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Changes in β-carotene bioaccessibility and concentration during processing of carrot puree

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ABSTRACT

The effect of some process and product factors (addition of olive oil, high pressure homogenisation, subsequent thermal or high pressure pasteurisation) on β-carotene bioaccessibility and isomerisation of carrot puree was investigated. High pressure homogenisation could improve β-carotene bioaccessibility by disrupting cells, but only at a pressure higher than 50 MPa. Softening of the cell walls during a subsequent thermal pasteurisation resulted in a further increase in β-carotene bioaccessibility. Unfortunately, the high temperature also induced formation of some undesirable *cis*-isomers. As cell walls are probably strengthened by high pressure, high pressure pasteurisation could not positively affect the amount of bioaccessible β-carotene. Moreover, the high pressure process induced β-carotene oxidation. A positive effect of olive oil on β-carotene bioaccessibility could only be noticed in combination with a thermal pasteurisation process when β-carotene was solubilised in the oil droplets. Under high pressure however, oil can be crystallised which hinders the solubilisation of β-carotene.

1. Introduction

Carotenoids are an important group of nutrients in fruit and vegetable based products. In carrots, β-carotene is present in a high concentration and can be considered as one of the most essential micronutrients because of its antioxidant activity and its property to act as a provitamin A. These health promoting properties are the result of the specific structure of β-carotene, which consists of a polyene system with 11 conjugated double bonds and a β-ring at each end of the chain (Britton, 1995). However, the conjugated system of double bonds makes β-carotene also very susceptible to isomerisation and oxidation. The *cis*-isomers of β-carotene have a decreased provitamin A activity and an altered antioxidant activity. In the literature, both a lower (Conn, Schalch, & Truscott, 1991) and a higher (Levin & Mokady, 1994) antioxidant activity of β-carotene-*cis*-isomers compared to all-*trans*-β-carotene have been reported. Oxidation causes complete degradation of β-carotene.

As only the fraction of a nutrient that is absorbed by the human body can effectively contribute to human health, the bioaccessibility and bioavailability of a nutrient are more important than its total concentration when evaluating the nutritional value of fruits and vegetables. The bioaccessibility of a nutrient is defined as the amount of a nutrient that is released from its food matrix during digestion and made accessible for absorption into mucosa (Hedrén, Diaz, & Svanberg, 2002a). The bioavailability is defined as the fraction of an ingested food that is available for utilisation in normal physiological functions or storage (Castenmiller & West, 1998).

A number of factors can affect the bioaccessibility and/or bioavailability of carotenoids and can be described by the term "SLAMENGHI" which includes Species of carotenoids, Linkages at molecular level, Amount of carotenoid, Matrix, Effectors, Nutrient status, Genetics, Host related factors and Interactions among these variables (Van het Hof, West, Weststrate, & Hautvast, 2000b). One of the important factors is the food matrix. In carrots, β-carotene is located in the chromoplasts (surrounded by a double bilayer membrane) of the plant cells (surrounded by a cell membrane and a cell wall), where it is often associated with proteins and/or residual membranes (Hornero-Méndez & Mínguez-Mosquera, 2007). As a result, several physical barriers have to be broken before β-carotene can be released from the carrot matrix and made accessible for absorption. As processing can have an effect on the food matrix and on these barriers, it can affect the β-carotene bioaccessibility and bioavailability. It has already been shown in several studies that thermal processing can improve β-carotene bioaccessibility in carrots (Hedrén et al., 2002a; Knockaert et al., 2011; Lemmens, Van Buggenhout, Oey, Van Loey, & Hendrickx, 2009). On the other hand, the effect of high pressure processing, which is a new preservation technique developed as an alternative to conventional thermal treatment, on β-carotene bioaccessibility is less clear in the literature. Depending on the applied treatment conditions

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and on the matrix, both positive (Knockaert et al., 2011) and negative (Butz et al., 2002; Knockaert et al., 2011) effects or no effects at all (McInerney, Seccafien, Stewart, & Bird, 2007) of high pressure on β-carotene bioaccessibility have been described. In addition to this, mechanical processing like mixing or homogenising can also affect the bioaccessibility and bioavailability of carotenoids. By reducing the particle size and removing the barriers, the contact surface for interaction with digestive enzymes can be enlarged, which may improve the release of carotenoids from the food matrix (Hedrén et al., 2002a; Van het Hof et al., 2000a). Moreover, the degree of homogenisation prior to thermal or high pressure processing might affect the possible effect of these treatments.

Some effectors, for example the presence of oil, can also have an influence on the bioaccessibility and/or bioavailability of carotenoids. As carotenoids are lipophilic molecules, they have to be incorporated in mixed micelles in the duodenum before they can be absorbed in the mucosa. Mixed micelles are built up of triacylglycerols, hydrolysis products of triacylglycerols, phospholipids, cholesterol esters and bile acids. The presence of dietary fat in the duodenum is important because it regulates the level of pancreatic lipase, which hydrolyses triacylglycerols into free fatty acids and monoglycerides and stimulates the release of bile acids from the gall bladder (Deming & Erdman, 1999).

In the present study, the effect of different processing techniques of carrot puree and the effect of the addition of oil on β-carotene concentration, β-carotene isomerisation and β-carotene *in vitro* bioaccessibility were investigated. The particle size distribution and the microstructure of carrot purees were also examined as structural properties.

