirm the role of emulsifier as second plasticizer besides water in dough formation. Hence, it compensates the total amount plasticizer required in zein dough system, resulting in lower optimum water amount for dough mixing and consequently also making the water content of doughs lower. The more noticeable T of G' transition depression in doughs with SSL might also confirm that combination of SSL and water could performed better plasticizing effect than PATCO-3™ combined with water and zein becomes more mobile with the presence of SSL than when PATCO-3™ is present. Furthermore, lower T of G' transition of zein doughs with emulsifier might suggest that their plasticizer activities increase the zein mobility and its interaction in dough formation.

As discussed above in the effect of emulsifier on dough mixing properties (sub-chapter 4.1), the gel theory is thought as appropriate plasticization theory to explain the mechanism of plasticization by emulsifier and water in this study. According to Sothornvit and Krochta (2005), the gel theory proposes that the action of a plasticizer is to interpose itself between the polymer chains and alter the forces holding the chains together (hydrogen bonds and van der Waals or ionic forces). That the hydrogen bonds of zein polymer chains surrounded by the highly hydrophobicity of α-helices secondary structures (Argos et al. 1982) suggests that the totally hydrophilic water, even though it possesses small molecule structure, might find difficulty to access and break the hydrogen bonds. On the other hand, emulsifier with

its amphiphilic structure might have higher chance to go through that zein's structures and to break the hydrogen bonds. Moreover, SSL together with water might also take role as lubricant due to its better compatibility to water compared to PATCO-3™. The final result is higher degree of plasticization effect and more mobile zein protein chains. In addition, linear structure and more accessible polar part of SSL suggests that SSL provides higher degree of plasticization than PATCO-3™ with its bigger structure and less accessible polar part. The higher degree of plasticization, the more mobile zein protein polymers and the more interactions may be perceived. Furthermore, the mobility of zein polymers can be maintained at lower temperature before the T of G' transition is obtained.

The findings of this testing also show that SSL or PATCO-3™ incorporation could be employed in lowering dough mixing temperature. Based on T_g's results, two mixing temperature were chosen for next studies: 1) 35°C, representing mixing at above room temperature, 2) 28°C, representing mixing at below room temperature (30°C).

4.3   Viscoelatic properties

The complex modulus (G*) provides information about the strength of the material as it relates information on viscous modulus (G'') and elastic modulus (G') of material. The material with low G* indicates low strength of the system. The effect of emulsifier addition on complex modulus of dough samples with different zein-emulsifier composites (SSL or PATCO-3™) at two different temperatures (28°C and 35°C) are given in Fig. 4.3. At both mixing temperatures, the control doughs exhibited low G* values, indicating low strength of doughs. Addition of emulsifier generally
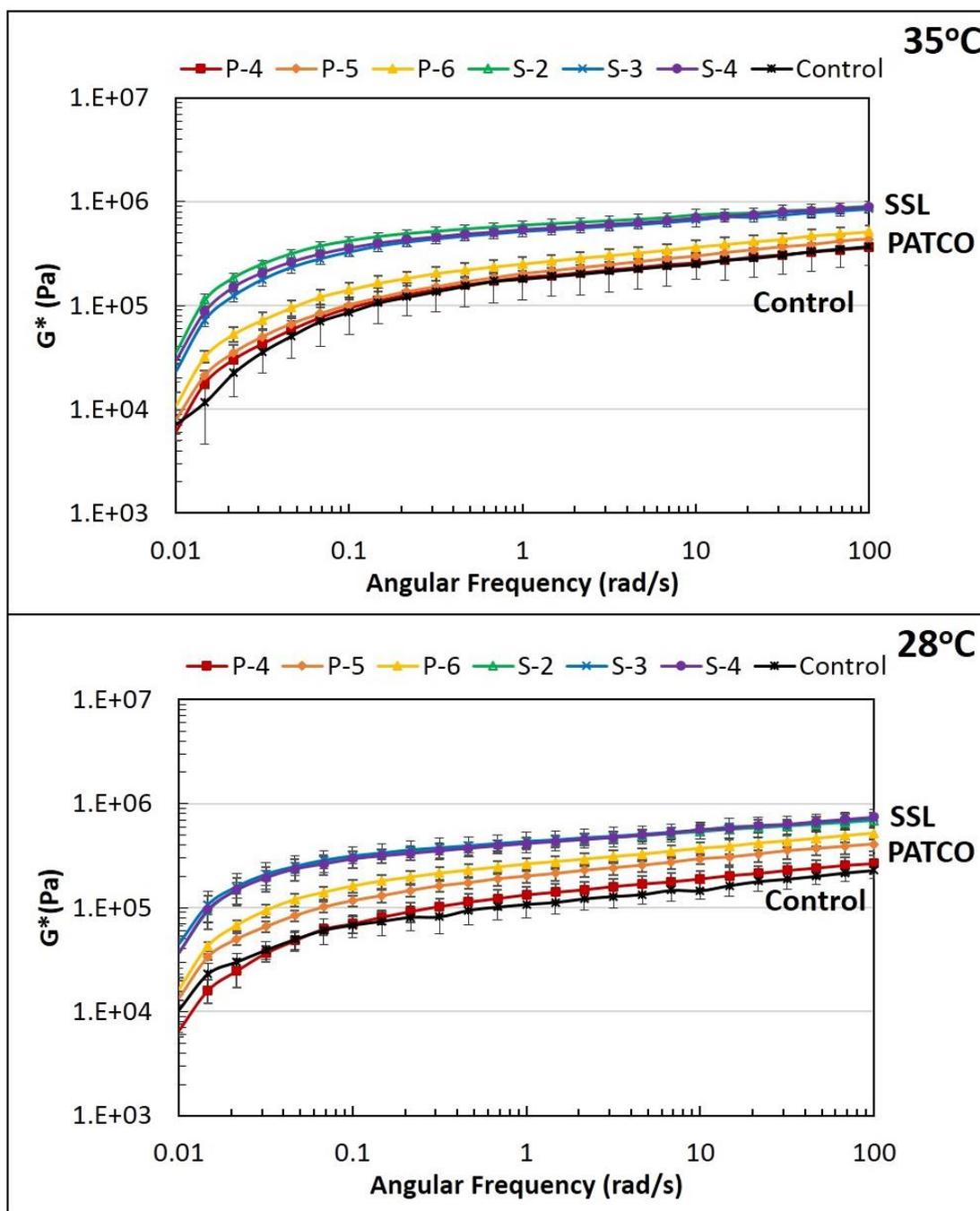
Figure 4.3   Complex modulus (G*) as a function of angular frequency for the zein-starch dough and the zein-starch dough with emulsifier mixed and tested at at 28°C and 35°C.

caused increase in the G* values which meant higher dough strength. These results indicated that the interaction between emulsifier and zein improved the viscoelastic properties of zein-starch doughs. These data is in accordance to the similar results observed in wheat flour and composite rice flour. Shiau (2004) observed that the G* values significantly increased as a result of addition of SSL with the concentrations from 0.5 to 1.5% to wheat dough. On the other hand, Sciarini et al. (2012) found that incorporation of 1% SSL to the dough based on composite flour made from rice flour, cassava starch and full-fat active soy led to a substantial increasing in the G* values.

As seen in Fig. 4.3, the G* values of all dough samples were increased as a function of frequencies or deformation rates. At both mixing temperatures, the control doughs exhibited the lowest G* values, indicating the lowest strength of doughs compared to the remaining dough samples. Furthermore, at the higher angular frequency, the G* value of the control dough at 28°C was slightly higher than that of the control dough at 35°C. As described in the previous section, the possible explanation might be that mixing at lower temperature (28°C) resulted in poorer hydration than at 35°C, thus less protein network formation was obtained. Moreover, at the beginning of lower angular frequency, the control doughs were indicated by unsmooth curves, especially for dough mixed at 28°C. This might be just a non-uniformly mixed dough due to a poor hydration, thus given not smooth curve and had some error on the measurement. The phase separation also occurred during measurement for both dough mixed at 28°C and 35°C (Appendix C.5).

