JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 9855–9860 9855
DOI:10.1021/jf1010353

Effect of Carrot (*Daucus carota*) Microstructure on Carotene Bioaccessibility in the Upper Gastrointestinal Tract. 2. In Vivo Digestions

Elizabeth A. Tydeman,[†] Mary L. Parker,[†] Richard M. Faulks,[†] Kathryn L. Cross,[†] Annette Fillery-Travis,[†] Michael J. Gidley,[‡,§] Gillian T. Rich,[†] and Keith W. Waldron*,[†]

[†]Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, United Kingdom, and [‡]Unilever R&D Colworth, Sharnbrook MK44 1LQ, United Kingdom. [§]Present address: Centre for Nutrition and Food Sciences, The University of Queensland, St. Lucia, Brisbane 4072, Australia.

Nutrient bioaccessibility and subsequent absorption will be directly influenced by changes in food structure during gastrointestinal processing. The accompanying paper (Tydeman et al. *J. Agric. Food Chem.* **2010**, *58*, doi: 10.1021/jf101034a) reported results on the effect of carrot processing on the release of carotene into lipid phases during in vitro gastric and small intestinal digestions. This paper describes results from in vivo digestion of two of the types of processed carrot used previously, raw grated carrot and cooked carrot mashed to a purée. Ileostomy effluents from human volunteers fed meals containing the carrot material were used to study tissue microstructure and carotene release. Raw carrot shreds and intact cells that had survived the puréeing process were identifiable in ileal effluent. The gross tissue structure in the shreds had not changed following digestion. Carotene-containing particles remained encapsulated in intact cells, but were absent from ruptured cells. Microscopy revealed marked changes to the cell walls including swelling and pectin solubilization, which increased in severity with increasing residence time in the upper gut. These observations were entirely consistent with the in vitro observations. It was concluded that a single intact cell wall is sufficient to reduce carotene bioaccessibility from a cell by acting as a physical barrier, which is not broken down during upper gut digestion.

KEYWORDS: In vivo digestion; ileostomy; carrot; *Daucus carota*; cell wall; carotene; bioaccessibility; microstructure

INTRODUCTION

Epidemiological evidence has suggested that people who eat more plant-derived foods have better health and lower risk of chronic diseases (*1*, *2*). The potential for bioactive components within plant-based foods to exert their effects in the body is dependent on their release from the food matrix during digestion, conversion to absorbable species, and subsequent absorption. Recent studies have shown that plant structure can be a controlling factor in the bioaccessibility (proportion of ingested micronutrient available for absorption by the gut) of a range of nutrients from plant-based foods. Examples include carotenoids from vegetables (*3*, *4*), lipids from almonds (*5*, *6*), and starches from bananas and beans (*7*, *8*). Although food structure is considered to be of importance with regard to the metabolic response to starchy food, there remains a lack of information on the effect of plant structure on the bioaccessibility of many other nutrients and bioactive compounds such as carotenoids.

Carotenoids are associated with a decreased risk of cardiovascular disease (*9*) and some cancers (*10*), and some, notably β-carotene, act as precursors to vitamin A. However, carotenoid bioavailability is greatly variable, with a wide range of values (2−90%) reported depending on the meal given and the model used (*11*). In our accompanying paper (*12*) we reported the results from experiments measuring carotene transfer from carrot tissue to lipid phases during simulated upper gastrointestinal (GI) tract digestions. Such transfer is believed to be a necessary prerequisite for absorption owing to the hydrophobic nature of carotene (*13*, *14*). We showed that although there was marked swelling of the cell walls during the digestions, they remained intact and did not allow release of carotene from the cells. Consideration of the structural changes of plant foods during digestion in vivo has not been studied in much detail because of the difficulty in aspirating plant tissue samples from the GI tract and, in some cases, a desire to demonstrate absorption through changes in plasma concentration elicited by feeding nonphysiological doses of pure compounds, juices, and purées.

To improve our understanding of the structural characteristics of plant tissue that determine carotenoid bioaccessibility, we used samples of raw carrot and puréed cooked carrot to examine the changes in structure and carotenoid location induced by digestion in the upper gut of ileostomists. Ileostomists have had their colon removed for medical reasons and the terminal ileum is brought to a stoma in the abdominal wall. Although the stoma may be

*Corresponding author (phone 0044 1603 255385; fax 0044 1603 507723; e-mail keith.waldron@bbsrc.ac.uk).

