

## Review

# The relation between the *in vitro* bioaccessibility of $\beta$ -carotene and structural characteristics of carrot pieces during thermal processing (sterilization and pasteurization) in a retort

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Accepted 21 November, 2011

It has been shown that thermal processing, which is intended to extend the shelf life of food products, may affect the quality of fruit and vegetable based products negatively (e.g. vitamin destruction, texture degradation). However, it has also been highlighted that the nutrient bioaccessibility is increased during thermal processing, possibly by the structural changes occurring during thermal processing. Understanding these quality changes could help to identify optimal processing conditions for better quality retention during thermal processing. This review is a case on carrots whereby the textural changes as well as the nutritional changes ( $\beta$ -carotene concentration and  $\beta$ -carotene bioaccessibility) are considered.

**Key words:** Carrots,  $\beta$ -carotene, processing, texture,  $\beta$ -carotene concentration and bioaccessibility.

## INTRODUCTION

### Carrots as source of $\beta$ -carotene

Carotenoids are natural pigments that occur in many plants, fruits and flowers. The carotenoid composition of foods varies substantially in quality and quantity. Even with a particular food, compositional variation occurs (Rodriguez-Amaya and Kimura, 2004). A large group of food products containing carotenoids are fresh fruits and vegetables.  $\beta$ -Carotene is an important micronutrient in the group of carotenoids. Sweet potatoes, carrots, cassava, spinach, yellow squash, turnip greens, collard greens, beet greens, red peppers, tomatoes, apricots, peaches, prunes and oranges are excellent sources of  $\beta$ -carotene. Carrot roots are the richest source of carotenes particularly  $\beta$ -carotene (M. Hendrickx, K.U.Leuven, Belgium, Unpublished observations).

### $\beta$ -CAROTENE STRUCTURE AND FUNCTION

$\beta$ -carotene is a carotenoid and describing the structure of

carotenoids is the basis for understanding the structure of  $\beta$ -carotene. Carotenoids are isoprenoid compounds, biosynthesized by tail-to-tail linkage of two  $C_{20}$ -geranylgeranyl diphosphate molecules. This produces a  $C_{40}$ -polyene chain from which all the individual variations are derived. These modifications could be due to cyclization of the terminal end, changes in hydrogenation level and complementation with oxygen containing groups. The hydrocarbon carotenoids are known as carotenes, while oxygenated derivatives of these hydrocarbons are known as xanthophylls (Britton et al., 1995).

The structure of carotenoids, that is, the typical conjugated double bond chain and the specific functional groups, determines its potential biological function(s) such as absorbing excess of energy from other molecules and the solubility in fat, which may account for antioxidant properties and interaction with biological membranes, respectively. The reaction between

carotenoids and oxidizing agents is important for evaluating their antioxidant activity (Britton, 1995). The ability of singlet oxygen quenching and free radicals trapping by carotenoids is discussed more in detail.

Reactive oxygen species (ROS), like  $^1\text{O}_2$  (singlet oxygen),  $\text{O}_2^{\cdot-}$  (superoxide radical),  $\text{H}_2\text{O}_2^{-2}$  (Hydrogen peroxide ion) and  $\text{HO}\cdot$  (hydroxyl radical), are powerful oxidizing agents which can be formed during exposure to UV and radioactive radiation (Halliwell, 1996; Stahl et al., 2000; De Proft, 2008). During photo-oxidative damage, these oxidizing agents may react with cellular components such as proteins, nucleic acids, lipids, carbohydrates and poly-unsaturated fatty acids (PUFAs). Consequently, an abstraction of a hydrogen atom from cellular components results in the formation of free radicals (Stahl et al., 2000; De Proft, 2009).

The free radical can then react with a carotenoid. As a result, carotenoid radicals, which are short-lived and harmless products, are formed. The addition of a radical species, such as a peroxy radical ( $\text{ROO}\cdot$ ) or hydroxyl radical ( $\text{HO}\cdot$ ), to the polyene chain of a carotenoid could also generate carotenoid-adduct radicals (Britton, 1995). Many of the most serious human diseases, like cancer, cardiovascular disease and macular degeneration, at some stage involve oxidation processes mediated by free radicals ( $\text{ROO}\cdot$ ,  $\text{HO}\cdot$ ,...). In these cases, the consumption of carotenoids could help to remove these radicals from the system and prevent the diseases. Thus, carotenoids act as antioxidants (Britton, 1995; Fraser and Bramley, 2004).

