1 **Impact of pectin properties on lipid digestion under simulated**
2 **gastrointestinal conditions: Comparison of citrus and banana**
3 **passion fruit (*Passiflora tripartita* var. *mollissima*) pectins**

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20 **Journal:** Food Hydrocolloids

21 **Type:** Original Research Paper

22 **Submitted:** February 2015

23 **Revised:** April 2015
24
25

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29 **Abstract**

30 Medium methoxylated pectin (52 % *mol/mol*, MMP) was isolated from banana passion
31 fruit (*Passiflora tripartita* var. *mollissima*) by hot acidic extraction. The impact of MMP on
32 lipid digestion was compared to that of commercial citrus pectins with high (71% *mol/mol*,
33 HMP) and low (30% *mol/mol*, LMP) methoxylation degree. A static *in vitro* digestion
34 model was used to elucidate the impact of pectin properties (methoxylation degree and
35 molecular weight) on the gastrointestinal fate of emulsified lipids. A 2.0% (w/w) oil-in-
36 water emulsion stabilized with 0.2% (w/w) Tween 80 was prepared, mixed with 1.8% (w/w)
37 pectin samples, and then subjected to the static *in vitro* digestion model (37 °C): initial (pH
38 7.0); oral (pH 6.8, 10 min, mucin); gastric (pH 2.5, 120 min, pepsin); and intestinal (pH 7.0,
39 120 min, bile salts, and pancreatic lipase) phases. The impact of the three pectin samples on
40 surface particle charge (ζ -potential), particle size distribution of lipid droplets,
41 microstructure, rheology, and lipid digestion (free fatty acids (FFAs) released) was
42 determined. The rate and extent of lipid digestion decreased with increasing simultaneously
43 both the molecular weight and pectin methoxylation, with the FFAs released after 120 min
44 of intestinal digestion being 47, 70, and 91% (w/w) for HMP, MMP, and LMP,
45 respectively. These results have important implications for understanding the influence of
46 pectin on lipid digestion. The control of lipid digestibility within the gastrointestinal tract
47 might be important for the designing and development of novel functional foods to control
48 bioactive release or to modulate satiety.

49 **Keywords:** Pectin, *Passiflora tripartita* var. *mollissima*, emulsion, lipid digestion,
50 gastrointestinal tract, depletion flocculation.

1. Introduction

Tropical fruits are a good source of bioactive agents suitable for utilization in the food, pharmaceutical, and cosmetic industries (Schieber, Stintzing, & Carle, 2001). Certain bioactive agents found in tropical fruits have been shown to inhibit cardiovascular diseases and some types of cancer (Rufino, et al., 2010). Banana passion fruit (*Passiflora tripartita* var. *mollissima*) may be a particularly good source of bioactive agents because of its relatively high levels of phenolics, carotenoids, and dietary fibers (Gil, Restrepo, Millán, Alzate, & Rojano, 2014), which are known to be beneficial to human health and wellbeing (Wootton-Beard & Ryan, 2011). Previous studies have shown that dietary fibers from fruits have a positive effect on the treatment of diseases such as hyperlipidemia, coronary heart disease, and certain types of cancer (Kumar, Sinha, Makkar, de Boeck, & Becker, 2011)- The major source of non-cellulosic dietary fiber in fruits are pectins (F. J. Voragen, Timmers, Linssen, Schols, & Pilnik, 1983). Pectins are acidic hetero-polysaccharides composed mainly of α -(1,4) linked D-galacturonic acid (GalA) residues (Ridley, O'Neill, & Mohnen, 2001). The carboxyl moieties of the GalA unit may be esterified with methanol, which alters the electrical characteristics of the molecule. Overall, the degree and patterning of methoxylation, as well as the molecular weight, are important parameters determining the functional attributes of different pectins (Funami, et al., 2011).

Although is usually accepted that pectin cannot be digested by the human gastrointestinal tract (GIT), it is possible to get some nutrients from pectins due to the presence of symbiotic bacteria in the GIT. Some bacteria are able to produce a group of enzymes that break down pectin into simple sugars (mainly galacturonic acid), which are then in turn fermented to create short chain fatty acids that human cells can absorb and which can contribute as much as 10 percent of the calories required by cells. It has been established that two species of gut bacteria in mammals have evolved to break down some particular kinds of foods. *Bacteroides ovatus* and *Bacteroides thetaiotaomicron* are able to break down hemicelluloses and pectins, as well as other complex carbohydrates that human intestinal cells secrete as mucus. These bacteria are a crucial part of the bacterial cells that make up our gastrointestinal tract, so further understanding of the metabolism of these gut bacteria could help to improve the influence of pectin on human nutrition (Inman, 2011).

Overconsumption of fat is a major contributing factor to obesity, cardiovascular disease, and diabetes (Bray & Popkin, 1998). For this reason, there has been considerable interest in the development of effective strategies to reduce the caloric content of foods, or to reduce the spike in blood lipids that occurs after consuming a fatty meal. Several studies have suggested that certain types of dietary fibers can inhibit the digestion and absorption of lipids (Beysseriat, Decker, & McClements, 2006; Edashige, Murakami, & Tsujita, 2008; Tsujita, et al., 2003; Yonekura & Nagao, 2009). Numerous physicochemical and physiological mechanisms may contribute to this effect, including the ability of dietary fibers to alter the rheology of the gastrointestinal fluids, to bind digestive components (such as bile salts and digestive enzymes), to alter the aggregation state of lipid droplets, to form protective coatings around lipid droplets, and to be fermented within the large intestine by colonic bacteria (Grabitske & Slavin, 2009; Lattimer & Haub, 2010; D.J. McClements, Decker, & Park, 2009). In a recent study, we showed that pectin reduced the rate and extent of the digestion of emulsified lipids (Espinal-Ruiz, Parada-Alfonso, Restrepo-Sanchez, Narvaez-Cuenca, & McClements, 2014). Increased consumption of pectin may therefore prove to be one strategy of reducing the caloric content of fatty food products or of modulating blood lipid levels (Mesbahi, Jamalain, & Farahnaky, 2005).

The lipids in food may be consumed in a wide variety of different physical structures such as oils (edible oils), bulk fats (margarine and butter), or emulsified fats (milk, cream, soups, and sauces). Nevertheless, most fatty foods are broken into oil-in-water emulsions within mouth during mastication and within the stomach and small intestine during the digestion process (D. J. McClements & Li, 2010). Consequently, lipid digestion within the gastrointestinal tract typically involves digestion of emulsified fats. Lipid digestion involves several sequential steps that include various physicochemical and biochemical events (Torcello-Gomez, Maldonado-Valderrama, Martin-Rodriguez, & McClements, 2011).

In the mouth, an ingested food is mixed with saliva ($\text{pH} \approx 7$), undergoes temperature changes ($T \approx 37^\circ\text{C}$), and is subjected to mechanical forces that may alter the structure, physical state, and interfacial properties of the lipid phase (Li, Kim, Park, & McClements, 2012). In the stomach, the lipids are mixed with a highly acidic aqueous solution that

contains minerals, biopolymers, surface active compounds, and digestive enzymes (Singh, Ye, & Horne, 2009). The lipid phase may undergo further changes in structure due to droplet disruption and coalescence processes, as well as changes in the nature and composition of the surface active materials adsorbed at the lipid-water interface (Singh & Sarkar, 2011). In particular, gastric lipase adsorbs to the lipid-water interface and initiates the lipid digestion process, converting some of the triacylglycerols (TAGs) to diacylglycerols (DAGs), monoacylglycerols (MAGs), and free fatty acids (FFAs) (Wilde & Chu, 2011). In the small intestine, the emulsified lipids are mixed with digestive juices that contain pancreatic lipase, colipase, bile salts, and phospholipids (Golding & Wooster, 2010). The bile salts and phospholipids compete and displace any surface active material present at the lipid-water interface, and the lipase-colipase complex binds to the lipid droplet surfaces (Reis, Holmberg, Watzke, Leser, & Miller, 2009). The pancreatic lipase converts TAGs into MAGs and FFAs, which leave the lipid droplet surfaces and are incorporated into mixed micelle structures consisting of phospholipids and bile salts, which then transport them to the epithelial cells, where they are adsorbed (Yao, Xiao, & McClements, 2014).

