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Mastication effects on carotenoid bioaccessibility from mango fruit tissue

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ABSTRACT

The release of carotenoids from fresh fruits or vegetables is determined by the encapsulating plant tissue matrix, intracellular carotenoid location within the cell, and the mastication process. The objectives of this study were to assess the particle sizes obtained after mastication of mango fruit tissue, and how the resulting degree of plant tissue rupture affects carotenoid bioaccessibility. A fine and a coarse chewer were selected after screening 20 healthy volunteers for *in vivo* human mastication, and the collected chewed boluses were subjected to wet sieving fractionation, followed by an *in vitro* gastric and small intestinal digestion model. Confocal micrographs show that the smallest particle size fraction (0.075 mm) consists mostly of fragmented cells and the largest size fraction (2.8 mm) contains bulky clusters of whole cells and vascular fibers. Higher amounts of total carotenoids (211–320 μg/100 g) were observed in the larger particle size fraction (2.8 mm) relative to the 1 mm (192–249 μg/100 g) and 0.075 mm fractions (136–199 μg/100 g). Smaller particles showed a greater % release of total carotenoids after *in vitro* digestion. Xanthophyll derivatives are more bioaccessible than β-carotene for all particle sizes. The effects of particle size or degree of fine vs coarse chewing are unexpectedly small ($p > 0.05$), but the process of chewing substantially reduced the release of β-carotene and xanthophylls by 34% and 18%, respectively. While there is a (small) particle size effect, this appears to not be the primary factor controlling bioaccessibility for soft tissues such as mango, in contrast to previous reports that a single cell wall appears to be enough to prevent bioaccessibility of carotenoids in more robust carrot tissues.

1. Introduction

Epidemiological studies have shown an inverse correlation between consumption of carotenoid-rich fruits and vegetables, and the incidence of cancers of the gastrointestinal tract (Kant, Block, Schatzkin, & Nestle, 1992; Kiokias & Gordon, 2004; Mayne, 1996; Rock & Swendseid, 1992), cardiovascular diseases (Krinsky, 1998; Murr et al., 2009), diabetes (Yeum & Russell, 2002), some inflammatory diseases (Perera & Yen, 2007), and age-related macular degeneration (Snodderly, 1995). The most documented function of β-carotene is its provitamin A activity, with consequent health benefits, such as maintenance of epithelial function, embryonic development, and immune system function (Diplock, 1991). Xanthophylls are only present in human retinal pigment epithelia, in contrast to other body sites where all other carotenoids occur (Bone, Landrum, Hime, Cains, & Zamor, 1993), and probably function as blue light filters and singlet oxygen quenchers (Seddon et al., 1994).

Human studies are most appropriate to predict nutrient bioavailability, but these studies have technical and ethical limitations (Netzel et al., 2011). Metabolic and physiological factors have been reported to influence the absorption, distribution and elimination of carotenoids (Bowen, Mobarhan, & Smith, 1993; Johnson, Qin, Krinsky, & Russell, 1997; Kostic, White, & Olson, 1995), resulting in inter-individual variability in plasma concentrations. In addition, host-related factors such as gut health, nutritional status or discrepancies, and genotype are typically encountered in most laboratory rodent models (Van Buggenhout et al., 2010). However, these factors can be avoided through the use of *in vitro* models. *In vitro* models are relatively easy to apply to large sample numbers, and are suitable for studying the effects of various digestion conditions or other factors linked to nutrient bioaccessibility (Fernandez-Garcia et al., 2012). *In vitro* digestion models can be used to simulate the physiological conditions of gastric and intestinal digestion. In addition, nutritional recommendations are often based on intakes or concentrations present in extracts of raw plant material, not taking into account bioaccessibility and any changes during gastrointestinal digestion. This could result in nutrient overestimation and emphasizes the importance of estimating bioaccessibility.

