Food Research International 43 (2010) 1449–1454

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Bioavailability of β-carotene isomers from raw and cooked carrots using an in vitro digestion model coupled with a human intestinal Caco-2 cell model

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ARTICLE INFO

Article history:
Received 8 March 2010
Accepted 28 April 2010

Keywords:
In vitro digestion
Micelles
β-Carotene isomers
Carrots
Caco-2 cells
Uptake
Transport

ABSTRACT

Not only is there limited information in the literature regarding the β-carotene (BC) isomer profile of micelles from digested foods; few studies have looked at their subsequent uptake and transport by human intestinal Caco-2 cells. Therefore, the aims of the present study were, first, to assess the profile of BC isomers in micelles from digested raw and cooked carrots; and, second, to determine their cellular uptake and transport. Greater amounts of all-*trans*-, 13-*cis*- and 15-*cis*-BC isomers were present in the micelles of cooked carrots compared with raw carrots. Furthermore, micelle fractions obtained from the most highly processed (pureed) carrots had greater ($P < 0.05$) amounts of all-*trans*-, 13-*cis*- and 15-*cis*-BC compared with those derived from raw and boiled carrots. A similar trend was seen with BC isomer uptake and transport. Our data suggest that the food matrix and degree of processing play important roles on carotenoid isomerization and bioavailability.

1. Introduction

Vitamin A must be obtained from the diet, in the form of either preformed retinol or provitamin A carotenoid precursors (During & Harrison, 2007). β-Carotene (BC) is the most commonly detected carotenoid in human tissue and plasma (Khachik et al., 1992; Krinsky & Johnson, 2005). After crossing the gut wall, BC is either cleaved to vitamin A and related metabolites, returned to the intestinal lumen via bile, or absorbed intact into circulation with lymph chylomicrons (Burri & Clifford, 2004). Hence, the bioavailability of BC is of particular interest owing to its provitamin A activity (Boileau & Erdman, 2004). The physicochemical state of a carotenoid in a food, the type of processing/cooking, the presence of other nutrients and non-nutrients within the food as well as seasonal variation and geographical origin can affect the different stages of carotenoid bioavailability such as: (i) the transfer of carotenoids from the food matrix to the micelle fractions during intestinal digestion, (ii) uptake of carotenoids by intestinal cells, (iii) incorporation of carotenoids into chylomicrons, and/or (iv) secretion of carotenoids into the lymph system for delivery to tissues (Aherne, Jiwan, Daly, & O'Brien, 2009; Failla, Thakkar, & Kim, 2009; O'Connell, Ryan, O'Sullivan, Aherne, & O'Brien, 2008; Ryan, O'Connell, O'Sullivan, Aherne, & O'Brien, 2008). Therefore it is important to consider influential factors such as food matrix, cooking, and isomerization when determining the bioavailability and vitamin A (retinol) value of BC-rich foods (Boileau & Erdman, 2004).

All-*trans* BC is the predominant isomer found in unprocessed carotene-rich plant foods (Castenmiller & West, 1998; Failla et al., 2009); however, several different geometric isomers of BC exist in foods (Chandler & Schwartz, 1988; Cortes, Esteve, Frigola, & Torregrosa, 2004; During, Hussain, Morel, & Harrison, 2002; Pott, Marx, Neidhart, Muhlbauer, & Carle, 2003). Cooking carotenoid-rich vegetables can lead to the degradation and/or isomerization of carotenoids (Castenmiller & West, 1998; Failla, Huo, & Thakkar, 2008; Faulks & Southon, 2005; Schieber & Carle, 2005). Although low concentrations are found in circulating human serum, *cis*-BC isomers are present in human tissues where it is thought that they exert their biological function(s) (Deming, Baker, & Erdman, 2002; Khachik et al., 1997; Stahl, Schwarz, von Laar, & Sies, 1995; Stahl & Sies, 1993). The provitamin A activity of *cis*-BC isomers is much lower than that of all-*trans* BC. 9-*Cis*-BC has a relative bioconversion to retinol of 38%, 13-*cis*-BC 53% whereas the all-*trans* form is 100% (Castenmiller & West, 1998; Yeum & Russell, 2002).

