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Current Opinion in Food Science

The colourless carotenoids phytoene and phytofluene: sources, consumption, bioavailability and health effects

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Phytoene and phytofluene are rarities among carotenoids as they are colourless, have a less rigid conformation and differ in their reactivity compared to other bioavailable carotenoids. Although they have been traditionally ignored, there is an expanding interest in them as recent studies indicate that they are present in some widely consumed foods, are bioavailable and may be involved in health-promoting biological actions. According to some reviews associations between lycopene intake from tomato products and health should be revised to include other compounds present in tomato, including colourless carotenoids, because there are still obscure points in the possible health benefits of pure lycopene. Basics about colourless carotenoids are summarized together with recent studies in the context of agro-food and health.

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Current Opinion in Food Science 2021, **41**:201–209

This review comes from a themed issue on **Food chemistry and biochemistry**

Edited by **Lilian R Barros Mariutti**

For complete overview of the section, please refer to the article collection, "Food Chemistry and Biochemistry 2021"

Available online 29th April 2021

https://doi.org/10.1016/j.cofs.2021.04.013

Introduction

Carotenoids are essential for photosynthesis as well as for plant development and propagation, hence their relevance in life on Earth. Apart from some carotenoids serving as a source of vitamin A precursors, and most serving as pigments, the presence of carotenoids in foods is important because they and/or their derivatives can contribute to health-promoting biological actions that could result in decreased risk of certain conditions. They are therefore very interesting for the development of products including functional foods, nutraceuticals, nutricosmetics, supplements or novel foods, among others [1••,2••]. Probably due to their lack of colour, phytoene (PT) and phytofluene (PTF) have been largely overlooked in studies dealing with carotenoids in the context of agro-food, nutrition and health. However, recent original studies indicate that they are major dietary carotenoids, readily bioavailable and may be involved in health-promoting biological actions [2••] (Figure 1). Given the importance that these carotenoids are currently raising, several reviews focused on them have been published. In particular, in 2015 the state of art regarding these carotenoids was exhaustively reviewed [3] and in 2019 another review focused on the relationship between these compounds and skin health and appearance was published [2••]. The objective of the present work is to build on them by reviewing recent findings (mostly reported in articles published in the last three years) about the colourless carotenoids.

Distinctive structure and properties

The system of conjugated double bonds (c.d.b.) is the main structural characteristic of carotenoids and is largely responsible for key physicochemical characteristics. PT and PTF have much fewer c.d.b. relative to other major dietary carotenoids (Figure 2), as a result of which they exhibit important differences in some physico-chemical properties, for instance in terms of light absorption (absorption maxima and specific absorption coefficient in Refs. [2••,3]), interaction with certain radicals or conformation [2••]. While most carotenoids are found in nature in the *trans* configuration, colourless carotenoids are found primarily in the *cis* configuration. In particular, PT is mostly found as 15-*cis*-PT, while PTF is usually found as a mixture of different isomers (Figure 3).