Published on Web 08/12/2010

colonized by a small population of organisms, microbiological degradation is usually minimal because there is no storage and the effluent is discharged from the stoma by the normal peristalsis of the ileum, allowing convenient, real-time collection of effluent. Carotene, the major carotenoid in carrot cells, can be directly visualized by microscopy with minimal sample preparation, as it is stored in a concentrated highly-colored form in chromoplasts. The samples examined were from the study of Livny et al. (*15*), who recovered 59 and 35% of total ingested β-carotene in ileostomy effluents from raw and cooked forms, respectively. The microscopic techniques reported here identify the features responsible for the retention of carotene within foods after upper gut digestion. They demonstrate, as did our in vitro studies (*12*), the importance of cell walls in limiting carotene bioaccessibility.

MATERIALS AND METHODS

In Vivo Digestion of Carrot Tissue. The protocol is described in detail by Livny et al. (*15*). In brief, eight volunteers (38−75 years of age) with an average BMI of 20.5 kg/m^2 gave informed consent to the study, which was conducted at the Soroka Medical Centre, Israel. The Israeli Ministry of Health Ethics committee approved the study. Volunteers were at least 2.5 years postsurgery, in otherwise good health, and had not had more than 15 cm of the terminal ileum removed. Apart from the ileostomy, all subjects had entirely normal ingestion and digestion of food with no indications of abnormal health conditions.

The volunteers arrived at 8:00 a.m. having fasted overnight from 7:00 p.m. the previous evening. After changing their effluent collection bag, the subjects were given a breakfast meal of either 200 g of raw grated carrot or 240 g of carrot purée (prepared from boiled carrot, mashed and sieved through 0.5 mm^2 mesh) followed by a standard meal of 400 g of skimmed milk yogurt containing 40 g of sunflower oil, 20 g of sucrose, and chocolate flavoring. The volunteers were instructed to refrain from chewing the carrot. Stomal effluents were collected every 2 h up to 12 h and then as timed samples up to 24 h at the convenience of the volunteers. The effluents were rapidly frozen on collection and stored at −80 °C until analysis. Control (undigested) tissue was frozen and stored in the same way.

Microscopy. Pieces of carrot tissue were present in effluents up to 10 h after the meal for all volunteers. The effluents were defrosted at 5 °C and thoroughly mixed. From the effluents taken from volunteers fed raw grated carrot, a number of carrot shreds were removed at random from each of the serial samples and briefly rinsed in distilled water to remove excess digesta. Time course samples from three subjects (chosen at random) were examined by light microscopy as detailed below. For the volunteers fed puréed carrot, an aliquot of the serial samples of the whole effluent was examined after each was mixed thoroughly.

Preparation of Carrot Tissue for Light Microscopy. Carrot shreds were sectioned transversely by hand, mounted in water, and examined and photographed unstained with bright field optics as described previously (*12*). The walls of the carrot cells and the location of carotene were clearly visible in these samples.

For examination of carrot purée, a small amount of thoroughly-mixed effluent or control material was placed onto a glass slide and covered with a coverslip. Microscopy was conducted as above.

Preparation of Sections of Resin-Embedded Carrot Tissue for Light Microscopy of Cell Walls. Carrot shreds were fixed, dehydrated, embedded in resin, and sectioned transversely into 1 μm thick slices. Sections were stained with toluidine blue and examined and photographed as described (*12*).

RESULTS

Raw Carrot Shreds Digested in Vivo. Intact shreds were positively identified within effluent samples obtained from the ileostomy volunteers fed raw grated carrot. There were no obvious differences in the gross structure of raw (**Figure 1A**) or digested (**Figure 1B**) shreds. Light microscopy of hand-cut sections was used to determine the effect of digestion at the cellular level. Carotene aggregates (commonly termed "crystals") in undigested carrot tissue are shown in **Figure 2A**. After 10 h in the upper gut, carotene

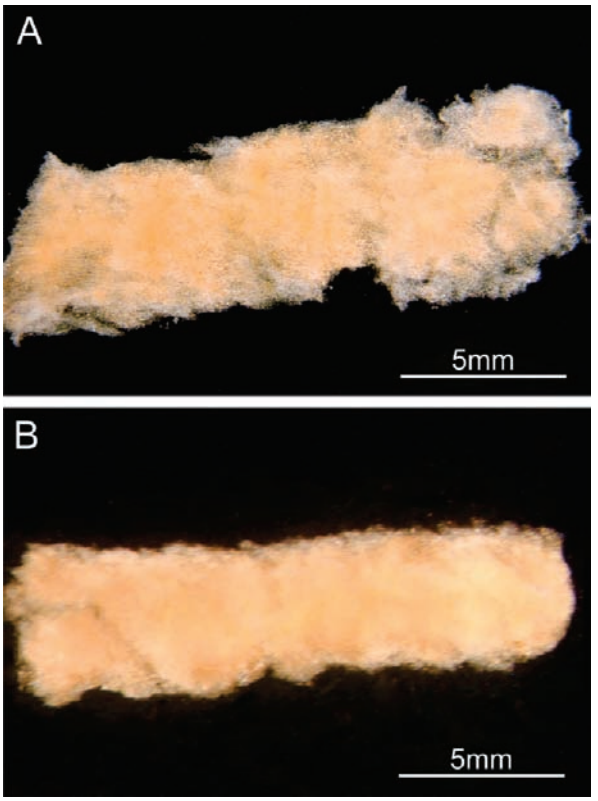

Figure 1. Raw grated carrot shreds: (**A**) undigested control; (**B**) material recovered at the terminal ileum 10 h postingestion. Note the limited change to gross structure.