In this study, a simulated *in vitro* gastrointestinal model was used to evaluate the impact of commercial high (HMP) and low (LMP) methoxylated pectins from citrus, and medium methoxylated pectin (MMP) isolated from banana passion fruit (*Passiflora tripartita* var. *mollissima*) on the gastrointestinal fate of emulsified lipids. These three pectin samples were selected because of their different charge (methoxylation) and size (molecular weight) characteristics, and because they can be used as functional ingredients in food and beverage products (Willats, Knox, & Mikkelsen, 2006). **We hypothesized that these three pectins would have different effects on lipid digestion due to their different molecular and physicochemical characteristics.** In particular, we focused on their influence on the rheology of the gastrointestinal fluids, the aggregation stability of lipid droplets in different stages of the gastrointestinal tract, and the rate and extent of lipid digestion. The aim of the study was to obtain a better understanding of the role of pectin characteristics on the gastrointestinal fate of ingested lipids. The knowledge obtained in this study might be useful for the design, fabrication, and implementation of pectin-based functional foods designed to promote health and wellness by modulating lipid digestion.

2. Materials and methods

2.1. Chemicals

Corn oil was purchased from a commercial food supplier (Mazola, ACH Food Companies Inc., Memphis, TN) and stored at 4 °C until use. The manufacturer reported that the corn oil contained approximately 14, 29, and 57% (w/w) of saturated, monounsaturated, and polyunsaturated fatty acids, respectively. Commercial powdered high methoxylated pectin (HMP, Genu Citrus Pectin USP/100) was kindly donated by CP Kelco Co. (Lille Skensved, Denmark) and was used without further purification. The methoxylation degree of this material was 71% (mol/mol) and the average molecular weight 181 kDa. Commercial powdered low methoxylated pectin (LMP) was kindly donated by TIC Gums Inc. (Belcamp, MD) and was also used without further purification. The methoxylation degree of this material was 30% (mol/mol) and the average molecular weight 130 kDa. Fat soluble fluorescent dye Nile Red (N3013), lipase from porcine pancreas (Type II, L3126, triacylglycerol hydrolase E.C. 3.1.1.3), bile extract (porcine, B8631), mucin from porcine stomach (Type II, M2378, bound sialic acid $\leq 1.2\%$), and pepsin A from porcine gastric mucose (P7000, endopeptidase E.C. 3.4.23.1, activity ≥ 250 units mg^{-1} solid) were purchased from Sigma-Aldrich Chemical Company (St Louis, MO). **One unit of activity of pepsin A will increase a $\Delta A_{280\text{nm}}$ of 0.001 per min at pH 2.0 and 37 °C, using hemoglobin as substrate. The supplier reported that the lipase activity at 37 °C was 100-400 units mg^{-1} protein (pH 7.7 using olive oil) and 30-90 units mg^{-1} protein (pH 7.4 using triacetin) for 30 min incubation (one unit of activity of lipase corresponds to the release of 1 μeq of free fatty acids).** The composition of the bile extract has been reported as 49% (w/w) total bile salt (BS), containing 10-15% glycodeoxycholic acid, 3-9% taurodeoxycholic acid, 0.5-7% deoxycholic acid, 1-5% hydrodeoxycholic acid, and 0.5-2% cholic acid; 5% (w/w) phosphatidyl choline (PC); $\text{Ca}^{2+} \leq 0.06\%$ (w/w); critical micelle concentration of bile extract 0.07 ± 0.04 mM; and mole ratio of BS to PC being around 15:1. Dextran analytical standards (25, 150, and 410 kDa) for high performance size exclusion chromatography (HPSEC) were purchased from Sigma-Aldrich Chemical Company (St Louis, MO). All other chemicals were purchased from Sigma-Aldrich Chemical Company (St Louis, MO). Double distilled water was used to prepare all solutions.

2.2. Extraction of pectin from banana passion fruit and characterization of the pectin samples (LMP, MMP, and HMP)

2.2.1. Extraction of pectin from banana passion fruit (MMP)

Five grams of homogenized banana passion fruit epicarp (*Passiflora tripartita* var. *mollissima*) were mixed with 30 mL of 0.1 M HCl (pH 1.0) and stirred for 60 min at 90 °C. The mixture was neutralized to pH 7.0 with 1 M NaOH solution and then 100 mL of 95% (v/v) ethanol were added to induce pectin precipitation. The pectin obtained after 12 h of precipitation was filtered, washed with 100 mL of 70% (v/v) ethanol, and then dried at 45 °C for 12 h. The extraction yield was 64% (w/w). Pectin samples (LMP, MMP, and HMP) were characterized as follows:

2.2.2. Molecular weight and gyration radius

The average molecular weight, the molecular weight distribution, and the gyration radius (r_g) were determined using high performance size exclusion chromatography (HPSEC), using a 1260 Infinity liquid chromatograph (Agilent Technologies Inc., Santa Clara, CA). One-hundred microliters of 0.5% (w/w) pectin samples were injected into a packed column (OHpak SB-806M HQ, 8.0 mm × 300 mm, Shoko America Inc., Torrance, CA) and the elution was performed using 200 mM NaCl at a flow rate of 1 mL min⁻¹ for 25 min at 20 °C. An Optilab T-rex differential refractive index detector (Wyatt Technology Co., Santa Barbara, CA) at 40 °C; and a Dawn Heleos-II multi-angle laser light scattering detector (MALLS, Wyatt Technology Co., Santa Barbara, CA) at 30 °C were used to monitor the eluents. Both multi-angle laser light scattering signal at 90° and dextran analytical standards (25, 150, and 410 kDa; 0.5% w/w) were used to estimate the average molecular weight, molecular weight distribution, and gyration radius of the pectin samples. Although the multi-angle light scattering detector has 18 optimized scattering angles, the scattered light signal at 90° was selected for the quantification of the average molecular weight because it allows to obtain a reliable and accurate measure of the scattered light (Yoo & Jane, 2002).

2.2.3. Fourier transform-infrared (FT-IR) spectra

Pectin samples were dried in a desiccator containing blue silica gel prior to FT-IR analysis. KBr-pectin disc mixtures (90:10 w/w) were prepared and then the FT-IR spectra were collected at the transmittance mode in a Nicolet iS10 FT-IR Spectrometer (Thermo Fisher Scientific, Waltham, MA) at a 4 cm⁻¹ resolution. Eighty interferograms were measured to obtain a high signal to noise ratio.