2. Materials and methods

2.1. Sample preparation

Fresh carrots (*Daucus carota* cv Nerac) were purchased in a local shop in Belgium and stored at 4 °C. Carrots were peeled, cut into pieces and blended with water (1:1) for 1 min in a kitchen blender. The dry matter content of the puree was 4.94 ± 0.22% (g/g). To selected carrot purees, 5% (w/w) olive oil was added, after which the purees were stirred for 15 min at room temperature.

2.2. High pressure homogenisation

Carrot puree was homogenised at 10, 50 or 100 MPa for 1 cycle using a high pressure homogeniser (Panda 2K; Gea Niro Soavi, Mechelen, Belgium). Sample collection was started 1 min after the beginning of the high pressure homogenisation. The pressure could be monitored continuously on a digital display. The sample inlet was thermostated at 4 °C. As a result of the high pressure homogenisation, the product temperature normally increases by 2–2.5 °C per 10 MPa. Therefore, the carrot puree was cooled to 4 °C in a heat exchanger immediately after being homogenised.

2.3. Intense pasteurisation

Thermal pasteurisation was carried out in a water bath. Stainless steel cylindrical tubes (13 mm internal diameter, 1 mm thickness, 110 mm length) were filled with (homogenised) carrot puree and placed in a temperature controlled water bath at 90 °C. The temperature profile in the carrot puree was measured using type T thermocouples connected to a thermocouple box (TR9216; Ellab, Hilleroed, Denmark) and a CMC-92 data acquisition system (Ellab). After reaching a process value of $^{10^{\circ}C}P_{90^{\circ}C}$ = 10 min, the tubes were cooled in ice water.

High pressure pasteurisation was performed in a laboratory scale single vessel high pressure equipment (SO. 5-7422-0, Engineered Pressure Systems International, Temse, Belgium). Pressure medium was a mixture of 60% DowCalN in water (Dow Chemical Company, Horgen, Switzerland). A high pressure process of 20 min at 600 MPa and 45 °C was performed. Flexible polyethylene plastic flasks (LDPE, ~100 mL, Medisch Labo Service, Menen, Belgium) were filled with (homogenised) carrot puree and were placed in the high pressure vessel which was already equilibrated at 45 °C. Pressure was built up automatically to 600 MPa in a few seconds. After 20 min, pressure was released manually to atmospheric pressure and the samples were cooled in ice water. During the process, temperature and pressure in the pressure vessel were registered.

Both thermal and high pressure treatments were performed three times.

2.4. Determination of total β-carotene content and β-carotene isomers

To determine the total β-carotene concentration, β-carotene was first extracted from the carrot puree. The extraction procedure was based on the procedure described by Sadler, Davis, and Dezman (1990), with some modifications. To 1 g carrot puree, 1 g $CaCl_2{\cdot}2H_2O$ and 50 mL extraction solution (50% hexane, 25% acetone, 25% ethanol, 0.1% BHT) were added. The mixture was then stirred for 20 min at 4 °C. After adding 15 mL reagent grade water, the mixture was stirred for another 10 min at 4 °C. The organic phase, containing the carotenoids, was separated from the water phase using a separating funnel and was filtered (Chromafil PET filters, 0.20 μm pore size, 25 mm diameter; Macherey–Nagel, Düren, Germany). The absorbance of the filtrate was measured spectrophotometrically at 450 nm (= λ_{max} for β-carotene in hexane) using hexane + 0.1% BHT as a blank. To calculate the total carotenoid concentration, the following equation was used:

$$\text{Carotenoid concentration}(\mu g/g) = \frac{A \times volume(ml) \times 10^4}{E^{1\%}_{1cm} \times \text{sample weight}(g)}$$

where A = absorbance at λ_{max}, volume = total volume of extract, $^{1\%}E_{1cm}$ = extinction coefficient (= 2560 for β-carotene in hexane (Hart & Scott, 1995)). The whole procedure was carried out under subdued red light. Analyses were performed three times.

To identify and quantify β-carotene isomers in the carrot purees, the filtrate obtained after the extraction procedure was also analysed by HPLC. An HPLC system equipped with a reversed phase C_{30}-column (5 μm × 250 mm × 4.6 mm, YMC Europe, Dinslaken, Germany) and a diode array detector (Agilent Technologies 1200 Series, Diegem, Belgium) was used. During analysis, the temperatures of the autosampler and the column were kept at 4 °C and 25 °C, respectively. To separate the different isomers, linear gradient elution was used. The gradient was built up in 20 min from 81% MeOH, 15% methyl-*t*-butyl-ether and 4% reagent grade water to 41% MeOH, 55% methyl-*t*-butyl-ether and 4% reagent grade water at a flow rate of 1 mL/min. The absorbance of the different isomers was measured at 450 nm (= λ_{max} for all-*trans*-β-carotene in hexane). To quantify the concentration of the different isomers, calibration curves of all-*trans*-β-carotene, 15-*cis*-β-carotene, 13-*cis*-β-carotene and 9-*cis*-β-carotene standards (CaroteNature, Lupsingen, Switzerland) were used.

2.5. Measurement of in vitro β-carotene bioaccessibility

The β-carotene bioaccessibility was measured by simulating human digestion in the stomach and small intestine *in vitro*. All enzymes used in the procedure were obtained from Sigma–Aldrich (Bornem, Belgium). The procedure was based on the procedure