Acknowledgement

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Abstract

Part 1

Research Title	Development of rice bran oil emulsions by using pectin and zein as emulsifiers
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Research Grants	Fiscal Year 2015, Research and Development Institute, Silpakorn University
Year of completion	2016
Type of research	Applied research
Subjects (based on NRCT)	Chemical and Pharmaceutical Science

Part 2 Abstract

The objective of this study was to investigate the physical properties of pectin-zein polyelectrolyte complexes and the stability of rice bran oil-in-water emulsions stabilized by pectin–zein complexes. First, the formation of pectin-zein polyelectrolyte complexes was investigated with emphasis on the effect of pectin type and zein concentration. The pectin-zein complexes were formed by mixing pectin solution with zein solution, at pH 4, where zein and pectin had opposite charges. The formation of complexes was evidenced by an increased turbidity, a slight decrease of negative charges, and an aggregation of polymers as observed by digital images, microscopic images and scanning electron micrographs. Fourier transform infrared spectra suggested the possibility of weak physical interaction between pectin and zein. The pectin-zein complexes demonstrated better oil/water interfacial tension lowering properties than pectin alone. Next, the stability of emulsions stabilized by pectin–zein complexes was investigated. The effects of concentration of pectin, zein and rice bran oil as well as order of mixing on physical properties, such as droplet size and zeta potential, and emulsion stability after storage at ambient temperature and under environmental stress conditions, i.e., temperature cycling and centrifugation test, were observed. The stable emulsions could be prepared by homogenizing pectin with rice bran oil and then adding zein solution at pH 4. It was found that the pectin–zein complexes could improve stability of emulsions by decreasing droplet size and increasing viscosity of the emulsion system. The stability of emulsions also increased as concentration of zein was increased. In addition, applying high-pressure homogenization after mechanical homogenization caused only a small additional decrease in emulsion droplet size. The droplet size of emulsions was influenced by the type of pectin used; emulsions using high methoxy pectin (HMP) were smaller than those using low methoxy pectin (LMP). The emulsions stabilized by HMP–zein showed good physical stability with lower percent creaming index than those using LMP, both before and after passing through high-pressure homogenizer. These results suggested that the pectin–zein complexes within optimal condition could be used to improve the physical properties and stability of rice bran oils emulsions. It suggested that pectin and zein are promising materials, thus extending the range of applications of these natural polymers.

Key words: rice bran oil, emulsion, pectin, zein, stability

บทคัดย่อ

ส่วนที่ 1

ชื่อโครงการ การพัฒนาอิมัลชันของน้ำมันรำข้าวโดยใช้เพกตินและเซอินเป็นสารก่ออิมัลชัน

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แหล่งทุนอุดหนุนการวิจัย งบประมาณแผ่นดินประจำปี 2558 .
สถาบันวิจัยและพัฒนา มหาวิทยาลัยศิลปากร

ปีที่เสร็จ 2559

ประเภทการวิจัย การวิจัยประยุกต์

สาขาวิชา (อ้างอิงตามวช.) วิทยาศาสตร์เคมีและเภสัช

ส่วนที่ 2 บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาสมบัติทางกายภาพของสารประกอบเชิงซ้อนระหว่างเพกตินและเซอินและผลของสารประกอบเชิงซ้อนต่อสมบัติทางกายภาพและความคงสภาพของอิมัลชันน้ำมันรำข้าว ขั้นแรกศึกษาการเกิดสารประกอบเชิงซ้อนระหว่างเพกตินและเซอิน โดยเน้นผลของชนิดเพกตินและความเข้มข้นของเซอิน สารประกอบเชิงซ้อนระหว่างเพกตินและเซอินเกิดขึ้นโดยการผสมสารละลายเพกตินและสารละลายเซอินที่พีเอชเท่ากับ 4 ซึ่งเซอินและเพกตินมีประจุตรงข้ามกัน พบว่าการเติมเซอินลงในสารละลายเพกตินเกิดเป็นสารประกอบเชิงซ้อนระหว่างเพกตินและเซอินที่ไม่ละลายน้ำ โดยมีความขุ่นเพิ่มขึ้นและประจุที่เป็นลบลดลงเล็กน้อยเมื่อทดสอบศักย์ไฟฟ้าซีตา ภาพการเกาะกลุ่มกันจากกล้องจุลทรรศน์ และกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด ผลจากอินฟาเรดสเปกโทรสโกปีชนิดฟูเรียร์ทรานสฟอร์มแสดงให้เห็นว่าอาจมีอันตรกิริยาทางกายภาพอย่างอ่อนระหว่างเพกตินกับเซอิน แรงตึงระหว่างผิวประจันของสารประกอบเชิงซ้อนระหว่างเพกตินและเซอินต่ำกว่าสารละลายเพกตินเดี่ยวเล็กน้อย จากนั้นศึกษาความคงตัวของอิมัลชันที่มีส่วนประกอบของเพกตินและเซอิน ศึกษาอิทธิพลของความเข้มข้นเพกติน เซอิน และน้ำมันรำข้าว ลำดับการผสมต่อสมบัติทางกายภาพ เช่น ขนาดหยดอนุภาค ศักย์ไฟฟ้าซีตาและความคงสภาพของอิมัลชันหลังจากเก็บไว้ที่อุณหภูมิห้องและภายใต้สภาวะความเครียดทางสิ่งแวดล้อม ได้แก่ การเปลี่ยนแปลงของวงจรอุณหภูมิและการปั่นเหวี่ยง พบว่าอิมัลชันที่มีความคงสภาพสูงเตรียมได้จากการปั่นผสมเพกตินกับน้ำมันรำข้าวก่อนแล้วจึงเติมสารละลายเซอินที่พีเอชเท่ากับ 4 ผลการศึกษาชี้ให้เห็นว่าสารประกอบเชิงซ้อนระหว่างเพกตินและเซอินสามารถปรับปรุงความคงสภาพของอิมัลชันได้โดยการลดขนาดหยดอนุภาคและการเพิ่มความหนืดของระบบอิมัลชัน ความคงสภาพของอิมัลชันเพิ่มขึ้นเมื่อความเข้มข้นของเซอินเพิ่มขึ้น นอกจากนี้พบว่าการนำอิมัลชันไปผ่านเครื่องปั่นผสมแรงดันสูงทำให้ขนาดของอนุภาคลดลงอีก

เล็กน้อยทั้งนี้ขึ้นกับชนิดของเพกตินที่ใช้ โดยอิมัลชันที่มีเพกตินชนิดเมทอกซีสูงจะมีขนาดเล็กกว่าเพกตินชนิดเมทอกซีต่ำ ความคงสภาพของอิมัลชันที่ใช้สารประกอบเชิงซ้อนระหว่างเพกตินชนิดเมทอกซีสูงและเซอิน สูงกว่าอิมัลชันที่ใช้สารประกอบเชิงซ้อนระหว่างเพกตินชนิดเมทอกซีต่ำและเซอิน ทั้งก่อนและหลังจากผ่านเครื่องปั่นผสมแรงดันสูง ดังนั้นสารประกอบเชิงซ้อนระหว่างเพกตินและเซอินภายใต้สภาวะที่เหมาะสมสามารถนำมาใช้เพื่อปรับปรุงสมบัติทางกายภาพและความคงสภาพของอิมัลชันน้ำมันรำข้าวได้ เพกตินกับเซอินจึงเป็นพอลิเมอร์ธรรมชาติที่มีแนวโน้มที่จะนำไปประยุกต์ใช้งานได้กว้างขวางมากขึ้น

คำสำคัญ : น้ำมันรำข้าว อิมัลชัน เพกติน เซอิน ความคงตัว

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Introduction

Since the excipients used in pharmaceutical industries in Thailand were quite expensive and had to be imported from overseas. Therefore, the development of the new natural pharmaceutical excipient was interesting. This would reduce the cost in pharmaceutical industries in Thailand, add value to local agricultural products and replace imports from abroad and might also help Thai economic. Rice bran oil, which is extracted from germ and inner husk of rice, has been used extensively in several Asian countries and United States. India and Thailand are the most important producers of rice bran oil, together accounting for more than 225,000 metric tons of rice bran oil per year. Rice bran oil is used as additives in foods, pharmaceuticals, cosmetics and animal feeds. The major fatty acid compositions of rice bran oil are oleic, linoleic and palmitic fatty acids, similar to peanut oil, except that there are more long-chain fatty acids than in peanut oil. Rice bran oil has long been considered to be very health-promoting and has a high nutritional value. It contains γ-oryzanol and phytosterols, which have the capacity to lower blood cholesterol and decrease cholesterol absorption in laboratory animals and humans. The powerful antioxidants are tocotrienols and γ-oryzanol, which associate with prevention of cardiovascular diseases and some cancers. Because of these beneficial effects, rice bran oil is very appealing as specialty oil or healthy oil in niche markets. However, rice bran oil is distasteful and strong-smelling. This may be improved by preparing the rice bran oil as oil-in-water (o/w) emulsion which is easy and low-cost in terms of production. It also provides better sensory properties (without an oily mouthfeel, unpalatable taste and unpleasant odor). The o/w emulsions have been used in a variety of products such as foods, paints, cosmetics and pharmaceutical products.

Emulsions are known to be thermodynamically unstable systems and hence emulsifiers are needed to produce emulsions that are kinetically stable under specific environmental conditions. During homogenization, emulsifiers adsorb to surface of freshly formed droplets. It can reduce interfacial tension and overall free energy or form around the droplets to prevent droplets from aggregation by generating repulsive interactions. There is a growing trend within the food industry to replace synthetic emulsifiers with natural emulsifiers. The most common natural emulsifiers used in the food industry are amphiphilic proteins, polysaccharides and phospholipids. The most widely used polysaccharides emulsifiers in food applications are gum arabic, modified starches, modified celluloses, some kinds of pectin and galactomannans while protein ingredients derived from milk and eggs are the commonly used protein emulsifiers. The main stabilizing action of polysaccharides is via viscosity modification or gelation in the aqueous continuous phase whereas proteins have a strong tendency to adsorb at oil-water interface to form stabilizing membrane around oil droplets and, therefore, they are able to fulfill both the emulsifying and stabilizing roles. Many studies have shown the advantages of combining protein and polysaccharide, by formation of protein-polysaccharide complexes, to emulsify and stabilize emulsions under appropriate conditions, leading to increased emulsion stability. Previous studies have shown that pectin alone at low concentration is not good emulsifying agent for stabilizing emulsion. This may be due to its low adsorption ability, which is not sufficient to provide effective steric stabilization over long-term period and against harsh conditions. In this study, the emulsion system containing pectin, an anionic polysaccharide, and zein as a cationic charge under appropriate conditions has been proposed in order to improve the physical properties and stability of o/w emulsions.

Review literature

Rice bran oil, which is extracted from germ and inner husk of rice, has been used extensively in several Asian countries and United States [1]. India and Thailand are the most important producers of rice bran oil, together accounting for more than 225,000 metric tons of rice bran oil per year. Rice bran oil is used as additives in foods, pharmaceuticals, cosmetics and animal feeds [2]. The major fatty acid compositions of rice bran oil are oleic, linoleic and palmitic fatty acids, similar to peanut oil, except that there are more long-chain fatty acids than in peanut oil. Rice bran oil has long been considered to be very health-promoting and has a high nutritional value. It contains γ-oryzanol and phytosterols, which have the capacity to lower blood cholesterol and decrease cholesterol absorption in laboratory animals and humans [3-5]. The powerful antioxidants are tocotrienols and γ-oryzanol, which associate with prevention of cardiovascular diseases and some cancers [6-7]. Because of these beneficial effects, rice bran oil is very appealing as specialty oil or healthy oil in niche markets [1-3]. However, rice bran oil is distasteful and strong-smelling. This may be improved by preparing the rice bran oil as oil-in-water (o/w) emulsion which is easy and low-cost in terms of production. It also provides better sensory properties (without an oily mouthfeel, unpalatable taste and unpleasant odor) [8]. The o/w emulsions have been used in a variety of products such as foods, paints, cosmetics and pharmaceutical products.

Emulsions are known to be thermodynamically unstable systems and hence emulsifiers are needed to produce emulsions that are kinetically stable under specific environmental conditions (pH, temperature, storage time). During homogenization, emulsifiers adsorb to surface of freshly formed droplets. It can reduce interfacial tension and overall free energy or form around the droplets to prevent droplets from aggregation by generating repulsive interactions [9-10]. There is a growing trend within the food industry to replace synthetic emulsifiers with natural emulsifiers. The most common natural emulsifiers used in the food industry are amphiphilic proteins, polysaccharides and phospholipids [11]. The most widely used polysaccharide emulsifiers in food applications are gum arabic, modified starches, modified celluloses, some kinds of pectin and galactomannans while protein ingredients derived from milk and eggs (such as β-lactoglobulin, lecithin) are the commonly used protein emulsifiers [12-13]. The main stabilizing action of polysaccharides is via viscosity modification or gelation in the aqueous continuous phase whereas proteins has a strong tendency to adsorb at oil-water interface to form stabilizing membrane around oil droplets and, therefore, they are able to fulfill both the emulsifying and stabilizing roles [12].

Many studies have shown the advantages of combining protein and polysaccharide, by formation of protein-polysaccharide complexes, to emulsify and stabilize emulsions under appropriate conditions, leading to increased emulsion stability, e.g., xanthan + whey protein [14], β-lactoglobulin + gum arabic [15], lecithin + chitosan [16], β-lactoglobulin + carrageenan [17], etc. A two-component interfacial membrane is produced by adding a charged biopolymer to an emulsion containing oppositely charged droplets, which can provide electrostatic and steric stabilization, thus, improving thermal stability and resistance to external treatment. Previous studies have shown that pectin alone at low concentration is not good emulsifying agent for stabilizing emulsion. This may be due to its low adsorption ability, which is not sufficient to provide effective steric stabilization over long-term period and against harsh conditions [18].

Zein is one of interesting proteins that could be used to form complexes with polysaccharide. It is a prolamine, a major storage protein of corn. Zein contains many hydrophobic amino acid residues, including many sulfur-containing amino acids, but is deficient in ionizable and polar amino acids. The zein proteins are hydrophobic and insoluble in water and, thus, ethanol at high concentrations (60–95%) is required to maintain their molecular conformations [19]. The properties of zein are not only dependent on the amino acid composition, but also their molecular structures on the nanometer scale [20]. Due to its water insolubility, zein has been used in food and packaging industry to form a moisture barrier. In pharmaceutical application, zein nanoparticles have been studied for sustained drug delivery applications for drug molecules [21].

Pectin, a naturally occurring water-soluble polysaccharide, is a well-known food additive which is mainly used for its gelling and stabilizing abilities. Due to its biocompatibility, biodegradability and non-toxicity, pectin represents an attractive biopolymer for a variety of pharmaceutical and biomedical applications [22]. It also has several unique properties that have enabled it to be used potentially as an emulsifier and a carrier for drug delivery to gastrointestinal tract, such as matrix tablets, gel beads, film-coated dosage forms [23,24]. Chemically, pectin contains linear chains of (1-4)-linked α-D-galacturonic acid residues. The linear structure of pectin is partly interrupted by (1-2)-linked side chains consisting of L-rhamnose residues and some other neutral sugars [22]. The galacturonic acids have carboxyl groups, some of which are naturally presented as methyl esters and others, which are reacted with ammonia to produce carboxamide groups. Pectin is divided into two major groups on the basis of their degree of esterification (DE) of the galacturonic acid residues. Pectin with DE less than 50% is so-called low methoxy pectin (LMP) while that with DE more than 50% is so-called high methoxy pectin (HMP).

Recently, combination of zein and pectin has been used as drug delivery carrier in the form of complex hydrogel beads [25], microspheres [26], or nanoparticles [27,28]. Therefore, in this study, pectin-zein polyelectrolyte complexes were prepared and the physical properties of pectin-zein complexes were characterized and the emulsion system containing pectin, an anionic polysaccharide, and zein as a cationic charge under appropriate conditions has been proposed in order to improve the physical properties and stability of o/w emulsions.

Objectives

This research was aimed to:

1. Develop pectin-zein complexes as emulsifier in order to increase emulsion stability in pharmaceutical products
2. Develop products that are used as health supplement, prepared from rice bran oil, as oral emulsions using pectin and zein as emulsifier, and determine physicochemical properties and stability of prepared products.

Expected Outcome

1. To develop natural substances including pectin and zein as emulsifier, apply emulsion process technology and develop a new manufacturing process (knowledge)
2. To publish research in international journals and present the work in the academic conferences (knowledge)
3. To obtain prototype/production process of rice bran oil emulsion using natural substances as emulsifier in order to be an alternative in using supplements to promote good health (commercial)

Keywords

rice bran oil
emulsion
pectin
zein
stability

List of Symbols and abbreviations

°C	degree celcius
cps	centipoises
µm	micrometer
mV	millivolt

rpm	round per minute
%w/w	percent weight by weight
LMP	low methoxy pectin
HMP	high methoxy pectin
A	absorbance
cm^{-1}	wavenumbers
min	minute
FTIR	fourier transform infrared spectroscopy
H	hours
kDa	kilodalton
kg	kilogram
mg	milligram
mL	milliliter
MW	molecular weight
N	normality
NaOH	Sodium hydroxide

Conceptual Framework

To develop the emulsion system containing pectin, an anionic polysaccharide, and zein, a cationic charge as emulsifier under appropriate conditions in order to improve the physical properties and stability of o/w emulsions and to develop products that are used as health supplement prepared from rice bran oil in oral emulsion using pectin and zein as emulsifier and determine physicochemical and stability of prepared products

Materials and Methods

Materials

1. Glacial acetic acid (Lot No. 6M387197A, Carlo Erba, Italy)
2. High methoxy pectin, 200 kDa, 70% DE (Lot No. 00501087, Herbstreith & Fox KG, Germany)
3. Hydrochloric acid, 37% (Lot No. E15W66, J.T. Baker, USA)
4. Low methoxy pectin, 70 kDa, 38% DE (Lot No. 00412072, Herbstreith & Fox KG, Germany)
5. Methyl paraben (Lot No. MP-163/10-11, P.C. Drug Center, Thailand)
6. Propyl paraben (Lot No. n.a., P.C. Drug Center, Thailand)

7. Refined rice bran oil for food industry (Thai Edible Oil, Thailand)
8. Sodium hydroxide (Lot No. B0035298, Merck, Germany)
9. Sodium acetate (Lot No. AF604190, Ajax Finechem, Australia)
10. Zein, 35 kDa (Lot No. F4000262-L, Freeman Industries, USA)

Equipments

1. Glassware
2. Analytical balance
3. Magnetic stirrer
4. Texture analyzer
5. Water bath
6. Refrigerator
7. Oscillatory rheometer
8. Brookfield viscometer
9. Du Nouy tensiometer
10. Fourier transform infrared spectroscopy
11. Shaker with temperature control system
12. Scanning electron microscope
13. Zeta potential Analyzer
14. Vortex mixer
15. UV-visible spectrophotometer
16. High pressure homogenizer
17. Hot air oven
18. Freeze dryer
19. pH-meter
20. Centrifuge
21. Particle size analyzer
22. Dynamic light scattering analyzer

Methods

1. Preparation of biopolymer solutions and complexes

1.1 Preparation of pectin solution

Stock solution (4% w/w LMP or HMP) was prepared by dissolving pectin powder in distilled water and stirring at ambient temperature (25°C) for at least 2 h to ensure complete hydration. The pectin solution was centrifuged (model Universal 320R, Hettich, Germany) at 8,500 rpm for 30 min to remove any insoluble particles. Only supernatant was used and pH was adjusted to the appropriate pH value.

1.2 Preparation of zein solution

Stock solution of zein was prepared by dispersing zein powder (10 g) in aqueous alcohol solution (consisting of 80% v/v ethanol), adjusting to 100 g, and stirring at ambient temperature (25°C) for at least 2 h to ensure complete hydration. The solution was then treated in the same manner as described in section 1.1.

1.3 Preparation of pectin-zein complexes

Pectin-zein complexes were prepared by mixing 1% w/w pectin (LMP or HMP) and 0-0.5% w/w zein. The pH of solution was adjusted to desired pH (pH 4). The resulting solutions were mixed for 1 min using a vortex mixer and then stored at ambient temperature (25°C) for 24 h prior to analysis.

2. Characterization of biopolymer solutions

2.1 Zeta potential measurement

The zeta potential of biopolymer solutions (pectin and zein) at various pHs (pH 3 to 7) and mixtures of 1% w/w pectin (HMP or LMP) and zein at various concentrations was measured by zeta potential analyzer (model Zetaplus, Brookhaven, USA). The pectin-zein complexes were dispersed in 5 mM acetate buffer (pH 4) with gentle stirring before measurement. The electric field applied was 1 V. The average and standard deviation of the measurement of three batches of samples were reported.

2.2 Turbidity measurement

The turbidity of pectin, zein and mixtures of pectin and zein at various pHs (pH 3 to 7) was determined using a UV-visible spectrophotometer (model T60, PG Instrument, USA) at 600 nm. The quartz cuvettes with a cell path length of 1.0 cm were used. Distilled water was used as a blank reference. The transmission and absorbance data (at 600 nm) were analyzed.

2.3 Optical microscopy

The microstructure of pectin-zein complexes was observed using a light microscope (model CX41, Olympus, Japan). The pectin-zein complexes were dropped on a glass slide and covered afterward with a coverslip, then the photos of sample were taken.

