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Microstructure and bioaccessibility of different carotenoid species as affected by high pressure homogenisation: A case study on differently coloured tomatoes

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ABSTRACT

The effect of high pressure homogenisation (HPH) on structure (Bostwick consistency, particle size distribution and microstructure) and carotenoid *in vitro* bioaccessibility of different tomato pulps was investigated. HPH decreased tomato particle size due to matrix disruption and increased product consistency, probably due to the formation of a fibre network. Homogenisation also resulted in a decrease of *in vitro* bioaccessibility of lycopene, ζ-carotene, and lutein. Such decrease was attributed to the structuring effect of HPH. An inverse relation between tomato consistency and carotenoid *in vitro* bioaccessibility was found. This dependency was affected by carotenoid *species* and its localisation within the matrix. It could be observed that one matrix (e.g. (homogenised) red tomato pulp) can contain carotenoids with a very low bioaccessibility (lycopene) as well as carotenoids with a very high bioaccessibility (lutein), indicating that carotenoid bioaccessibility is not solely dependent on the matrix.

1. Introduction

High pressure homogenisation (HPH) is commonly used to increase physical stability of pumpable tomato derived products (Den Ouden & Van Vliet, 2002; Thakur, Singh, & Handa, 1995). During homogenisation, tomato pulp is forced through a narrow gap undergoing high turbulence, shear, cavitation and impact. As a consequence, tomato particle structure is modified by disaggregation of cell clusters and disruption of cells (Stang, Schuchmann, & Schubert, 2001). Homogenisation has also been shown to affect the particle interactions. For the specific case of tomato, this may possibly lead to the formation of a fibre network accounting for an increase in viscosity (Bayod, Mansson, Innings, Bergenstahl, & Tornberg, 2007; Beresovsky, Kopelman, & Mizrahi, 1995; Lopez-Sanchez, Svelander, Bialek, Schumm, & Langton, 2011a; Lopez-Sanchez et al., 2011b). It has been suggested that such structural modifications may affect the release and bioaccessibility of carotenoids from the food matrix (van het Hof et al., 2000).

Carotenoids are naturally occurring fat-soluble pigments responsible for tomato colour. They are well known to have beneficial effects on human health by reducing the risk of chronic diseases, including cardiovascular and gastrointestinal diseases, obesity, diabetes and certain types of cancer (Rao & Rao, 2007). Health protective effects of carotenoids strongly depend on their bioavailability and bioaccessibility. The latter is defined as the fraction of the ingested carotenoids that is released from the food matrix, incorporated into mixed micelles and thus available for intestinal absorption (Parada & Aguilera, 2007).

Among the several factors affecting carotenoid bioaccessibility, the structure of the food matrix, as well as the *species* of carotenoid, and their localisation within the plant tissue, seem to play a major role (Castenmiller & West, 1998). The reduction in particle size due to plant tissue disruption has been shown to enhance carotenoid bioaccessibility (Knockaert, Lemmens, Van Buggenhout, Hendrickx, & Van Loey, 2012; Parada & Aguilera, 2007; Svelander, Lopez-Sanchez, Pudney, Schumm, & Alminger, 2011; van het Hof et al., 2000). On the other hand, the increase in consistency, attributed to the formation of a fibre network by polymer–polymer interaction, has been demonstrated to decrease carotenoid bioaccessibility (McClements, Decker, & Park, 2009). With regard to carotenoid species, the extent of incorporation into micelles is considered to be inversely related to their hydrophobicity. For example, lutein and other xanthophylls are known to be better absorbable into micelles and thus to have higher bioaccessibility than β-carotene or

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lycopene, which are more hydrophobic (Borel et al., 1996;). In plant tissues, carotenoids are generally deposited in various types of chromoplasts in which they occur in crystalline or lipid-dissolved form (Rosso, 1968; Vásquez-Caicedo, Heller, Neidhart, & Carle, 2006). In general, the latter are easily incorporated into micelles and thus more accessible than those stored in crystalline form (Schweiggert, Steingass, Heller, Esquivel, & Carle, 2011; Schweiggert, Mezger, Schimpf, Steingass, & Carle, 2012; Vásquez-Caicedo et al., 2006).

In recent years, several studies have focussed on the effect of HPH on carotenoid bioaccessibility in tomato pulps as well as in other matrices. Upon HPH, a decrease in lycopene bioaccessibility was detected in both tomato pulps and tomato based emulsions. Such decrease was attributed to the formation of a fibre network (Colle, Van Buggenhout, Van Loey, & Hendrickx, 2010; Svelander et al., 2011). On the other hand, β-carotene bioaccessibility was found to increase in both carrot purees and carrot based emulsions following HPH. In these cases, the improvement in β-carotene bioaccessibility was associated to the disruption of the matrix (Knockaert et al., 2012; Svelander et al., 2011). Given these apparently contradictory evidences, it is largely unclear whether carotenoid bioaccessibility depend either on the carotenoid species or the structure of the matrix.

The aim of the present study was therefore to investigate the effect of HPH on different carotenoid *species* within a similar matrix. To this purpose, tomatoes with different colour (i.e. red- orange- and yellow-fleshed), and thus different prevalent carotenoids, were chosen. Samples were homogenised at increasing pressure levels and analysed for carotenoid bioaccessibility and concentration. To understand the relations between changes in carotenoid bioaccessibility and plant tissue structure upon HPH, samples were also submitted to evaluation of Bostwick consistency, particle size distribution and microstructure.