aggregates were still present within intact cells (**Figure 2B**,**C**), and it was only ruptured cells at the surface of the carrot shreds that were devoid of carotene (**Figure 2B**,**C**). Indeed, carotene within intact cells was positively identified at all time points. Spherical lipid droplets were associated with the carotene (**Figure 2D**, arrow heads). It is possible these droplets result from coalescence of intracellular lipids and membranes. These droplets were present throughout the in vivo residence time.

To investigate the effect of digestion on the microstructure of carrot cell walls, shreds were embedded in resin, sectioned transversely, and stained with toluidine blue prior to light microscopy. This method is ideal for visualizing changes in cell-wall morphology, but it does not preserve the carotene and lipid content of the cells (*12*). The shreds from ileal effluents from three volunteers were examined and found to be similar. The findings from one subject are illustrated in **Figure 3**. The cell walls of parenchyma cells in undigested, control tissue (**Figure 3A**) are characteristically thin-walled, and the intercellular spaces between cells are small. After a 2 h residence time in the upper gut (**Figure 3B**), there was no apparent change in the appearance of the cell walls. However, after 6 h, the walls showed evidence of swelling (**Figure 3C**) and a concomitant reduction in staining with toluidine blue. The enhanced purple stain in the intercellular spaces between the cells suggested that pectin has accumulated in these regions. The changes progressed through residence times of 8 h (**Figure 3D**) and 10 h (**Figure 3E**,**F**) until the whole of the cell wall appears swollen and diffuse and the intercellular spaces were full of pectin (**Figure 3F**, arrows). Despite the changes to the walls, the cells remained physically intact with only the cells at the surface of the shreds disrupted as a result of the initial grating procedure.

Cooked Carrot Purée Digested in Vivo. Light microscopy of the cooked purée prior to digestion revealed not only a significant number of free carotene-containing particles (**Figure 4A**) but also

