

Lycopene: A Review of Chemical and Biological Activity Related to Beneficial Health Effects

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INTRODUCTION

Carotenoids were first discovered in biological tissues during the nineteenth century, when W.H. Wackenroder isolated carotene for the first time in 1831 [1], from carrot roots. According to a review by Davies *et al.* [2] and Isler [3], in 1837, J.J. Berzelius obtained yellow pigments from leaves, called xanthophylls. Officially, the class of carotenoids received its name in 1911 from M.S. Tswett, who was able to isolate and purify xanthophylls and carotenes using chromatography. It was during the period 1873–1927 that lycopene was separated from other carotenoids by Harsten and R. M. Willstätter, who isolated 11 g of lycopene from 75 Kg of tomatoes. Later, research conducted by P. Karrer, R. Kuhn, L. Zechmeister, and I.M. Heilbron led to the elucidation of the chemical structures of lycopene and other carotenoids, establishing relationships between the presence of conjugated double bonds, their color, and spectroscopic properties, as well as their similarities to retinol molecule. Following these studies, P. Karrer received the Nobel Prize in 1937 for the elucidation of the chemical structure of β -carotene [2,3]. From these first findings regarding the structure and properties of lycopene, substantial knowledge has been accumulated about its functions in plant and animal tissues, especially related to its role in the maintenance or improvement of human health.

Different biochemical functions of lycopene in preventing chronic diseases have been proposed: lycopene may act as an antioxidant, trapping reactive oxygen species (ROS), increasing the overall antioxidant potential, or reducing the oxidative damage to lipids, proteins, and deoxyribonucleic acid (DNA), thereby lowering oxidative stress, which may lead to a reduced risk for cancer and cardiovascular disease (CVD) with an anticarcinogenic and antiatherogenic effect. Alternatively, the increased lycopene status in the body may regulate gene functions and metabolism, improve intercellular communication, and modulate hormone and immune response, thus lowering the risk for different chronic diseases [4]. These mechanisms may also be interrelated and may act simultaneously to provide health benefits. However, the exact mechanisms of action of lycopene are still not clearly understood.

In this sense, critical reviews by experts, several epidemiological studies, cell culture, and animal and human intervention studies provide scientific evidence to support the role of lycopene in human health [5,6] and its protective effects against CVD (one of the main causes of mortality in developed countries), as well as hypertension, atherosclerosis, diabetes, certain types of cancer (prostate, lung, cervix, breast, pancreas, stomach), eye diseases, and skin damage among others [7–12].

On the other hand, the developments in lycopene analysis by spectroscopic and chromatographic techniques together with mathematical modeling have opened up new possibilities to evaluate and determine the concentration of this carotenoid in natural products. Methods for efficient lycopene extraction from food samples are reported, taking into account the importance of sample

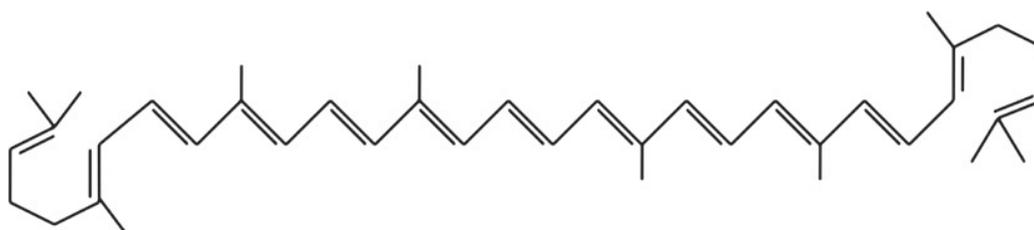
preparation, the selection of the extraction solvent, and the extraction procedure. The identification and quantification of lycopene using different advanced techniques and methods are also discussed. A very large number of reviews and chapters dealing with lycopene analysis exist in the literature. A personal selection has been made in this review, which covers the developments in the analysis of lycopene during the last decades. The review is the result of scanning through a large number of references. Nevertheless, many more exist and those listed reflect the personal choice of the authors.

