Phytochemicals in *Daucus carota* and their importance in nutrition – Review article

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Background. Carrot is a multi-nutritional food source. It is an important root vegetable, rich in natural bioactive compounds with health-promoting properties, such as antioxidants that have anti-carcinogenic properties. **Aim**. This review summarises the occurrences and biosynthesis of phytochemicals and factors affecting their concentration in carrot and their pharmacological functions related to human health. **Method.** 155 articles including original research papers, books, book chapters were downloaded and 94 articles (most relevant to the topic) were selected for writing the review article. The rejected research papers were too old or irrelevant. **Results.** Carrot contains important phytochemicals i.e. phenolic compounds, carotenoids, polyacetylenes and ascorbic acid which are bioactive compounds and recognised for their nutraceutical effects and health benefits. These chemicals aid in the prevention of cancer and cardiovascular diseases due to their antioxidant, anti-inflammatory, plasma lipid modification and anti-tumour properties. This vegetable can be used to improve the health of poor people, especially in developing countries. **Discussion.** We recommend carrot to be promoted as a food security and food safety crop in the future to meet the global food demands in developed as well as in developing countries. Future cultivation programmes should focus on the cultivation of carrot for its phytochemicals to improve the health of impoverished people.

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Abstract

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Aim. This review summarises the occurrences and biosynthesis of phytochemicals and factors affecting their concentration in carrot and their pharmacological functions related to human health.

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Results. Carrot contains important phytochemicals i.e. phenolic compounds, carotenoids, polyacetylenes and ascorbic acid which are bioactive compounds and recognised for their nutraceutical effects and health benefits. These chemicals aid in the prevention of cancer and cardiovascular diseases due to their antioxidant, anti-inflammatory, plasma lipid modification and anti-tumour properties. This vegetable can be used to improve the health of poor people, especially in developing countries.

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Introduction

Fruits and vegetables are rich sources of nutrients that are directly or indirectly associated with homeostasis in human beings (Allende, Tomás-Barberán & Gil, 2006). They contain a variety of phytochemicals (also known as bioactive compounds) recognised for their nutraceutical effects and health benefits (Tiwari & Cummins, 2013). These chemicals aid in the prevention of cancer and cardiovascular diseases due to their antioxidant (Leja et al., 2013; Alarcón-Flores et al., 2015), anti-inflammatory (González-Gallego et al., 2010; Vincent, Bourguignon & Taylor, 2010), plasma lipid modification (Perez-Vizcaino & Duarte, 2010; Wang et al., 2011), and anti-tumor properties (Prasain & Barnes, 2007; Stan et al., 2008). In addition, phytochemicals are also responsible for the smell, flavour, and colour of agricultural commodities (Miglio et al., 2008; Alarcón-Flores et al., 2015). Carrot (*Daucus carota* L.) plays a major role in human nutrition because of its high dietary value and good storage attributes (Leja et al., 2013; Umar et al., 2015). However, poor post-harvest handling and delayed marketing significantly alter the concentrations of its bioactive compounds (Alasalvar et al., 2005). Among 39 fruits and vegetables, it has been ranked 10th due to its multiple nutritional benefits. In addition to the nutritional antioxidants (vitamins A, C, and E), carrot also possesses a valuable amount of non-nutritional antioxidants that include β-carotene, flavonoids, and phenolics (Leja et al., 2013). Carrot pigments such as carotenoids, polyacetylenes, and phenolic acids are effective antioxidants. Foods such as carrot that contain natural antioxidants enhance resistance to oxidative damage (Leiss et al., 2013) and have a substantial impact on human health. Keeping in mind the beneficial properties of carrot phytochemicals in the human diet, it is necessary to make policy makers aware of the promotion of carrot as a food security crop in the future to meet global food demands and to improve the health of poor people through natural resources. This

review article comprehensively describes the pharmacological importance of phytochemicals present in carrot and the factors affecting their regulation during pre- and post-harvest conditions.

Survey Methodology

The literature for this review paper was retrieved from Google Scholar by using following key words: occurrence of phenolics or phenols or phenolic acids, carotenoids, polyacetylenes and ascorbic acid or vitamin C in carrot; biosynthesis of phenolics or phenols or phenolic acids, carotenoids, polyacetylenes and ascorbic acid or vitamin C in carrot; Factors affecting the concentration of phenolics or phenols or phenolic acids, carotenoids, polyacetylenes and ascorbic acid or vitamin C in carrot; Nutritional importance or nutritional benefits of phenolics or phenols or phenolic acids, carotenoids, polyacetylenes and ascorbic acid or vitamin C in carrot. Structures of phenols or phenolic acids, carotenoids, polyacetylenes and ascorbic acid or vitamin C in carrot were searched from NCBI website and redrawn in the MS word using TIF format.155 articles including orignal reserch papers, books, book chapters were downloaded, and 94 articles (most relevant to the topic) were selected for writing the review article. The rejected research papers were too old or irrelevant.

Carrots mainly contain four types of phytochemicals, *i.e.* phenolics, carotenoids, polyacetylenes, and ascorbic acid, which are reviewed in succession in the following sections.

Phenolic compounds

Phenolic compounds in fruits and vegetables affect their appearance, taste, smell, and oxidative stability (Naczk & Shahidi, 2006). Phenolic compounds impart bitterness (Kreutzmann,

Christensen & Edelenbos, 2008) and astringent flavours to horticultural commodities. The bitter taste in carrot is caused by terpenoids and water-soluble phenolics (Czepa & Hofmann, 2004). Isocoumarins and phenolic acids are potential bitter compounds found in the peel of carrot. Therefore, their presence can be used as biological markers to assess the quality of fruits and vegetables during postharvest operations (Sharma et al., 2012). Phenols represent a class of compounds that are mainly comprised of an aromatic ring bearing one or more hydroxyl substituents, thus creating an extended range of simple molecules to highly polymerised phenolics (Balasundram, Sundram & Samman, 2006). Phenolic acids found in carrot are chlorogenic acid, p-hydroxybenzoic, caffeic acid, and cinnamic acid derivatives (see Figure 1) (Gonçalves et al., 2013).

