A Thesis for the Degree of Master of Science

Influence of Charge Distribution and Structure of Pectin on Bioaccessibility of Curcumin Loaded in Pectin-based Emulsions

펙틴의 구조 및 전하 분포도가
펙틴 기반 에멀션 내 커큐민의 생체접근율에 미치는 영향

August, 2018

Park, Su Yeon

Department of Agricultural Biotechnology

Seoul National University

농학석사학위논문

Influence of Charge Distribution and Structure of Pectin on Bioaccessibility of Curcumin Loaded in Pectin-based Emulsions

펙틴의 구조 및 전하 분포도가
펙틴 기반 에멀션 내 커큐민의 생체접근율에 미치는 영향

지도교수 문 태 화
이 논문을 석사학위 논문으로 제출함

2018년 8월
서울대학교 대학원
농생명공학부
박 수 연

석사학위 논문을 인준함
2018년 8월

위 원 장 장 판 식 인

부위원장 문 태 화 인

위 원 하 남 출 인

A Thesis for the Degree of Master of Science

Influence of Charge Distribution and Structure of Pectin on Bioaccessibility of Curcumin Loaded in Pectin-based Emulsions

by

Park, Su Yeon

Advisor: Tae Wha Moon, Professor

Submitted in Partial Fulfillment of the Requirement
for the Degree of Master of Science

August, 2018

Department of Agricultural Biotechnology

Seoul National University

ABSTRACT

Pectin is an important functional material in the food industry as a gelling agent, a viscosity modifier, and an emulsifier. Recent studies revealed that not only the degree of methylesterification (DM) but also the charge distribution of unmethylesterified region has great impacts on functional properties of pectin. The objectives of this study were to access the potentials of pectin as a mono-emulsifier, and to investigate the influence of pectin type and oil concentration on physicochemical properties, lipid digestibility, and *in vitro* curcumin bioaccessibility in emulsions stabilized by pectin as an emulsifier. Commercial high methyl-esterified pectin (CP72; DM=72), medium methyl-esterified pectin (CP50; DM=50), and low methyl-esterified pectin (CP7; DM=7) were used as emulsifiers. MP50 (DM50) having consecutive demethylesterified galacturonic acid residues were prepared by enzymatic demethylesterification of CP72 using pectin methylesterase from papaya to induce differed charge distribution to CP50 while having the same DM. Emulsions containing 0.015 mg of curcumin with 1% or 3% (w/w) oil were prepared by emplying a homogenizer and a microfluidizer consecutively. The initial oil droplet size was clearly increased in accordance to the oil concentration; however, no significant difference (p>0.05) was observed among pectin types. CP72 emulsion showed the lowest surface

electrical charge at both oil concentrations. MP50 emulsion displayed a more negative electrical charge than CP50 emulsion did, and this might be attributed to the ordered distribution of galacturonic acid residues with carboxylic groups. After *in vitro* mouth and gastric digestion, CP72 and CP50 emulsions retained their initial droplet structure, whereas MP50 and CP7 emulsions displayed coalescence and bridging flocculation during digestion. Although CP72 emulsion showed great emulsion stability due to a more proportion of methylesterified galacturonic acid having hydrophobic character, it displayed a low degree of final lipid digestion similar to CP7 emulsion. In contrast, MP50 emulsion revealed a high degree of final lipid digestion and high bioaccessibility of curcumin as well. Because of the consecutively demethylesterified galacturonic acid residues, MP50 might adsorb onto the oil droplet with regular arrangement resulting in geometrically empty space within the clusters. Even though CP50 has the same DM and Mw as MP50, it exhibited a low bioaccessibility of curcumin which might be contributed to the fast lipid digestion profiles. It is thus suggested that curcumin micellarization might be related to the lipid digestion profile and the charge distribution of pectin as well as DM. These observations implied that the emulsion stability and bioaccessibility of lipophilic neutraceuticals could differ depending on the molecular features and the charge distribution of pectin emulsifiers. Emulsion stabilized by

pectin with consecutive negative charges displayed lipid digestion to a greater extent compared to high methoxyl pectin, and even a higher *in vitro* bioaccessibility of curcumin compared pectin with random electrical charges, suggesting a potential as a carrier of lipophilic nutraceuticals in human digestive system. It is thus manifested a great potential of pectin as an emulsifier for the food industry because pectin is a biodegradable compound naturally present in plants and preferred over artificial emulsifiers by consumers.

Keywords: Pectin methylesterase, bioaccessibility

Student Number: 2016-25843

CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Curcumin is the principal curcuminoid of turmeric (*Curcuma longa*), a member of the ginger family, Zingiberaceae, and one of the most biologically active components. The health benefits of curcumin are attributed to a broad spectrum of biological effects, including anti-inflammatory, antioxidant, antiviral, antibacterial, antifungal, and antitumor activities (Sahdeo Prasad, 2014)

However, many studies have shown that no or little amount of curcumin is detected in serum or tissue after administration, even with high doses of curcumin (Shen, Liu, An, & Ji, 2016). The low bioavailability of curcumin is mainly caused by the high melting point, poor water solubility, poor stability (due to chemical transformation), and low bioaccessibility within the gastrointestinal tract (Anand, Kunnumakkara, Newman, & Aggarwal, 2007; Fu et al., 2015).

To improve the bioavailability of curcumin, numerous approaches have been undertaken by many researchers including encapsulation technology within food-grade delivery systems such as nanoparticles, hydrogel beads, biopolymer complexes, and emulsions (Aditya et al., 2015; Bergonzi, Hamdouch, Mazzacuva, Isacchi, & Bilia, 2014; Chen et al., 2014; Guri, Gülseren, & Corredig, 2013; Loo et al., 2015; Sari et al., 2015). Among the

encapsulation systems, nanoemulsion is particularly suited for the design and fabrication of delivery systems for encapsulating bioactive lipids, and widely accepted for food grade applications (McClements, Decker, & Weiss, 2007; Sari et al., 2015). The main advantages of nanoemulsion are its small droplet size and high kinetic stability. The kinetically stable suspension can be manufactured by adding stabilizers or emulsifiers into the emulsion systems.

Pectin is a family of heterogeneous polysaccharides mainly located in the primary cell wall and middle lamella of higher plants. Generally, pectin is used as an important functional material in the food industry as a gelling agent, a viscosity modifier, and an emulsifier. The functional properties of pectin are reported to be governed by structural features (D.N. Sila, 2009; Ngouémazong, Christiaens, Shpigelman, Van Loey, & Hendrickx, 2015; Thakur, Singh, & Handa, 1997; Van Buggenhout, Sila, Duvetter, Van Loey, & Hendrickx, 2009).

The most abundant pectic polysaccharide accounting for about 65% of the total pectin content of plant tissues is heterogeneous homogalacturonan (HG) consisting of 1,4-linked α-D-galacturonic acid. This linear structural element of pectin is methyl esterified at the C-6 position (Schols, Vierhuis, Bakx, & Voragen, 1995), thereby assigning a specific degree of methyl esterification (DM) to the polymer.

Conventionally, pectins are classified by their sample averaged DM into high (>50% DM) or low methoxyl pectin (<50% DM). The demethylesterification of pectin can be obtained through chemical saponification and enzymatic conversions using pectin methylesterase (PME) of either fungal or plant origin (Hunter & Wicker, 2005; Ngouémazong et al., 2015). In addition, origin of PME as well as each demethylesterification method cause different charge distribution on pectin which means the different pattern of demethylesterification .

The DM is generally used to demonstrate the different functionality of pectins as gelling, thickening and stabilizing agents, along with molecular weight (Mw) of pectin (Ngouémazong et al., 2015). However, some studies addressed that not only DM and Mw, but also the pattern of methyl esterification has great impacts on functional properties of pectin (Savary, Hotchkiss, Fishman, Cameron, & Shatters, 2003; Thibault, Renard, Axelos, Roger, & Crépeau, 1993; Van Buggenhout et al., 2009).

Recently, it is desirable to use natural ingredients to formulate emulsions to create “label-friendly” products in many applications. Pectin has been gradually gaining acceptance as a label-friendly food grade emulsifier, and a number of studies have focused on revealing the factors that affect pectin’s emulsifying and emulsion-stabilizing abilities (Burapapadh, Kumpugdee-

Vollrath, Chantasart, & Sriamornsak, 2010; Makoto Nakauma, 2008; Ngouémazong et al., 2015; Schmidt, Schmidt, Kurz, Endreß, & Schuchmann, 2015; Tina A.J. Verrijssen 2016; Verrijssen, Verkempinck, Christiaens, Van Loey, & Hendrickx, 2015). In addition, pectin could be used in emulsion-based foods including reduced-fat dairy products and emulsified meat products (Dartey, Trainor, & Evans, 1990; Fernández-Ginés, Fernández-López, Sayas-Barberá, & Pérez-Alvarez, 2005; Lobato-Calleros et al., 2008). The hydrodynamic properties (conformation, surface charge density, and molecular weight) and hydrophobic character of pectins have been suggested to affect its emulsifying and emulsion-stabilizing properties (Jiyoung Jung & Wicker, 2012; Lutz, Aserin, Wicker, & Garti, 2009; Nakauma et al., 2008; Ngouémazong et al., 2015). However, a clear mechanism of the influence of structural features of pectin on the polymer emulsifying potentials is yet to be fully determined, because the main structural characteristics of pectin are complicated to elucidate the mechanisms involved in this phenomenon. Especially, the influence of the pattern of methyl esterification on the emulsifying and emulsion-stabilizing potential of pectin has not been compared to DM and Mw at once. (Ngouémazong et al., 2015) addressed that a limited number of studies, demonstrating the influence of individual structural characteristics of pectin on functional properties, represents a

major technical challenge.

The main objective of this study was to comprehensively discuss the potentials of pectin as a mono-emulsifier, with highlights on the effect of molecular structure and charge distribution of pectin on physicochemical properties. In addition, the influence of pectin type and oil concentration on the microstructure, lipid digestibility, and *in vitro* curcumin bioaccessibility of pectin-based emulsions was examined using a simulated gastrointestinal tract (GIT).

MATERIALS AND METHODS

1. Materials

All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA) unless otherwise indicated.

GENU pectins were provided by CP Kelco (Atlanta, GA, USA). The low-methoxyl pectin was type explorer-65CS with DM 50 (CP50) and LM-5CS with DM 7 (CP7). For modification, the high-methoxyl pectin, type YM-100H with DM 72 (CP72), was used as pectin source, and Liquipanol® T-200 from Enzyme Development Corporation (New York, USA) was used as PME source driven from the dried latex of the fruit of *Carica papaya L* (CpL-PME, GRAS – generally recognized as safe – 21 CFR 184.1585). Canola oil was purchased from local market.

2. Methods

2.1. Demethylesterification

Pectin with defined DM 72 was used for enzymatic treatment adopted from the study of Hotchkiss et al. (2002) with some modifications. Pectin was prepared at 1% (w/v) in 0.2 M LiCl, and then the mixture was placed in a water-jacketed reaction beaker at 30°C. The pH of the mixture was adjusted to 7.0 by the addition of 0.1M LiOH. The CpL-PME (175 U/L) was added to the reaction mixture (Randall G. Cameron, Luzio, Goodner, & Williams, 2008; R. G. Cameron, Luzio, Vasu, Savary, & Williams, 2011), and pH was maintained with a pH-stat automatic titration system (842 Titrando, Metrohm, Switzerland) using 0.1M LiOH as the titrant at 30°C. The PME-treatment was terminated when desired DM 50 was reached. After deesterification, the reaction mixture was added to 3 volumes of ethanol, and adjusted to pH 3.8 with 1M HCl to inactivate the enzyme and precipitate the pectin. The precipitated pectin was stored at 4°C and centrifuged (12,000 x g, 30 min, 4°C), and the supernatant was discarded. The pellet was placed in liquid nitrogen, lyophilized, and homogenized in a blender (BL142AKR, Tefal, Rumilly, France). The pectin was frozen and kept at -80°C until future analysis.

2.2. Measurement of sucrose contents

Commercial pectin for food use may contain up to almost 50% sucrose (May, 1990). For the comparison of the structural properties of pectins under equal process conditions, the amount of pectin and sucrose should be equalized by adjusting it at a given pectin concentration using sucrose. The determined sucrose content was taken into consideration for the preparation of pectin-stabilized emulsions.

Pectin was dissolved at 1% (w/v) in distilled water and put into Amicon® Ultra (0.5 PL 10K, Merck, Darmstadt, Germany) placed in an Eppendorf tube and then centrifuged (1248R, Labogene, Seoul) at 8,000 x g for 30 min. Analysis of soluble sugars in pectin was carried out using high-performance anion-exchange chromatography (HPAEC) system equipped with a Carbo-pak PA1 anion-exchange column (4x250 mm, Dionex, Sunnyvale, CA, USA) and a pulsed amperometric detector (PAD). Samples were filtered prior to injection with a 0.2 μm nylon syringe filter (Whatman, Kent, UK) and diluted 10-fold with deionized water. This analysis was performed using 200 mM NaOH for sample elution with isocratic flow rate of 1 mL/min for 15 min. Sucrose was quantified by comparing the peak areas with peak areas of the standard solutions of known concentration.

