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Physicochemical parameters that influence carotenoids bioaccessibility from a tomato juice

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ABSTRACT

In vitro digestion models have been developed to estimate carotenoid bioavailability but most do not consider that their diffusion from fruit matrix to the lipid phase of the bolus could be a limiting step. Therefore we designed a model in which tomato juice is mixed with oil or oil/water emulsions, and the carotenoids diffusing to oil are measured by spectrometry. Temperature, pH and tomato juice/peanut oil ratio were evaluated for their influence on carotenoid diffusion. When oil/tomato ratio was between 0.11 and 1, extraction of lycopene was limited by the saturation of the oil phase. With a large excess of oil, diffusion was also limited, as only 31 ± 1% of lycopene could be extracted from the juice. Diffusion did not vary significantly with pH but doubled when temperature rose from 10 °C to 37 °C.

When the juice was mixed in an emulsion stabilised with bovine serum albumin or phospholipids the maximum extraction decreased to 14.5 ± 0.2% and 18.5 ± 1.5% respectively, indicating that in addition to the saturation of the oil phase at low oil/tomato ratio and in addition to intrinsic properties of the tomato juice in non-saturating conditions, lycopene diffusion was limited by the structure of the interface in emulsions.

1. Introduction

Carotenoids are natural fat-soluble pigments synthesised by plants, found in relatively high amounts in numerous fruits and vegetables, which are the source of the four main carotenoids in the human diet: lycopene, β-carotene, β-cryptoxanthin and lutein, but also of many of the 750 identified members of this broad family. These compounds have been identified as being beneficial to health by epidemiological studies (Bugianesi et al., 2004; Rao & Rao, 2007). Some activities are straightforward (provitaminic (vit A) properties for some of them, (especially β-carotene) and protection against macular degeneration for lutein), but remain less understood for others. However, they are poorly absorbed from plant sources. Their bioavailability is increased by processing (Hedren, Mulokozi, & Svanberg, 2002; Svelander et al., 2010), which sparked an interest in understanding mechanisms which modify this bioavailability (Borel, 2009), and the effects of the presence of oil (Hedren et al., 2002).

The absorption of carotenoids follows four steps: (i) they are extracted from the food matrix during mastication, (ii) they enter the lipid phase of the gastric emulsion, (iii) they are incorporated in micelles stabilised by biliary salts in the gut, and (iv) micelles are absorbed by gut cells in order to reach the plasma circulation. It was long suggested that this last step consisted of a simple passive diffusion through gut cells (Hollander, 1978). However, recent advances indicate that carotenoid absorption consists of a set of complex mechanisms involving specific transporters (some being shared with cholesterol routes), incorporation in chylomicrons, transportation to liver, incorporation in lipoproteins before distribution to peripheral tissues. All these steps involve molecular changes of carotenoids, such as esterification or cleavage (Borel et al., 2011) The factors limiting absorption during this process have been summarised in the mnemonic SLAMENGHI where every letter identifies a limiting factor (Castenmiller & West, 1998). Among them, the "M" stands for the interactions with matrix in which the carotenoids are incorporated. This factor is the focus of our study.

Tomato is one of the main dietary sources of carotenoids in the human diet, due to its high lycopene content, and to its large consumption across the world (40 million T in 2009 (FAO)). Lycopene is an unsaturated acyclic lipophilic carotenoid with 11 linearly conjugated double bonds (Castro 2007).

Models were developed to evaluate bioavailability by reproducing the digestion time course (Liu, Glahn, & Liu, 2004; Epriliati, D'Arcy, & Gidley, 2009; Van Buggenhout et al., 2010). Most include

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steps enabling the transfer of carotenoids from the food matrix to micelles by mimicking the food maceration and its sequential changes which occur in the gastrointestinal tract. Micelles are then recovered by centrifugation and quantified for their carotenoid contents (Garrett, Failla, & Sarama, 1999). Various gastric juices were tested, including or not digestive enzymes (pepsin, lipase at varying concentrations). Simulated gastric juices can be composed of porcine pepsin at 40 mg ml^{-1} in 0.1 M HCl (Garrett et al., 1999), or pepsin solution at 5 g l^{-1} in saline at physiological concentrations (Hedren, Diaz, & Svanberg, 2002), or 300 mg ml^{-1} pepsin solution in 0.2 M HCl–KCl (Serrano, Goñi, & Saura-Calixto, 2005).The digestion is then completed with the use of intestinal juices containing pancreatin (2–4 g l^{-1}) and bile salt (from extract or porcine bile salt) (Garrett et al., 1999; Hedren et al., 2002), to allow for the micelle formation. Buffer composition and sequential change of pH were also tested (from 1.5 to 7.5), to better simulate the evolution of pH during intestinal digestion (Hedren et al., 2002; Serrano et al., 2005). For some models, a second step consisting of applying these isolated micelles to a monolayer of Caco-2 human intestinal cells is used to evaluate the efficiency of the intestinal uptake.

Whatever their complexity, none of these models studied specifically the release of the carotenoids from food matrix to the lipid phase. In particular, they often begin with a fine grinding of the food to be tested, while vegetables are often ingested as rather large particles (Borel, 2003; Jalabertmalbos, Mishellanydutour, Woda, & Peyron, 2007). Similarly, the proportion and type of fat present is rarely addressed, though it has been shown to affect bioavailability and may vary in the bolus according to meal composition. However, the influence of oil in meal has been assessed by Colle, Van Buggenhout, Lemmens, Van Loey, and Hendrickx (2012), and the lipid/matrix ratio appears as a limiting factor for diffusion from the matrix to the lipid phase. Lastly, all the process is carried out at 37 °C, to mimic the human gut, but diffusion may start in the food before digestion at higher temperature. Therefore, we developed (using a commercial tomato juice as example) a simple model that can be used to assess the impact of physicochemical conditions on the limits to carotenoid diffusion from food to a lipid phase.

