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The effect of pectin on *in vitro* β-carotene bioaccessibility and lipid digestion in low fat emulsions

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ABSTRACT

In this work, we investigated how pectin with different DM, with or without the presence of an additional emulsifier (L-α-phosphatidylcholine), influences on the one hand the *in vitro* bioaccessibility of β-carotene, loaded in the oil phase of an oil-in-water emulsion, and on the other hand the lipid digestion. As a consequence, the relation between the β-carotene bioaccessibility and the lipid digestion was investigated as well. For this research, two types of oil-in-water emulsions have been investigated. The first type contained 5% olive oil enriched with β-carotene and water in which only 2% citrus pectin (CP) (with a DM of 99%, 66% or 14%) was dissolved. In this type, only pectin is present that can function as emulsifier. The second type contained 5% enriched oil and water in which 1% L-α-phosphatidylcholine and 0 or 2% CP (with a DM of 99%, 66% or 14%) were dissolved.

Results show that the influence of pectin DM on the *in vitro* β-carotene bioaccessibility (incorporation of β-carotene in the micelles) and the lipid digestion (incorporation of free fatty acids (FFAs) and monoacylglycerols (MAGs) in the micelles) was dependent on the presence of phosphatidylcholine but was less dependent on the particle size (distributions) or the viscosity. In the emulsions with phosphatidylcholine, an increase of on the one hand the incorporation of β-carotene and on the other hand the incorporation of FFAs and MAGs in the micelles was seen by decreasing the DM of the citrus pectin from 99% to 66%, whereas both incorporations decreased again by decreasing the DM further to 14%. In the emulsions without phosphatidylcholine, an increase of the incorporation of β-carotene into the micelles was seen by decreasing the DM. On the contrary, the incorporation of FFAs and MAGs into the micelles remained. This means that there was a clear relation between the incorporation of β-carotene and the incorporation of FFAs and MAGs in the micelles for the emulsions without phosphatidylcholine, whereas this was not the case for the emulsions containing phosphatidylcholine.

1. Introduction

Soups and sauces, full of vegetables like tomatoes and carrots, are good sources of water, fibres (e.g. pectin), micronutrients such as vitamins and/or pro-vitamins (e.g. carotenoids) and lipids. These lipids are often added to increase the palatability of the food. Besides the risk of obesity when too much lipids are taken, lipids are also important macronutrients, providing energy, essential fatty acids and lipid soluble nutrients (e.g. carotenoids) which are needed in a human diet. A balance should be found between too large intake and uptake of lipids and not taking up essential fatty acids and lipid soluble nutrients like carotenoids (Armand et al., 1996; Bauer, Jakob, & Mosenthin, 2005; Lowe, 1994).

Carotenoids are lipid soluble nutrients, present in fruits and vegetables, which have benefical health effects. Besides their antioxidant properties, they seem to be of interest due to their influence on the modulation of immune responses and the regulation of cell growth (Rock, 1997). Some carotenoids like β-carotene also have a provitamin A-activity. Vitamin A is an important micronutrient for preventing night blindness, good immune function, growth, development and gastrointestinal functioning of the body (Grune et al., 2010; Haskell, 2012). Only a certain amount of the dietary carotenoids will be absorbed and used by the human body due to several factors, e.g. the presence of lipids or fibres (e.g. pectin)

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(McClements & Decker, 2009; Xu et al., 2014). The terms bioavailability or bioaccessibility of a nutrient are used to describe the fraction of this nutrient that is respectively available for utilization in physiological functions and for storage or available for absorption into the small intestinal mucosa (Castenmiller & West, 1998). For lipid soluble nutrients like carotenoids, the conversion into a water-soluble form, like micelles, is important before they can be absorbed into the mucosa. Therefore, the knowledge of lipid digestion is important to understand the absorption of carotenoids. Lipid digestion can be divided into two steps: hydrolysis of the dietary lipids and formation of micelles. Lipase hydrolyses the lipids, mostly triacylglycerols (TAGs), into the hydrolysed products diacylglycerols (DAGs), monoacylglycerols (MAGs) and free fatty acids (FFAs). The efficiency and rate of this step are depending on several factors including the properties of the surrounding medium of the lipid droplets (e.g. viscosity or presence of interacting compounds) and properties of the oil-water-interface (e.g. droplet size, droplet composition, oil type, surface active compounds) (Hu, Li, Decker, & McClements, 2010; McClements & Decker, 2009). McClements, Decker, Park, & Weiss, 2008; McClements, Decker, & Park, 2008 described a number of factors why fibres might interact with this step. Fibres may (i) directly interact with lipase or with co-lipase, reducing the enzyme activity, (ii) form a protective membrane around the lipid droplets, preventing lipase or co-lipase to interact, (iii) increase the viscosity which can increase the duration in the stomach and small intestinal phase but also decrease the transport between substrate and enzymes. Besides these possibilities, some fibres can also function as emulsifiers depending on their structure and properties (Akhtar, Dickinson, Mazoyer, & Langendorff, 2002; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003; Leroux et al., 2003). An emulsifier can form smaller oil droplets by adsorbing at the oil-water interface and decreasing the surface tension, thereby enlarging the surface area available for lipase to interact, resulting in an increase of lipase binding and activity. On the other hand, competitive adsorption processes can occur between the surface active compounds (emulsifiers) and lipase, which can interfere with the binding of lipase to the droplet surface or with the lipase activity (McClements & Decker, 2009; Singh & Ye, 2013).