Besides, the incorporation of SSL at all different levels increased G* at higher values than PATCO-3™ addition, which suggested that SSL had more marked effect in dough strengthening than PATCO-3™. At 35°C the dough with 2% SSL exhibited

slightly higher G* value than the doughs with 3% or 4% of SSL addition. However, at 28°C the gap among all SSL doughs became slightly narrower. Furthermore, the doughs with 4% added PATCO-3™ at both mixing temperatures were attributed by similar dough strength as compared to the control, while the doughs with 6% incorporated PATCO-3™ resulted in the highest dough strength among the PATCO-3™ doughs. At 28°C, slightly higher G* for the doughs with either added 5% or 6% PATCO-3 were observed as compared to mixing at 35°C.

Fig. 4.4 displays elastic modulus (G') and viscous modulus (G") of all dough samples mixed at 35°C and 28°C, respectively. G' value indicates the energy storage of materials and is useful to explain the elastic nature of material. On the other hand, G" value is related to the viscous nature of the material. At initial frequency all dough samples at both mixing temperatures possessed slightly higher G" values than G'. Moreover, the G' values increased in higher rate than G" values at higher frequency. Theses indicate that all samples at initial frequency had a viscous-like behavior and became to elastic-like behavior at higher deformation.

Overall, G' and G" values for all dough samples at both mixing temperatures showed similar trend like G* values, such as 1) generally the control dough showed low G' and G" values with dough at 28°C exhibited slightly higher value than that of at 35°C, 2) G' and G" values of SSL doughs were higher than PATCO-3™ doughs. Likewise, the trends of G' and G" magnitudes among SSL doughs or PATCO-3™ doughs at different concentration were also in accordance to G* values. It is reasonable since G* value is derived from G' and G" value.

The effects on phase angles (δ) of zein dough samples as results of emulsifier
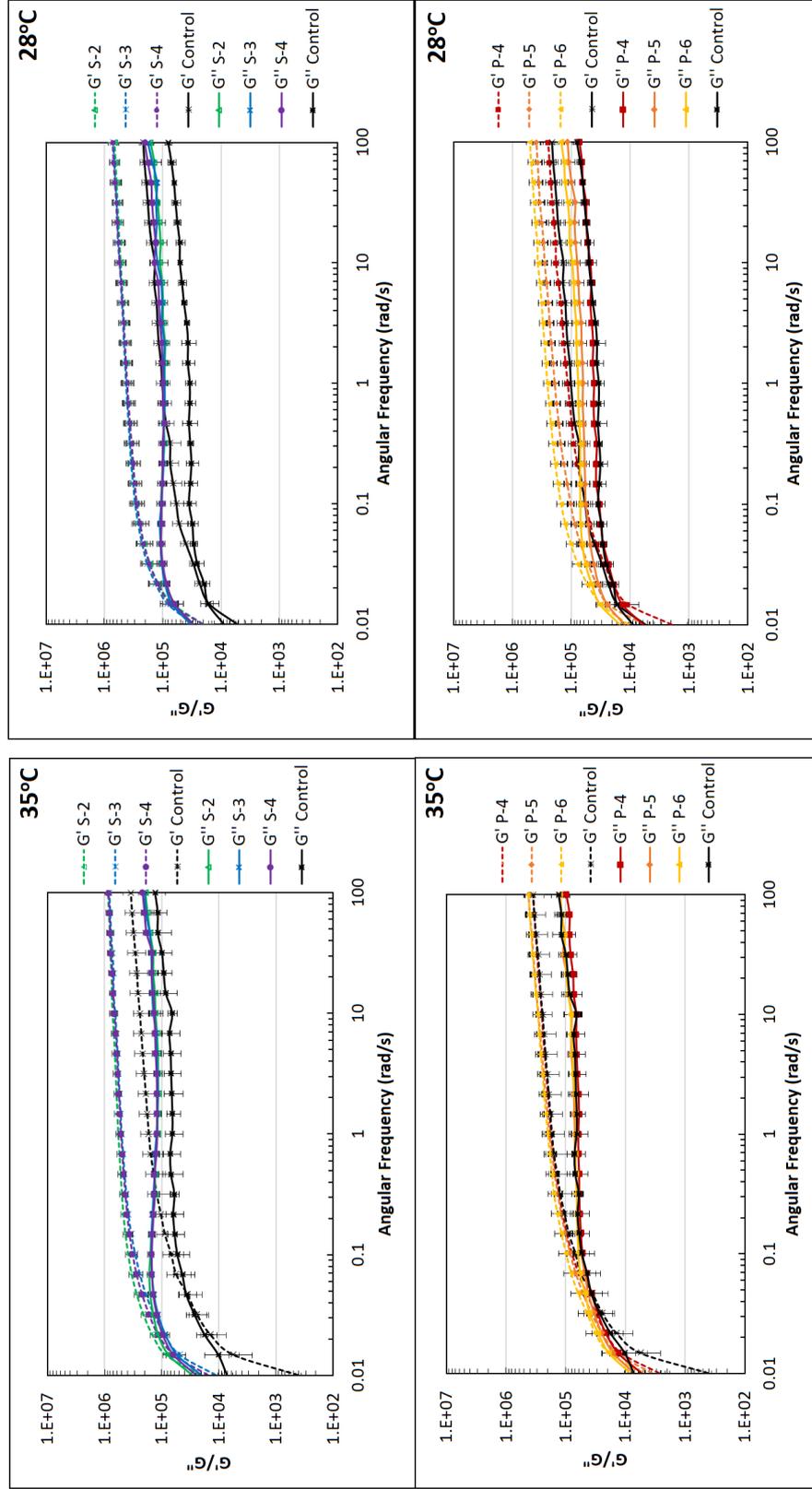
Figure 4.4 Elastic modulus (G') and viscous modulus (G") as a function of angular frequency for the zein-starch dough and the zein-starch dough with emulsifier mixed and tested at at 35°C and 28°C.

incorporation at two different temperatures (28°C and 35°C) are presented in Fig. 4.5. In rheological measurement, phase angle is employed to estimate the material's elasticity. Smaller phase angle indicates more elastic or more solid-like character of the sample (Steffe 1996). All dough samples showed more liquid-like behavior at low frequencies and more solid-like behavior at high frequencies. It suggested that the doughs became more elastic upon increasing in deformation rate (higher frequency). This trend was probably as a consequence of oscillatory motion during the measurement resulting in more hydrogen bonds formed among zein molecules.

In accordance to the complex modulus data, the control at 28°C was observed to have lower phase angle value than the control dough at 35°C, indicating the higher dough strength for the dough mixed at 28°C. Furthermore, the phase angle values of the control at both mixing temperatures was not getting lower constantly during the initial increasing frequency, as shown by inconsistent increasing in phase angle values; and the trend was more noticeable at 35°C. These findings were believed as indication of a weaker structure. According to Khatkar et al. (1995), weak gluten showed an increase in phase angle values indicating increasing viscous behavior with increasing frequency, while strong gluten exhibited a consistent elastic behavior as a function of frequency.