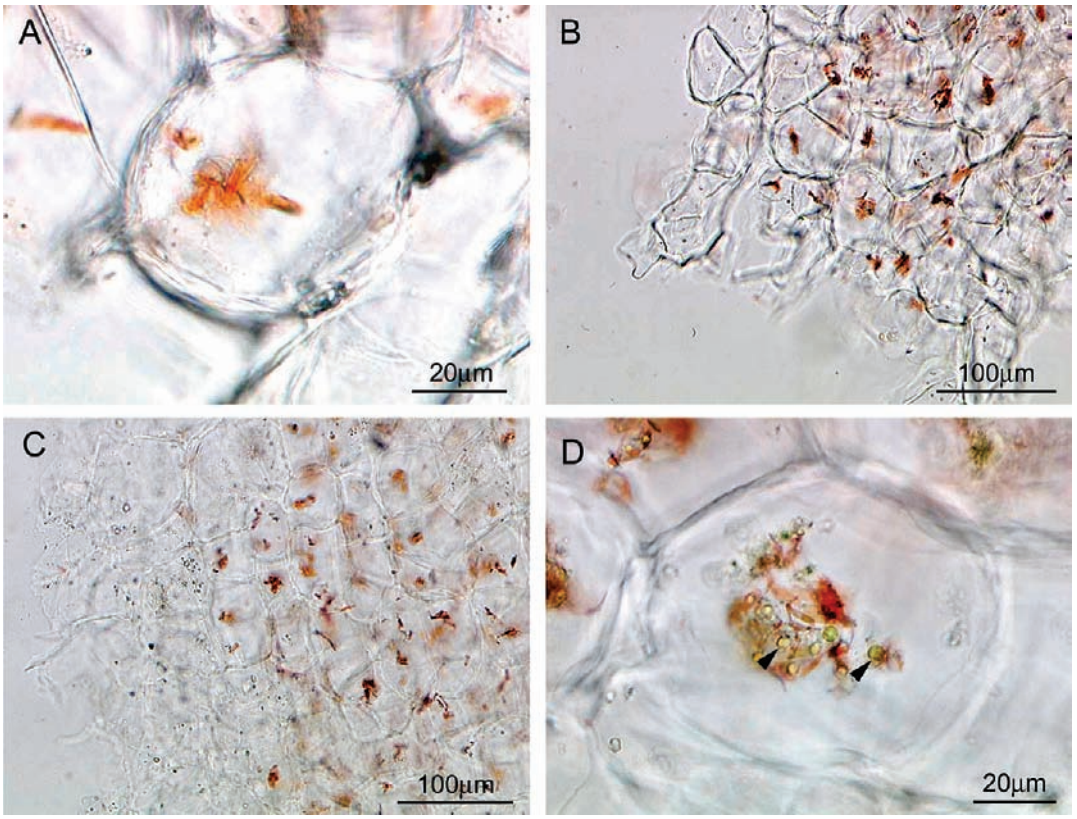

Figure 2. Effect of in vivo residence time on raw grated carrot shreds recovered from ileostomy patients: (**A**) edge of section of undigested raw shreds (control); (**B**−**D**) edge of sections of shreds after residence of 10 h. Note the presence of yellow lipid droplets (arrowheads in **D**) and orange carotene crystals. Samples were unstained.

a large number of intact cells in small clumps (**Figure 4B**). Following digestion, intact cell clumps encapsulating cell contents were found at all time points up to 10 h (**Figure 4C**,**D**). Carotene was retained within intact cooked cells despite being subjected to in vivo digestion for up to 10 h (**Figure 4D**). Clearly, the cell walls do not break down sufficiently to release carotene during digestion in the upper gut despite having been first cooked in boiling water.

DISCUSSION

Raw carrot shreds were identified within ileostomy effluents up to 10 h postfeeding and showed no apparent change in gross structure. Furthermore, the clumps of cells in the cooked purée in effluent collected 10 h after feeding were also found to be largely unaffected by digestion. Comparison of samples prior to and during digestion suggests that cells that had not been ruptured during the grating or puréeing step remain intact and retain both carotene and droplets of lipid. The comparison also indicates that the freezing and storage of the samples did not affect the microstructure of the tissue. The presence of carotene in uncut cells close to the surface of raw carrot pieces and in cooked purée in ileostomy effluents, together with our in vitro results (*12*), suggests that a single intact cell wall is an effective barrier to the release of carotene from food structures, even after cooking. Therefore, we first discuss the details of the change of this barrier during digestion.

Swelling of the cell walls increased in extent with increasing residence time in the gut. This swelling, observed and discussed previously in our in vitro work (*12*), is associated with movement of water into voids left in the cellulose−hemicellulose network by solubilized pectin (*16*, *17*). We have considered the possibility that it may be a result of bacterial fermentation from bacterial colonization of the terminal ileum. However, any significant invasion causes medical complications (*18*, *19*), which were absent in the volunteers. The effluent collections were made every 2 h and immediately frozen to minimize bacterial growth and fermentation within the bags. This method has been used extensively, and bacterial degradation of nonstarch polysaccharides has been found to be small (*20*, *21*). No adherent bacteria were observed associated with the cell walls despite bacteria being generally well preserved during preparation for microscopy (*5*, *22*). In addition, bacterial degradation generally involves pitting, erosion, and thinning of the cell walls, which were not identified here. It is therefore unlikely that the cell-wall changes observed during upper gut digestion are a consequence of bacterial fermentation.

We know of no endogenous enzymes present in the human upper gut that can degrade plant cell walls. We consider first the raw carrot, where changes in cell walls due to cooking (*16*, *23*) are absent. Solubilization of cell-wall pectin is most likely to occur in the gastric conditions, where acid hydrolyzes glycosidic linkages and stabilizing Ca^{2+} is displaced by H^{+} (*24*). Certainly, for raw carrot, the most significant cell-wall swelling we observed in vitro occurred after the 2 h simulated gastric digestion (*12*). In contrast, the 2 h samples from the ileostomists showed no cell-wall swelling (**Figure 3B**), because this material had had the shortest residence time in the stomach and the effect of the time in the small intestine on the cell walls was relatively small. After 6 h, the cell walls in ileostomy effluent appeared to be similar to those from the in vitro 2 h gastric followed by 2 h duodenal digestion, except that the intercellular staining of the solubilized pectin was more uniform in the in vivo samples, presumably because of the longer time for the pectin to diffuse into the spaces. These results are in accord with other work. For example, Hoebler et al. (*25*) found that the conditions of the digestive tract can partially solubilize pectic substances from the cell walls of beet fiber following

