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# Novel targeted approach to better understand how natural structural barriers govern carotenoid *in vitro* bioaccessibility in vegetable-based systems

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ABSTRACT

An experimental approach, allowing us to understand the effect of natural structural barriers (cell walls, chromoplast substructures) on carotenoid bioaccessibility, was developed. Different fractions with different levels of carotenoid bio-encapsulation (carotenoid-enriched oil, chromoplasts, small cell clusters, and large cell clusters) were isolated from different types of carrots and tomatoes. An *in vitro* method was used to determine carotenoid bioaccessibility.

In the present work, a significant decrease in carotenoid *in vitro* bioaccessibility could be observed with an increasing level of bio-encapsulation. Differences in cell wall material and chromoplast substructure between matrices influenced carotenoid release and inclusion in micelles. For carrots, cell walls and chromoplast substructure were important barriers for carotenoid bioaccessibility while, in tomatoes, the chromoplast substructure represented the most important barrier governing bioaccessibility. The highest increase in carotenoid bioaccessibility, for all matrices, was obtained after transferring carotenoids into the oil phase, a system lacking cell walls and chromoplast substructures that could hamper carotenoid release.



## 1. Introduction

Epidemiological studies often relate a healthy lifestyle, in which the consumption of adequate amounts of fruit and vegetables is important, to a reduced risk of chronic diseases such as cardiovascular diseases and cancers (Key, 2011; Ness & Powles, 1997; Steinmetz & Potter, 1996; Van't Veer et al., 2000). The health-related benefits that are associated with the consumption of fruit and vegetables, can be attributed to the presence of various bioactive compounds, of which micronutrients form an important class (Key, 2011; Van't Veer et al., 2000). Carotenoids, a group of natural food pigments, are major contributors to the nutritional value of several fruits and vegetables (Rao & Rao, 2007).

Carotenoids are isoprenoid compounds which typically have a tetraterpenoid structure, implying a long chain of conjugated double bonds. Due to their chemical structure, carotenoids are highly lipophilic molecules (Britton, 1995). In fruit and vegetable tissues, specific structures are developed in chloroplasts and chromoplasts to sequester and store large amounts of carotenoids (Vishnevetsky, Ovadis, & Vainstein, 1999). Their lipophilic nature and their specific localisation in plant tissues (i.e. attached to cellular components and surrounded by organelle membranes, cell membrane and cell wall), hamper the absorption of carotenoids from fruits and vegetables in the human tract (Rich et al., 2003). This has been and still is an issue for food technologists and food processors. In this context, the concepts of carotenoid bioaccessibility and bioavailability are to be defined. Carotenoid bioaccessibility refers to the fraction of ingested carotenoids that is released from the food matrix and incorporated into micelles during digestion in the gastrointestinal tract, and thus becomes available for intestinal absorption. The amount of carotenoids that is bioavailable is always less than the amount that is bioaccessible, since carotenoid bioavailability additionally takes into account the fraction that is available for utilisation in normal physiological functions or for storage in the human body (Holst & Williamson, 2008; Parada & Aguilera, 2007).

In the literature, several studies describe the carotenoid bioaccessibility in fruit- and vegetable-based food products (e.g. Granado-Lorencio et al., 2007; O'Connell et al., 2007; O'Sullivan et al., 2010; Reboul et al., 2006; Ryan, O'Connell, O'Sullivan,

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Aherne, & O'Brien, 2008; Veda, Kamath, Platel, Begum, & Srinivasan, 2006; etc.). However, only a few authors have linked the observations for carotenoid bioaccessibility to structural characteristics of the food products (e.g. Lemmens, Van Buggenhout, Oey, Van Loey, & Hendrickx, 2009 (carotenoid bioaccessibility – pectin properties); Bengtsson, Brackmann, Enejder, Alminger, & Svanberg, 2010 (carotenoid bioaccessibility – microstructure); Colle, Van Buggenhout, Van Loey, & Hendrickx, 2010 (carotenoid bioaccessibility – strength of fibre network); Lemmens, Van Buggenhout, Van Loey, & Hendrickx, 2010 (carotenoid bioaccessibility – particle size); Tydeman et al., 2010 (carotenoid bioaccessibility – microstructure); Knockaert, Lemmens, Van Buggenhout, Hendrickx, & Van Loey, 2012 (carotenoid bioaccessibility – microstructure)). Reviews by Waldron, Parker, and Smith (2003), Parada and Aguilera (2007) and Van Buggenhout et al. (2010) have stressed the importance of the association between food structure and nutrient bioaccessibility. However, studies identifying and directly investigating the role of structural barriers against carotenoid bioaccessibility in fruit- and vegetable-based food products are lacking. In our opinion, such studies might give useful explanations and better insights of how crucial structural parameters can determine carotenoid bioaccessibility in fruit- and vegetable-based food products. Recently, some initiatives in this direction have been taken. Schweiggert, Mezger, Schimpf, Steingass, and Carle (2012) studied the relationship between chromoplast morphology and carotenoid bioaccessibility in different matrices, and found a strong correlation between the physical state of the chromoplast substructures and the efficiency of carotenoid release during digestion. Jeffery, Holzenburg, and King (2012) and Jeffery, Turner, and King (2012) studied different fruit and vegetable purees microscopically (cell wall thickness, cell size) (Jeffery, Holzenburg, et al., 2012) and they tried to link these observations with the carotenoid bioaccessibility results obtained for the same purees (Jeffery, Turner, et al., 2012). They concluded that the cell wall and the chromoplast substructures form the most important barriers against carotenoid release during digestion. Moreover, it turned out that a high amount of large plant cells, a low density of cell wall material and a high concentration of plastoglobuli (containing the carotenoids) are factors favouring carotenoid bioaccessibility (Jeffery, Turner, et al., 2012). However, for these experiments, it should be kept in mind that, during the mixing/blending process to obtain fruit and vegetable purees, a complex environment is created, which could lead to difficulties in the interpretation of the results. For example, it is possible that additional structural networks are being formed during mixing which entail new processed-induced barriers against carotenoid release during digestion, next to and/or replacing the natural barriers present in the fruit and vegetable matrix. Such examples have already been cited by Colle, Van Buggenhout, Lemmens, Van Loey, and Hendrickx (2012) and Anese, Mirolo, Beraldo, and Lippe (2013), respectively, in the framework of the effect of high pressure homogenisation and ultrasound treatments on tomato pulp microstructure and lycopene *in vitro* bioaccessibility.