2.4 Viscosity measurement

The viscosity of pectin, zein and pectin-zein complexes was measured using a cone and plate viscometer (model DV-III Ultra, Brookfield, USA) with spindle No.51 at a speed of 50 rpm, at ambient temperature (25°C). Sample (0.5 mL) of each formulation was added on the plate.

2.5 Surface and interfacial tensions measurement

Interfacial tension is determined by fitting a shape of drop (in a captured video image) to the Young-Laplace equation which relates interfacial tension to drop shape. Equilibrium surface tension (liquid-air interfacial tension) was determined by static drop shape analysis (model FTA 100, Data Physics Corporation, USA) operated with the FTA32 v2.0 software. All measurements were conducted at ambient temperature (25°C). Pendant aqueous solution drops were formed in the air using a needle with inner diameter of 0.635 mm. Surface tension of pectin (HMP or LMP) and zein solutions at various concentrations were determined immediately after drop formation. The mean values of three measurements were reported. The oil-water interfacial tension was also determined using drop shape analysis method. An aqueous droplet containing 0.5-2% w/w pectin (HMP or LMP) was automatically formed at the tip of needle which was immersed in a glass cuvette containing the oil phase. The droplet shape was automatically analyzed to record the changes in the interfacial tension over time. The interfacial tension of the system containing pectin-zein complexes, prepared at pH 4 or 7, was also determined. All the measurements were carried out in triplicate.

2.6 Morphological observation

The morphology of pectin-zein complexes was investigated by a scanning electron microscope (model Maxim-2000, CamScan Analytical, England), under an accelerating voltage of 15 keV. All samples were fixed on SEM stubs with double-sided adhesive tape and then coated, in a vacuum, with thin gold layer before investigation.

2.7 Fourier transform infrared (FTIR) spectroscopic analysis

The FTIR spectra of samples were recorded by FTIR (model 4700, Thermo Nicolet, Japan). All samples were prepared using KBr disc method. Each sample was blended with KBr powder and compressed to a disc with a pressure of 5 tons for 60 s before placing in a sample holder. The spectral value of the samples was obtained by scanning from 4000 to 400 cm-1. FTIR spectra of the samples were obtained using a software package (Omnic FTIR software, version 7.2a, Thermo Electron Corporation, USA).

3. Preparation of o/w emulsions

In this study, the term primary emulsion is used to refer to the emulsion prepared by using only zein or pectin as an emulsifier whereas the term secondary emulsion is used to refer to the primary emulsion to which another emulsifier has been added.

3.1 Effect of pectin and rice bran oil concentration

To study the effect of pectin and rice bran oil concentration. Emulsion was prepared by homogenizing the oil phase (5-30% w/w rice bran oil) and the aqueous phase (0.5-2% w/w HMP) at a speed of 12,000 rpm for 3 min. The obtained emulsion containing 0.1% w/w methyl-propyl paraban as an antimicrobial agent. The resulting emulsions were then stored at ambient temperature (25°C) for 24 h before analysis.

3.2 Effect of mixing order

Emulsions stabilized by pectin and zein were prepared by different mixing order using homogenizer (model T25 digital Ultra-Turrax, IKA, Germany) performed in an ice-bath to avoid over heating.

Method I: The o/w emulsion was prepared by homogenizing the oil phase (20% w/w rice bran oil) and the aqueous phase containing 2% w/w zein at a speed of 12,000 rpm for 3 min.

Method II: The o/w emulsion was prepared by homogenizing the oil phase (20% w/w rice bran oil) and the aqueous phase containing 2% w/w HMP at a speed of 12,000 rpm for 3 min.

Method III: The o/w emulsion was prepared by homogenizing the oil phase (20% w/w rice bran oil) and the aqueous phase containing a mixture of 2% w/w pecin and 0.1% w/w zein at a speed of 12,000 rpm for 3 min.

Method IV: The primary emulsion was prepared by homogenizing the oil phase (20% w/w rice bran oil) and the aqueous phase containing 2% w/w HMP at a speed of 12,000 rpm for 3 min. The weighed amount of zein (0.1% w/w) was added to primary emulsion. The obtained mixture was homogenized for another 3 min.

All emulsions were then stored at ambient temperature (25°C) for 24 h before analysis.

3.3 Effect of pH

To study the effect of pH of emulsion, method IV was used to prepare pectin-zein stabilized emulsions. Primary emulsion was prepared by mixing rice bran oil with pectin using homogenizer at a speed of 12,000 rpm for 3 min. Zein was then added to primary emulsion in order to obtain secondary emulsion. The composition of secondary emulsion was 20% w/w rice bran oil, 2% w/w pectin and 0.1 or 0.5% w/w zein. In this study, the pH condition (pH 4 or 7) for preparation of emulsions was compared. The effect of pH change from 7 to 4 was also investigated. The obtained emulsions were then stored at ambient temperature (25°C) for 24 h before analysis.

3.4 Effect of zein concentration

To study the effect of zein concentration, method IV was used to prepare pectin-zein stabilized emulsions. Primary emulsion was prepared by homogenizing the oil phase (20% w/w rice bran oil) and the aqueous phase (2% w/w HMP) at a speed of 12,000 rpm for 3 min. The weighed amount of zein (0-0.5% w/w) was added to primary emulsion. The obtained mixture containing 0.1% w/w methyl-propyl paraban as an antimicrobial agent was homogenized for 3 min. The resulting emulsions were then stored at ambient temperature (25°C) for 24 h before analysis.

3.5 Effect of high-pressure homogenizer

The effects of homogenization pressure and number of pass through the high-pressure homogenizer on the droplet size of emulsion were studied.

4. Characterization of o/w rice bran oil emulsions

4.1 Measurement of drop size: The size of emulsion droplets was measured by using laser scattering particle size distribution analyzer (model LA-950, Horiba, Japan).

4.2 Zeta potential measurement: The zeta potential of the obtained emulsions was measured by zeta potential analyzer

4.3 Measurement of viscosity: The viscosity of the obtained emulsions was measured by Brookfield viscometer:cone and plate (DV III, Brookfield, Germany)

4.4 Optical microscopy: The morphology of droplet of the emulsions was investigated by light microscope (model CX41, Olympus, Japan).

4.5 Determination of %creaming or cracking after keeping in various conditions (detail in 4.6)

4.6. Stability of o/w emulsion

All emulsions were transferred into glass vials and then stored at ambient temperature (25°C) for 14 days The emulsions were also stored under environmental stress condition, i.e., temperature cycling at 4°C/40°C (6 cycles), and subjected to centrifugation test at 3,000 rpm for 10 min. After storage or test, a number of emulsions separated into optically opaque "cream layer" at the top and a transparent (or turbid) "serum layer" at the bottom. The total height of the emulsions (HE) and the height of the serum layer (HS) were measured. The extent of creaming was characterized by creaming index using the following Equation:

$$\%\ \text{Creaming index} = \left(\frac{H_S}{H_E}\right) \times 100$$

Results and Discussion

1. Characterization of pectin and zein solutions

The zeta potential of zein, HMP or LMP at various pHs was determined as shown in Figure 1. The zeta potential of zein was about zero at pH ~ 5.5, indicating the isoelectric point (pI) of zein. At pH below pI value (pH<5.5), zein was positively charged while at pH above pI (pH>5.5) it was negatively charged. This effect is attributed to a change in the ionization of amino and carboxyl groups at low pH ($-NH_3^+$; -COOH) and high pH ($-NH_2$; $-COO^-$). The zeta potential of pectin solutions was negatively charged at all pHs (from pH 2.5 to 7).

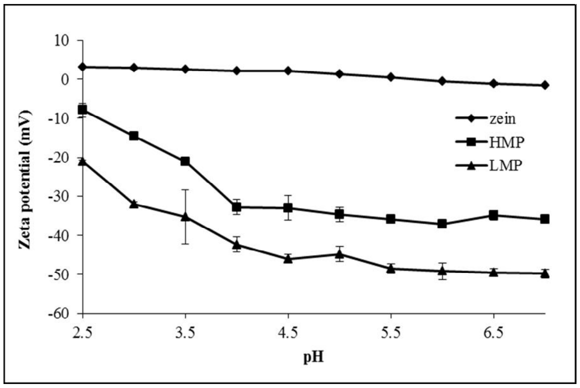


Figure 1 Zeta potential of 1% w/w LMP, 1% w/w HMP and 0.125% w/w zein at various pHs.

The particle size of zein solution at various pHs was determined as shown in Figure 2. The results demonstrated that the particle size of zein increased sharply when pH closes to pH ≈ 5.5, i.e., the particle size changed from 2 µm at pH 4 to 45 µm at pH 5.5. This indicated that the extensive droplets aggregation may occur at pH close to the pI of zein [20]. This is confirmed by the photograph of zein solution at various pHs (Figure 3). The zein solution was turbid with some precipitates at pH ≈ 5.5. Clear solution of zein was observed at pH below 4.5 and pH above 7. Therefore, it is confirmed that the pI value of zein was at pH 5.5.

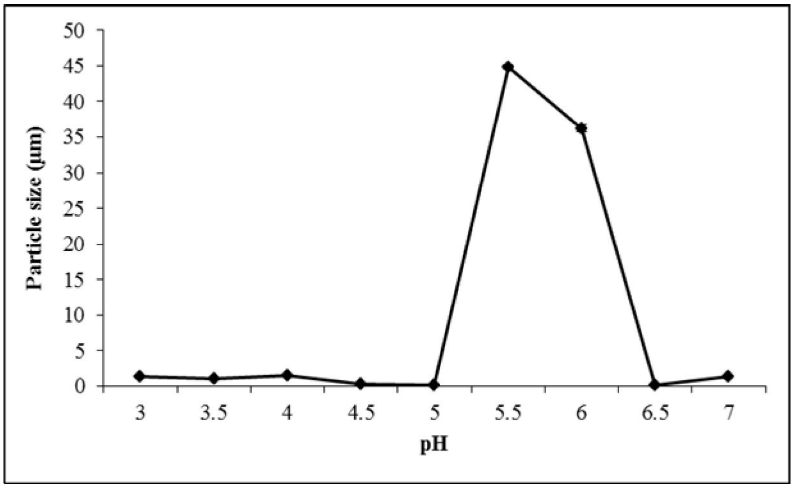


Figure 2 Particle size of zein solution (0.125% w/w) at various pHs.

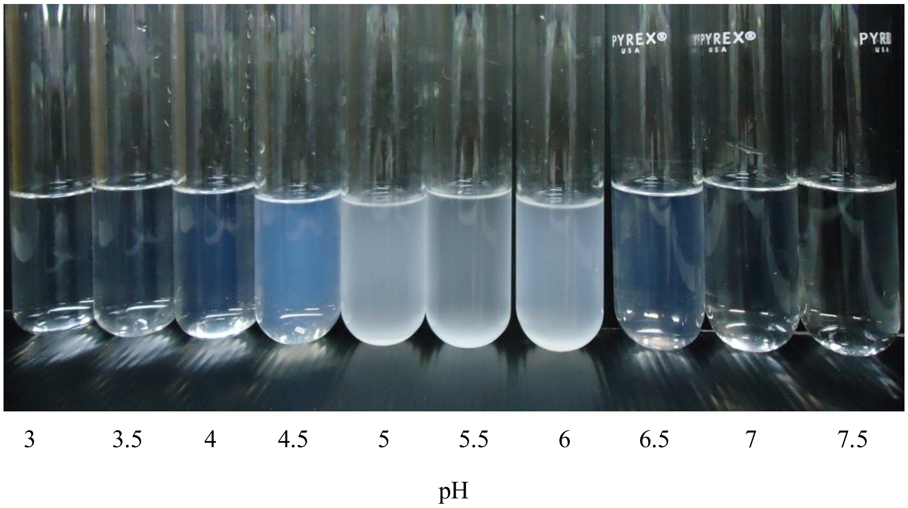


Figure 3 Photograph of 0.125% w/w zein solution at various pHs.

Figure 4 shows turbidity of zein and pectin solutions at various pHs. The results showed that zein solutions were more turbid than pectin solutions. High absorbance at pH ranged from 4.5 to 6.5 was observed. Moreover, the absorbance of zein solution at pH 4.5-6.5 tended to increase with increased concentration of zein. This may result from the increased amount of insoluble form of zein [29]. The turbidity of pectin solution at various pHs was also investigated. The results showed that the turbidity of HMP was not significantly different at the investigated pHs (from 2.5 to 7.5). However, the turbidity of

LMP, at low pH (2.5 - 3.5), was more than that of HMP and gave a clear solution at pH 4 and above. It is possible that the ionization of carboxylic acid is suppressed at low pH, resulting in a reduction in hydration of the carboxylic acid group.

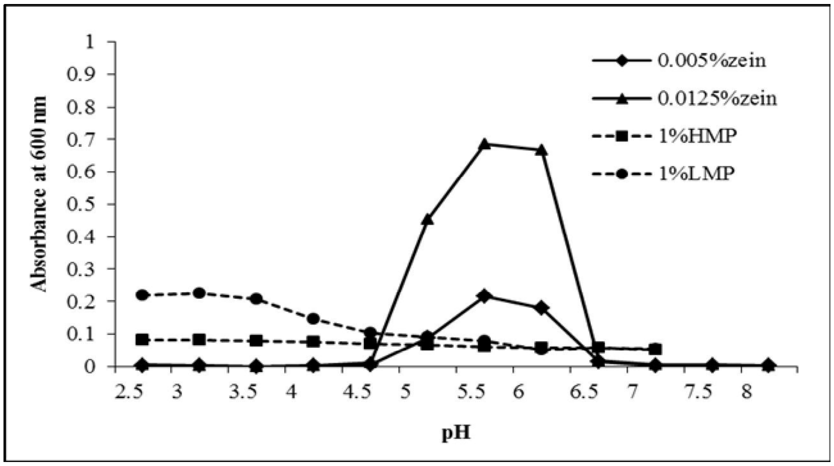


Figure 4 Turbidity of zein and pectin at various pHs.

Figure 5 shows the viscosity of pectin (LMP or HMP) solution at various concentrations. The viscosity of pectin solution increased in a pseudoplastic fashion when the concentration of pectin was increased. This may result from the increase in hydrogen bonding of water with hydroxyl groups of pectin structure and the distortion in the velocity pattern of the liquid by hydrated molecules of the solute [30].

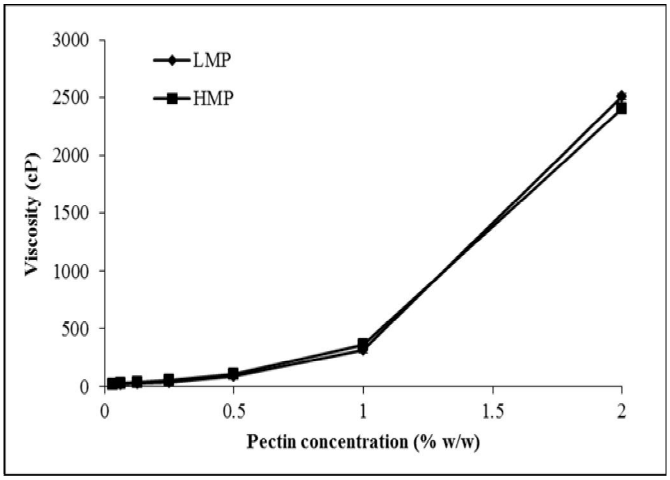


Figure 5 Viscosity of pectin (LMP or HMP) solution at various concentrations.

Surface tension of pectin (LMP or HMP) at various concentrations was investigated as shown in Figure 6. The results demonstrated that, at low concentration, the surface tension of LMP and HMP solutions was not significantly different. In addition, at higher concentration 1% w/w or more, the surface tension of LMP solution tended to decrease. This may be due to the fact that concentration of LMP was already excess, above or close to the interfacial saturation [15]. However, the expansion of the air-water interface was not practical in the preparation of o/w emulsion, so the interfacial tension between oil and water was also investigated.

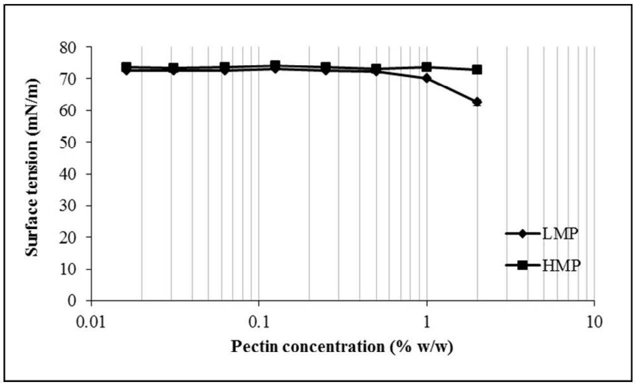
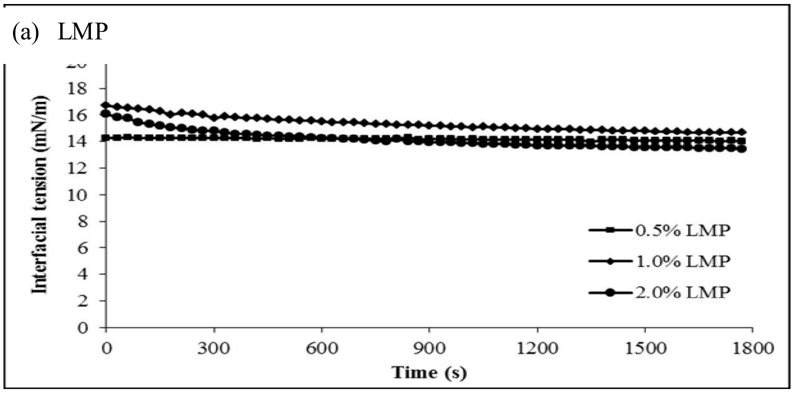


Figure 6 Surface tension of pectin (LMP or HMP) at various concentrations.

Time evolution of the interfacial tension for rice bran oil and 0.5-2% w/w LMP or HMP was investigated (Figure 7). The interfacial tension slightly decreased with increasing of time and the constant value was observed at more than 600 s. This might be due to time consuming for adsorption of macromolecule on the interface followed by rearrangement in the surface film [31]. Moreover, the interfacial tension of pectin decreased with the increased pectin concentration, resulting from the more surface active character of pectin molecules.

2. Characterization of pectin-zein complexes

Zeta potential is an analytical tool that could point to existence of strong charge-charge interactions between two biopolymers. Therefore, experiments were carried out under conditions where protein and polysaccharide had opposite charge [32], i.e., at pH 4. Figure 8 shows zeta potential of mixtures prepared from various concentrations of zein (0-0.01% w/w) and 1% w/w pectin (LMP or HMP), at pH 4. The results showed that, for HMP, the zeta potential increased from -17 mV to -13mV in the presence of zein concentration in range of 0.001-0.004% w/w, resulting from neutralization of cationic zein molecules to pectin surface [32]. The zeta potential of the complexes in the presence of 0.006-0.010% w/w zein was slightly more negative than in the absence of zein. It is possible that low surface charge of zein molecule could form bridges between pectin molecules and show the result negatively charged [18]. Furthermore, the zeta potential of LMP in the presence of zein was highly negative and close to that of pure LMP solution (-45 mV). This may be because the concentration of zein is not enough for adsorption to the surface of LMP molecules. Therefore, it is suggested that the concentration of zein primarily influenced the formation of zein-pectin complexes, especially those using HMP.


(a) LMP

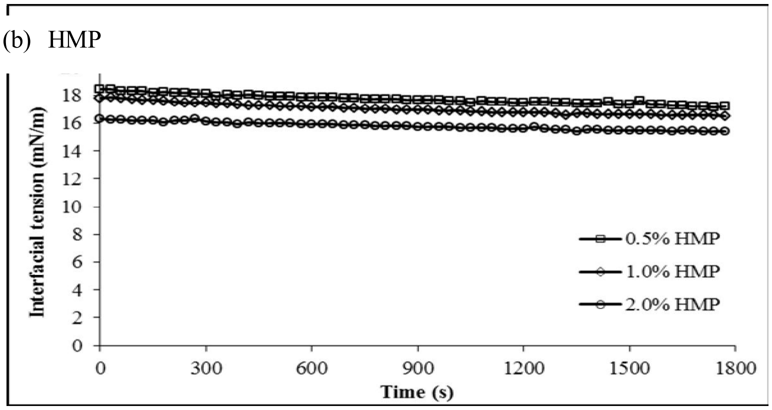

(b) HMP

Figure 7 Time evolution of the interfacial tension for rice bran oil and 0.5-2% w/w (a) LMP or (b) HMP, at 25°C.

The solutions containing 1% w/w pectin and different concentrations of zein were left to settle overnight and formation of complexes was assessed by turbidity measurement at pH 4. The pure zein solution was clear with about 100% transmittance. The turbidity of pectin-zein solutions increased with the increased concentration of zein (Figure 9). This may be due to the increased amount of insoluble pectin-zein complexes in the system, resulting from the strong interaction between the two oppositely charged biopolymers [29].