It was also found that more elastic properties were observed in all dough samples with emulsifier addition. They were indicated by lower phase angle values at both mixing temperatures and these results implied marked improvement in the elasticity of zein dough. Overall the effect of SSL on phase angle values was more noticeable compared to PATCO-3™ at two different mixing temperatures, as shown by lower phase angle values for all SSL doughs.
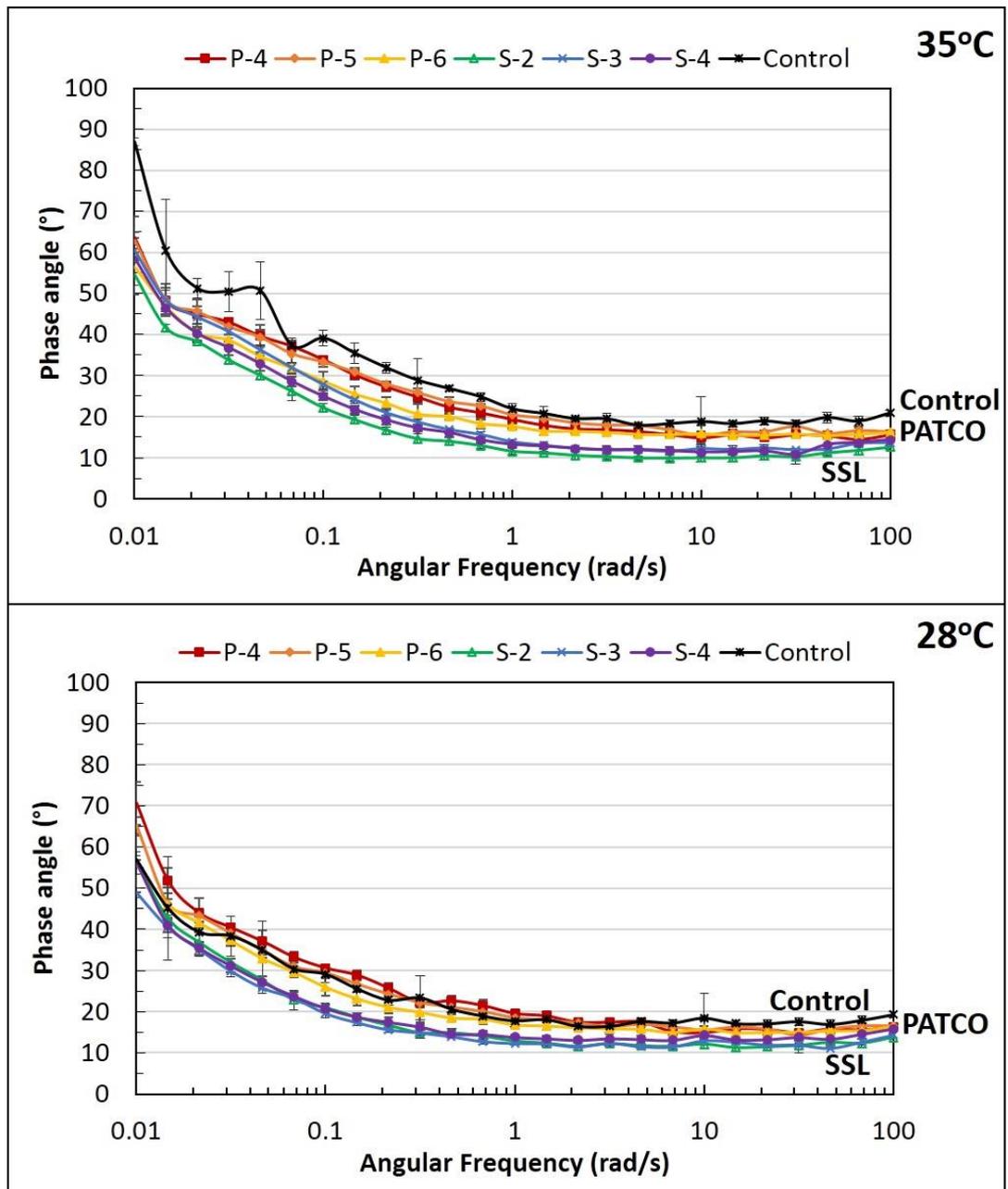
Figure 4.5   Phase angle (δ) as a function of angular frequency for the zein-starch dough and the zein-starch dough with emulsifier mixed and tested at at 28°C and 35°C.

Furthermore, the phase angle value of 2% SSL dough mixing at 35°C was slightly lower than dough with 3% or 4% SSL. However, at 28°C the gap between phase angles of those dough samples became narrower and the different levels of addition seemed to give similar effect on the elasticity of doughs. On the other hand, incorporation of 6% PATCO-3™ at 35°C resulted in the lowest phase angle magnitude among doughs with PATCO-3™, while 4% and 5% incorporated PATCO-3™ showed similar magnitudes. A similar trend was also observed at 28°C. Among all of the treatments, incorporation of 2% SSL might be the optimum.

In addition, generally similar trend and magnitudes of phase angles and complex modulus data were observed at two different mixing temperatures, with the exception of the control dough. Uneven result of the control dough mixed at 28°C might be due to lacking of hydration and unevenly mixing. Temperature of 28°C was chosen based on the T of G' transition data and this temperature was considered as not only the temperature below room temperature (30°C) but also the temperature above the T of G' transition of all dough samples (Table 4.2). These results suggested that mixing below room temperature (28°C) resulted in similar viscoelastic dough properties with the dough samples mixed above room temperature (35°C). The results also confirmed that in this present study the emulsifiers were able to depress the T of G' transition.

In regards to the molecular level, the elasticity/strength of wheat dough is related to its amino acid profile, polymeric nature, and structure (Lasztity 1995). It is believed that formation of β-sheet secondary structures by high molecular weight (HMW) glutenin subunits of gluten plays the most important role to the elastic behavior of gluten in wheat dough (Belton et al. 1995). Upon water addition to wheat

flour during mixing, large glutenin polymers develop β-sheets structures through physical entanglements of aligned peptide chains (uncoiled protein structures) and through formation of end-to-end disulphide bonds and intra and intermolecular hydrogen bonds (Belton 1999). Even though it is known that individual hydrogen bond is relatively weak but the presence of large numbers of them contributes overall strength to the inter-chain interactions (Stauffer 1998). The main contributor of this bonding is the high of hydrophilic amino acids content as the nature of gluten, especially from the high percentage of glutamine, which accounts for about 35% of all residues (Shukla and Cheryan 2001; Arendt et al. 2008).

Zein dough is also reported to form β-sheets secondary structures upon hydration and mixing at 35°C (Mejia et al. 2007). However, zein differs from gluten in structure and amino acid composition, suggesting the difference in its chain interactions. As discussed in the previous section, gluten is high of hydrophilic amino acids content, resulting in its good water-binding capacity (Shukla and Cheryan 2001; Arendt et al. 2008). In contrast, zein is rich in non-polar amino acids and deficient in charged amino acids (Shukla and Cheryan 2001; Arendt et al. 2008), which are responsible to its high hydrophobicity. Due to its hydrophobic nature, zein is more difficult to hydrate by water (Padua and Wang 2002). For this reason, it is thought that the similar phenomenon to that of gluten system could not take place in zein system.

Given the hydrogen bonds of the tertiary structures of zein surrounding by the highly hydrophobicity of α-helices secondary structures as modeled by Argos et al. (1982), the totally hydrophilic water, even though it possesses small molecule structure, might find difficulty to access and break the hydrogen bonds, resulting in

lacking of hydration. Since hydration is prerequisite facilitating subsequent steps to occur, the lacking of hydration in this initial step definitely affects the remaining steps and ends up with small amount of β-sheet secondary structures and less intra- and inter- hydrogen molecular bonds to strengthen the structures. Moreover, zein also lacks of HMW glutenin, thereby formation of stable β-sheet through disulfide bonds are not able to happen (Mejia et al. 2007). That lacking of intra and inter- hydrogen bonds which is responsible to stabilize the β-sheets secondary structures in zein dough contributes to weak and brittle properties of zein dough even at 35°C since the β-sheets secondary structures tends to fold back into the α-helix secondary structures. Hence, the consistency of the control dough is thought to be associated with the frictions of the flour components during mixing rather than the role of protein network and zein alone in the dough system results in lower dough strength or elasticity and weaker viscoelastic properties.

As discussed above, the higher degree of plasticization in the presence of emulsifier consequently will result in higher degree of conformational change from the tertiary structures to the linear strands and amount of β-sheets secondary structures. Moreover, it also may increase intra- and inter- hydrogen bonds that are largely responsible to strengthen β-sheets secondary structures. The fact that glutamine constitutes a large part of the total residues (~ 20%) in zein (Shewry and Tatham 1990) and its high propensity to interact with water (Wellner et al. 1996) may support an increase in intra- and inter- molecular of hydrogen bonds. As a consequence the increase in dough strength and elasticity were observed.

The higher dough strength and elasticity of doughs with SSL than that of doughs with PATCO-3™ may be related with the difference in molecule structure and

size between SSL and CSL. The molecular weight of SSL and CSL are 450.58 g/mol and 895.26 g/mol, respectively (EFSA 2013; US-NLM 2015). Moreover, SSL has linear and small structure, while CSL in PATCO-3™ has bigger structure (EFSA 2013; US-NLM 2015). Following the gel theory, the linear structure and smaller molecular weight of SSL will disrupt the hydrogen bonds of tertiary structures and α-helix secondary structures at ease and higher chance. On the contrary, CSL might be more difficult to occupy the zein polymer sites due to more complicated chemical structure. Moreover, polar side (Na^+) of SSL that is adjacent to only one hydrocarbon chain provides more accessible interaction for water as surrounding environment. Besides, SSL molecules that are not attached to polymer together with water might act like plasticizer; whereas CSL with its polar side attached between two hydrocarbon chains is more difficult to interact with water for acting as lubricant. For this reason, it is believed that SSL provides higher degree of conformational change into unfolded linear strands, resulting in more linear strands during formation of SSL dough than that of PATCO-3™ dough.