Therefore, in this study, a specific experiment (including different matrices, different types of carotenoids, and different levels of bio-encapsulation) was designed to evaluate the role of natural barriers in carotenoid bioaccessibility. The fruit and vegetable matrices included in this study (orange carrots, red carrots, orange tomatoes, red tomatoes) were not used as such (e.g. as a puree), but specific fractions were isolated, each representing a different number of barriers encapsulating the carotenoids. The fractions included a carotenoid-enriched oil, a chromoplast, a small/single cell cluster and a large cell/multicellular cluster. In this way, a systematic and detailed understanding of the role of different structural barriers for the carotenoid bioaccessibility could be obtained. Carotenoids with different polarities (β-carotene, ζ-carotene and lycopene) in different matrices (e.g. carrot, tomato) were included.

## 2. Materials and methods

### 2.1. Materials

Red tomatoes (*Lycopersicon esculentum* cv. Patrona) were obtained from a Spanish supplier. Orange tomatoes (*Lycopersicon esculentum* cv. Bolzano) were purchased in an auction in Mechelen, Belgium. The two types of tomatoes were cut, frozen with liquid nitrogen, and stored at −40 °C, until the start of the experiments.

Orange carrots (*Daucus carota* cv. Nerac) and red carrots (unknown Indian variety) were obtained fresh from local shops in Belgium and stored briefly at 4 °C, prior to their use for the experiments.

### 2.2. Experimental set-up

In order to study the different physical barriers that determine the carotenoid *in vitro* bioaccessibility, various fractions from red tomatoes, red carrots, orange carrots and orange tomatoes were isolated. The fractions prepared include a carotenoid-enriched oil fraction, a chromoplast fraction, a small cell cluster fraction, and a large cell cluster fraction. The concomitant physical barriers surrounding the carotenoids were assumed to be the chromoplast structure/organisation, and (multiple) cell membranes and cell walls. An *in vitro* bioaccessibility assay was performed on each fraction. Light microscopy was used to visualise the chromoplast and small cell cluster fractions and to observe the structural characteristics of barriers involved. Fig. 1 represents a schematic overview of the experimental set-up.

### 2.3. Preparation of carotenoid-containing fractions with different barrier properties

#### 2.3.1. Carotenoid-enriched oil fraction

Lycopene from red carrots and red tomatoes, β-carotene from orange carrots and ζ-carotene from orange tomatoes were extracted in olive oil.

To produce carotenoid-enriched oil fractions, carrots were peeled, cut into small pieces, mixed with deionized water (1:1) and blended (Waring Commercial, Torrington, CT, USA) for 1 min. Tomatoes were thawed, peeled, mixed three times (Büchi B-400 mixer, Flawil, Switzerland) for 5 s and then sieved to remove the seeds.

According to the method described by Colle et al. (2010), the obtained carrot and tomato purees were homogenised (Panda 2 K, Gea Niro Soavi, Parma, Italy) at 1000 bar for one cycle to decrease the particle size and facilitate the release of carotenoids from the matrix. Purees were then mixed with olive oil (ratio puree-oil 5:1) for 5 h while rotating, end-over-end. The carotenoid-enriched oil fraction was separated by centrifugation (J2 – HS centrifuge, Beckman, J2 – HS centrifuge, Palo Alto, CA, USA) at 18,900*g*, 4 °C for 15 min. The carotenoid-enriched oil fraction was isolated and emulsified (5% carotenoid-enriched oil in water emulsion) with a 1% of L-α-phosphatidylcholine solution. The emulsion was stabilized by homogenisation (Gea Niro Soavi, Parma, Italy) at 1000 bar for one cycle.

#### 2.3.2. Chromoplast fraction

The chromoplast fraction was obtained by following the procedure of Hansen and Chiu (2005) with some modifications. Both types of carrots and tomatoes (previously defrosted) were cut into pieces and mixed in a blender (Waring Commercial, Torrington, CT, USA) for 5 s with 0.05 M EDTA solution (1:1 ratio).

The obtained purees were filtrated using a cheesecloth. The filtrate was centrifuged (Beckman, J2-HS Centrifuge, Palo Alto, CA,