Sources of carotenoids

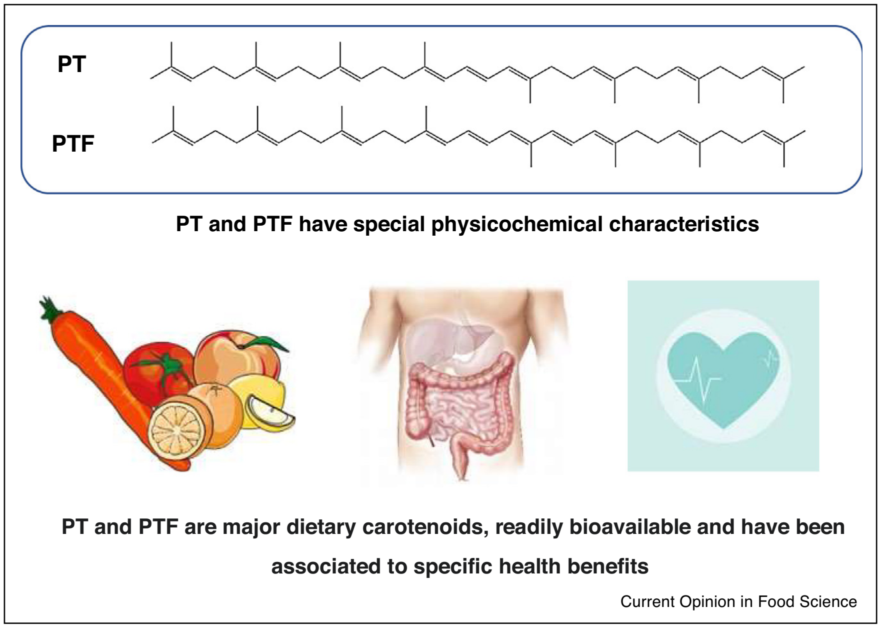
PT and PTF are present in common foods of Western diets (tomatoes, red grapefruits, watermelon, apricot, carrots, some peppers, cantaloupe, banana, melon, some citrus, avocado, nectarine, peach, etc.) and in foods less common in the diet such as caja, buriti, mamey, marimari, physalis, gac, rosehips and fruits of *Acrocomia aculeate* [2••,4,5]. PT can also occur as major carotenoid in microalgae treated with herbicides (for instance norflurazon or chlorpropham) [6,7]. Some of the most important dietary sources of colourless carotenoids are shown in Figure 4 and the concentration of PT and PTF in these sources can be consulted on Meléndez-Martínez *et al.* [3].

Factors affecting the levels of food carotenoids

Genetic factors

In the case of PT and PTF there are colour mutants with remarkable increased levels such as the yellow-fleshed orange fruits (*Citrus sinensis* L. Osbeck 'Pinalate') [8] or

Figure 1



Phytoene and phytofluene in the context of agro-food and health.

the *tangerine FG04-167* tomato (orange) [9]. Important differences have also been reported in tomatoes. PT was the predominant carotenoid in 'Cherry pera clásico', 'Cherry pera naranja', 'Green Zebra', 'Sunchocola' and 'Orange' and its concentrations ranged from 0.3 ('Cherry amarillo') to 252.6 mg/100 g dry weight ('Orange') (ca. 840-fold difference). PTF fluctuated between non detectable levels and 12.3 mg/100 g dry weight ('Orange') [10].

Effect of light

In a study in which the effect of light on fruit pigmentation in tomato and bell peppers was evaluated, it was observed that total carotenoids, PT and PTF in ripe control fruits were higher than those in the covered fruits. More specifically, total carotenoids in covered tomatoes and peppers were 50% and 25% lower than that in control, respectively [11]. Red light (660 $\pm$ 10 nm at 100 μmol/m^2 s) can result in an overaccumulation of PT in tomatoes (*Solanum lycopersicum* L. 'Micro-Tom') [12]. It has been observed that the greater the intensity (50−1500 μmol/m^2 s) of a white LED light, the greater the biosynthesis of PT in *Dunaliella salina* and that this biosynthesis was greater in the algal culture grown under red light (625−680 nm at 200 μmol/m^2 s) than in the culture grown under white light (at 200 μmol/m^2 s) [13].

Nitrogen reduction

It has been observed that a reduction of N (50 and 78%) imposed from transplant led to a reduction in the concentration of PT, PTF and lycopene in tomatoes, while when this reduction was imposed from anthesis (i.e. flowering period) an increase in the concentration of PT and lycopene was noticed [14].