Moreover, the dissimilarity in the molecule structures between SSL and CSL might affect the formation of β-sheet secondary structures. Nylander et al. (2008) explained that in wheat dough system, the mechanism of dough strengthening is related with interaction between lipophyllic portions of emulsifier with hydrophobic parts of proteins, causing subsequent contribution to unfold the protein. As a consequence, it will increase the degree of gluten–gluten binding sites that supplement disulfide linkages and hydrogen bonds. The final result is the stronger gluten structures. SSL has one tail of hydrophobic chain whereas CSL has two tails of hydrophobic chain, in which the length of each hydrophobic chain of SSL and CLS is

same. Based on Nylander et al. (2008), this difference in hydrophobic chain length suggests that smaller hydrophobic part of SSL might occupy less space on the unfolded protein strands than that of CSL. As a result, in the presence of SSL, there will be more remaining space in the zein's linear strands to form protein-protein interactions via hydrogen bonds. Thus, it leads to more elastic and stronger properties of SSL dough compared to CSL dough counterpart.

It is interesting to note that among all dough samples with SSL, 2% of SSL addition exhibits the highest dough strength, indicating by the highest value of G* or the lowest value of phase angle. This concentration might be optimum level of addition to unfold all coiled structures of zein into the linear strands and beyond this concentration is considered excessive. Too much of SSL addition might result in much more occupied space on the unfolded protein strands by hydrophobic tails of SSL, leaving much less remaining space for intermolecular hydrogen bondings with subsequent lower dough strength obtained. On the other hand, for the doughs with added PATCO-3™, the higher concentration is required compared to the concentration of added SSL. The possible reason might be due to the lower degree of unfolding of coiled structures of zein by CSL in PATCO-3™. As discussed above, the bigger structure of CSL is like a barrier to access the hydrogen bonds of zein's tertiary structures, resulting in smaller number of linear strands for β-sheets formation. Thus, the higher level of PATCO-3™ incorporation is expected to give higher number of linear strands leading to more protein networks can be build up. This is also a reason why the increasing level of incorporated PATCO-3™ is linear with the increasing value of dough strength. Besides, from all results, it seems that hydrophilic part and hydrophobic part of emulsifier plays different role in affecting the viscoelastic

properties of zein-starch dough. The hydrophilic part of emulsifier seems to positively affect the degree of unfolding of protein chains through hydrogen bonds cleavage, while the hydrophobic part of emulsifier negatively affects the available interaction site on the unfolded protein strand for intermolecular hydrogen bondings. The proper balance between hydrophobic and hydrophilic parts of emulsifier might be required to get optimum viscoelastic properties of zein-starch dough.

4.4   Secondary structural analysis

Secondary structural changes in zein-starch doughs with SSL or PATCO-3™ addition are shown in Fig. 4.6 and Fig. 4.7. According to the small deformation results, S-2 dough supposedly had the highest amount of β-sheet secondary structures at the 0 min and all SSL dough samples were expected to have higher values in β-sheet secondary structures than PATCO-3™ dough samples. Nevertheless, as a result of a very unstable character and delay of measurement, these trend were not happened. In this study, measurement of secondary structure immediately after mixing was impossible to do since collection of background spectrum and sample preparation onto the stage of the crystal were required prior to analysis using FT-IR. Therefore, in this case, comparison of the initial amount of β-sheet secondary structures between SSL doughs and PATCO-3™ could not be discussed. Furthermore, at 0 min, the increasing in β-sheet secondary structures with the higher concentration of PATCO-3™ was still able to be observed. More obvious trend was shown for dough mixed at $35^{o}C$. This confirmed the result from small deformation test that the higher incorporated PATCO-3™ might result in the higher dough strength as the concentration increased.
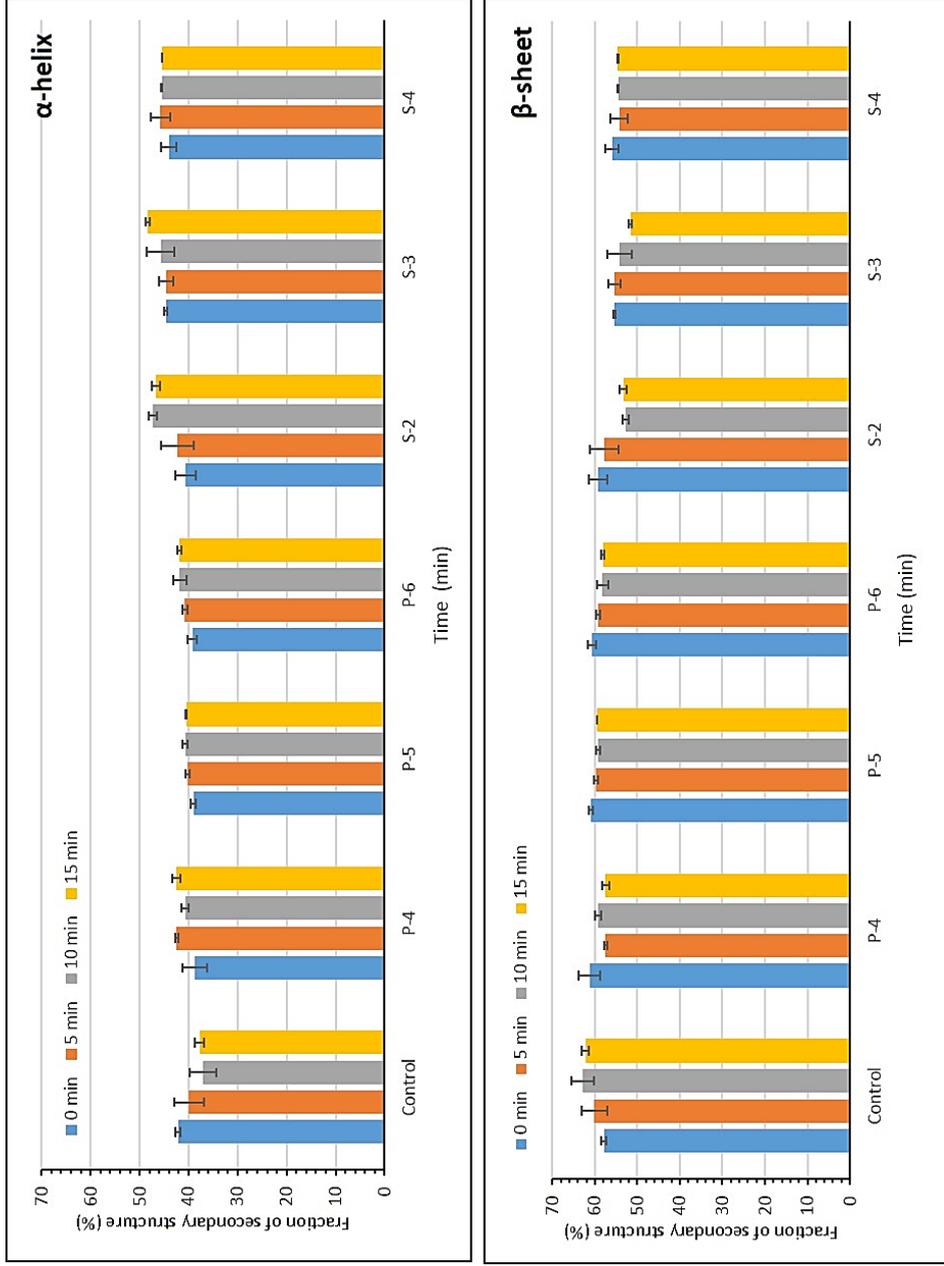
Figure 4.6   Secondary structural contents of proteins in dough state in Amide I when mixed at 35°C and stored at room temperature (25-28°C) for 15 min.
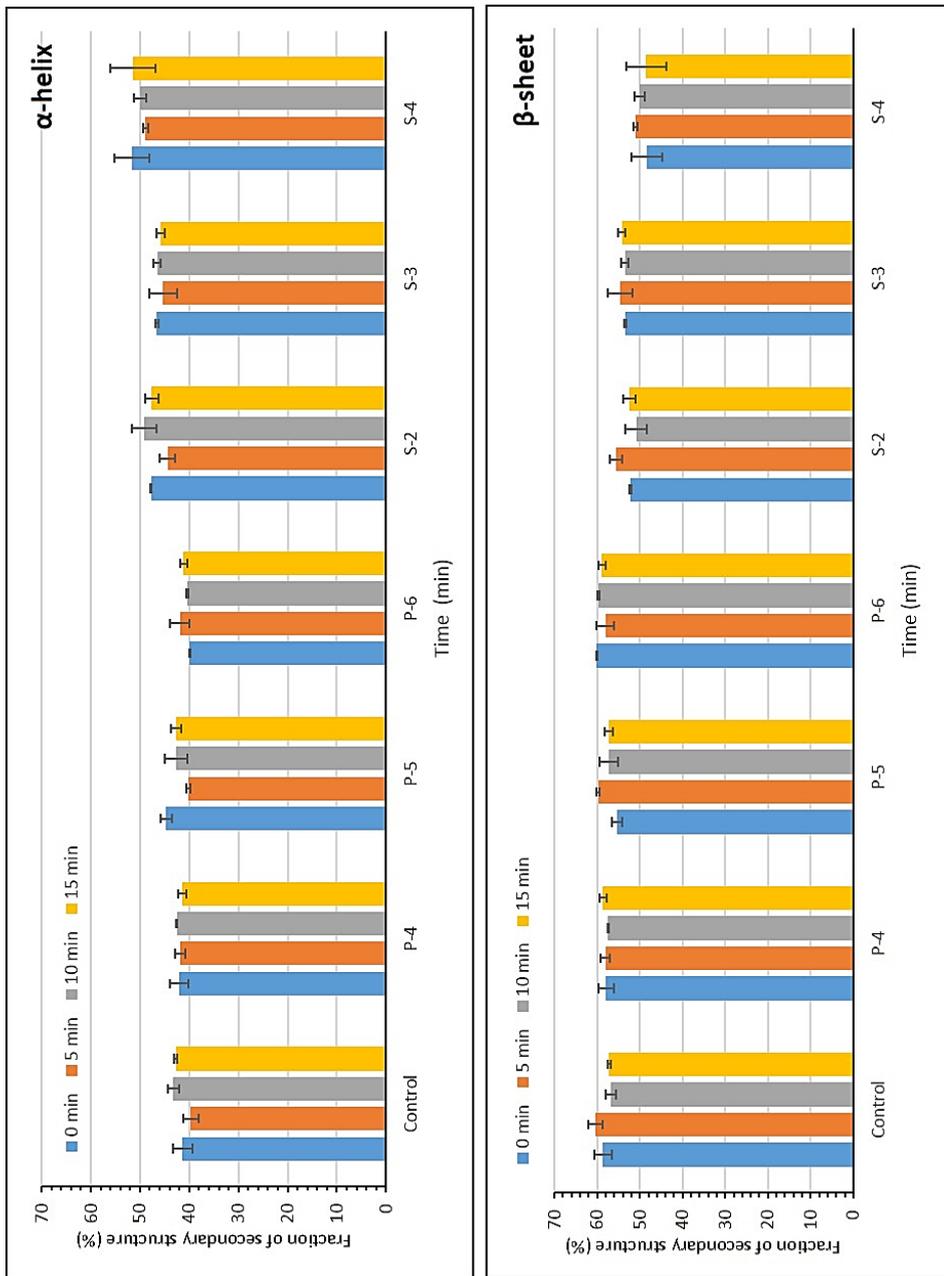
Figure 4.7   Secondary structural contents of proteins in dough state in Amide I when mixed at 28°C and stored at room temperature (25-28°C) for 15 min.