Occurrence

Phenolic compounds are present in high concentrations in the root periderm tissue of carrot. Carrot root particularly consists of hydroxycinnamic acids and derivatives (Zhang & Hamauzu, 2004). These compounds enhance sensory characters, namely the aroma, colour, and bitterness. (Naczk & Shahidi, 2006; Kreutzmann, Christensen & Edelenbos, 2008; Kreutzmann et al., 2008). Carrot tissue may be similar to phenolic composition, although individual phenolic compounds decrease from the outer peel to the interior inside xylem (Naczk & Shahidi, 2006; Gonçalves et al., 2013). The peel contains 54.1% phenolics, followed by phloem (39.5%) and xylem (6.4%). Carrot mainly contains chlorogenic acid, accounting for 42.2% to 61.8% of total phenolics as identified in different carrot tissues (Sharma et al., 2012). However, the concentration depends on the cultivar, the extraction method, the manner of expressing the results, and post-harvest and processing circumstances (Zhang & Hamauzu, 2004; Alasalvar et al., 2005). Carrots of different colours demonstrated high variation in antioxidant properties

(Gajewski et al., 2007). The results consistently indicated that among different carrot colours, purple carrot exhibited the highest antioxidant capacity due to its higher phenolic compound concentration (Leja et al., 2013). In general, carrot contains 26.6±1.76 μg/g phenolic compounds (Oviasogie, Okoro & Ndiokwere, 2009; Sharma et al., 2012).

Biosynthesis

Phenols are synthesised along acetyl coenzyme A in the shikimic acid pathway. Hydroxycinnamic acid and its derivatives are the most common phenolic compound reported in carrot. The carbon skeleton of cinnamic acid forms from the aromatic amino acid L-phenylalanine. Further studies on *Salvia splendens* (McCalla & Neish, 1959) revealed that cinnamic acid passes through ring substitution in a series of hydroxylation and methylation steps to produce various hydroxycinnamic acids (El-Seedi et al., 2012; Gross, 2016).

Factors affecting phenolic concentration

Polyphenols are affected by multiple factors such as the cultivar (Vågen & Slimestad, 2008), the time of harvest, and processing procedures (Manach et al., 2004). Red/purple carrots exhibited more antioxidant capacity than orange, yellow, and white carrots and, in low rainfall seasons, they stored higher quantities of phenolics (Leja et al., 2013). Nutrients and secondary plant metabolites are also affected by growing conditions and the type of fertilisers applied (Dangour et al., 2009). A deficiency of boron increases the accumulation of phenolics while wounding stress significantly affects phenolic concentration in carrots (Singh et al., 2012). Jacobo-Velázquez et al. (2011) found an increase of 287% and 349% in phenolic contents when carrots were stored for 48 hours at 20 ºC and when wounded carrots were exposed to low oxygen conditions respectively. Wounding stress (23.5 cm^2/g) produces approximately 2.5 times more soluble phenols in carrots than in undamaged carrots. It mostly stimulates the synthesis of chlorogenic acid (5-CQA) and 3, 5-dicaffeoylquinic acid, which enhances the antioxidant capacity of carrots (Surjadinata & Cisneros-Zevallos, 2012). The application of different doses of fertiliser and fertiliser types at different growth stages also increased phenolic contents significantly (Søltoft et al., 2010). An example is the application of N fertiliser that could change phenolic concentration in carrot roots (Singh et al., 2012). Maximum variation at different growth stages was observed for chlorogenic acid in carrots (RSD, 12%) (Søltoft et al., 2010). Unblanched frozen and blanched (to put carrots in water at 95 ºC for three minutes) frozen treatments non-significantly affected phenolic concentration in carrots even after seven days of storage at 4 ºC (Patras, Tiwari & Brunton, 2011).

Human health benefits

The mean nutritional ingestion of polyphenolics that contains 50% of hydroxycinnamic acids, 20-25% flavonoids, and 1% anthocyanin, is 1058 mg/day for males and 780 mg/day for females (Soto-Vaca et al., 2012). These compounds possess multiple biological functions such as antioxidant, anticarcinogenic, and anti-inflammatory properties. Many of these biological properties have been credited to their basic reducing capabilities (Stan et al., 2008). They have attained immense attention because of their strong antioxidant and health-promoting properties (Kaur & Kapoor, 2001; Balasundram, Sundram & Samman, 2006). Due to their antioxidant properties, the risk of cardiovascular diseases is minimised, and they also possess anti-ageing properties as well as anti-carcinogenic properties by functioning as free-radical scavengers. Polyphenols also potentially protect against diabetes and Alzheimer's disease (Soto-Vaca et al., 2012). They enhance bile secretion, decrease cholesterol and lipid levels in the blood, and promote the antimicrobial movement against *Staphylococcus aureus* (Ghasemzadeh & Ghasemzadeh, 2011). Preclinical and epidemiological studies suggest that polyphenols might be helpful in reversing neurodegenerative pathogenic actions and ageing in neurocognitive development. However, there is no evidence on the role of polyphenols in the improvement of neurological health. Their potential roles are due to their capability of interrelating with intracellular neuronal and glial signalling, affect peripheral and cerebrovascular blood flow, and lessen neural injury and damage caused by neurotoxins and the inflammation of neurons (Del Rio et al., 2013).