Industrial practices

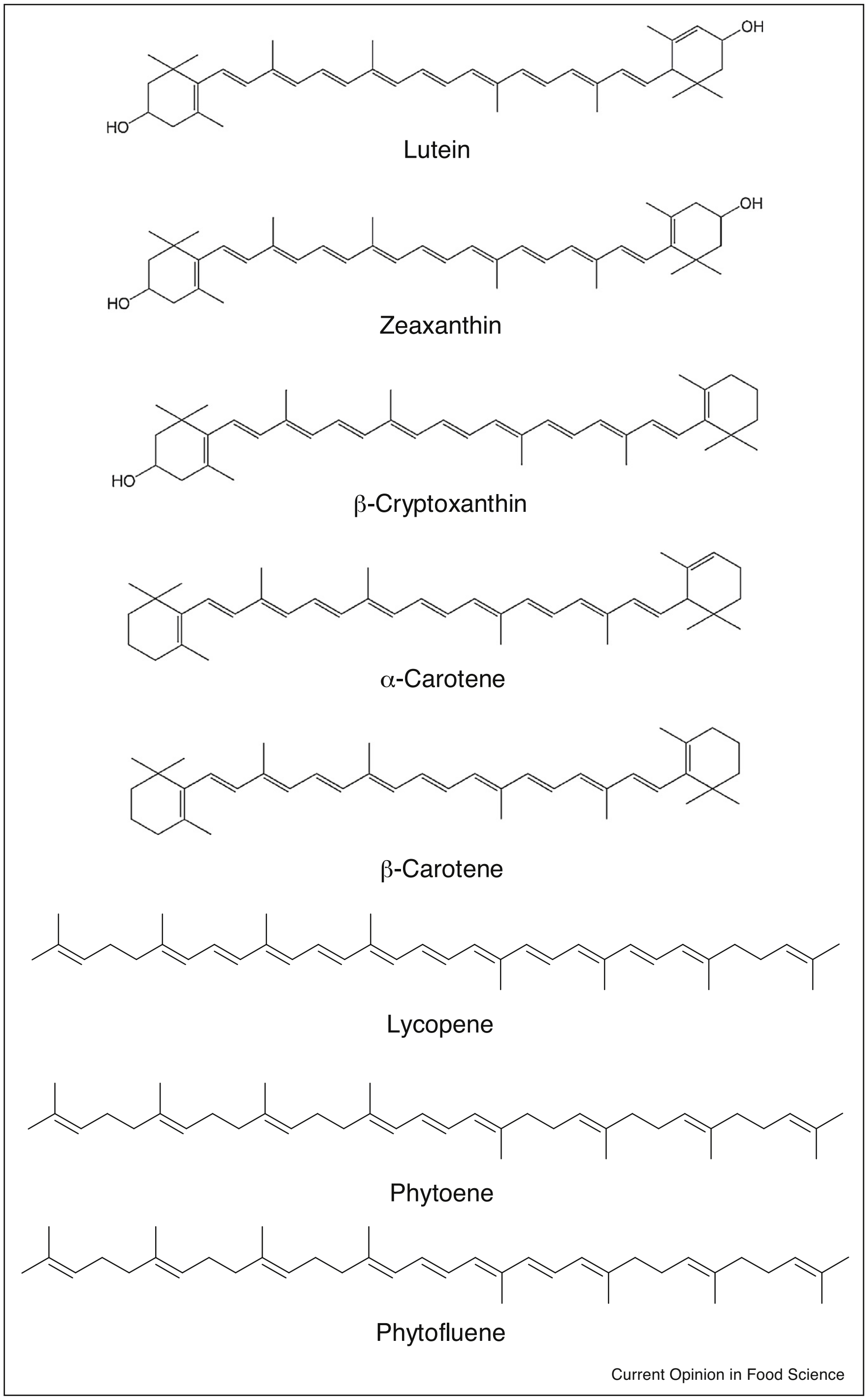
Thermal treatments

The effect of pasteurization (30 s at 90°C) and thawing of ultrafrozen juices under different conditions (at 20°C, at 4°C, and in microwave oven during 20 s at 800 W) on the carotenoid levels and bioaccessibility in orange (*Citrus sinensis* L. Osbeck 'Pinalate') juices has been evaluated [8]. As a result of the pasteurization, all the carotenoids suffered a similar degree of degradation (68% for both PT and PTF). During the ultrafrozen/thawing processes, the colourless carotenoids were more stable than xanthophylls [8].

High-pressure homogenization

The effect of high-pressure homogenization (HPH, 150 MPa, 68°C/15 s) as compared to traditional pasteurization (92°C/30 s or 85°C/15 s) on the carotenoid levels and bioaccessibility in orange (*Citrus sinensis* L. Osbeck 'Lane Late') juice have been evaluated by Stinco *et al.* [15••]. Furthermore, the pulpy fraction of the juice was subjected to the same HPH conditions and then blended with serum and pasteurized (85°C/15 s). While pasteurization did not have a significant effect on the total carotenoid concentration, HPH led to a decrease of 27%. With HPH, PT concentration decreased by 25%, while that of PTF apparently increased by 10%. The combination of HPH and pasteurization on the pulpy