LYCOPENE: CHEMISTRY, METABOLISM, AND BIOAVAILABILITY

Lycopene is a lipophilic red-colored carotenoid pigment, composed of eight isoprene units (octaprene) joined by regular head to tail bindings, except in the middle of the molecule where the binding is tail to tail, giving rise to a symmetric structure (Fig. 1). Lycopene is the prototype of other carotenoids, which may be formed through cyclizations, oxidations, or reductions. The presence of a system of 11 conjugated double bonds confers on lycopene its ability to absorb light in the UV–visible region.

Lycopene is insoluble in water, almost insoluble in methanol and ethanol, and soluble in organic solvents such as carbon disulphide, ethyl ether, petroleum ether, chloroform, and benzene.

More than 72 lycopene isomers have been identified, the most important being all-*trans*-lycopene, neolycopene A (6-*cis*-lycopene), 5-*cis*-lycopene, prolycopene (1-, 3-, 5-, 7-, 9-, 11-*cis*-lycopene), and *cis*-lycopene (1-, 3-, 5-, 6-, 7-, 9-, 11-*cis*-lycopene) [13]. Naturally, lycopene is found mainly in the all-*trans* form and can be isomerized to mono-*cis* or poly-*cis* through exposure to high temperatures, light, oxygen, acids, catalysts, and metal ions, with the *cis* form being less thermostable. Previous studies have shown a better stability of lycopene from natural matrices, such as tomatoes, than in its isolated form [14,15].



Lycopene

Molecular weight: 536.89

Molecular formula. C₄₀H₅₆

Molecular composition: C: 9.49%; H: 10.51%

FIGURE 1 Chemical structure of lycopene.

After ingestion, lycopene is quickly absorbed in the intestine, at a rate of 7–10%, together with dietary fat, and distributed to corporal tissues. The existence of protein-mediated transport mechanisms, which are saturable at low doses of lycopene (<30 mg), have been suggested. In an intervention study in humans, the maximum plasma concentrations of 4–11 $\mu\text{g/dL}$ were reached after 15–32 h with an intake of 10–30 mg/day; higher intakes of up to 120 mg/day did not give rise to higher lycopene plasma concentrations [16,17].

Although the major form in food products is all-*trans*-lycopene, the bioavailability is higher for *cis*-lycopene isomers; this fact has been attributed to the lower length of the molecules, a lower tendency to precipitate in the gastrointestinal tract, and/or higher solubility in bile micelles, which make it easy for them to be incorporated into chylomicrons. Some authors have also indicated intense isomerization during or after absorption, which explains the higher levels of *cis*-lycopene found in plasma [18,19].

The food matrix, food processing, and the presence of different substances in the diet could also have an influence on lycopene availability. Some studies have shown equal bioavailability from different tomato products, such as lycopene-rich tomato juice, tomato oleoresin, and lycopene supplements, as well as from a synthetic lycopene source [20–22]. However, lycopene absorption can be improved by low heating as well as by reducing particle size, as is the case in many tomato products due to the technological processes applied [23,24]. The superior availability of lycopene from processed tomatoes than from raw tomatoes is probably due to the release of lycopene from the cellular matrix and the isomerization of all-*trans*-lycopene into *cis*-isomers induced by heating processes [25–27].

The presence of fat in tomato products can also improve the intestinal absorption of lycopene, as well as the simultaneous ingestion of β -carotene. On the other hand, any factor that decreases fat absorption will also decrease lycopene absorption, as is the case with the synthetic oil Olestra in food or drug formulations. Koonsvitsky *et al.* [28] reported a reduction of nearly 30% over 16 weeks in patients consuming Olestra together with lycopene, and the presence of very high levels of phytosterols in some functional foods, which have been demonstrated to reduce carotenoid absorption. Thus, consumption of lycopene and this kind of products should be separated by several hours [29,30].

In the serum, lycopene is one of the most abundant carotenoids, together with lutein, β -carotene, and γ -carotene, with normal levels in the range of 220–1168 nmol/L in healthy subjects (585 nmol/L on average). Variations may be influenced by factors such as ingestion of alcohol or smoking [29,31,32]. The maximum levels of lycopene in serum are found to be at 6 h after its ingestion with an average half-life of 12–20 h [24,33].