2.2.4. Methoxylation and acetylation degrees

The methoxylation and acetylation degrees of pectin samples were determined according to (A. G. J. Voragen, Schols, & Pilnik, 1986). Pectin samples (30 mg) were suspended in 1 mL of an isopropanol-water mixture (1:1 v/v) containing 0.4 M NaOH and held at room temperature for 2 h. The suspension was centrifuged (20 min, 18000 g, 4 °C) and then 20 µL of the clear supernatant was injected into the column. A model LC-20AT liquid chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with an Aminex HPX-87H column (300 × 7.8 mm × 9 µm, Bio-Rad Laboratories, Hercules, CA) was used. The column was operated at room temperature and a flow rate of 0.6 mL min⁻¹ with 4 mM H₂SO₄ as the eluent. Components eluting from the column were detected using a RID-10A refractive index detector (Shimadzu Corporation, Kyoto, Japan) thermostated at 40 °C. The amounts of methanol and acetic acid released after saponification were determined using an external standard method. Calibration lines were obtained at concentrations ranging from 5 to 40 mM, and from 0.1 to 0.8 mM for methanol and acetic acid, respectively. The methoxylation and acetylation degrees were expressed as moles of methyl and acetyl esters, respectively, per 100 mol of uronic acid, and were corrected for free methanol and acetic acid. The uronic acid content was determined according to (van den Hoogen, et al., 1998). Briefly, an aliquot of 400 µL of each pectin sample solution (100 µg mL⁻¹) was mixed with 2 mL of 98% (w/w) H₂SO₄ containing 120 mM sodium tetraborate (Na₂B₄O₇•10H₂O) and incubated for 60 min at 80 °C. After cooling to room temperature, the background absorbance of the samples was measured at 540 nm. Then, 400 µL of *m*-hydroxydiphenyl reagent (prepared by mixing 100 µL of 100 mg mL⁻¹ *m*-hydroxydiphenyl in dimethyl sulfoxide with 4.9 mL of 80% (w/w) H₂SO₄) was added and mixed with the samples. After

15 min, the absorbance of the pink-colored samples was measured at 540 nm. A calibration line was obtained using GalA at final concentrations ranging from 0.1 to 1.0 $\mu\text{g mL}^{-1}$.

2.3. Solutions and emulsions preparation

2.3.1. Pectin stock solutions

Pectin stock solutions (2.0% *w/w*) were prepared by dispersing 1 g of powdered pectins (LMP, MMP, and HMP) into 49 g of 5 mM phosphate buffer (pH 7). The solutions were stirred at 800 rpm overnight at room temperature to ensure complete dispersion and dissolution. Stock solutions were finally adjusted to pH 7 using 1 M NaOH solution, and were equilibrated for 10 min before the analysis.

2.3.2. Stock emulsion

A stock emulsion was prepared by mixing 20% (*w/w*) corn oil and 80% (*w/w*) buffered emulsifier solution (5 mM phosphate buffer pH 7, containing 2.5% (*w/w*) Tween 80) together for 5 min using a bio-homogenizer (Speed 2, Model MW140/2009-5, Biospec Products Inc., ESGC, Switzerland). The coarse emulsion obtained was then passed 5 times through a high-pressure homogenizer (Microfluidizer M-110L processor, Microfluidics Inc., Newton, MA) operating at 11,000 psi (75.8 MPa).

2.3.3. Pectin-emulsion mixtures

Pectin-emulsion mixtures were prepared by mixing the stock emulsion containing 20% (*w/w*) corn oil with buffered stock solutions of 2% (*w/w*) pectin (mass ratio 1:9), to obtain emulsions containing 2.0% (*w/w*) corn oil and 1.8% (*w/w*) pectin. The pectin-emulsion mixtures were then stirred with a high-speed stirrer (Fisher Steadfast Stirrer, Model SL 1200, Fisher Scientific Inc., Pittsburgh, PA) at 800 rpm and stored overnight (**approximately 12 h**) at room temperature. The pectin-emulsion mixtures were characterized to obtain the initial phase, prior to subjection to the static *in vitro* digestion model.

2.4. Static *in vitro* digestion model

Each emulsion sample (initial phase) was passed through a simulated static *in vitro* digestion model that consisted of oral, gastric, and intestinal phases. Measurements of emulsion microstructure and stability, particle size distribution, particle charge, and viscosity were performed after each phase. The standardized static *in vitro* digestion model used in this study was a slight modification of that described previously (Espinal-Ruiz, Parada-Alfonso, Restrepo-Sanchez, et al., 2014; Minekus, et al., 2014).

2.4.1. Oral phase

Simulated saliva fluid (SSF, pH 6.8) containing 3.0% (w/w) mucin was prepared according to the composition shown in **Table 1**. Each emulsion (20 mL of initial phase) was mixed with 20 mL of SSF and the resulting mixture containing 1.0% (w/w) corn oil and 0.9% (w/w) pectin was used for characterization after the incubation period. The oral phase model consisted of a conical flask containing emulsion-SSF mixture incubated at 37 °C with continuous shaking at 100 rpm for 10 min in a temperature controlled air incubator (Excella E24 Incubator Shaker, New Brunswick Scientific Co., New Brunswick, NJ) to mimic the conditions in the mouth. Although 10 min of incubation time is somewhat longer than *in vivo* (approximately 1 min), however, accuracy and reproducibility in a laboratory situation may be compromised if using any shorter digestion time. In addition, it has been recommended an oral digestion time of 10 min in order to compensate the lack of a proper mechanical action for static models, which in most cases is difficult to simulate (Minekus, et al., 2014). The resulting oral phase (bolus) was used in the gastric phase.

2.4.2. Gastric phase

Simulated gastric fluid (SGF) was prepared by adding 2 g NaCl, 7 mL concentrated HCl (37% w/w), and 3.2 g pepsin A (from porcine gastric mucose, 250 units mg⁻¹) to a flask and then diluting with double distilled water to a volume of 1 L, and finally adjusting to pH 1.2 using 1 M HCl. Samples taken from the oral phase (20 mL bolus) were mixed with 20 mL of SGF so that the final mixture contained 0.50% (w/w) corn oil and 0.45% (w/w) pectin. These mixtures were then adjusted to pH 2.5 using 1 M NaOH and incubated at 37 °C with continuous shaking at 100 rpm for 2 h. Samples were taken for

characterization at the end of the incubation period (gastric phase). The resulting gastric phases (chyme) were used in the intestinal phase.

2.4.3. Intestinal phase

Samples obtained from the gastric phase (30 mL chyme containing 0.50% (w/w) corn oil and 0.45% (w/w) pectin) were incubated for 2 h at 37 °C in a simulated small intestine fluid (SIF) containing 2.5 mL pancreatic lipase (24 mg mL⁻¹), 3.5 mL bile extract solution (54 mg mL⁻¹), and 1.5 mL salt solution containing 0.25 M CaCl₂ and 3.0 M NaCl, to obtain a final composition of the intestinal fluid in the reaction vessel of 0.40% (w/w) corn oil and 0.36% (w/w) pectin. The lipolytic reaction was conducted at constant pH (≈ 7.0) using an automatic titration unit (pH stat titration unit, 835 Titrand, Metrohm USA, Inc., Riverview, FL) and then the FFAs released were monitored by determining the amount of 0.1 M NaOH needed to maintain the constant pH within the reaction vessel. All additives were dissolved in 5 mM phosphate buffer solution (pH 7.0) before use. Lipase addition and initialization of the titration program were carried out only after the addition of all pre-dissolved ingredients and balancing the pH to 7.0. Samples were taken for physicochemical and structural characterization at the end of the digestion period (intestinal phase). The volume of 0.1 M NaOH added to the emulsion was recorded over time and then was used to calculate the concentration of FFAs generated by lipolysis. The amount of FFAs released was calculated using the following equation:

$$\text{FFA (\% w/w)} = 100 \times \left(\frac{V_{\text{NaOH}} \times C_{\text{NaOH}} \times \text{MW}_{\text{Lipid}}}{2 \times w_{\text{Lipid}}} \right) \quad (1)$$

Here, 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, C_{NaOH} is the concentration of the sodium hydroxide (0.1 M), MW_{Lipid} is the average molecular weight of corn oil (872 g mol⁻¹), and w_{Lipid} is the initial weight of corn oil in the intestinal phase (0.15 g). Titration blanks were performed by inactivating pancreatic lipase solution in boiling water for 15 min prior to initialization of the titration program.