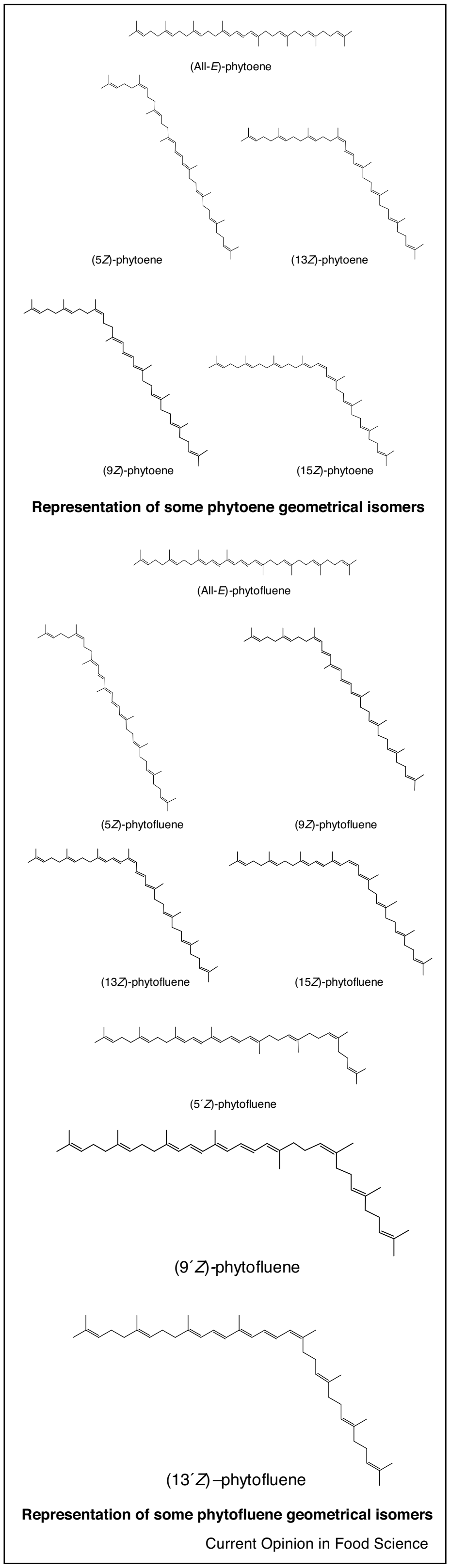
Figure 2



Chemical structures of major dietary carotenoids found in human fluids and tissues.

fraction led to a decrease in the total carotenoid, PT and PTF concentration of 2.6-fold, 3.8-fold, and 1.5-fold, respectively [15••]. A similar study was conducted on juice from 'Ortanique' mandarin (*Citrus reticulata x Citrus sinensis*), which were subjected to traditional pasteurization (65°C/15 s, 85°C/15 s, or 92°C/30 s) or to HPH (150 MPa, 68°C/15 s). Both processes led to a significant decrease in the total carotenoid concentration (13% for 65°C/15 s, 22% for 85°C/15 s, 30% for 92°C/30 s and 40% for HPH). PT was more stable than PTF under

Figure 3



Geometrical isomers of phytoene and phytofluene.

all treatments evaluated. The percentage of degradation ranged from 1.7% (65°C/15 s) to 34.3% (HPH) for PT and from 15.2% (65°C/15 s) to 38.7% (HPH) for PTF [16].

Storage practices

It has been shown that the levels of PT and PTF were not markedly altered by household refrigeration conditions (three weeks, 7°C, 85% relative humidity) in sweet red peppers (*Capsicum annuum* L. 'Lamuyo') [17]. In addition, it has been observed how the storage of cherry tomatoes at 10°C (7 days) leads to a higher concentration of PT and PTF and a lower concentration of lycopene compared to the storage at 20°C. This fact could be related to an inhibition of enzymatic desaturation required for lycopene biosynthesis in the storage at 10°C [18].

Intakes

The daily intakes of PT and PTF have been assessed in Luxembourg (2.0 mg for PT and 0.7 mg for PTF). The intake of PT turned out to be superior to that of most of the major carotenoids detected in humans, specifically lycopene (1.8 mg/day), lutein (1.5 mg/day), β-cryptoxanthin (1.4 mg/day) and zeaxanthin (0.3 mg/day) [19].