2.3. Determination of molecular weight

Molecular weight (Mw) of commercial and PME-treated pectins were determined using high performance gel permeation chromatography (HPGPC) combined with a refractive index detector (Thermo Dionex HPLC Ultimate3000 RI system, Sunnyvale, CA, USA). Pectin samples were placed in dialysis tubing (MWCO 12000-14000, Sigma Aldrich, St. Louis, MO, USA) to remove sucrose and other sugars within samples and lyophilized. Lyophilized pectin samples (0.25% w/v) were dispersed in distilled water and then filtered with syringe filters (0.45 μm) prior to injection (10-100 μL). The HPGPC system used running columns (Waters Ultrahydrogel 120, 500, 1000 serial) at 40°C. The flow rate of mobile phase (0.1M sodium azide in water) was 1 mL/min. The molecular weight was measured with the pullulan standards. The results were analyzed by Chromeleon 6.8 Extention-pak software (Thermo Fisher Scientific, Sunnyvale, CA, USA).

2.4. Preparation of emulsion

Pectin stock solutions were prepared by dispersing pectins (CP72, CP7, CP50, and MP50) into 10 mM phosphate buffer (pH 7), followed by adjustment of the amount of sucrose to 28 % in each pectin sample. The solutions were stirred at 300 rpm overnight at room temperature to ensure complete dispersion and dissolution. An oil phase was prepared by

dispersing curcumin (1.5 mg/g oil) in canola oil heating (~60°C for 10 min) on a magnetic stirrer and then sonifying using a sonicating water bath (ULTRA-TURRAX model T25 digital, IKA, Staufen, Germany) for 20 min to ensure complete dissolution. Pectin-stabilized emulsions were therefore prepared by homogenizing 1 or 3% (w/w) corn oil and 99 or 97% (w/w) emulsifier solution using a high shear mixer for 1 min at 12,000 rpm (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland), and then fine emulsions were formed by passing the coarse emulsions through a microfluidizer three times at 12 kpsi (Picomax MN 300-25P, Micronox, Seongnam, Korea).

2.5. Characteristics of the pectin based-emulsions

The mean particle diameter, particle size distribution, and surface electro charge of the samples were measured at various stages in the simulated gastrointestinal process and during storage (0, 3, 7, 15, and 30 days).

2.5.1 Mean particle size and particle size distribution

The particle size distribution was measured using a dynamic light scattering instrument (Z390, Malvern Instruments Ltd., Worcestershire, United Kingdom). The emulsions were diluted to a droplet concentration of approximately 0.002% (w/w) using 0.1M phosphate buffer solution prior to analysis. Particle sizes were reported as particle size distribution profiles

(number fraction (%) vs. particle diameter (mm)) and surface-weighted mean diameter (d_{32}, nm).

2.5.2 Surface electrical charge

The surface electrical charge (ζ-potential, mV) of emulsions was determined using a particle micro-electrophoresis instrument (Z390, Malvern Instruments Ltd., Worcestershire, United Kingdom). The emulsions were diluted to a droplet concentration of approximately 0.002% (w/w) using 0.1M phosphate buffer solution prior to analysis. Diluted emulsions were injected into the measurement chamber, equilibrated for 10 s and then the ζ-potential was determined by measuring the direction and velocity that the droplets moved in the applied electric field. Each ζ-potential measurement was calculated from the average of 100 continuous readings made per sample.

2.6. Simulated gastrointestinal tract model

A simulated gastrointestinal tract (GIT) consisting of oral, gastric, and intestinal phases was used to access the biological fate of ingested samples.

2.6.1. Oral phase

Simulated saliva fluid (SSF) was prepared according to a previous study (Sarkar, Goh, & Singh, 2009). Mucin (30 g/L) was dissolved in SSF on a

magnetic stirrer for overnight. The mixture was adjusted to pH 6.8 with 3M NaOH and equilibrated in a water bath at 37°C prior to use. The samples were mixed with SSF at a 50:50 ratio, and the mixture was then adjusted to pH 6.8. The mixture was incubated at 37ºC for 10 min with continuous agitation at 100 rpm.

2.6.2. Gastric phase

Simulated gastric fluid (SGF) was prepared using a method reported previously (Sarkar, Goh, Singh, & Singh, 2009) by dissolving 2 g of NaCl and 7 mL of HCl (37%) in 1 L of water and then adding 3.2 g of pepsin. The sample from the oral phase was mixed with SGF at a 50:50 ratio, and the pH of the sample was adjusted to 2.5 using 1M NaOH. The sample was then incubated at 37 °C for 2 h with continuous agitation at 100 rpm.

2.6.3. Small intestinal phase

A pH-stat automatic titration unit (842 Titrando, Metrohm, Herisau, Switzerland) was used to simulate the conditions in the small intestinal phase of the GIT (Salvia-Trujillo, Qian, Martin-Belloso, & McClements, 2013). An aliquot (30 mL) of sample from the gastric phase was placed in a temperature-controlled (37 °C) chamber, and the pH was set at 7.0. Then 3.5 mL of bile extract solution (187.5 mg/3.5 mL) and 1.5 mL of salt solution (10 mM of $CaCl_2$ and 150 mM of NaCl) were added to the sample, and the

mixture was adjusted to pH 7.0. Afterwards, 2.5 mL of freshly prepared pancreatin suspension (187.5 mg/ 2.5 mL) in phosphate buffer was added to the mixture. The pH of the mixture was monitored, and the volume of 0.25 M NaOH (mL) added to the emulsion was recorded over 2h and then was used to calculate the concentration of FFAs generated by lipolysis. The percentage of FFA released was calculated using the following equation:

$$\% \text{ FFA } = 100 \times \frac{V_{\text{NaOH}} \times \text{m}_{\text{NaOH}} \times M_{\text{lipid}}}{w_{\text{lipid}} \times 2}$$

where, V_{NaOH} is the volume of NaOH (in L) titrated into the reaction vessel to neutralize the FFAs released, assuming that all TAGs are hydrolyzed in two molecules of FFAs and one molecule of MAG, m_{NaOH} is the molarity of sodium hydroxide, M_{lipid} is the average molecular weight of canola oil (877 g/mol), and w_{lipid} is the weight of oil in the digestion system in grams. Blanks (samples without oil) were run, and the volume of titrant used for these samples was subtracted from the corresponding samples that contained oil.

2.7. Microstructure of pectin-based emulsions

The microstructure of the samples was observed at 1,000× magnification by using a light microscope (Carl Zeiss, DE/Axio Imager A1, Obserkochen, Germany). A small amount of aliquot of each sample was placed on a

microscope slide and covered with a cover slip prior to observation.

2.8. Curcumin bioaccessibility

The bioaccessibility of curcumin was determined by using a method described previously (Zou et al., 2016). After the *in vitro* digestion process, 20 mL of digesta from each sample was centrifuged (1236R, Labogene, Daejeon, Korea) at 3,100 x g for 20 min at 25°C. The clear supernatant was collected and assumed to be the "mixed micelle" fraction in which the curcumin was solubilized. In some samples, a layer of non-digested oil was observed at the top of the test tubes, which was excluded from the micelle fraction. Aliquots of 5 mL of digesta or micelle fraction were mixed with 5 mL of chloroform, vortexed, and centrifuged at 800 x g for 10 min at ambient temperature. The bottom layer containing the solubilized curcumin was collected, while the top layer was mixed with an additional 5 mL of chloroform, and the same procedure was repeated. The two collected chloroform layers were mixed together, and then diluted to an appropriate concentration to be analyzed by a UV–VIS spectrophotometer at 419 nm. The concentration of curcumin in the digesta or in the mixed micelle phase was calculated from the absorbance using a standard curve.

The bioaccessibility of curcumin at the end of the GIT model was calculated using the following expression:

$$\text{Bioaccessibility} = 100 \times \frac{C_{\text{Micelle}}}{C_{\text{Digesta}}}$$

Here, C_{Micelle} and C_{Digesta} are the concentrations of curcumin in the mixed micelle fraction, and in the overall digesta after the pH-stat experiment, respectively.

2.9. Statistical analysis

All experimental data were analyzed using analysis of variance (ANOVA) and expressed as mean ± standard deviation of replicate measurements. Significant differences ($p<0.05$) among mean values were compared using the Duncan's multiple range test. These statistical analyses were conducted using IBM SPSS statistics version 21.0 (IBM, Armonk, NY, USA).

RESULTS AND DISCUSSION

1. Molecular weights of commercial and PME-modified pectins

The molecular weight of pectin is one of the important features to determine the functionality of pectin. Thus, Mw of commercial and PME-modified pectins were examined using high performance size exclusion chromatography. The results are shown in Table 2. The deesterification using PME enables to control DM of pectin without a decrease of Mw (Hills, Mottern, Nutting, & Speiser, 1949). Although MP50 which was produced via PME reaction showed a slight decrease in Mw, it still maintained high Mw similar to Mw of CP50. It is possible to compare the effect of different charge distribution because of the same molecular weight of MP50 and CP50. The commercial pectin with the lowest DM, CP7, showed the smallest Mw. This result was possibly caused by chemical demethylation common to commercial pectins inducing a decrease of Mw by cleaving the homogalacturonan backbone of pectin (J. Jung, Arnold, & Wicker, 2013; Ngouemazong et al., 2012).

Table 1. Molecular weights of commercial and PME-modified pectins

Samples[1)]	CP72	MP50	CP50	CP7
Peak Mw	6.3×10^5	5.9×10^5	5.9×10^5	2.5×10^5

1) CP72 = commercial high methoxyl pectin (DM72); MP50 = PME-modified pectin (DM50); CP50 = commercial medium methoxyl pectin containing reduced sucrose by alcohol precipitation (DM50); CP7 = commercial low methoxyl pectin (DM7)

2. Sucrose contents of commercial and PME-modified pectins

In general, for the production of commercial pectins, sucrose is added to pectin for standardization purpose during the manufacturing process (May, 1990). The results are shown in Table 1. In commercial pectins, 28.22−34.94% of sucrose was quantified.

MP50 had only 0.83% sucrose because of the PME treatment process while the mother pectin CP72 had 28.3 %. The main reason of this decrease was the ethanol addition to quench the PME reaction. In the presence of sucrose, both emulsifying activity and emulsion-stabilizing effectiveness of pectin could be reduced. Thus, ethanol precipitation of medium methoxyl pectin (DM=50) was conducted to remove excess sucrose and the resulting pectin, CP50 having only 6.39% of sucrose was used as an emulsifier.

Then, the amount of sucrose in each pectin sample was adjusted to about 28% when making the aqueous phase of pectin to eliminate the effect of sucrose on emulsion forming and stabilizing properties of pectin.

Table 2. Sucrose contents of commercial and PME-modified pectins

Samples[1)]	CP72	MP50	CP50	CP7
Sucrose[2)] (%)	28.22 ± 0.19	0.83 ± 0.14	6.39 ± 0.42	28.44 ± 0.25

[1)] CP72 = commercial high methoxyl pectin (DM72); MP50 = PME-modified pectin (DM50); CP50 = commercial medium methoxyl pectin having reduced sucrose conten by alcohol precipitation (DM50); CP7 = commercial low methoxyl pectin (DM7).

[2)] Mean±Standard deviation; means of triplicates.

3. Emulsifying and emulsion stabilizing ability

The 1% and 3% oil emulsions were used to investigate the influence of DM, Mw, and charge distribution of pectin on the emulsifying and emulsion stabilizing abilities for 30 days.

3. 1. Particle size and electrical charge

The results of the particle size distribution analysis of the different emulsions showed a clear increase in oil droplet size with increasing oil concentration (Table 3). Further, Fig. 3 and 4 revealed that the extent of oil droplet coalescence increased in accordance to an increase of lipid. High frequency of droplet-droplet encounter was evoked by the increase in the number of droplets, thereby promoted fast droplet coalescence.

Although CP72 and MP50 had the smallest droplet size in 1% oil and 3% oil emulsion, respectively, the initial droplet size was not significantly different ($p > 0.05$) among pectin emulsions with 1% ($474 - 623$nm) and 3% ($865 - 1143$nm) oil concentration. Pectin type seemed to have a slight influence on the initial average droplet size since the emulsion contained a relatively small amount of oil.

Table 3. Peak particle size of the initial pectin based-curcumin emulsions.

[unit; nm]

% Oil in emulsion	CP72	MP50	CP50	CP7
1%	588.3 ± 138.0	474.0 ± 51.9	627.7 ± 14.9	523 ± 19.1
3%	884.5 ± 109.9	1142.7 ± 152.7	1036 ± 148	1023.5 ± 53.0

[1)] CP72 = commercial high methoxyl pectin (DM72); MP50 = PME modified pectin (DM50); CP50 = commercial medium methoxyl pectin that contains reduced sucrose by alcohol precipitation (DM50); CP7 = commercial low methoxyl pectin (DM7).