Lycopene is stored mainly in the liver, where it can be mobilized when serum levels decrease. Significant amounts have also been found in other tissues, especially those rich in lipids, such as adipose tissue, adrenal glands,

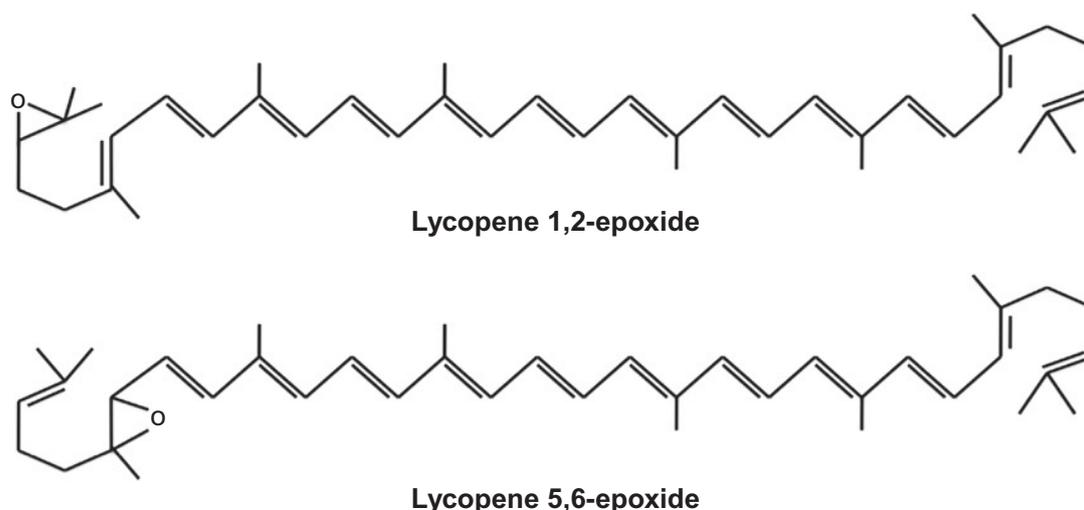


FIGURE 2 Chemical structure of the main lycopene metabolites.

prostate, kidney, and ovaries [14,30,34]. Although most of the ingested lycopene is in the all-*trans* form, between 50% and 88% of lycopene is found as *cis*-isomers [14]. During its metabolism, the oxidation of lycopene first produces lycopene 1,2-epoxide and then lycopene 5,6-epoxide as the main metabolites (Fig. 2), together with other minor metabolites such as lycopene 1,2;5,6-diepoxide; lycopene 1,2;5',6'-diepoxide; lycopene 5,6;5',6'-diepoxide; and lycopene 1,2;1',2'-diepoxide, followed by ring opening and rearrangement to cyclic structures. [35].

Excretion of lycopene takes place through the feces and in much lower amounts through urine; some lycopene can also be excreted via sebaceous glands. Due to its lipidic nature, breast milk is a good vehicle for lycopene, solubilized in the lipid fraction, providing for lycopene intake for breastfed children. In general, lycopene is found in human milk at approximately 3.8 $\mu\text{g}/100\text{ g}$, or 10% of the serum concentrations [29,36], although a low correlation has been found between the amount of lycopene in the diet of tested mothers and concentrations in breast milk.

Lycopene from either natural sources or supplements is generally well tolerated by the population. Only one manifestation of excessive lycopene intake (about 2 L of tomato juice per day) has been described as lycopenemia, consisting of a high concentration of lycopene in the blood that produces a yellowish-orange pigmentation of the skin, with no health alterations, and reversibility after administration of a lycopene-poor diet [37]. Although there is no scientific evidence available for the metabolism of lycopene during pregnancy, animal studies have revealed the absence of adverse effects associated with the consumption of lycopene-rich foods during pregnancy, taking into account acute, subacute, and chronic toxicity studies, as well as reproductive and geno-toxicity studies [17].

