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Role of structural barriers for carotenoid bioaccessibility upon high pressure homogenization

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ABSTRACT

A specific approach to investigate the effect of high pressure homogenization on the carotenoid bioaccessibility in tomato-based products was developed. Six different tomato-based model systems were reconstituted in order to target the specific role of the natural structural barriers (chromoplast substructure/cell wall) and of the phases (soluble/insoluble) in determining the carotenoid bioaccessibility and viscosity changes upon high pressure homogenization. Results indicated that in the absence of natural structural barriers (carotenoid enriched oil), the soluble and insoluble phases determined the carotenoid bioaccessibility upon processing whereas, in their presence, these barriers governed the bioaccessibility. Furthermore, it was shown that the increment of the viscosity upon high pressure homogenization is determined by the presence of insoluble phase, however, this result was related to the initial ratio of the soluble:insoluble phases in the system. In addition, no relationship between the changes in viscosity and carotenoid bioaccessibility upon high pressure homogenization was found.

1. Introduction

Carotenoids are naturally occurring lipophilic pigments responsible for the characteristic colors of some fruits and vegetables. They have received particular attention due to their health-related functions, including pro-vitamin A activity, prevention and protection against certain types of cancers, cardiovascular diseases, and macular degeneration, as well as enhancing the immune function (Bendich, 1989; Rao & Rao, 2007). In order for carotenoids to exert their bioactivity, their absorption and uptake by specific target organs and compartments of the human body is a prerequisite. In this regard, carotenoids have to be released from the food matrix, solubilized in the oil phase, and eventually incorporated into micelles in the small intestine for further absorption. The release from the matrix and subsequent incorporation into micelles is commonly referred to as carotenoid bioaccessibility (Castenmiller & West, 1998). As carotenoids are located inside the plant cells within the chromoplast organelles, the release of carotenoids from a fruit/vegetable food matrix represents a crucial step in determining their bioaccessibility. In this context, it has been shown that the organization and localization within the chromoplast substructure, as well as the cell wall, constitute the two main natural structural physical barriers governing carotenoid release (Jeffery, Holzenburg, & King, 2012; Palmero et al., 2013). In light of these considerations, the disruption of these structural barriers could favor the release of carotenoids, thereby enhancing their bioaccessibility.

High pressure homogenization is a common unit operation used in the food industry. This process forces a fluid through a narrow gap valve, resulting in cavitation, turbulence and high shear stress (Bayod, Månsson, Innings, Bergenståhl, & Tornberg, 2007). As a consequence, the microstructure of the matrix is disrupted, generating particles with a more uniform and smaller size, leading to better texture characteristics and improved physical stability, as well as enhanced release of the micronutrients, such as carotenoids (Thakur, Singh, & Handa, 1995; van het Hof, West, Weststrate, & Hautvast, 2000).

The effect of high pressure homogenization on carotenoid bioaccessibility of tomato-based products has been largely investigated in the recent years. However, a limited effect of high pressure homogenization on lycopene *in vitro* bioaccessibility in tomato has been reported (Colle, Van Buggenhout, Van Loey, & Hendrickx, 2010; Panozzo et al., 2013; Svelander, López-Sánchez, Pudney, Schumm, & Alminger, 2011). According to these studies, a fiber network is formed upon high pressure homogenization that limits/hinders carotenoid micellarization. The formation of this

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network was supported by the increase in tomato pulp viscosity upon high pressure homogenization (Bayod et al., 2007). According to Gallaher, heir David, and heir Kelly (1999), the homogenization process modifies the cell structures by unraveling the fibers and allowing their end groups to bind higher amounts of liquids, resulting in tomato-based products having a thicker consistency. In addition, upon mechanical processing also pectins are released and uniformly distributed within the product, thus contributing to the gel formation that accounts for the increment in consistency. High pressure homogenization might also increase the linearity of the cells, exerting more resistance to flow (Thakur et al., 1995).

The limited effect of high pressure homogenization on lycopene *in vitro* bioaccessibility has been also related to the presence of soluble and insoluble fibers that form a barrier preventing carotenoid bioaccessibility (Tibäck et al., 2009). Similarly, Fernández-García, Butz, and Tauscher (2001) suggested that high hydrostatic pressure processing of tomato puree induced structural changes in the matrix, which resulted in the formation of a barrier surrounding the carotenoids limiting their extraction.

Based on the results reported in the literature, further research is needed to better understand the individual contribution of the soluble and insoluble phases, and the different natural structural barriers, in limiting carotenoid bioaccessibility in tomato-based products upon high pressure homogenization. In this context, insight in the specific role of the soluble and/or insoluble phases in modifying the viscosity upon high pressure homogenization, and its relationship to carotenoid bioaccessibility would provide useful information for controlling these parameters. Likewise, information targeting the role of each natural structural barrier (cell wall/chromoplast substructure) as it interplays with the soluble/insoluble phases in limiting carotenoid bioaccessibility during high pressure homogenization, is needed.