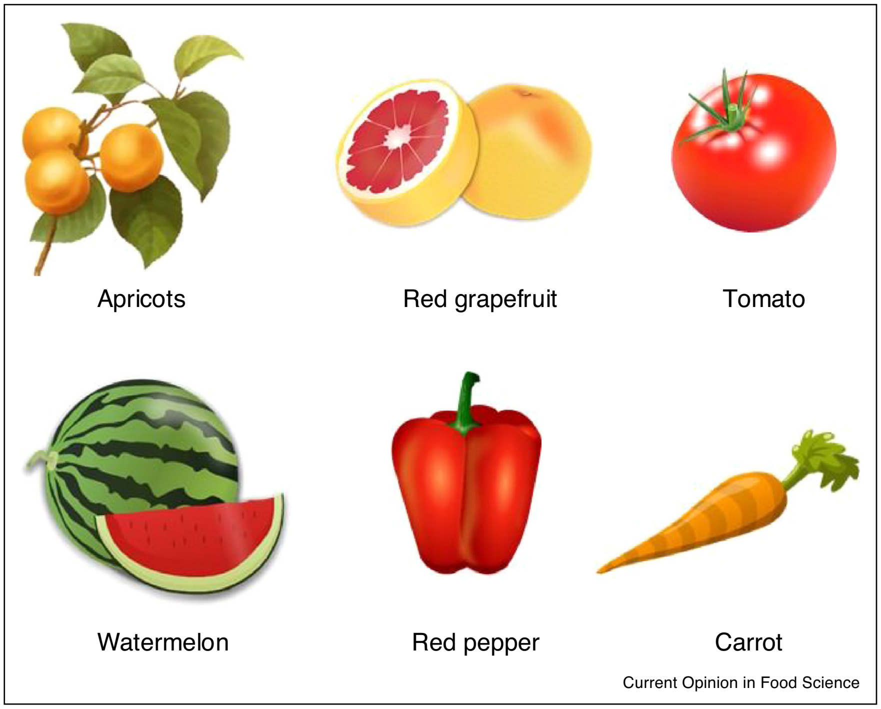
Bioavailability

Carotenoid bioavailability is influenced by many factors and is difficult to assess accurately, hence the use of standardized simulated *in vitro* digestion to assess bioaccessibility has become commonplace. Bioaccessibility usually refers to the percentage of a carotenoid that is incorporated into mixed micelles and can be subsequently taken up by enterocytes. Another concept, 'carotenoid bioaccesible content' (CBC) is being recently used and refers to the actual amount of a carotenoid that is potentially absorbable from a given food portion, that is, the absolute carotenoid levels in the micellar fractions referred to such portion [1••].

The *in vitro* behaviour of PT, PTF, lycopene, β-carotene and lutein was studied during two key steps governing bioavailability (micellization and uptake by Caco-2 cells). PT and lutein were the carotenoids with the highest incorporation efficiencies into micelles. Interestingly, PT and PTF showed a much higher incorporation efficiency than lycopene [20]. Noticeably, it was observed that approximately 14% of the PT and PTF taken up by Caco-2 cells was effluxed, this being one of the scarce (if any) pieces of evidence of carotenoid efflux reported to-date. Furthermore, enough evidence has been obtained to assume that the SR-BI protein is involved, at least in part, in the intestinal uptake of PT and PTF [20].

Concerning the uptake of colourless carotenoids along the small intestine it has been shown in an *ex-vivo* study with mice that whilst lycopene accumulated similarly along the intestine, the colourless carotenoids were principally found in the distal part of the intestine [21••].

Figure 4



Important common dietary sources of colourless carotenoids.

