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Pectin influences the kinetics of *in vitro* lipid digestion in oil-in-water emulsions

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Lipase (PubChem CID: 54603431)
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ABSTRACT

Oil-in-water emulsions were prepared with 5% (w/v) carrot-enriched olive oil and stabilized with Tween 80 (TW), phosphatidylcholine (PC), citrus pectin (CP) or a combination of these emulsifiers. Additionally, the methylesterification degree (DM) of citrus pectin was modified, resulting in three different studied pectin structures: CP82, CP38 and CP10. All initial emulsions presented small initial oil droplet sizes and were submitted to an *in vitro* simulated gastric and small intestinal phase. The latter was executed in a kinetic way to determine the time dependency of the lipolysis reaction, micelle formation and carotenoid bioaccessibility. The results showed that the pectin DM mainly influenced the reaction rate constants, while the emulsifier (combination) determined the extent of lipolysis and carotenoid bioaccessibility. Moreover, a direct relation was observed between the lipolysis reaction and bioaccessibility extent. The presented study showed that targeted emulsion design can be used to tailor lipid digestion kinetics.

1. Introduction

Lipids are the macronutrients with the highest energy density, which deliver essential fatty acids and can be carriers of lipophilic micronutrients, such as carotenoids (Golding & Wooster, 2010). In the human diet, lipids are frequently consumed as oil-in-water (o/w) emulsions, such as soups and sauces, being natural sources of fibers and micronutrients. Lipid digestion predominantly occurs in the small intestine, where triacylglycerol (TAG) can be hydrolyzed by pancreatic lipase into diacylglycerol (DAG), monoacylglycerol (MAG), free fatty acids (FFA) and glycerol (GLY). Subsequently, these lipid digestion products form mixed micelles together with bile salts excreted from the liver. These mixed micelles are amphiphilic structures which can incorporate lipophilic components in the core and easily migrate in the aqueous intestinal environment towards the intestinal mucosa (McClements & Decker, 2009). The fraction of ingested lipids and

Abbreviation: bioaccessibility, BAC; citrus pectin, CP; citrus pectin with a methylesterification degree of 82%, 38% and 10%, respectively, CP82 CP38 or CP10; 5% (w/v) olive oil-in-water emulsion stabilized with 1% (w/v) citrus pectin with a methylesterification degree of 82%, CP82 emulsion; 5% (w/v) olive oil-in-water emulsion stabilized with 1% (w/v) citrus pectin with a methylesterification degree of 38%, CP38 emulsion; 5% (w/v) olive oil-in-water emulsion stabilized with 1% (w/v) citrus pectin with a methylesterification degree of 10%, CP10 emulsion; diacylglycerol, DAG; methylesterification degree, DM; free fatty acids, FFA; glycerol, GLY; high methoxylated pectin, HMP; joint confidence region, JCR; low methoxylated pectin, LMP; monoacylglycerol, MAG; medium methoxylated pectin, MMP; oil-in-water emulsion, o/w emulsion; phosphatidylcholine, PC; 5% (w/v) olive oil-in-water emulsion stabilized with 1% (w/v) phosphatidylcholine, PC emulsion; 5% (w/v) olive oil-in-water emulsion stabilized with 1% (w/v) phosphatidylcholine and 1% (w/v) citrus pectin with a methylesterification degree of 82%, PCCP82 emulsion; 5% (w/v) olive oil-in-water emulsion stabilized with 1% (w/v) phosphatidylcholine and 1% (w/v) citrus pectin with a methylesterification degree of 38%, PCCP38 emulsion; 5% (w/v) olive oil-in-water emulsion stabilized with 1% (w/v) phosphatidylcholine and 1% (w/v) citrus pectin with a methylesterification degree of 10%, PCCP10 emulsion; triacylglycerol, TAG; Tween 80, TW; 5% (w/v) olive oil-in-water emulsion stabilized with 0.5% (w/v) Tween 80, TW emulsion; 5% (w/v) olive oil-in-water emulsion stabilized with 0.5% (w/v) Tween 80 and 1% (w/v) citrus pectin with a methylesterification degree of 82%, TWCP82 emulsion; 5% (w/v) olive oil-in-water emulsion stabilized with 0.5% (w/v) Tween 80 and 1% (w/v) citrus pectin with a methylesterification degree of 38%, TWCP38 emulsion; 5% (w/v) olive oil-in-water emulsion stabilized with 0.5% (w/v) Tween 80 and 1% (w/v) citrus pectin with a methylesterification degree of 10%, TWCP10 emulsion

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lipophilic components which are micellarized and consequently are available for absorption into the blood, is called the 'bioaccessible' fraction.

