

# Two-step sequential cross-linking of sugar beet pectin for transforming zein nanoparticle-based Pickering emulsions to emulgels



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## ABSTRACT

Nanosized (<100 nm) zein spheres were employed for fabrication of a series of fish O/W Pickering emulsions. Although surface tension measurement informed the high potency of zein particles for interfacial adsorption, the prepared emulsions destabilized shortly. Electrostatic deposition of sugar beet pectin onto zein particles interfacial layer at pH 4.0 increased significantly the emulsion stability which was attributed to enhanced steric and electrostatic repulsions based on  $\zeta$ -potential measurements. Pectin enrichment also increased the viscosity of the continuous phase of emulsion. Injection of the oxidative enzyme laccase into the pectin-enriched emulsion to cross-link the feruloyl-bearing pectin molecules and the subsequent addition of  $\text{CaCl}_2$  to set ion-mediated cross-linkages between carboxyl groups of pectin chains transformed Pickering emulsions to emulgels. The higher the pectin content, the shorter was the gelation time of emulsions. The obtained emulgels were self-standing and became harder along with increasing pectin content.

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## 1. Introduction

Emulsions are thermodynamically unstable but kinetically-stabilized mixtures of immiscible liquids. These systems are conventionally stabilized by means of (chemically-synthesized) surface active agents to reduce the dispersed phase/continuous phase interfacial tension (Destribats, Rouvet, Gehin-Delval, Schmitt, & Binks, 2014). Over the past decades, surfactant-free emulsions have been studied and developed enthusiastically by food scientists due to the documented adverse effects of some small-molecule surfactants on human health (Frelichowska, Bolzinger, & Chevalier, 2010). In the so-called Pickering emulsions, solid particles of tendency for partial wetting by both oil and aqueous phases stabilize the disperse phase droplets by a combination of steric, electrostatic and hydration repulsion mechanisms (McClements, 2005).

Various kinds of particles have been employed for stabilization of Pickering emulsions including silica and clay particles (Chen

et al., 2011). Starch particles (Marku, Wahlgren, Rayner, Sjöö, & Tingren, 2012; Tan et al., 2012), chitin nanocrystals (Tzoumaki, Moschakis, Kiosseoglou, & Biliaderis, 2011), polymer-grafted cellulose nanocrystals (Zoppe, Venditti, & Rojas, 2012), chitosan nanoparticles (Wei, Wang, Zou, Liu, & Tong, 2012) and whey protein microgels (Destribats et al., 2014) are examples of bioparticles employed at Pickering emulsions preparation and stabilization. Being an amphiphilic and generally recognized as safe (GRAS) biomacromolecule, zein is of great potential for fabrication of particle emulsifiers with biocompatible and biodegradable characteristics. Zein nano/micro particles can be prepared readily by liquid-liquid dispersion process which is a scale-upable and cost-effective procedure (Zhong & Jin, 2009). In a study by de Folter, van Ruijven, and Velikov (2012) a series of protein-stabilized Pickering emulsions with different concentrations of zein and NaCl at pH 4.0 (lower than the *pI* of zein) were prepared. The emulsions were stable only for a relatively short period of time and underwent coalescence and/or creaming within days. This suggests requirement for supplementary stabilizing mechanisms for zein particles-based emulsions. In order to overcome problems associated with both coalescence and creaming of zein-based Pickering emulsions, Gao et al. (2014) employed ultrasonically-prepared water soluble complexes of zein particles and sodium

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stearate. The complexes packed efficiently at the oil–water interface, producing Pickering emulsions with superior stability against both coalescence and creaming. The partial unfolding of zein particles and hydrophobic domains exposure that improved the diffusive mobility and interfacial wettability of zein particles were argued as responsible mechanisms for enhanced surface particle coverage.