Furthermore, carotenoids especially  $\beta$ -carotene, cryptoxanthin,  $\alpha$ -carotene and  $\gamma$ -carotene are known to be provitamin A carotenes, whereas lutein and lycopene are not (Olson, 1989; Bendich and Olson 1989). Structurally, vitamin A (retinol) is essentially one half of the  $\beta$ -carotene molecule (Rodriguez-Amaya and Kimura, 2004). Vitamin A is important nutrient for human health. Vitamin A deficiency causes night blindness in childhood, reduced immune response, reduced senses of balance and taste, and affects the reproduction and growth of human being (Vansant, K.U.Leuven, Belgium, Unpublished observation). Provitamin A carotenes can be converted into vitamin A and the major pathway of conversion (central cleavage of carotenoids) is catalyzed by the enzyme 15,15'- $\beta$ -carotenoid dioxygenase (Bendich and Olson, 1989; Castenmiller and West, 1998). Primarily, carotenoids are converted into retinal (a precursor of vitamin A) in the intestinal mucosa, but also to some extent in the liver and other organs.

#### Localization of $\beta$ -carotene in the plant cell

Carotenoids are ubiquitous natural pigments that serve essential functions in higher plants photosystems. Moreover, they occur in non-photosynthetic tissues of some flowers and fruits (Baranska et al., 2006).

Carotenoids in plants are synthesized in all types of plastids but accumulate in chloroplasts and chromoplasts in large quantities. In chloroplasts, carotenoids are located in photosynthetic membranes and integrated with chlorophyll-binding proteins, whereas in chromoplasts, carotenoids are associated with polar lipids and carotenoid associated proteins to form carotenoid-lipoprotein complexes (Lu and Li, 2008).

Carrot storage root is a rich source of carotenoids. Carrot root has a very complex carotenogenesis and various carotenes may be synthesized and deposited there. Raman spectroscopy can provide insight into carotenoid accumulation directly in living tissue (Baranska et al., 2006). It has been stated by Parada and Aguilera (2008) that  $\beta$ -carotene of carrots is localized in chloroplasts as carotenoid-protein complexes or inside chromoplasts in a crystalline form.

#### Thermal processing

Thermal processing is a common technique used for food preservation. During thermal processing, heat is applied to the food product in order to obtain microbiologically safe products with acceptable eating quality. Depending on the intensity of heating, different processes can be distinguished (e.g. pasteurization and sterilization) (Holdsworth and Simpson, 2007).

The aim of a pasteurization process is to produce safe products by destruction of pathogenic microorganisms and to extend the shelf life of food products by destruction of the main part of the spoilage microorganisms (Dewettinck and Depypere, UGent, Belgium, Unpublished observations). Pasteurization processes should be designed to achieve a reduction of the number of the most heat resistant pathogenic or spoilage microorganisms relevant to the type of product by a pre-set level and to ensure that the production or formulation and the storage conditions applied inhibit the growth of any surviving cells during the intended shelf life of the product (Van Loey, K.U. Leuven, Belgium, Unpublished observations). Pasteurization is performed in a temperature range of 60 to 100°C, with different treatment times (Depypere, 2008). Since pasteurization is a mild heat treatment, most of the time, a pasteurization process is combined with other inhibitory factors (e.g. acidification, refrigerated storage) to ensure inhibition of the growth of any surviving microorganisms or the germination of spores during the anticipated shelf life (Van Loey, K.U. Leuven, Belgium, Unpublished observations).

The purpose of heat sterilization is to extend the shelf life of food products and to produce commercially sterile products, that is, no microorganisms will be able to grow after sterilization. This heat treatment inactivates both vegetative cells and spores of microorganisms. The shelf life of sterilized products can be more than six months

without cooling. In most cases, the processing temperature is higher than 100°C for different treatment times. The required treatment time is influenced by the pH of the food, the heat resistance of the microorganisms or enzymes, the heating conditions, the size of the container (heat transfer), the physical state of the food and the storage conditions after the heat treatment (Dewettinck and Depypere, UGent, Belgium, Unpublished observations). Next to the desired effects, the quality of food products can be affected considerably during sterilization (e.g. change in colour, flavour and aroma or in viscosity, degradation of nutrients and texture) (Dewettinck and Depypere, UGent, Belgium, Unpublished observations).