[2)] Mean± Standard deviation; means of triplicates.

In general, the ability of pectin to facilitate the formation and retention of fine droplets during emulsification is attributed to two reasons (Ngouémazong et al., 2015). First, the ability to promptly reduce the interfacial tension at the oil/water interface is critical, with the hydrophobic portions adsorbing strongly to the interface, while the hydrophilic part extends into the aqueous phase. Second, the adsorbed pectin could provide stabilization by forming an effective protective layer against flocculation or coalescence. In addition, during emulsification, only a small proportion of pectin actually adsorbs onto oil droplets to form a protective layer which decreases the oil/water interfacial tension, thereby reducing the emulsion droplet size. The similar size of oil droplets indicates that the surface of oil droplets is completely covered with an emulsifier. This means that all types of pectin with different Mw, DM, and DB could action as an efficient emulsifier at 1%t oil concentration.

A schematic representation of the pectin adsorption behavior during emulsification at the oil/water interface is shown in Fig. 5. CP72 having more hydrophobic features than other types of pectins and large Mw might adsorb strongly to the interface covering the droplet completely. As a result, it produced small and uniform oil droplets which could stay stable during the storage. MP50 having introduced negatively charged demethylesterified

blocks (DMB) and large Mw might adsorb to the oil droplet forming a regular layer. (Lutz et al., 2009) also observed a pronounced decrease in surface and interfacial tensions for enzymatically demethylesterified pectin having large blocks by improving pectin assembly into a layer at the oil/water interface. CP50 having randomly distributed negative charge and similar Mw and DM with MP50 might adsorb to the oil droplet forming irregular arrangement at the interface. CP7 with low methylesterified regions and low Mw might adsorb to the oil droplet weakly and protect the droplet by electrostatic repulsion caused by high negative charge, not producing a layer. Owing to the small Mw of CP7, its ability was not enough to protect the oil droplet completely to retain stability against flocculation, coalescence, and creaming for an extended period (Fig. 3-D).

Initial droplet size showed only a slight increase until 15 days except for MP50 emulsions (Fig 1 and Fig 3). Fig. 3B-c and Fig. 3D-c showed that the CP7 emulsions extremely produced flocculation and coalescence as compared with MP50, but the result displayed in Fig. 1 did not entirely reflect the different behavior of oil droplets. It is known that if the large particles present in even small quantities, they may be accounted by dynamic light scattering (DLS) during data analysis, resulting in overestimation and large deviation (James & Driskell, 2013). Therefore, the real shape of

particles and distribution within emulsions should comprehensively consider the DLS values and microscopic pictures at the same time.

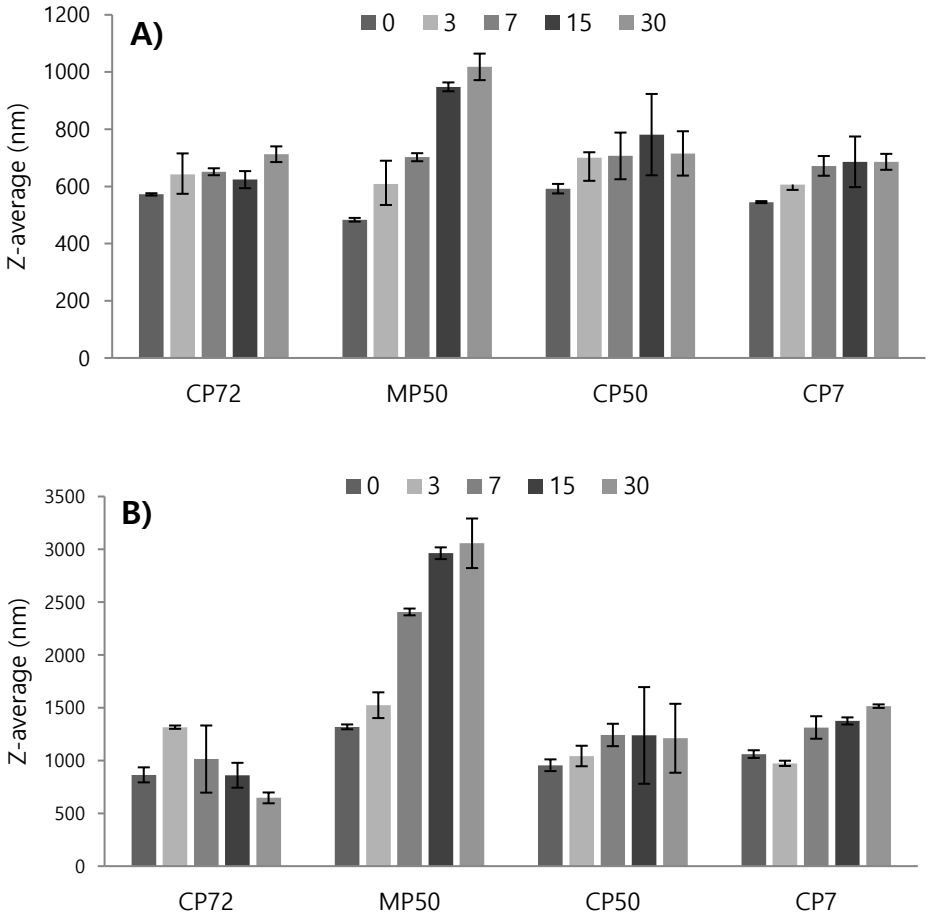


Figure 1. The average particle size of pectin based-curcumin emulsions containing A) 1% oil and B) 3% oil during 0 to 30 days of storage at room temperature.

3. 2. Surface electrical charge

Generally, pectin carries a negative charge because of carboxyl groups embedded on its molecules. Table. 4 and Fig. 2 shows that CP72 has less negative electrical charge in both oil concentrations because of the relatively high proportion of hydrophobic methyl-esterified regions (about -30.6 and -29.7mV, respectively). MP50, which is demethylesterified in blockwise manner and has Mw similar to CP50, has more negative electrical charge than CP50 with similar DM, and even bigger than CP7 (-49.1, -45.3, and 49.0 mV at 1% oil concentration; -52.3, -41.8, and -51.1 mV at 3% oil concentration, respectively). It means that more negative surface electrical charge was attributed to the ordered distribution of carboxylic groups. The initial surface electrical charge remained fairly constant for 30 days in all emulsions (Fig. 2).

Previous studies have shown that during hydrocolloid adsorption to the oil/water interface, the predominance of electrostatic repulsion over attractive van der Waals force promotes emulsion stabilization through repulsive interaction between particles. Nevertheless, in case of CP72 emulsions which exhibited less negative electrical charge than the emulsions stabilized by other pectins showed high stability during storage test. This result corresponded to the report by (Ngouémazong et al., 2015) which

suggested that the contribution of electrostatic repulsion to emulsion-stabilization effectiveness is equivocal in pectin.

Table 4. The surface electrical charge (ζ-potentials) of the initial pectin based-curcumin emulsion.

[unit; mV]

% Oil in emulsion	CP72	MP50	CP50	CP7
1%	-30.5 ± 7.4	-49.1 ± 3.2	-45.3 ± 0.2	-49.0 ± 2.0
3%	-29.6 ± 7.4	-52.3 ± 9.9	-41.8 ± 4.3	-51.1 ± 1.9

[1)] CP72 = commercial high methoxyl pectin (DM72); MP50 = PME-modified pectin (DM50); CP50 = commercial medium methoxyl pectin that contains reduced sucrose by alcohol precipitation (DM50); CP7 = commercial low methoxyl pectin (DM7).

[2)] Mean± Standard deviation; means of triplicates.

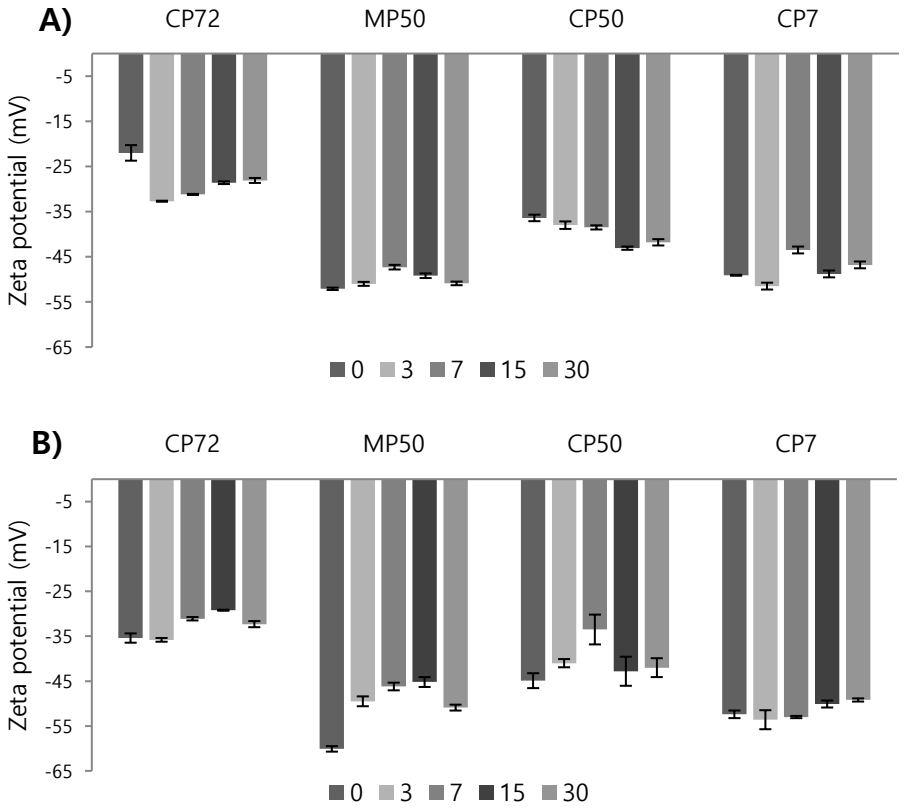


Figure 2. The electrical charge of pectin based-curcumin emulsions containing A) 1% oil and B) 3% oil during 0 to 30 days of storage at room temperature.

3. 3. Microstructure of pectin-based emulsions

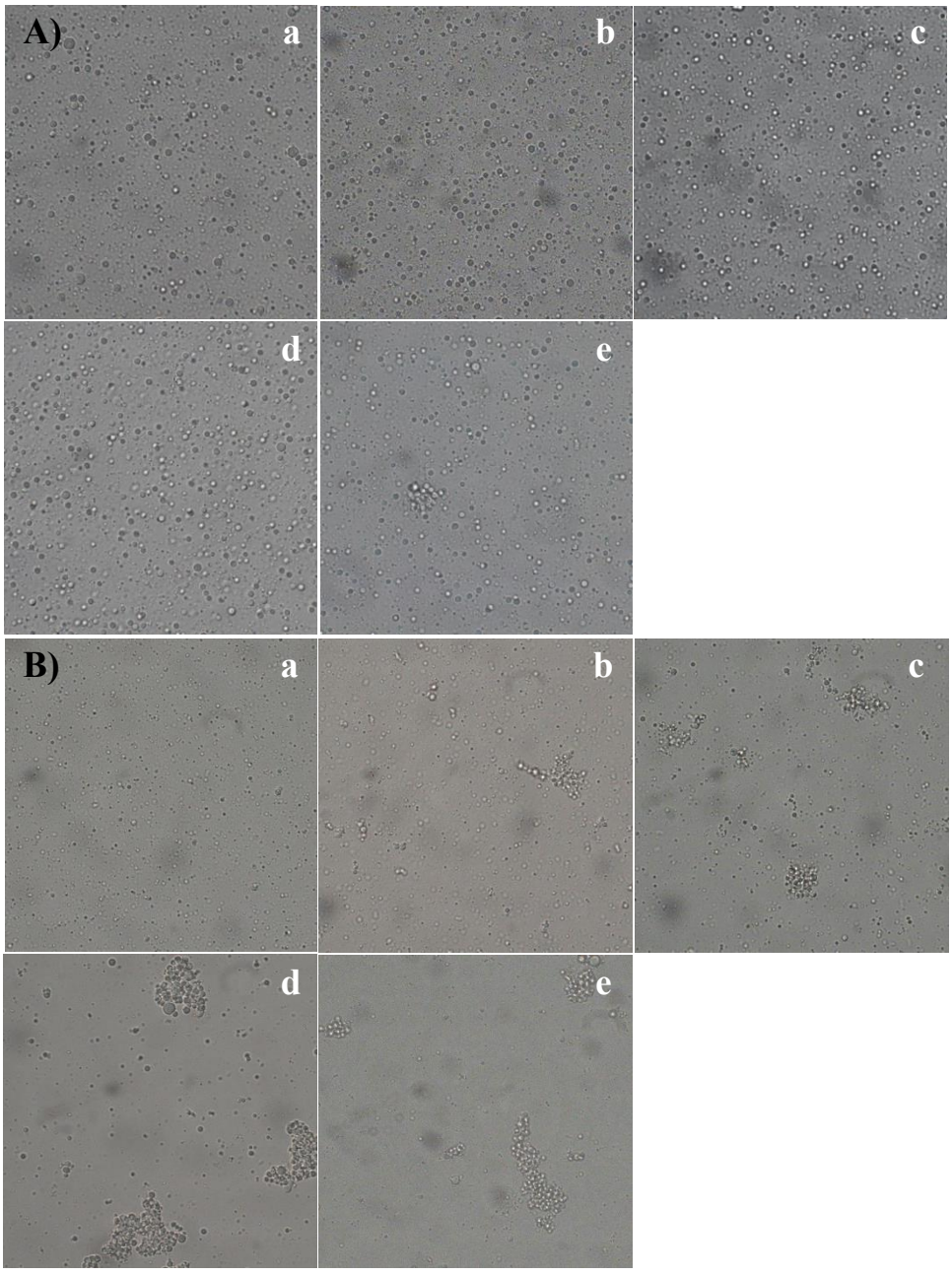
The changes in the microstructure of the emulsions were examined by light microscopy for 30 days (Fig. 3 and Fig. 4). Initially, small lipid droplets were evenly dispersed throughout the aqueous phase of all emulsions (Fig. 3-a and Fig. 4-a). A clear increase of oil droplet size with increasing oil concentration was observed corresponding to the results of particle size obtained by DLS (Fig. 1).