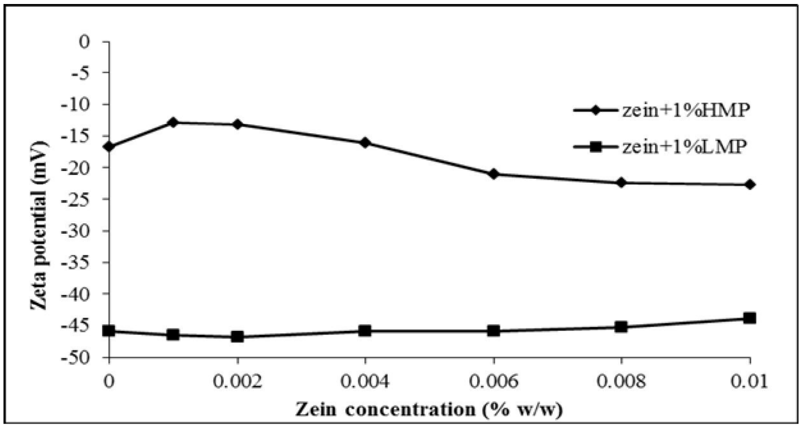


Figure 8 Zeta potential of mixtures prepared from various concentrations of zein, (0-0.01% w/w) and 1% w/w pectin (LMP or HMP), at pH 4.

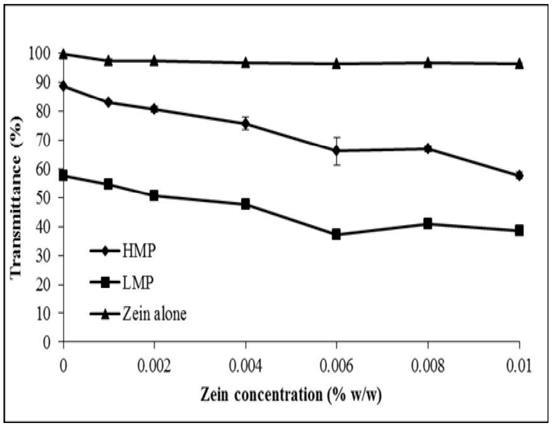


Figure 9 Turbidity of mixtures prepared from various concentrations of zein (0-0.01% w/w) and 1% w/w pectin (LMP or HMP), at pH 4.

To confirm the complex formation between pectin and zein at pH 4, zein with various concentrations was mixed with pectin solution and then investigated visually, as shown in photographs of pectin-zein complexes (Figure 10). It is clearly observed that the solutions were turbid. It is possible that the bridging flocculation occurred in the mixed solutions. Moreover, the turbidity of pectin-zein complexes increased when zein concentration was increased, resulting from the increased amount of pectin-zein complexes, as discussed above. It is apparent that a mixture of LMP and zein was extensively aggregated whereas a mixture of HMP and zein was fairly homogeneous. This is probably due to the electrostatic bridging phenomena of LMP, leading to the formation of large particle size.

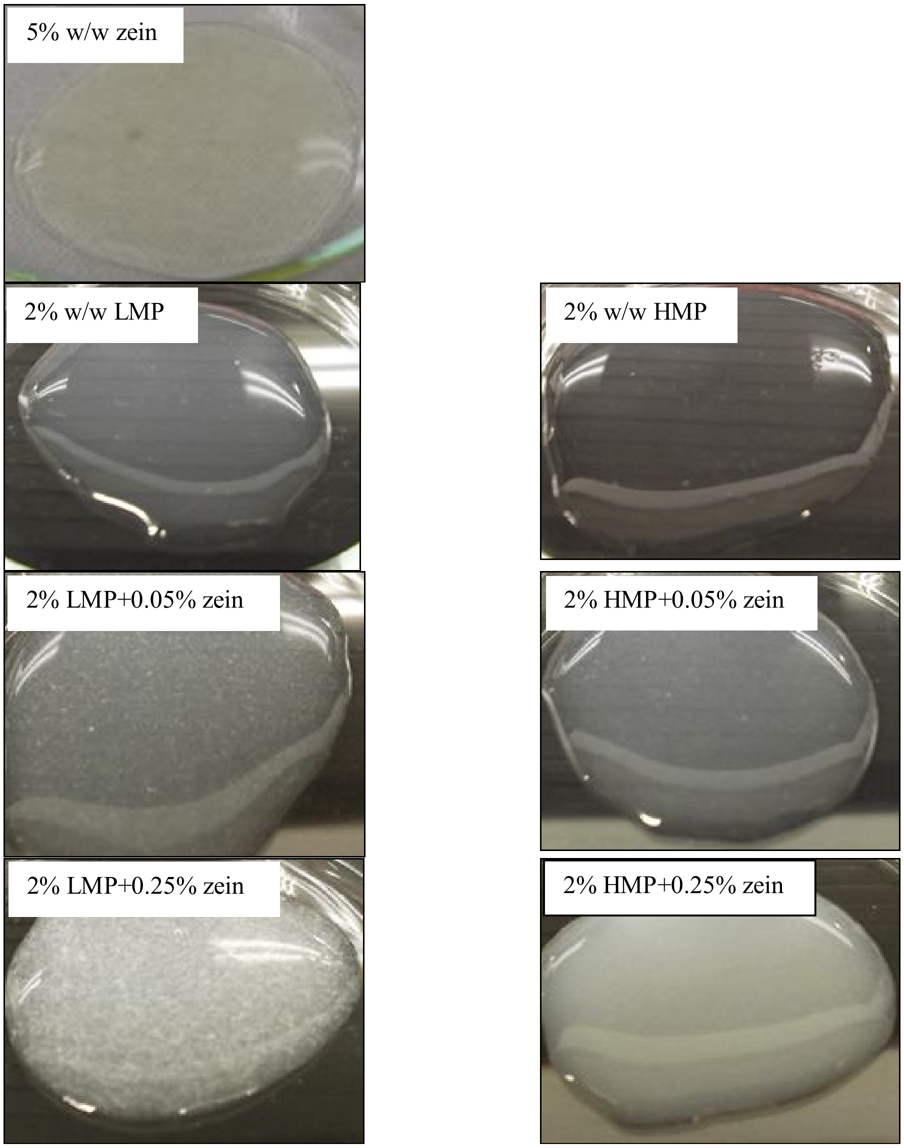
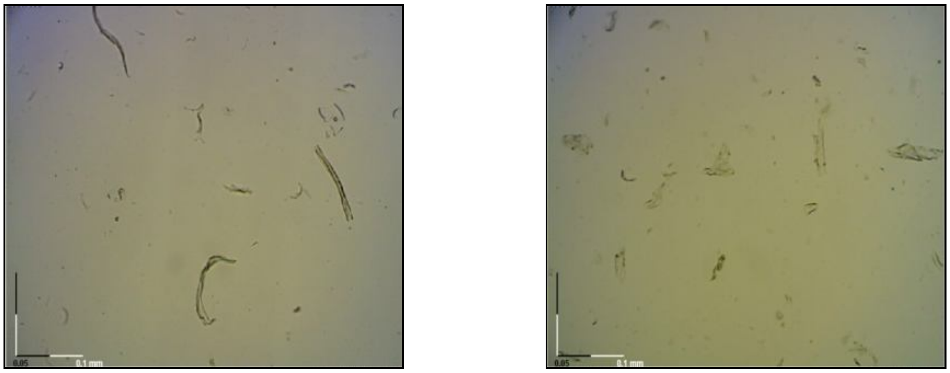
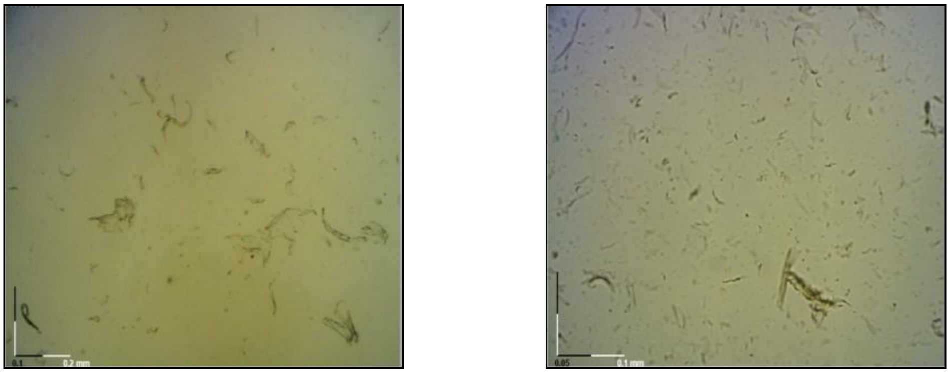


Figure 10 Photographs of zein, pectin and pectin-zein complexes containing 2% w/w pectin (LMP or HMP) and zein (0.05 or 0.25% w/w), at pH 4.

Figure 11 shows optical microscopic images of pectin-zein complexes containing 2% w/w pectin (LMP or HMP) and zein (0.05 or 0.25% w/w). The particulates and fibrous materials were observed in the mixtures. It is likely that the formation of insoluble complexes between protein (i.e., zein) and polysaccharide (i.e., pectin) was occurred via electrostatic interaction [32].



2% w/w LMP + 0.05% w/w zein   2% w/w HMP + 0.05% w/w zein



2% w/w LMP + 0.25% w/w zein   2% w/w HMP + 0.25% w/w zein

0.1 mm

Figure 11 Optical microscopic images of pectin-zein complexes containing 2% w/w pectin (LMP or HMP) and zein (0.05 or 0.25% w/w), at pH 4.

The surface morphology of the pectin-zein complexes was also investigated by SEM, as shown in Figures 12 and 13. The SEM micrographs demonstrated the smooth surface of pectin film, both LMP and HMP. In the presence of zein, the round-shaped, submicron-sized globules spreading over surface film were observed. Moreover, the surface of pectin-zein complexes was rougher when the concentration of zein was increased.

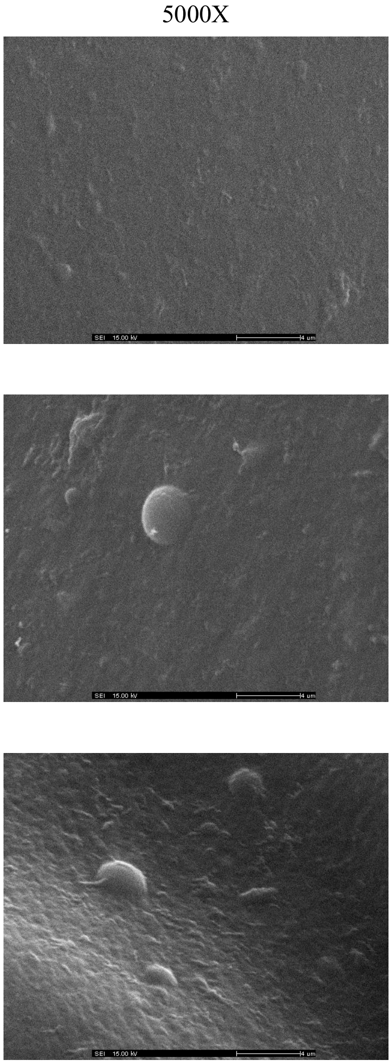


Figure 12 SEM micrographs of pectin-zein complexes containing 2% w/w LMP and zein (0.05 or 0.25% w/w), at pH 4.

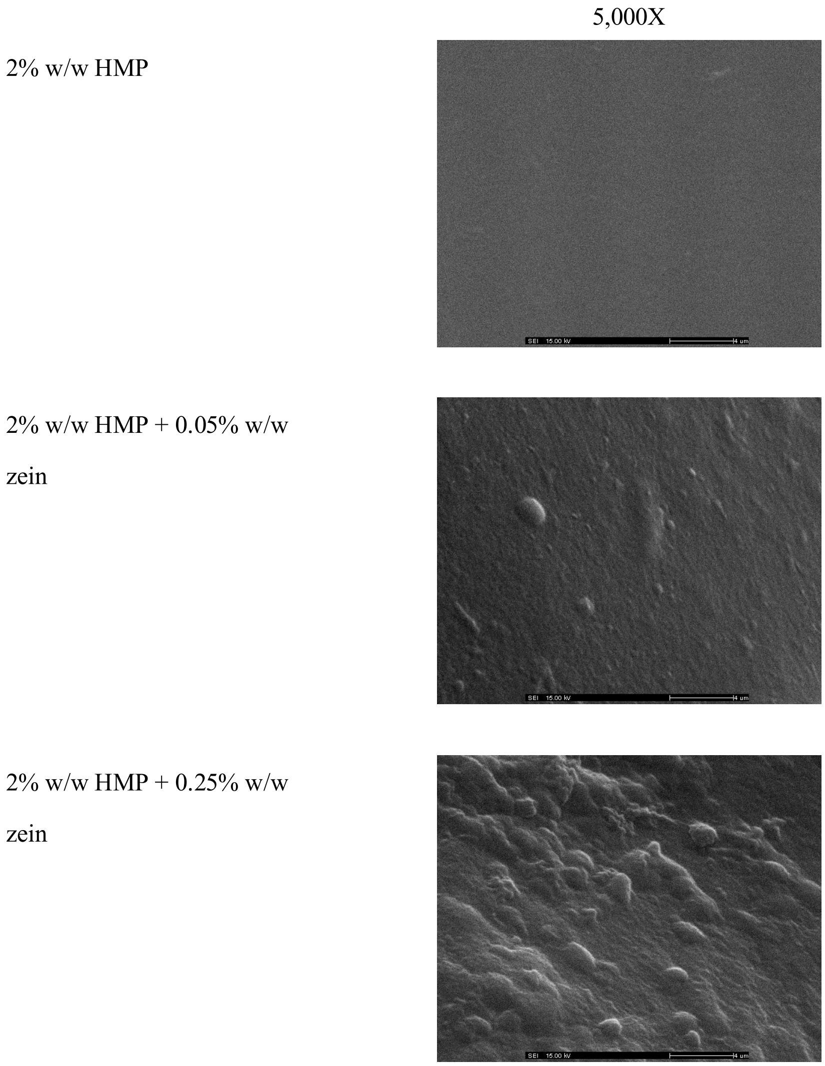


Figure 13 SEM micrographs of pectin-zein complexes containing 2% w/w HMP and zein (0.05 or 0.25% w/w), at pH 4.

FTIR spectra of pectin-zein complexes are shown in Figures 14 and 15. The obtained spectra were analyzed by comparing the following characteristic regions; O-H stretching (3,100-3,600 cm^{-1}), C-H stretching (2,800-3,000 cm^{-1}), carboxylic group stretching region (1,200-1,800 cm^{-1}) and the fingerprint region (under 2,000 cm^{-1}) which reflects the monosaccharide composition of the analyzed pectin [33]. FTIR spectrum of zein showed O-H stretching from H-bond between 3,200 and 3,400 cm^{-1}, and the peaks at 1,550-1,500 cm^{-1} and 1,700-1,600 cm^{-1} corresponded to the characteristic transmission of primary amide and secondary amide, respectively, which are typical protein transmission bands [34]. Figure 15 showed that no new transmission peak or shifting was found from the FTIR analysis, indicating no or minor interaction between pectin and zein molecules after the formation of pectin-zein complexes. Moreover, the fingerprint region of the pectin-zein complexes was similar to pectin. However, there were some differences in the FTIR spectra between physical mixture and pectin-zein complexes. After pectin-zein complex formation, the transmission peak at 1,735-1,750 cm^{-1} corresponding to C=O of pectin increased, compared to the physical mixture of pectin and zein in the same ratio. Similar results were observed in case of HMP (Figure 15). These observations suggested the possibility of weak physical interaction between pectin and zein.

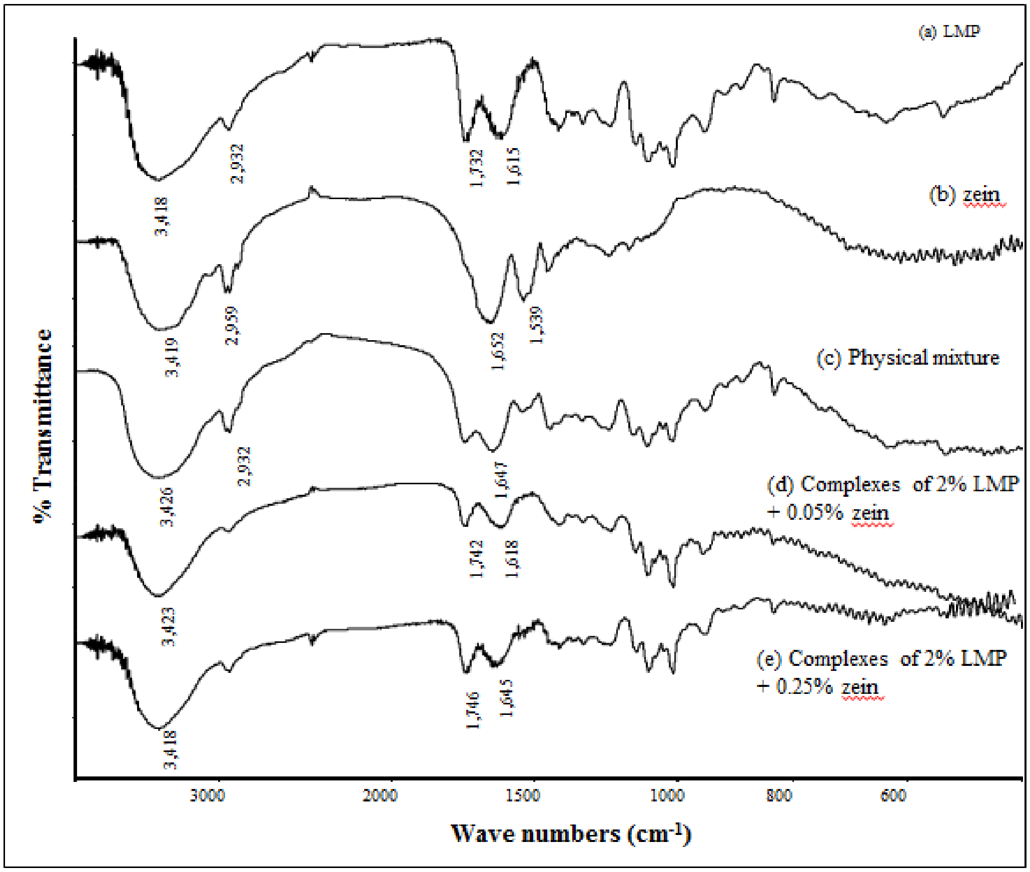


Figure 14 FTIR spectra of LMP-zein complexes, at pH 4; (a) LMP, (b) zein, (c) physical mixture of 2% w/w LMP and 0.25% zein, (d) complexes of 2% w/w LMP and 0.05% w/w zein, and (e) complexes of 2% w/w LMP and 0.25% w/w zein.

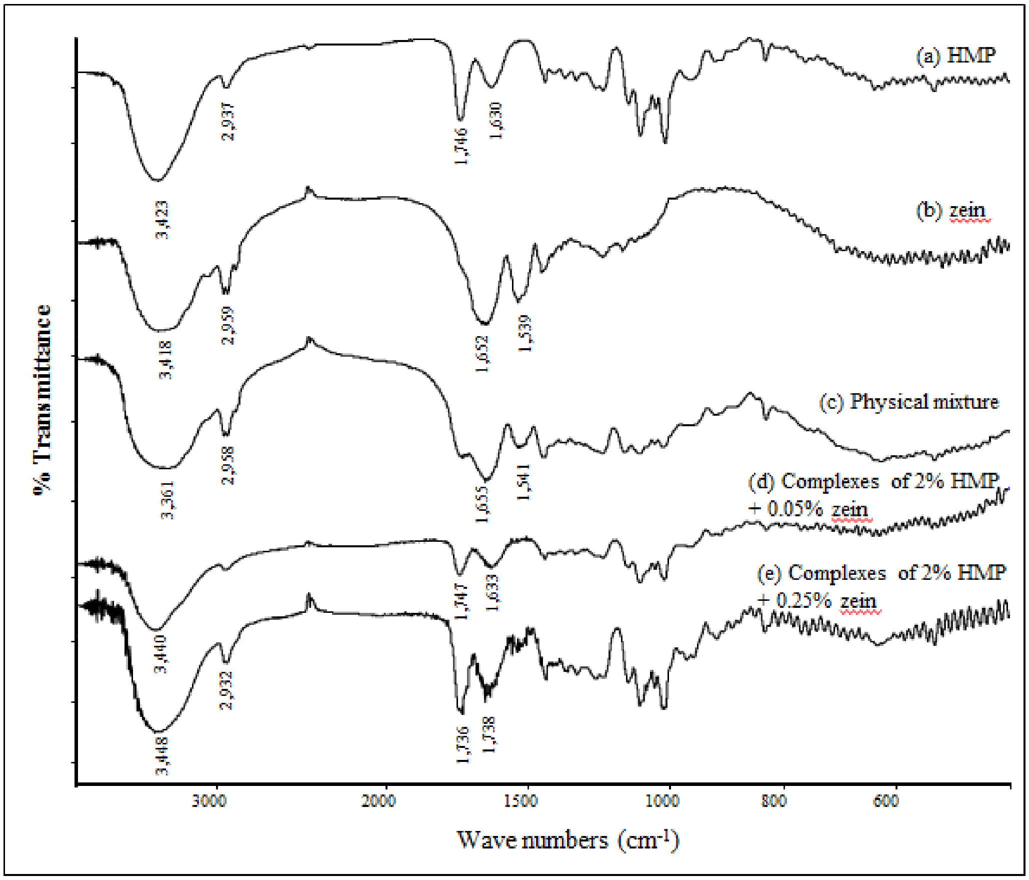


Figure 15 FTIR spectra of HMP-zein complexes, at pH 4; (a) HMP, (b) zein, (c) physical mixture of 2% w/w HMP and 0.25% zein, (d) complexes of 2% w/w HMP and 0.05% w/w zein and, (e) complexes of 2% w/w HMP and 0.25% w/w zein.