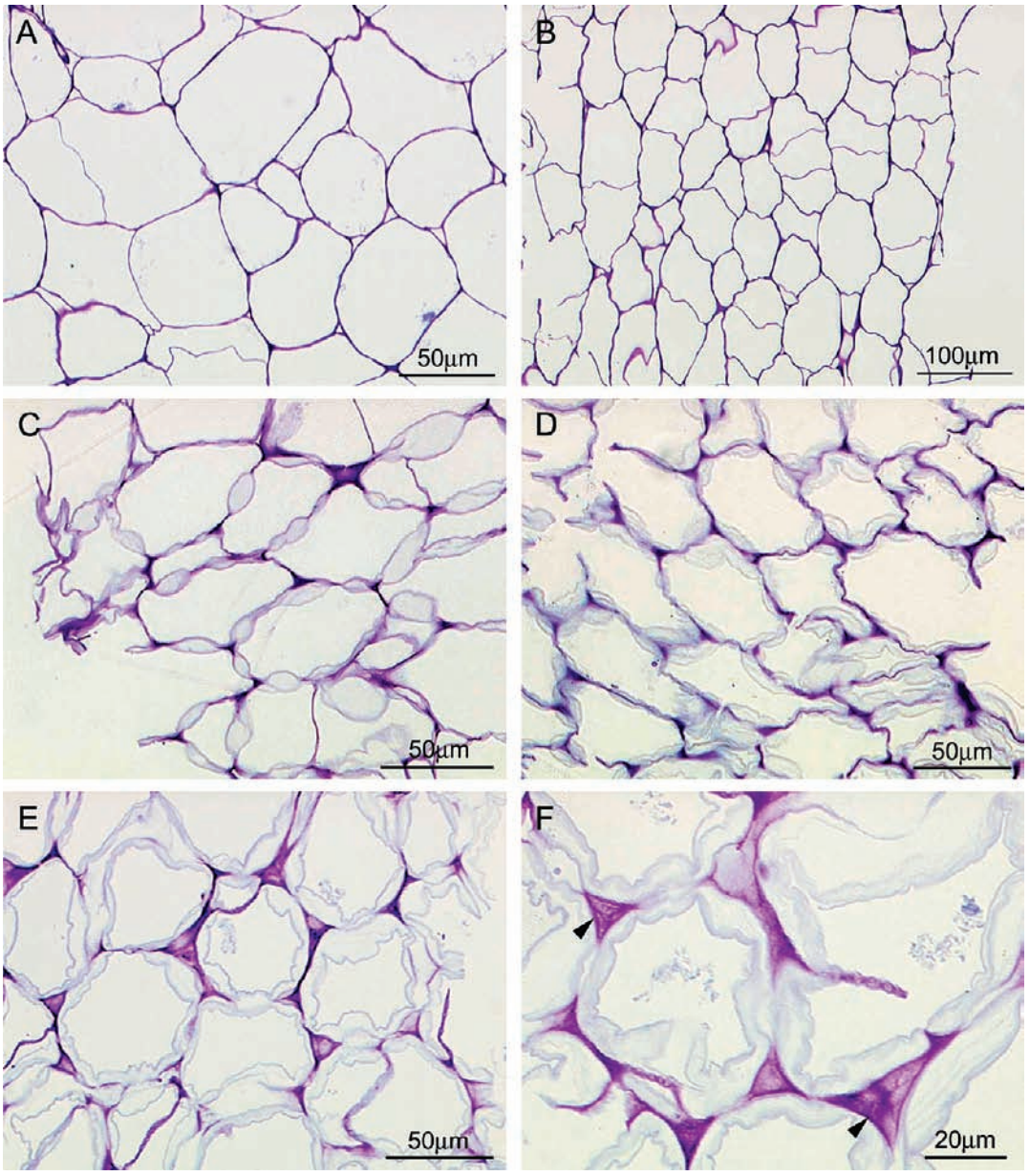

Figure 3. Effect of in vivo residence time on the cell walls of raw carrot recovered from ileostomy effluents: (**A**) undigested raw carrot (control); (**B**) 2 h; (**C**) 6 h; (**D**) 8 h; (**E**) 10 h; (**F**) 10 h. Note severity of cell-wall swelling as residence time increases and solubilized pectin in intercellular spaces, arrowed in (**F**). Samples were embedded in LR White resin and 1 μm thick sections stained with toluidine blue.

digestion in the ileal-cannulated pig. Zhang and co-workers (*26*) found that up to 10% L-arabinose was released from cell-wall hemicelluloses (corn hull, larch wood, and banana peel) during gastric simulation. Progressive cell-wall swelling has also been observed during the digestion of almond seeds (*6*).