2. Material and methods

2.1. Chemicals

Lycopene (98.8%) was from Extrasynthèse (Genay, France); β-carotene (97%), (all-*E*)-β-apo-8′-carotenal (96%), bovine serum albumin (BSA), egg phospholipids (PL) and all buffers were from Sigma–Aldrich (Taufkirchen, Germany). Methanol, and methyl *tert*-butyl ether were HPLC grade from Merck (Darmstadt, Germany). Ethyl acetate, dichloromethane, hexane were analytical grade from VWR international (Fontenay-sous-bois, France).

2.2. Food products

One-litre bottles of peanut oil and 33-ml bottles of tomato juice were purchased at a local supermarket taking care to have products from a single production batch. They were stored at 4 °C in the dark. Oil composition and stability along the experiments were controlled by gas chromatography–mass spectrometry (GC–MS) (see conditions below). Carotenoids concentration and composition of tomato juice were controlled by HPLC throughout the experiments (see condition below).

2.3. Tomato juice analysis

Particle sizes of the juice were measured using a Mastersizer 2000 (Malvern Instruments, Great Malvern, UK). Approximately 1 g of tomato juice was introduced in the stirred tank filled with water corresponding to a laser obscuration of 20%. Volumetric particle size distributions were calculated from the distribution intensity.

Carotenoid extraction was carried out using the method described by Serino et al. (2009) with few modifications: about 400 mg of tomato puree (determined to the nearest 10 mg) were introduced to a 2-ml Eppendorf tube containing 80 μg of 0.1-mm zirconia/silica beads (BioSpec Products, Bartlesville OK) and 10 μl of a solution at 0.2 mg ml^{-1} of β-apo-8′-carotenal used as internal standard. Three reagents were then successively added, each time followed by a 40-s shaking using a FastPrep® homogeniser (Thermo Scientific, Waltham, A) and 2 min centrifugation at 10,000*g* (4 °C). Reagents were (i) 100 μl of saturated aqueous NaCl solution/50 μl *n*-hexane, (ii) 200 μl of dichloromethane and (iii) 800 μl of ethyl acetate. The organic fraction was filtered and 20 μl were injected into an HPLC equipped with a diode array detector (SPD-M20A; Shimadzu, Kyoto, Japan). The column used was C30 (250 × 4.6 mm, particle size 3 μm; YMC Co, Kyoto, Japan). The temperature of the column oven was 30 °C, flow rate 1.4 ml min^{-1}, injection volume 20 μl. Mobile phase was methanol and a gradient was achieved with MTBE, starting with 100% methanol, and increasing to 60% MTBE in 20 min, remaining isocratic for 5 min, then back to 100% methanol in 5 min and finally remaining at 100% methanol for 10 more minutes. Compounds were detected from 280–600 nm, and quantified at 290 nm (for phytoene), 350 nm (for phytofluene), 450 nm (for apo-carotenal, β-carotene and lutein) and 503 nm (for lycopene). β-Carotene, (all-*E*)-lycopene and β-apo-8′-carotenal were identified by co-elution of standards. The others, and especially (*Z*)-lycopene isomers, were tentatively identified according to their UV spectra and quantified as equivalent of (all-*E*)-lycopene. Quantification was performed relative to the peak area of the internal standard (β-apo-8′-carotenal). Response factors were calculated for lycopene and β-carotene from standard solutions.

2.4. Oil analysis

Oil was analysed by GC–MS after saponification: 20 mg of oil were mixed with 500 μl of toluene, 1 ml of BF_3/methanol (14% w/w) in a vial, which was heated at 80 °C for 1 h. Then, 1 ml of hexane was added, and the mix was flushed with 0.2 M sodium bicarbonate. The organic phase was injected into a QP 2010 GC–MS (Shimadzu). The column was a UBWAX (30 m × 0.25 mm, 0.50 μm). Carrier gas was hydrogen (flow rate 36 cm s^{-1}). Ionisation was achieved by electron impact at 70 eV and mass scanned from 50 to 360 atomic mass units at 2 scan s^{-1}. The injector was heated at 250 °C and injection was in split mode with a ratio of 1/20. The temperature program was 50 °C for 1 min, increasing to 150 °C at 10 °C min^{-1}, increasing to 230 °C at 3 °C min^{-1}, isothermal at 230 °C for 5 min, increased to 240 °C at 20 °C min^{-1} and finally isothermal at 240 °C for 10 min. Total duration of the analysis was 53 min.

2.5. Preparation of oil/water emulsions

Quantity of oil in emulsion, quantity of energy applied with ultrasound for emulsification, and concentrations of surfactant were studied.

Bovine serum albumin (BSA) emulsion: BSA was dissolved in acetate buffer (5 mM, pH 4.7) at 125 mg l^{-1}, 250 mg l^{-1} or 500 mg l^{-1} prior to mixing with oil. The BSA solution (20 ml) was mixed with oil (5 g, 7.5 g or 15 g). The mixing included first a treatment with an Ultra Turrax T25 Basic at 11,000 rpm (1 min), then emulsification was completed using an ultrasonic (US) probe Vibra Cell 75022 (Bioblock Scientific, Illkirch, France) at 30 W, 20 kHz.