The current *in vitro* digestion procedures have proven useful for the analysis of carotenoid release and/or bioaccessibility (Castenmiller & West, 1998; Tydeman, Parker, Faulks et al., 2010). However, the reliability of the two-phase (stomach and small intestine) *in vitro* digestion model would be expected to be improved by including a "real" chewing phase, or a phase that more closely mimics actual chewing behavior and mechanics, which has been excluded in most digestion studies.

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Mastication is often the first step of food digestion, where the process of breaking down solid foods into smaller particle sizes and mixing with saliva takes place. During simulated or real oral chewing, the physical barriers to the release of nutrients from plant cells may be ruptured. Therefore, the degree of cellular intactness could be indicative of their potential bioaccessibility, particularly as cell breakage is likely to be a major requirement for carotenoid bioaccessibility (Lemmens, Van Buggenhout, Van Loey, & Hendrickx, 2010; Tydeman, Parker, Wickhan et al., 2010). Ideally, the structural properties of a food product digested *in vitro* should be similar to that of a chewed food bolus, since mastication varies subjectively between individuals, which impacts on food matrices and the structural properties of food boluses. Currently, simulated oral chewing has been mimicked using techniques such as pulverizing, sieving, chopping, or mincing (Woolnough, Monro, Brennan, & Bird, 2008) and the occasional inclusion of (salivary) α-amylase for starch digestion (Bornhorst, Hivert, & Singh, 2014; Miao et al., 2014). However, such mechanical steps do not adequately reflect the heterogeneous nature of chewed food. Epriliati, D'Arcy, and Gidley (2009) demonstrated the importance of the simultaneous punch and gentle squash action of teeth, while Hoerudin (2012) found that mastication has a considerable effect on the cellular architectures of vegetables. In addition, mastication involves lubrication, softening, and dilution with saliva (Lucas et al., 2006; Prinz & Lucas, 1995) and the formation of a cohesive bolus (Barry et al., 1995).

Mangoes are the second most important tropical fruit in terms of production and consumption and have high carotenoid contents, particularly of β-carotene (Chen, Tai, & Chen, 2004; Yahia, Soto-Zamora, Brecht, & Gardea, 2007), which is responsible for the yellow-orange color of ripe mango flesh (Pott, Breithaupt, & Carle, 2003). Current carotenoid studies have focused on the compositional profile or content (de la Rosa, Alvarez-Parrilla, & Gonzalez-Aguilar, 2010; Manthey & Perkins-Veazie, 2009; Mercadante & Rodriguez-Amaya, 1998; Robles-Sanchez et al., 2009), the impacts of ripening stages (Ornelas-Paz, Yahia, & Gardea, 2008), the presence of fat (Veda, Platel, & Srinivasan, 2007), and the effects of processing (dried, fresh, juice) (Epriliati et al., 2009). However, mastication effects on carotenoid gastrointestinal release from mango fruit have not been reported. Comparisons of the carotenoid content before and after *in vitro* digestion can provide information on their stability during gastrointestinal digestion. *In vitro* digestion models can be adapted to estimate the bioaccessibility of carotenoids by quantifying the fractions of phytonutrients transferred from the food matrix into the aqueous digesta or micellar phase, which then represents their potential for absorption or bioavailability. Studies have shown that the bioaccessibility of carotenoids can be as inefficient as 1.7% or as high as 100% (Tydeman, Parker, Faulks, et al., 2010), depending on the type of carotenoids as well as raw versus cooked conditions. The different solubility of polar xanthophylls and apolar carotenes can also affect their ability to be incorporated into micelles and thus affect both release and absorption efficiency.

It is hypothesized that the mechanism limiting carotenoid release involves intact cell walls (Tydeman, Parker, Faulks, et al., 2010), which prevent the passage of carotenoids into lipid-soluble components or micelles, thus affecting bioaccessibility. The objective of this study was to investigate how the degree of mastication results in varying size distributions of ready to swallow bolus particles, and how this affects subsequent simulated gastrointestinal release of carotenoids from masticated mango tissue.