Carotenoids

Carotenoids are a group of isoprenoid molecules present in all photosynthetic plants, including carrot. Some non-photosynthetic fungi and bacteria also possess carotenoids. Several conjugated double bonds of a polyene chain that function as a chromophore are responsible for the yellow, orange, and red colours of carotenoids (Águila Ruiz-Sola & Rodríguez-Concepción, 2012; Rodriguez-Concepcion & Stange, 2013). There are two types of carotenoids, *i.e.* carotenes and xanthophylls, which impart red or yellow colour and enhance food quality. The major carotenoids (see Figure 2) in carrot roots are β-carotene (75%), α-carotene (23%), and lutein (1.9%) (Søltoft et al., 2010), β-cryptoxanthin, lycopene, and zeaxanthin (Stan et al., 2008). Carotenoids are acyclic or have five or six C rings on one or both ends of the molecule (Sharma et al., 2012).

In many developing countries, including Pakistan, the public lacks the money to purchase animal products and pharmaceutical supplements and relies on proper access to carotenoid-rich vegetables and fruits for their nutritional needs.

Occurrence

Carotenoids are named after carrot because carrot accumulates an enormous amount of carotenoids in its roots. Beta-carotene makes up 80% of total carotenoids contained in domestic carrot root (Kim et al., 2010). Carrot contains 16-38 mg/100 g carotenoids (Mustafa, Trevino & Turner, 2012). The orange colour is also a good indicator of the nutritional value of carotenoids, as in the case of carrots. Thus, it is a reliable marker for carotenoid identification in processed carrots.

Biosynthesis

Carotenoids are formed in plastids from isoprenoid precursors via the methylerythritol 4-phophate (MEP) pathway (Rodríguez-Concepción, 2010). In the first step, 15-cis-phytoene (colourless carotenoid) is produced by the catalytic action of phytoene synthase (PSY). This compound is desaturated, isomerised, and converted into reddish all-trans lycopene through the catalytic actions of enzymes; phytoene desaturase (PDS), 15-cis-z-carotene isomerase (ZISO), z-carotene desaturase (ZDS), and carotenoid (pro-lycopene) isomerase (CRTISO). In the next step, lycopene splits into two orange carotenes β (LCYB) and/or ε (LCYE) by lycopene cyclases: β-carotene (with two β-rings on two ends of the lycopene molecule) or α-carotene (by cyclisation of one β-ring on one end and one ε-ring on the other). Zeaxanthin is produced by hydroxylation of β-carotene by carotenoid β-hydroxylase (CHYB) enzymes, particularly of the nonheme diiron (BCH) type, while yellowish xanthophyll lutein is formed by hydroxylation of α-carotene catalysed by β- and ε-hydroxylase (CHYB and CHYE) enzymes, primarily of the cytochrome P450 (CYP97) type (Águila Ruiz-Sola & Rodríguez-Concepción, 2012; Britton, 2012; Rodriguez-Concepcion & Stange, 2013).

Factors affecting carotenoid concentration

Carotenoids are mainly influenced by two main factors, *i.e.* inherited characteristics and the environment (Seljasen et al., 2001; Kidmose et al., 2004; Gajewski et al., 2007). A difference of seven to eleven times difference in β-carotene was observed in cultivars with different genetic makeup (Seljåsen et al., 2013). Environmental conditions during growth and packaging alter the level of carotenoids, sugars, and volatiles (Seljasen et al., 2001; Gajewski & Dąbrowska, 2007); however, results may vary when research is conducted under different conditions. Some studies also demonstrated slight variations in α- or β-carotene when carrots were stored at 0 ºC, even for

six months (Koca & Karadeniz, 2008). Carrots contain reduced carotenoids after harvest, due to the prolonged exposure to direct sunlight in the open field (Fuentes et al., 2012). Also, the form of a product, such as fresh, boiled, frozen, or canned, has a significant impact on carotenoid concentration. Similarly, variations in the analytical techniques also confer characteristic margins onto nutritional bio data in processed food. This phenomenon makes it rather more complex to compare the results of multiple geographical locations (Hedges & Lister, 2005). Suggestions for retaining carotenoids include the usage of cultivars known to have higher ranges of the useful compounds and which might be more suited to the local weather and geographical location. Other researchers (Martín-Diana et al., 2007; Rico et al., 2007) emphasise that crops grown in sandy soil tend to build up fewer vitamins than those grown in clay soils. Retail storage of carrots often takes place at a temperature range of 18-22 ºC. Carrots can be subjected to these temperatures for a few days. According to Imsic et al. (2010), α- and β-carotene concentration increased in Nantes carrot stored at 2 ºC and 90% R.H., with up to 35% and 25% increases respectively after three days of storage and increased even more after ten days to 42% and 34%. Longer storage periods of 21 days at 20 ºC have a negative effect on α- and β-carotene, although not extensively (Imsic et al., 2010). Significant increases in β-carotene were observed in both Nevis and Kingston cultivars stored at 20 ºC for seven days. Increases in carotene contents at some stage in storage at 4 ºC or 20 ºC may be because of improved extractability of the carotenes after enzymatic degradation of macromolecular matrix compounds (Imsic et al., 2010).