To this end, in the present study simplified tomato-based model systems were designed in order to identify the structure/phase responsible for limiting carotenoid bioaccessibility during processing. The model systems designed were obtained by mixing different carotenoid containing fractions (carotenoid enriched oil, chromoplast or cell cluster fractions) with a soluble/insoluble phase. The effect of high pressure homogenization on the microstructure (microscopy and laser diffraction analyses), viscosity, and carotenoid bioaccessibility of the different model systems was studied in order to gain insight in the correlation between the presence/absence of a specific structural barrier and/or a specific phase and the carotenoid bioaccessibility during mechanical processing.

2. Materials and methods

2.1. Materials

Red tomatoes (*Lycopersicon esculentum* cv. Prunus) and yellow tomatoes (*L. esculentum* cv. Lorenzo) were purchased in a local shop in Leuven and in an auction in Mechelen, Belgium, respectively. The tomatoes were washed, cut into slices of 0.5–1 cm and vacuum-packed in plastic bags. The bags were blanched in a temperature-controlled water bath at 95 °C for 8 min (to inactivate enzymes), and cooled. Thereafter, the bags were immersed into liquid nitrogen to freeze the tomato slices, and stored at −40 °C until use.

2.2. Puree preparation

Tomato pieces were thawed and the peel was removed. The pieces were mixed three times (Büchi B-400 mixer, Flawil, Switzerland) for 5 s and then sieved to remove the seeds. Purees from red and yellow tomatoes were first prepared and then used to isolate carotenoid-containing fractions (except for the chromoplast fraction), and the soluble and insoluble phases, respectively.

2.2.1. Soluble and insoluble phases

2.2.1.1. Soluble phase. The soluble phase corresponded to the serum phase from yellow tomatoes. Yellow tomato puree was centrifuged (Beckman, J2-HS, Centrifuge Palo Alto, CA, USA) at 12,400*g* for 30 min at 20 °C. The supernatant, which corresponds to the serum phase, was collected and filtered (MN 615, pore size 8 μm) under vacuum. The serum was used immediately.

2.2.1.2. Insoluble phase. The insoluble phase corresponded to cell clusters from yellow tomatoes. Yellow tomato puree was sieved using wet sieving equipment (Retsch AS200, Haan, Germany). The sizes corresponding to 160–500 μm were isolated and the cell clusters were reconstituted with deionized water according to the calculated yield.

2.2.2. Carotenoid containing fractions

2.2.2.1. Carotenoid enriched oil fraction. The carotenoid enriched oil was obtained according to the method described by Palmero et al. (2013) with some modifications. The puree obtained from red tomato was mixed with olive oil at a ratio of 5:1 for 5 h at room temperature 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.

2.2.2.2. Chromoplast fraction. The chromoplast fraction was obtained following the procedure described by Palmero et al. (2013). The pieces of tomatoes were thawed and mixed in a blender (Waring Commercial, Torrington, CT, USA) for 5 s at low speed with 0.05 M EDTA solution (1:1 ratio). The purees were filtered using a cheesecloth. The filtrate was centrifuged (Beckman, J2-HS Centrifuge, Palo Alto, CA, USA) at 27,200*g*, 4 °C for 30 min. The pellet, consisting of the chromoplast fraction, was retained for the experiments.

2.2.2.3. Cell cluster fraction. Red tomato puree was sieved with the Retsch AS200 wet sieving equipment, using the same sizes as for the insoluble phase.

2.2.3. Preparation of tomato-based model systems

Six tomato-based model systems consisting of a carotenoid containing fraction, and a soluble or insoluble phase isolated from red and yellow tomatoes, respectively, were prepared. The carotenoid containing fractions consisted of samples with different levels of carotenoid bio-encapsulation within the natural structural barriers. These samples corresponded to, in increasing order of bio-encapsulation, a carotenoid enriched oil, a chromoplast, and a cell cluster fraction isolated from the red tomatoes. The soluble and insoluble phases consisted of the isolated serum and cell clusters, respectively. Yellow tomatoes were used to obtain soluble/insoluble phases in order to have a representative phase from a tomato matrix without any lycopene or β-carotene content. Thereby, changes in carotenoid bioaccessibility upon high pressure homogenization could be attributed to the effect of the process on the individual natural structural barriers from the carotenoid containing fractions and/or the specific soluble and insoluble phase. A schematic representation of the designed tomato-based model systems is depicted in Fig. 1.

The carotenoid enriched oil (CEO) (5%) emulsified with L-α-phosphatidylcholine (1%) was dissolved in the serum or the reconstituted cell cluster fraction from yellow tomatoes to prepare