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The type and quantity of lipids present during digestion influence the *in vitro* bioaccessibility of lycopene from raw tomato pulp

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ABSTRACT

This study elucidates the impact of the type and quantity of lipids, added upon digestion of raw tomato pulp, on the bioaccessibility of lycopene. Lycopene bioaccessibility was studied by measuring the micellarization during *in vitro* digestion. Coconut oil, palm oil, cocoa butter, olive oil, sunflower oil and fish oil were selected because of their distinctly different fatty acid composition. Upon adding 5% of lipid to raw tomato pulp, all tested lipids significantly improved the lycopene bioaccessibility. The largest increase in lycopene bioaccessibility was noticed after supplying 5% of sunflower oil, followed by olive oil and cocoa butter (not all differences were significant). A slightly smaller increase was observed when fish oil, coconut oil and palm oil were used. In addition, the effect of different quantities (0–10%) of coconut oil, olive oil and fish oil was examined. Over the entire concentration range, increasing the amount of coconut oil increased the lycopene bioaccessibility, while the highest bioaccessibility was found using 1 and 2% of respectively fish oil and olive oil. Moreover, depending on the amount of added lipid, the type of lipid resulting in the highest lycopene bioaccessibility differed. The results obtained clearly indicate that lycopene bioaccessibility depends both on the type and on the quantity of the lipid present during *in vitro* digestion of raw tomato pulp.

1. Introduction

Lycopene is one of the dietary carotenoids relevant for human health. Carotenoids such as β-carotene and β-cryptoxanthin serve as a source of vitamin A (Fernández-García et al., in press). However, since lycopene is devoid of provitamin A activity, its promising implications for human health must be attributed to other mechanisms of actions. Due to its polyene nature consisting of 11 conjugated double bounds, lycopene is found to be an effective antioxidant (Conn, Schalch, & Truscott, 1991; Di Mascio, Kaiser, & Sies, 1989). Moreover, Erdman, Ford, and Lindshield (2009) reviewed that lycopene or lycopene containing products have the ability to decrease cancer cell growth and inflammation, increase gap junctional communication, inhibit androgen/estrogen signaling, induce detoxification enzymes, decrease cell surface adhesion and intima-media thickness, decrease serum cholesterol and decrease C-reactive protein. All these mechanisms of action can contribute to reduce the risk of chronic diseases (Bramley, 2000; Rao & Agarwal, 1999) such as cancers (Giovannucci, 1999) and cardiovascular diseases (Willcox, Catignani, & Lazarus, 2003). Humans are unable to synthesize lycopene *de novo* and therefore depend on their diet to supply this compound. The main dietary source of lycopene are tomatoes and tomato-based products (Bramley, 2000). In this regard, bioavailability of lycopene is of importance. Carotenoid bioavailability includes (i) release of carotenoids from the food matrix and conversion into a potentially absorbable form (=bioaccessibility), (ii) absorption, (iii) metabolism, (iv) transport and tissue distribution, and (v) bioactivity (Faulks & Southon, 2005; Fernández-García et al., in press). Carotenoid uptake follows the same fate as lipids and they need to be incorporated into micelles to be absorbable (Borel, 2003). Next to bile salts and biliary phospholipids, mixed micelles contain free fatty acids and monoglycerids (MGs) resulting from hydrolysis of triglycerides (TGs) (Yonekura & Nagao, 2007). Therefore ingestion of fat along with carotenoids is thought to be crucial for the absorption of carotenoids (van het Hof, West, Weststrate, & Hautvast, 2000). The importance of dietary fat for the bioaccessibility and bioavailability of β-carotene is already well established. *In vitro*, it was demonstrated that the presence of rapeseed oil during cooking of carrot pulp and the presence of sunflower oil during cooking of green leafy vegetables increased the release of β-carotene during digestion (Hedrén, Diaz, & Svanberg, 2002; Hedrén, Mulokozi, & Svanberg, 2002). Similarly, the micellarization of β-carotene was found to increase by the addition of olive oil to carrot samples during cooking (Hornero-Méndez & Mínguez-Mosquera, 2007) and by the presence of a minimal amount of lipid in a salad puree (Huo, Ferruzzi, Schwartz, & Failla, 2007). *In vivo*, Brown et al. (2004) observed that the appearance of β-carotene in plasma chylomicrons was higher after the ingestion of salads with full fat than with reduced fat salad dressing. In addition, higher plasma

Abbreviations: TG, triglyceride; MG, monoglyceride.