Human health benefits

Carotenoids are powerful antioxidants that help in maintaining a healthy skin and also prevent many diseases like cancer (lung, pancreas, and gastrointestinal tract), cardiovascular diseases, muscle degeneration in old age, and cataracts (Søltoft et al., 2011; Mustafa, Trevino & Turner,

2012; Sommer & Vyas, 2012). Beta-carotene is the most widely studied carotenoid so far due to its significance in medical science. The major precursor of carotenoids in the human body is β-carotene. Less than one-third of total retinol intake in developed countries is attributed to pro-vitamin A. Vitamin A is essential for normal organogenesis, immune functions, tissue differentiation, and eyesight (Sommer & Vyas, 2012). Alpha-carotene, β-carotene, and β-cryptoxanthin are the carotenes that are converted into retinol in the human body. Lutein and its isomer, zeaxanthin, both accumulate in the centre of the retina (also known as the macula) of the eye. These are the only carotenoids that pass through the retinal barrier and form the macula in the eye. The macula enhances eyesight through its light-filtering characteristics. They are also powerful antioxidants and essential for healthy eyes. They protect eyes from diseases by absorbing harmful blue light that enters the eye. Lutein is also the most dominant carotenoid in brain tissue and the predominant carotenoid in the developing primate brain and retina. The amount of lutein is twice as much in paediatric brains than in the adult brain, indicating its role in neural growth, and may play a role in biological functions, including anti-oxidation, anti-inflammation, and structural activity. It shields neural tissue, especially during infancy when the retina and brain are continuously in a state of change after birth. In adults, it is linked to cognitive health, and its supplement enhances cognition. High ingestion (near 6 mg/day) of lutein is associated with low risk of muscular degeneration during old age, although actual intake of lutein varies between 1-2 mg/day in adults. It can also prevent the production of harmful free radicals such as reactive oxygen species (ROS) via physical or chemical quenching of singlet oxygen (Vílchez et al., 2011; Johnson, 2014; Vishwanathan et al., 2014).

Polyacetylenes

C_{17}-polyacetylenes include biologically active compounds and are scientifically valuable due to their cytotoxicity towards cancer cells (Rawson et al., 2012). These are secondary metabolites found in plant foods. They are well-known antifungal agents which could develop at some point of storage in crop plants (Christensen & Kreutzmann, 2007). More than 1400 unique polyacetylenes and related compounds have been identified from higher flora (Christensen & Brandt, 2006). Plants of the *Apiaceae* and *Araliaceae* families contain aliphatic C_{17}-polyacetylenes of the falcarinol type. Falcarinol, falcarindiol, and falcarindiol-3-acetate (see Figure 3) are essential polyacetylenes found in carrot roots.

Occurrence

These are mainly present in the pericyclic parenchyma of root and phloem near the secondary cambium (Baranska & Schulz, 2005a; Kjellenberg et al., 2010). Falcarinol is uniformly distributed in the peel and the peeled carrot (Kreutzmann, Christensen & Edelenbos, 2008). Falcarinol is allocated to all parts of carrot root, while falcarindiol and falcarindiol-3-acetate are more abundant inside the higher and outer segments respectively ((Czepa & Hofmann, 2004; Kjellenberg et al., 2010).

The amount of falcarindiol and falcarindiol-3-acetate correlates with growing carrot root, but it does not apply to falcarinol (Kjellenberg et al., 2010). Falcarinol has been restricted to secondary phloem and the pericycle channels in the area of the periderm (Baranska & Schulz, 2005b). Kjellenberg (2007) indicated that the falcarinol level was slightly enriched after harvesting (with a short storage span), ultimately reaching a stabilised stage. In carrot roots, the concentrations of falcarindiol, falcarinol, and falcarindiol-3-acetate are 16-84 mg kg^{-1}, 8-40 mg kg^{-1}, and 8-27 mg

kg^{-1} of fresh weight respectively, based on the cultivar (Czepa & Hofmann, 2003, 2004). Dawid et al. (2015) recently reported on additional polyacetylenes isolated from carrot, namely (E)-isofalcarinolone, falcarindiol-8-acetate, 1,2-dihydrofalcarindiol-3-acetate, (E)-falcarindiolone-8-acetate, (E)-falcarindiolone-9-acetate, 1,2-dihydrofalcarindiol, (E)-1-methoxy-falcarindiolone-8-acetate, (E)-1-methoxy-falcarindiolone-9-acetate, and panaxydiol.

Biosynthesis

The crepenynate pathway is involved in the biosynthesis of falcarinol-type polyacetylenes. In this pathway, acetyl-CoA and malonyl-CoA reacted in the presence of fatty acid synthase and Δ^9-desaturase enzymes and converted into oleic acid. Oleic acid undergoes dehydrogenation to C_{18}-acetylenes; linoleic acid, crepenynic acid, and dehydrocrepenynic acid by the catalytic action of Δ^{12}-desaturase, Δ^{12}-acetylenase, and Δ^{14}-desaturase enzymes respectively. Dehydrocrepenynic acid is further transformed into C_{17}-acetylenes in the presence of Δ^{14}-acetylenase through β-oxidation (Hansen & Boll, 1986; Dawid et al., 2015).

Factors affecting polyacetylenes concentrations

The amount of falcarinol-type polyacetylenes in carrots is significantly affected by cultivar, harvesting dates (Kjellenberg et al., 2010), geographic area (Kidmose et al., 2004), storage conditions (Hansen, Purup & Christensen, 2003), and industrial processing ((Minto & Blacklock, 2008). The concentration of falcarindiol and falcarindiol-3-acetate reduces during early harvesting dates and increases during late harvesting dates, while falcarinol concentration does not change significantly. Similar effects were observed during storage (Kjellenberg et al., 2010). Temperature and duration of treatment significantly affect polyacetylenes concentration in carrot disks. Their concentration decreases at low temperatures (50-60 ºC) and increases at high