Architectural manipulation of oil–water interface through conjugation of proteins with polysaccharides is a widely attended approach for augmenting emulsion stability (Zeeb, Gibis, Fischer, & Weiss, 2012). In this manner, a protein-stabilized interface is deposited electrostatically with an oppositely charged polysaccharide (Dickinson, 2008) resulting in increased steric hindrance. Previous studies (Chen, McClements, Gray, & Decker, 2010; Li et al., 2012; Zaidel, Chronakis, & Meyer, 2013; Zeeb et al., 2012) indicated that coating of protein molecules-stabilized oil droplets with pectin and subsequent cross-linking of the pectin increases emulsion stability. However, to be the best of authors' knowledge, the layer-by-layer electrostatic deposition technique had not been employed for increasing Pickering emulsions stability. One of the aims of the present study was therefore to interact negatively charged sugar beet pectin molecules with positively charged zein nanoparticles at pH 4.0 to increase the storage stability of zein particles-based Pickering emulsions. The fabricated emulsions were subsequently charged with laccase and calcium. Sugar beet pectin was chosen to interact with zein particles due to being a good substrate for enzymatically oxidation-induced gelation reactions (Zaidel, Chronakis, & Meyer, 2012). This kind of pectin contains considerable amounts of feruloyl groups ester-linked mainly on O-2 of arabinose and O-6 of galactose residues in pectin side-chains (Saulnier & Thibault, 1999). The exploited oxidative enzyme, laccase oxidizes feruloyl groups and cross-links sugar beet pectin chains (Jung & Wicker, 2012). Kuuva, Lantto, Reinikainen, Buchert, and Autio (2003) employed laccase-triggered cross-linking of sugar beet pectin to form a viscoelastic gel. They found that the gel prepared enzymatically from calcium-enriched pectin solution was harder than calcium-deficient counterpart.  $\text{Ca}^{2+}$  ions interact with carboxyl groups of pectin and inter-connect galacturonan chains. The sequential employment of laccase and calcium transformed emulsions to emulsion gels (emulgels). The gelation rate of emulsions and the characteristics of the obtained emulgels were studied. Emulgels are emulsion systems at which the continuous aqueous phase is gelled (Alexander et al., 2013) enjoying of the functional advantages of both hydrogels and emulsions. These dispersions are effective controlled-release delivery systems for bioactives including poorly water-soluble compounds (Yang, Liu, & Tang, 2013a, 2013b). The procedure employed in the present study yielded chemical oxidants- and sugar-free fish oil-in-water emulgels.

## 2. Materials and methods

### 2.1. Materials

Zein from maize, laccase (from *Trametes versicolor*) and ethanol were obtained from Sigma–Aldrich Co. (Taufkirchen, Germany). High-methoxyl sugar beet pectin (Betapec Ru 301) was donated by Herbstreith and Fox (Werder/Havel, Germany) and used with no purification. The protein content and esterification degree of the pectin according to the information by the manufacturer were 3.25% and 55%, respectively. Total phenolics content of the pectin was  $\approx 11.50 \mu\text{g mg}^{-1}$ . Omega 3 fish oil (Ho 307-Batch VO 10045) was gifted by LYSI (Reykjavik, Iceland). All other reagents were of analytical grade.

### 2.2. Preparation and characterization of emulsion continuous phase

#### 2.2.1. Preparation of zein nanoparticles and pectin solutions

Zein nanoparticles were prepared by the solvent evaporation method (Wang & Padua, 2010) following the procedure reported in a previous study (Soltani & Madadlou, 2014). Briefly, zein powder was dissolved ( $2 \text{ mg mL}^{-1}$ ) in 80% v/v aqueous ethanol solution and stirred at 700 rpm for 15 min at  $25^\circ\text{C}$ . To evaporate ethanol, dispersion (100 mL) was placed at  $50^\circ\text{C}$  under hood until volume decreased to 20 mL. The pH of the zein nanoparticles dispersion ( $10 \text{ mg mL}^{-1}$ ) was adjusted onto 4.0 by 1 M HCl. Pre-experiments indicated that at higher pH values, emulsions destabilized immediately.

Five concentrations of sugar beet pectin solution (5, 10, 15, 20 and  $25 \text{ mg mL}^{-1}$ ) were prepared by dissolving pectin powder in distilled water and stirring at 500 rpm overnight to warrant complete hydration. Then pH was adjusted onto 4.0 by 1 M NaOH.