2.5. Emulsion characterization

2.5.1. Gravitational separation

Ten milliliters of each sample were transferred into a glass test tube, sealed with a plastic cap, and then stored at room temperature for 24 h. Digital photographs (Lumix DMC-ZS8 Digital Camera, Panasonic Corporation, Newark, NJ) of the samples were taken after storage to record their stability to gravitational separation.

2.5.2. Microstructure

The microstructure of the samples was characterized by confocal fluorescence microscopy. An optical microscopy (C1 Digital Eclipse, Nikon Co., Tokyo, Japan) with a 60× objective lens was used to capture images of the emulsions. **Emulsions were gently stirred to form a homogeneous mixture without introducing air bubbles and then the emulsions were stained with fat soluble fluorescent dye Nile Red (0.1% (w/w) dissolved in 90% (v/v) ethanol) to visualize the location of the oil phase. A small aliquot of the stained emulsions (5 µL) was then transferred to a glass microscope slide and covered with a glass cover slip.** The cover slip was fixed to the slide using nail polish to avoid evaporation. A small amount of immersion oil (Type A, Nikon Co., Melville, NY) was placed on the top of cover slip. All fluorescence confocal images were taken using an excitation argon laser (543 nm) and emitted light was collected between 555 to 620 nm, and then characterized using the instrument software (EZ CS1 version 3.8, Niko Co., Melville, NY).

2.5.3. Apparent viscosity

The apparent viscosity of samples was measured using a dynamic shear rheometer (Kinexus Rotational Rheometer, Malvern Instruments Ltd., Worcestershire, United Kingdom). A cup and bob geometry consisting of a rotating inner cylinder (diameter 25 mm) and a static outer cylinder (diameter 27.5 mm) was used. The samples were loaded into the rheometer measurement cell and allowed to equilibrate at 37 °C for 5 min before the beginning of all experiments. Samples underwent a constant shear treatment (10 s^{-1} for 10 min) prior to analysis to standardize the shear rate of each sample. **The apparent**

viscosity (η) was then obtained from measurements with a shear rate of 10 s^{-1} selected to mimic oral conditions (Pal, 2011).

2.5.4. Particle size distribution

The samples were diluted to a droplet concentration of approximately 0.005% (w/w) using buffer solution at the appropriate pH prior to analysis to avoid multiple scatterings effects. The particle size distribution of emulsions was then measured using a static light scattering instrument (Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, United Kingdom). Refractive indices of 1.47 (corn oil) and 1.33 (water) were used for the calculations of the particle size distribution. Background corrections and system alignment were performed prior to each measurement when the measurement cell was filled with the appropriate buffer solution. Particle sizes were reported as particle size distribution profiles (volume fraction (%) vs. particle diameter (μm)) and surface-weighted mean diameter (d_{32} , nm).

2.5.5. Surface electrical charge

The surface electrical charge (ζ -potential, mV) of emulsions was determined using a particle micro-electrophoresis instrument (Zetasizer NanoSeries, Malvern Instruments Ltd., Worcestershire, United Kingdom). The emulsions were diluted to a droplet concentration of approximately 0.005% (w/w) using buffer solution at the appropriate pH prior to analysis. Diluted emulsions were injected into the measurement chamber, equilibrated for 120 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 20 continuous readings made per sample. To determine the effect of pH on the surface electrical charge (ζ -potential) of pectin solutions (0.5% w/w), a titration between pH 2.0 to 8.0 with 0.25 M NaOH was performed with an automatic titration unit (Multi Purpose Titrator MPT-2, Malvern Instruments Ltd., Worcestershire, United Kingdom). The ζ -potential was recorded at each pH after 60 s equilibrium.

2.6. Data analysis

All digestions and measurements were performed at least three times using freshly prepared samples. Averages and standard deviations were calculated from these triplet measurements.

3. Results and discussion

3.1. Characterization of the pectin samples

3.1.1. FT-IR analysis of functional groups

The FT-IR spectra of the different pectin samples are shown in **Supplementary Fig. 1**. It was observed the presence of the monosaccharide units making up pectin such as GalA, xylose, arabinose, and rhamnose, which exhibit intense signals between 1200 and 950 cm^{-1} wavenumber values and constituting the fingerprint region specific for each polysaccharide. However, the most representative signals of the FT-IR spectra of pectin samples are those related to the carboxyl ($-\text{COOH}$) and carbomethoxyl ($-\text{COOCH}_3$) groups (Manrique & Lajolo, 2002). Common features of all the spectra were: a peak around 3410 cm^{-1} due to an O-H stretching vibration; a peak around 2900 cm^{-1} due to C-H stretching of $-\text{CH}_2$ groups; and two peaks at 1610 and 1410 cm^{-1} due to symmetrical stretching vibrations of the $\text{O}=\text{C}-\text{O}$ structure. The signal that appears at 1730 cm^{-1} can be assigned to the C=O stretching vibration of carbomethoxyl group (and also, if present, of protonated carboxylic group) and shows clear evidence that HMP and MMP (higher signal strength) were more methoxylated than LMP. FT-IR spectra of aliphatic carboxylic acids (anionic form) exhibit a characteristic pair of strong intensity signals at 1610 and 1410 cm^{-1} corresponding, respectively, to asymmetrical and symmetrical stretching vibrations of the carboxylate group ($-\text{COO}^\ominus$). Considering that a total ionization of $-\text{COOH}$ groups could be attained in the partially methoxylated pectin (*e.g.* MMP), the 1730 cm^{-1} signal would be generated exclusively by the carbomethoxyl group (Kačuráková, Capek, Sasinková, Wellner, & Ebringerová, 2000). The similarity of the fingerprint region of MMP with commercial HMP and LMP pectins, and the relative intensity of the signal at 1730 cm^{-1} (intermediate intensity between LMP and HMP) demonstrated that the polysaccharide obtained by acidic

extraction from banana passion fruit (*Passiflora tripartita* var. *mollissima*) corresponds to medium methoxylated pectin (MMP).

3.1.2. Electrical characteristics of pectin samples

In this series of experiments, we used chemical analysis and micro-electrophoresis to establish differences in the electrical characteristics of the three pectins. The degree of methoxylation of the three pectins was 71, 52, and 30% (*mol/mol*) for the HMP, MMP and LMP samples, respectively (**Table 2**). Measurements of the ζ -potential *versus* pH profiles of the three different pectin samples are shown in **Supplementary Fig. 2**. In general, all of the samples had their highest negative charges at pH 8, and became less negatively charged as the pH was decreased with the steepest change in charge occurring below pH 4.5. This effect can be attributed to protonation of the carboxylic acid groups ($-\text{COO}^\ominus + \text{H}^\oplus \rightleftharpoons -\text{COOH}$) on the pectin molecules when the pH is reduced below their pK_a values (typically around pH 3.5). As expected, the magnitude of the negative charge increased with decreasing methoxylation (HMP, $\zeta = -28.2$ mV; MMP, $\zeta = -34.8$ mV; and LMP, $\zeta = -47.5$ mV), since then there were more non-esterified carboxyl groups present that could be ionized (**Table 2**). It is also important to consider that only MMP had a considerable degree of acetylation (6.6 % *mol/mol*) compared to HMP and LMP. Because of the neutral nature of the acetyl group ($-\text{COCH}_3$) this parameter is not expected to contribute to the overall charge of pectin. Acetyl groups, however, play an important role in the structural conformation of pectin since this residue is related to controlling the formation of branched structures (Mohnen, 2008).

