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Studying the real-time interplay between triglyceride digestion and lipophilic micronutrient bioaccessibility using droplet microfluidics. 2 application to various oils and (pro)vitamins

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ABSTRACT

The kinetics of micellar solubilization of lipophilic micronutrients (bioaccessibility) in relation with triglyceride digestion remains poorly known. To study this interplay in real-time, a droplet microfluidic method was designed and used as reported in the first part of this article series. In this second part, the interplay between the micellar solubilization of (pro)vitamins (beta-carotene or retinyl palmitate) and the digestion of triglyceride oils (tricaprylin TC, or high-oleic sunflower seed oil HOSO, or fish oil FO) during simulated gastrointestinal digestion was investigated. The relation between the release of both micronutrients and of triglyceride lipolytic products was found to be non-linear. The kinetics of beta-carotene was found to follow the kinetics of lipolytic products, depending on the oil type (TC > HOSO > FO). The effect of the gastric phase on the intestinal phase was also found to follow this order, mostly due to partial lipolysis during the gastric phase.

1. Introduction

Micronutrients (minerals, vitamins) are essential to maintain normal functions of human body. However, their absorption, especially that of lipophilic vitamins and carotenoids, is much more variable than that of macronutrients, due to biological and physicochemical factors (Borel, 2003). In the fat-soluble micronutrient class, vitamin A has received an intensive research attention due to its multiple functions in normal growth and development of human body. Vitamin A is notably involved in immune system maintenance, vision health and regulation of cell division (Grune et al., 2010; Haskell, 2012). Vitamin A is present in food in two forms: pre-formed vitamin A (mostly as retinyl palmitate) from animal sources, and provitamin A carotenoids (carotenes, beta-cryptoxanthin) from plant sources. Among provitamin A carotenoids, beta-carotene has the highest vitamin A activity thanks to its unique symmetrical structure (Grune et al., 2010; Haskell, 2012). Nevertheless, in order to achieve their vitamin activity, they need to be available in tissues (bioavailability), what requires many processes: i) release from the food matrix and incorporation in triglyceride droplets, ii) co-digestion with triglycerides, then co-solubilization into mixed micelles (bioaccessibility), iii) transport, processing, and secretion by intestinal cells iv) circulation in the lymph or blood system in lipoprotein. Among these processes, the micellar solubilization is an important prerequisite for transport. However, because fat-soluble micronutrients are poorly soluble in the aqueous gastrointestinal environment, their bioaccessibility may be low and variable depending on many factors involving the food matrix structure and composition (Borel, 2003). Improving bioaccessibility is thus a strategy to enhance the bioavailability of these lipophilic micronutrients.

For the last couple of decades, many works based on *in vitro* digestion were carried out to study the bioaccessibility of beta-carotene in relation with triglyceride digestion (Huo, Ferruzzi, Schwartz, & Failla, 2007; Yi, Zhong, Zhang, Yokoyama, & Zhao, 2015). However, the interplay between the micellar solubilization of lipophilic micronutrients and of lipolytic products remains poorly known. For that matter, emulsion kinetic studies provided insights into the mechanisms of micellar solubilization of beta-carotene (Borel et al., 1996; Nik, Corredig, & Wright, 2011; Mutsokoti et al., 2017). Better than a single end-point measurement, the release profile of bioactive molecules can be obtained by analyzing different incubation time points, but this is challenging due to difficulties in the control of experimental parameters using emulsion, the amount of materials needed, and the required high number of time points. Alternative approaches are scarce and the simultaneous real-time kinetics were established only once, using multiplex coherent Anti-Stokes Raman scattering microspectroscopy (Day, Rago, Domke, Velikov, & Bonn, 2010).

These issues can also be solved using droplet microfluidics. In the first part of this article series, we proposed a lab on a chip method

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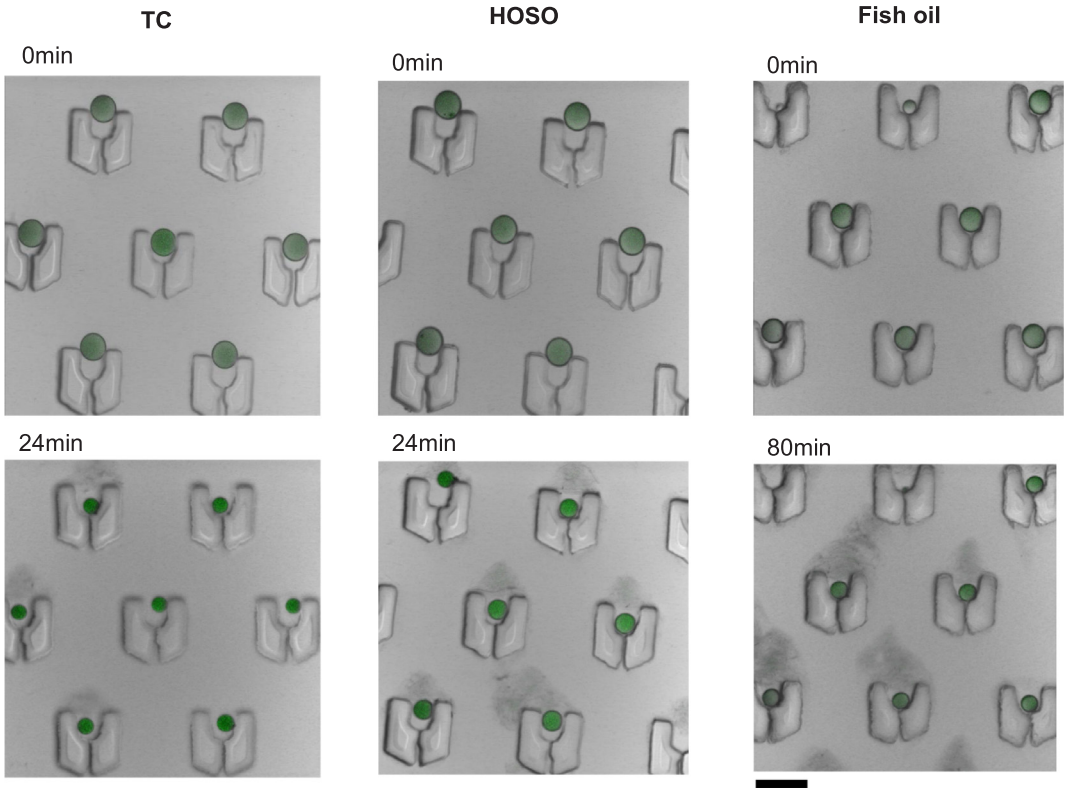