#### 2.2.2. Size, morphology and $\zeta$ -potential of zein particles and pectin

The hydrodynamic size, polydispersity index and  $\zeta$ -potential of zein particles and pectin were measured by using a ZetaPlus Nano range instrument (Brookhaven Instruments Co., NY, USA). The hydrodynamic radius and polydispersity index were analyzed by cumulant method and  $\zeta$ -potential was calculated by using the Smoluchowski equation. Sample characteristics were measured at  $25^\circ\text{C}$ .

Zein nanoparticles were imaged for their morphology and size by a field emission scanning electron microscope (FE-SEM) system (Mira 3-XM, Tescan, USA) at  $50,000\times$  magnification. Powdered sample was coated with gold prior to examination.

#### 2.2.3. Apparent viscosity measurement

The viscosity of zein particles dispersion and zein particles-pectin mixed dispersion (at the same ratios applied in emulsion formula) was measured by a Brookfield viscometer (LV/DV-II Pro, Brookfield Engineering, Inc., USA) equipped with the LV spindle by using 16 mL sample at  $25^\circ\text{C}$ . Shear rate was set to increase from 12 to  $110 \text{ s}^{-1}$  within 12 s intervals.

#### 2.2.4. Surface tension measurements

The equilibrium surface tension of zein particles dispersion and pectin-enriched zein particles dispersion (zein concentration of  $6.6 \text{ mg mL}^{-1}$  and pectin/zein ratio of 0.76) was measured using the Wilhelmy plate method (Krüss processor tensiometer K100, Hamburg, Germany) at  $25^\circ\text{C}$ . A thin platinum plate was dipped into the sample vessel and the force exerted on the plate was recorded.

### 2.3. Preparation of pectin-enriched zein particles-based Pickering emulsions

A series of zein nanoparticles-based O/W Pickering emulsions with different ratios of fish oil to zein particles dispersion (10:90, 20:80, 30:70, 40:60 and 50:50 w/w) was prepared by mixing the oil and particles dispersion in a sealed container (with 5% head space volume) to avoid air introduction at  $\sim 2000 \text{ rpm}$  for 10 min. The ratio 30:70 (0.3 g fish oil and 0.7 g zein dispersion) that yielded the highest stability of emulsion was identified (see Section 2.4) and then employed for preparation of pectin-enriched zein-based Pickering emulsions. For this purpose, based on a series of preliminary experiments pectin solutions of different concentrations (5, 10, 15, 20 and  $25 \text{ mg mL}^{-1}$ ) at the fixed volume ratio of 1:1.95 were added to the primary emulsion followed by mixing for 15 s. The final concentration of zein at emulsion continuous aqueous phase was  $6.6 \text{ mg mL}^{-1}$  and that of pectin was 1.7, 3.4, 5.0, 6.8 and  $8.5 \text{ mg mL}^{-1}$ ,

giving emulsions with pectin/zein ratios of 0.26, 0.5, 0.76, 1.03 and 1.29.

The oil and zein particles dispersion was also homogenized by a high shear homogenizer (Wise Tis HG-15D, Korea) at 13,500 rpm for 5 min to investigate homogenization intensity influence on pectin-deficient emulsion stability. This procedure failed to lengthen emulsion stability considerably and was excluded from the experiments.

#### 2.4. Emulsion stability and droplet size determination

For stability study, prepared Pickering emulsions were thermostated at 40 °C. Stability was assessed by visual observation and monitoring the appearance of samples so that any deviation from monophasic to biphasic system was considered as instability.

Emulsions were imaged 30 min after preparation with an optical microscope (BX51, OLYMPUS, Tokyo, Japan) equipped with a camera (Olympus DP25). Samples were diluted with continuous phase and a drop of diluted emulsion was placed on a microscopic slide and covered gently with a cover slip. Samples were imaged at 25 °C. Droplets average diameter was determined by size measurement of 100 droplets in each micrograph with Image-Pro Plus software (version 6, Media Cybernetic, Inc.).