Despite the cell-wall swelling, carotene is lost only from cells that have been ruptured prior to digestion. This is true for both raw and cooked carrot and reflects the hydrophobic nature of carotene and its stability in the crystalline state so that during digestion it can only be solubilized in lipid phases, which cannot penetrate the cell walls. The fact that more carotene was absorbed from the cooked carrot purée than from the raw shreds (*15*), whereas our in vitro work showed cooking reduced carotene release, is a reflection of the smaller particle size of the carrot purée consumed by the ileostomists. Also, as **Figure 4A** shows, the undigested purée contained many free carotene crystals that are easily solubilized in a lipid phase (*27*). If the latter (micelles and oil droplets) cannot penetrate the cell walls, which is clearly the case despite the swelling, the carotene cannot be released. The cell wall acting as a barrier so that only cut cells at the surfaces of tissue can release their nutrients has been noted for other plant material: the aleurone and endosperm of wheat (*28*) and almond seeds (*5*, *6*). Furthermore, the cell wall has been found to act as a barrier to the effective diffusion of carbohydrates during in vitro rumen digestion of forage plants (*29*).

A number of studies have been conducted on the recovery of dietary fiber following upper gut digestion, and the current findings are in general agreement with these. Studies on potatoes and beans found that nonstarch polysaccharides and dietary fiber are almost totally recovered in the ileostomy effluents of humans (*21*,*30*). Recent work by Serrano et al. (*31*) found a negative correlation between an indigestible fraction (Klason lignin) of green vegetables (spinach, chaya, and macuy) and the release of carotene during enzymatic digestion.

We have found that cell rupture is an absolute requirement for carotene release and that intact plant cells can survive digestion in

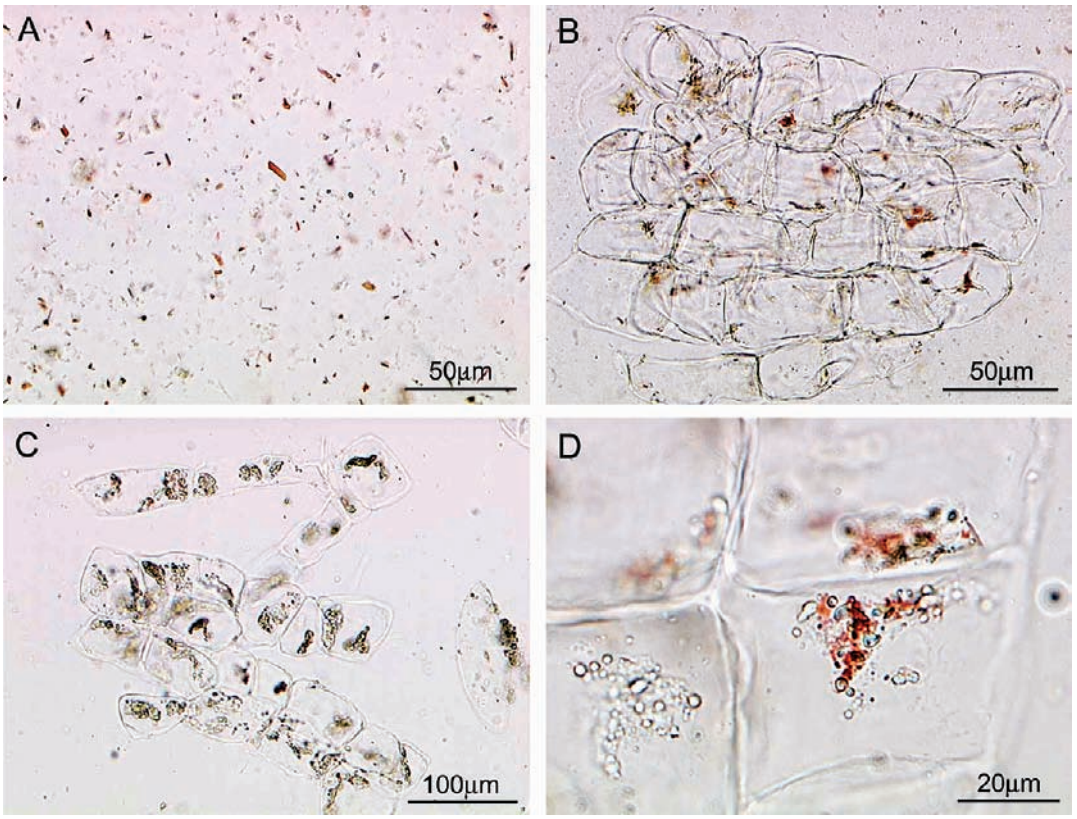

Figure 4. Effect of in vivo residence time on cooked carrot purée recovered from ileostomy effluents: (**A**) undigested purée showing free crystalline carotene; (**B**) intact cells; (**C**) 6 h (note that intact cells remain); (**D**) 10 h (note carotene presence). Samples were unstained.

the upper gastrointestinal tract, resulting in fiber and encapsulated carotene being carried to the colon for fermentation. It appears carotenes are made bioaccessible during food processing and/or mastication and not during digestion in the upper gastrointestinal tract. Although heating will affect cell-wall integrity, this process was found not to facilitate carotene release from intact cells. As only a minority of carotene is released from carrot tissue by the end of the small intestine both in vitro (*12*) and in vivo (*15*), the majority of carotenes will enter the colon. Here bacterial fermentation is expected to break down most carrot cell walls, thereby allowing the release of carotenes, which can provide protection against free radical formation and may subsequently be metabolized by colonic bacteria. It remains to be determined whether intact carotenes or their fermentation metabolites are taken up from the colon.