### Stability of $\beta$ -carotene during thermal processing and storage

During processing and storage, carotenoid stability differs in different foods. Accordingly, the optimal conditions for carotenoid retention during preparation or processing vary from one food to another. Longer processing times, higher processing temperatures and cutting or pureeing, the contact with oxygen of food products increase carotenoid destruction. The carotenoids retention is improved by applying short processing times and by lowering the temperature, so high temperature short time (HTST) technique might be a good alternative (Rodriguez-Amaya and Kimura, 2004). Due to their highly unsaturated structure, they can be degraded by oxidation and isomerization leading to the formation of epoxy-carotenoids, apo-carotenoids and hydroxy-carotenoids. Further, these products are also degraded to yield low molecular mass compounds.

$\beta$ -Carotene is highly susceptible to oxidation during processing and storage (Lee and Coates, 1999; Rodriguez-Amaya and Kimura, 2004). The  $\beta$ -carotene oxidation may occur during exposure to oxygen traces, light, heat, metals, enzymes, peroxides and oxidizing species or free radicals resulting in carotenoid bleaching (Britton, 1995; Rodriguez-Amaya, 1997). The oxidation can be inhibited by antioxidants, such as tocopherols (vitamin E) and ascorbic acid (vitamin C). Epoxides and apo-carotenoids (carotenoids with a shortened carbon skeleton) are believed to be the initial products of the oxidative degradation. As a result of  $\beta$ -carotene oxidation, its characteristic color, provitamin A activity and apparent absorption may be affected (Rodriguez-Amaya, 1997; Henry et al., 1998). Development of off-flavor in dehydrated carrots has also been associated with degradation of carotenoids. Unit operations that can disrupt the natural protection of carotenoids in plant tissue, such as cutting, shredding, chopping and pulping, increase exposure to oxygen and bring together carotenoids and enzymes that catalyze carotenoid oxidation. Oxidation is often accompanied by isomerization and both the *cis*- and *trans*-isomers are

subject to oxidation (Rodriguez-Amaya and Kimura, 2004).

Naturally, carotenoids exist as *trans*-isomers. Isomerization of *trans*-carotenoids to the *cis*-isomers occurs during heat treatment, contact with acid and exposure to light (Henry et al., 1998; Marx et al., 2003; Rodriguez-Amaya and Kimura, 2004; Schieber and Carle, 2005). Greater degree of isomerization occurs during thermal processing. A few studies dealt with thermally induced isomerization of  $\beta$ -carotene. It has been described by Marx et al. (2003) that heat treatment of carrot juice below 100°C predominantly results in the formation of 13- and 15-*cis*- $\beta$ -carotene, whereas above 100°C, 9-*cis*- $\beta$ -carotene is mainly formed. Recent investigations indicated that pasteurization at 100°C and sterilization at 121°C caused only minor amounts of *cis*-isomers, whereas excessive blanching of carrots and sterilization of carrot juice at 130°C resulted in increased levels of *cis*-isomers (Schieber and Carle, 2005).

The ability of *cis*- $\beta$ -carotene to quench singlet oxygen has been demonstrated to be less effective than the ability of all-*trans*- $\beta$ -carotene to quench singlet oxygen. Raw carrot roots have been demonstrated to be devoid of carotene *cis*-isomers (Marx et al., 2000, 2003). Schieber and Carle (2005) stated that the *cis*-isomers of carotenoids are less stable and have lower melting points than their all-*trans*-counterparts, due to a decreased tendency to crystallization.

### BIOACCESSIBILITY AND BIOAVAILABILITY OF $\beta$ -CAROTENE

It is not possible to get a complete digestion and absorption of  $\beta$ -carotene, present in carrots. Understanding the  $\beta$ -carotene bioaccessibility and bioavailability is thus important. Bioaccessibility is defined as the amount of an ingested nutrient that is available for absorption in the gut after digestion (Parada and Aguilera, 2007). It is the amount of nutrients that can be released from the food matrix. The bioavailability of nutrients is termed as the rate and extent to which the nutrients, contained in a food, are absorbed and become available at the site of action. It is the amount of the nutrients that can be used effectively by human tissues (Failla and Chitchumroonchokchai, 2005; Parada and Aguilera, 2007).