In general, the higher β-sheet and lower α-helix structure observed at all dough samples mixing at 35°C than that of at 28°C at 0 min clearly indicate the higher dough strength due to higher degree of hydration at higher temperature. These results were linear with the results of small deformation tests indicated by higher complex modulus.

At both dough mixing temperatures, the control exhibited quite high amount of β-sheet and of α-helix secondary structures. Furthermore, it was maintained during storage time. However, during analysis the severe phase separation occurred, thus the results might be not reliable (Appendix C.6). Moreover, the interpretation of protein spectra at Amide I region is somewhat sensitive to the interference of O-H vibrations caused by liquid water or surrounding water vapor. For this reason, starchy paste around the the sample might highly affect the result of measurement.

The substantial decreased in the stability of SSL doughs mixed at 35°C was obviously seen by significant changes in β-sheet secondary structural amount in FT-IR. However, this trend for dough mixed at 28°C somewhat was not that clear. It seemed that the stability all SSL dough samples at both mixing temperatures were diminished substantially within the first five minutes and has started to level off over storage time. As discussed above, the change in secondary structural of SSL doughs could not be observed at the first five minutes immediately after mixing due to a gap time during measurement. Thus, the secondary structural changes for SSL dough samples could not be interpreted completely. However, it was still quite obvious that the amount of β-sheet secondary structures were decreasing with the higher level of SSL addition, especially at mixing temperature of 35°C. This trend indicated that the higher incorporated SSL, the lower dough strength, in accordance to the result of

small deformation test. In term of viscoelasticity, S-2 dough was characterized by the highest dough strength, but contrary it had the lowest dough stability than other SSL doughs.

In addition, all dough samples with incorporated PATCO-3™ showed more stable property as indicated by maintained β-sheet amounts than all doughs with SSL during the storage time. The relatively constant amount of β-sheet secondary structures was also concomitant with the constant amount of α-helix secondary structures. The possibly reason for this findings might be related to the effect of different molecule structures between SSL and CSL during the formation of β-sheet secondary structures. As mentioned above, the hydrophilic side is thought to play a key role in unfolding coiled secondary and tertiary structures. SSL attributed by its linear structure, smaller molecular weight, and more accessible hydrophilic parts will disrupt the hydrogen bonds of tertiary structures and α-helix secondary structures at ease and higher chance than CSL. For this reason, it is believed that SSL provides higher degree of conformational change into unfolded linear strands, resulting in more linear strands during formation of SSL dough than that of PATCO-3™ dough. However, once all coiled structures become unfolded, hydrophobic chain of emulsifier is believed being responsible to retard the linear strands of protein coiling back into folded structures through hydrophobic interactions (Nylander et al. 2008). SSL has one tail of hydrophobic chain whereas CSL has two tails of hydrophobic chain, in which the length of each hydrophobic chain of SSL and CLS is same. This difference in hydrophobic chain length suggests that smaller hydrophobic part of SSL might occupy less space for hydrophobic interaction than that of CSL. Consequently, in the presence of SSL, the unfolded structures of zein linear strands might be much

more prone to coil back. For this reason, 2% SSL incorporated to zein dough resulted in the most unstable dough among all dough samples since the amount of hydrophobic parts were not sufficient to retard the linear strands of zein from coiling back. While for concentration of 3% and 4% SSL, there were more hydrophobic chain to stabilize the zein linear strands. This is why, even though S-3 and S-4 doughs had lower dough strength, they had more stable character than S-2 doughs. On the contrary, CSL in PATCO-3™ with twice longer of hydrophobic chains could occupy more hydrophobic sites of zein's linear strands, resulting in more stable β-sheet structures for all PATCO-3™ doughs.

It seems that the role of hydrophobic part of emulsifier in dough stability is in contrast to the viscoeleasticity of zein dough. Hydrophobic part of emulsifier affects negatively to the viscoelastic properties of zein-starch dough, while conversely it affects positively to dough stability. The pictorial model is proposed to explain the possible different role of hydrophilic and hydrophobic part of SSL and CSL on viscoelasticity and stability of zein-starch doughs (Fig. 4.8). Thereby, the proper balance between hydrophobic and hydrophilic parts of emulsifier is necessary not only to get optimum viscoelastic properties of zein-starch dough but also good dough stability.