3.1.3. Molecular weight of pectin samples

The molecular weight distribution of the three pectin samples was determined by HPSEC (**Supplementary Fig. 3**) and the average molecular weight was calculated (**Table 2**). The average molecular weight values reported in the **Table 2** were obtained from the multi-angle laser light scattering detector signal at 90° (**MALLS**). The values obtained from the dextran analytical standards were fairly similar than those obtained from **MALLS**. All three pectins had mono-modal distributions, but the width of the distribution was broader for MMP than for LMP and HMP. The average molecular weights of the three pectins were

fairly similar, with all being in the range of 130 to 181 kDa. In general, HMP had the higher average molecular weight (181 kDa), followed by MMP (148 kDa) and LMP (130 kDa).

3.2. Influence of pectin type on gastrointestinal fate of emulsified lipids

In this section, the influence of pectin type on the potential gastrointestinal fate of corn oil-in-water emulsions was determined using an *in vitro* digestion model that simulated oral, gastric, and small intestinal phases. Changes in particle size, electrical charge, microstructure, appearance, and rheology of the emulsions were measured after their exposure to each stage of the gastrointestinal tract (**Fig. 1 to 5**).

3.2.1. Particle size, microstructure, and appearance of emulsions

Initially, all of the emulsions had similar particle size distributions and mean particle diameters (**Fig. 1 and 5a**), which suggested that the droplets were stable to coalescence or Ostwald ripening. However, the confocal microscopy images indicated that all the emulsions containing pectin were highly flocculated (**Fig. 2**), and photographs of the emulsions showed that they were highly susceptible to creaming (**Fig. 3**). The initial oil droplets were coated with a non-ionic surfactant (Tween 80), and therefore it seems likely that the origin of aggregation was depletion flocculation rather than bridging flocculation (Blijdenstein, Winden, Vliet, Linden, & van Aken, 2004). Indeed, calculations of the strength of the osmotic attraction between the droplets in the presence of pectin support this hypothesis (**Section 3.3**). When the emulsions were diluted for particle size analysis by laser light scattering the flocs would have been disrupted because the amount of pectin present would have fallen below the critical flocculation concentration (D. J. McClements, 2000).

After exposure to oral conditions, all of the emulsions (including the ones containing no pectin) were highly flocculated (**Fig. 2**) and exhibited some creaming (**Fig. 3**), but the individual droplets remained relatively small after dilution (**Fig. 1 and 5a**). This result suggests that the mucin molecules present within the SSF promoted droplet flocculation through depletion and/or bridging flocculation (Vingerhoeds, Blijdenstein, Zoet, & van Aken, 2005). **Again, the most likely mechanism is depletion flocculation due to the fact that**

the fat droplets had a low negative charge, and the flocs easily dissociated upon dilution for particle size measurements (Jenkins & Snowden, 1996; Klinkesorn, Sophanodora, Chinachoti, & McClements, 2004; D. J. McClements, 2000).

After exposure to gastric conditions, the particle size distribution became broader with many larger particles being present (**Fig. 1 and 5a**), and there was evidence of flocculation in the confocal microscopy images (**Fig. 2**) and creaming in the photographs (**Fig. 3**). The nature of the flocs in the gastric phase was quite different from those present in the oral phase (**Fig. 2**). In the gastric phase, there appeared to be a greater number of smaller flocs than in the oral phase. This effect may have occurred because of the dilution of the emulsions or because of changes in environmental conditions that changed the nature of the colloidal interactions, such as pH and ionic strength (Hur, Lim, Decker, & McClements, 2011; Singh, et al., 2009).

After exposure to small intestine conditions, there was evidence of a broad range of different sized particles in both the particle size distributions (**Fig. 1d**) and confocal microscopy images (**Fig. 2**). Lipid digestion may result in numerous different kinds of colloidal particles being present in the intestinal fluids, including undigested fat droplets, mixed micelles (micelles and vesicles) assembled from FFAs, DAGs, bile salts, and phospholipids, and insoluble calcium soaps formed from long chain FFAs and calcium (Golding, et al., 2010; D. J. McClements, et al., 2010). However, it is not possible to discern the exact nature of these different particles from the light scattering or confocal images.

3.2.2. Rheological properties of emulsions

The presence of dietary fibers in the emulsions is likely to alter the rheological properties of the gastrointestinal fluids, which may impact the rate and extent of lipid digestion by altering mixing and mass transport processes (Langhout, Schutte, Van Leeuwen, Wiebenga, & Tamminga, 1999). The apparent viscosity of the gastrointestinal fluids was therefore measured after the emulsions were exposed to each stage of the simulated GIT (**Fig. 4**). Initially, all of the emulsions containing pectin had a higher viscosity than the control emulsions due to the ability of pectin molecules to increase the

effective volume fraction of the dispersed phase (Mohnen, 2008; Ridley, et al., 2001). The extent of the increase in viscosity decreased in the following order HMP > MMP > LMP. This effect may have been due to differences in the average molecular weight of the different pectins: HMP > MMP > LMP (Table 2), which led to corresponding differences in the radius of gyration (Table 3). Extended polymers with higher molecular weights tend to have higher effective volume fractions in aqueous solutions, and therefore cause larger increases in viscosity (D. J. McClements, 2000).

As the emulsions passed through the successive stages of the simulated gastrointestinal system there was a progressive decrease in the apparent viscosities of the emulsions, which can be attributed to the dilution of the systems leading to a lower effective disperse phase volume fraction. However, in each phase the viscosities still decreased in the same order for the different pectins: HMP > MMP > LMP. The relatively high viscosities of the emulsions containing pectin may also have been due to some flocculation of the emulsion droplets promoted by the biopolymer (*e.g.* depletion or bridging flocculation) or due to formation of hydrogel particles (*e.g.*, calcium pectinate) (Jenkins, et al., 1996; D. J. McClements, 2000). Interestingly, the viscosities of all the samples were relatively low once they reached the small intestine phase, which can be attributed to the fact that the emulsions had undergone appreciable dilution. Consequently, one might not expect a large influence of viscosity on the lipid digestion process in the small intestine (Golding, et al., 2010).