Figure 16 shows the surface tension of zein and HMP-zein complexes prepared from 1% w/w HMP and various concentrations of zein (0.0001-0.1% w/w), at pH 4. The results demonstrated that surface tension of zein solution rapidly decreased from 75 to 55 mN/m with increased concentration of zein from 0.01 to 0.05% w/w. Similarly, in the case of pectin-zein complexes, surface tension tended to decrease when zein concentration was increased. It is likely that zein, which has amphiphilic character, can adsorb at the oil-water interface and thus decrease the surface tension [35]. However, the decrease in surface tension of pectin-zein complexes was less extent than that of zein solution alone. This might be due to the fact that pectin molecules having low surface active were located at the air-water interface by replacing on some molecules of the zein [36]. This finding indicated that the concentration of pectin-zein complexes could influence the adsorption properties at the interface between oil and water phases.

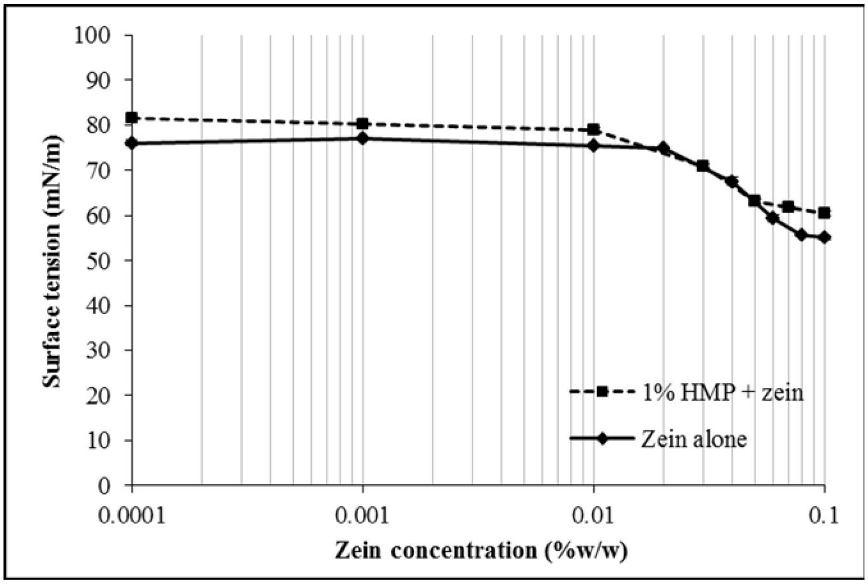


Figure 16 Surface tension of zein alone and HMP-zein complexes prepared from 1% w/w HMP and various concentrations of zein (0.000-0.1 %w/w), at pH 4.

The time evolution of the interfacial tension for rice bran oil containing zein, pectin, or a mixture of pectin and zein was investigated, as shown in Figure 17. The interfacial tension of pectin-zein complexes was higher than that of zein alone and slightly less than that pectin alone. In order to compare the pH condition on pectin-zein complexes, interfacial tension of rice bran oil and zein mixture at pH 4 and 7 was investigated. The result showed that the interfacial tension at pH 4 and 7 was not significantly different. In contrast, the interfacial tension of HMP-zein complexes at pH 7 decreased slightly when time progressed. The constant value (about 11 mN/m) was observed at more than 600 s. It might be due to the fact that the electrostatic interaction was not occurred at pH 7 because HMP and zein exhibited the same charge. Hence, independent pectin and zein need time for adsorption of macromolecule on the interface followed by rearrangement at the interface [15]. The interfacial tension of complexes using HMP more decreased than those using LMP. This was because HMP had more hydrophobic group than LMP, thus, exhibited a good emulsifying property [18].

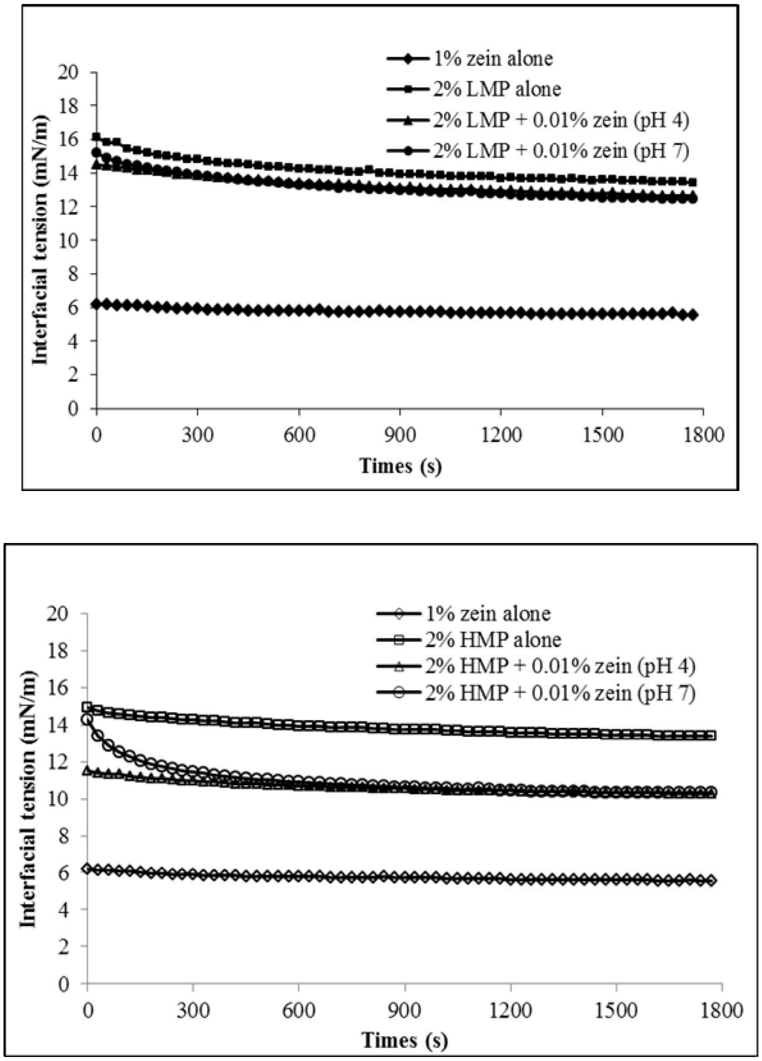


Figure 17 Time evolution changes of the interfacial tension for rice bran oil containing zein, pectin, or a mixture of pectin and zein; (a) LMP or (b) HMP, at 25°C.

3. Preparation of o/w emulsions stabilized by pectin-zein complexes

3.1 Effect of pectin and rice bran oil concentration

In the preliminary studies, it was found that emulsions prepared by using LMP alone were not stable. Therefore, only HMP was used in this experiment. Figure 18 shows droplet size of emulsions stabilized by HMP, prepared by using various concentrations of rice bran oil. The results showed that, increasing of rice bran oil concentration led to an increase in droplet size of emulsions, resulting from an increase in the amount of internal phase of emulsion droplets. The results also showed that the droplet size decreased with the increase in pectin concentration. The results suggested that higher amount of hydrophobic groups in the HMP may result in a greater emulsifying property capable of making smaller droplets [12]. Pectin at low concentration (e.g., 0.5 and 1% w/w) could not be used to prepare emulsions which contained high concentration of rice bran oil (e.g., 25 and 30% w/w). This is probably pectin was not sufficient to prepare stable emulsions.

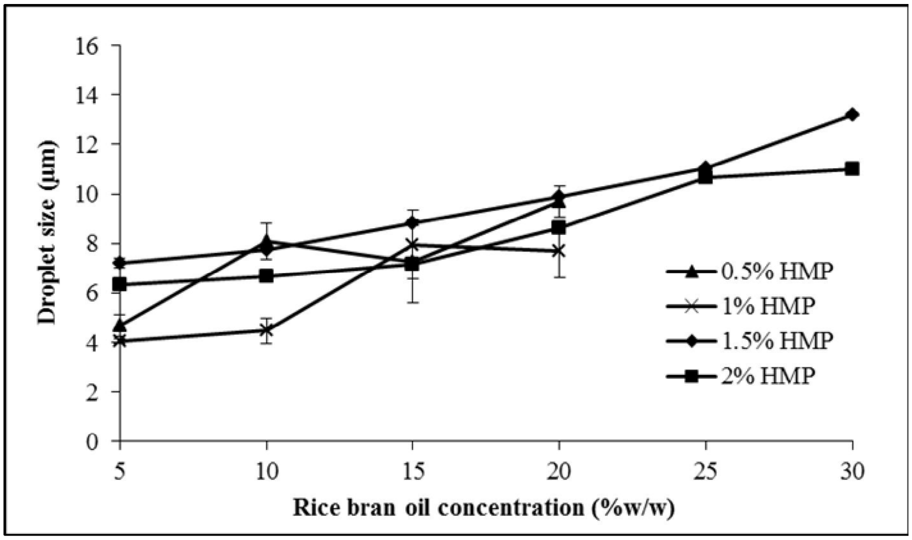


Figure 18 Droplet sizes of o/w emulsions stabilized by HMP, prepared by using various concentrations of rice bran oil.

Figure 19 shows the zeta potential of o/w emulsions containing 0.5-2% w/w HMP and various concentrations of rice bran oil. The results showed that zeta potential of all emulsions was negative due to carboxyl groups of pectin. However, there was no change upon increasing of rice bran oil concentration, suggesting that the concentration of internal phase did not influence the zeta potential. In case of the increased pectin concentration in the formulation, the zeta potential was not significantly changed about (-22 mV), resulting from a small change in pectin concentration in the formulation.

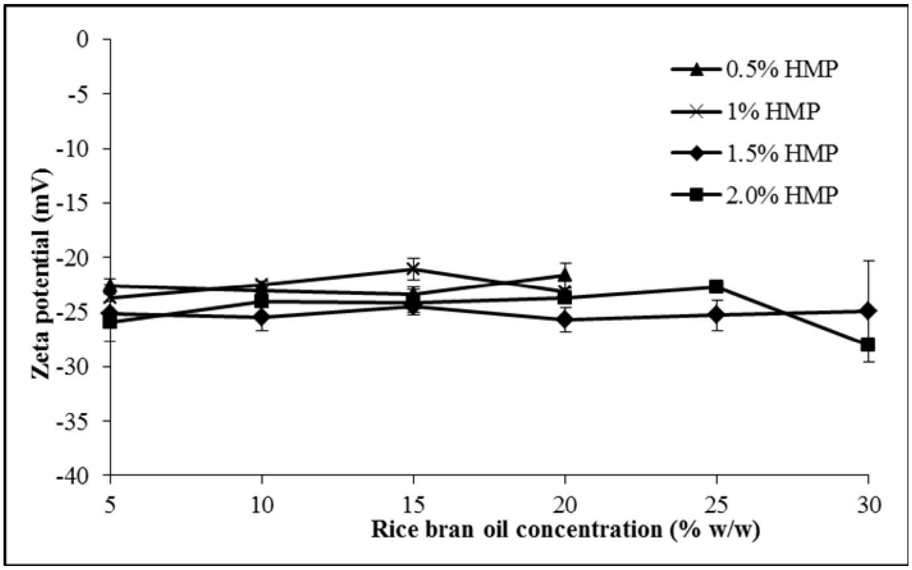


Figure 19 Zeta potential of o/w emulsions containing 0.5-2% w/w HMP and various concentrations of rice bran oil.

The stability of o/w emulsions at various concentrations of rice bran oil was investigated using percent creaming index. When the concentration of rice bran oil was increased from 10 to 20% w/w, the emulsion stability increased (percent creaming index decreased), as shown in Figure 20, 21, and 22. These suggested that the concentration of pectin in bulk is sufficient to allow rapid diffussion and adsorption of pectin to newly formed droplets [13]. At concentration of rice bran oil 25-30% w/w demonstrated low stability of emulsion (percent creaming index increased). This is probably pectin was not sufficient to prepare stable emulsions. Using at least 1.5% w/w of HMP could produce the stable o/w emulsions with low percent creaming index at the rice bran oil concentration of 15 and 20% w/w, when kept at ambient temperature (25°C) for 14 days. Figure 21 also appears that the formations using 1.5 and 2% w/w HMP produced the stable o/w emulsions with the lowest percent creaming index, after centrifugation test at 3,000 rpm for 10 min, while the emulsions using low concentration of pectin, e.g., 0.5 and 1% w/w, were separated into two phases with high percent creaming index. The stability of emulsions tested by temperature cycling also demonstrated a good stability with low percent creaming index when rice bran oil concentration of 15-20% w/w and a high concentration of HMP (e.g., 1, 1.5 and 2% w/w) were used (Figure 22). This suggested that the pectin concentration at least 1.5% w/w had sufficient to produce stable emulsion. From the above results, therefore, the o/w emulsions prepared by using 2% w/w HMP and 20% w/w rice bran oil was selected for further investigation.

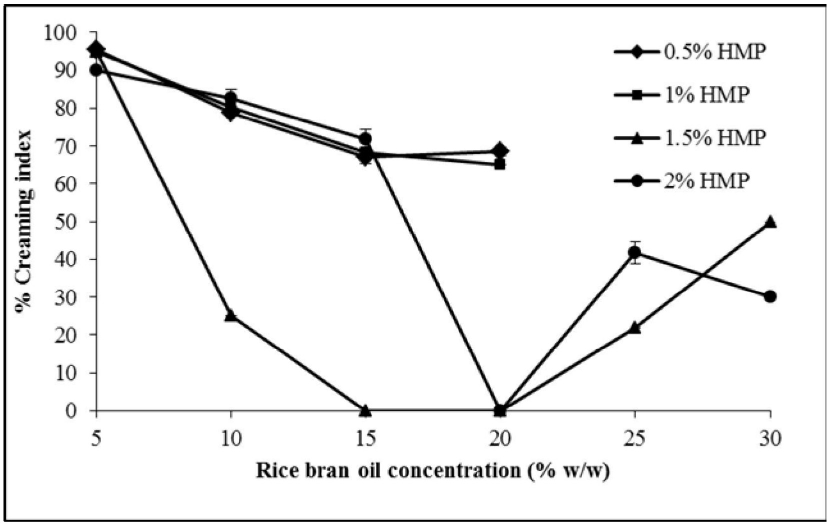


Figure 20 Percent creaming index of o/w emulsions stabilized by pectin, prepared at various concentrations of rice bran oil, when kept at ambient temperature (25°C) for 14 days.

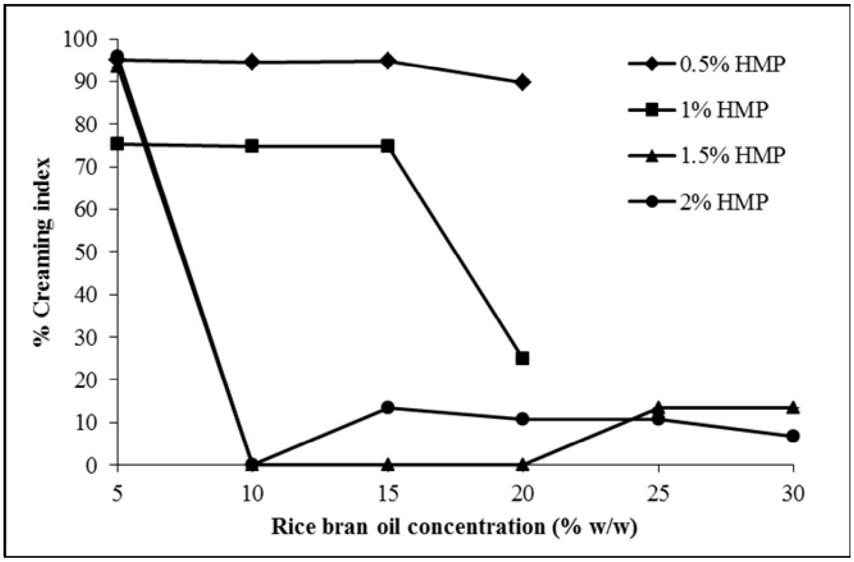


Figure 21 Percent creaming index of o/w emulsions stabilized by pectin, prepared at various concentrations of rice bran oil, after centrifugation test at 3,000 rpm for 10 min.

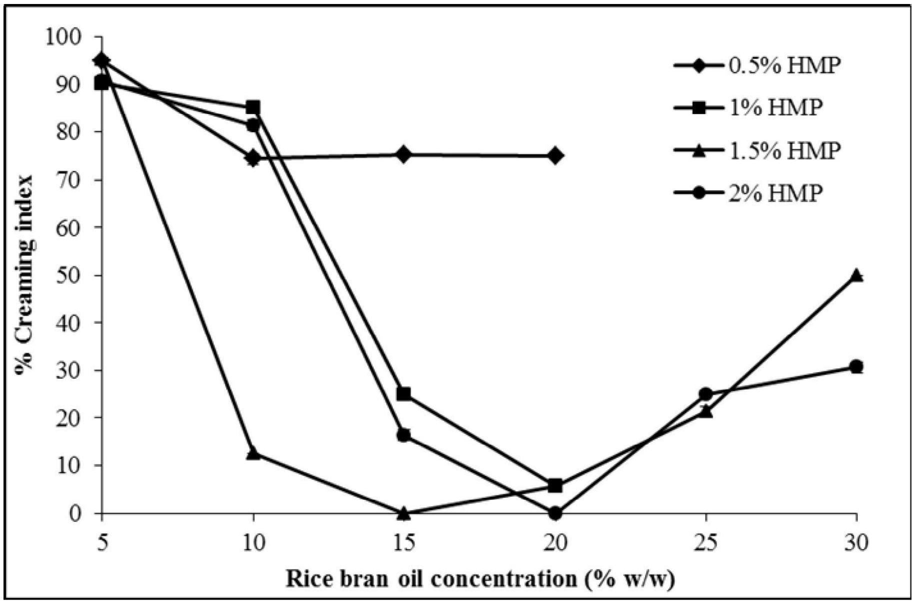


Figure 22 Percent creaming index of o/w emulsions stabilized by pectin, prepared at various concentrations of rice bran oil, after temperature cycling test (4°C/40°C) for 6 cycles.

3.2 Effect of order of mixing

In order to compare the order of mixing for emulsion preparation using pectin and/ or zein as emulsifiers, four methods were used. According to the preliminary studies, 2% w/w HMP and 0.1 or 2% w/w zein were used, and the preparation pH was fixed at pH4. In case of zein alone (method I), stable emulsions could not be obtained. Zein alone has insufficient surface active property to produce emulsion. The zeta potential of emulsions prepared by different methods was not significantly change (Table 1). In method II, emulsions prepared by HMP alone showed that the mean droplet size of o/w emulsions was about 8 µm. In method III and method IV, which used both HMP and zein as co-emulsifier, the mean droplet size of o/w emulsions was smaller than those prepared by method II (Table 1). This suggested that zein and HMP showed opposite charge and then electrostatic interaction could form. However, method III and method VI were different in term of order of mixing. In method III, HMP was combined with a positive charge zein while in method IV, emulsion was formed using (HMP) as primary emulsifier and then zein was added to adsorb onto the pectin layer. Table 1 also shows that the span value of emulsions prepared by method IV was slightly lower than those prepared by method III. This indicated that method IV could produce uniform-sized emulsions. Hence, based on this finding, method IV was selected for further investigation.

Table 1 Droplet size and zeta potential of o/w emulsions prepared by using different orders of mixing.

Method		**$d_{4,3}$ (μm) ± S.D.**	**Span value**	**Zeta potential (mV) ± S.D.**
I	0.1 or 2% zein + 20% rice bran oil	-	-	-
II	2% HMP + 20% rice bran oil	8.925 ± 0.55	0.824	-25.46 ± 0.35
III	2% HMP + 0.1% zein + 20% rice bran oil	6.404 ± 0.09	0.925	-27.34 ± 0.49
IV	(2% HMP + 20% rice bran oil) + 0.1% zein	6.192 ± 0.08	0.761	-26.59 ± 1.43

Figure 23 shows microscopic images of o/w emulsions prepared by different methods. The results demonstrated that the oil droplets dispersed in water phase and not coalesced. This may be due to the fact that their surfaces were completely saturated with biopolymers. Moreover, method II which prepared by using HMP alone showed a slightly larger in droplet size than those prepared by other methods (method III and method IV). It is possible that the complexes of HMP and zein may improve emulsification properties by reducing interfacial tension, resulting in smaller droplet size than those used HMP alone.