During the storage for 30 days, CP72 emulsion fairly maintained the initial oil droplets at both oil concentrations, but a slight flocculation was shown after 30 days. MP50 emulsion containing 1% oil showed bridging flocculation after 3 days, and the extent of flocculation steadily increased during the storage (Fig. 3-B). MP50 emulsion containing 3% oil also showed bridging flocculation after 3 days, and the particle size increased sharply because of coalescence. In addition, the increased size and irregular structure were maintained roughly for 30 days.

The droplet size of initial MP50 emulsions was small enough to be classified as nano-emulsions, but Fig. 4-B showed that the bridging flocculation and coalescence increased faster than that of other types of pectin emulsions, especially in those containing 3% oil concentration. Owing to the regular DMB, the time taken for the interface to be covered with MP50 might be

relatively short during emulsification. Moreover, the MP50 might be contained more than necessary for emulsification, thereby resulting the nonadsorbing pectin in the aqueous phase. By these effects, the large molecular weight of MP50 and the concentration of nonadsorbing pectin in the aqueous phase seemed to be enough to stimulate excessive polymer chain entanglements and interactions. It is known that the intermolecular interactions of pectin chains within the adsorbed pectin layer result in an increase of the layer thickness and, thus, an increase in the diameter (size) of emulsion droplets (Nakauma et al., 2008). The increased crosslinking of pectin chains (adsorbed and nonadsorbed) could result in bridging flocculation and increased instability of emulsions.

CP50 emulsions also showed a little bridging flocculation and coalescence after 7 days, and the extent was increased with the increasing oil concentration (from 1% to 3%). In contrast, CP7 emulsion showed poor storage stability forming much coalescence and bridging flocculation even after 3 days at both oil concentrations. As mentioned before, CP7 has too low methylesterified regions and Mw to be adsorbed onto the oil droplet completely. Therefore, it has limited emulsifying ability and stabilizing ability, causing significantly increased oil droplet and flocculation.


A)
a
b
c
d
e
B)
a
b
c
d
e

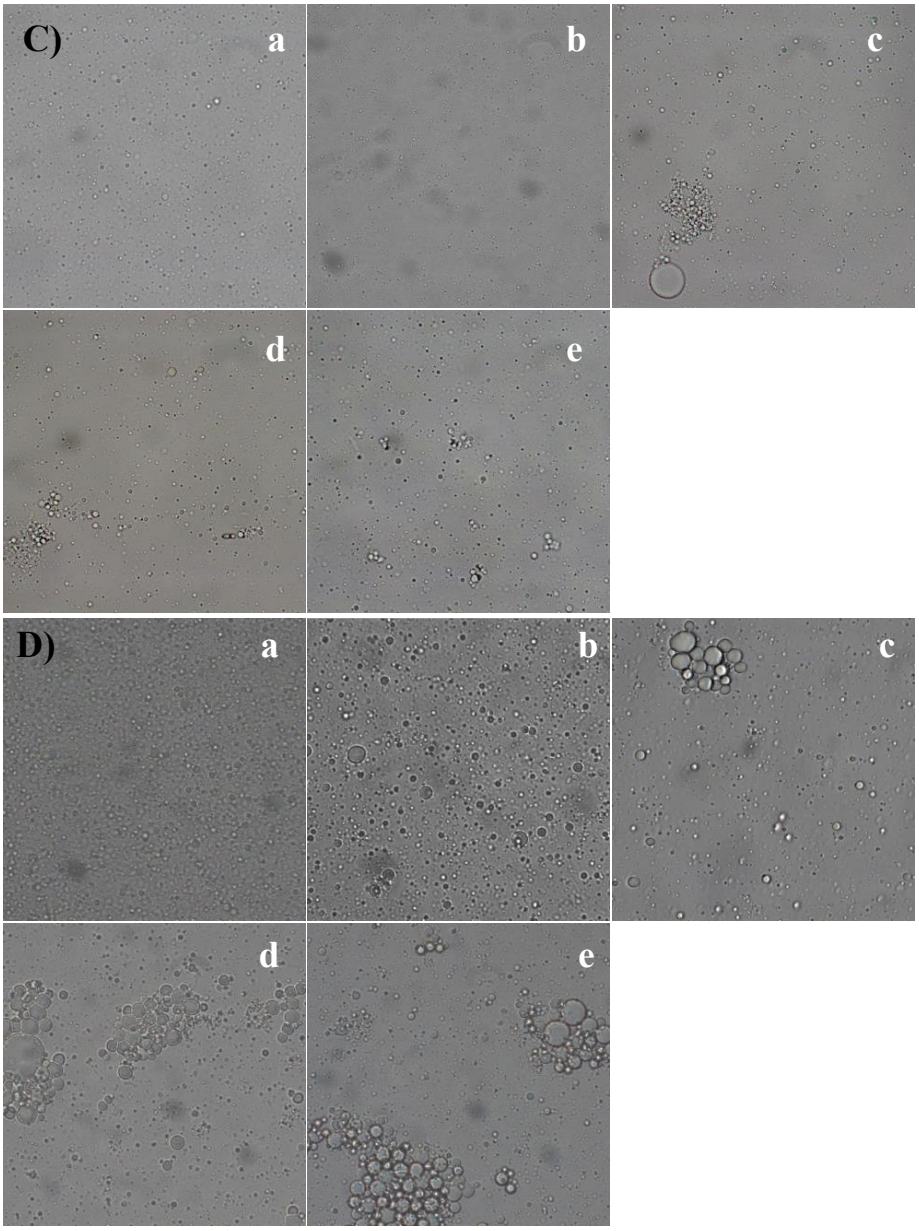
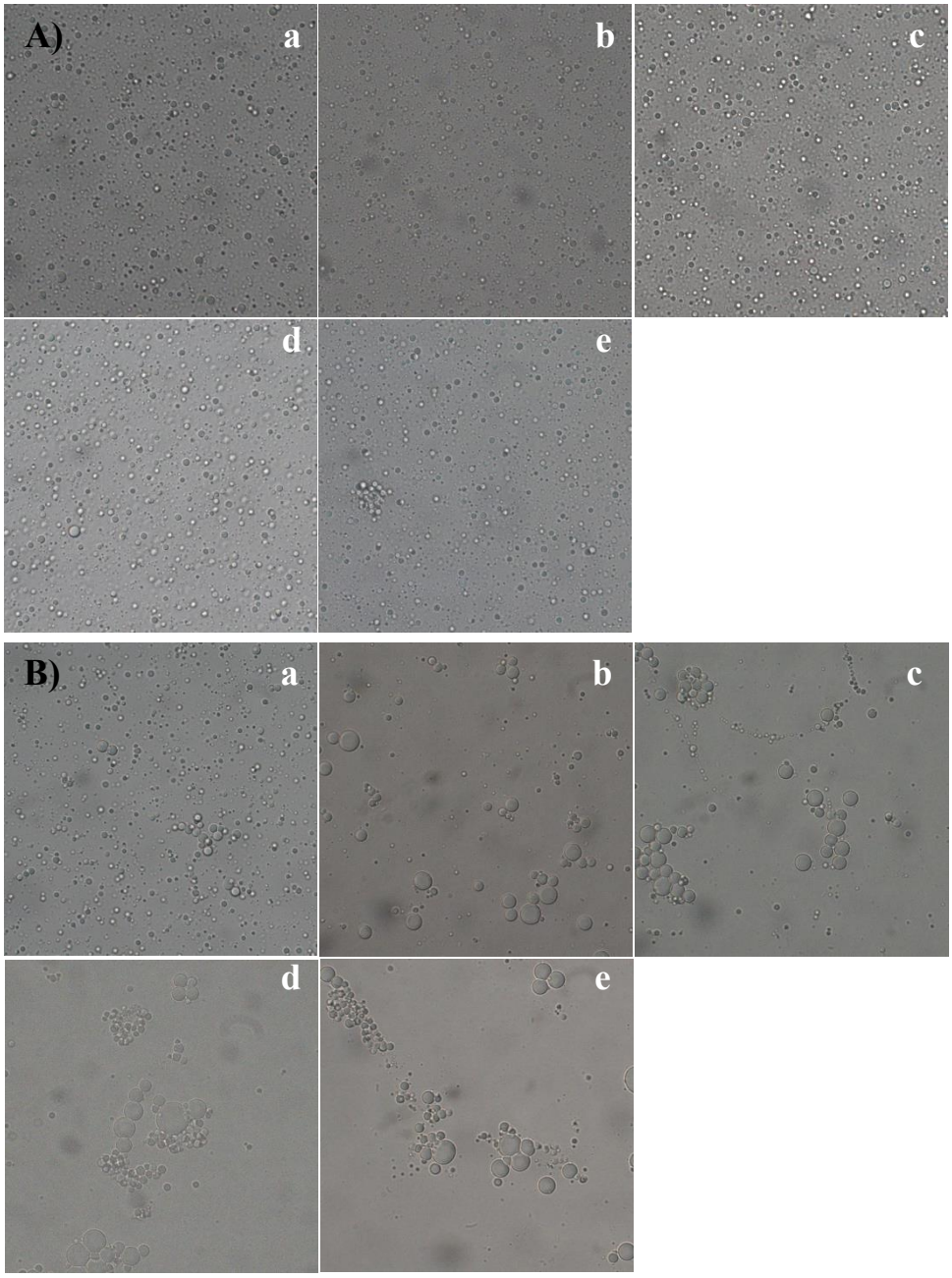


Figure 3. Light micrographs of oil droplet in A) CP72, B) MP50, C) CP50, and D) CP7 pectin-based emulsions containing 1% oil stored for a) 0, b) 3, c) 7, d) 15, and e) 30 days.