Besides being essential for vision, the vitamin A derivative retinoic acid (RA) is a major signal pathway controlling centre which regulates a wide range of biological processes (von Lintig et al., 2005). RA is the ligand of two classes of nuclear receptors, the RA receptors (RARs) and the retinoid X receptors (RXRs) (von Lintig et al., 2005). All-*trans* BC is a precursor of all-*trans* RA, which preferentially binds to RARs, whereas 9-*cis*-BC is a precursor of 9-*cis* RA – the preferred ligand for RXRs (Ferruzzi, Lumpkin, Schwartz, & Failla, 2006). The binding of these ligands to their respective

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doi:10.1016/j.foodres.2010.04.026

receptors is vital for growth, reproduction, maintenance of skin and mucous membranes, and immune function (Ferruzzi et al., 2006). In addition, *cis* isomers of BC have been shown to possess in vitro antioxidant activity (Bohm, Puspitasari-Nienaber, Ferruzzi, & Schwartz, 2002; Levin & Mokady, 1994). For instance, Bohm et al. (2002) reported that all-*trans*-, 9-*cis*-, 13-*cis*- and 15-*cis*-BC showed antioxidant activity, using the TEAC assay, with values ranging from 1.0 to 1.4 mM. It has also been suggested recently that *cis*-BC isomers may also have a role in preventing atherogenesis (Harari et al., 2008); however, studies are still ongoing to fully elucidate these findings.

Information in the literature on the bioavailability and function of individual BC isomers is scarce. Therefore, in the present study, we determined the cellular uptake and overall transport (i.e. bioavailability) of BC isomers from both raw and cooked carrots using an in vitro digestion model coupled with the use of human intestinal Caco-2 cells.

2. Materials and methods

2.1. Materials

BC isomers were purchased from CaroteNature (Lupsingen, Switzerland, >96% purity). All other reagents including β-carotene, Dulbecco's modified eagle's medium, Hanks balanced salt solution (HBSS), and non-essential amino acids were purchased from Sigma–Aldrich Chemical Co. (Dublin, Ireland). Fetal bovine serum was purchased from Biosciences Ltd. (Invitrogen Ltd., Paisley, Scotland). Cell culture plastics were obtained from Cruinn Diagnostics Ltd. (Greiner Bio-One, Kremsmunster, Austria). Transwell plates were purchased from Costar (New York, USA). All solvents employed were of HPLC grade.

2.2. Sample preparation

On five separate days during one season, undamaged carrots (*Daucus carota* L.) were purchased from Dennigans fruit and vegetable wholesalers (Lenaghmore, Cork, Ireland). The carrots originated from the same supplier and geographical location (The Netherlands). All manipulations with the carrots were performed under subdued (yellow) light to minimize photo-decomposition of the phytochemicals. Prior to cooking, carrots were peeled and sliced. The cutting dimensions were standardized to ensure replication of conditions. For boiling, carrots were added to 500 ml of boiling water (ca. 100 °C) and cooked at a steady rolling boil for 6 min. For the puree method, vegetables were added to 500 ml boiling water (ca. 100 °C) and were cooked at a steady rolling boil for 20 min, then mashed using a standard kitchen mashing utensil.

2.3. Sample homogenization and in vitro digestion

The in vitro digestion procedure was performed by the method of Garrett, Failla, and Sarama (1999) with minor modifications as previously described by Aherne, Jiwan, Daly, and O'Brien (2009). Briefly, 2 g of each sample was weighed and homogenized twice (Janke and Kunkel, Ultra-Turrax T25; IKA-Labortechnik, Staufen, Germany) in 5 ml of HBSS for 10 s. Digestion was carried out by using porcine pepsin (0.04 g/ml 0.1 M HCl) to acidify the sample homogenate to pH 2 followed by incubation at 37 °C in a Grant OLS 200 orbital shaking water bath (Grant Instruments, Cambridge, UK) for 1 h. After gastric digestion, 0.9 M sodium bicarbonate was added to adjust the pH to 5.3, followed by the addition of glycodeoxycholate (0.04 g/ml), taurodeoxycholate (0.025 g/ml), taurocholate (0.04 g/ml), porcine pancreatin (0.04 g/ml) and cholesterol esterase (1 U/ml). The pH of each sample was increased to 7.4 followed by incubation at 37 °C for 2.5 h in the orbital shaking water bath. After the intestinal phase, 5 ml of the digesta were overlaid with a layer of nitrogen gas and were frozen at −80 °C. The remainder of the digesta was ultracentrifuged at 194,270*g* (Beckman L7-65 ultracentrifuge, Palo Alto, CA, USA) for 95 min. The resulting supernatants (micelle fractions) were collected with a syringe and filter-sterilized using a surfactant free cellulose acetate filter (0.2 μm; Millipore, Bedford, MA, USA) to remove any microcrystalline aggregates. Samples were stored at −80 °C, after overlaying the headspace with nitrogen gas, until further analysis.