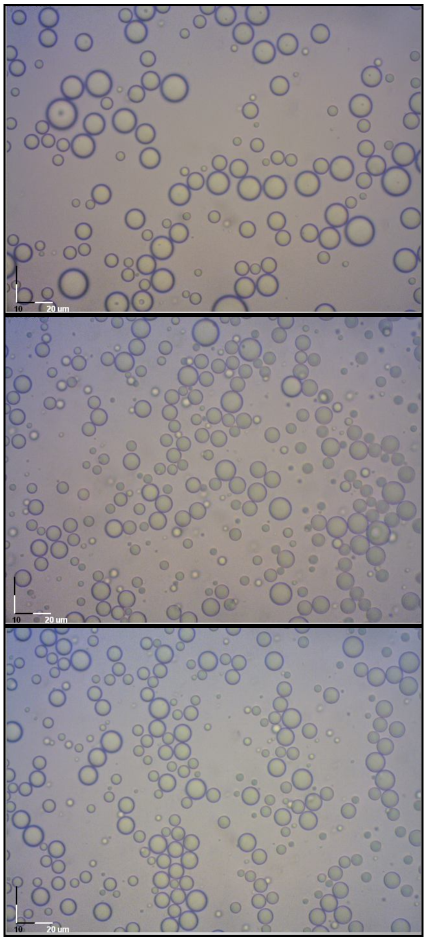
The viscosity of o/w emulsions prepared by different mixing orders was investigated (Figure 24). The results demonstrated that the viscosity of emulsions prepared by method IV was slightly higher than those prepared by other methods, implying the more stable o/w emulsions. This indicated that the order of mixing influenced the emulsificasion properties. The increased viscosity of o/w emulsions prepared by method IV was probably due to electrostatic interaction between positive charge of zein and negative charge of pectin at the surface of oil droplets [32].

In general, the physical stability of the emulsions can be improved according to Stroke'law by increasing viscosity of continuous phase. The creaming rate can be slowed down by adding stabilizer or reducing and maintaining the droplet size, as expressed in Equation (1):

$$v = \frac{2}{9}\frac{(\rho_p-\rho_f)}{\mu}\mathrm{g}\mathrm{R}^{2} \qquad (1)$$

where v is the particle setting velocity (m/s), ρ_p and ρ_f are mass density of particle and fluid, μ is gravitational acceleration (m/s^2), and R is radius of the spherical object (m).

The Stoke'law states that the velocity at which droplet moves is directly proportional to the square of its radius and the difference between oil droplet and external phase density. Therefore, the stability of emulsions can be enhanced by reducing the droplet size along with increasing internal and external phase density. In this experiment, using high concentration of pectin was preferred for the preparation of stable emulsions [31].



(a) Method II

(b) Method III

(c) Method VI

Figure 23 Microscopic images of o/w emulsions prepared by different methods; (a) method II, (b) method III, (c) method IV.

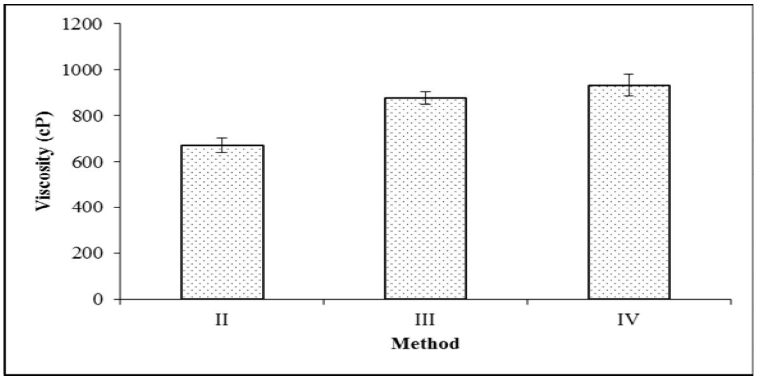


Figure 24 Viscosity of o/w emulsions prepared different mixing order.

Percent creaming index of o/w emulsions prepared by different mixing orders after storage at ambient temperature (25°C) for 14 days and after testing under environmental stress, i.e., temperature cycling at 4°C/40°C (6 cycles) and centifugation at 3,000 rpm for 10 min (Figure 25). The results showed that the emulsions prepared by method IV had the highest stability. This may result from the complexation between zein and pectin at the interfacial layer of oil droplets as well as the viscosity of emulsions. In case of the method III, it was shown that the stability of o/w emulsions was not as good as that prepared by method IV. This was because the formation from method III, the emulsifying properties was not sufficently improved for protecting emulsion stability. Emulsions prepared by method II, using pectin as emulsifier, demonstrated lower viscosity and stability than emulsions prepared by other methods. It is possible that the emulsifying property of pectin was not sufficient to prevent the coalescence of oil droplets. Therefore, in this study the method IV was the suitable order of mixing for preparation of o/w emulsions.

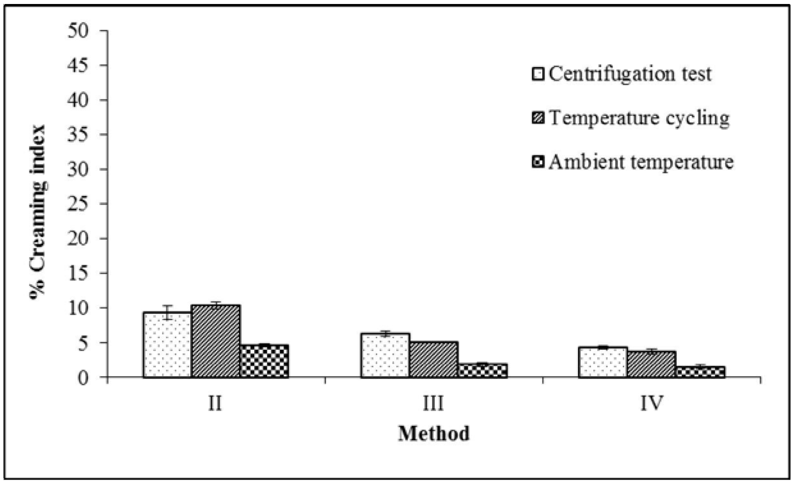


Figure 25 Percent creaming index of o/w emulsions prepared by different mixing orders, tested by various techniques.

3.3. Effect of pH

To investigate the effect of preparation pH on emulsion stability, the emulsions were prepared at pH 4 and 7 as by well as pH change from 7 to 4. The droplet size of emulsions stabilized by pectin-zein complexes prepared at various pHs was investigated, as shown in Table 2. The results showed that the droplet size of emulsions prepared at pH 4 was smaller and the zeta potential was less negative than those prepared at pH 7 and by changing pH from 7 to 4. Moreover, the highest viscosity was obtained in emulsions prepared at pH 4. This indicated that, at pH 4, zein and pectin were oppositely charged and the complexes were then formed between them, which the pectin-zein complexes was improved the physical properties i.e., droplet size, zeta potential and viscosity of emulsion system [32]. At pH 7, the zeta potential of emulsion droplets was more negative than those prepared at pH 4 and changing pH from 7 to 4. It was suggested that, both zein and pectin were negatively charged and no electrostatic interaction took place, hence, both existed as mixed individual soluble polymers and favored the bridging of two oil droplets [31], leading to flocculation and coalescence of the emulsion droplets (Figure 26c). In case of pH change from 7 to 4, the zeta potential was slightly less negative than that of emulsion droplets prepared at pH 7. The viscosity of emulsions was less than those prepared at pH 4 but was not significantly different from those prepared at pH 7 moreover, the extensive droplets aggregation also occurred (Figure 26b).

Table 2 Droplet size, zeta potential and viscosity of o/w emulsions prepared by different mixing orders.

pH	**Droplet size (µm)**	**zeta potential (mV)**	**Viscosity (cP)**
4	6.49 ± 0.33	-21.44 ± 2.38	846.43 ± 45.55
7 to 4	10.05 ± 0.09	-29.58 ± 0.08	654.68 ± 20.23
7	12.42 ± 0.08	-32.53 ± 0.08	663.17 ± 44.77

Figure 27 shows percent creaming index of o/w emulsions stabilized by pectin-zein complexes, prepared at various pHs. Emulsions prepared at pH 4 were more stable (low percent creaming index) after stability test than those prepared at pH 7 and by changing pH from 7 to 4. The results suggested that zein may help to stabilize the emulsions by improving the rigidity of coated layer on oil droplets. Moreover, at pH 4, emulsions were smaller in size and higher in viscosity (Table 2). In agreement with Stoke s'law , the stability of emulsions can be enhanced by reducing the droplet size along with increasing internal and external phase density [18]. In the case of emulsion prepared at pH 7 showed low emulsion stability (high percent creaming index). It is probably due to the fact that both pectin and zein represented as negative charge and no complex was formed. Furthermore, at pH 7, it showed larger droplets, lower viscosity than prepared at pH 4 and also aggregation of droplet (Figure 26c) which leading to the less stable emulsions. Emulsions prepared by changing pH from 7 to 4 demonstrated a low stability (high percent creaming index). This may be due to, during pH adjustment from 7 to 4, the change of negative charge of zein to positive charge. Subsequently, the droplets were aggregated and coalesced (Figure 26b).

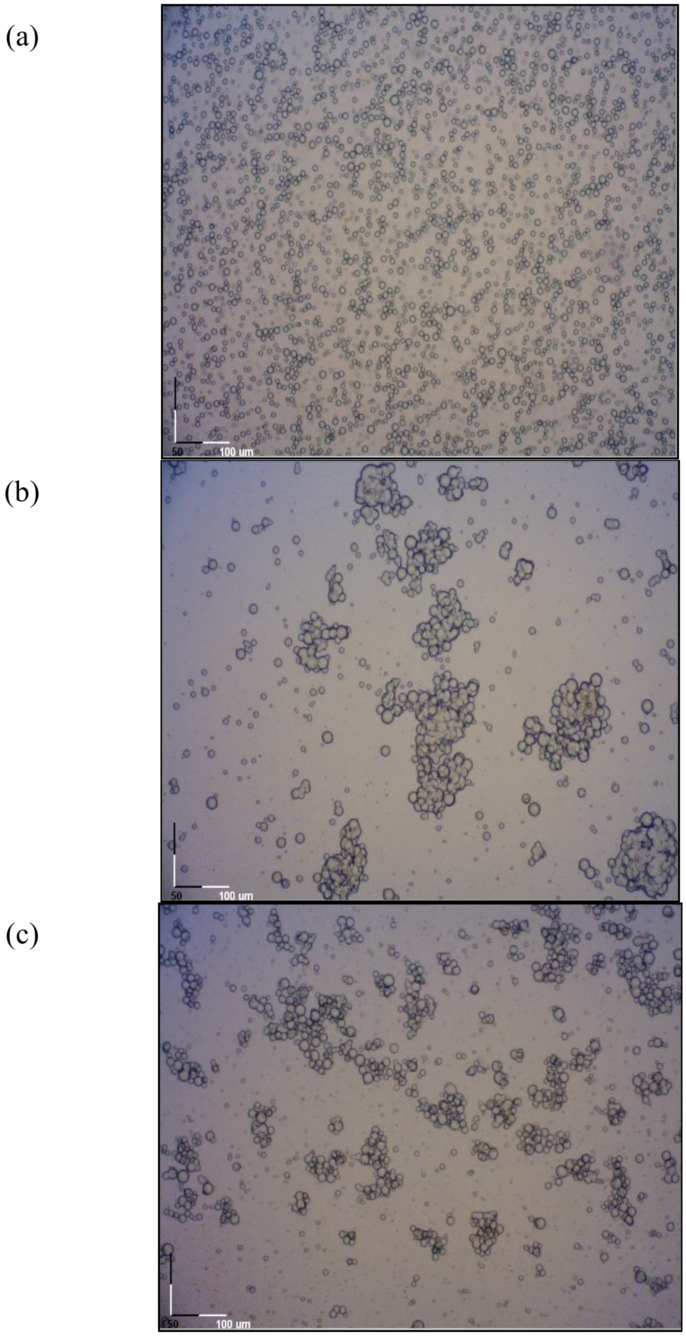


Figure 26 Microscopic images of o/w emulsions stabilized by pectin-zein complexes, prepared at various pHs; (a) pH 4 (b) pH change from 7 to 4 (c) pH 7.

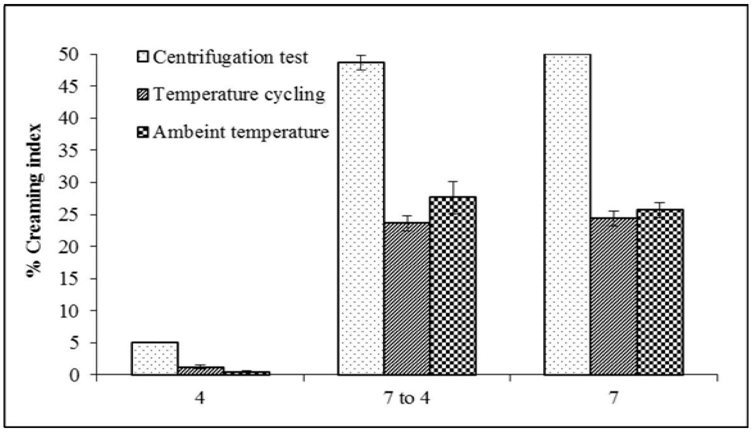


Figure 27 Percent creaming index of o/w emulsions stabilized by pectin-zein complexes, prepared at various pHs.

3.4 Effect of zein concentration

As discussed in 3.2, the more stable emulsions were achieved when 2% w/w HMP was used, at pH 4. As emulsions containing 20% w/w rice bran oil showed good stability with low percent creaming index so 30% w/w rice bran oil was used to investigate the effect of zein concentration on physical properties and stability of o/w emulsions. The emulsions were prepared by mixing the pectin emulsions with the weighed amount of zein (0-0.5% w/w), at pH 4. The results demonstrated that the droplet size decreased from about 9 µm in the absence of zein to about 5 µm, in the presence of 0.5% w/w zein (Figure 28).

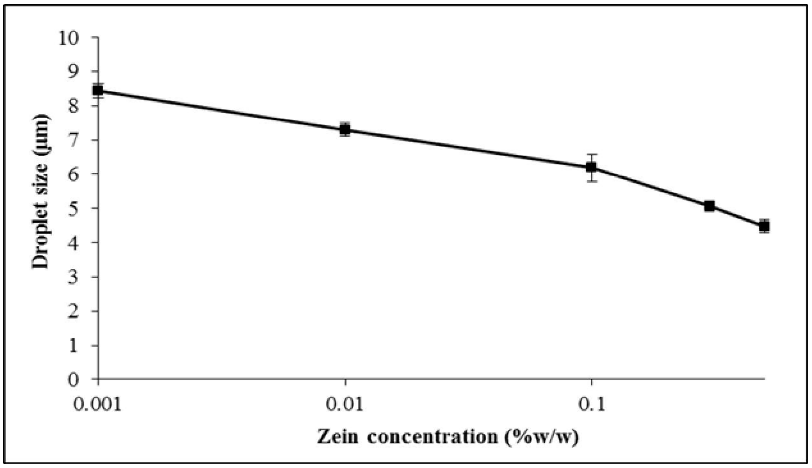
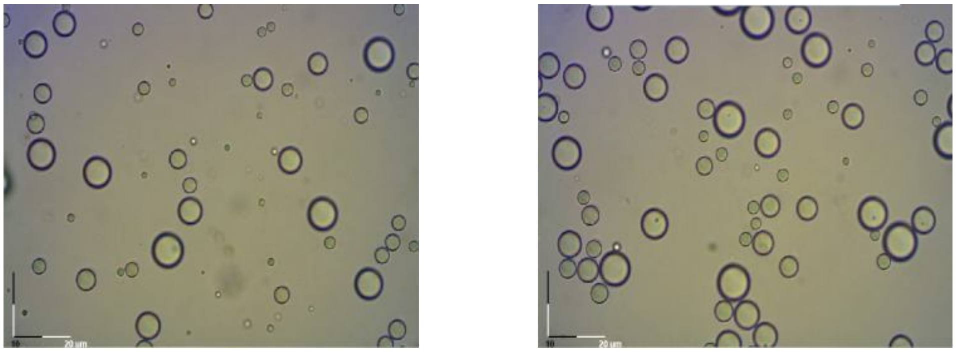


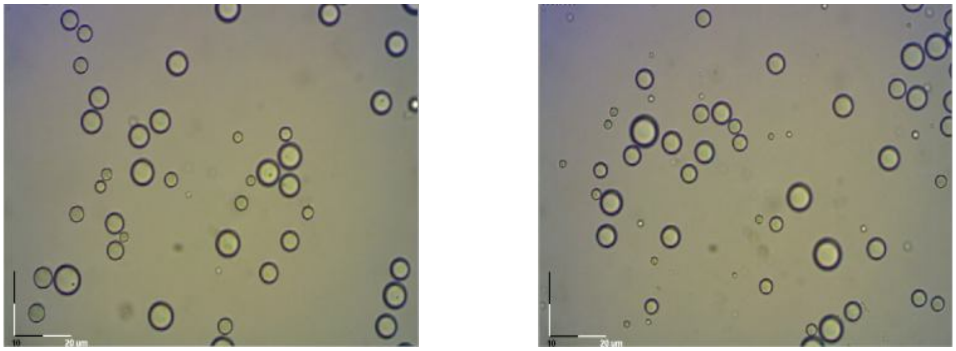
Figure 28 Droplet sizes of o/w emulsions stabilized by pectin-zein complexes, prepared at various zein concentrations.

Figure 29 shows microscopic images of o/w emulsions containing 2% w/w HMP and zein at various concentrations. The results demonstrated that the oil droplets dispersed in water phase and did not flocculate. It is likely that their surfaces were completely saturated with biopolymers. Moreover, the droplet size tended to decrease with the increased zein concentration. This may be due to the complexes of HMP and zein that could improve emulsification properties, resulting from an increase in hydrophobic behavior from zein molecules.



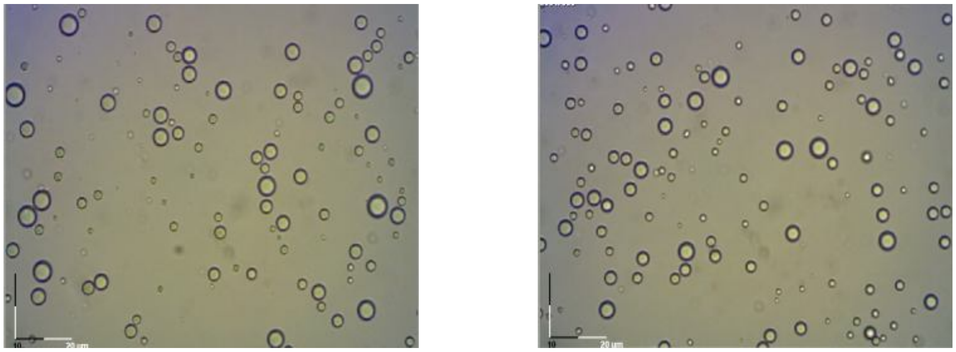
0% w/w zein

0.001% w/w zein



0.01% w/w zein

0.1% w/w zein



0.3% w/w zein

0.5% w/w zein

Figure 29 Microscopic images of o/w emulsions containing 2% w/w HMP and zein at various concentrations.

Figure 30 shows the zeta potential and viscosity of o/w emulsions containing 2% w/w HMP and various concentrations of zein. It was found that the negative charge of pectin decreased with the concentration zein increased. The increase in zein concentration resulted in the increase of pectin-zein complexes in the emulsion system which could neutralize the negative charge of pectin. Only slight charge in zeta potential was observed when the zein concentration ranged from 0.01-0.03% w/w. It is possible that the concentration of zein in this range was insufficient to cover the oil droplets containing pectin. In contrast, at 0.5% w/w zein, the zeta potential was significantly changed. It is likely that zein sufficiently adsorbed to the surface of the negative charge of oil droplets containing pectin.

The viscosity was also slightly increased with the increased concentration of zein (Figure 30), resulting from the increased pectin-zein complexes. Dickinson and co-workers also showed that the higher viscosity of pectin-casein stabilized emulsions attributes to significant attractive protein-polysaccharide interaction at the surface of the emulsion droplets [37].

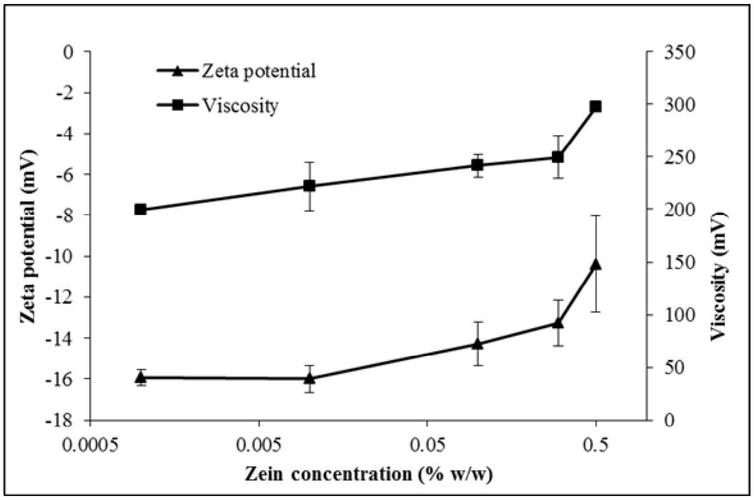


Figure 30 Zeta potential and viscosity of o/w emulsions containing 2% w/w HMP and various concentrations of zein.

Figure 31 demonstrates the percent creaming index of o/w emulsions containing 2% w/w HMP and various concentrations of zein. The results showed that percent creaming index of o/w emulsions decreased as concentration of zein was increased, resulting from pectin-zein complex formation. Moreover, when adding zein into the formulations, the hydrophobicity of oil droplets containing pectin was increased, leading to an improve in emulsifying functionality [12]. The droplets size of emulsions decreased and the viscosity was increased (Figure 30) when zein concentration was increased. The viscosity of continuous contributed to emulsion stability by slowing the creaming rate, leading to the stable emulsions [34].