Effect of thermal treatments

Mapelli-Brahm *et al.* [8] studied the effect of pasteurization (30 s at 90°C) and thawing of ultrafrozen juices (2 min in liquid nitrogen) under different conditions (at 20°C, at 4°C, and in microwave oven during 20 s at 800 W) on the carotenoid bioaccessibility from orange (*Citrus sinensis* L. Osbeck 'Pinalate') juices. In all the juices, the bioaccessibility of PT was higher than that of PTF. In addition, both showed a bioaccessibility higher than that of ζ-carotene. The bioaccessibility of the colourless carotenoids in pasteurized juice (32% for PT and 27% for PTF) was over threefold higher than that found in fresh juice. The juices that gave the highest and lowest amounts of bioaccessible PT, PTF, and ζ-carotene were those thawed in the microwave and at 4°C, respectively [8].

Effect of high pressure

The effect of traditional pasteurization (92°C/30 s or 85°C/15 s) and high-pressure treatments (HPH, 150 MPa, 68°C, 15 s) on the bioaccessibility of carotenoids was studied in orange (*Citrus sinensis* L. Osbeck 'Lane Late') juice. In addition, the pulpy fraction of the juice was also subjected to the same HPH conditions and then blended with serum (i.e. the low pulp fraction) and pasteurized (85°C/15 s). Pasteurization did not have a significant effect on the bioaccessibility of most of the carotenoids, including the colourless carotenoids. The bioaccessibility content of PT, PTF and total carotenoids in the HPH juice was ∼4-times higher than that found in the fresh juice. The combination of HPH and pasteurization on the pulpy fraction led to a significant increase in total carotenoid bioaccessibility and in the CBC [15••]. A similar study in 'Ortanique' mandarin (*Citrus reticulata x Citrus sinensis*) juices subjected to traditional pasteurization (65°C/15 s, 85°C/15 s, or 92°C/30 s) or to HPH (150 MPa, 68°C /15 s) revealed that pasteurization did not have a significant effect on carotenoid bioaccessibility. Contrastingly, CBC for PT, PTF and total carotenoids in the HPH juice was 4.4, 4.7 and 4.9 times higher relative to the fresh juice [16].

Effect of other meal components

The effect of the addition of sunflower oil (5%) on the bioaccessibility of colourless carotenoids from two tomato powders, fresh tomato and fresh cherry, has been evaluated. PT bioaccessibility in the tomato powder samples ranged between 27 and 30%, while that of PTF ranged between 16 and 21%. In turn, the bioaccessibility of PT in the tomato and cherry samples was 102 and 82%, respectively, and that of PTF was 95 and 79%, respectively. As expected given their richness in colourless carotenoids, the CBC was markedly higher in the powders. For example, in the tomato powder with higher carotenoid concentration the bioaccessible content was 2.0 mg of PT and 0.5 mg of PTF per g of powders, while the tomato pulp provided 0.6 and 0.3 mg of bioaccessible PT and PTF, respectively, per 100 g. Upon addition of sunflower oil to the tomato powder samples, the bioaccessibility of PT and PTF increased ~3-fold and 4-fold, respectively [22].

Presence in human samples

Plasma levels of PT and PTF in the range of 0.1–1.0 μM are typically reported, with higher concentrations of PTF [2••]. Both have also been reported in human milk and the concentrations of PTF was noticeably higher (ranging from 0.4 to 1.4 μg/dL) compared to those of PT (0.1 μg/dL) [23]. Their presence has been reported in lung, breast, liver, prostate, cervix, colon and skin at concentrations of ng/g, with important variations across organs and, again, with higher amounts of PTF in most cases [24]. Interestingly, PT and PTF has been reported to account for 25% total carotenoids in adipose tissue [25••]. Median contents of PT and PTF in faeces of healthy adults (n = 101) of 11.4 and 2.9 μg/g dry weight, respectively, have also been reported [26].

Health-promoting biological actions

There is evidence that PT and PTF could protect from light damage and oxidative stress, exhibit antiinflamatory activity or exert anticarcinogenic activity, as recently reviewed [2••,27].

Colourless-carotenoid containing extracts

The *in vitro* anti-proliferative and anti-inflammatory activities of a *tangerine* tomato variety were greater than those of other varieties, which could be related to its high content of tetra-*cis* lycopene and the presence of other major compounds, such as PT and PTF. The highest and lowest *in vitro* antioxidant capacity (determined by the ferric reducing antioxidant power (FRAP) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays) were found in varieties with a similar concentration of tetra-*cis* lycopene and so it reasonable to think that this capacity is not due to this carotenoid alone. To evaluate the anti-proliferative properties of the tomato extracts in in androgen-dependent prostate cancer cells (LNCaP), the cell proliferation was measured through the sulforhodamine-B (SRB) assay after incubate (96 hours) the cells with the extracts diluted in DMSO (0.94–20 mg dry tomato equivalent/mL).The half-maximal inhibitory concentration (IC50) values of the *tangerine* tomatoes LNCaP cells were approximately 2–3 fold lower than those of the red tomatoes [28].