#### 2.5. Gelation of emulsions

A two-step sequential gelation procedure was established based on a series of preliminary tests. For this purpose, pectin-enriched Pickering emulsions were injected with laccase (12 units g<sup>-1</sup> pectin) and incubated at 40 °C for 4 h. Then, emulsions were charged with 5 M calcium chloride to obtain a Ca<sup>2+</sup> concentration of 0.84 mM in emulsions aqueous phase. Emulsions were subsequently stored at 40 °C for 1 day. Those Pickering emulsions that transformed to emulgels were subjected to further characterization.

#### 2.6. Characterization of emulgels

##### 2.6.1. Gelation time and rate determination

A Paar-Physica rheometer (Physica MCR 300, Anton Paar GmbH, Stuttgart, Germany) fitted with cone-and-plate geometry was utilized for measuring the development of the storage modulus (*G'*) of the emulsion samples that underwent gelation. Measurements were performed immediately after adding CaCl<sub>2</sub> at 40 °C. The cone angle of 1°, plate diameter of 50 mm and gap of 0.05 mm were applied. Frequency and constant strain were 1 Hz and 0.01%, respectively.

##### 2.6.2. Measurement of emulgels firmness

The firmness of emulgels was measured by using a texture analyzer (M350-10CT, Testometric Co., Ltd, Rochdale, Lancashire, England) equipped with a cylindrical probe of 4.5 mm diameter. Emulgels were stored at 40 °C for 24 h after gelation and penetrated to a depth of 20 mm at 5 mm s<sup>-1</sup> at 25 °C. The recorded force was considered as firmness index.

##### 2.6.3. Water holding capacity (WHC)

WHC of the O/W emulgels after 24 h-storage was measured according to the method described by Yang et al. (2013a, 2013b) and Tang, Chen, and Foegeding (2011) with slight modification. The emulgel samples prepared in 1.5 mL microtubes were centrifuged at 8000 × g for 15 min at 25 °C. Separated water was weighed and WHC was measured by using the following equation:

$$\text{WHC}(\%) = 1 - (\text{separated water weight} / \text{water weight in the emulgel}) \times 100$$

#### 2.7. Statistical analysis

The results reported as means ± standard deviations were performed at least in triplicate. Analysis of variance (ANOVA) carried out on the data using IBM SPSS Statistics 21.0 software. Significant differences between treatments were examined at *p* level of 0.05.

### 3. Results and discussion

#### 3.1. Pickering emulsions

The mean hydrodynamic size and polydispersity index of zein particles was 64 nm and 0.32, respectively. An exemplar SEM image of nanoparticles is shown in Fig. 1. Particles are observed as individual entities with nanoscale size and spherical shape. Hydrodynamic size measurements indicated bimodal (75–104 nm and 327–385 nm) size distribution of pectin in solution which suggests a non-spherical extended shape. The intramolecular electrostatic repulsion at pH 4.0 could impose an extended conformation on pectin molecules.

The surface tension of zein particles dispersion was ~28 mN m<sup>-1</sup> indicating particles high affinity to locate on hydrophile-hydrophobe phases interface and reduce their contact area. This property is given by the amphiphilic nature of zein (Wang & Padua, 2010). All the zein-based Pickering emulsions prepared with different oil/aqueous phases ratios were however unstable considerably and broken down in <45 min. Our findings are in accordance with those reported by de Folter et al. (2012) who observed that the emulsions formed with different concentrations of zein particles destabilized shortly (within days) and underwent coalescence/creaming. The ζ-potential of zein particles measured at pH 4.0 was +7 mV. This low ζ-potential value was caused by the interaction of negatively-charged chlorine ions with positively-charged zein particles at pH 4.0 and subsequent electrostatic screening. It is remembered that HCl was added into the zein particles dispersion to decrease pH from the inherent value of 5.2 onto 4.0. For electrostatic stabilization, ζ-potentials over |30| mV are needed and progressive flocculation occurs between |5| and |15| mV (Marianecci, Marzio, Rinaldi, Esposito, & Carafa, 2013; Poletto, Beck, Guterres, & Polmann, 2011). The instability of the zein-based Pickering emulsions is therefore attributed to the insufficient electrostatic repulsion among zein-covered oil droplets. The steric repulsion of zein particles could not provide sufficient stability due most probably to the extensive hydrophobic nature of zein