The factors that affect the bioaccessibility and the bioavailability of carotenoids are explained more in detail by Castenmiller and West (1998), Oslon (1999), Huang et al. (2000), Dutta et al. (2005) and Failla and Chitchumroonchokchai (2005). All the factors are combined and abbreviated as "SLAMENGI", where each letter stands for an effect. These factors include:

a. Speciation: This is related to the type of carotenoids in food (e.g. xanthophylls versus carotenes). Xanthophylls have a higher bioavailability than the hydrocarbon

carotenoids. A very recent investigation also demonstrated that all-*trans*- $\beta$ -carotene is more bioavailable than 9-*cis*- and 13-*cis*- $\beta$ -carotene in gerbils (Schieber and Carle, 2005).

b. Linkage at molecular level. The binding to proteins and esterification of carotenoids has an effect on the bioavailability.

c. Amount of carotenoids consumed. High consumption of carotenoids is related to high plasma concentration of carotenoids and retinol. The absorption of carotenoids is likely to be dependent on the vitamin A status.

d. Matrix in which the carotenoid is incorporated. The bioaccessibility in raw products is different from the bioaccessibility in processed products (e.g. pureeing, chopping, heating leads to a disruption of the food structure), hence a higher bioaccessibility is attained (Hedren et al., 2002). The same trend is observed in studies dealing with the bioavailability (Rodriguez-Amaya and Kimura, 2004).

e. Effectors of absorption (fat, fiber and interaction of carotenoids). Dietary fat increases carotenoid bioavailability by providing a storage area for hydrophobic compounds released from the food matrix, by stimulating the secretion of bile salts and pancreatic lipases required for micelles formation and by inducing chylomicron synthesis. Fibers decrease carotenoid bioavailability by decreasing micellization due to binding of fibers with bile acids and phospholipids, due to inhibition of lipase activity and due to an increased viscosity and volume of luminal contents. Concerning the interaction between carotenoids, it has been shown that  $\beta$ -carotene decreased lutein absorption, whereas lutein decreased  $\beta$ -carotene absorption (Failla and Chitchumroonchokchai, 2005). Positive effects are also well known due to antioxidant activity of the carotenoids (one carotenoid is oxidised while the other is protected) (Britton, 1995; Stahl et al., 2000; Fraser and Bramley, 2004; De Proft, K.U.Leuven, Belgium, Unpublished observations).

a. Nutrient status of the host: The amount of retinol present in the human body and protein deficiency (impaired protein synthesis means low synthesis of retinol binding proteins, which are important during absorption of vitamin A) may affect the absorption of  $\beta$ -carotene (Failla and Chitchumroonchokchai, 2005; Vansant, K.U.Leuven, Belgium, Unpublished observations).

b. Genetic factors: Failure to split  $\beta$ -carotene in humans is rare but can lead to metabolic carotenemia (even when the intake of carotenoids is normal) and to vitamin A deficiency if retinol intake is low.

c. Host related factors: Sex, age, illness and protein-energy malnutrition are also known to influence the bioavailability of carotenoids.

d. Interaction or combination of the above factors.

Nevertheless, in this review, the focus point is on the influence of heat treatments (sterilization and

pasteurization) on the bioaccessibility of  $\beta$ -carotene.

## ABSORPTION OF $\beta$ -CAROTENE BY HUMANS

Generally, the absorption of dietary carotenoids involves the transfer of carotenoids from the food matrix to micelles during digestion, the delivery of carotenoids to the apical surface of absorptive epithelial cells and the packaging of the carotenoids within chylomicrons for secretion into lymph (Furr and Clark, 1997; Failla and Chitchumroonchokchai, 2005; Ryan et al., 2008). It is stated by Furr and Clark (1997), Failla and Chitchumroonchokchai (2005) and Ryan et al. (2008) that the digestion of carotenoids occurs in the same manner as fat-soluble compounds.

During the digestion, carotenoids must first be released from the food matrix, emulsified in the lipid phase of the chyme and solubilized in mixed micelles, so that they can be absorbed by epithelial cells (Furr and Clark, 1997; Failla and Chitchumroonchokchai, 2005; Ryan et al., 2008). Hydrochloric acid, pepsin and gastric lipase are secreted into the gastric lumen and mixed with the ingested foods. Then, the carotenoids are released partially from the food matrix into the emulsified oil droplets. Non-polar carotenoids such as  $\beta$ -carotene reside in the core of lipid droplets, polar carotenoids are distributed at the surface (Furr and Clark, 1997; Failla and Chitchumroonchokchai, 2005). During intestinal digestion, the chyme enters into the small intestine, while pancreatic secretions and bile are released into the lumen. Bile salts are required for the partitioning of the lipophilic products into mixed micelles. The mixed micelles diffuse across the unstirred water layer and deliver carotenoids and other fat-soluble compounds to the apical surface of the mucosal epithelium (Furr and Clark, 1997; Failla and Chitchumroonchokchai, 2005; Ryan et al., 2008).