Colourless-carotenoid containing foods

Associations with cardiovascular benefits

The distribution of carotenoids in human serum and adipose tissue between obese (n = 64) and nonobese individuals (n = 16) was compared and related to some parameters. The level of PT and PTF in adipose tissue was inversely correlated with diastolic blood pressure. Inverse relationships between total body fat content and central fat and most of the carotenoids detected in serum and in adipose tissue, including PT and PTF, were found [25••].

Lycopene is found together with phytoene and phytofluene in most tomato products, which raises the question whether these colourless carotenoids are overlooked in studies finding associations between the consumption of lycopene from tomatoes and health benefits [2••,3]. As this review is based on the analysis of the progress made in recent years, two key recent studies in this respect are summarized below.

Forty-six non-smoker hypertensive subjects followed one of the following treatments during eight weeks: tomato-based supplement (TBS) (5, 15, or 30 mg lycopene/d), synthetic lycopene (15 mg/d) or placebo. The supplement with 15 mg of lycopene (6%), also contained other phytonutrients commonly present in tomato, such as PT (1%), PTF (1%), and β-carotene (0.15−0.2%). In the eight-week treatments, only the supplementations with TBS containing 15 and 30 mg of lycopene reduced significantly the systolic blood pressure (SBP). PT and PTF plasma levels changed more drastically than lycopene between TBS 5 mg (non-effective dose) and TBS 15 mg (effective doses), which implies that colourless carotenoids may help to reduce the SBP [29].

A meta-analysis was performed considering studies from four controlled trials from Europe, one from Western Asia Pacific and one from South America to evaluate the effects of tomato on several parameters. Pooled results indicated that tomato intervention can reduce significantly cholesterol, plasma triglyceride and low-density lipoprotein cholesterol (LDL) as well as increase high-density lipoprotein cholesterol (HDL) levels [30]. It is important to note that in addition to carotenoids, fibre, polyphenols and other bioactive compounds present in tomatoes could be involved in the effects.

Cancer

Key studies published in last years on this topic are summarized below, while references to previous studies can be found elsewhere [3].

Some biological actions attributed to carotenoids are thought to occur via transcription factors and nuclear receptors. In fact, it has been proposed that the initial effect of carotenoids in cancer prevention may involve modulation of transcription. Regarding lycopene, it seems that this carotenoid could suppress the proliferation of androgen-dependent human prostate tumour cells (LNCaP) through activation of the PPARγ-LXRα-ABCA1 pathway and that could also inhibit the cell adhesion and migration properties in androgen-independent prostate cancer cells (PC3 and DU145). In addition, during early prostate carcinogenesis in a murine model for prostate cancer (TRAMP), it has been shown that dietary tomato and lycopene may have an influence on androgen signalling-related and carcinogenesis-related gene expression [31].

A recent umbrella review of meta-analyses and systematic reviews on the relationship between tomato and lycopene intake and human health stated that although the dietary lycopene or serum lycopene and tomato intake were inversely associated with prostate cancer, the quality of most of evidence was low or very low [32].

The relationship between the consumption of tomato and lycopene and cancer mortality among US adults has been studied in a research in which data from two-year US National Health and Nutrition Examination Surveys (NHANES) were analysed. Dietary intake was assessed from a 24 hours recall. 22 835 participants were included (average age: 48 years). The mortality from cancer in the group with the highest intake of tomato and lycopene (42.5%) was significantly lower than that found in the group with the lowest consumption (45.9%). A strong inverse association was found between tomato and lycopene and cancer mortality when clinical, dietary and anthropometrical confounders were adjusted and this association was also significant when deaths from other causes were considered as competitors to cancer mortality [31].