295 temperatures (70-100 ºC), particularly the concentration of falcarinol (Rawson, Brunton &
296 Tuohy, 2012). High pressure-temperature (HPT) processing enhances the retention of
297 polyacetylenes in carrot. Four hundred MPa at 50 ºC and 60 ºC for 10 minutes and 400 MPa at
298 50 ºC for 10 minutes are HPT combinations for falcarinol, falcarindiol, and falcarindiol-3-acetate
299 respectively that yield the highest retention in 10 to 30 minutes (Rawson, Brunton & Tuohy,
300 2012). Refrigeration of the roots for four months at 1 ºC before processing leads to restored
301 polyacetylenes in comparison with frozen storage of processed carrots. The falcarinol contents
302 multiplies and the falcarindiol and falcarindiol-3-acetate contents decrease for the duration of
303 steam blanching of the carrots before freezing (Kidmose et al., 2004). Rapid freezing increases
304 the retention rate of polyacetylenes in carrots, and blanching before freezing is also effective for
305 this purpose for storage in cool conditions (Kramer et al., 2012; Rawson et al., 2012). Ultrasound
306 and blanching pre-treatments affect the concentration of polyacetylenes in freeze-dried and hot-
307 air-dried carrots. An ultrasound followed by hot-air drying results in the higher retention of
308 polyacetylenes in dried carrot discs than blanching. Moreover, freeze-dried samples exhibit a
309 better retention of polyacetylenes than those of hot-air-dried samples (Rawson et al., 2011).
310 Conventional and natural farming systems confirm no difference in the content of the falcarinol,
311 falcarindiol, and falcarindiol-3-acetate (Søltoft et al., 2010; Seljåsen et al., 2013). Peeling affects
312 the retention of polyacetylenes in carrots. Peeled carrots have a higher amount of polyacetylenes,
313 but when washed after cutting, the contents decrease substantially due to leakage (Koidis et al.,
314 2012).

315 ***Human health benefits***

316 Polyacetylenes have been described as being associated with advantages for people's health
317 (Christensen & Brandt, 2006) and limitations due to their undesirable bitter taste (Czepa &

Hofmann, 2004). A few polyacetylenes are useful skin-sensitising compounds at lower concentrations, while others are neurotoxic at excessive levels. These are extremely cytotoxic against several cancer cell lines and have revealed antifungal, anti-inflammatory, and anti-platelet-aggregatory characteristics (Baranska et al., 2013). The hydroxyl group (OH^-) at C_3 may be accountable for these polyacetylenes activities (Purup, Larsen & Christensen, 2009). A group of falcarinol-type polyacetylenes shields against cancer (Kreutzmann, Christensen & Edelenbos, 2008). It was recently reported that C_{17}-polyacetylenes inhibit breast cancer resistance protein BCRP/ABCG2 when used as a multidrug resistance reversal agent (Tan et al., 2014). Falcarinol activates mammalian cell differentiation but also shows toxic effects against human cancer cells and may cause allergic inflammation of the skin (Kjellenberg et al., 2012). Falcarinol and falcarindiol may be used as antidiabetic agents in the treatment of diabetes due to their ability to arouse basal or insulin-dependent glucose absorption in adipocytes and porcine myotube cell cultures based on different doses (El-Houri et al., 2015).

Ascorbic acid

Ascorbic acid (AA or vitamin C) (see Figure 4) is a major component of the kingdom Plantae. It is water soluble due to its polar nature. It may be accumulated up to 20 m*M* in chloroplasts and occurs in almost all parts of the cell. It is known to play a role in photosynthesis as an enzyme cofactor and controller of cell growth. It is suggested that vitamin C is important in the rehabilitation process of human bodies (Rickman, Barrett & Bruhn, 2007).

Occurrence

Vitamin C is a vital dietary supplement for people. It is obtained as an essential vitamin from vegetables and fruits. Its importance can be estimated from the fact that more than 90% of the

vitamin C in human diets is obtained from fresh fruit and vegetables. In carrots, it ranges from 21 mg kg^{-1} (Pokluda, 2006) to 775 mg kg^{-1} (Matějková & Petříková, 2010).

Biosynthesis

In plants, four alternative pathways for AA biosynthesis are reported in the literature, namely the D-Mannose/L-Galactose (D-Man/L-Gal) pathway, myoinositol pathway, galacturonate pathway, and L-glucose pathway. Of these pathways, the D-Man/L-Gal pathway is the main and the most acceptable for AA biosynthesis in carrot. It consists of nine steps (Wang et al., 2015) as specified in Figure 5.

Factors affecting the concentration of ascorbic acid

Trimming of leaves in Chinese cabbage is associated with a more rapid reduction of vitamin C content than storing it at 4 ºC for 11 days (Lee & Kader, 2000). Vitamin C is sensitive to adverse handling. Frozen storage lessened ascorbic acid concentration by 4.1% (Cortés et al., 2005). The effect of prolonged storage indicates considerable losses of vitamin C up to 15-49% (Singh, Kawatra & Sehgal, 2001; Matějková & Petříková, 2010). After eight days of storage, AA concentrations decreased by 38% at 7.5-8.5 º C and by 70% at 22-37.5 ºC. The maximum decrease in AA contents was observed during local storage at 25-28 ºC (Seljåsen et al., 2013). Treatment of carrots at high temperatures (98 ºC for 10 minutes) inactivates AA oxidase and enhances the stability of AA (Leong & Oey, 2012). Boron deficiency enhances ascorbic acid contents from 45-70% (Singh et al., 2012). Conventional blanching and ultrasound affect ascorbic acid concentration in carrots. Conventional blanching at higher temperatures enhances vitamin C contents from 37.5% to 85% in carrots, while it lowers to <4% when blanched at 60 ºC and ultrasound at 60 and 70 ºC (Gamboa-Santos et al., 2013).