The lipase hydrolysis products can form micelles together with bile acids, phospholipids and lipid-soluble compounds, like carotenoids. The efficiency and micelle formation rate depend on the properties of the surrounding medium and of the interface as well. Also for this step, McClements et al. (2008) described the reasons why fibres might interact. On the one hand, fibres may increase the system viscosity increasing the residence time in the small intestinal phase and decreasing the transport between the different compounds thereby influencing micelle formation. On the other hand, some fibres can bind bile salts thereby preventing them from incorporation into the micelles or emulsifying the lipids.

The role of the fibre structure on lipid digestion is although not fully understood and seems important because different fibre properties (e.g. length, embranchment, hydrophobicity, or charge) result in differences in e.g. binding properties, pH and viscosity which might be important for lipid digestion (Eastwood & Mowbray, 1976; Falk & Nagyvary, 1982; McClements & Decker, 2009; Verrijssen, Balduyck, et al., 2014). Pectin is a fibre located in the cell wall and middle lamellae of dicotyledonous plants, so present in fruits and vegetables together with carotenoids. Pectin structure largely depends on the plant source, the ripening stage, the processing and storage (Sila et al., 2009). The degree of methyl-esterification (DM) of pectin is of interest because it determines the pectin functionality, such as its hydrophobicity and its charge density which might influence the interactions with all compounds present in the sample or digestion juices (e.g. ions, lipids, lipase, bile acids or micelles).

It has to be noted that besides the influence fibres are assumed to have on lipid digestion, intake of dietary fibre can also protect against diseases like coronary artery diseases, hypertension, colon cancer and diabetes (Mehta & Kaur, 1992; Reiser, 1987).

The aim of this work was to investigate the relation between the *in vitro* β-carotene bioaccessibility and lipid digestion in model systems which might represent (simplified) soups or sauces and allow to study the interactions between different compounds. To better understand the effect of pectin (DM) and phosphatidylcholine on the β-carotene bioaccessibility and the lipid digestion, also structural characteristics, such as the particle size distributions and the viscosity of the different emulsions, were investigated at different stages of digestion. The model systems contain water, enriched olive oil (with β-carotene from carrots), 0 or 1% emulsifier (L-α-phosphatidylcholine, PHC) (from egg yolk) and (0 or 2%) citrus pectin with different structures in terms of DM. In plant-based food products, extra emulsifiers are often added, which makes it of interest to investigate the effect of its addition. Phosphatidylcholine is choosen as extra emulsifier because more and more consumers prefer foods with natural ingredients (like phosphatidylcholine from plant sources) instead of chemically prepared additives. In addition, phospholipids play a role in the micelle formation (McClements & Decker, 2009) and Marisiddaiah, Rangaswamy, and Vallikannan (2011) found improvement of β-carotene bioavailibility in rats by phospolipids. Besides the fact that the tested emulsions represent simplified soups and sauces, it is known that carotenoids can be isolated from natural sources and can be used as nutraceutical ingredients. Because of the hydrophobicity of carotenoids, emulsions are suitable for successfully incorporating the carotenoids into a wide range of food and beverage products. It is therefore interesting to investigate emulsions as study object.

2. Material and methods

2.1. Materials

Citrus pectin (CP) (Sigma Aldrich) was used for the preparation of pectin with different degree of methyl-esterification (DM). Carrots (*Daucus carota* cv. Nerac) were purchased in a local shop and stored at 4 °C before used. Olive oil (extra virgin) was kindly donated by Vandemoortele (Ghent, Belgium). All chemicals and reagents were from Sigma Aldrich, except for NaCl, HCl, urea, anhydrous sodium sulphate and ethanol (from VWR); $CaCl_2.2H_20$, NH_4Cl and $MgCl_2$ (from Merck); hexane, sulphuric acid and acetone (from Chem Lab); glucose and $NaHCO_3$ (from Fisher Scientific); heptane (from Fluka); KCl (from MP Biomedicals) and diethylether (from Riedel-De Haën). All chemicals and reagents were of analytical grade.

2.2. Preparation of citrus pectin with different DM

Citrus pectin (CP) with different DM was prepared by incubating high methyl-esterified CP (DM of 98.6%) (Sigma Aldrich) with purified carrot pectin-methyl-esterase (PME) for 4 min or 30 h, as described by Verrijssen, Balduyck, et al. (2014). The DM values of the pectin samples were measured by using Fourier transform-infrared (FT-IR) spectroscopy (IRAffinity-1, Shimadzu) and were 98.6% (±1.5), 65.6% (±5.8) and 14.1% (±1.1). Therefore, the different pectin samples will be further called “CP99”, “CP66” and “CP14”.

2.3. Preparation of oil-in-water emulsions enriched with β-carotene

The procedure to prepare olive oil enriched with β-carotene from carrots is described by Verrijssen, Vanierschot, et al. (2014).