CONTROL

Uncoiling
Δ/H$_2$O, mixing

β-sheet formation
Δ/H$_2$O, mixing

Less interaction
H$_2$O H$_2$O
H$_2$O
H$_2$O H$_2$O
H$_2$O H$_2$O

NOT STABLE

Stop mixing
Coiling

SSL
CSL

Uncoiling
Δ/H$_2$O, mixing

SSL
H$_2$O H$_2$O
H$_2$O H$_2$O

CSL
H$_2$O H$_2$O
H$_2$O H$_2$O

β-sheet formation
Δ/H$_2$O, mixing

Strengthening effect

SSL
H$_2$O
H$_2$O
H$_2$O H$_2$O
Stabilize linear structure

Strengthening effect

CSL
H$_2$O H$_2$O H$_2$O
H$_2$O H$_2$O
Stabilize linear structure

SSL
CSL
Hydrogen bond

Figure 4.8 Proposed pictorial models of role of hydrophilic and hydrophobic parts of SSL and CSL on viscoelasticity and stability of zein-starch dough.

CHAPTER V

CONCLUSIONS

In zein plasticization, the effective and efficient plasticizer of zein requires a proper balance between polar and nonpolar functional groups which is recognized as its amphiphilic property (Di Gioia et al. 1999; Lawton 2002, 2004). The addition of amphiphilic plasticizer to zein system was found not only decreasing the glass transition temperature, but also increasing the β-sheet secondary structures amount (Di Gioia et al. 1998, 1999; Lawton 2002; Mizutani et al. 2003). Sodium stearoyl lactylates (SSL) and calcium stearoyl lactylates (CSL) by their amphiphilic nature have been acknowledged as widely used dough strengthener in breadmaking process. In this study, zein composite flour was mixed with water and either with PATCO-3™ (an emulsifier blend of SSL and CSL) or SSL.

In the regards of mixing properties, it was found that dough with emulsifiers required less optimum water amount and incorporation of SSL in zein dough resulted in less optimum water amount and faster development of protein network than PATCO-3™ dough. This might be related with the ability of SSL to improve hydration better than PATCO-3. The difference degree of hydration between SSL and PATCO-3™ is related with the better ability of SSL to work together with water as plasticizer or as a medium in which protein polymers become flexible and mobile.

Additionally, decreases were determined in the T of G' transition of zein doughs with emulsifier. Doughs with added SSL had lower T of G' transition compared to dough with PATCO-3™. This suggests that PATCO-3™ and SSL might play as plasticizer during dough mixing through disrupting polymer-polymer

interactions (hydrogen bonds) and also as lubricant to facilitate the movement of zein polymers. Smaller molecule structure and more accessible polar group of SSL facilitate easier disruption to the zein protein structure, resulting higher degree of plasticizing effect compared to PATCO-3™.

Similar and general improvement on viscoelasticity of zein dough mixed with emulsifier (SSL or PATCO-3™) was observed under small deformation test. SSL incorporation resulted in more notably increase in dough strength and elasticity than PATCO-3. However, this effect was decreasing with increasing level of SSL addition, while on the contrary the higher concentration of PATCO-3™ results in more pronounced effect. For this reason, it is thought that hydrophilic part and hydrophobic part of emulsifier are believed to play different role in affecting the viscoelastic properties of zein-starch dough and dough stability. The hydrophilic part of emulsifier seems to positively affect the degree of unfolding of protein chains through hydrogen bonds cleavage, while the hydrophobic part of emulsifier might negatively affect the available interaction site on the unfolded protein strands for intermolecular hydrogen bondings.

Furthermore, the results of FTIR results showed that SSL doughs exhibited more inferior dough stability than PATCO-3™ doughs. It is believed that the hydrophobic part of emulsifier helps to prevent the unfolded protein strands return to their folded structures. Thus, it is reasonable that SSL with its shorter hydrophobic tail than CSL in PATCO-3™ results in inferior dough stability.

As conclusion, PATCO-3™ addition was found to have more positive effect on the T of G' transition, viscoelastic properties, and stability of zein dough. Incorporation of 6% PATCO-3™ showed the optimum results based on those dough

properties. Dough with SSL even though showed lower T of G' transition and more improved viscoelastic nature, the stability of dough could not be maintained over the time. Therefore, structural improvement in zein cannot be attributed only by the effect of glass transition but also the interaction between zein and emulsifier. The proper balance between hydrophobic and hydrophilic parts of emulsifier might be required to get optimum T of G' transition depression, viscoelastic properties, and stability of zein-starch dough.

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APPENDICES

APPENDIX A

DETAILED INFORMATION OF MATERIALS USED IN THE EXPERIMENT

A.1   Detail information of commercial zein

FLO CHEMICAL CORPORATION
"Continuously Making Freeman Zein For Over 30 Years"

CERTIFICATE OF ANALYSIS

Test Material: **Zein, F4400C SPECIAL GRADE:** (USP24/NF19)
Corn Protein. (Made in Ashburnham, MA Plant)

Lot #: **F44004021C**   (non GMO **IP Zein**)
Covers Sub-Lots: **3**

Physical Properties

Color	yellow to gold
Form	sand-like powder
Solubility	insoluble in water

Organoleptic Properties

Odor	none
Taste	bland, nondescript

Chemical Properties	**Results**	**Specifications**
Identification: A, B, C	conforms	*
Loss on Drying	2.80%	less than 8.00%
Residue on Ignition	0.99%	less than 2.00%
Nitrogen Content	13.94%	min 13.10% - 16.00% max
Heavy Metal	0.002% max	0.002% max**
OVI	meets the requirements	***

The material covered by this Certificate of Analysis does not trigger GM labeling according to Regulation (EC) NO. 1830/2003 concerning tractability and labeling of food and feed products produced from genetically modified organisms.

Based upon knowledge of our manufacturing, handling, shipping and storage processes there is no potential for specific toxic solvents to be present, and that the material, if tested, will comply with established standards.

*   These tests are performed on raw materials and therefore pass on finished product.
**   Test not performed on a regular basis as the results always conform.
*** The solvents in question are never present in our facility, therefore cannot be involved.

Shelf life: ZEIN (corn protein) is stable for 5 years, if it is stored in a **"WELL-CLOSED CONTAINER AT ROOM TEMPERATURE"**. See USP24/NF19.

Release Date: 02/28/14

A.2   Detail information of native maize starch

General Information	
Physical state	Powder
Appearance	White
Odor	No odor

Factory Division
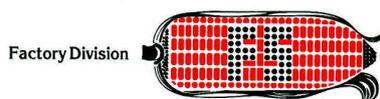
บริษัท เฟรนด์ชิพ คอร์น สตาร์ช จำกัด
Friendship Corn Starch Co., Ltd.

110 Sukhumvit Rd., 47.5 Km. Bangpoo, Muang, Samutprakarn 10280 Thailand Tel. (662) 7077201-4 Fax: (662) 7077205

QCD-FM-009
Edit. A

NO. QCA 0813/15   APRIL 1, 2015

Certificate Of Analysis

Sample of	: CORN STARCH (FOOD GRADE)
Bag No.	: 024220
Amount	: 1 Bag. x 25 Kgs.
Date of Manufacturing	: 29 / 03 / 15
Date of Expiring	: 28 / 03 / 16
Date of Checking	: 31 / 03 / 15

ANALYSIS	RESULT	UNIT
Viscosity	370	B.U.
Moisture	12.50	%
SO_2	20	ppm
pH	5.09	
Protein Content	0.22	%

A.3   Detail information of sodium stearoyl lactylate (SSL)

General Information	
Physical state	Powder
Appearance	White to off-white
Odor	Slight odor of fatty acid

DANISCO

First you add knowledge...

Certificate of Analysis

Date:	09 Sep 2014
Our ref. no.:	0
Your ref.	