3.2.3. Electrical characteristics of emulsions

Initially, the electrical charge on the control emulsions containing no pectin was around -7 mV (Fig. 5b), which can be attributed to the presence of some anionic impurities in the oil or surfactant ingredients (such as FFAs or phospholipids), to the ionization of the hydroxyl groups of Tween 80, or due to the preferential adsorption of hydroxyl ions (rather than hydronium ions) from water by the lipid droplet surfaces (Nikiforidis & Kiosseoglou, 2011). The addition of pectin to the emulsions led to an increase in the measured negative charge, with the magnitude of the effect increasing with decreasing degree of methoxylation. This effect can be attributed to the contribution of the anionic pectin molecules to the measured signal used to calculate the ζ -potential. The electrical charge became slightly more negative in all of the emulsions after exposure to the oral conditions,

which can be attributed to the presence of anionic mucin molecules in the simulated saliva (Espinal-Ruiz, Parada-Alfonso, Restrepo-Sanchez, et al., 2014; Vingerhoeds, et al., 2005). The magnitude of the negative charge on the particles decreased appreciably when exposed to simulated stomach phase, which may be due to the relatively low pH and high ionic strength of the gastric fluids (Singh, et al., 2009). The acidic pH of the gastric fluids reduced the negative charge on the pectin molecules, as well as on the non-ionic surfactant coated oil droplets. Finally, the negative charge increased appreciably after exposure to the simulated small intestine conditions, which can be attributed to the anionic nature of the molecules that assemble the colloidal particles in this phase, *i.e.*, FFAs, bile salts, and phospholipids (Hur, et al., 2011; D. J. McClements, et al., 2010). The electrical properties of the emulsions were affected by pectin samples according to their methoxylation degree (related to electrical charge). For all gastrointestinal phases, the measured negative charge of the emulsions increased with decreasing the degree of pectin methoxylation, which can be attributed to the higher negative charge density of the pectin molecules (**Fig. 5b**). In addition, the pectin molecules are not digested within the upper gastrointestinal tract, and therefore, they should remain in the gastrointestinal fluids of each phase, thereby contributing to the measured electrical properties (Ridley, et al., 2001).

3.2.4. Digestion of emulsified lipids

In this section, the influence of the different kinds of pectin on the rate and extent of lipid digestion was determined. In general, there was a rapid increase in the amount of FFAs released within the first few minutes, followed by a more gradual increase at later times (**Fig. 6a**). For the control sample, the amount of FFAs formed eventually reached around 100% (*w/w*) indicating that all of the TAGs were hydrolyzed by the lipase. For the emulsions containing pectin samples, the lipid digestion profile depended on the nature of the pectin molecules in the system. The final extent of lipid digestion decreased in the following order: 100, 92, 70 and 47% (*w/w*) for the control, LMP, MMP, and HMP samples, respectively (**Fig. 6b**). These results suggest that the extent of lipid digestion decreased as the degree of methoxylation and the molecular weight of the pectin molecules increased. **It should be stressed that the results obtained using simple simulated GIT models should be treated with caution, since they cannot represent the compositional, structural,**

and dynamic complexity of the processes occurring within the human GIT. Nevertheless, they may provide some useful insights into the potential physicochemical mechanisms occurring within the GIT.

In principle, there are numerous ways that pectin methoxylation can alter the lipid digestion process. An increase in methoxylation leads to an increase in the number of non-polar (hydrophobic) groups on the molecules, and a decrease in the number of negative groups (Dongowski, Lorenz, & Proll, 2002). An increase in the number of non-polar groups may lead to increased binding of bile salts through hydrophobic attraction (Dongowski, 1995; Espinal-Ruiz, Parada-Alfonso, Restrepo-Sánchez, Narváez-Cuenca, & McClements, 2014; Wilde, et al., 2011). The binding of bile salts by pectin may retard lipid digestion because they can no longer interact with the fat droplet surfaces (thereby inhibiting lipase adsorption) or because they can no longer solubilize FFAs generated at the fat droplet surfaces and thereby, inhibiting lipase activity (Espinal-Ruiz, Parada-Alfonso, Restrepo-Sánchez, et al., 2014). An increase in methoxylation would therefore be expected to decrease the rate of lipid digestion through this effect (Reis, et al., 2009). A decrease in the number of anionic carboxyl groups on the pectin chains (*e.g.*, increased methoxylation) may lead to decreased binding with cations, such as calcium ions (Willats, et al., 2006). Calcium ions play an number of important roles in the lipid digestion process: (i) a minimum level is required for proper lipase functioning; (ii) they precipitate long chain FFAs, thereby removing them from the fat droplet surfaces and avoiding the adsorption of lipase; and (iii) they form insoluble soaps with long chain FFAs thereby decreasing their absorption (Wilde, et al., 2011). An increase in methoxylation degree (decreased negative charge) might therefore be expected to alter the rate of lipid digestion. Differences in the ability of pectin molecules to promote droplet flocculation may have altered the digestion rate (Espinal-Ruiz, Parada-Alfonso, Restrepo-Sanchez, et al., 2014). Flocculated fat droplets may be digested more slowly than non-flocculated ones, because the surface area of lipids exposed to the lipase in the aqueous phase is reduced (Reis, et al., 2009). In this study, all of the pectins used promoted flocculation in the mouth, stomach, and small intestine and therefore had potential to inhibit digestion through this mechanism. Nevertheless, there may have been differences in the nature of the flocs formed, *e.g.*, the packing of the fat droplets within the flocs (**Fig. 2**). There are a number of physicochemical

phenomena that might account for the observed decrease in lipid digestion with increasing methoxylation of the pectin molecules, such as binding of bile salts to the non-polar groups. However, further studies would be required to characterize the importance of this mechanism.

Besides the contribution of the methoxylation degree, the molecular weight (as stated above in section 3.2.2) is also an important parameter that contributes to the overall inhibition of lipid digestion. The viscosity of the gastrointestinal phases increased with increasing molecular weight of pectin samples (HMP > MMP > LMP, **Fig. 4**). It is well known that the higher the molecular weight of pectin, the greater its capacity to form complex structures (gels) which are able to trap water and other components such as lipids in their inner structures (Willats, et al., 2006). An increase in the viscosity of the gel causes a restriction on the diffusive processes of lipids and lipases, inhibiting their capacity to interact to each other and consequently, reducing the lipolytic reaction rate. Furthermore, as the lipids are trapped inside pectin gels, lipases will not be able to access the lipid surfaces and thus, the lipolytic reaction rate will be reduced (Espinal-Ruiz, Parada-Alfonso, Restrepo-Sanchez, et al., 2014). **Although both the methoxylation degree and molecular weight are important parameters determining the physicochemical properties of pectin molecules, other structural parameters such as monosaccharide composition and degree of branching may also influence their functionality, and therefore their influence on the gastrointestinal fate of lipids. Further studies are therefore needed to clarify the importance of specific molecular characteristics on pectin functionality.**

3.3. Calculation of the depletion attraction between the lipid droplets

In this section we provide a theoretical rationalization for the influence of pectin type (HMP, MMP, and LMP) on the depletion flocculation of the emulsions in terms of the characteristics of the different pectin molecules (Klinkesorn, et al., 2004; D. J. McClements, 2000). The presence of non-adsorbed pectin molecules in the aqueous phase (bulk solution) of an emulsion is known to increase the osmotic attraction between the lipid droplets through a depletion mechanism (D. J. McClements, 2000). The magnitude of this attractive interaction can be calculated using the following equations (Klinkesorn, et al., 2004):

$$w_{\text{depletion}}(h) = -\frac{2}{3}\pi r^3 P_{\text{Osm}} \left[2 \left(1 + \frac{r_g}{r} \right)^3 + \left(1 + \frac{h}{2r} \right)^3 - 3 \left(1 + \frac{r_g}{r} \right)^2 \left(1 + \frac{h}{2r} \right) \right] \quad (2)$$

$$P_{\text{Osm}} = \frac{ckTN_A}{MW} \left(1 + \frac{2cR_V}{\rho_m} \right) \quad (3)$$