LITERATURE CITED

(1) Block, G.; Patterson, B.; Subar, A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr. Cancer* **1992**, *18*, 1–29.

(2) Bazzano, L. A.; He, J.; Ogden, L. G.; Loria, C. M.; Vupputuri, S.; Myers, L.; Whelton, P. K. Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first national health and nutrition examination survey epidemiological follow-up study. *Am. J. Clin. Nutr.* **2002**, *76*, 93–99.

(3) van het Hof, K. H.; West, C. E.; Weststrate, J. A.; Hautvast, J. G. A. T. Dietary factors that affect the bioavailability of carotenoids. *J. Nutr.* **2000**, *130*, 503–506.

(4) Castenmiller, J. J. M.; West, C. E.; Linssen, J. P. H.; van het Hof, K. H.; Voragen, A. G. J. The food matrix of spinach is a limiting factor in determining the bioavailability of β-carotene and to a lesser extent of lutein in humans. *J. Nutr.* **1999**, *129*, 349–355.

(5) Ellis, P. R.; Kendall, C. W. C.; Ren, Y.; Parker, C.; Pacy, J. F.; Waldron, K. W.; Jenkins, D. J. A. Role of cell walls in the bioaccessibility of lipids in almond seeds. *Am. J. Clin. Nutr.* **2004**, *80*, 604–613.

(6) Mandalari, G.; Faulks, R. M.; Rich, G. T.; Lo Turco, V.; Picout, D. R.; Lo Curto, R. B.; Bisignano, G.; Dugo, P.; Dugo, G.; Waldron, K. W.; Ellis, P. R.; Wickham, M. S. J. Release of protein, lipid, and vitamin E from almond seeds during digestion. *J. Agric. Food Chem.* **2008**, *56*, 3409–3416.

(7) Faisant, N.; Buleon, A.; Colonna, P.; Molis, C.; Lartigue, S.; Galmiche, J. P.; Champ, M. Digestion of raw banana starch in the small intestine of healthy humans: structural features of resistant starch. *Br. J. Nutr.* **1995**, *73*, 111–123.

(8) Noah, L.; Guillon, F.; Bouchet, B.; Buleon, A.; Molis, C.; Gratas, M.; Champ, M. Digestion of carbohydrate from white beans (*Phaseolus vulgaris* L.) in healthy humans. *J. Nutr.* **1998**, *128*, 977–985.

(9) Gey, K. F.; Moser, U. K.; Jordan, P.; Stahelin, H. B.; Eichholzer, M.; Ludin, E. Increased risk of cardiovascular disease at suboptimal plasma concentrations of essential antioxidants: an epidemiological update with special attention to carotene and vitamin C. *Am. J. Clin. Nutr.* **1993**, *57*, 787S–797S.

(10) Astorg, P. Food carotenoids and cancer prevention: an overview of current research. *Trends Food Sci. Technol.* **1997**, *8*, 406–413.

(11) Faulks, R. M.; Southon, S. Carotenoids, metabolism and disease. In *Handbook of Nutraceuticals and Functional Foods*; CRC Series in Modern Nutrition; Wildman, R. E. C., Ed.; CRC Press: Boca Raton, FL, 2001; pp143−156.

(12) Tydeman, E. A.; Parker, M. L.; Wickham, M. S. J.; Rich, G. T.; Faulks, R. M.; Gidley, M. J.; Fillery-Travis, A.; Waldron, K. W. Effect of carrot (*Daucus carota*) microstructure on carotene bioaccessibility in the upper gastrointestinal tract. 1. In vitro simulations of carrot digestion. *J. Agric. Food Chem.* **2010**, *58*, doi: 10.1021/jf101034a.

(13) Borel, P.; Grolier, P.; Armand, M.; Partier, A.; Lafont, H.; Lairon, D.; Azais-Braesco, V. Carotenoids in biological emulsions: solubility, surface-to-core distribution, and release from lipid droplets. *J. Lipid Res.* **1996**, *37*, 250–261.

(14) Tyssandier, V.; Reboul, E.; Dumas, J.-F.; Bouteloup-Demange, C.; Armand, M.; Marcand, J.; Sallas, M.; Borel, P. Processing of vegetable-borne carotenoids in the human stomach and duodenum. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2003**, *284*, G913–G923.