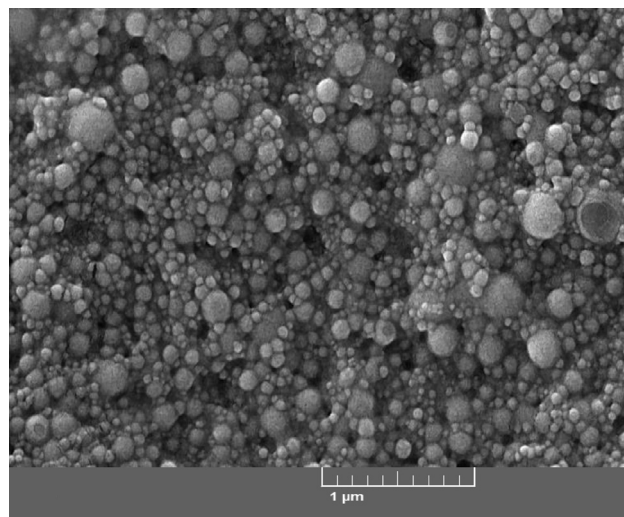


Fig. 1. FE-SEM image of zein nanoparticles formed by ethanol evaporation method.

**Table 1**  
Stability duration and gelation capability of zein-stabilized Pickering emulsions charged with different concentrations of pectin.

Pectin concentration at continuous phase (mg mL <sup>-1</sup> )	Pectin/zein ratio	Stability (days) <sup>a</sup>	Emulgel formation
1.7	0.26	<1	Negative
3.4	0.5	60	Negative
5.0	0.76	60	Positive (soft emulgel)
6.8	1.03	60	Positive (soft emulgel)
8.5	1.29	60	Positive (soft emulgel)

Zein concentration was 6.6 mg mL<sup>-1</sup> at the continuous phase of all emulsion samples.

<sup>a</sup> Stability was monitored up to 60 days.

that arises from its unusual amino acid sequence; more than 50% of residues are hydrophobic (Wang & Padua, 2010). Hydration repulsion of interfaces was also negligible since monovalent Cl<sup>-</sup> ions that possess weak electric field (McClements, 2005) perched onto zein particles layer.

Amongst the short-lived zein particles-based Pickering emulsions, the sample with the oil to aqueous phase ratio of 30:70 was more stable and therefore was selected for further stabilization by pectin enrichment. Depletion flocculation and insufficient interface coverage are argued as the responsible mechanisms, respectively for the ultra-short stability of the emulsion samples with lower and higher oil/aqueous phases ratios. Pectin enrichment of the zein particles-based Pickering emulsion (with the phases ratio of 30:70) lengthened the emulsion stability considerably (Table 1). The surface tension of pectin-enriched zein particles dispersion was ~31 mN m<sup>-1</sup> which is indifferent statistically ( $p > 0.05$ ) from that of pectin-deficient zein dispersion (~27). This suggests that pectin did not contribute significantly to the interfacial tension modulation of zein particles-based emulsions upon the subsequent addition. The  $\zeta$ -potential of pectin was -30 mV; thus upon enrichment of zein particles-based emulsions, pectin molecules interacted electrostatically with positively charged zein particles that located on interface. The  $\zeta$ -potential of pectin-deposited zein particles-based oil droplets (at the emulsion with pectin/zein ratio of 0.76) was almost equal to that of pectin solution. We hypothesize therefore that electrostatic attachment of pectin molecules onto zein particles occurred through one-point anchoring mode and pectin molecules did not spread over zein particles layer to wrap it up. Pectin molecules competed for interacting with zein particles located on interface and the electrostatic repulsion amongst pectins prevented their compaction over the particles layer. The one-point anchoring of each pectin molecule onto zein particles did not neutralize pectin charge and forced pectin molecules to protrude into the aqueous phase of emulsion. The increased stability of the pectin-enriched emulsions is therefore attributed to enhanced electrostatic repulsion among droplets, as well as, long-range steric stabilization by protruded pectin molecules. Then we can argue the shorter stability of the emulsion enriched with the lowest pectin content (1.7 mg mL<sup>-1</sup>; Table 1). At this low concentration, pectin molecules did not compete notably to interact with zein particles and interacted multi-pointly with interfacial particles layer. Therefore, both electrostatic and steric repulsion mechanisms did not work significantly for stabilizing the emulsion for a longer period of time. It is expected that at very low concentrations of pectin ( $\leq 1.7$  mg mL<sup>-1</sup>), zein particles located on the interface of separate droplets would compete to interact with pectin molecules to result hypothetically in bridging of emulsion droplets *i.e.* bridging flocculation (Li et al., 2012).