A)
a
b
c
d
e
B)
a
b
c
d
e

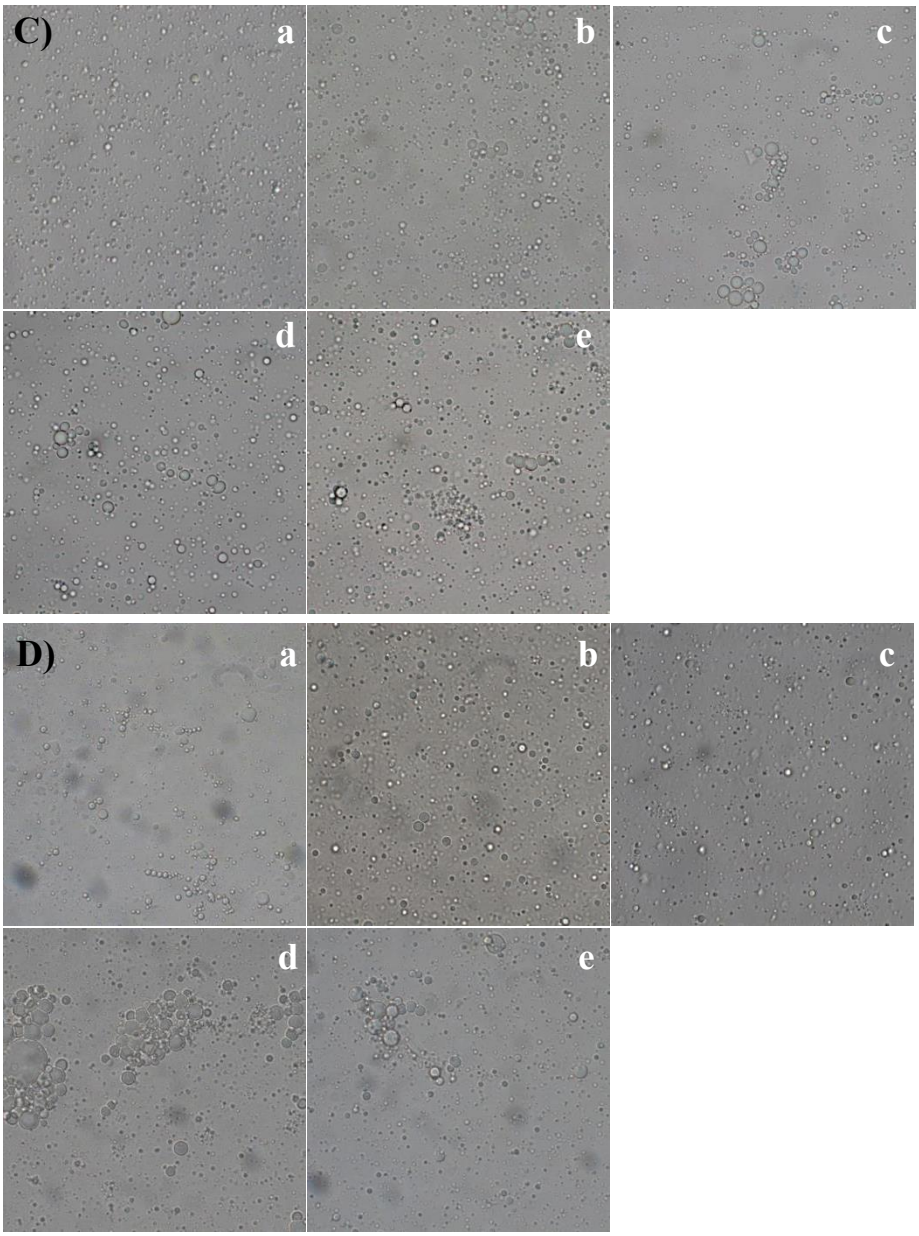


Figure 4. Light micrographs of oil droplet in A) CP72, B) MP50, C) CP50, and D) CP7 pectin-based emulsions containing 3% oil stored for a) 0, b) 3, c) 7, d) 15, and e) 30 days.

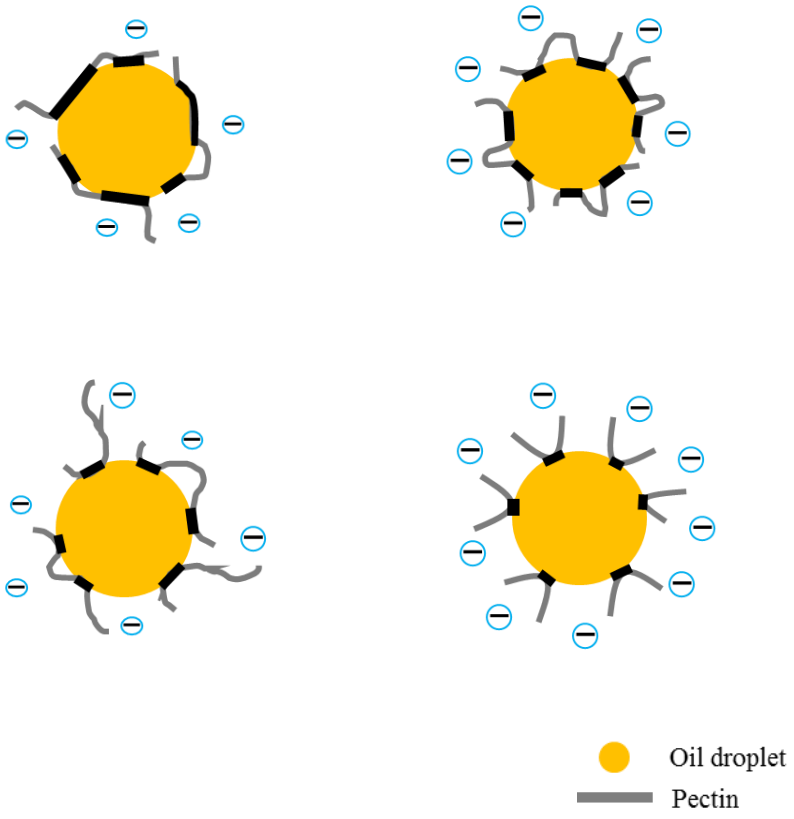


Figure 5. Illustration of the pectin adsorption during emulsification onto the oil/water interface in oil-in-water emulsions stabilized by A) CP72, B) MP50, C) CP50, and D) CP7 pectin.

4. Impact of pectin structure on behavior of the emulsions in simulated gastrointestinal tract (GIT)

During and after the digestion of the emulsions containing 1% and 3% oil, DM, Mw, and charge distribution of pectin were determined to investigate their influence on behavior of the emulsions in GIT.

The CP7 emulsion containing 3% oil was excluded from the *in vitro* digestion, since it had shown excessive aggregation after the oral phase. This result can be attributed to the fact that low methoxyl pectin (LMP) is sensitive to acidity and ions, and vulnerable to varying environment, causing gelation with divalent cation (Ca^{2+}) in the stomach juice and bridging flocculation with other various salts (Kim, Yoo, Kim, Park, & Yoo, 2008; Ngouemazong et al., 2012; Tina A.J. Verrijssen 2016; Verrijssen et al., 2015).

4.1. Microstructure of pectin-based emulsions

The changes in the microstructure of the emulsions during digesion were examined by light microscopy (Fig. 6 and 7).

At 1% oil concentration, the oil droplets within CP72 and CP50 emulsions retained the initial droplets even after the intestinal phase (Fig. 6).

On the other hand, slight changes were observed in MP50 emulsion when the initial emulsions went through the simulated oral phase, and the aggregation increased after the gastric phase (Fig. 6-B and C). In addition, extreme

aggregation which looks like gel-like structure was shown on the micrographs of the CP7 emulsion even after the oral phase (Fig. 6-B). This behavior was also observed by (Verrijssen et al., 2015) who found that both high and medium methyl-esterified pectins might bind to the hydrophobic oil droplets strongly because of its hydrophobic, methoxylated groups. Therefore, the CP72 and CP50 emulsions could preserve the initial droplet structure during *in vitro* digestion. Further, they reported that the aggregation, which was exhibited in MP50 and CP7 emulsions, might be caused by the addition of ions at the start of stomach digestion, causing the interaction between the demethylated negatively charged regions of pectin and ions like Ca^{+2}. In other words, the different behavior of MP50 and CP50 in oral and gastric phases was attributed to the fact that the MP50 is more sensitive to the ions owing to the introduced charge distribution blocks.

The aggregation of CP7 emulsion after the oral phase can be attributed to mucin, a complex mixture of biopolymers containing cationic, anionic, and hydrophobic groups. The bridging flocculation may have occurred between charged groups of pectin on the lipid droplet surfaces and mucin, a highly glycosylated protein, present in the artificial saliva fluid (Anwesha Sarkar, 2009; Dongowski, 1997; Duvetter et al., 2009; Salvia-Trujillo, Qian, Martin-Belloso, & McClements, 2013).

Large and irregular clusters of lipid droplets were also observed in MP50 emulsion after the oral phase, when it contained 3% oil. These gel-like pectin clusters embedding oil droplets were visualized by microscopy (Fig. 7-B). CP50 emulsion also showed a little bridging flocculation after the gastric phase. In contrast, CP72 emulsion presumably retained its original structural properties even at in the 1% oil concentration. Although CP50 and MP50 properly acted as emulsifiers at both oil concentrations, when the oil content increased from 1% to 3%, these pectin emulsions had less stable emulsion environment while *in vitro* digestion resulted in the aggregation and flocculation.

After the small intestinal phase, some reserved oil droplets were visualized by microscopy (Fig. 6-D and 7-D). Besides, the emulsions containing 3% oil seemed to have more remaining oil droplets. It corresponded to the result of lipid digestion which revealed that a decrease in the final amount of FFA occurred as the total oil concentration was increased (Fig. 9).

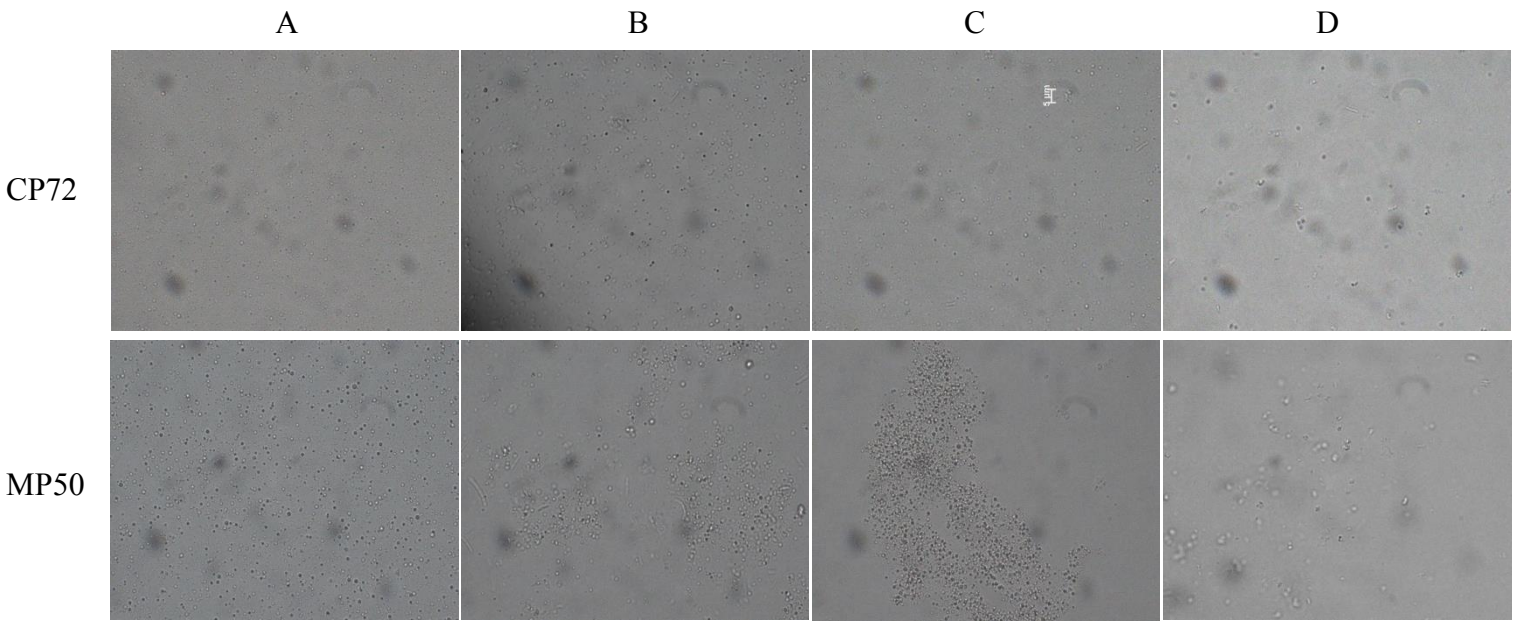


Figure 6. Continued

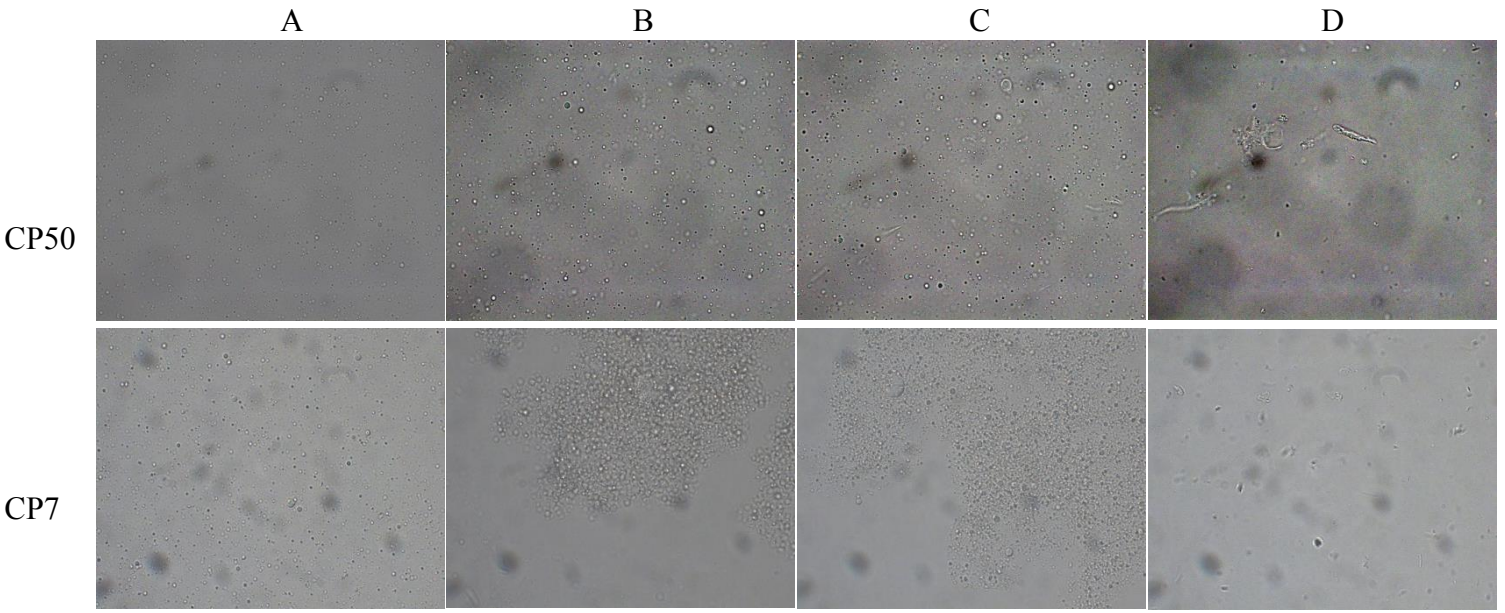


Figure 6. Light micrographs of oil droplet in the 1% oil emulsion A) initial, and after B) oral phase, C) gastric phase, and D) intestinal phase.