Human health benefits

Vitamin C prevents scurvy and maintains healthy skin, gums, and blood vessels. It also aids in collagen formation, inhibition of nitrosamine, absorption of iron, reduction of plasma cholesterol, the vitality of the immune system, and reaction with ROS. Vitamin C can reduce the risk of cancer, arteriosclerosis, and other cardiovascular diseases (Lee & Kader, 2000; Leong & Oey, 2012). Vitamin C is a non-protein portion, essential for the proper functioning of various enzymes that are involved in carnitine and catecholamine biosynthesis, the metabolism of tyrosine and peptide amidation, the post-translational hydroxylation of collagen, and in the conversion of the neurotransmitter dopamine to norepinephrine. It plays a vital role in Fe absorption from the gut by reducing Fe^{3+} to Fe^{2+} and maintains the structure of Fe-binding proteins. Vitamin C is involved in the regulation of hypoxia-inducible factor 1α (HIF 1α, a transcription factor that activates genes that control several mechanisms at the cell level, like cell survival, development of new blood vessels, Fe transport, and glycolysis) that induces cell responses to hypoxic conditions. It can cure neurodegenerative diseases like Alzheimer's disease, Huntington's disease, ischemic stroke, and Parkinson's disease (Duarte & Lunec, 2005; Li & Schellhorn, 2007; Harrison & May, 2009). In high concentrations, it acts as a prodrug, and transports a high flux of H_2O_2 to cancer cells and plays a role in the treatment of cancer (Du, Cullen & Buettner, 2012).

Conclusion and future outlook

Cancer, cardiac issues, and ageing are currently common themes in medical science. The role of antioxidants to combat these problems is indispensable. Carrot is a multi-nutritional source of food. Its phytochemicals are excellent sources of antioxidants that can prevent the deterioration of cells in the human body. Ascorbic acid, phenolics, polyacetylenes, and carotenoids from

carrot roots can provide unparalleled support to combat these global health challenges. This vegetable is available to the consumer in almost all possible forms of food on the market, *i.e.* raw, canned, frozen, extracted, pickled, etc. Moreover, it is available at low prices in all temperate regions throughout the globe. Hence, it is emphasised that carrot should be incorporated as an essential part of the diet for the prevention of diseases and a prolonged and healthy lifespan. Carrot must be promoted as a food security and food safety crop in the future to meet food demands in developed as well as in developing countries. Future cultivation programmes should focus on the carrot's phytochemicals to improve the health of local people.

Disclosure of interest

The authors declare no conflicts of interest.

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682

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694 phosphomannose mutase (PMM), GDP-d-mannose pyrophosphorylase (GMP),
695 GDP-d-mannose-3′,5′-epimerase (GME), GDP-l-galactose phosphorylase (GGP),
696 l-galactose-1-P phosphatase (GPP), l-galactose dehydrogenase (GalDH), and l-
697 galactono-1,4-lactone dehydrogenase (GalLDH). Myo-inositol oxygenase
698 (MIOX) is the only enzyme identified in the myo-inositol pathway, and d-
699 galacturonate reductase (GalUR) is involved in the galacturonate pathway. The l-
700 gulose pathway is a hypothetical side pathway. Enzymes implicated in recycling
701 pathway include ascorbate oxidase (AO), ascorbate peroxidase (APX),
702 monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase
703 (DHAR), and glutathione reductase (GR) (Wang et al., 2015).

Figure 1(on next page)

Structures of phenolic acids: p-hydroxybenzoic acid, cinnamic acid, caffeic acid, and chlorogenic acid

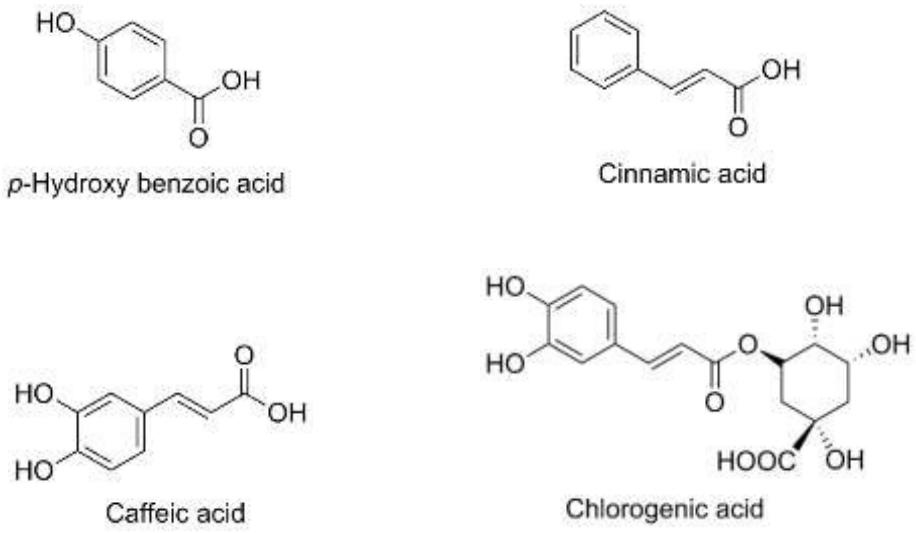

Fig. 1: Structures of phenolic acids: p-hydroxybenzoic acid, cinnamic acid, caffeic acid, and chlorogenic acid

Figure 2(on next page)

Structures of carotenoids: β-carotene, α-carotene, β-cryptoxanthin, lutein, lycopene, and zeaxanthin