Apparent viscosity measurements indicated that zein particles dispersion was Newtonian but became shear-thinning due to

**Table 2**  
Rheological models fitted to continuous phase (pectin charged zein dispersion) with different pectin concentration.

Pectin/zein ratio	Model	$K$ (pa s <sup>n</sup> )	$n$	Confidence of fit (%)
0	Newtonian	0.03 <sup>b</sup> ± 0.01	1 <sup>a</sup>	99
0.26	Newtonian	0.06 <sup>b</sup> ± 0.02	0.96 <sup>a</sup>	99
0.5	Power law	0.71 <sup>ab</sup> ± 0.05	0.36 <sup>b</sup>	98
0.76	Power law	0.78 <sup>ab</sup> ± 0.08	0.31 <sup>bc</sup>	98
1.03	Power law	1.5 <sup>a</sup> ± 0.18	0.29 <sup>bc</sup>	98
1.29	Power law	2.1 <sup>a</sup> ± 0.23	0.19 <sup>c</sup>	98

Means with different superscripts in the same column differ significantly ( $p < 0.05$ ).

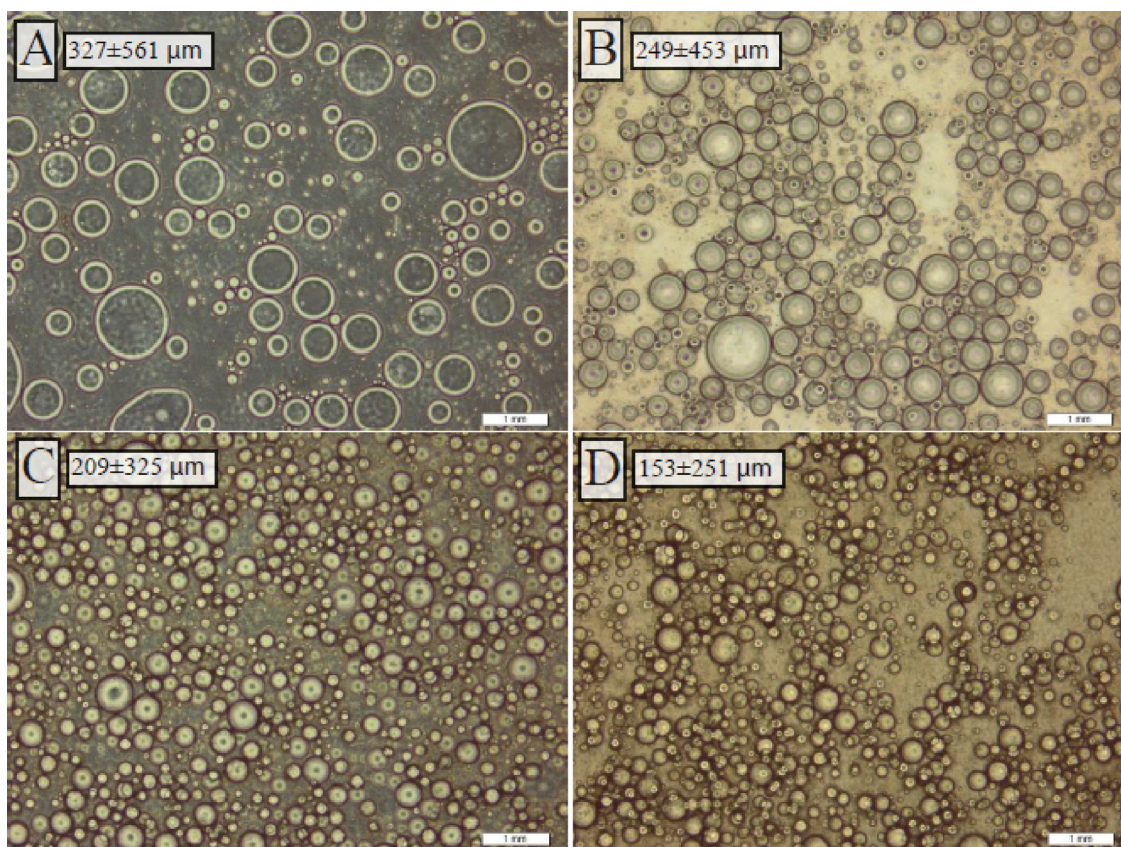
pectin enrichment at ratios  $\geq 0.5$  (Table 2). The higher the pectin concentration, the lower was the flow index of mixed solution. The de-flocculation of aggregated particles by increasing shear, as well as, alignment of the electrostatically-formed zein particles-pectin molecules hyper-assemblies in direction of applied shear field (Wagner & Brady, 2009) accounts for the shear-thinning character of the samples. Pectin enrichment thickened the zein dispersion and resulted in progressively higher consistency coefficient with increasing pectin content (Table 2). At a highly viscous continuous phase, emulsion droplets mobility is suppressed and instability is delayed.