2.4. Cellular uptake, secretion, and overall transport of carotenoids

Human colon adenocarcinoma Caco-2 cells were purchased from the European Collection of Animal Cell Cultures (Salisbury, Wilts, UK). Cells were maintained in Dulbecco's modified eagle's medium supplemented with 10% (v/v) FBS and 1% (v/v) non-essential amino acids. Cells were grown at 37 °C in a humidified incubator with 5% CO_2, in the absence of antibiotics. For experiments, cells were seeded at a density of 5×10^4 cells/cm^2 on transwell plates that had a 0.4 μm pore size membrane. Cells were grown for 21–25 d to obtain a differentiated cell monolayer. Transepithelial electrical resistance (TEERS) measurements were taken twice weekly by a TEERS voltohmmeter to ensure the monolayer was intact. At the beginning of each experiment, the apical side of the transwell plate received 2 ml micelle-containing media that were obtained from the digested test carrot samples. The basolateral chamber received 2 ml serum-free media. Treatment with the micelles was for 4 h after which time the micelle-enriched media was removed and the cell layer was washed with warm sterile HBSS. Preliminary work showed that the micelle-enriched media was not toxic to the cells (data not shown). Media (2 ml) containing taurocholate (0.5 mM), oleic acid (1.6 mM) and glycerol (45 mM) was added to the apical chamber and incubated for 16 h for the stimulation and secretion of chylomicrons (O'Sullivan, Ryan, & O'Brien, 2007). After incubation, media from each side of the membrane were removed, the monolayer was washed twice with HBSS, and cells were scraped in HBSS. Samples were sonicated for 30 s on ice and were stored at −80 °C, after overlaying the headspace with nitrogen gas, until further analysis.

2.5. Carotenoid extraction and HPLC analysis

Carotenoids were extracted from all samples according to the method of Olives-Barba, Camara-Hurtado, Sanchez Mata, Fernandez Ruiz, and Lopez Saenz de Tejada (2006), as previously reported by O'Sullivan, Ryan, Aherne and O'Brien (2008). Briefly, samples were extracted twice with hexane/ethanol/acetone (50:25:25, v/v/v) and centrifuged (Sorvall TC6, H400 rotor, DuPont Instruments, Herts, UK) at 1499*g* for 5 min. The resulting supernatants were removed, pooled, and evaporated using a solvent evaporation system (miVac, Genevac Ltd., Suffolk, UK). The residues were re-constituted in 200 μl of methanol:dichlorormethane (1:1, v/v), and the carotenoid content of the samples was analyzed by reversed-phase HPLC. The HPLC method used was based on the methodology of Inbaraj, Chien, and Chen (2006) with slight modifications. The HPLC system (Finnigan SpectraSYSTEM; Thermo Scientific, Philadelphia, PA) consisted of a P2000 pump connected to an AS3000 auto-injector and a UV6000LP photodiode array (PDA) detector. Separation of carotenoids was achieved using a YMC C30 reversed phase column (TMC Europe GmbH, Dinslaken, Germany) (250 × 4.6 mm–5 μm particle size) and column temperature was maintained at 33 °C by an internal column oven. Prior to use, the mobile phase was filtered through a 0.45 μm filter and degassed using ultrasonic agitation. Carotenoids were separated by gradient elution. Mobile phase A was composed of methanol:acetonitrile:water