(15) Livny, O.; Reifen, R.; Levy, I.; Madar, Z.; Faulks, R.; Southon, S.; Schwartz, B. β-Carotene bioavailability from differently processed carrot meals in human ileostomy volunteers. *Eur. J. Nutr.* **2003**, *42*, 338–345.

(16) Waldron, K. W.; Parker, M. L.; Smith, A. C. Plant cell walls and food quality. *Comp. Rev. Food Sci. Food Saf.* **2003**, *2*, 128−146 (web based).

(17) Redgwell, R. J.; MacRae, E.; Hallett, I.; Fischer, M.; Perry, J.; Harker, R. In vivo and in vitro swelling of cell walls during fruit ripening. *Planta* **1997**, *203*, 162–173.

(18) Warner, B. W. Short- and long-term complications of colectomy. *J. Pediatr. Gastroenterol. Nutr.* **2009**, *48*, S72–S74.

(19) Johnson, M. W.; Das, P.; Dewar, D. H.; Forbes, A.; Ciclitira, P. J.; Nicholls, R. J. Medical management of patients with ileal pouch anal anastomosis after restorative procto-colectomy. *Eur. J. Gastroenterol. Hepatol.* **2009**, *21*, 9–17.

(20) Lia, A.; Sundberg, B.; Aman., P.; Sandberg, A.-S.; Hallmans, G.; Andersson, H. Substrates available for colonic fermentation from oat, barley and wheat bread diets. A study in ileostomy subjects. *Br. J. Nutr.* **1996**, *76*, 797–808.

(21) Schweizer, T. F.; Andersson, H.; Langkilde, A. M.; Reimann, S.; Torsdottir, I. Nutrients excreted in ileostomy effluents after consumption of mixed diets with beans or potatoes. II. Starch, dietary fiber and sugars. *Eur. J. Clin. Nutr.* **1990**, *44*, 567–575.

(22) Garrood, M.; Wilson, P. D. G.; Brocklehurst, T. F. Modeling the rate of attachment of *Listeria monocytogenes*, *Pantoea agglomerans*, and *Pseudomonas fluorescens* to, and the probability of their detachment from, potato tissue at 10 °C. *Appl. Environ. Microbiol.* **2004**, *70*, 3558–3565.

(23) Waldron, K. W.; Smith, A. C.; Parr, A. J.; Ng, A.; Parker, M. L. New approaches to understanding and controlling cell separation in relation to fruit and vegetable texture. *Trends Food Sci. Technol.* **1997**, *8*, 213–221.

(24) Diaz, J. V.; Anthon, G. E.; Barrett, D. M. Nonenzymatic degradation of citrus pectin and pectate during prolonged heating: effects of pH, temperature, and degree of methyl esterification. *J. Agric. Food Chem.* **2007**, *55*, 5131–5136.

(25) Hoebler, C.; Guillon, F.; Fardet, A.; Cherbut, C; Barry, J.-L. Gastrointestinal or simulated *in vitro* digestion changes dietary fibre properties and their fermentation. *J. Sci. Food Agric.* **1998**, *77*, 327–333.

(26) Zhang, P.; Zhang, Q.; Whistler, R. L. L-Arabinose release from arabinoxylan and arabinogalactan under potential gastric acidities. *Cereal Chem.* **2003**, *80*, 252–254.

(27) Rich, G. T.; Fillery-Travis, A.; Parker, M. L. Low pH enhances the transfer of carotene from carrot juice to olive oil. *Lipids* **1998**, *33*, 985–992.

(28) Parkkonen, T.; Tervila-Wilo, A.; Hopeakoski-Nurminen, M.; Morgan, A.; Poutanen, K.; Autio, K. Changes in wheat microstructure following in vitro digestion. *Acta Agric. Scand., Sect. B* **1997**, *47*, 43–47.

(29) Kingston-Smith, A. H.; Bollard, A. L.; Thomas, B. J.; Brooks, A. E.; Theodorou, M. K. Nutrient availability during the early stages of colonization of fresh forage by rumen micro-organisms. *New Phytol.* **2003**, *158*, 119–130.

(30) Englyst, H. N.; Cummings, J. H. Digestion of polysaccharides of potato in the small intestine of man. *Am. J. Clin. Nutr.* **1987**, *45*, 423–431.

(31) Serrano, J.; Goni, I.; Saura-Calixto, F. Determination of β-carotene and lutein available from green leafy vegetables by an in vitro digestion and colonic fermentation method. *J. Agric. Food Chem.* **2005**, *53*, 2936–2940.

Received for review September 23, 2009. Revised manuscript received June 22, 2010. Accepted June 23, 2010. This work was supported by a BBSRC-CASE studentship with Unilever R&D Colworth, U.K.