Based on the available toxicology studies, a no-observed-adverse-effect level (NOAEL) of 3 g/kg/day is assumed, which is much higher than the

estimated lycopene intake among the general population. Because of the lack of adverse-effect data for lycopene in animals or healthy humans, the Institute of Medicine has not set a tolerable upper intake level for lycopene. Synthetic lycopene, tomato lycopene extracts, and crystallized lycopene extracts are generally recognized as safe (GRAS) for use as ingredients in a variety of foods at levels of 0.5–7% [17].

SOURCES OF LYCOPENE

Substances included in the denomination of carotenoids are natural pigments that can be synthesized by plants, algae, and bacteria, and reach animal tissues only through the feeding process. Thus, humans are unable to synthesize carotenoids and the level of these compounds in plasma depends on the consumption of different carotenoid sources in the diet [38].

Lycopene is not as widespread in nature as other carotenoids, contributing to the attractive colors of some animals (e.g., flamingo feathers) or plant tissues (flowers and specially fruits). Lycopene is naturally located in the chromoplasts of plant cells, and it is found primarily in tomato fruits (about 80% of their total carotenoid content) and transformed tomato products. Some other edible fruits may also be sources of lycopene for the diet, mainly watermelon, guava, and pink grapefruit (Table 1); it is found in minor amounts in other food products such as apricot, cloudberry, cranberry, eggplant, grape, papaya, and peach [39–41,45,46].

TABLE 1 Main Dietary Sources of Lycopene and Their Range of Contents

Fruit	Range of Lycopene Content (mg/100 g of Edible Portion)
Tomato (<i>Solanum pimpinellifolium</i> L.)	0.594–43.09
Tomato (<i>Solanum lycopersicon</i> L.)	1.60–18.46
Pink guava (<i>Psidium guajava</i> L.)	5.43–7.02
Watermelon (<i>Citrullus lanatus</i> (Thumb.) Mansf.)	2.45–7.30
Pink grapefruit (<i>Citrus paradisi</i>)	3.32–3.36
Papaya (<i>Carica papaya</i> L.)	2.54–3.72
Persimmon (<i>Diospyros kaki</i> L.)	0.158–0.359
Mango (<i>Mangifera indica</i> L.)	0.072–0.082
Wild cherry (<i>Prunus avium</i> L.)	0.010

Data obtained from Refs. [38–44].

Tomato is one of the vegetables that are in great demand, and tomato products have a great economical relevance worldwide. The importance of tomato lies in its highly appreciated sensory properties, its being able to integrate into diverse food preparations, either cooked or raw, and also in its nutritional properties, as an important source of bioactive compounds with antioxidant activity, lycopene being one of the more interesting ones. These features contribute to significantly increasing the value of these tomato products from the consumer point of view [42,47,48]. Furthermore, there is a special interest in tomato as it is a major component in the “Mediterranean diet,” which has recently been associated with a healthier lifestyle.

Fresh tomato and its processed products are the main contributors to the total lycopene intake in the diet, present mainly in the all-*trans* configuration in variable amounts between 3.1 and 43.1 mg/100 g depending on the species, variety, and ripening stage. These contents may be different in varieties with different colors, the very red varieties having as much as 44 mg/100 g, and the yellow varieties as little as 5 mg/100 g [29,49]. The major part of the lycopene content in tomato is associated with the water-insoluble fraction, the exocarp or skin of the fruit being the part with higher contents, ranging from 35.4 to 53.56 mg/100 g.

Lycopene degradation in commercial tomato-based products not only affects the attractive color, but also the nutritional and functional value as an antioxidant in the final product. In fact, the stability of lycopene is critical for health benefits; therefore, it is essential to preserve its content in food products. Most commercial tomato products have quite a long shelf life, which makes them susceptible to lycopene losses [14,50,51].

There are some studies related to the effect of processing on lycopene stability that show that lycopene instability phenomena in biological tissues occur through *cis*-isomerization or oxidation. Lycopene autoxidation may occur at ambient temperature, in the presence of O₂, and can be accelerated with light, heat, oxygen, moisture, extreme pH, or the catalytic action of some metals. Oxidation may also take place by the action of lipoxygenase, with the formation of hydroperoxides, and the presence of some antioxidants may inhibit this reaction. These processes may be reduced by using techniques that avoid contact with oxygen in air, such as freeze-drying, microwave, or others [25,26,52–58].