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β-carotene levels were found after intake of a high fat diet compared with a low fat diet (Dimitrov et al., 1988) and after consuming salad with avocado oil compared with without oil (Unlu, Bohn, Clinton, & Schwartz, 2005). For lycopene, some recent studies indicated that lipids can positively influence its bioaccessibility and bioavailability. After the *in vitro* digestion of a salad puree (Huo et al., 2007), a meal containing courgette, red pepper and spinach (O'Connell, Ryan, O'Sullivan, Aherne-Bruce, & O'Brien, 2008) and a tomato soup (Colle et al., 2011), a significantly enhanced micellarization of lycopene was found in the presence of oil. Likewise, in human subjects, the lycopene bioavailability was improved by adding avocado oil to a salad (Unlu et al., 2005) and by using full fat salad dressing instead of reduced fat salad dressing (Brown et al., 2004). What is less clear, is the amount of dietary fat needed for optimal carotenoid bioavailability. Only a small amount of fat is needed to ensure uptake. The minimum amount of fat required depends, however, on the physicochemical characteristics of the fat-soluble compounds (Roodenburg, Leenen, van het Hof, Weststrate, & Tijburg, 2000). Only one study (Huo et al., 2007) suggested that the bioaccessibility of carotenoids increases more by the consumption of long chain-triglycerides than when short/medium chain triglycerides are ingested. Furthermore, controversial results have been published on the effect of the fatty acid degree of saturation on the carotenoid bioavailability (Clark, Yao, She, & Furr, 2000; Hu, Jandacek, & White, 2000; Huo et al., 2007; Lee, Thurnham, & Chopra, 2000).

Therefore, the present study was designed to elucidate the effect of the type and quantity of lipids, added upon digestion of raw tomato pulp, on the bioaccessibility of lycopene. A tomato pulp containing 5% of coconut oil, palm oil, cocoa butter, olive oil, sunflower oil or fish oil was prepared to investigate the effect of the type of lipids. Moreover, different amounts (0–10%) of coconut oil, olive oil and fish oil were added to evaluate the effect of the quantity of the lipids. Lycopene bioaccessibility was studied by quantifying the amount of lycopene that was transferred from the food matrix to the aqueous micellar phase during *in vitro* digestion.

2. Materials and methods

2.1. Materials

A number of oils and fats with different fatty acid composition were selected. Coconut oil, palm oil, olive oil, sunflower oil and fish oil were donated by Vandemoortele (Gent, Belgium). Cocoa butter was a kind gift of Barry Callebaut (Lebbeke-Wieze, Belgium). The fatty acid composition of the lipids is listed in Table 1. None of the lipids contained lycopene (analysed using RP-HPLC) (data not shown). Red ripe tomatoes (var. Patrona) were harvested in Spain in 2010. They were washed, dried, quartered, frozen in liquid nitrogen and stored at −40 °C. Upon use, a tomato pulp free from seeds and skin was prepared. Hereto, the tomato quarters were thawed, mixed (3 times 5 s) (Büchi Mixer B-400, Flawil, Switzerland) and sieved (pore diameter 1 mm) in the absence of added lipids. All chemicals and reagents used were of analytical or HPLC grade.

Table 1
Fatty acid composition of lipids used.

Fatty acid (%)	Coconut oil	Palm oil	Cocoa butter	Olive oil	Sunflower oil	Fish oil
C8	7.0	nd	nd	nd	nd	nd
C10	6.0	nd	nd	nd	nd	nd
C12	46.0	0.2	nd	0.0	0.0	nd
C14	19.0	1.0	0.1	0.0	0.1	5.6
C16	9.0	43.0	24.9	11.4	6.0	15.4
C18	3.0	4.5	37.8	2.9	4.0	3.3
C18:1	7.5	40.4	33.3	74.4	28.6	13.9
C18:2	2.0	9.0	2.7	8.9	60.0	1.6
C18:3	nd	0.1	1.2	0.6	0.3	1.0
C20	nd	0.4	nd	0.5	0.1	1.8
C20:1	nd	0.1	nd	0.3	0.1	5.3
C20:5	nd	nd	nd	nd	nd	9.4
C22	nd	0.1	nd	0.2	0.8	1.4
C22:6	nd	nd	nd	nd	nd	13.5
Others	0.5	1.2	nd	0.8	nd	27.8

nd: not detected.

