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Role of carotenoid type on the effect of thermal processing on bioaccessibility

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ABSTRACT

Cell walls and chromoplast substructures constitute natural structural barriers governing carotenoid bioaccessibility. In order to enhance carotenoid bioaccessibility, thermal processes were applied to fractions surrounded by different levels of structural barriers. The matrices studied were orange carrots, red carrots, red tomatoes and atomic red carrots. In the case of carrots, no effect of thermal treatments on carotenoid bioaccessibility at the chromoplast level was obtained. However, in the case of tomatoes, lycopene bioaccessibility decreased upon thermal processing of chromoplasts. At the cell cluster level, low intensities of thermal processing resulted in a decrease of β-carotene and lycopene bioaccessibility. Nonetheless, at high intensities of thermal processing, only β-carotene bioaccessibility was increased. This observation was confirmed by the results obtained in the matrix rich in both types of carotenoids (atomic red carrots). It was therefore suggested, that the type of carotenoid constitutes an important factor determining the effect of thermal processing on their bioaccessibility.

1. Introduction

Carotenoids are lipophilic molecules that occur naturally in fruits and vegetables, accounting for their characteristic colors. These compounds possess important biological activities such as antioxidant capacity and pro-vitamin A activity (in the case of α-carotene and β-carotene), characteristics related to health promoting effects such as a reduced chance to develop cancer and cardiovascular diseases (Astorg, 1997; Krisnky, 1993). To exert these positive effects, carotenoids should be readily absorbed to reach their site of action. However, before being absorbable during digestion, carotenoids have to overcome some physical structural barriers within the food matrix that hinder their release. As carotenoids are located within the chromoplast organelles, the organization and localization within the chromoplast substructure, as well as the cell wall, constitute the two main natural structural physical barriers that govern carotenoid release (Jeffery, Holzenburg, & King, 2012; Palmero et al., 2013). Once carotenoids are released from the matrix, they can be solubilized in the oil droplets in the stomach and then incorporated into micelles in the small intestine for further absorption (Castenmiller & West, 1998). The release from the matrix and subsequent incorporation into micelles is commonly known as carotenoid bioaccessibility.

Recently, a new experimental approach has been developed in order to gain a more detailed insight in the effect of the different structural barriers on carotenoid bioaccessibility (Palmero et al., 2013). Fractions containing carotenoids with different levels of structural barriers were isolated. In that way, it was possible to evaluate directly the effect of specific barriers on carotenoid bioaccessibility. The chromoplast substructure and the cell wall were both suggested to be important natural physical structural barriers limiting, at different levels, lycopene and β-carotene bioaccessibility in red tomatoes, red carrots and orange carrots, respectively. Nonetheless, additional research is required to investigate whether and how these structural barriers could be modified to facilitate carotenoid release from the matrix, without creating process induced barriers.

Thermal processing disrupts cellular structures and organelle membranes (Van Buggenhout et al., 2010). In literature, several studies investigated the effect of thermal treatments on carotenoid bioaccessibility. For example, Lemmens, Van Buggenhout, Oey, Van Loey, and Hendrickx (2009) mentioned that β-carotene bioaccessibility in carrot pieces is improved upon thermal treatments due to induced β-eliminative pectin degradation. Knockaert et al. (2011) also found an increase of β-carotene bioaccessibility in carrots after pasteurization and sterilization

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processes. Within the same context, β-carotene release and micellarisation were found to be enhanced by cooking carrot pieces according to Rock et al. (1998), Hedren, Diaz, and Svanberg (2002) and Hornero-Méndez and Mínguez-Mosquera (2007). On the other hand, Tydeman et al. (2010) found a decrease in carotenoid bioaccessibility upon heating carrots due to induced separation of intact cells that encapsulate carotenoids. Regarding lycopene bioaccessibility upon processing, in the study of Colle, Lemmens, Van Buggenhout, Van Loey, and Hendrickx (2010), an enhancement of lycopene bioaccessibility was found after applying intense thermal treatments to tomato puree. Similarly, lycopene serum concentrations were found to be higher when consuming processed tomato products compared to unprocessed products (Stahl & Sies, 1992).

It should be remarked that the cited studies have been performed by applying thermal treatments to a complex matrix. To the best of our knowledge, the effect of thermal treatments targeting a specific structural barrier for carotenoid bioaccessibility (e.g. cell wall, chromoplast substructure) has not been previously investigated. Cell walls and chromoplasts substructures from different matrices might be affected differently by heating conditions due to structural differences among them.

Therefore, the objective of the present study is to investigate whether thermal treatments could modify specific structural barriers leading to changes in carotenoid bioaccessibility. For this reason, a large cell cluster fraction, a small cell cluster fraction, and a chromoplast fraction were isolated from four different matrices. The surrounding cells in the large cell cluster fraction, the cell wall in the large and small cell cluster fractions, and the chromoplast substructure in the three fractions, constitute the main barriers governing carotenoid bioaccessibility, respectively.