Material:	452234	GRINDSTED SSL P 70 VEG	
Batch No.:	7112374448	Best before date:	28 Feb 2016
		Production date:	29 Aug 2014

Test	Result	Unit
Acid Value	73	mg KOH/g
Ester Value	156	mg KOH/g
Saponification Value	229	mg KOH/g

Comments

A.4   Detail information of PATCO-3™

Product characteristics	
Material	SSL-CSL blend
Brand	PATCO-3™
Form	Free flowing powder
Color	Light tan
Odor	Mild

A.5   Modified hand mixer equipped with power inverter for dough preparation

APPENDIX B

ANALYTICAL METHOD

B.1   Example of window pane test
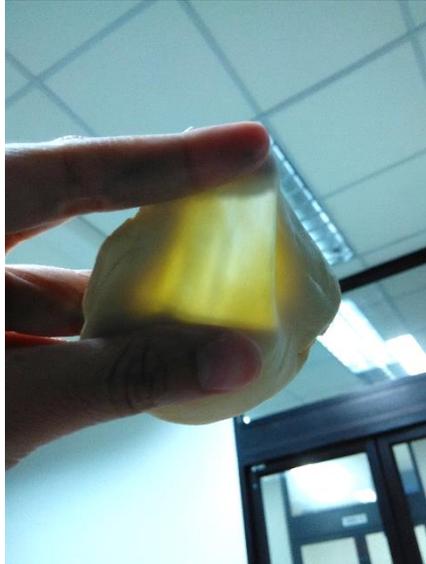
B.2   Test configuration of rheometer for determination of G' transition temperature

1. Test configuration for amplitude sweep test

2. Test configuration for single frequency sweep test
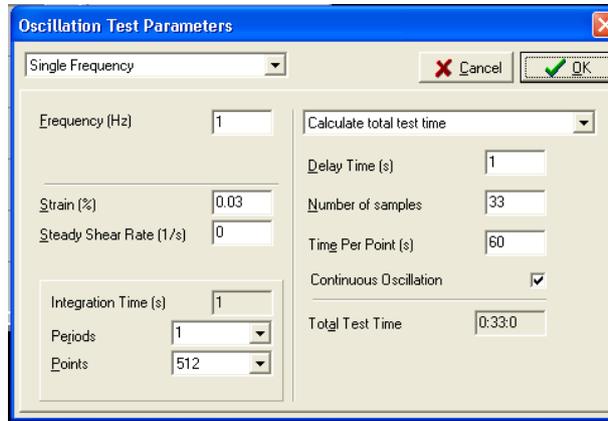
3. Test configuration for temperature gradient
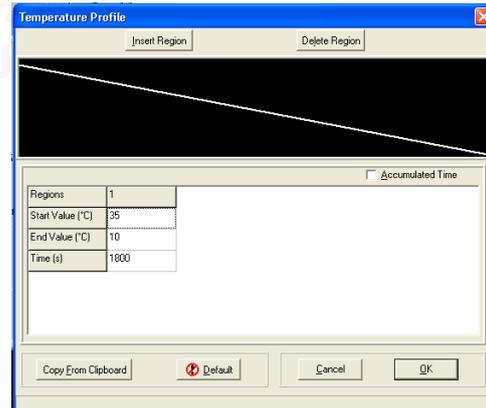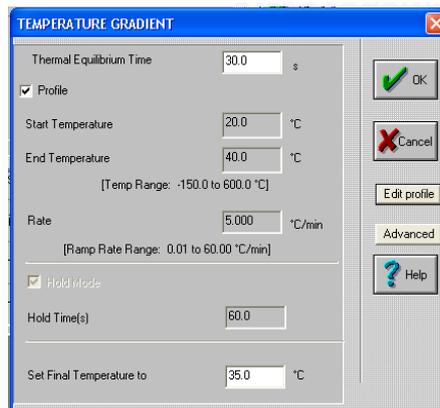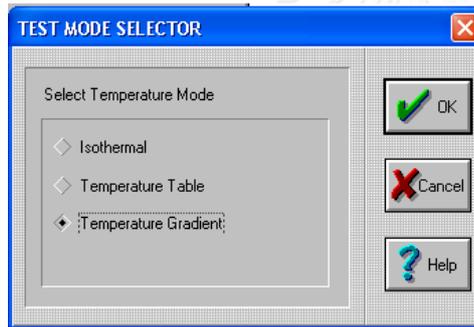
B.3   Test configuration of rheometer for small oscillatory test

1. Test configuration for amplitude sweep test

2. Test configuration for frequency sweep test

B.4   Example of result of peak substraction
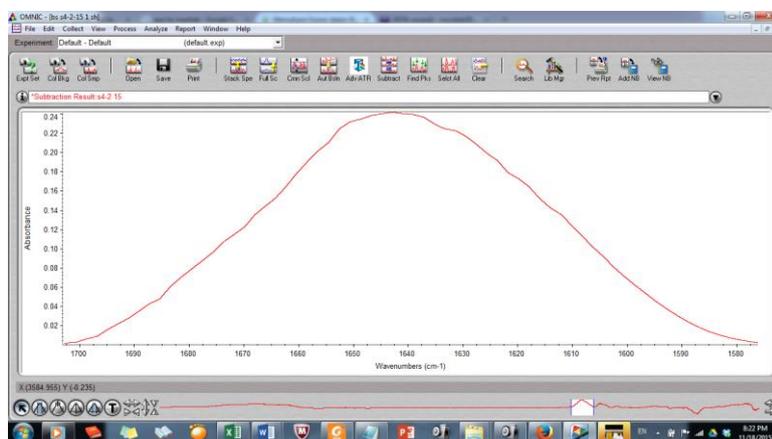
B.5   Example of peak assignment and calculation of % fraction of secondary
       structures.
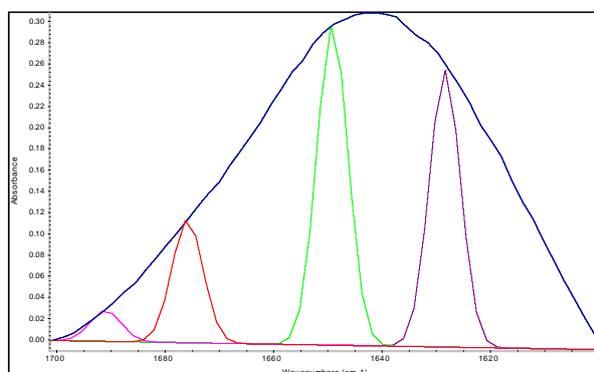
| s4-1	Peak #	Peak Type	Center X	Height	FWHH	Area	Total Area	%	%β-sheet
0	1	Gaussian	1630.815	0.18	6.75	1.288	3.373	38.19	57.07
	2	Gaussian	1652.729	0.2025	6.75	1.448		42.93	
	3	Gaussian	1674.124	0.0892	6.75	0.637		18.89	
	4	Gaussian	1695.497	0	0	0		0.00	
5	Peak #	Peak Type	Center X	Height	FWHH	Area	Total Area	%	
	1	Gaussian	1628.081	0.1774	6.75	1.273	3.669	34.70	55.66
	2	Gaussian	1652.757	0.2266	6.75	1.627		44.34	
	3	Gaussian	1674.311	0.0993	6.75	0.712		19.41	
	4	Gaussian	1695.497	0.0117	6.75	0.057		1.55	
10	Peak #	Peak Type	Center X	Height	FWHH	Area	Total Area	%	
	1	Gaussian	1628.068	0.1632	6.75	1.144	3.403	33.62	54.42
	2	Gaussian	1652.725	0.2265	6.75	2		45.58	
	3	Gaussian	1674.237	0.0652	6.75	0.664		19.51	
	4	Gaussian	1695.51	0.0085	6.75	0.044		1.29	
15	Peak #	Peak Type	Center X	Height	FWHH	Area	Total Area	%	
	1	Gaussian	1628.137	0.1591	6.75	1.142	3.371	33.88	54.55
	2	Gaussian	1652.79	0.2137	6.75	1.532		45.45	
	3	Gaussian	1674.342	0.0913	6.75	0.652		19.34	
	4	Gaussian	1695.512	0.0081	6.75	0.045		1.33	