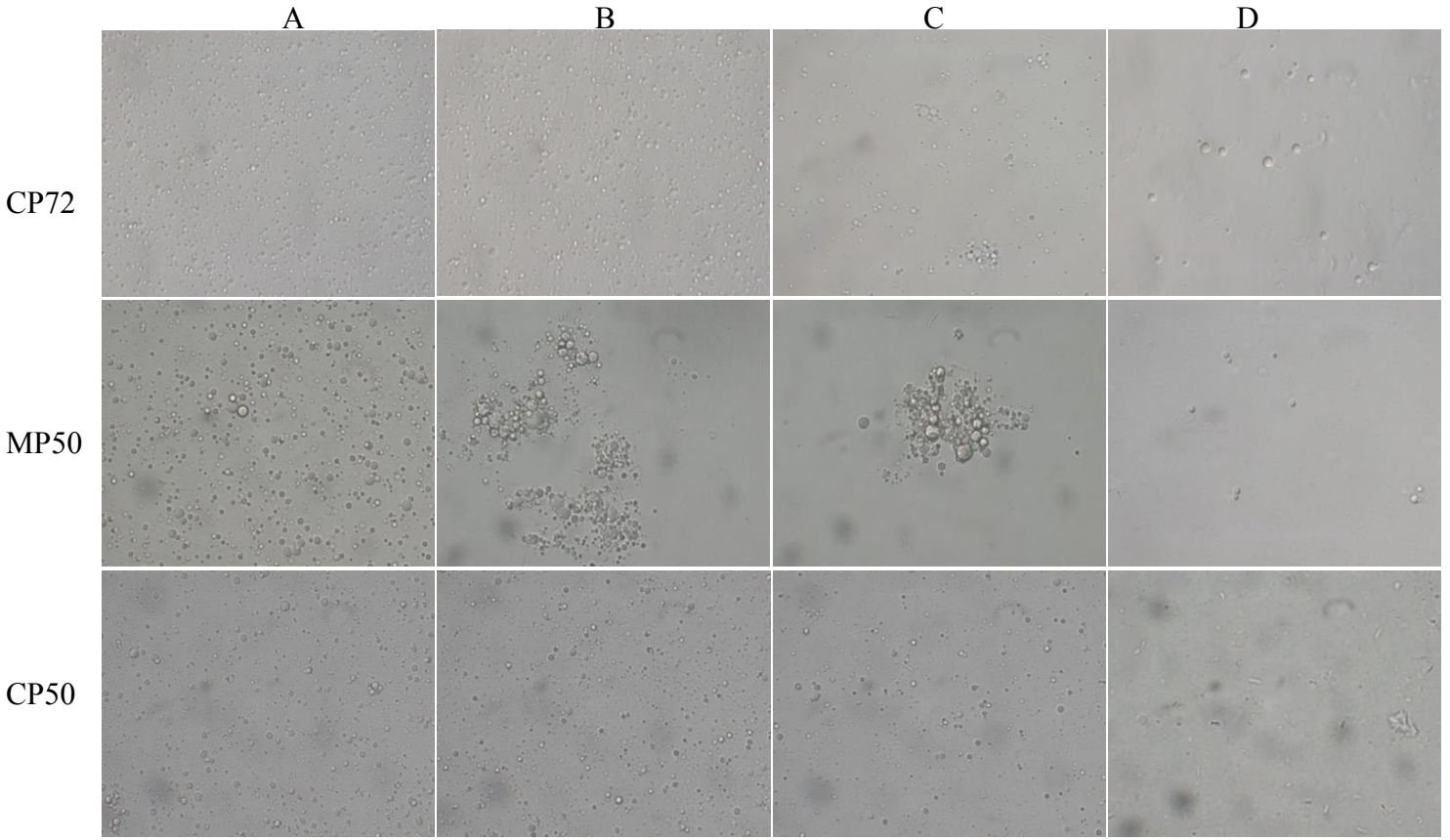


Figure 7. Light micrographs of oil droplet in the 3% oil emulsion A) initial, and after B) oral phase, C) gastric phase, and D) intestinal phase.

4. 4. Lipid digestion

The influence of pectin type and oil concentration on the rate and extent of lipid digestion was quantified by using an *in vitro* lipid digestion model. A series of oil-in-water pectin-based emulsions containing 15 mg/g wt.% curcumin in the lipid phase was produced with different amount of oil. The kinetics of lipid digestion was monitored by measuring the amount of free fatty acids released over 2 hr while the emulsions reacted with the simulated small intestinal fluid. The amount of FFA released from curcumin-loaded emulsions containing no oil was also examined as a control, and this value was subtracted from those of the pectin-based emulsions.

The full digestion profiles are shown in Fig. 9. In general, there was a steep increase in the amount of FFA released during the first few minutes of digestion, followed by a more gradual increase. However, the shape of the full digestion profiles depended on the type of pectin and oil concentration. The final extent of lipid digestion decreased in the following order CP50≈MP50>CP72≫CP7 emulsions when they contained 1% oil, and CP50≈MP50 >CP72 emulsions when they contained 3% oil.

Although CP72 and CP50 emulsions showed similarly retained oil droplets after the gastric phase (Fig. 7), CP72 showed a lower degree of lipid

digestion than CP50 emulsion at both oil concentrations. This observation might be explained by the high DM and Mw of CP72, which means that the pectin molecules cause higher steric hindrance and protect the oil droplet by adsorbing tightly. Therefore, the enzyme could not access the oil droplet effectively, as much as in medium-methoxyl pectin-based emulsions. CP7 emulsion showed the lowest amount of released FFA in 1% oil containing emulsions, which may be contributed by extreme aggregation after the exposure to oral phase. The cluster might have reduced the surface area of lipid droplets exposed to the lipase.

Interestingly, in case of MP50 emulsion, a much higher extent of lipid digestion was shown than CP7 emulsion when they contained 1% oil, even if both emulsions revealed aggregation and bridging flocculation during the digestion. This result indicated that, in respect of the mechanism, the gel-like structure of the two emulsions was quite different. The clusters within MP50 emulsion were formed by weak interaction which were dissociated by dilution and attacked by lipase. The aggregation might be attributed to the polymer chain entanglements and interactions caused by the nonadsorbing high molecular pectin in the aqueous phase. Moreover, the gel-like structure within MP50 emulsion seems to have geometrically empty room, facilitating the dilution and an attack by lipase (Fig. 8). In contrast, CP7 having low

methoxyl groups and Mw is believed to be insufficient to act as an efficient emulsifier during the digestion, and form gel by the 'egg box' mechanism (Grant, Morris, Rees, Smith, & Thom, 1973).

In addition, there was a decrease in the final amount of FFA produced as the total oil concentration increased from 1 to 3 wt.%. (Ahmed, Li, McClements, & Xiao, 2012) suggesting that this effect can be attributed to a few mechanisms. The proposed mechanisms are (i) the amount of lipase per unit surface area of oil droplets decreased as the amount of lipid increased, (ii) the amount of bile salts might be deficient to solubilize all of the lipid digestion products, free fatty acid (FFA) and monoacylglycerol (MAG), and (iii) the amount of calcium might be deficient to precipitate all the FFAs produced by digestion and remove them from the droplet surfaces if the lipid was present at a high concentration.

The lipolysis of oil droplets within 1% oil emulsions was almost completed during the initial 5 min showing sharply released FFAs, while the amount of released FFAs increased steadily at 3% oil concentration. It implies that the rate of lipolysis of 3% oil emulsions was slower than that of 1% emulsions. Besides, CP50 emulsions released FFAs faster than MP50 emulsions did, even if the final extent of lipolysis was similar. This result suggested that the cluster of oil droplets within MP50 emulsions was steadily disassembled

from the surface to the inner part, owing to the dilution effect and the attack from lipase in the small intestinal phase.

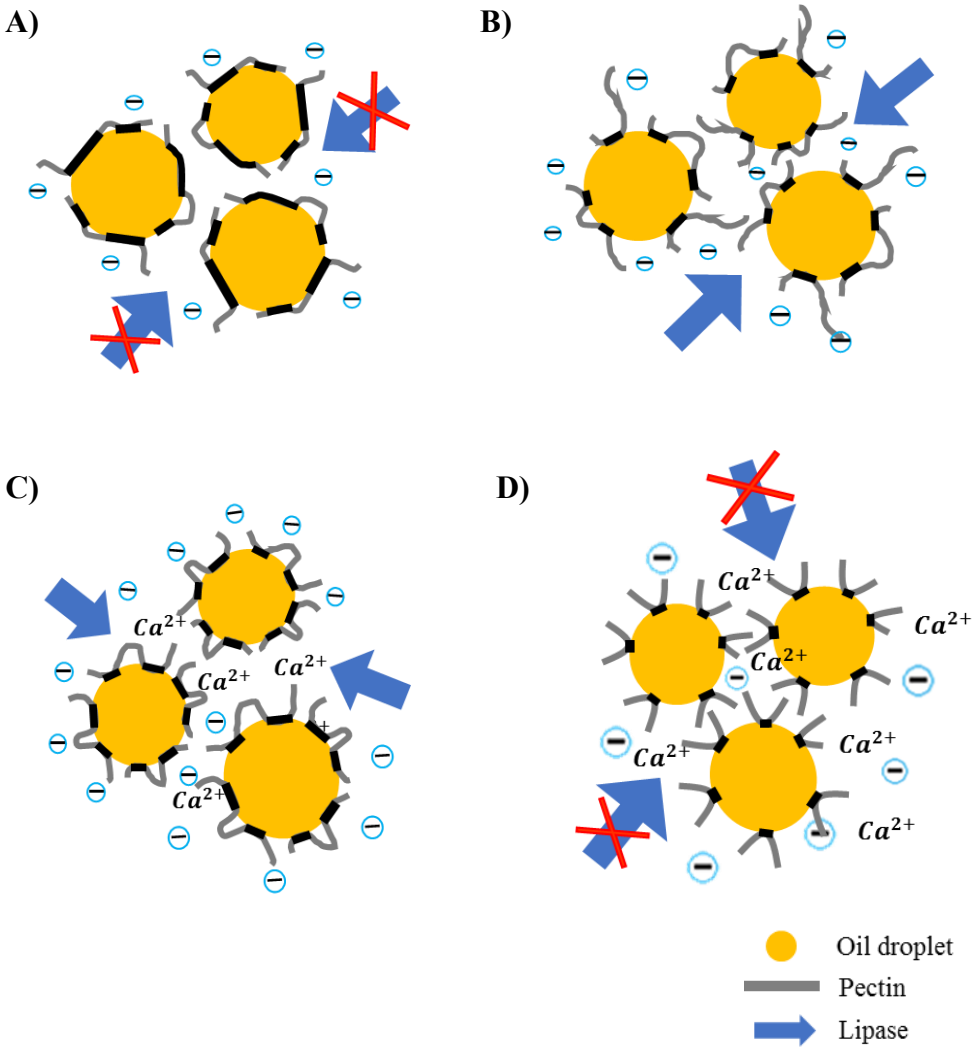


Figure 8. Illustration of the protective action against lipase in the small intestinal phase of oil-in-water emulsions stabilized by A) CP72, B) MP50, C) CP50, and D) CP7 pectin

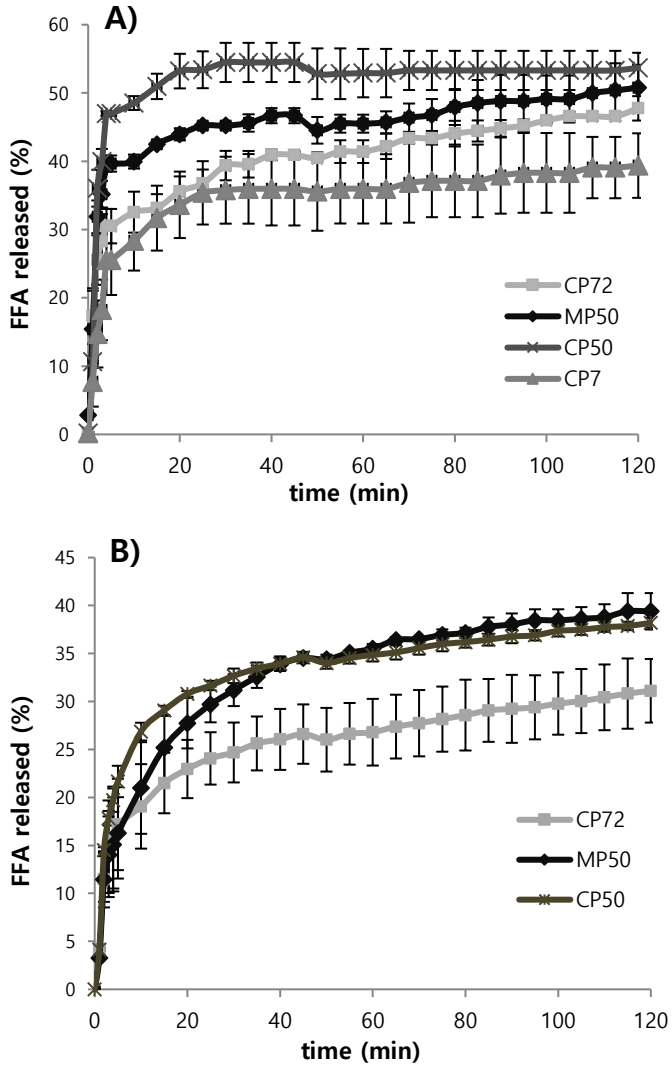


Figure 9. Amount of fatty acids released from pectin based-emulsions with different lipid concentrations as measured by using a pH-stat method: A) 1% oil and B) 3% oil.