Fig. 1. Images of droplets containing beta-carotene for different oils (TC, HOSO, fish oil) at various intestinal digestion times. The scale bar represents 200 μm.

enabling the simultaneous monitoring of beta-carotene and of tricaprylin lipolytic products in real time. In this second part, we extend this microfluidic approach to other oils and lipophilic micronutrients in order to understand their roles on the kinetic solubilization interplay. Three oils and two (pro)vitamins were tested separately, among which 5 systems were investigated. The full relation between the micellar solubilization of oil lipolytic products and of these micronutrients was established. The effect of the gastric phase on the subsequent intestinal phase was investigated as well.

2. Experimental section

2.1. Materials

Pancreatic lipase (L3126, lipase from porcine pancreas type II, 1.7–8.3 U mg^{-1}), Amano lipase A (534781, lipase from Aspergillus niger, 12 U mg^{-1}, protease activity ≤ 2.5 U mg^{-1}), pepsin (P7012, pepsin from porcine gastric mucosa, 2500 U mg^{-1}), sodium glycodeoxycholate (G9910), tricaprylin TC (T9126), beta-carotene (22040), retinyl palmitate RP (R1512) were provided by Sigma-Aldrich. High-oleic sunflower seed oil HOSO was provided by Vandamme (Belgium). Fish oil FO (1050 TG) was provided by Polaris (France).

2.2. Droplet digestion and lipid monitoring

In this work, digestion of oil droplets containing an added micronutrient was performed using the same microfluidic method described in detail in the first part of this article series. Briefly, monodisperse oil droplets of 100 μm containing an added micronutrient were generated/immobilized in a lab on chip device and then subjected to a semi-dynamic gastrointestinal digestion in the same chip, with a continuous flow (and thus renewal) of the digestive fluids at a flow rate of 50 μL min^{-1}. The digestion of the trapped oil droplets was carried out under controlled temperature of 37 °C inside the digestion chamber, and monitored in real-time (2 min time steps) using a confocal fluorescence microscope (Nikon A1 +) with a 10× objective. All optical parameters were optimized to obtain auto fluorescence intensity of the different micronutrients for quantitative analysis. A laser with an excitation wavelength of 488 nm and a channel with emission window of 500–530 nm were used to obtain the autofluorescence image of BC inside the oil droplets. A transmitted light image for the droplet size was obtained simultaneously using the same excitation beam. Due to its different absorption and emission properties compared to those of BC, a laser with an excitation wavelength of 375 nm and a channel with an emission window of 425–475 nm were used to obtain the autofluorescence image of RP. A transmitted light image for the droplet size was obtained simultaneously using the 488 nm laser already used for BC. The droplet size and fluorescence were measured by image analysis. Micronutrient concentration and release were calculated from these values using a fluorescence calibration curve as explained in the first part of this series.

The digestion was run with either an intestinal phase alone or a gastric phase followed by an intestinal phase. The intestinal fluid was prepared by mixing a buffer solution (100 mM NaH_2PO_4 adjusted to pH 7.0) with pancreatic lipase at 4 mg mL^{-1} and a bile salt (sodium glycodeoxycholate) at 5 mg mL^{-1}. When a gastric phase was performed prior to the intestinal phase, it was carried out for 2 h with a gastric fluid prepared by mixing 0.03 mg mL^{-1} lipase from *Aspergillus niger* (lipase AN), and 0.6 mg mL^{-1} pepsin in a 100 mM KCl buffer adjusted to pH 3.0.

Three triglycerides composed of different fatty acids were tested: pure tricaprylin (TC, C8:0), or high-oleic sunflower seed oil (HOSO, mainly C18:1), or a fish oil rich in DHA (FO, mainly C22:6). Two micronutrients were tested separately (same initial concentration of 0.2 wt % in the oils): beta-carotene (provitamin A) or retinyl palmitate (preformed vitamin A). For each system, two to three independent digestions were conducted with the monitoring of seven individual droplets for each digestion. A distinct microfluidic device was used for each digestion to ensure identical initial conditions. The variability of the measurements was very low between the seven droplets monitored