## Nutritional quality related to food structure

Nutrients are located in or attached to cellular compartments and need to be released so that their uptake can be guaranteed. Food processing (e.g. grinding, fermentation, heating) denatures proteins and breaks down the cell walls, making easier the release of carotenoids from the food matrix during digestion. Plant cell walls are important structural components of plants and determine partially the quality characteristics of many plant based foods (e.g. texture) (Waldron et al., 2003).

Texture is an important quality attribute for fruits and vegetables. It gives an indication on the firmness or hardness of the food products. The texture of fruits and vegetables is strongly dependent on the composition of the cell wall. In fruits and vegetables products, changes

in texture are directly related to structural changes in the cell wall polymers, in particular pectin changes and to a lesser extent changes in hemicellulose and cellulose material (Waldron et al., 2003; Sila et al., 2004; Sila et al., 2009).

Pectin is a heterogeneous group of polysaccharides containing acidic sugars, such as galacturonic acid, and neutral sugars, such as rhamnose, galactose and arabinose (Taiz and Zeiger, 2002; Waldron et al., 2003; Sila et al., 2009). Structurally, three major pectic polysaccharides are well known: Homogalacturonan (HG), rhamnogalacturonan I (RG I) and rhamnogalacturonan II (RG II).

Pectin is the most soluble cell wall polysaccharide. It can be extracted with hot water or with calcium chelators. The quality characteristics, more specifically the textural properties of many plant based foods depend largely on pectin and its stability in the cell wall (Waldron et al., 2003; Sila et al., 2009). Both endogenous and exogenous enzymes are involved in pectin modification. Successive demethoxylation by pectin methyltransferase (PME) and depolymerization by polygalacturonase (PG) and pectin lyase (PL) are well known enzymatic degradations (Taiz and Zeiger, 2002; Waldron et al., 2003; Sila et al., 2004).

It has been described that PME catalyzes the specific demethoxylation of HG within plant cell walls by releasing methanol and protons. Consequently, negatively charged carboxyl groups are formed which can form cross-links with divalent ions such as  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  and this leads to an improvement of the texture. Demethoxylated HG is also a substrate for pectin depolymerising enzymes (PG and PL). Depolymerisation of pectin is associated with texture loss (Sila et al., 2009). In carrots, research has been focused on PME and its influence on texture. Endogenous PG occurs in low amounts in carrots. Therefore, it has a minor influence on the texture of carrots (Sila et al., 2005).

Pectin conversions can also occur non-enzymatically. The most important conversion reaction is the base catalyzed (heated at  $\text{pH} > 4.5$ ) splitting of pectin chains by  $\beta$ -elimination (Sila et al., 2005; Daiz et al., 2007). Most plant based foods that have a pH above 4.5 and that are processed at a temperature above  $80^\circ\text{C}$ , are susceptible to  $\beta$ -eliminative pectin degradation. Pectin degradation during thermal processing by acid hydrolysis ( $\text{pH} < 3.0$ ) is another possible conversion reaction and is less important since fruits and vegetables products with low pH are not often consumed (Daiz et al., 2007; Sila et al., 2009).

Generally, texture changes during thermal processing of carrots can be controlled in different ways like controlling the pH (reducing  $\beta$ -eliminative degradation), stimulating the activity of PME through low temperature blanching prior to the main thermal process (reducing  $\beta$ -elimination), infusing calcium ions (cross-linking between demethoxylated pectins) (Waldron et al., 2003; Sila et al., 2009).

## Structural quality in relation to nutritional quality

Recently, the importance of the link between structural characteristics of foods and carotenoid *in vitro* bioaccessibility and bioavailability is highlighted (Waldron et al., 2003; Parada and Aguilera, 2007; Epriliati et al., 2009; Hedren et al., 2009; Lemmens et al., 2009). Since nutrients are located in or attached to cellular compartments, the limiting factors for the bioaccessibility of the nutrients might be structural elements (Huang et al., 2000; Failla and Chitchumroonchokchai, 2005; Parada and Aguilera, 2007; Ryan et al., 2008; Lemmens et al., 2009; Hendrickx, 2009). Studies on the bioaccessibility of  $\beta$ -carotene also indicated that cooking increases the amount of  $\beta$ -carotene that can be released: Hedren et al. (2002) observed a two fold increase due to cooking of carrot pieces, while Lemmens et al. (2009) observed a two to three fold increase. Moreover, in the studies of Epriliati et al. (2009) and Lemmens et al. (2009), the relation between the increased  $\beta$ -carotene bioaccessibility and pectin changes occurring during thermal processing is highlighted.

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