4. 5. *In vitro* curcumin bioaccessibility

It has been explained that the fraction of the curcumin existing in the micelle phase after digestion is a measure of curcumin bioaccessibility (Carbonell-Capella, Buniowska, Barba, Esteve, & Frígola, 2014). The transparent micelle phase was collected after ultracentrifugation of the digested emulsion, and the curcumin incorporated into mixed micelles was extracted via chloroform. If less micelle is formed, it is expected that the incorporation of curcumin into micelles, which means curcumin bioaccessibility, is lower for those samples.

Previous studies suggested that the type and DM of pectin might influence the lipid incorporation, determining which components can be incorporated into the micelles (Braulio Cervantes-Paz et al., 2016; B. Cervantes-Paz et al., 2017; Dongowski, 1997; Verrijssen et al., 2014; Verrijssen et al., 2015).

At the 1% oil concentration, the curcumin bioaccessibility was markedly high in the CP7 emulsion (77.8%). This result was attributed to the insignificant interaction of CP7 and bile salt owing to the gel-like cluster, which can reduce the amount of free pectins. Moreover, pectin is known to bind bile salts through hydrophobic interactions, and binding of low DM pectin with bile acids is difficult because of the eletrostatic repulsions (B. Cervantes-Paz et al., 2017; Espinal-Ruiz, Parada-Alfonso, Restrepo-Sanchez,

Narvaez-Cuenca, & McClements, 2014; Falk & Nagyvary, 1982; Jeffrey D. Falk, 1982). This implies that the intensity of the interaction between CP7 and bile salts would not be enough strong. For these reasons, a large proportion of released and solubilized curcumin could be incorporated into the micelles without interruption from pectin.

CP50 emulsions revealed low curcumin bioaccessibility at both oil concentrations, resulting in bioaccessibility of 70.2 and 66.0% at 1% and 3% oil concentration, respectively. Meanwhile, the bioaccessibility of CP72 and MP50 emulsions was not significantly ($p > 0.05$) different (77.0 and 75.3% at 1% oil, and 78.2 and 79.0% at 3% oil, respectively). This result was not in accordance with the report by (B. Cervantes-Paz et al., 2017)) which suggested that the low DM pectin can favor carotenoid micellarization and absorption. Therefore, the charge distribution of pectin as well as DM and Mw, should be considered for determining how strongly the pectin can interact with bile salts and how much the curcumin can be incorporated into the micelles.

There are two possible reasons for the high bioaccessibility of CP72 and MP50 emulsions. First is the different portions of hydrophobic and hydrophilic region in pectin structure. The micellarization of lipophilic neutraceuticals is favored by the electrostatic repulsion between pectin

chains and bile salts in the micelles, and hydrophobic interaction between pectin and the active surface of micelles (Braulio Cervantes-Paz et al., 2016). Therefore, CP72, which has more hydrophobic properties owing to the methylated carboxyl groups, could contribute to the increased curcumin bioaccessibility. In addition, more hydrophilic properties caused by the demethylesterified block could contribut to the enhanced curcumin bioaccessibility of MP50 emulsions.

Second, CP50 emulsions showed the fastest lipid digestion profiles at the first few minutes (Fig. 9). The faster digestion rate of the emulsion could increase the amount of time the curcumin spent in close contact with the aqueous phase rather than in the hydrophobic core of the lipid droplets (Zou, Liu, Liu, Xiao, & McClements, 2015). By this reason, the probability of degradation of curcumin might have increased, causing curcumin unstability and low bioaccessibility.

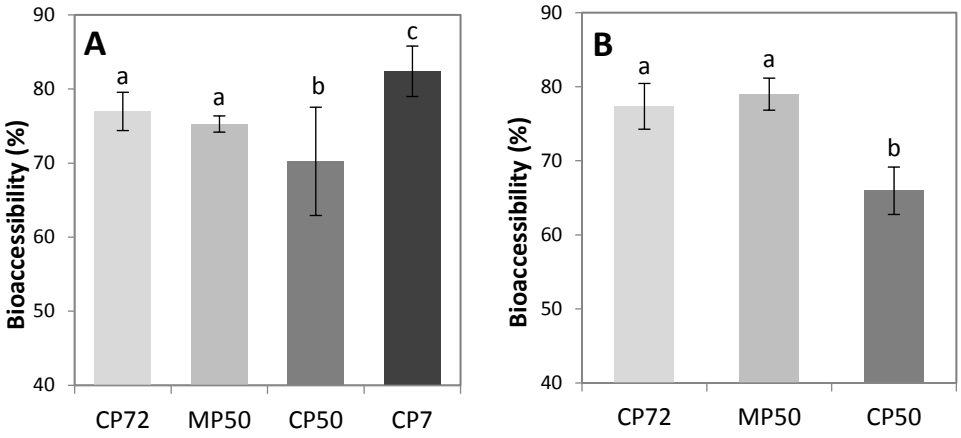


Figure 10. Percentage *in vitro* curcumin bioaccessibility A) 1% oil emulsions and B) 3% oil emulsions (mean ± standard deviation). Significant differences (Tukey test, $p < 0.05$) are indicated with different letters.

CONCLUSION

In this work, the potential of pectin as a mono-emulsifier and as an effective encapsulation material of lipophilic neutraceuticals was discussed with highlights on the effect of molecular structure and charge distribution of pectin.

Emulsion stabilized by MP50 which has demethylestrified blocks manifested a higher degree of final lipid digestion than CP72 emulsion did, and better *in vitro* bioaccessibility of curcumin than CP50 emulsion did. The tightly adsorbing behavior of CP72 onto the oil droplet was unfavorable to *in vitro* digestion, while it could be advantageous to emulsion stabilizing ability. Even if CP50 has the same DM and Mw, it showed low curcumin bioaccessibility which was attributed to rapid lipid digestion. In other words, modified pectin with blockwise negative charge distribution, which might be adsorbed to the oil droplet with regular arrangement, revealed advantages in *in vitro* lipid digestion and bioaccessibility.

The slow rate of lipid digestion, a high degree of final lipid digestion, and high bioaccessibility of MP50 emulsion might be useful for the small intestinal-target delivery system for lipophilic neutraceuticals.

In addition to its current applications, the functional properties of pectin

might be extended to plant-based food, cosmetics, and pharmaceutics by using pectin methylesterase which causes different pattern of demethlyesterification.

REFERENCES

Aditya, N., Aditya, S., Yang, H.-J., Kim, H. W., Park, S. O., Lee, J., & Ko, S. (2015). Curcumin and catechin co-loaded water-in-oil-in-water emulsion and its beverage application. *Journal of Functional Foods, 15*, 35-43.

Ahmed, K., Li, Y., McClements, D. J., & Xiao, H. (2012). Nanoemulsion- and emulsion-based delivery systems for curcumin: Encapsulation and release properties. *Food Chemistry, 132*(2), 799-807. doi:10.1016/j.foodchem.2011.11.039

Anand, P., Kunnumakkara, A. B., Newman, R. A., & Aggarwal, B. B. (2007). Bioavailability of curcumin: problems and promises. *Molecular Pharmaceutics, 4*(6), 807-818.

Anwesha Sarkar, K. K. T. G., Harjinder Singh (2009). Colloidal stability and interactions of milk-protein-stabilized emulsions in an artificial saliva. *Food Hydrocolloids, 23*(5), 1270-1278.

Bergonzi, M., Hamdouch, R., Mazzacuva, F., Isacchi, B., & Bilia, A. (2014). Optimization, characterization and in vitro evaluation of curcumin microemulsions. *LWT-Food Science and Technology, 59*(1), 148-155.

Burapapadh, K., Kumpugdee-Vollrath, M., Chantasart, D., & Sriamornsak, P. (2010). Fabrication of pectin-based nanoemulsions loaded with itraconazole for pharmaceutical application. *Carbohydrate Polymers, 82*(2), 384-393. doi:10.1016/j.carbpol.2010.04.071

Carbonell-Capella, J. M., Buniowska, M., Barba, F. J., Esteve, M. J., & Frígola, A. (2014). Analytical methods for determining bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables: A review. *Comprehensive Reviews in Food Science and Food Safety, 13*(2), 155-171.

Cervantes-Paz, B., Ornelas-Paz, J. d. J., Pérez-Martínez, J. D., Reyes-Hernández, J., Zamudio-Flores, P. B., Rios-Velasco, C., . . . Ruiz-Cruz, S. (2016). Effect of pectin concentration and properties on digestive events involved on micellarization of free and esterified carotenoids. *Food Hydrocolloids, 60*, 580-588. doi:10.1016/j.foodhyd.2016.04.038

Cervantes-Paz, B., Ornelas-Paz, J. J., Ruiz-Cruz, S., Rios-Velasco, C., Ibarra-Junquera, V., Yahia, E. M., & Gardea-Bejar, A. A. (2017). Effects of pectin on lipid digestion and possible implications for carotenoid bioavailability during pre-absorptive stages: A review. *Food Research Intternationl, 99*(Pt 2), 917-927. doi:10.1016/j.foodres.2017.02.012

Chen, L., Bai, G., Yang, S., Yang, R., Zhao, G., Xu, C., & Leung, W. (2014). Encapsulation of curcumin in recombinant human H-chain ferritin increases its water-solubility and stability. *Food Research International, 62*, 1147-1153.

D.N. Sila, S. V. B., T. Duvetter, I. Fraeye, A. De Roeck, A. Van Loey, M. Hendrickx. (2009). Pectins in Processed Fruits and Vegetables: Part II—Structure–Function Relationships. *Comprehensive Reviews in Food Science and Food Safety, 8,* 86-104.

Dartey, C. K., Trainor, T. M., & Evans, R. (1990). Low cholesterol mayonnaise substitute and process for its preparation. *U.S. Patent No. 4,948,617*. Washington, DC: U.S. Patent and Trademark Office.

Dongowski, G. (1997). Effect of pH on the in vitro interactions between bile acids and pectin. *European Food Research and Technology, 205*(3), 185-192.

Duvetter, T., Sila, D. N., Van Buggenhout, S., Jolie, R., Van Loey, A., & Hendrickx, M. (2009). Pectins in Processed Fruit and Vegetables: Part I - Stability and Catalytic Activity of Pectinases. *Comprehensive Reviews in Food Science and Food Safety, 8*(2), 75-85.

Espinal-Ruiz, M., Parada-Alfonso, F., Restrepo-Sanchez, L. P., Narvaez-Cuenca, C. E., & McClements, D. J. (2014). Interaction of a dietary fiber (pectin) with gastrointestinal components (bile salts, calcium, and lipase): a calorimetry, electrophoresis, and turbidity study. *Journal of Agricultural and Food Chemistry, 62*(52), 12620-12630. doi:10.1021/jf504829h

Falk, J. D., & Nagyvary, J. J. (1982). Exploratory studies of lipid-pectin interactions. *The Journal of Nutrition, 112*(1), 182-188.

Fernández-Ginés, J. M., Fernández-López, J., Sayas-Barberá, E., & Pérez-Alvarez, J. (2005). Meat products as functional foods: A review. *Journal of Food Science, 70* (2), 37-43.

Fu, S., Augustin, M. A., Shen, Z., Ng, K., Sanguansri, L., & Ajlouni, S. (2015). Bioaccessibility of curcuminoids in buttermilk in simulated gastrointestinal digestion models. *Food Chemistry, 179*, 52-59.