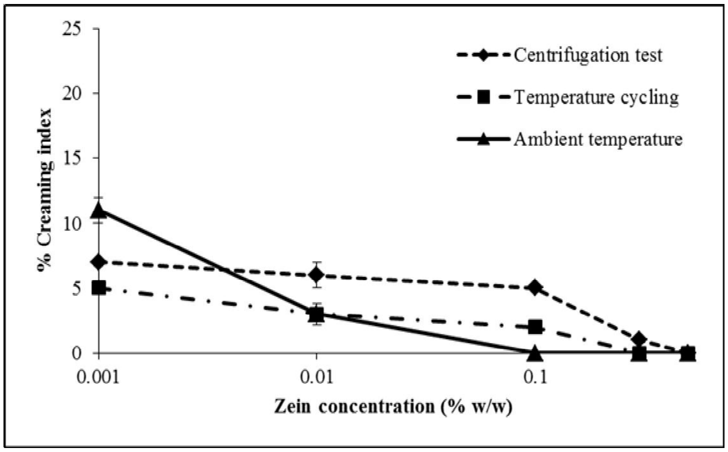


Figure 31 Percent creaming index of o/w emulsions containing 2% w/w HMP and various concentrations of zein.

Figure 32 shows photographs of o/w emulsions containing 30% w/w rice bran oil, 2% w/w HMP, and various concentrations of zein, after storage at ambient temperature (25 °C) for 30 days. The results showed that the o/w emulsions cracked at zein concentration of 0.001% w/w and below. It is probable that zein concentration was not sufficient to stabilize the emulsions, leading to phase separation. At zein concentration above 0.01% w/w, the creaming layer increased with increased zein concentration. This may result from the formation of three-dimensional network of aggregated droplets that retarded droplet movement [12].

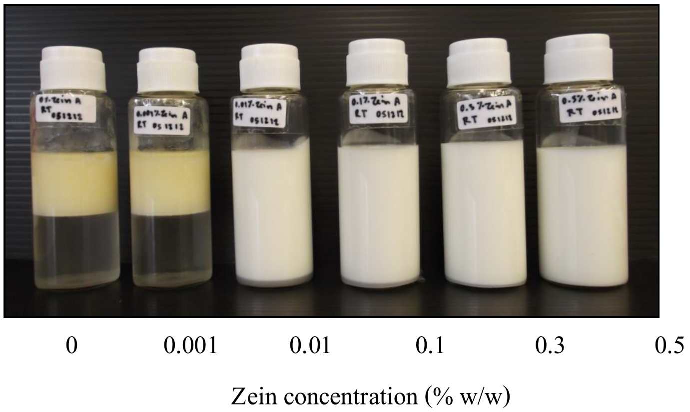


Figure 32 Photographs of o/w emulsions containing 30% w/w rice bran oil, 2% w/w HMP, and various concentrations of zein, after storage at ambient temperature (25°C) for 30 days.

3.5 Effect of high-pressure homogenization

The effect of high-pressure homogenization on droplet size and stability of emulsions was also studied. Different homogenization pressures and homogenization times were investigated, in order to adjust the suitable condition for the preparation of emulsions. HMP was used as emulsifier in a preliminary experiment. Figure 33 shows droplet size of o/w emulsions stabilized by HMP, using various homogenization pressures and times. Increasing of homogenization pressure from 50 to 100 or 150 MPa did not significantly affect the droplet size of emulsions. Therefore, the homogenization pressure of 100 MPa was chosen for further formulations because of its reproducibility.

The effect of homogenization cycles in high-pressure homogenization process was also investigated. Figure 34 shows the droplet size of o/w emulsions stabilized by HMP, using various homogenization cycles. It appears that when the emulsions were homogenized for three cycles, the droplet size was decreased from 5.5 to 4 μm and was not significantly decreased when the homogenization was more than three cycles (Figure 34). Besides, at 100 cycles, emulsions were separated to two phases (data not shown). It is possible that a long-term high-pressure homogenization broke pectin chain up into shorter chain pectin. Thus, the homogenization cycle for passing through the high-pressure homogenizer was set at three cycles for all formulations.

Various concentrations of pectin (both LMP and HMP) were used as an emulsifier, i.e., 0.5, 1.0 and 1.5% w/w. Low concentration of pectin (0.5% w/w) was not sufficient to prepare stable emulsions. The pectin concentration of greater than 2% w/w tended to obstruct the machine because of its high viscosity. Therefore, the concentration of pectin used in this study was fixed at 1.5% w/w for both LMP and HMP.

Figure 35 shows microscopic images of o/w emulsions containing 1.5% w/w LMP and various concentrations of zein, before and after passing through high-pressure homogenizer. Freshly prepared emulsions were milky white in color and all of emulsions showed spherical droplets. The droplet size of emulsions was not significantly decrease after passing through the high-pressure homogenizer. Besides, higher concentration of zein did not affect the droplet size. This may be due to a small difference in zein concentration.

Figure 36 shows microscopic images of o/w emulsions containing 1.5% w/w HMP and various concentrations of zein, before and after passing through high-pressure homogenizer. The droplet size of emulsions was obviously decreased after passing through the high-pressure homogenizer. It was also evident that the droplet size of emulsion using HMP was smaller than that using LMP, after passing through high-pressure homogenizer. This may be due to a greater emulsifying property of HMP, resulting from more hydrophobic group in the structure compared to LMP [38].

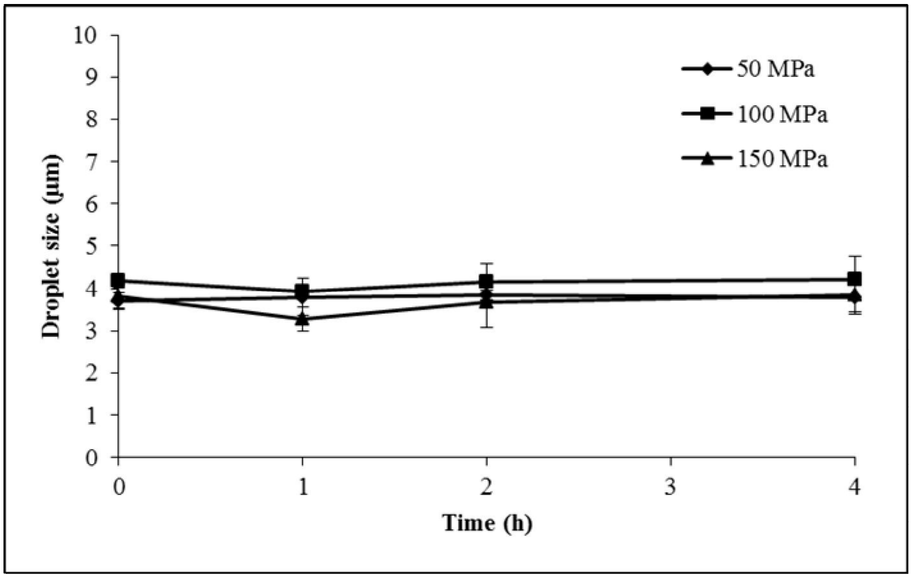


Figure 33 Droplet sizes of o/w emulsions stabilized by HMP, using various homogenization pressures and times.

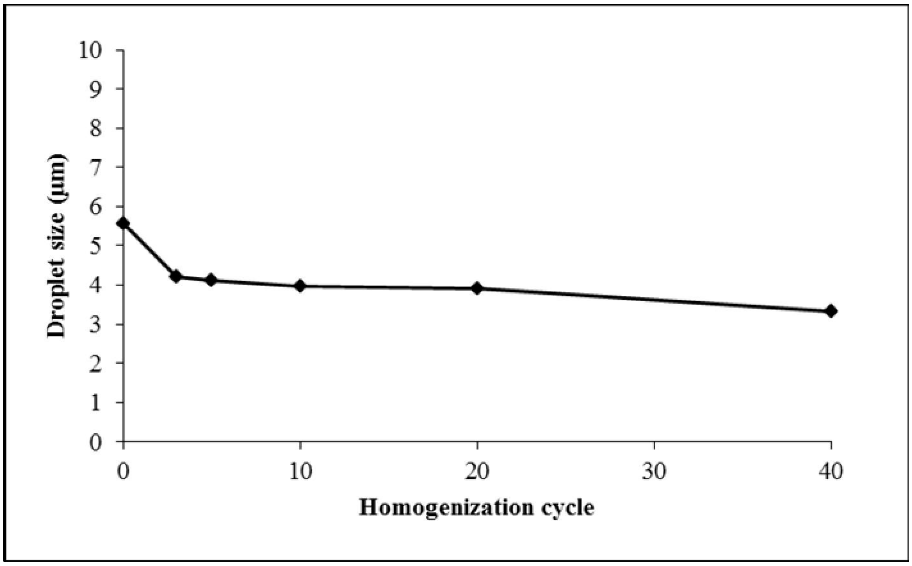


Figure 34 Droplet size of o/w emulsions stabilized by HMP, using various homogenization cycles.

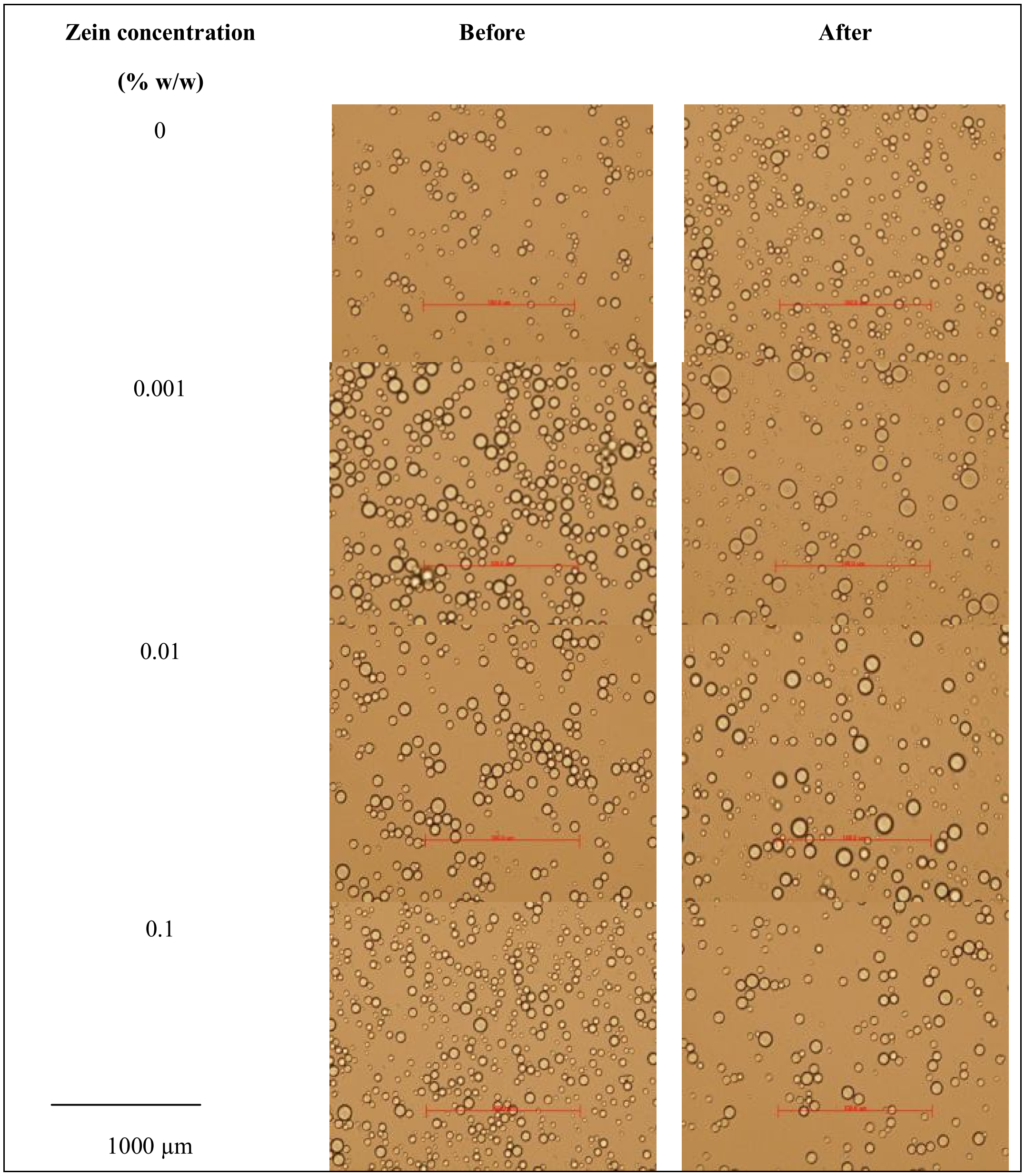


Figure 35 Microscopic images of o/w emulsions containing 1.5% w/w LMP and various concentrations of zein, before and after passing through high-pressure homogenizer.

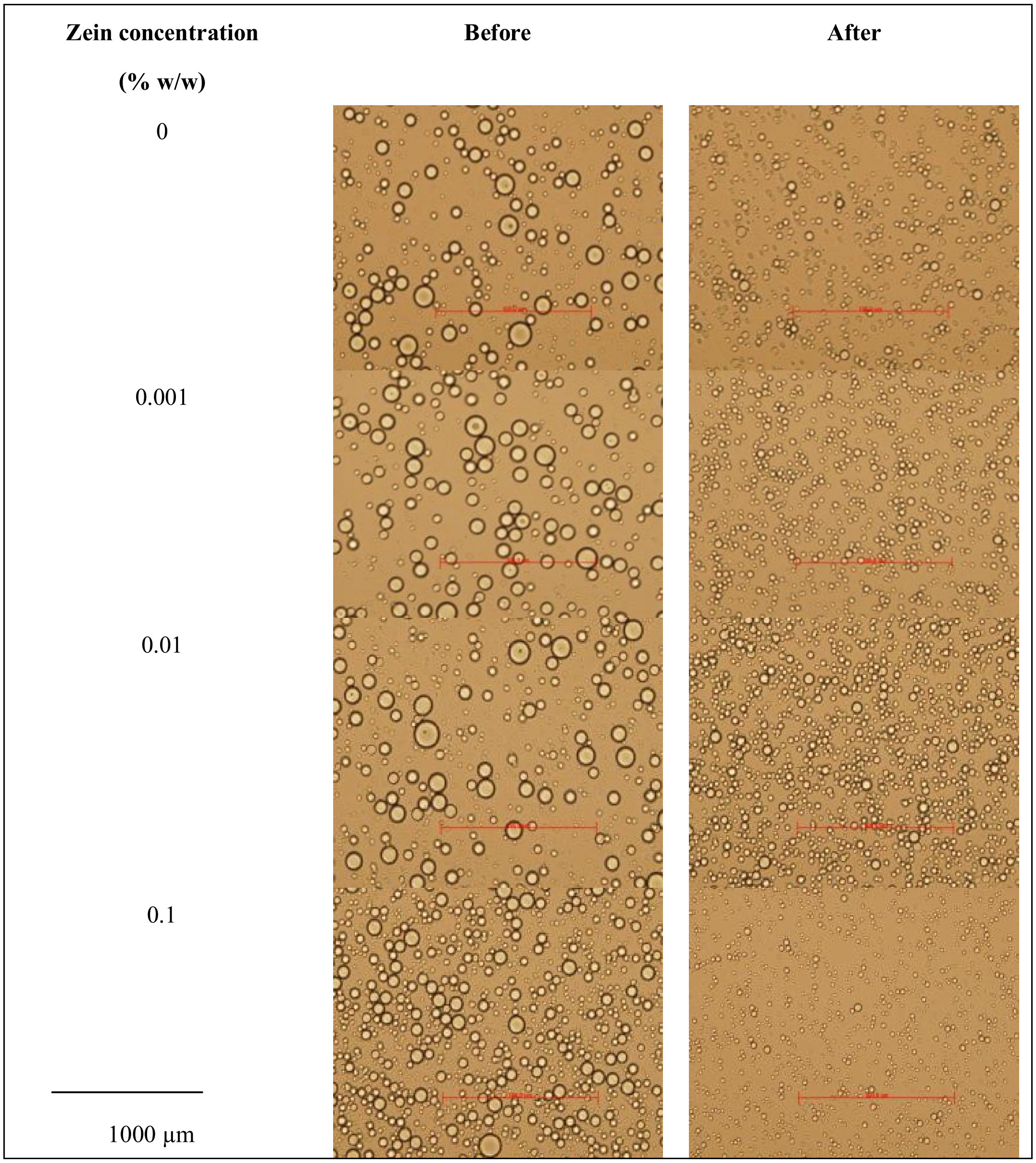


Figure 36 Microscopic images of o/w emulsions containing 1.5% w/w HMP and various concentrations of zein, before and after passing through high-pressure homogenizer.

Droplet size of o/w emulsions using LMP or HMP at various concentrations of zein, before and after passing through high-pressure homogenizer was shown in Figure 37. After passing through the high-pressure homogenizer, droplet size of o/w emulsions using HMP decreased. It is likely that the high-pressure homogenizer could reduce droplet size of the internal phase with forcing macro-emulsion through narrow gaps by imposing high pressure [39]. In addition, the droplet size decreased when the concentration of zein increased. In case of LMP, using high-pressure homogenizer or higher concentration of zein did not significantly affect the droplet size. It is possible that LMP has low emulsifying property. According to the high amount of hydrophilic group, which was not suitable to form small size emulsions.

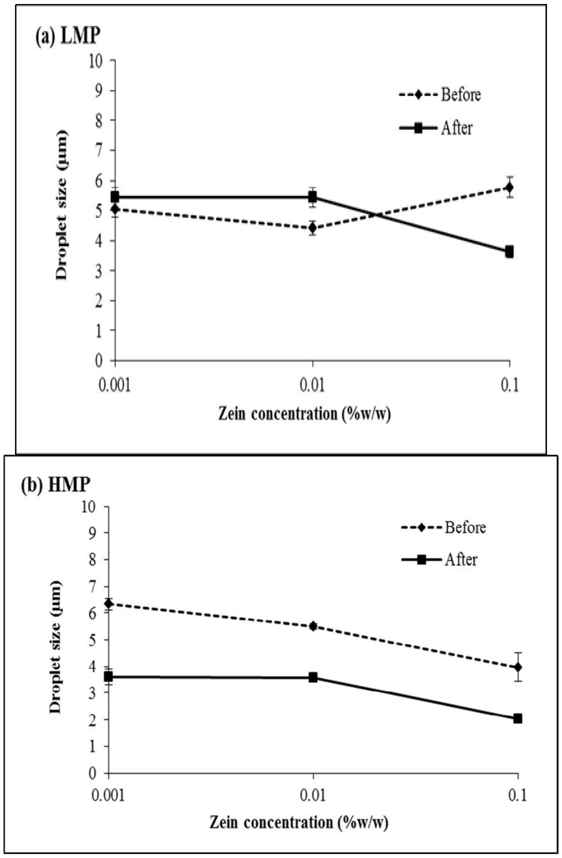


Figure 37 Droplet size of o/w emulsions using (a) LMP or (b) HMP and various concentrations of zein, before and after passing through high-pressure homogenizer.

Table 3 shows zeta potential of o/w emulsions using LMP or HMP and various concentrations of zein, before and after passing through high-pressure homogenizer. The zeta potential of the emulsions before and after passing through high-pressure homogenization was not significantly different. It is possible that the composition and structure of emulsions were not changed after passing through the high-pressure homogenizer. However, the zeta potential of emulsions using HMP was less nagative than those using LMP, resulting from the higher amout of free carboxyl group in HMP [22].

Table 3 Zeta potential of o/w emulsions using LMP or HMP and various concentrations of zein, before and after passing through high-pressure homogenizer (n=3).

Zein (% w/w)	Zeta potential (mV) ± S.D.			
	LMP		HMP	
	Before	After	Before	After
0	-37.90 ± 0.61	-36.90 ± 0.96	-22.90 ± 2.26	-23.67 ± 0.71
0.001	-38.57 ± 0.55	-38.47 ± 0.68	-24.00 ± 0.36	-24.20 ± 0.53
0.01	-37.47 ± 1.21	-37.13 ± 0.40	-23.00 ± 1.35	-23.73 ± 1.10
0.1	-38.00 ± 0.20	-37.37 ± 0.91	-23.27 ± 0.75	-23.97 ±0.74

The stability of o/w emulsions prepared by high pressure homogenizer was also investigated in this study. Figure 38 shows percent creaming index of o/w emulsions using LMP or HMP and various concentrations of zein, before and after passing through high-pressure homogenizer, determined by centrifugation test at 3,000 rpm for 10 min. Freshly prepared emulsions were milky white in color all preparations. The results showed a lower emulsion stability of both pectin types (with a higher percent creaming index) after passing through the high-pressure homogenizer as shown in Figure 38, 39, and 40. This might be due to the fact that high-pressure homogenizer might destroy the long chain molecules and then reduce the emulsifying property of pectin [39]. The results demonstrated that the stability of emulsions after passing through high-pressure homogenizer was slightly higher when using high zein concentration, compared to that using low zein concentration. This may be due to, HMP used in this study was higher in molecular weight than LMP. Therefore, the HMP may effectively cover on the oil droplet compared to LMP [40]. Moreover, emulsions using HMP were more stable (low percent creaming index) than those using LMP, the results showed that emulsions using LMP separated to two phases (data not shown) and demonstrated low stability (with 50 percent creaming index) of emulsions using LMP, both before and after passing through high-pressure homogenization. In addition, the percent creaming index was not influenced by zein concentration. It is probably due to the low emulsifying property and sensitivity to temperature of LMP [22].