Recently, digestion studies were more focusing on controlling the lipid digestion rate as it plays an important role in satiety control, which in turn is related to food intake and diseases of nutritional excess (Ohlsson, Rosenquist, Rehfeld, & Härröd, 2014). Previous research has shown that dietary fibers, such as chitosan, cellulose, guar gum and pectin, may affect lipid digestion based on analysis of FFA release (Espinal-Ruiz, Parada-Alfonso, Restrepo-Sanchez, Narvaez-Cuenca, & McClements, 2014a; Hur, Kim, Choi, & Lee, 2013; Mun, Decker, Park, Weiss, & McClements, 2006; Pasquier et al., 1996). It can be postulated that by increasing the intake of natural fibers, the lipid digestion mechanism can be modified and subsequently lipid intake can be controlled. In addition, lipid digestion is strongly related to the uptake of lipophilic carotenoids (Borel et al., 1996; Deming & Erdman, 1999), so modulating the lipolysis extent might also have influence carotenoid uptake. Therefore, the effect of the presence of fibers in food systems must be investigated on the kinetics of both lipid digestion as well as carotenoid bioaccessibility.

Pectin is one of the most abundant fibers present in the primary cell wall and middle lamella of all higher plants (Willats, McCartney, Mackie, & Knox, 2001). It is a diverse group of polysaccharides rich in galacturonic acid (GalA) units which recently has shown emulsifying and emulsion-stabilizing potential (Schmidt, Schwab, & Schuchmann, 2017). Since pectin is a natural ingredient, it can be interesting to explore its role as emulsifier or when it is present in the aqueous phase of o/w emulsions on the lipolysis kinetics. It is known that the properties of the oil droplet, oil-water interface and surrounding medium can have a major effect on the lipolysis kinetics. In this context, it was proven that initial small oil droplet sizes led to a faster and higher lipolysis than larger initial oil droplets (Salvia-Trujillo et al., 2017). Not only initial small oil droplets are of importance, but also the stability of these oil droplets along the simulated digestive tract, which can be influenced by the emulsifier type used for emulsion stabilization. The emulsion stability during digestion will determine the available surface area for lipase adsorption in the small intestinal phase and the subsequent lipolysis kinetics (Verkempinck et al., 2018b). However, little is known about the specific influence of the presence of pectin in o/w emulsions on the kinetics of lipid digestion and carotenoid bioaccessibility. In this sense, pectin can be added in emulsions, being present at the oil-water interface or pectin can be located in the aqueous phase of these o/w emulsions. In addition, the structural characteristics of these pectin structures might play an important role during digestion. For example, the methylesterification degree of pectin influences its charge density (Celus et al., 2018) and interactions with different components during digestion (such as calcium, lipase, bile salts and lipophilic components) (Espinal-Ruiz, Parada-Alfonso, Restrepo-Sánchez, Narváez-Cuenca, & McClements, 2014b; McClements, Decker, & Park, 2008; Tsujita et al., 2007; Verrijssen et al., 2014). Therefore, the presented work, explored the effect of the presence of citrus pectin with different methylesterification degree in an emulsified system on the lipid digestion, using the kinetic digestion approach presented by Verkempinck et al. (2018a). Moreover, not only the effect of citrus pectin as mono-emulsifier was evaluated, but also the combination of citrus pectin and a conventional emulsifier stabilizing o/w emulsions was studied. Consequently, the link between lipid digestion and carotenoid bioaccessibility can be quantitatively proven. This allows to evaluate the proposed hypothesis throughout the whole digestion process based on the resulting kinetic parameters. So far in this research field, lipid digestion is mostly studied by evaluation of FFA release in the small intestinal phase. By contrast, this study aimed to quantify multiple lipid digestion species, namely TAG, DAG, MAG and FFA, both in the digest as well as in the micellar fraction. The obtained results can eventually contribute to the development of predictive mathematical (*in silico*) models needed for simulation of lipid digestion of particular food products and to create products for specific consumer groups.