Grant, G. T., Morris, E. R., Rees, D. A., Smith, P. J., & Thom, D. (1973). Biological interactions between polysaccharides and divalent cations: the egg-box model. *FEBS letters, 32*(1), 195-198.

Guri, A., Gülseren, I., & Corredig, M. (2013). Utilization of solid lipid nanoparticles for enhanced delivery of curcumin in cocultures of HT29-MTX and Caco-2 cells. *Food & Function, 4*(9), 1410-1419.

Hills, C. H., Mottern, H., Nutting, G., & Speiser, R. (1949). Enzyme-

demethylated pectinates and their gelation. *Food Technology, 3*(3), 90-94.

Hunter, J. L., & Wicker, L. (2005). De-esterification of pectin by alkali, plant and fungal pectinmethylesterases and effect on molecular weight. *Journal of the Science of Food and Agriculture, 85*(13), 2243-2248. doi:10.1002/jsfa.2221

James, A. E., & Driskell, J. D. (2013). Monitoring gold nanoparticle conjugation and analysis of biomolecular binding with nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS). *Analyst, 138*(4), 1212-1218.

Jeffrey D. Falk, J. D. F. (1982). Exploratory studies of lipid-pectin interactions. *The Journal of Nutrition, 112*(1), 182-188.

Jung, J., Arnold, R. D., & Wicker, L. (2013). Pectin and charge modified pectin hydrogel beads as a colon-targeted drug delivery carrier. *Colloids Surf B Biointerfaces, 104*, 116-121. doi:10.1016/j.colsurfb.2012.11.042

Jung, J., & Wicker, L. (2012). Laccase mediated conjugation of sugar beet pectin and the effect on emulsion stability. *Food Hydrocolloids, 28*(1), 168-173.

Kim, Y., Yoo, Y. H., Kim, K. O., Park, J. B., & Yoo, S. H. (2008). Textural properties of gelling system of low-methoxy pectins produced by demethoxylating reaction of pectin methyl esterase. *Journal of Food Science, 73*(5), C367-372. doi:10.1111/j.1750-3841.2008.00771.x

Lobato-Calleros, C., Sosa-Pérez, A., Rodríguez-Tafoya, J., Sandoval-Castilla, O., Pérez-Alonso, C., & Vernon-Carter, E. (2008). Structural and textural characteristics of reduced-fat cheese-like products made from W1/O/W2 emulsions and skim milk. *LWT-Food Science and Technology, 41*(10), 1847-1856.

Loo, C.-Y., Rohanizadeh, R., Young, P. M., Traini, D., Cavaliere, R., Whitchurch, C. B., & Lee, W.-H. (2015). Combination of silver nanoparticles and curcumin nanoparticles for enhanced anti-biofilm activities. *Journal of Agricultural and Food Chemistry, 64*(12), 2513-2522.

Lutz, R., Aserin, A., Wicker, L., & Garti, N. (2009). Structure and physical properties of pectins with block-wise distribution of carboxylic acid groups. *Food Hydrocolloids, 23*(3), 786-794.

Makoto Nakauma, T. F., Sakie Noda, Sayaka Ishihara, Saphwan Al-Assaf, Katsuyoshi Nishinari , Glyn O. Phillips (2008). Comparison of sugar beet pectin, soybean soluble polysaccharide, and gum arabic as food

emulsifiers. 1. Effect of concentration, pH, and salts on the emulsifying properties. *Food Hydrocolloids, 22*(7), 1254-1267.
May, C. D. (1990). Industrial pectins: sources, production and applications. *Carbohydrate Polymers, 12*(1), 79-99.
McClements, D., Decker, E., & Weiss, J. (2007). Emulsion-based delivery systems for lipophilic bioactive components. *Journal of Food Science, 72*(8).
Nakauma, M., Funami, T., Noda, S., Ishihara, S., Al-Assaf, S., Nishinari, K., & Phillips, G. O. (2008). Comparison of sugar beet pectin, soybean soluble polysaccharide, and gum arabic as food emulsifiers. 1. Effect of concentration, pH, and salts on the emulsifying properties. *Food Hydrocolloids, 22*(7), 1254-1267.
Ngouemazong, D. E., Jolie, R. P., Cardinaels, R., Fraeye, I., Van Loey, A., Moldenaers, P., & Hendrickx, M. (2012). Stiffness of Ca(2+)-pectin gels: combined effects of degree and pattern of methylesterification for various Ca(2+) concentrations. *Carbohydrate Research, 348*, 69-76. doi:10.1016/j.carres.2011.11.011
Ngouémazong, E. D., Christiaens, S., Shpigelman, A., Van Loey, A., & Hendrickx, M. (2015). The Emulsifying and Emulsion-Stabilizing Properties of Pectin: A Review. *Comprehensive Reviews in Food Science and Food Safety, 14*(6), 705-718. doi:10.1111/1541-4337.12160
Sahdeo Prasad, S. C. G., Amit K. Tyagi, Bharat B. Aggarwal. (2014). Curcumin, a component of golden spice: From bedside to bench and back. *Biotechnology Advances, 32*(6), 1053-1064.
Salvia-Trujillo, L., Qian, C., Martin-Belloso, O., & McClements, D. J. (2013). Influence of particle size on lipid digestion and beta-carotene bioaccessibility in emulsions and nanoemulsions. *Food Chemistry, 141*(2), 1472-1480. doi:10.1016/j.foodchem.2013.03.050
Sari, T. P., Mann, B., Kumar, R., Singh, R. R. B., Sharma, R., Bhardwaj, M., & Athira, S. (2015). Preparation and characterization of nanoemulsion encapsulating curcumin. *Food Hydrocolloids, 43*, 540-546. doi:10.1016/j.foodhyd.2014.07.011
Savary, B., Hotchkiss, A., Fishman, M., Cameron, R., & Shatters, R. (2003). Development of a Valencia orange pectin methylesterase for generating novel pectin products. In *Advances in pectin and pectinase research* (pp. 345-361). Springer, Dordrecht.
Schmidt, U. S., Schmidt, K., Kurz, T., Endreß, H. U., & Schuchmann, H. P. (2015). Pectins of different origin and their performance in forming

and stabilizing oil-in-water-emulsions. *Food Hydrocolloids, 46*, 59-66. doi:10.1016/j.foodhyd.2014.12.012

Schols, H. A., Vierhuis, E., Bakx, E. J., & Voragen, A. G. (1995). Different populations of pectic hairy regions occur in apple cell walls. *Carbohydrate Research, 275*(2), 343-360.

Shen, L., Liu, C.-C., An, C.-Y., & Ji, H.-F. (2016). How does curcumin work with poor bioavailability? Clues from experimental and theoretical studies. *Scientific Reports, 6*, 20872.

Thakur, B. R., Singh, R. K., & Handa, A. K. (1997). Chemistry and uses of pectin--a review. *Critical Reviews in Food Science & Nutrition, 37*(1), 47-73. doi:10.1080/10408399709527767

Thibault, J.-F., Renard, C. M., Axelos, M. A., Roger, P., & Crépeau, M.-J. (1993). Studies of the length of homogalacturonic regions in pectins by acid hydrolysis. *Carbohydrate Research, 238*, 271-286.

Tina A.J. Verrijssen , S. C., Sarah H.E. Verkempinck, Jeroen Boeve, Tara Grauwet, Ann M. Van Loey, Laura Salvia-Trujillo, Marc E. Hendrickx. (2016). In vitro β-Carotene Bioaccessibility and Lipid Digestion in Emulsions: Influence of Pectin Type and Degree of Methyl-Esterification *Journal of Food Science, 81*(10), C2327-C2336.

Van Buggenhout, S., Sila, D. N., Duvetter, T., Van Loey, A., & Hendrickx, M. (2009). Pectins in Processed Fruits and Vegetables: Part III - Texture Engineering. *Comprehensive Reviews in Food Science and Food Safety, 8*(2), 105-117.

Verrijssen, T. A. J., Balduyck, L. G., Christiaens, S., Van Loey, A. M., Van Buggenhout, S., & Hendrickx, M. E. (2014). The effect of pectin concentration and degree of methyl-esterification on the in vitro bioaccessibility of β-carotene-enriched emulsions. *Food Research International, 57*, 71-78. doi:10.1016/j.foodres.2014.01.031

Verrijssen, T. A. J., Verkempinck, S. H. E., Christiaens, S., Van Loey, A. M., & Hendrickx, M. E. (2015). The effect of pectin on in vitro β-carotene bioaccessibility and lipid digestion in low fat emulsions. *Food Hydrocolloids, 49*, 73-81. doi:10.1016/j.foodhyd.2015.02.040

Zou, L., Liu, W., Liu, C., Xiao, H., & McClements, D. J. (2015). Designing excipient emulsions to increase nutraceutical bioavailability: emulsifier type influences curcumin stability and bioaccessibility by altering gastrointestinal fate. *Food Function, 6*(8), 2475-2486. doi:10.1039/c5fo00606f

국문초록

펙틴은 젤화제, 증점제, 유화제 등으로 이용되는 다기능성 고분자물질로 메틸화 수준(DM)과 분자량에 따라 기능적 특성이 달라진다. Pectin methylesterase (PME) 효소 처리를 통해 탈 메틸화한 펙틴은 분자량이 유지되며, 규칙적으로 탈 메틸화되어 블록을 가지게 된다. 최근 펙틴의 DM과 분자량뿐만 아니라, 탈 메틸화 영역의 규칙성이 야기하는 블록 또한 펙틴의 기능적 성질에 영향을 미친다는 것이 알려졌다. 이 연구에서는 펙틴의 단일유화제로서 활용 가능성을 확인하고, 펙틴이 가진 다양한 구조의 결정 인자에 따라 달라지는 분산계 내에서 작용 방식을 분석하고자 하였다. 펙틴의 종류 및 기름 함량에 따라 오일 입자의 크기 및 분포도, 소화과정에서 나타나는 변화, 유지 분해율, 그리고 커큐민의 생체 접근율을 조사하였다. 특히 DM 비교에 한정되었던 이전 연구들의 한계에서 벗어나 블록과 분자량을 종합적으로 고려하여 비교 분석하였다. 상업용 고메톡실펙틴 (CP72; DM=72, CP50; DM=50), 상업용 저메톡실펙틴 (CP7; DM=7), PME 변형 펙틴(MP50; DM=50)을 유화제로 사용하여 1% 와 3% 카놀라 기름을 함유하는 유화액을 제조하였다. 기름의 함량이 증가함에

따라 초기 에멀션 입자 크기가 증가하였으나, 펙틴의 종류에 따른 유의적인 차이가 없었다 ($p > 0.05$). CP72 에멀션은 가장 낮은 제타포텐셜 값을 나타내었고, MP50 에멀션은 같은 DM을 가지는 CP50 에멀션에 비해 큰 제타포텐셜 값을 보였다. 이는 블록이 야기하는 규칙적인 전하의 분포에 의한 것으로 추정된다. 소화를 마친 후, CP72와 CP50 에멀션은 초기 입자의 구조를 안정하게 유지하였으나, MP50과 CP7 에멀션은 합체와 응집을 보였다. 하지만 CP72는 뛰어난 에멀션 안정화능을 보였음에도 불구하고, CP7과 유사한 정도의 낮은 유지 분해율을 나타내었다. 이에 비해, MP50 에멀션은 높은 유지 분해율을 보임과 동시에 우수한 커큐민 생체접근율을 보였다. 이는 MP50에 형성된 블록이 규칙적인 배열로 오일 입자에 흡착하도록 야기하면서, 효소가 침투할 공간을 형성하고 있음을 시사하였다. CP50은 MP50과 같은 DM 및 분자량을 가짐에도 불구하고, 빠른 유지 분해 속도로 인해 커큐민의 생체 접근율이 낮았다. 이러한 결과는 펙틴의 DM뿐만 아니라 탈 메틸화의 규칙성과 유지 분해 속도 또한 커큐민의 마이셀화에 영향을 미친다는 것을 시사하였다. 또한 펙틴을 유화제로 사용하였을 때, 펙틴의 구조적 특징 및 전하의 분포도가 에멀션의 안정성과 지용성 생

리활성물질의 생체 접근율에 영향을 미침을 의미하였다. 블록 형태로 탈 메틸화되어 규칙적인 전하 분포를 가지는 펙틴은 불규칙적으로 탈 메틸화된 펙틴에 비해 높은 커큐민 생체 접근율을 나타내었고, 고메톡실펙틴에 비해 높은 유지 분해율을 보였다. 따라서 지용성 생리활성물질을 효과적으로 체내로 전달하는 물질전달 소재로의 가능성을 시사하였다. 소비자들의 천연 유화제에 대한 선호도가 증가함에 따라 식물 내 존재하는 생분해성 고분자인 펙틴은 식품 산업에서 폭넓게 이용될 수 있는 우수한 다기능성 소재임을 확인하였다.

주요어: 펙틴메틸에스터레이즈, 생체 접근율

학번: 2016-25843