The final concentration of lycopene in processed tomato products depends on the kind of technological treatment and the original raw material, and usually ranges between 0.85 and 94.0 mg/100 g [59–61]. Tomato juices may contain between 5.95 and 20.10 mg/100 g of lycopene, while ketchups usually contain a significantly higher content of lycopene, between 15.37 and 24.60 mg/100 g [62] (Table 2).

Consumption of lycopene through the diet and supplements can contribute to half of the carotenoids in the human serum [63]. Mackinnon *et al.* [64] studied the frequency of consumption of lycopene (from various sources) in a week among people aged between 25 and 79 years. The results showed that

TABLE 2 Lycopene Content (Average \pm Standard Deviation) Found in Different Brands of Common Tomato-Based Foods During 2 Years' Storage Time [62]

		Tomato Products									
		Juices			Sauces			Ketchups			
Code of tomato brands		J1	J2	J3	J4	S1	S2	S3	K1	K2	K3
Lycopene (mg/100 g)		13.16 a \pm 0.568	16.72 b \pm 0.114	20.10 c \pm 1.019	12.56 a \pm 0.479	20.25 a \pm 0.710	20.67 a \pm 0.288	19.75 a \pm 0.593	15.37 a \pm 0.177	21.95 b \pm 0.266	24.60 b \pm 0.825

For each type of product, different letters mean significant statistical differences (ANOVA Tukey test, $p < 0.05$).

the consumption of lycopene is higher in the form of fresh tomato, followed by sauces, pizza, and ketchup. It was also shown that subjects between 25 and 49 years were significantly the greatest consumers of ketchup and pizza sauce.

Tucker *et al.* [65] used the Willett 126-item food-frequency questionnaire to determine lycopene intake. The reliability of this method was tested in elderly patients, showing that this questionnaire does not provide a reasonable evaluation of lycopene status in such patients since the effects of smoking, age, energy intake, and body mass index may alter the absorption and subsequent plasma levels of lycopene.

To date, no international standard recommendation for daily lycopene intake has been made, although different studies have been carried out with this purpose. Rao [12] proposed a lycopene intake of 7–8 mg/day as the recommended amount to achieve antioxidant properties in the human body. Considering this, the ingestion of 100 g of most of the varieties of tomato and tomato-based products would guarantee the intake of this recommended lycopene amount: 100 g of tomato sauce provides approximately 6.20 mg of lycopene, and 100 g of pizza sauce may contain about 12.71 mg of lycopene. Also, the daily ingestion of a glass of tomato juice (200–250 mL); 5 portions (10 mL) of ketchup; 120 g of tomato sauce; or 60 g pizza sauce would provide for 7–8 mg/day of lycopene, as indicated by Rao [12].

Regarding dietary lycopene supplements, lycopene is commercially available in many formulations, mainly used for prostate treatment, but it is also used by the general population. Its content is highly dependent on a variety of factors such as quality of the raw materials, manufacturing process, and packaging. Since no official standards have been established to regulate the production of lycopene dietary supplements, dosage ranges must be employed as guidelines. Generally, a daily dose of 5–10 mg is taken for general recommendations. In adults with hypercholesterolemia, 60 mg of daily intake may cause a 14% reduction in plasma low-density lipoprotein (LDL) cholesterol concentrations [66].

LYCOPENE MECHANISMS OF ACTION

Although different physiological mechanisms of action have been reported for lycopene in the scientific literature, some of the most studied have been those related to protection against oxidative damage, effects on cardiovascular health, and antineoplastic activity.