APPENDIX C

ADDITIONAL DATA

C.1   Farinograms for all dough samples
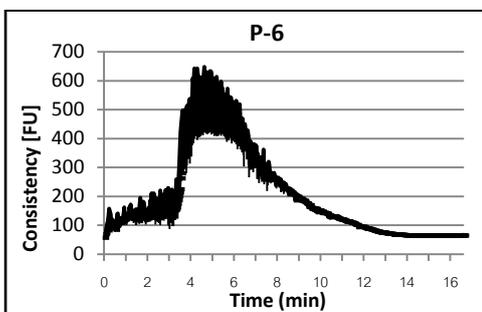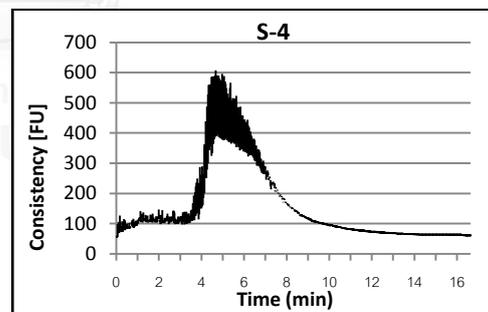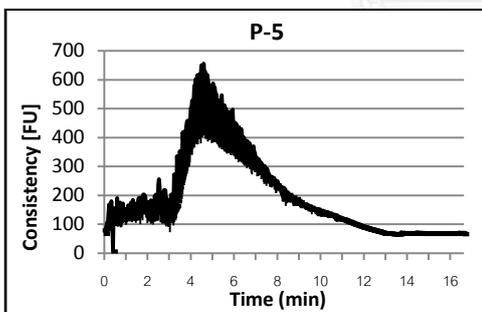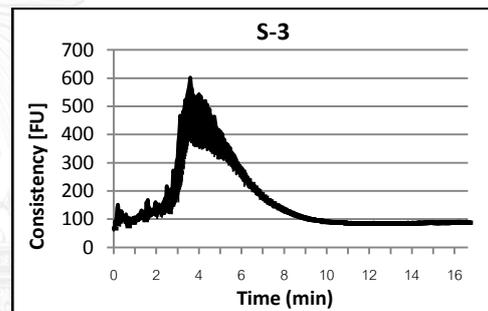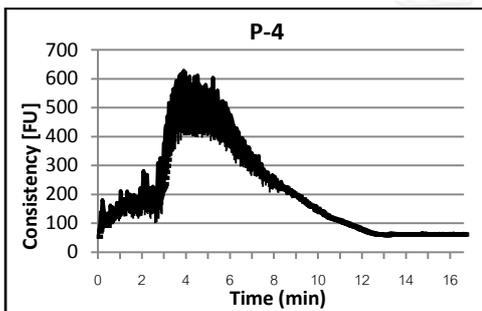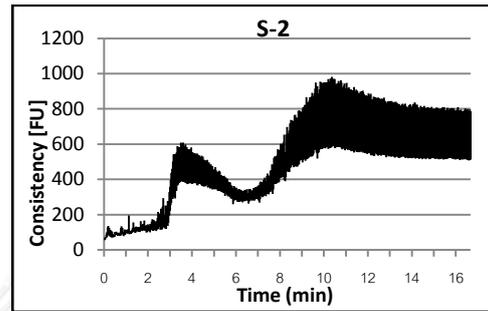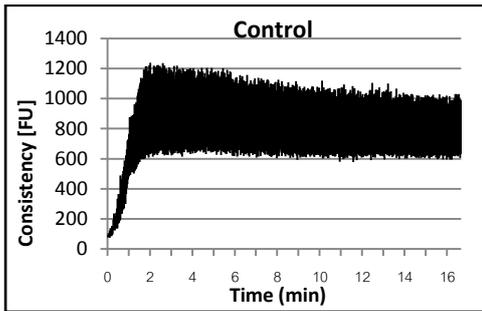
C.2 Starchy liquid of phase separation in the control dough.

C.3 Data of G' transition temperatures and dough moisture contents

| Trt	Moisture content	Average	SD	T_g	Average	SD
c	46.67	46.35	1.12	25.9	25.03	0.85
c	47.28			24.2		
c	45.11			25		
p4-1	43.78	43.62	0.18	26.7	26.17	0.92
p4-2	43.66			26.7		
p4-3	43.43			25.1		
p5-1	43.05	42.90	0.22	25.9	25.33	0.98
p5-2	43.01			24.2		
p5-3	42.65			25.9		
p6-1	41.62	41.13	0.49	25.1	24.50	0.52
p6-2	41.14			24.2		
p6-3	40.64			24.2		
s2-1	40.54	40.88	0.30	22.5	22.53	0.85
s2-2	41.08			21.7		
s2-3	41.02			23.4		
s3-1	39.10	38.90	0.21	17.5	18.67	1.32
s3-2	38.68			20.1		
s3-3	38.91			18.4		
s4-1	37.41	37.34	0.07	13.4	13.93	0.92
s4-2	37.26			15		
s4-3	37.35			13.4		

C.4   Thermo-dynamical curves of all dough samples for T of G' transition determination
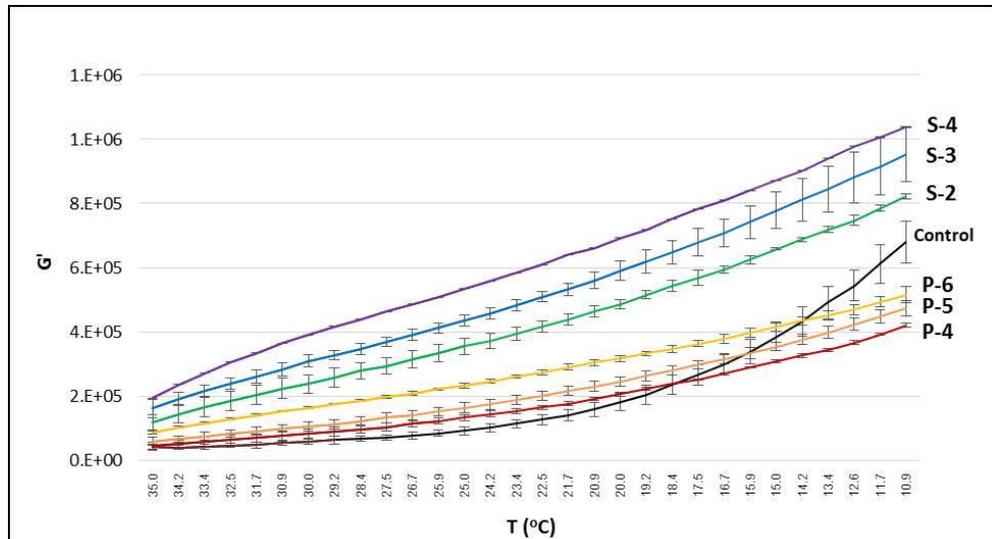
C.5   Phase separation during small amplitude oscillatory test

C.6   Phase separation during secondary structural analysis
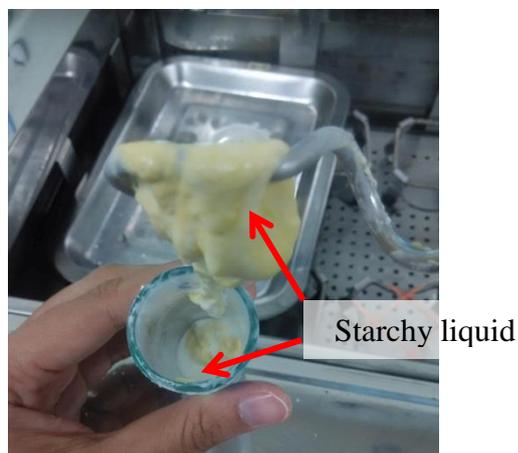
APPENDIX D

ANALYSIS OF VARIANCE

D.1   ANOVA for G' transition temperatures of all dough samples

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	326.225	6	54.371	36.572	.000
Within Groups	20.813	14	1.487		
Total	347.038	20			

D.2   ANOVA for moisture contents of all dough samples

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	163.733	6	27.289	111.692	.000
Within Groups	3.421	14	.244		
Total	167.154	20			

VITA

Murdiati was born on June 3, 1989, in Jakarta, Indonesia. She graduated with Bachelor of Science in Food Technology from Bogor Agricultural University, Indonesia in 2012. In 2012-2015 she received scholarship for her master's study from the Ministry of Finance of the Republic of Indonesia under Indonesian Endowment Fund for Education (IEFE) scholarship program. She was also awarded as the best female participant in IEFE Leadership Training Program (Batch 2) from the same scholarship program.

Publication/Presentation: Murdiati, M., Kuakpetoon, D., & Lumbudwong, N. 2015. Effects of emulsifier on mixing properties and glass transition temperatures of zein-starch doughs. Presented at the 9th Center for Research and Development of Tropical and Sub-tropical Crops Conference (oral presentation), 3-4 September 2015, Bangkok, Thailand.