Here, $w_{\text{depletion}}(h)$ is the inter-droplet pair potential due to depletion interactions at a surface-to-surface droplet separation of h , r is the lipid droplet radius, P_{Osm} is the osmotic pressure arising from the exclusion of non-adsorbed pectin molecules from a narrow region ($\approx r_g$) surrounding the lipid droplets, and r_g is the effective radius of the pectin molecules in solution (**gyration radius**). In addition, c , MW , and ρ_m are the concentration, molecular weight, and molecular density of the pectin molecules in the aqueous solution, respectively. N_A is the Avogadro's number, k is the Boltzmann's constant, and T is the absolute temperature. The parameter, R_V , is referred to as the *volume ratio*, which is equal to the effective volume of a pectin molecule in solution divided by the actual volume of the constituent atoms making up the molecule (D. J. McClements, 2000). If a pectin molecule adopts a compact spherical conformation, like a globular protein, then $R_V \approx 1$. However, pectin molecules entrain large quantities of water as they rotate in solution, then $R_V \gg 1$. The effective volume of pectin in solution should be considerably greater than the volume occupied by the atoms that make up the pectin chain because it sweeps out a large volume of solvent as it rapidly rotates due to Brownian motion (Jenkins, et al., 1996; D. J. McClements, 2000). **In this model, we have assumed that pectin molecules behave like random coil in solution, so that:**

$$R_V = \frac{\pi n^{3/2} l^3 \rho_m N_A}{6MW} \quad (4)$$

Here, n is the number of monomer units per molecule ($n = MW/MW_0$), MW is the molecular weight of the whole pectin molecules, MW_0 is the molecular weight of a GalA monomer unit ($\approx 180 \text{ g mol}^{-1}$), l is the length of the monomer unit ($\approx 0.47 \text{ nm}$), and ρ_m is the density of the pectin chain ($\approx 2000 \text{ kg m}^{-3}$). The molecular characteristics of the pectin molecules used in our calculations are shown in **Table 3**. It should be noted that $w(h)/kT = 0$ for $h \geq 2r_g$ and that the strongest interaction between the fat droplets occurs when they

come into contact ($h = 0$). So that, equation 2 is applicable for $h < 2r_g$ and when the separation between the lipid droplets is small compared to their size ($h \ll r$).

The equations 2, 3, and 4 were used to calculate the influence of pectin type (HMP, MMP, and LMP) on the depletion attraction between lipid droplets ($r = 100$ nm), assuming that pectin molecules behave as random coil in aqueous solution. The variation of the droplet attraction potential ($w(h)/kT$) with the droplet separation (h) for different pectin types, but the same overall aqueous concentrations (1.8% (w/w) equivalent to the initial phase) is shown in **Fig. 7a**.

Both the magnitude (depletion attraction $w(h)/kT$) and the range (lipid droplet separation h) of the attractive depletion attraction between lipid droplets increased with increasing molecular weight and methoxylation degree: HMP > MMP > LMP > control. An estimate of the overall strength of the depletion attraction in a particular system can be obtained by calculating the magnitude of $w_{\text{depletion}}(h = 0)$ when the droplets are in contact:

$$w_{\text{depletion}}(h = 0) = -2\pi r_g^2 P_{\text{Osm}} \left[r + \frac{2}{3} r_g \right] \quad (5)$$

The dependence of $w_{\text{depletion}}(h = 0)/kT$ on pectin type was calculated (**Fig. 7b**). The strength of the depletion attraction increases progressively with the simultaneous increase of the molecular weight and methoxylation degree (HMP > MMP > LMP > Control). In the absence of pectin (control), the lipid droplets are prevented from flocculating because the repulsive droplet-droplet interactions (*e.g.*, steric, electrostatic, and hydration repulsion) dominate the attractive interactions (*e.g.*, van der Waals) (D. J. McClements, 2000). Addition of the pectin molecules to the emulsion increases the depletion attraction between the lipid droplets, until eventually the overall attractive interactions overcome the repulsive interactions and the droplets flocculate. As the molecular weight and the methoxylation degree of the pectin molecules increased simultaneously, a smaller amount of pectin needs to be added to the emulsion in order to generate the additional attraction required to promote droplet flocculation (Jenkins, et al., 1996). These calculations suggest that pectin can promote depletion flocculation in the emulsions used in this work. **In particular, Equation 2 suggests that the strength of the depletion interaction is highly dependent on the molecular weight of the pectin molecules. However, the electrical properties of pectin**

molecules may also indirectly influence the depletion interaction by altering the effective size (gyration radius) of the colloidal particles and the depletion zone. For example, increasing the number of negative charges on a pectin molecule by either decreasing the methoxylation degree or increasing the pH, can increase its effective size. In the one hand, increasing the number of negative groups usually causes the pectin molecules to become more extended because of electrostatic repulsion between negatively charged groups ($\text{COO}^\ominus$). On the other hand, decreasing the number of negative charges on a pectin molecule by either increasing the methoxylation degree or reducing the pH, can decrease its effective size. Thus, the strength of the depletion interaction may depend on the electrical properties of the pectin molecules and the solution conditions, *i.e.*, pH, and ionic strength (Furusawa, Ueda, & Nashima, 1999).

Additional calculations were conducted to evaluate the relative contribution of the molecular weight and the methoxylation degree to the overall magnitude of the depletion attraction. These calculations were performed by fixing the r_g value ($r_g = 4.7$ nm) and changing the molecular weight (according to **Table 3**), and by fixing the molecular weight (MW = 153 kDa) and changing the r_g value (according to **Table 3** as well). These calculations allowed use to establish that the molecular weight of pectin molecules had a significant impact on the magnitude of the depletion attraction ($w(h=0)/kT$ of -0.3, -1.1, and -1.7 for LMP, MMP, and HMP, respectively) while the degree of methoxylation (represented by r_g) had a smaller contribution to the overall magnitude of the depletion attraction ($w(h=0)/kT$ of -0.04, -0.07, and -0.09 for LMP, MMP, and HMP, respectively). Finally, we can suggest that both HMP and MMP form a closed structure around the lipid droplets (through a mechanism of depletion flocculation) which restricts the access of lipase to their surfaces and therefore, preventing lipid digestion (**Fig. 8a**), whereas LMP forms an open structure due to its repulsion with negatively charged lipid droplets, allowing the access of lipase to their surfaces and promoting lipid digestion (**Fig. 8b**).

4. Conclusions

The objective of this work was to study the impact of different types of pectin on the physicochemical characteristics and microstructure of emulsified lipids during passage

through a simulated gastrointestinal model. Three pectins with different molecular characteristics were studied: LMP and HMP from citrus fruit and MMP from banana passion fruit. These pectins differ in their molecular weights and degrees of methoxylation, which led to differences in their molecular dimensions (radius of gyration) and electrical characteristics (ζ -potential). All three pectins promoted flocculation of the fat droplets in the emulsions, which was attributed to a depletion flocculation mechanism, associated with exclusion of the biopolymers from the fat droplet surfaces. The pectin molecules decreased the extent of lipid digestion with increasing degree of methoxylation and molecular weight: HMP > MMP > MLP. These effects may have been due to the impact of the pectin molecules on the rheological properties of the gastrointestinal fluids, binding of key digestive components (such as calcium, free fatty acids, and bile), alteration in the droplet aggregation state, or entrapment of the lipid droplets by pectin microgels. Further studies are clearly required to establish the relative contribution of the methoxylation degree and the molecular weight to the overall inhibitory effect, as well as to identify the precise molecular origin of this inhibition. This information may be useful for the design of emulsion-based functional foods that give healthier lipid profiles and thereby promote health and wellness.