Antioxidant Activity

Oxidation processes involve electron transference reactions, with the participation of molecules, atoms, or ions. These processes usually occur enzymatically, as in the case of catalase or dehydrogenase reactions in mitochondria, acting in several stages of the respiratory chain. During the breathing process, oxygen is

consumed and adenosine triphosphate (ATP) is generated, with the production of carbon dioxide and water. However, this process is not perfect, since other pollutant molecules also take part, and ROS are produced. The very reactive free radicals formed normally as a result of the cellular aerobic activity have an unpaired electron with the potential to cause damage to a great number of biological molecules. Between 1% and 3% of the oxygen that our cells consume is transformed into ROS, which are free radicals or take part in their formation. The permanent production of free radicals is opposed by the organism through the production of antioxidants. When the balance between free radicals and antioxidants is lost, harmful processes associated with the development of several diseases are triggered.

Human antioxidant systems are grouped into two principal classes, enzymatic and nonenzymatic endogenous antioxidants. The endogenous system is quite efficient; however, it does not suffice and the human body depends on various types of antioxidants present in the diet to maintain free radical concentration at low levels.

The most important antioxidants present in the diet are carotenoids (such as lycopene), phenolic compounds, vitamin C (ascorbic acid), and vitamin E (α -tocopherol) [67,68]. Lycopene is one of the most powerful antioxidants among dietary carotenoids, two times more efficient than β -carotene [69]. Lycopene has the highest singlet oxygen-quenching capacity *in vitro*, which results in excited lycopene molecules that dissipate the newly acquired energy through a series of rotational and vibrational interactions with the solvent, thus returning to the unexcited state and allowing them to quench more radical species. This is the reason why its presence in the diet is considered of great significance [12,60,61,70]. Due to the presence of conjugated double bonds in the chemical structure of lycopene, it can capture ROS, and work as an antioxidant at low oxygen pressure [35,71].

Lycopene's antioxidant action is beneficial in the prevention and improvement of certain pathologies, since most of them start with a cellular oxidation process. Results obtained by Wenli *et al.* [72] showed the greater antioxidant capacity of lycopene (and all the isomeric forms of lycopene) in human tissues in comparison with other antioxidant substances. Böhm *et al.* [69] also reported higher trolox equivalent antioxidant capacity (TEAC) values for lycopene than for other carotenoids.

Modulation of Lipid Metabolism

Lipoprotein metabolism plays an important role in the development of several human diseases, including coronary artery disease and the metabolic syndrome. A good comprehension of the factors that regulate the metabolism of the various lipoproteins is therefore a key factor for a better understanding of the variables associated with the development of these diseases.

Related to CVD, elevated blood LDL-cholesterol concentrations are associated with increased risk of coronary heart disease (CHD); there is strong evidence for the biological basis through which it can contribute to the development of atherosclerosis (one pathway to CHD). Therefore, a decrease in blood LDL-cholesterol concentration may be considered beneficial in the context of reduction of disease risk for CHD. Similarly, reduction in systolic blood pressure may be considered beneficial in the reduction of disease risk for CHD or stroke.

It is now well recognized that atherosclerosis is an inflammatory disease that begins with the development of fatty streaks, formed when macrophages that have invaded the artery wall scavenge lipid from plasma lipoproteins in the subendothelial space, and eventually become so engorged that they can form foam cells. LDL plays a major role in macrophage foam cell formation; however, uptake of native LDL via the LDL receptor (LDLr) is down-regulated when intracellular cholesterol levels begin to rise, and thus does not lead to foam cell formation. The modified LDL, however, is taken up mainly by unregulated scavenger receptors, allowing large amounts of lipid to accumulate intracellularly [73].

Lycopene as an isoprenoid is synthesized in plant and animal cells from mevalonate via the 3-hydroxy-3-methyl glutaryl-CoA reductase (or HMG-CoA reductase) pathway, an enzyme that produces cholesterol and other isoprenoids, regulated by an end-product repression. Competitive inhibitors of reductase induce the expression of LDL receptors in the liver, which in turn increase the catabolism of plasma LDL, lowering the plasma concentration of cholesterol.

Lycopene has anticholesterol effects as it is able to reduce the expression of HMG-CoA reductase in a dose- and time-dependent manner. The inhibition of HMG-CoA reductase by lycopene is also accompanied by a reduction in intracellular cholesterol levels [74], both in cultured macrophages [66] and in human subjects [75]. In agreement with these *in vitro* observations, dietary supplementation of lycopene in human subjects resulted in a significant reduction in plasma LDL-cholesterol concentrations [76].