2. Material and methods

Carotenoid enriched oil-in-water (o/w) emulsions were formulated with different emulsifiers (Tween 80, phosphatidylcholine or citrus pectin) or combinations of emulsifiers (Section 2.4). Emulsions stabilized with one emulsifier type will be called 'mono-emulsifier emulsions', while emulsions stabilized with pectin and another emulsifier will be called 'di-emulsifier emulsions'. More specifically, the effect of pectin present at the oil-water interface or in the aqueous phase of o/w emulsions on the kinetics of lipid digestion and carotenoid bioaccessibility was studied. Therefore, all emulsions were *in vitro* digested by simulating a gastric and small intestinal phase (Section 2.5). The latter was performed using a kinetic approach to evaluate the time dependency of the lipolysis reaction and micellarization of multiple lipolysis products and carotenoids. Digested emulsions will be further referred to as 'digest'. All digests were ultracentrifuged to harvest the aqueous, 'micellar fraction' containing the bioaccessible lipid species and carotenoids. Initial emulsions, digests and micellar fractions were characterized in terms of lipid and carotenoid content (Sections 2.7 and 2.8). Additionally, the particle charge and size of the initial emulsion, after the gastric phase (chyme) and after 2 h of small intestinal phase (digestion end point) were determined to evaluate the behavior of the oil droplets during gastrointestinal conditions (Section 2.6).

2.1. Materials

Orange carrots (*Daucus carota* cv. Nerac) were bought in a local shop and stored at 4 °C until use. Olive oil was purchased in a local shop. Citrus pectin (CP), Tween 80 (TW) and phosphatidylcholine (PC) were obtained from Sigma Aldrich (Diegem, Belgium). All used chemicals and reagents were of analytical or HPLC-grade and were purchased from Sigma Aldrich (Diegem, Belgium) except for KCl, $MgCl_2(H_2O)_6$, NaOH, heptane, methanol, methyl-*tert*-butyl-ether and ethyl acetate (Acros Organics, Geel, Belgium); KH_2PO_4; $NaHCO_3$, NaCl, H_2SO_4, ethanol, acetone and trimethylamine (Fisher Scientific, Merelbeke, Belgium); HCl, diethylether and isopropanol (VWR, Leuven, Belgium); acetone (Carlo Erba, Val-de-Reuil, France); $CaCl_2(H_2O)_2$ (Chem-Lab, Zedelgem, Belgium) and lipid standards (Larodan, Solna, Sweden). Sample preparations were performed with reagent water (organic free, 18.2 MΩ cm resistance), supplied by a Simplicity™ 150 water purification system (Millipore, Billerica, USA).

2.2. Pectin preparation

High methylesterified CP (HMP) was enzymatically demethylesterified according to the procedure described by Ngouémazong et al. (2011). It was opted use citrus pectin as study vehicle since it is commercially available and has a linear structure, facilitating the interpretation of the obtained data and attributing that to differences in degree of methylesterification only.

Briefly, 0.8% (w/v) HMP was incubated with purified extract of carrot pectin methylesterase (PME) at 30 °C for different time periods, resulting in CP with a medium and low degree of methylesterification (DM) (MMP and LMP, respectively). A thermal treatment was used to inactivate PME (4 min, 85 °C) and was followed by a 48-h dialysis to remove present ions (Spectra/Por®, Molecular weight cut-off = 12–14 kDa). In a final step, pectin samples were lyophilized (Christ Alpha 2–4 LSC, Germany) and stored in a desiccator at room temperature until use. Fourier transform infra-red spectroscopy (IRAffinity-1, Shimadzu, Japan) was used to determine the pectin DM, according to the method described by Kyomugasho, Christiaens, Shpigelman, Van Loey, and Hendrickx (2015). The resulting values for the DM of HMP, MMP and LMP were 82.2% (± 1.2), 38.3% (± 0.9) and 10.4% (± 1.0), respectively and these pectin structures will be