Optical microscopy images of O/W pectin-enriched zein particles-based Pickering emulsions 30 min after preparation are shown in Fig. 2. Pectin-deficient and minimally-enriched emulsion samples were so unstable that were ignored for imaging. The rapid progress of droplets coalescence within minutes was observable under microscope for pectin-free emulsions. The higher the pectin content, the smaller were the oil droplets and the more homogenous was the emulsion (Fig. 2). It is concluded that oil droplets in zein particles-based Pickering emulsions coalesce quickly unless are stabilized by a supplementary approach. The high sensitivity of zein-covered oil droplets to coalescence rises likely from the considerable hydrophobic nature of zein which enhances hydrophobic attraction between zein particles located on separate droplets interface. The particles then fuse and emulsion breaks down.

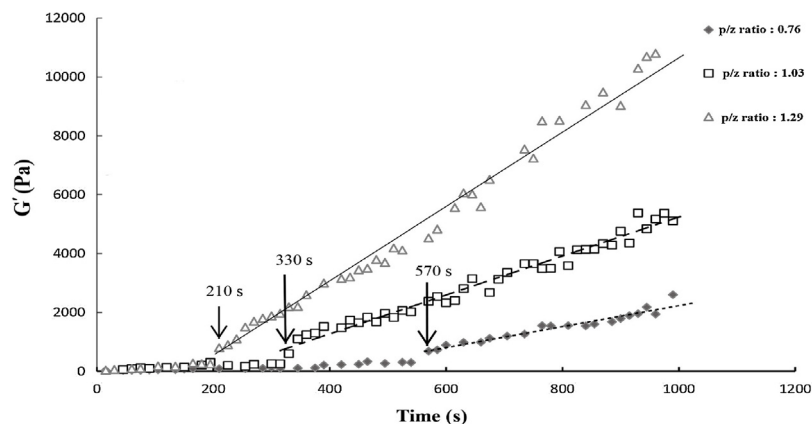
### 3.2. Emulgels

The existence of feruloyl groups in sugar beet pectin provides a special way for enzyme-promoted cross-linking of pectin chains and eventual gelation (Zaidel et al., 2012). In this research, we intended to transform produced Pickering emulsions to emulgels. For this purpose, at first, emulsions were treated with laccase and stored at 40 °C for 4 h. This process did not gelify pectin due to the inadequacy of enzymatic cross-linking to promote gel formation at the pectin concentration range studied in the present study. On the other hand, the emulsions that were not cross-linked enzymatically but enriched with CaCl<sub>2</sub> also failed to gel. The high acetyl content of sugar beet pectin limits the extent of Ca<sup>2+</sup>-mediated cross-linking of pectin chains and subsequent gelation (Oosterveld, Beldman, Searle-van Leeuwen, & Voragen, 2000). However, calcium and laccase showed a synergistic action and promoted pectin gelation when enzymatically-cross-linked emulsion was charged with CaCl<sub>2</sub>. Emulsion gelation was observed for emulsion samples with pectin/zein ratios  $\geq 0.76$  (Table 1). Calcium associated the neighboring pectin chains via carboxyl groups bridging (Sriamornsak, Thirawong, & Puttipatkhachorn, 2005) and decreased the electrostatic repulsion among the co-charged chains, thus allowed the chains to pack closely and eventually form gel.

Fig. 3 indicates the time evolution of  $G'$  for laccase- and calcium-treated Pickering emulsions with pectin/zein ratio  $\geq 0.76$ . The  $G'$