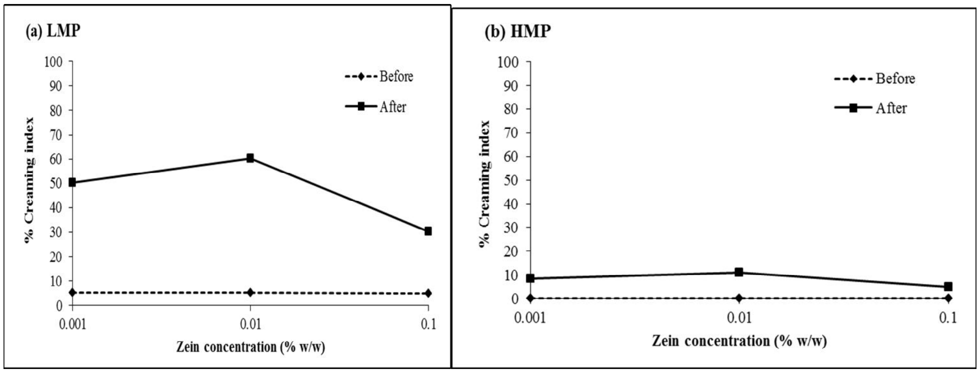


Figure 38 Percent creaming index of o/w emulsions using (a) LMP or (b) HMP and various concentrations of zein, before and after passing through high-pressure homogenizer, determined by centrifugation test at 3,000 rpm for 10 min.

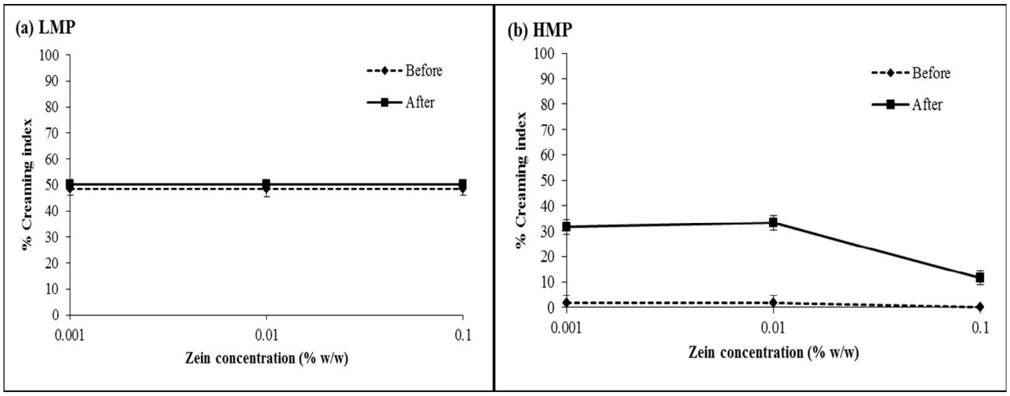


Figure 39 Percent creaming index of o/w emulsions using (a) LMP or (b) HMP and various concentrations of zein, before and after passing through high-pressure homogenizer, after temperature cycling test (4°C/40°C) for 6 cycles.

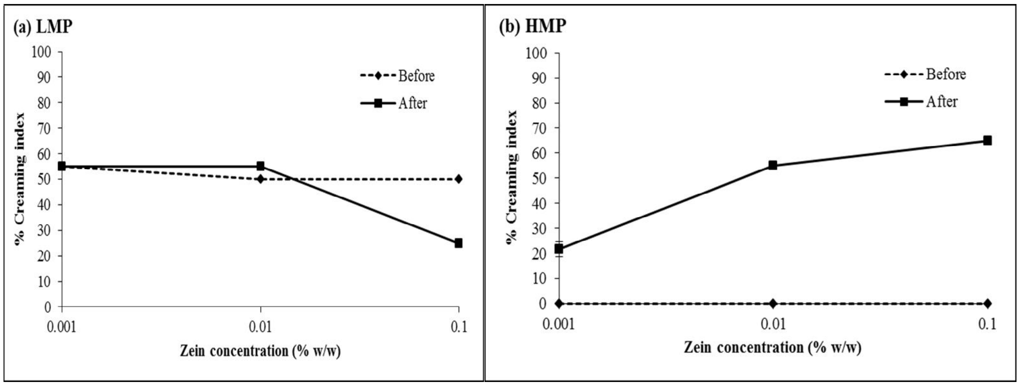


Figure 40 Percent creaming index of of o/w emulsions using (a) LMP or (b) HMP and various concentrations of zein, before and after passing through high-pressure homogenizer, when kept at ambient temperature (25°C) for 14 days.

CONCLUSIONS

Emulsions are known to be thermodynamically unstable systems and the biopolymers are widely used as emulsifier. Previous studies have shown that pectin alone at low concentration is not good emulsifier for stabilizing emulsion. In this study, the system containing pectin-zein complexes under appropriate conditions has been proposed in order to improve the physical properties and stability of o/w emulsions. To gain more understanding about the pectin-zein complexes, the properties of the complexes were studied. Various concentrations of zein was mixed with pectin solution, at pH 4, and the morphology of pectin-zein complexes was observed from digital images, optical microscopic images and SEM micrographs. When the concentration of zein was increased, the turbidity of pectin-zein mixture increased but the surface tension of pectin-zein mixture decreased. The interfacial tension of pectin-zein mixture was slightly lower than that of pectin alone. FTIR results confirmed the possibility of weak interaction between pectin and zein.

Emulsions stabilized by pectin-zein complexes were evaluated. Various concentrations of rice bran oil, pectin, and zein were investigated. The effect of order of mixing, pHs and high-pressure homogenization on physical properties, e.g., droplet size, zeta potential, viscosity and emulsion stability (i.e., percent creaming index) at ambient temperature and under environmental stress conditions. The emulsions were prepared by using pectin as primary emulsifier and zein as secondary emulsifier (method IV) showed small droplet size, high viscosity and the highest stability. Emulsions prepared at pH 4 showed small droplet size, high viscosity and stability while at pH 7 and pH change from 7 to 4, the extensive droplets aggregation occurred and large droplet size, low viscosity and low stability emulsions were obtained. When the concentration of zein was increased, the droplet size of emulsions decreased, the viscosity increased, the zeta potential was less negative and the emulsion stability was improved. Only using high-pressure homogenizer, emulsions containing 1.5% w/w pectin (i.e., LMP or HMP), 20% rice bran oil at various concentrations of zein were prepared and investigated using optical microscopy, droplet size, zeta potential and emulsion stability at ambient temperature and under environmental stress conditions. The droplet size of emulsions was influenced by the type of pectin used; emulsions using high methoxy pectin (HMP) were smaller than those using low methoxy pectin (LMP). The emulsions stabilized by HMP–zein showed good physical stability with lower percent creaming index than those using LMP, both before and after passing through high-pressure homogenizer.

In conclusion, this study provides valuable insights into the pectin-zein complexes and their emulsifying properties. The selection of suitable conditions, such as polymer and oil concentration, order of mixing, pH and emulsification equipment, may help to improve the rice bran oil emulsion stability. This information may be useful in designing materials with novel properties for commercial products, such as foods, cosmetics or personal care products.

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Outputs

Article

1. Piriyaprasarth S, Juttulapa M, Sriamornsak P. Stability of rice bran oil-in-water emulsions stabilized by pectin-zein complexes: Effect of composition and order of mixing. Food Hydrocolloids 2016; 61:589-598.
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Presentation

1. Sriamornsak P, Juttulapa M, Piriyaprasarth S, Takeuchi H. Influence of homogenization parameters on droplet size of o/w emulsions containing zein and pectin. Proceedings of the 10th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology 2016; 10: 130-133. [Glasgow, 3 – 7 April 2016]

Curriculum Vitae

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2. Education

2000-2003	Ph.D. (Pharmaceutical Sciences) Department of Drug Delivery Research, Faculty of Pharmaceutical Sciences Kyoto University, Japan (supervisor: Prof. Mitsuru Hashida) Thesis title "Quantitative structure/property relationship modeling of pharmacokinetic properties using genetic algorithm-combined partial least squares method"
1994-1996	M.Sc.(Pharmaceutical Technology) GPA 4.00 Department of Pharmacy, Faculty of Pharmacy, Mahidol University, Thailand (supervisor: Associate Prof. Pimolpan Pitayanukul) Thesis title "Evaluation of hair care products against sunlight"
1989-1994	B.Sc. in Pharm. (First class honor, Gold medal) GPA 3.71 Faculty of Pharmacy, Mahidol University, Thailand
1986-1989	Certificate of high school Triumudomsuksa-Pathanakarn School

3. Working experiences

- Lecturer, Department of Pharmaceutical technology, Faculty of Pharmacy, Silpakorn University, Nakorn pathom 73000, Thailand (1996-present)
- Head of Department, Department of Pharmaceutical technology, Faculty of Pharmacy, Silpakorn University (2011-2015)

- Chairman of Master of Pharmacy Program in Pharmaceutical Sciences. Faculty of Pharmacy, Silpakorn University (2009-2015)
- Assistant Dean of Academic Affair, Faculty of Pharmacy, Silpakorn University (2004-2008)
- Member of Thai Industrial Pharmacist Association (2013-present)

4. Research Interests

- Bioinformatic, pharmacokinetic analysis and its application for drug delivery design and advanced drug delivery such as transdermal drug delivery system including formulation and controlled release approaches, plant extraction and evaluation for theit activities, as well as pharmaceutical polymer sciences and its application.
- Development of nanoemulsion and evaluation for effective drug delivery
- Evaluation of various starches as excipients in pharmaceuticals using design of experiment

5. Technique Speacialisation

- Quantitative structure activity/property relationship analysis on pharmacokinetic properties
- Bioinformatic, Pharmacokinetic analysis and its application for drug delivery design
- Development of nanoemulsion and evaluation for effective drug delivery
- Evaluation of various starches as excipients in pharmaceuticals using design of experiment
- In vitro skin penetration measurement
- Release control with polymeric matrixes and membranes
- Plant Extraction and evaluation for theit activities

6. Scientific Publication

Article

1. Piriyaprasarth S, Juttulapa M, Sriamornsak P. Formation and Characterization of Polyelectrolyte Complexes containing Pectin and Zein. Walailak J Sci & Tech 2016 (In Press)
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1. Piriyaprasarth S, Sriamornsak P, Chaimaneec P, Jiaranai C, Chuchan S, Sakunpakdi S, Panomsuk S, Chaichiangkong J and Patomchaiviwat V. Effect of tamarind (Tamarindus indica L.) seed polysaccharide on physical properties of itraconazole-loaded nanoemulsions. The 5th International Conference on Natural products for Health and Beauty. May 6-8, 2014, Phuket, Thailand: E-P-025:317-321.
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Book/ Review article

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Abstract

1. Sriamornsak P, Juttulapa M, Piriyaprasarth S, Takeuchi H. Influence of homogenization parameters on droplet size of o/w emulsions containing zein and pectin. Proceedings of the 10th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology 2016; 10: 130-133. [Glasgow, 3 – 7 April 2016]

2. Latt S, Boontara K, Teeraprasatkul T, Yangngam W, Patomchaiviwat V, Sriamornsak P, Piriyaprasarth S. Preparation and physical properties of itraconazole-loaded nanoemulsions using pineapple starch as co-emulsifier. AFPS 2015, Bangkok.
3. Patomchaiviwat V, Bunlikitkul M, Piriyaprasarth S. Prediction of pregelatinized arrowroot starch as tablet disintegrants using artificial neural network. Abstract of 7th International Conference on material Science and Technology. June 7-8, 2012 Swissotel Le Concorde, Bangkok, THAILAND.
4. Piriyaprasarth S, Sriamornsak P, Chansiri G, Limmatvapirat S, Thienthiti K, Maneenual P, Sataburusawong W. Effect of pectin as co-emulsifier on physical properties of itraconazole-loaded nanoemulsions. Abstract of 7th International Conference on material Science and Technology. June 7-8, 2012 Swissotel Le Concorde, Bangkok, THAILAND.
5. Piriyaprasarth S, Techaarpornkul S, Opanasopit P, Nunthanid J and Apirakaramwong A. In silico modeling of the evaluation of the chitosan derivatives for siRNA delivery into the cells by using artificial neural network method. Abstract of the Fifth Indochina Conference on Pharmaceutical Sciences 21-24 November 2007 at Siam City Hotel, Bangkok, Thailand.
6. Piriyaprasarth S, Sriamornsak P and Numnual S. Flocculation properties of pectin extracted from pomelo peel in indomethacin suspension. Proceeding of the 23rd Annual Research Conference in Pharmaceutical Sciences & JSPS 1st Medical Chemistry Seminar of Asia/Africa Science Platform Program 14-15 December 2006, Bangkok, Thailand: PT22.
7. Piriyaprasarth S, Opanasopit P and Luangtanaanan M. Stability of extracts from fruit hull of mangosteen. Proceeding of the 23rd Annual Resaerch Conference inPharmaceutical Sciences & JSPS 1st Medical Chemistry Seminar of Asia/Africa Science Platform Program 14-15 December 2006, Bangkok, Thailand: PN-4.
8. Sukma M, Piriyaprasarth S, Awale S, Tohda M, Kadota S. Neuroprotective activity and cytotoxicity of some extracts from *Ocimum sanctum*. Abstract of the 21st Congress of Federation of Asian Pharmaceutical Associations (FAPA) November 18-21, 2006, Pacific Yokohama, Yokohama, Japan:SC-P-23:307.
9. Piriyaprasarth S, Sriamornsak P and Numnual S. Flocculation properties of pectin extracted from pomelo peel in indomethacin suspension. Abstract of the 21st Congress of Federation of Asian Pharmaceutical Associations (FAPA) November 18-21, 2006, Pacific Yokohama, Yokohama, Japan: SC-P-15:115.
10. Piriyaprasarth S, Ngawhirunpat T, Panomsuk S and Puttipipatkhachorn S. Prediction of permeation across shed snake skin using genetic algorithm combined partial least squares and

artificial neural network method. 22nd Annual Research Conference in Pharmaceutical Sciences, Faculty of Pharmacy, Chulalongkorn University, P66.

8. Award/Grant

1. First Class Honor (Gold Medal): Faculty of Pharmacy, Mahidol University.
2. JSPS research Scholars 1999: Japan Society for Promotion of Sciences.
3. PhD candidate, Graduate School of Pharmaceutical Sciences, Kyoto University, Japan, Japan Government Grant (2000-2003)
4. (JSPS)March 2002: APSTJ/The Nagai Foundation Graduate Student Award, Shizuoka, Wanchana S, Shin-ichi Fujiwara, Miki Akamatsu, Yamashita F, Hashida M. 2D- and 3D-QSAR of carrier-mediated transport of beta-lactam antibiotics in Caco-2 cells. The Japanese pharmaceutical sciences conference (Yakuzaigaku) 17th, March 29-31, 2002. Shizuoka, Japan.
5. The Nagai Award Thailand for Research (2002) Sriamornsak P, Wanchana S, Nunthanid J, Luangtana-Anan M. Composite film-coated tablets intended for colon-specific delivery of 5-aminosalicylic acid: using deesterified pectin.
6. JSPS research Scholars 2005: Japan Society for Promotion of Sciences (JSPS).
7. Poster Presentation Award, Microwave-assisted modification of arrowroot starch for pharmaceutical matrix tablets, International Conference on Functionalized and Sensing Materials, December 7-9, 2009 at Chulabhorn Convention Center, Bangkok.
8. Poster Presentation Award, Microwave-assisted modification of arrowroot starch for pharmaceutical matrix tablets, National Conference of 1st Silpakorn Reseach by Silpakorn University Research and Development Institute, December, 2009 at Silpakorn University, Nakhon-Pathom.
9. Science & Technology Research Grant (2010): Thailand Toray Science Foundation
10. Research Award in the field of Science and Applied Science (2012): Institute of Research and Development, Silpakorn University.
11. Science & Technology Award (2013): Thailand Toray Science Foundation
12. Award of the reputation of the University (2012), from Silpakorn University
13. AFPS Nagai Best Paper Award (Bronze Medal), 2015, from Asian Federation of Pharmaceutical Sciences Conference
14. Award of the reputation of the Faculty (2016), from Faculty of Pharmacy, Silpakorn University

Curriculum Vitae

Name Mr. Pornsak Sriamornsak
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1. Personal data

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2. Education

☐ PhD in Pharmaceutics

Charles Sturt University, Australia, 1999 – 2002

Thesis: Analysis of selected physico-chemical properties of pectin and alginate gels intended for drug delivery (Supervisor: Dr. Ross A. Kennedy)

☐ MSc in Pharmacy (Pharmaceutics)

Mahidol University, Thailand, 1994 – 1996

Thesis: The utilization of pectin for the development of sustained release theophylline pellets

(Supervisors: Prof. Sompol Prakongpan & Prof. Satit Puttipipatkhachorn)

☐ BSc in Pharmacy (Honors)

Mahidol University, Thailand, 1989 – 1994

☐ BAg (Animal Production Management)

Sukhothai Thammathirat Open University, Thailand, 2004 – 2007

3. Research papers (refereed)

1. Sriamornsak P, Prakongpan S, Puttipipatkhachorn S, Kennedy RA. Development of sustained release theophylline pellets coated with calcium pectinate. *Journal of Controlled Release* 1997; 47(3): 221-232. (impact factor 2013 = 7.261)

2. Sriamornsak P, Puttipipatkhachorn S, Prakongpan S. Calcium pectinate gel coated pellets as an alternative carrier to calcium pectinate beads. *International Journal of Pharmaceutics* 1997; 156(2): 189-194. (impact factor 2013 = 3.785)
3. Sriamornsak P. Investigation of pectin as a carrier for oral delivery of proteins: using calcium pectinate gel beads. *International Journal of Pharmaceutics* 1998; 169(2): 213-220. (impact factor 2013 = 3.785)
4. Sriamornsak P. Preliminary investigation of some polysaccharides as a carrier for cell entrapment. *European Journal of Pharmaceutics and Biopharmaceutics* 1998; 46(2): 233-236. (impact factor 2013 = 4.245)
5. Sriamornsak P, Nunthanid J. Calcium pectinate gel beads for controlled release drug delivery: I. Preparation and *in-vitro* release studies. *International Journal of Pharmaceutics* 1998; 160(2): 207-212. (impact factor 2013 = 3.785)
6. Sungthongjeen S, Pitaksuteepong T, Somsiri A, Sriamornsak P, Katsawang K, Khruatanapaiboon S, Prakanrat O. Development of pectin matrix tablets: I. Effect of compression pressure and ratio on drug release. *Naresuan University Journal* 1998; 6(2): 24-37.
7. Sriamornsak P. Effect of calcium concentration, hardening agent and drying condition on release characteristics of oral proteins from calcium pectinate gel beads. *European Journal of Pharmaceutical Sciences* 1999; 8(3): 221-227. (impact factor 2013 = 3.005)
8. Sriamornsak P, Nunthanid J. Calcium pectinate gel beads for controlled release drug delivery: II. Effect of formulation and processing variables on drug release. *Journal of Microencapsulation* 1999; 16(3): 303-313. (impact factor 2013 = 1.878)
9. Sungthongjeen S, Pitaksuteepong T, Somsiri A, Sriamornsak P. Studies on pectins as potential hydrogel matrices for controlled-release drug delivery. *Drug Development and Industrial Pharmacy* 1999; 25: 1271-1276. (impact factor 2013 = 2.006)
10. Nunthanid J, Wanchana S, Sriamornsak P, Limmatavapirat S, Luangtana-anan, Puttipipatkhachorn S. Effect of heat on characteristics of chitosan film coated on theophylline tablets. *Drug Development and Industrial Pharmacy* 2002; 28: 919-930. (impact factor 2013 = 2.006)
11. Sriamornsak P, Nunthanid J, Wanchana S, Luangtana-anan A. Composite film-coated tablets intended for colon-specific delivery of 5-aminosalicylic acid: using deesterified pectin. *Pharmaceutical Development and Technology* 2003; 8: 311-318. (impact factor 2013 = 1.335)