Recent findings suggest that PT, PTF and lycopene protected against the nicotine-induced oxidative stress-mediated pancreatic islet dysfunctioning in rats and that the effect was higher when the carotenoids were administered simultaneously [33].

The association of carotenoids intake and their food sources with the risk of prostate cancer in Vietnamese adults has been evaluated in 244 prostate cancer patients and of 408 controls (average age: 68) through interviews using a questionnaire. A dose-responsive inverse relationship between the intake of lycopene, tomato, and carrot and risk of prostate cancer was found and this association was independent of factors usually related with prostate cancer, such as age, family history of prostate cancer, and body mass index. The inverse relationship between tomato intake and cancer could be due to the biological activity of lycopene or to the anticancer effect of other compounds also present in tomatoes. Very interestingly, although the association between carrot intake and cancer might be related with its high content in α-carotene and β-carotene, no relationships between these carotenoids and cancer were found. In addition, no associations between prostate cancer risk and the intake of β-cryptoxanthin, lutein, zeaxanthin, and major food sources of these carotenoids were observed [34]. It is noteworthy that PT and PTF were not studied although they are major carotenoids in both tomatoes and carrots [19,2••].

UV-light induced erythema
To evaluate if a tomato-based supplement (TBS) can protect against UV-induced erythema 145 healthy volunteers received TBS (15 mg lycopene, 5.8 mg PT and PTF, 0.8 mg β-carotene, and 5.6 mg tocopherols from tomato extract, and 4 mg carnosic acid from rosemary extract per day) or placebo during 12 weeks. At the end of each phase, the minimal erythemal dose (MED) was measured. Lycopene, PT and PTF level in serum increased 1.9, 1.8, and 3.0 times after the TBS supplementation, and no changes in these carotenoids levels were observed in the control group. MED did not change significantly with TBS and placebo intake. However, TBS seemed to protect against UVB-induced erythema, as the decrease in a* colour parameter after a 1.25 MED irradiation in the group supplemented with TBS was less than that observed in the control group. In addition, the results of this study indicated that the intake of TBS protect significantly against UVB-induced upregulation of IL6 and TNFα. This protection was not observed in the control group [35].

More information on the relationship between colourless carotenoids and skin health can be found somewhere [2••].

Conclusions

There is compelling evidence that the largely overlooked colourless carotenoids PT and PTF are major dietary carotenoids and readily bioavailable. Their intakes as well as levels in foods and human samples are comparable and sometimes higher than those of the carotenoids commonly studied in the context of agro-food and health (lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene and lycopene). Noticeably, evidence about their health-promoting biological actions continues to be produced. Within this scenario, it is very important to revisit the studies finding associations about tomato consumption and health benefits, as these are very often attributed to lycopene and not to the presence of phytoene and phytofluene, among other bioactives. Given their long ostracism, distinctive properties and recent trendiness, PT and PTF are amenable to the development of innovative products for the agro-food, cosmetic and even pharmacological industry. Lacking colour, these carotenoids can be especially useful for the development of products where such property is not desired. Their higher bioavailability and stability under certain conditions are also advantageous. On the other hand, new endeavours should focus on their sustainable production and management in alignment with the United Nation's Sustainable Development Goals, the circular economy concept and global socioeconomic challenges, as recently reviewed for carotenoids in general.

Conflict of interest statement

Nothing declared.

Acknowledgements

AJMM acknowledges funding from the Spanish State Secretariat of Research, Development and Innovation (Ministry of Economy and Competitiveness, project ref. AGL2012-37610, co-funded by FEDER) and the Andalusian Council of Economy, Innovation, Science and Employment (project ref. CAROTINCO-P12-AGR-1287). PMB held a predoctoral fellowship funded by the Andalusian Council of Economy, Innovation, Science and Employment and a postdoctoral fellowship funded by Consejería de Economía, Conocimiento, Empresas y Universidad de la Junta de Andalucía (project ref. CAROTINCO-P12-AGR-1287).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

•• of outstanding interest

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A comprehensive review stemming from the COST Action EUROCAROTEN (www.eurocaroten.eu) participated by 37 countries. The usefulness of carotenoids in the context of the development of diverse products is dealt with. Research needs are pinpointed and the need to works towards sustainable production and processing is stressed.

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