**Fig. 2.** The morphology and droplets average diameter of zein-stabilized Pickering emulsions formed with pectin/zein ratios of (A) 0.5, (B) 0.76, (C) 1.03, and (D) 1.29.



**Fig. 3.** Storage modulus ( $G'$ ) development of enzymatically cross-linked and calcium-treated pectin-enriched zein-based Pickering emulsions. The p/z ratio abbreviates pectin to zein ratio.

increment is indicative of gel formation with time (Zaidel et al., 2012). The first time at which a sudden rise in  $G'$  occurred was considered as gelation onset time. The higher the pectin content, the shorter was the gelation onset time. The initial rate of gelation was determined as the rate of changes in  $G'$  over the early linear region of curve after gelation onset time. Gelation rates of 4.5, 6.8 and 13.3 Pa s<sup>-1</sup> were calculated for emulsions with ratios of 0.76, 1.03 and 1.29, respectively. It is obvious that gelation rate increased with increasing pectin concentration. Zaidel et al. (2012) reported similarly a faster gelation rate for a high pectin concentration. The magnitude of  $G'$  is attributed to the number of cross-linkages (Kuuva et al., 2003). Therefore, increasing  $G'$  values with increasing pectin concentrations is ascribed to the more extensive cross-linking of the gel network.

The emulgels fabricated from Pickering emulsions were stored at 40 °C for 24 h, yielding self-standing and soft gels. The higher the pectin content of emulgels, the higher was the required force to penetrate into (Table 3). Higher pectin concentrations also provided higher WHC for emulgels (Table 3). Gels ability for holding

**Table 3**  
Water holding capacity (WHC) and penetration force of emulgels.

Pectin/zein ratio	WHC (%)	Penetration force (N)
0.76	53 <sup>b</sup> ± 4	0.15 <sup>c</sup> ± 0.02
1.03	68 <sup>a</sup> ± 7	0.24 <sup>b</sup> ± 0.03
1.29	72 <sup>a</sup> ± 7	0.32 <sup>a</sup> ± 0.03

Means within the same column with different superscripts differ significantly ( $p < 0.05$ ).

water is associated with the strength of gel and has relationship with  $G'$ . The water retention capability of hydrogels is influenced by the presence of charged groups; then a higher concentration of the poly-anion pectin caused entraining a higher amount of water through ion–dipole interactions (McClements, 2005) resulting in increased WHC.

#### 4. Conclusion

Pectin enrichment of zein particles-based Pickering emulsions at high enough concentrations stabilized the emulsions for a long period of time ( $\geq 60$  days). Sequential enzymatic and calcium cross-linking of pectin transformed the Pickering emulsions to self-standing emulsions with feasible exploitation as fish oil- and calcium-carrying delivery systems. A more comprehensive study for monitoring the oxidative stability of encapsulated fish oil during emulsions and emulsions storage at various conditions, as well as, the fate and bioavailability of various nutrients employed in emulsions and emulsions fabrication upon oral administration will be useful.

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