12. Sriamornsak P, Thirawong N. Use of back-scattered electron imaging as a tool for examining matrix structure of calcium pectinate. *International Journal of Pharmaceutics* 2003; 267: 151-156. (impact factor 2013 = 3.785)
13. Sriamornsak P. Chemistry of pectin and its pharmaceutical uses: A review [Review Article]. *Silpakorn University International Journal* 2003; 3(1-2): 206-228.
14. Sungthongjeen S, Sriamornsak P, Pitaksuteepong T, Somsiri A, Puttipipatkhachorn S. Effect of degree of esterification of pectin and calcium amount on drug release from pectin-based matrix tablets. *AAPS PharmSciTech* 2004; 5(1): 50-57. (Article 9) (http://www.aapspharmscitech.org) (impact factor 2013 = 1.776)
15. Nunthanid J, Luangtana-anan M, Sriamornsak P, Limmatvapirat S, Puttipipatkhachorn S, Lim LY, Khor E. Characterization of chitosan acetate as a binder for sustained release tablets. *Journal of Controlled Release* 2004; 99(1): 15-26. (impact factor 2013 = 7.261)
16. Sriamornsak P, Puttipipatkhachorn S. Chitosan-pectin composite gel spheres: Effect of some formulation variables on drug release. *Macromolecular Symposia* 2004; 216: 17-21. (impact factor 2005 = 0.913)
17. Sriamornsak P, Thirawong N, Puttipipatkhachorn S. Morphology and buoyancy of oil-entrapped calcium pectinate gel beads. *The AAPS Journal* 2004; 6(3): 65-71. (Article 24) (http://www.aapsj.org) (impact factor 2013 = 3.905)
18. Sriamornsak P, Thirawong N, Puttipipatkhachorn S. Emulsion gel beads of calcium pectinate capable of floating on the gastric fluid: Effect of some additives, hardening agent or coating on release behavior of metronidazole. *European Journal of Pharmaceutical Sciences* 2005; 24(4): 363-373. (impact factor 2013 = 3.005)
19. Luangtana-anan M, Opanasopit P, Ngawhirunpat T, Nunthanid T, Sriamornsak P, Limmatavapirat S, Lim LY. Effect of chitosan salts and molecular weight on a nanoparticulate carrier for therapeutic protein. *Pharmaceutical Development and Technology* 2005; 10: 189-196. (impact factor 2013 = 1.335)
20. Sriamornsak P, Kennedy RA. A novel gel formation method, microstructure and mechanical properties of calcium polysaccharide gel films. *International Journal of Pharmaceutics* 2006; 323: 72-80. (impact factor 2013 = 3.785)
21. Sriamornsak P, Burton MA, Kennedy RA. Development of polysaccharide gel coated pellets for oral administration. 1. Physico-chemical properties. *International Journal of Pharmaceutics* 2006; 326: 80-88. (impact factor 2013 = 3.785)

22. Sriamornsak P, Kennedy RA. Development of polysaccharide gel coated pellets for oral administration. 2. Calcium alginate. *European Journal of Pharmaceutical Sciences* 2006; 29: 139-147. (impact factor 2013 = 3.005)
23. Sriamornsak P, Sungthongjeen S, Puttipipatkhachorn S. Use of pectin as a carrier for intragastric floating drug delivery: Carbonate salt contained beads. *Carbohydrate Polymers* 2007; 67: 436-445. (impact factor 2013 = 3.916)
24. Sriamornsak P, Thirawong N, Korkerd K. Swelling, erosion and release behavior of alginate-based matrix tablets. *European Journal of Pharmaceutics and Biopharmaceutics* 2007; 66(3): 435-450. (impact factor 2013 = 4.245)
25. Sriamornsak P, Nunthanid J, Luangtana-anan M, Puttipipatkhachorn S. Alginate-based pellets prepared by extrusion/spheronization: A preliminary study on the effect of additive in granulating liquid. *European Journal of Pharmaceutics and Biopharmaceutics* 2007; 67(1): 227-235. (impact factor 2013 = 4.245)
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31. Thirawong N, Kennedy RA, Sriamornsak P. Viscometric study of pectin–mucin interaction and its mucoadhesive bond strength. *Carbohydrate Polymers* 2008; 71(2): 170-179. (impact factor 2013 = 3.916)

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33. Nunthanid J, Huanbutta K, Luangtana-anan M, Sriamornsak P, Limmatavapirat S, Puttipipatkhachorn S. Development of time-, pH-, and enzyme-controlled colonic drug delivery using spray-dried chitosan acetate and hydroxypropyl methylcellulose. *European Journal of Pharmaceutics and Biopharmaceutics* 2008; 68(2): 253-259. (impact factor 2013 = 4.245)
34. Thirawong N, Thongborisute J, Takeuchi H, Sriamornsak P. Improved intestinal absorption of calcitonin by mucoadhesive delivery of novel pectin-liposome nanocomplexes. *Journal of Controlled Release* 2008; 125(3): 236-245. (impact factor 2013 = 7.261)
35. Sriamornsak P, Thirawong N, Cheewatanakornkool K, Burapapadh K, Sae-Ngow W. Cryo-scanning electron microscopy (cryo-SEM) as a tool for studying the ultrastructure during bead formation by ionotropic gelation of calcium pectinate. *International Journal of Pharmaceutics* 2008; 352(1-2): 115-122. (impact factor 2013 = 3.785)
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42. Sriamornsak P, Wattanakorn N, Nunthanid J, Puttipipatkhachorn S. Mucoadhesion of pectin as evidence by wettability and chain interpenetration. *Carbohydrate Polymers* 2008; 74(3): 458-467. (impact factor 2013 = 3.916)
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44. Huanbutta K, Luangtana-anan M, Sriamornsak P, Limmatavapirat S, Puttipipatkhachorn S, Nunthanid J. Factors affecting preparations of chitosan microcapsules for colonic drug delivery. *Journal of Metals, Materials and Minerals* 2008; 18(2): 79-83.
45. Nunthanid J, Luangtana-anan M, Sriamornsak P, Limmatavapirat S, Huanbutta K, Puttipipatkhachorn S. Use of spray-dried chitosan acetate and ethylcellulose as compression coats for colonic drug delivery: Effect of swelling on triggering in vitro drug release. *European Journal of Pharmaceutics and Biopharmaceutics* 2009; 71(2): 356-361. (impact factor 2013 = 4.245)
46. Chaidedgumjorn A, Sotanaphun U, Kitcharoen N, Asavapichayont P, Satiraphan M, Sriamornsak P. Pectins from *Citrus maxima*. *Pharmaceutical Biology* 2009; 47(6): 521-526. (impact factor 2013 = 1.337)
47. Weerapol Y, Cheewatanakornkool K, Sriamornsak P. Effect of gastric pH on calcium availability of various calcium salts. *Drug Metabolism Reviews* 2009; 41(s2): 82. (impact factor 2013 = 6.286)
48. Kumpugdee-Vollrath M, Bilek H, Doganguezel M, Burapapadh K, Sriamornsak P. Herstellung von lipidemulsionen als drug-delivery-systeme mittels biopolymer pektin als stabilisator. *Chemie Ingenieur Technik* 2009; 81(8): 1178-1179. (impact factor 2013 = 0.661)
49. Sriamornsak P, Wattanakorn N, Takeuchi H. Study on mucoadhesive mechanism of pectin by atomic force microscopy and mucin-particle method. *Carbohydrate Polymers* 2010; 79(1): 54-59. (impact factor 2013 = 3.916)
50. Sriamornsak P, Kennedy RA. Effect of a small molecule on diffusion and swelling properties of selected polysaccharide gel beads. *Carbohydrate Polymers* 2010; 79(1): 219-223. (impact factor 2013 = 3.916)
51. Sriamornsak P, Juttulapa M, Piriyaprasarth S. Microwave-assisted modification of arrowroot starch for pharmaceutical matrix tablets. *Advanced Materials Research* 2010; 93-94: 358-361.
52. Piriyaprasarth S, Patomchaiviwat V, Sriamonsak P, Seangpongchawal N, Ketwongsa P, Akeuru P, Srijarreon P, Suttiiphratya P. Evaluation of yam (*Dioscorea* sp.) starch and arrowroot (*Maranta arundinacea*) starch as suspending agent in suspension. *Advanced Materials Research* 2010; 93-94: 362-365

53. Wlosnewski JC, Kumpugdee-Vollrath M, Sriamornsak P. Effect of drying technique and disintegrant on physical properties and drug release behavior of microcrystalline cellulose-based pellets prepared by extrusion/spheronization. *Chemical Engineering Research and Design* 2010; 88: 100-108. (impact factor 2013 = 2.281)
54. Wattanakorn N, Asavapichayont P, Nunthanid J, Limmatavapirat S, Chantasart D, Sungthongjeen S, Sriamornsak P. Pectin-based bioadhesive delivery of carbenoxolone sodium for aphthous ulcers in oral cavity. *AAPS PharmSciTech* 2010; 11(2): 743-751. (impact factor 2013 = 1.776)
55. Burapapadh K, Kumpugdee-Vollrath M, Chantasart D, Sriamornsak P. Fabrication of pectin-based nanoemulsions loaded with itraconazole for pharmaceutical application. *Carbohydrate Polymers* 2010; 82(2): 384-393. (impact factor 2013 = 3.916)
56. Weerapol Y, Cheewatanakornkool K, Sriamornsak P. Impact of gastric pH and dietary fiber on calcium availability of various calcium salts. *Silpakorn University Science and Technology Journal* 2010; 4(1): 15-23.
57. Sriamornsak P, Nunthanid J, Cheewatanakornkool K, Manchun S. Effect of drug loading method on drug content and drug release from calcium pectinate gel beads. *AAPS PharmSciTech* 2010; 11(3): 1315-1319. (impact factor 2013 = 1.776)
58. Piriyaprasarth S, Sriamornsak P. Flocculating and suspending properties of commercial citrus pectin and pectin extracted from pomelo (*Citrus maxima*) peel. *Carbohydrate Polymers* 2011; 83(2): 561-568. (impact factor 2013 = 3.916)
59. Thammachat T, Sriamornsak P, Luangtana-anan M, Nunthanid J, Limmatvapirat C, Limmatvapirat S. Preparation and characterization of shellac fiber as a novel material for controlled drug release. *Advanced Materials Research* 2011; 152-153: 1232-1235.
60. Piriyaprasarth S, Sriamornsak P, Juttulapa M, Puttipipatkhachorn S. Modeling of drug release from matrix tablets with process variables of microwave-assisted modification of arrowroot starch using artificial neural network. *Advanced Materials Research* 2011; 152-153: 1700-1703.
61. Huanbutta K, Sriamornsak P, Limmatvapirat S, Luangtana-anan M, Yoshihashi Y, Yonemochi E, Terada K, Nunthanid J. Swelling kinetics of spray-dried chitosan acetate assessed by magnetic resonance imaging and their relation to drug release kinetics of chitosan matrix tablets. *European Journal of Pharmaceutics and Biopharmaceutics* 2011; 77(2): 320-326. (impact factor 2013 = 4.245)

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63. Panjapornpon D, Limmatvapirat C, Luangtana-anan M, Nunthanid J, Sriamornsak P, Limmatvapirat S. Fabrication of thermally stabilized shellac through solid state reaction with phthalic anhydride. *Material Letters* 2011; 65: 1241-1244. (impact factor 2013 = 2.269)
64. Piriyaprasarth S, Sriamornsak P. Effect of source variation on drug release from HPMC matrix tablets: Linear regression modeling for prediction of drug release. *International Journal of Pharmaceutics* 2011; 411: 36-42. (impact factor 2013 = 3.785)
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66. Sriamornsak P. Application of pectin in oral drug delivery [Review Article]. *Expert Opinion on Drug Delivery* 2011; 8(8): 1009-1023. (impact factor 2013 = 4.116)
67. Sungthongjeen S, Sriamornsak P, Puttipipatkhachorn S. Design of floating HPMC matrix tablets: Effect of formulation variables on floating properties and drug release. *Advanced Materials Research* 2011; 311-313: 1140-1143.
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71. Panchapornpon D, Limmatvapirat C, Nunthanid J, Luangtana-Anan M, Sriamornsak P, Puttipipatkhachorn S, Limmatvapirat S. Development of shellac from source available in Thailand as an alternative polymer for postharvest treatment. *Thai Journal of Agricultural Science* 2011; 44(5): 224-229.
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74. Manchun S, Dass CR, Sriamornsak P. Targeted therapy for cancer using pH-responsive nanocarrier systems [Review Article]. *Life Sciences* 2012; 90(11-12): 381-387. (impact factor 2013 = 2.296)
75. Sotanaphun U, Chaidedgumjorn A, Kitcharoen N, Satiraphan M, Asavapichayont P, Sriamornsak P. Preparation of pectin from fruit peel of *Citrus maxima*. *Silpakorn University Science and Technology Journal* 2012; 6(1): 35-41. (Thai impact factor 2009 = 0.100)
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133. Manchun S, Dass CR, Sriamornsak P. Stability of freeze-dried pH-responsive dextrin nanogels containing doxorubicin. *Asian Journal of Pharmaceutical Sciences* 2016; in press.
134. Huanbutta K, Terada K, Sriamornsak P, Nunthanid J. Simultaneous X-ray diffraction-differential scanning calorimetry and physicochemical characterizations of spray dried drugs and chitosan microspheres. *Walailuk Journal of Science and Technology* 2016; in press.
135. Piriyaprasarth S, Juttulapa M, Sriamornsak P. Formation and characterization of polyelectrolyte complexes containing pectin and zein. *Walailuk Journal of Science and Technology* 2016; in press.

4. Awards and honours

- 2015
  - AFPS Nagai Best Paper Award 2015 “Gold Medal” from Nagai Foundation Tokyo (as co-worker) (at the Asian Federation for Pharmaceutical Sciences 2015 Conference, Bangkok)
  - Outstanding Pharmacist Award 2015 in Research and Development from Pharmaceutical Association of Thailand
  - Excellent Research Award from Office of the Higher Education Commission, Thailand (The Higher Education Research Promotion and National Research University Project of Thailand) (at the HERP Congress III, Nakhon Si Thammarat)

- 2014
  - * The Nagai Award Thailand for Pharmaceutical Science Research 2013 from the Nagai Foundation Tokyo, Japan (as co-worker)
    (at the 30th Annula Reseaech Conference in Pharmaceutical Sciences, Bangkok)
- 2013
  - * National Distinguished Researcher Award 2013 in Chemical and Pharmaceutical Sciences from National Research Council of Thailand
  - * 2012-2013 Thailand Distinguished Polymer Scientist from Polymer Society of Thailand.
  - * Outstanding Faculty Award 2012, Faculty of Pharmacy, Silpakorn University, Thailand
- 2012
  - * FAPA Ishidate Award for Pharmaceutical Research from Federation of Asian Pharmaceutical Associations (more than 20 countries)
  - * 2012 Outstanding Alumni Award, Pharmacist Mahidol University Alumni Club, Thailand
  - * Research Award 2012 (co-investigator) from Silpakorn University Research and Development Institute, Thailand
  - * Outstanding Faculty Award 2011, Faculty of Pharmacy, Silpakorn University, Thailand
- 2011
  - * 2010 Faculty of Graduate Studies Outstanding Alumni Award (Research) from Faculty of Graduate Studies, Mahidol University, Thailand
- 2010
  - * Research Council Award: Research Award 2009 in Chemistry and Pharmaceutical Sciences (Bronze medal) from National Research Council of Thailand
  - * Best Paper Award in Materials for Health and Medicine Session,
    National Metal and Materials Technology Center, National Science and Technology Development Agency, and Ministry of Science and Technology, Thailand *(at The Sixth Thailand Materials Science and Technology Conference (MSAT-6), Bangkok)*
- 2009
  - * Poster Presentation Award,
    Silpakorn University, Chulalongkorn University, and Rajamangala University of Technology Thanyaburi, Thailand *(at International Conference on Functionalized and Sensing Materials (FuSeM 2009), Bangkok)*

- ✱ The TRF-CHE-Scopus Researcher Award 2009 (in Health Sciences) for outstanding and exemplary mid-career researcher who have received grant from the Thailand Researcher Fund (TRF) and Commission on Higher Education (CHE), Thailand
- ✱ Outstanding Research Award 2009 (in Sciences and Applied Sciences) from Silpakorn University Research and Development Institute, Thailand
- ✱ Poster Presentation Award,
  The Pharmaceutical Association of Thailand under Royal Patronage, Thailand
  (at the 5th Thailand Pharmacy Congress, Bangkok)

- ▪ 2008
  - ✱ Poster Presentation Award in Science and Technology,
    Silpakorn University Research and Development Institute, Silpakorn University
    (at the 2nd Silpakorn University Research Fair, Bangkok)
  - ✱ Research Council Award: Research Award 2008 (Honourable Mention) in Chemistry and Pharmaceutical Sciences from National Research Council of Thailand
- ▪ 2007
  - ✱ Outstanding Poster Presentation Award (Pharmaceutical Sciences),
    Faculty of Pharmacy, Silpakorn University, Thailand
    (at the 1st Academic and Research Conference "Prachote Plengvidhya", Nakhon Pathom)
  - ✱ Poster Presentation Awards (3 posters) in Science and Technology,
    Silpakorn University Research and Development Institute, Silpakorn University
    (at the 1st Silpakorn University Research Fair, Nakhon Pathom)
- ▪ 2005
  - ✱ Research Council Award: Research Award 2005 (Honourable Mention) in Chemistry and Pharmaceutical Sciences from National Research Council of Thailand
  - ✱ Research Award 2005 from Silpakorn University Research and Development Institute, Thailand
  - ✱ Faculty Award for Teaching Excellence, Faculty of Pharmacy, Silpakorn University, Thailand (on the occasion of the 20th anniversary of Faculty of Pharmacy, Silpakorn University)
- ▪ 2004
  - ✱ TRF-CHE Outstanding New Researcher Award (Thailand's Postdoctoral Award) 2004 from The Thailand Research Fund and the Commission of Higher Education, Thailand
  - ✱ Young Scientist Award 2004 from the Foundation for the Promotion of Science and Technology under the Patronage of H.M. the King, Thailand

    * Travel Award for Post-doctoral Scholars, at the Pharmaceutical Sciences World Congress 2004, Japan
- 2003
    * TPEN Award 2003 (Best Poster Presentation) from the Thailand Pharmaceutics Education Network, Thailand (at the RGJ Seminar Series XXIV Meeting, Bangkok)
- 2002
    * Nagai Award Thailand 2002 (Outstanding Research) from the Nagai Foundation Tokyo, Japan (at the 19th Pharmaceutical Science Meeting, Bangkok)
    * Grant Winner, at the 29th Annual Meeting of the Controlled Release Society, Korea
    * Charles Sturt University Writing Up Award, Granted by Charles Sturt University, Australia

Curriculum Vitae

Name	Maneerat Juttulapa, Miss
Date of birth	March 15, 1985
Workplace	
2006-2008	Department of Biochemistry Technology, Faculty of Science, Mahidol University, Bangkok, Thailand
2009-2013	Pharmaceutical Biopolymer Group (PBiG), Department of Pharmaceutical Technology, Faculty of Pharmacy, Silpakorn University, Nakornpatom, Thailand
Institution Attended	
2007	Silpakorn University: Bachelor of Science (Biotechnology)
2013	Education in Silpakorn University: Master of Science in Pharmacy (Pharmaceutical Sciences)

Conference proceeding and conference abstracts

1. Sriamornsak P, Juttulapa M, Piriyaprasarth S. Microwave-assisted modification of arrowroot starch for pharmaceutical matrix tablets. *Advanced Materials Research* 2010; 93-94: 358-361.
2. Juttulapa M, Burapapadh K, Sriamornsak P. Effects of pectin type and oil type on stability of emulsions using pectin as an emulsifier. *Proceedings of the 3rd Silpakorn University Research Fair* 2010; 3: P210-P214. [Nakhon Pathom, 28-29 January 2010]
3. Piriyaprasarth S, Sriamornsak P, Juttulapa M, Puttipipatkhachorn S. Modeling of drug release from matrix tablets with process variables of microwave-assisted modification of arrowroot starch using artificial neural network. *Proceedings of the 2010 International Conference on Advances in Materials and Manufacturing Processes* 2010. [Shenzhen, China, 6-8 November 2010]
4. Sriamornsak P, Niyomsub R, Phunpruckocha P, Lengsiriwat M, Sakollimchareon R, Tiemsifa R, Manchun S, Juttulapa M. Disintegrating properties of physically modified starches: Yam starch and arrowroot starch. *The 5th Thailand Pharmacy Congress*, Bangkok, 27-28 December 2009.
5. Burapapadh K, Juttulapa M, Sirichiekawat K, Detwattanadet N, Srijareon P, Hengchamrat P, Aiemsuwan S, Piriyaprasarth S, Sriamornsak P. Enhanced dissolution of itraconazole by coprecipitation technique. *The Thai Journal of Pharmaceutical Sciences* 2009; 33(supp): 68.

6. Sriamornsak P, Juttulapa M, Piriyaprasarth S. Microwave-assisted modification of arrowroot starch for pharmaceutical matrix tablets. *International Conference on Functionalized and Sensing Materials (FuSeM 2009)*, Bangkok, 7-9 December 2009.
7. Juttulapa M, Burapapadh K, Manchun S, Sriamornsak P. Stability of oil-in-water emulsions using pectin as a polymeric emulsifier. *The 10th International Hydrocolloid Conference*, Shanghai, China, 20-24 June 2010.
8. Juttulapa M, Burapapadh K, Sriamornsak P. Surface properties and viscosity of emulsions using different pectins as an emulsifier. *Proceedings of the 4rd Silpakorn University Research Fair* 2011; 4: P210-P214. [Nakhon Pathom, 19-21 January 2011]
9. Juttulapa M, Sriamornsak P. Effect of zein concentration on the formation of pectin-zein complexes. *Advanced Materials Research* 2012; 506: 319-322.
10. Juttulapa M, Sriamornsak P. Effect of pH on stability of oil-in-water emulsions stabilized by pectin-zein complexes. *Advanced Materials Research* 2013; 747: 127-130.