After the isolation of the fractions, specific thermal treatments were applied in order to try to modify respectively the cell wall and the chromoplast substructure. The four matrices investigated in the present study consisted of orange carrots (rich in β-carotene), red carrots (rich in lycopene), red tomatoes (rich in lycopene) and atomic red carrots (rich in both lycopene and β-carotene). In this way, it was possible to compare the effect of heating on similar matrices containing different types of carotenoids (red and orange carrots) and on different matrices containing the same type of carotenoid (red tomatoes and red carrots). In this context, two important factors governing carotenoid bioaccessibility were included, i.e. the type of carotenoid and the matrix in where carotenoids are incorporated. In addition, by evaluating the effect of heating on a single matrix rich in both lycopene and β-carotene (atomic red carrots), the relevance of the type of carotenoid on determining the bioaccessibility, limiting the effect of the type of matrix, could be evaluated.

2. Materials and methods

2.1. Materials

Red tomatoes (*Lycopersicon esculentum* cv. Patrona) were obtained fresh from a Spanish supplier. They were cut into four pieces, 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 shortly at 4 °C, until further use for the corresponding experiments.

Seeds from a special variety of carrots (*D. carota* cv. Atomic red) were purchased from a seed supplier in the Netherlands. The carrots were grown during 3 months in a greenhouse. After harvesting, carrots were washed and stored shortly at 4 °C, until use for the experiments.

2.2. Experimental set-up

Fractions with different levels of structural barriers were obtained from the four matrices (red tomatoes, orange carrots, red carrots and atomic red carrots). The fractions obtained from each matrix corresponded to a chromoplast fraction, a small cell cluster fraction and a large cell cluster fraction.

In order to investigate the effect of thermal processing on the main structural barriers that hinder carotenoid release (chromoplast substructure and cell wall), specific heating conditions were selected and applied to the different fractions isolated from each matrix. Carotenoid bioaccessibility analysis was performed to all thermally treated fractions by applying an *in vitro* digestion method. Fig. 1 depicts schematically the experimental set-up performed in the present study.

2.3. Preparation of fractions with different levels of structural barriers

2.3.1. Chromoplast fraction

A chromoplast fraction, enriched with carotenoids, was isolated based on the procedure of Hansen and Chiu (2005) as described by Palmero et al. (2013). Firstly, a puree from each matrix was prepared. Tomatoes were initially thawed, while carrots were peeled and cut in small pieces. Afterwards the purees from the four matrices were obtained by mixing gently in a blender (Waring Commercial, Torrington, CT, USA) for 5 s with 0.05 M EDTA solution (1:1 ratio).

The obtained purees were then filtered using a cheesecloth and the filtrate was centrifuged (Beckman, J2-HS Centrifuge, Palo Alto, CA, USA) at 27,200 g, 4 °C for 30 min. The pellet, which corresponds to the chromoplast enriched fraction, was re-suspended in 100 ml of deionized water.

2.3.2. Small and large cell clusters fractions

The cell cluster fractions were obtained following the procedure described by Palmero et al. (2013). As a first step, a puree of red tomatoes was obtained by thawing, removing the peel and seeds and mixing three times (Büchi B-400 mixer, Flawil, Switzerland) for 5 s. Carrot purees were obtained by first peeling the carrots, cutting them in small pieces, and mixing with deionized water (1:1) (Waring Commercial, Torrington, CT, USA) for 1 min.

The cell clusters were obtained by separating the purees in different sizes with the use of a wet sieving equipment (Retsch AS200, Haan, Germany). The sizes for the small cell cluster fraction corresponded to 160–500 μm and 40–250 μm for tomatoes and carrots, respectively. For the large cell cluster fraction, particles ranging from 1000–1400 μm and from 800–2000 μm corresponded to the sizes of the clusters from tomatoes and carrots, respectively.

2.4. Thermal treatments

Thermal treatments were applied to the three fractions (chromoplast, small and large cell clusters) obtained from the four matrices (red tomatoes, orange carrots, red carrots and atomic red carrots), immediately after their isolation.

In order to perform the thermal treatments of the chromoplast fraction, 10 ml of the chromoplast solution was placed in a sealed plastic bag and subsequently immersed in a water bath at temperatures of 65, 75, 85 and 95 °C for 10 min.

Stainless steel tubes (5 mm internal diameter, 12 mm external diameter and 100 mm length) were used to perform the thermal treatments of the cell clusters. The tubes were filled with the cell clusters and immersed in an oil bath at temperatures of 95, 105, 115, and 125 °C for 25 min. All samples were immediately cooled in ice after the thermal treatments.