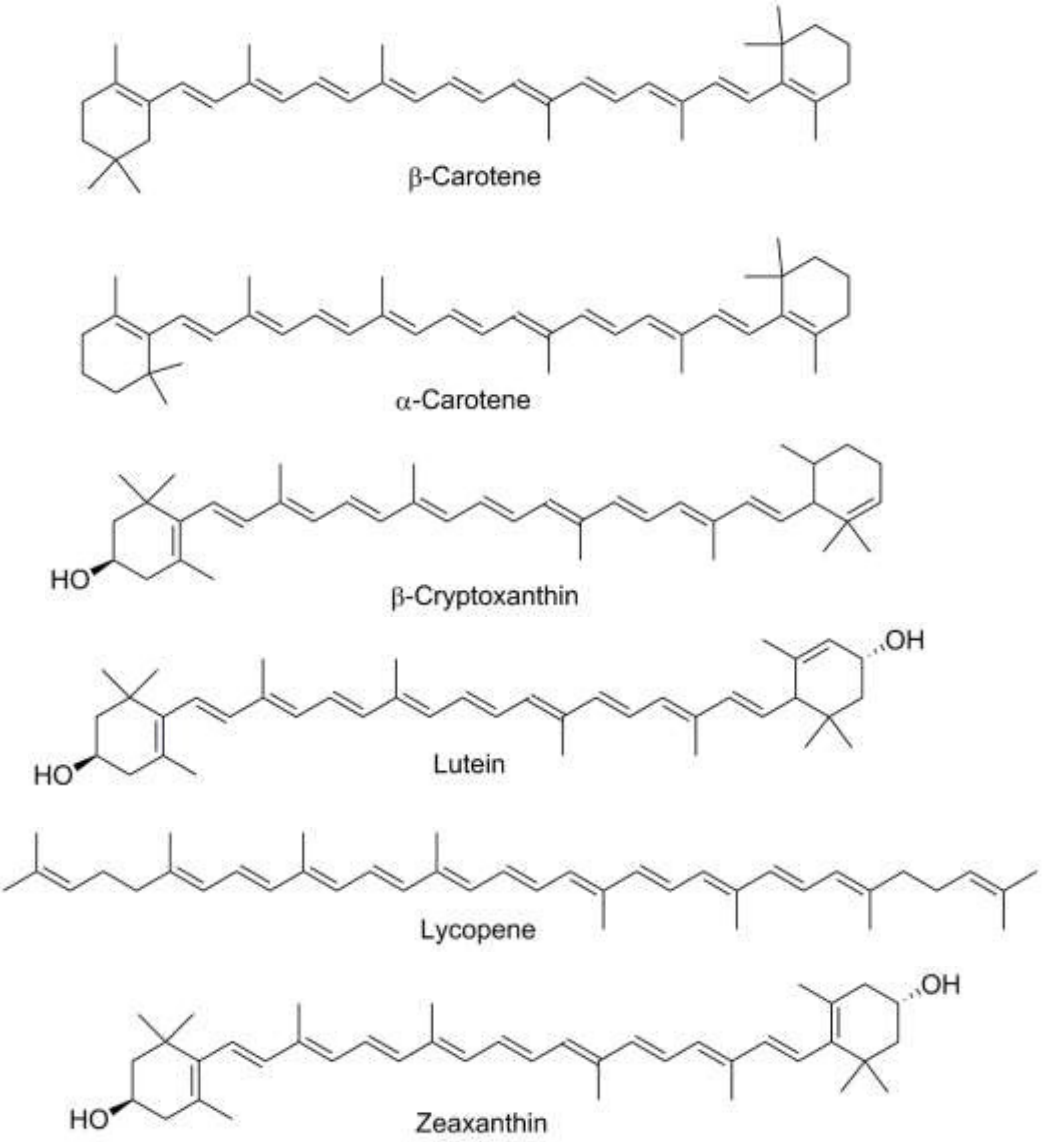

Fig. 2: Structures of carotenoids: β-carotene, α-carotene, β-cryptoxanthin, lutein, lycopene, and zeaxanthin

Figure 3(on next page)

Structures of the polyacetylenes: falcarinol, falcarindiol, and falcarindiol-three-acetate

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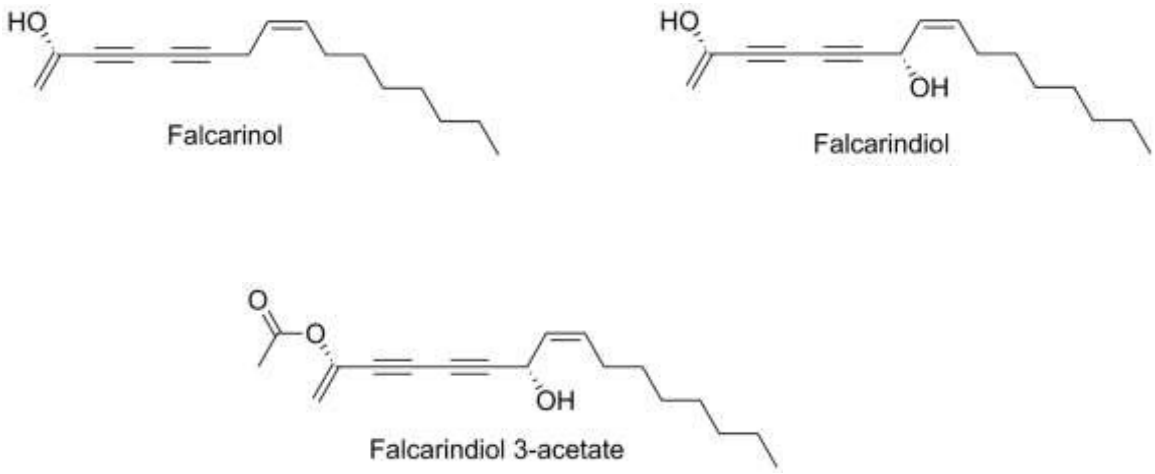

Fig. 3: Structures of the polyacetylenes: falcarinol, falcarindiol, and falcarindiol-three-acetate.

Figure 4(on next page)

Structure of ascorbic acid

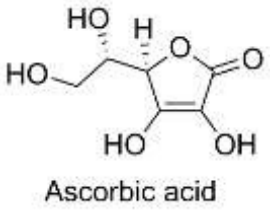

Fig. 4: Structure of ascorbic acid.

Figure 5(on next page)

Potential biosynthetic and recycling pathways of ascorbic acid in carrot.