2. Materials and methods

2.1. Materials

Red, orange and yellow ripe tomatoes (*Solanum lycopersicum* L.) of the cultivars Admiro, Bolzano and Lorenzo respectively, were purchased at a local greengrocer. They were washed, wiped, manually quartered with a sharp knife, frozen in liquid nitrogen and stored at −40 °C until use. The same batch of each tomato was used for the preparation of all samples. Extra virgin olive oil was purchased in a local supermarket and stored under dark. Lycopene, ζ-carotene and lutein standards were purchased from CaroteNature (Lupsingen, Switserland). β-Apo-8′-carotenal, was obtained from Sigma–Aldrich (Bornem, Belgium).

2.2. High pressure homogenisation

The frozen tomato quarters were thawed, peeled, mixed (3 times for 5 s) (Büchi Mixer B-400, Flawil, Switzerland) and sieved (pore size 1.0 mm) in order to remove the seeds. Tomato pulps were homogenised *via* a single pass at 20, 50 and 100 MPa using a high pressure homogeniser (Panda 2K; Gea Niro Soavi, Mechelen, Belgium) with inlet and outlet connected to a heat exchanger at a pre-set temperature of 4 °C. Non homogenised pulps were considered as control samples. All the samples were kept in the dark at 4 °C until analysis.

2.3. Bostwick consistency

The consistency of tomato pulps was measured using a Bostwick consistometer. This empirical test was conducted allowing the sample to flow under its own weight along a sloped stainless steel tray for 30 s at room temperature (23 °C). The distance the pulps flowed was recorded as the Bostwick consistency index (cm). High values correspond to a low consistency pulp with low resistance to flow, while lower values are associated with high consistency pulps resistant to flow. Measurements were done in triplicate.

2.4. Particle size distribution (PSD)

The PSD of tomato pulps was measured by laser diffraction using a Malvern Mastersizer S long bench instrument (Malvern Instrument Ltd., Worcestershire, UK). Tomato pulp was poured into a stirred tank filled with water until a laser obscuration of 20% was achieved. The diluted sample was pumped into the measuring cell, which was located in the optical path of the laser beam. The laser beam (He–Ne laser, wavelength 633 nm), collimated at 18 mm, was scattered to detector units (42 element composite solid state detector array), which detected particles in the range of 0.05–880 μm. Volumetric PSDs were calculated from the intensity distribution of the scattered light using the Mie theory by use of the instrument software.

2.5. Light microscopy

Micrographs of tomato pulps were taken using a digital camera mounted on a light microscope (Olympus BX-41, Optical Co. Ltd., Tokyo, Japan). To visualise the microstructure of the tomato pulps, the samples were diluted 1:10 (v/v) with 0.1% toluidine blue aqueous solution. The presence of starch was also evaluated by diluting 1:4 (v/v) tomato pulp with a iodine staining solution (0.2% iodine, 2% potassium iodide aqueous solution). Few droplets of the mixtures were placed on microscopic slides, covered with cover glasses and studied using a 10x magnification. To visualise the tomato chromoplasts, one droplet of tomato pulp was placed on a microscopic slide, covered with a cover glass and analysed using a 100× magnification.

2.6. In vitro bioaccessibility

The lycopene, ζ-carotene and lutein *in vitro* bioaccessibility was measured immediately after processing the tomato pulp by simulating human digestion in the stomach and small intestine *in vitro*. The procedure described by Moelants et al. (2012), based on Hedrén, Diaz, and Svanberg (2002), was followed. In particular, 5 g tomato pulp was weighted into a 50 mL capacity brown falcon tube. The sample was diluted with 5 mL NaCl/ ascorbic acid solution (0.9% NaCl, 1% ascorbic acid in water), 5 mL stomach electrolyte solution (0.30% NaCl, 0.11% KCl, 0.15% $CaCl_2{\cdot}2H_2O$, 0.05% $KHPO_4$, 0.07% $MgCl_2{\cdot}6H_2O$ in water) and 10 mL of freshly prepared oil emulsion. The latter was obtained by suspending 1% (w/v) L-α-phosphatidylcholine from egg yolk (Sigma) in water. 5% (v/v) extra virgin olive oil was then added and the mixture was homogenised (UltraTurrax® T25, IKA® – Werke GMBH & CO.KG, Staufen, Germany) at 9500 rpm during 10 min. A second homogenisation was performed at 100 MPa for one cycle using the high pressure homogeniser described above.

To simulate the first phase of gastric digestion, the pH of the mixture was adjusted to 4 ± 0.05 with 1 M HCl or 1 M $NaHCO_3$ and 5 mL pepsin solution (0.52% porcine pepsin, from Sigma, in electrolyte solution) was added. After flushing the headspace of the samples with nitrogen for 20 s, the mixture was incubated at 37 °C for 30 min while shaking end-over-end. The pH of the mixture was then acidified to 2 ± 0.05 to mimic the drop of the gastric pH after the intake of a meal (Tyssandier et al., 2003). The headspace of the samples was flushed again with nitrogen for 20 s and the incubation at 37 °C continued for another 30 min. To