2. Materials and methods

2.1. Plant material

Fully ripe mangoes (cv. Kensington Pride) were purchased from local stores in St. Lucia, Brisbane (Australia) 2–3 days before each of three chewing sessions, in the month of November 2012. Mango ripeness was selected based on typical eating maturity at stage 6 when the peel is yellow with pink-red blush and the flesh is slightly firm, according to the Department of Agriculture, Fisheries and Forestry (Queensland Government) mango-ripening guide (Primary Industries & Fisheries, 2012). Mangoes were stored at 4–6 °C prior to the chewing sessions.

2.2. Chewing, blending, and bolus collection

Chewing experiments were approved by the Medical Research Ethics Committee at The University of Queensland (ethical clearance no. 2012000683). Twenty healthy participants (aged 18–55 years) were recruited on the basis of frequent mango consumption and all gave informed consent to the study for mastication of fresh fruit. Individual mastication profiles from all the participants were studied for the selection of a fine and coarse chewer. Three independent chewing sessions were carried out on three different days of each consecutive week to account for inter-day variation. The chewing sessions were held between 9:00 and 11:00 a.m., after the chewers had consumed a light breakfast meal. 5–6 mangoes (300–600 g each) were cut into cubes and 300 g of cubes were randomly selected from the sample pile, and given to each of the fine and coarse chewer. The remaining cubes were combined and blended (Rocket blender DJL-1017, Cafe™ Essentials, China) for 1 min to a puree to determine the carotenoid composition of the fresh mango. The chewers were instructed to chew the mango as per their habitual chewing behavior, and to expectorate when they desired to swallow. The expectorated boluses were collected, washed with 70% ethanol to prevent further biochemical changes, and fractionated via a wet sieving method, where water was flushed through a stack of sieves of apertures 5.6, 2.8, 1, 0.5, and 0.075 mm (Fig. 1). The sieved particles were drained and collected for *in vitro* digestion. Chewing, fractionation, *in vitro* digestion, and blending processes were carried out in a single day.

2.3. In vitro gastrointestinal digestion

Gastrointestinal conditions were modified from Hoerudin (2012). Gastric digestion (1 h) of puree and bolus samples (2 ± 0.05 g) was initiated with 10 mL of emptying gastric secretion (130 mM NaCl, 5 mM KCl, 5 mM PIPES), followed by addition of 1 M HCl to reduce the pH to 2, and 1 mL porcine pepsin (1:2500 U/mg protein, Sigma-Aldrich, NSW, Australia) solution. Subsequently, transition from gastric to small intestinal phase was reflected by raising the pH to 6 with 1 M $NaHCO_3$. Small intestinal digestion (1 h) was mimicked by adding 5 mL pancreatin (lipase activity ≥8 USP U/mg, protease and amylase ≥4 USP U/mg, Chem Supply, Adelaide, Australia) bile (Sigma-Aldrich, NSW Australia) extract, adjusting the overall pH to 7, and diluting with 5 mL intestinal salt secretion (120 mM NaCl, 5 mM KCl). To simulate physiological movement, the mixtures were incubated in a shaking water bath at 37 °C, 55 rpm. Digesta samples were then centrifuged at 3000*g*, 10 min (Centrifuge 5702R, Eppendorf, USA) to separate the bioaccessible fraction from residual pellet, flushed with nitrogen and stored at -80 °C.

2.4. Carotenoid extraction

Carotenoid extractions of the puree, digesta, and residual pellets were carried out the very next day after chewing and digestion, as modified from Ornelas-Paz, Failla, Yahia, and Gardea (2008). Puree (0.8 g) and digested pellets were vortex mixed with 2.5 mL and 1.5 mL PBS respectively. Digesta supernatants were homogenized three times with an Ultra-Turrax® at 4200 rpm with 20 mL petroleum ether:acetone (2:1) containing 0.1% BHT, or until the digesta pellets turned white. In between each homogenization step, samples were centrifuged at 3000*g* for 5 min. Organic fractions were collected, combined, evaporated under nitrogen, dissolved in methanol:tetrahydrofuran