The enzymes' catalysing actions in the d-mannose/l-galactose (d-Man/l-Gal) pathway are phosphoglucose isomerase (PGI), phosphomannose isomerase (PMI), phosphomannose mutase (PMM), GDP-d-mannose pyrophosphorylase (GMP), GDP-d-mannose-3′,5′-epimerase (GME), GDP-l-galactose phosphorylase (GGP), l-galactose-1-P phosphatase (GPP), l-galactose dehydrogenase (GalDH), and lgalactono-1,4-lactone dehydrogenase (GalLDH). Myo-inositol oxygenase (MIOX) is the only enzyme identified in the myo-inositol pathway, and dgalacturonate reductase (GalUR) is involved in the galacturonate pathway. The lgulose pathway is a hypothetical side pathway. Enzymes implicated in recycling pathway include ascorbate oxidase (AO), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) (Wang et al. 2015)

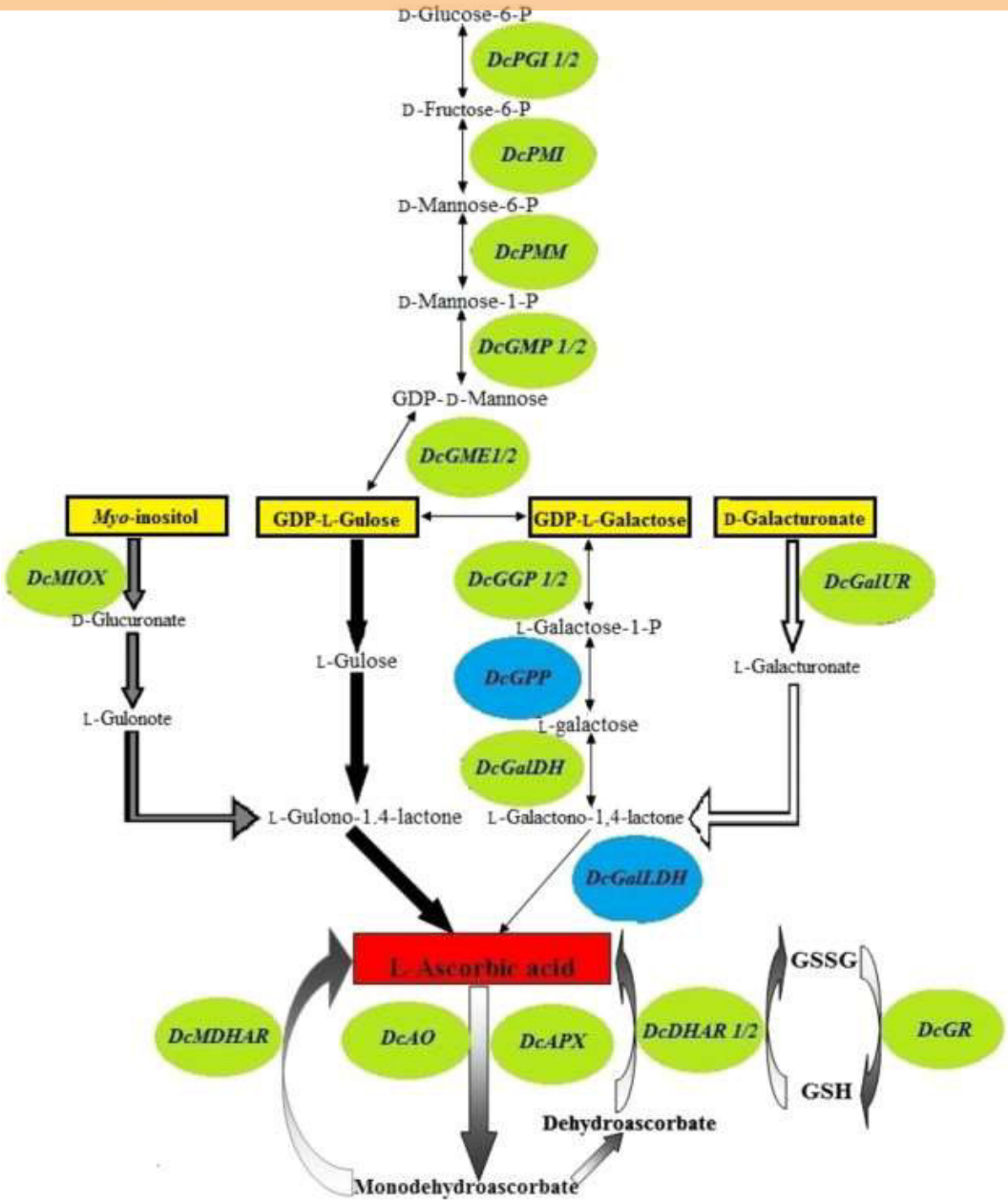

Fig. 5: Potential biosynthetic and recycling pathways of ascorbic acid in carrot. The enzymes' catalysing actions in the d-mannose/l-galactose (d-Man/l-Gal) pathway are phosphoglucose isomerase (PGI), phosphomannose isomerase (PMI), phosphomannose mutase (PMM), GDP-d-mannose pyrophosphorylase (GMP), GDP-d-mannose-3′,5′-epimerase (GME), GDP-l-galactose phosphorylase (GGP), l-galactose-1-P phosphatase (GPP), l-galactose dehydrogenase (GalDH), and l-galactono-1,4-lactone dehydrogenase (GalLDH). Myo-inositol oxygenase (MIOX) is the only enzyme identified in the myo-inositol pathway, and d-galacturonate reductase (GalUR) is involved in the galacturonate pathway. The l-gulose pathway is a hypothetical side pathway. Enzymes implicated in recycling pathway include ascorbate oxidase (AO), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) (Wang et al. 2015).