Acknowledgments

We are grateful to Departamento Administrativo de Ciencias, Tecnología e Innovación (COLCIENCIAS) and Vicerrectoría Académica of Universidad Nacional de Colombia for providing a fellowship to Mauricio Espinal-Ruiz supporting this work. We also thank the United States Department of Agriculture (USDA), NRI Grants (2011-03539, 2013-03795, 2011-67021, and 2014-67021); and Red Nacional para la Bioprospección de Frutas Tropicales COLCIENCIAS-RITFRUBIO (Contrato 0459-2013) for supporting this research. We are grateful to student Mayra Alejandra Quintero from Departamento de Química, Universidad Nacional de Colombia, for supporting both the extraction and characterization of *Passiflora tripartita* var. *mollissima* pectin (MMP) sample.

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Figure Captions

Fig. 1 Influence of high methoxylated pectin (HMP), medium methoxylated pectin (MMP) isolated from banana passion fruit (*Passiflora tripartita* var. *mollisima*), and low methoxylated pectin (LMP) on the particle size distribution of emulsions under simulated gastrointestinal conditions consisting of initial (a), oral (b), gastric (c), and intestinal phases (d). **Control corresponds to the emulsions without addition of pectin. The scale was shifted upwards by 25, 50, and 75% for HMP, MMP, and LMP respectively.**

Fig. 2 Influence of high methoxylated pectin (HMP), medium methoxylated pectin (MMP) isolated from banana passion fruit (*Passiflora tripartita* var. *mollisima*), and low methoxylated pectin (LMP) on the microstructure of emulsions observed by confocal fluorescence microscopy under simulated gastrointestinal conditions consisting of initial (a), oral (b), gastric (c), and intestinal (d) phases. **Control corresponds to the emulsions without addition of pectin.**

Fig. 3 Influence of high methoxylated pectin (HMP), medium methoxylated pectin (MMP) isolated from banana passion fruit (*Passiflora tripartita* var. *mollisima*), and low methoxylated pectin (LMP) on the creaming stability of emulsions under simulated gastrointestinal conditions consisting of initial (a), oral (b), gastric (c), and intestinal (d) phases. **Control (C) corresponds to the emulsions without addition of pectin.**

Fig. 4 Influence of high methoxylated pectin (HMP), medium methoxylated pectin (MMP) isolated from banana passion fruit (*Passiflora tripartita* var. *mollisima*), and low methoxylated pectin (LMP) on the apparent viscosity (η) of emulsions under simulated gastrointestinal conditions consisting of initial, oral, gastric, and intestinal phases. **Control corresponds to the emulsions without addition of pectin.**

Fig. 5 Influence of high methoxylated pectin (HMP), medium methoxylated pectin (MMP) isolated from banana passion fruit (*Passiflora tripartita* var. *mollisima*), and low methoxylated pectin (LMP) on the volume-surface mean diameter (d_{32} , a) and the electrical charge (ζ -potential, b) of emulsions under simulated gastrointestinal conditions consisting

of initial, oral, gastric, and intestinal phases. **Control corresponds to the emulsions without addition of pectin.**

Fig. 6 Influence of high methoxylated pectin (HMP), medium methoxylated pectin (MMP) isolated from banana passion fruit (*Passiflora tripartita* var. *mollissima*), and low methoxylated pectin (LMP) on free fatty acids (FFA) released after the digestion process. Kinetic profile of intestinal release of FFA (a), and FFA released after 2 hours of intestinal digestion (b). **Control corresponds to the emulsions without addition of pectin.**

Fig. 7 Inter-droplet pair potential attraction due to depletion interactions of lipid droplets containing high methoxylated pectin (HMP), medium methoxylated pectin (MMP) isolated from banana passion fruit (*Passiflora tripartita* var. *mollissima*), and low methoxylated pectin (LMP), related to the thermal energy (kT) of the system. Inter-droplet pair potential ($w(h)/kT$) due to depletion interactions at a surface-to-surface droplet separation of h (a), and inter-droplet pair potential ($w(h=0)/kT$) when the droplets are in contact (b). The model corresponds to the depletion interactions of lipid droplets in the initial phase (1.8% w/w pectins) at 37 °C, prior to subjection to the static *in vitro* digestion model. **Control corresponds to the emulsions without addition of pectin.**

Fig. 8 Schematic representation of the inhibition of lipid digestion by pectin. High methoxylated pectin (HMP) and medium methoxylated pectin (MMP) isolated from banana passion fruit (*Passiflora tripartita* var. *mollissima*) form a closed structure around the lipid droplets (depletion flocculation) which restricts the access of lipase to their surfaces and therefore, preventing the lipid digestion (a), whereas low methoxylated pectin (LMP) forms an open structure due to its electrostatic repulsion with negatively charged lipid droplets, allowing the access of lipase to their surfaces and promoting lipid digestion (b).

1

2 **Fig. 1**

Fig. 2

a. Initial

b. Oral

c. Gastric

d. Intestinal

5

6 **Fig. 3**

7

8 **Fig. 4**

9

10 **Fig. 5**

Fig. 6

Fig. 7

15

16 **Fig. 8**

Table Captions

Table 1. Chemical composition of simulated saliva fluid (SSF) used to simulate oral conditions.

Table 2. Physicochemical properties of high methoxylated pectin (HMP), medium methoxylated pectin (MMP) isolated from banana passion fruit (*Passiflora tripartita* var. *mollissima*), and low methoxylated pectin (LMP).

Table 3. Molecular characteristics of the high methoxylated pectin (HMP), medium methoxylated pectin (MMP) isolated from banana passion fruit (*Passiflora tripartita* var. *mollissima*), and low methoxylated pectin (LMP) molecules used in the theoretical calculations of the depletion interactions.

1 Tables

2 **Table 1.**

Compound	Chemical formula	Concentration (g/L) ¹
Sodium chloride	NaCl	1.594
Ammonium nitrate	NH ₄ NO ₃	0.328
Potassium dihydrogen phosphate	KH ₂ PO ₄	0.636
Potassium chloride	KCl	0.202
Potassium citrate	K ₃ C ₆ H ₅ O ₇ •H ₂ O	0.308
Uric acid sodium salt	C ₅ H ₃ N ₄ O ₃ Na	0.021
Urea	H ₂ NCONH ₂	0.198
Lactic acid sodium salt	C ₃ H ₅ O ₃ Na	0.146
Porcine gastric mucin (Type II)	----	30

3 ¹The SSF was prepared in double distilled water and then pH 6.8 was adjusted using 0.1 M NaOH.

4 **Table 2.**

Parameter	HMP	MMP	LMP
Average molecular weight (kDa) ¹	181	148	130
Methoxylation degree (% <i>mol/mol</i>)	71	52	30
Acetylation degree (% <i>mol/mol</i>)	0.4	6.6	0.1
Surface charge at pH 7 (ζ , mV)	-28.2	-34.8	-47.5

5 ¹Obtained from the multi-angle laser light scattering detector (reported according to the signal at 90°).

6 **Table 3.**

Parameter	HMP	MMP	LMP
Average molecular weight (kDa) ¹	181	148	130
n ²	1006	822	722
r _g (nm) ³	6.8	4.3	3.1
R _v ⁴	12	10	10

¹Obtained from the multi-angle laser light scattering detector (reported according to the signal at 90°).

²Average number of monomers per molecule ($n = MW/MW_0$). MW is the average molecular weight of the pectin molecules, and MW₀ is the molecular weight of a galacturonic acid monomer unit ($\approx 180 \text{ g mol}^{-1}$).

³Effective radius of the pectin molecules in solution (gyration radius). Obtained from the multi-angle laser light scattering detector (reported according to the signal at 90°).

⁴Volume ratio (dimensionless). It was assumed that pectin molecules were random coil in conformation.

1

2 **Graphical abstract**