2.2. In vitro digestion procedure

To determine the lycopene bioaccessibility, a two step (gastric and small intestinal) *in vitro* digestion procedure (Colle, Van Buggenhout, Van Loey, & Hendrickx, 2010) was carried out. Immediately prior the *in vitro* digestion, a certain amount (0–10%) of lipid was added to freshly prepared tomato pulp so that the total sample weight was 5.0 g (tomato pulp and lipid). This was gently mixed with 5.0 ml stomach electrolyte solution (0.30% NaCl, 0.11% KCl, 0.15% $CaCl_2$ $2H_2O$, 0.05% $KHPO_4$, 0.07% $MgCl_2$ $6H_2O$ in water) and 5.0 ml NaCl/ascorbic acid solution (0.9% NaCl, 1% ascorbic acid) in a brown falcon tube. The pH of this mixture was adjusted to pH 4 ± 0.05 with 1 M HCl or 1 M $NaHCO_3$. Next, 5.0 ml pepsin solution (0.52% porcine pepsin in electrolyte solution) was added and the headspaces of the tubes were flushed with nitrogen. The samples were incubated at 37 °C during 30 min, while shaking end-over-end. Subsequently, the pH was lowered to pH 2 ± 0.05. The headspaces were flushed again with nitrogen prior to continuing incubation for 30 min at 37 °C. To mimic the small intestinal digestion, the pH was raised to pH 6.9 ± 0.05 and a solution of pancreatin, lipase and bile salts (3 ml) (0.4% porcine pancreatin, 0.2% porcine pancreas lipase, 2.5% bile extract, 0.5% pyrogallol and 1% α-tocopherol in water) was added. Once again, the headspaces of the tubes were flushed and the samples were incubated during 2 h at 37 °C.

Following exposure to the digestive conditions, the aqueous micellar phase was separated from the undigested oil droplets and from the undigested tomato pulp by centrifugation (L7 Ultracentrifuge, Beckman, Palo Alto, Calif., U.S.A.) at 165,000 g during 1 h and 5 min at 4 °C. The aqueous fraction was collected and filtered (Chromfil PET filters, 0.20 μm pore size, 25 mm diameter) to remove any crystalline lycopene or lipid. For each sample, the *in vitro* digestion procedure was carried out in quadruple. The lycopene content in the aqueous phase was determined as described below.

2.3. Lycopene quantification

The total lycopene content in the tomato pulp and in the micellar phase after digestion was determined spectrophotometrically following extraction. The extraction procedure was based on the method described by Sadler, Davis, and Dezman (1990). The micellar phase or 2.0 g tomato pulp was stirred with 0.5 g NaCl and 50 ml extraction solvent during 20 min at 4 °C. The extraction solvent consisted of hexane, acetone, ethanol (50:25:25 v:v:v) and butylated hydroxytoluene (0.1%). Next, reagent grade water (15 ml) was added and stirring was continued for 10 min. The hexane layer (25 ml), containing lycopene, was separated from the polar phase in a separation funnel, collected and filtered (Chromfil PET filters, 0.20 μm pore size, 25 mm diameter). The extraction procedure was performed under subdued light to prevent lycopene degradation. Immediately after extraction, the absorbance of lycopene was measured at 472 nm using hexane as a reference. The total lycopene concentration was calculated using the Beer–Lambert law:

$$C = \frac{A \times 10^4}{E_{1\,cm}^{1\%} \times l}$$

With C the total lycopene concentration (μg/ml), A the absorbance at 472 nm, $E_{1\,cm}^{1\%}$ the extinction coefficient of lycopene in