Other variables in lipoprotein metabolism are regulators such as caveolins and caveolae. Caveolae are small plasma membrane invaginations that are observed in terminally differentiated cells. Their most important protein marker, caveolin-1, has been shown to play a key role in the regulation of several cellular signaling pathways and in the regulation of plasma lipoprotein metabolism by controlling plasma levels as well as their composition. Thus, caveolin 1 (cav-1) plays an important role in the development of atherosclerosis [77]. Palozza *et al.* [74] observed that lycopene induced the expression of both ATP-binding cassette, subfamily A member 1 (ABCA 1) and cav-1 in THP-1 macrophages in a dose-dependent manner, favoring cholesterol efflux through a potential mechanism involving Ras homolog gene family member

A (RhoA) inactivation and a subsequent increase in peroxisome proliferator-activated receptor-c (PPAR-c) and liver X receptor-a (LXRa) activity.

Other mechanisms that explain lycopene's effect in atherosclerosis prevention include prevention of endothelial injury; modulation of lipid metabolism through cholesterol synthesis control and oxysterol toxic activities; reduction of inflammatory response through changes in cytokine production; and inhibition of smooth muscle cell proliferation through regulation of molecular pathways involved in cell proliferation and apoptosis [74].

There is renewed interest in studying the possible beneficial effects of cytokines on the heart. The induction of most cytokine genes requires activation of the transcription factor, nuclear factor $\kappa\beta$ (NF- $\kappa\beta$). In cardiac myocytes, the stimulation of p38 mitogen-activated protein kinase (MAPK) by the mitogen-activated protein kinase kinase (MAPKK), mitogen-activated protein kinase kinase 6 (MKK6), activates the transcription factor, NF- $\kappa\beta$, and protects cells from apoptosis [78]. Palozza *et al.* [74] reported the effect of lycopene in reducing macrophage foam cell formation in response to modified LDL, by decreasing lipid synthesis in the cells and down-regulating the activity and expression of scavenger receptors. The mechanism by which lycopene was able to inhibit oxysterol-induced pro-inflammatory cytokine production, at both protein and messenger ribonucleic acid (mRNA) levels, was a redox mechanism involving an inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a decrease in ROS production and in MAPK, and NF- $\kappa\beta$ activation.

Lycopene is able to modulate the redox status, since it has been reported to chemically interact with ROS and undergo oxidation; to modulate ROS-producing enzymes, such as NADPH oxidase, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LO), and to control redox-sensitive molecular pathways. It has also been found that lycopene has a role in the inhibition of the pro-inflammatory cascade generated by macrophages since tumor necrosis factor alpha (TNF- α)-induced intercellular adhesion molecule 1 (ICAM-1) expression in human umbilical vein endothelial cells (HUVECs) was inhibited by lycopene, whereas COX-2 and platelet endothelial cell adhesion molecule (PECAM-1) expression was not affected [79].

These findings suggest that lycopene may act as an antiatherogenic agent through a mechanism involving, at least in part, an antioxidant mechanism.

Antineoplastic Activity

According to Clinton [29], much of lycopene's antineoplastic activity may be attributed to its antioxidant properties. However, other mechanisms underlying the inhibitory effects of lycopene on carcinogenesis have been described, such as upregulation of detoxification systems, interference with cell proliferation, induction of gap junctional communication (GJC), inhibition of cell cycle progression, and modulation of signal transduction pathways [80].

The antineoplastic activity of lycopene may be due to its inhibition of DNA synthesis [33]. Lycopene strongly inhibited proliferation of endometrial (Ishikawa), mammary (MCF-7), and lung (NCI-H226) human cancer cells with the half-maximal inhibitory concentration of 1–2 μM ; lycopene also suppressed insulin-like growth factor-I-stimulated growth. Inhibition of cell proliferation by lycopene may involve a modulation of protein kinase C (PKC) activity, which is important in the signal transduction pathway leading to cell proliferation [81]. Thus, inhibition of proliferation might also be linked to lycopene's antioxidant effect.

Modulation of intercellular communication, which has been demonstrated in cell cultures, may be another mechanism for the antiproliferative effect of lycopene. The scientific findings show that lycopene differentially modulates gap-junctional intercellular communication (GJIC) depending on the dose, with beneficial effects on cell communication [82,83]. Lycopene may stimulate GJC through stabilization of connexin43 mRNA [84].

Another postulated mechanism for the antiproliferative effect of lycopene is inducing differentiation of cancer cells. This induction of differentiation has been observed in leukemic cell cultures exposed to a combination of both lycopene and 1,25 dihydroxyvitamin D3 [85].

In breast and endometrial cancer, lycopene's mechanism of action is based on the inhibition of cell cycle progression associated with reduction in cyclin D levels and retention of p27Kip1 in the cyclin E binds to G1 phase—cyclin-dependent kinase 2 (E-cdk2) complexes. In prostate cancer, different mechanisms are proposed for the inhibition of cancerous cell proliferation at the G0–G1 cell cycle transition and protection of DNA [86].

BENEFICIAL HEALTH EFFECTS OF LYCOPENE

A beneficial effect may relate to maintenance or improvement of a function. This implies the reduction (or beneficial alteration) of a risk factor for the development of a human disease (not reduction of the risk of disease). In this sense, a risk factor is a factor associated with the risk of a disease that may serve as a predictor of the development of that disease.

The protection of body cells and molecules such as DNA, proteins, and lipids from oxidative damage, including photo-oxidative (UV-induced) damage, may be a beneficial physiological effect, assuming that any significant oxidative modification of the target molecule is potentially harmful [87]. For the purpose of classifying human diseases, we have used the World Health Organization (WHO) International Statistical Classification of Diseases and Related Health [88].

The physiological effects of lycopene have been the subject of study in a total of 1969 articles, 1067 of them published in the last 5 years as scientific publications of international scope [89]. Among these publications, 1680 studies refer to its antioxidant activity in relation to the reduction in the incidence

of different chronic diseases. Considering the different types of study, 186 are review articles and 189 correspond to clinical trials. The rest of the studies are chemical, epidemiological, *in vitro*, etc. In this review, those studies have been selected that provide the highest level of evidence for the health relationship with respect to the role of lycopene.

Hypercholesterolemia and CVD

In the scientific literature, different epidemiological studies and interventional trials have evaluated the association between lycopene (or tomato products) supplementation and CVD and/or progression of the risk factors. In the last 10 years (2002–2012), 1 meta-analysis [90] and 34 published clinical trials in humans (various randomized controlled trials and multicenter studies) have assessed the association between consumption of products rich in lycopene and a lower incidence of CVD [89]. Several reports have appeared supporting the role of lycopene in the prevention of CVD, based mostly on epidemiological studies showing a dose–response relationship between lycopene and CVD. However, a more complex situation emerges from the interventional trials, where several works have reported conflicting results.

In some studies, *in vitro* lipid oxidation was subjected to inhibition by lycopene, while endothelial cell-mediated oxidation of LDLs was not inhibited [91,92]. Furthermore, *in vitro* studies in J-774A cell line macrophages have been used to measure cellular cholesterol synthesis, showing its reduction (73%) by 10 μmol of lycopene [66].

In model animal studies, reduced atherosclerosis risk was found in mice treated with a tomato supplement for 4 months, attributed to reduced lipid oxidation [93].

There is scientific evidence showing that lycopene reduces cholesterol levels in human studies. A 14% reduction in plasma LDL-cholesterol concentrations was noted when six males were given a tomato lycopene supplementation of 60 mg daily for 3 months [66]. However, no significant change in lipids was detected with a daily lycopene supplementation of 5 mg in 22 female adults for 6 weeks, and the antioxidant activity of the subject's plasma was not altered [94].

Some epidemiological studies found an association between intake of lycopene from fresh tomatoes and tomato products and the prevention of CVD. As discussed before, an epidemiological study from 10 European countries [8] showed a correlation between lycopene lipid levels and a reduced risk of myocardial infarction. A multicenter case-control study was performed evaluating the content of lycopene in human tissue taken after myocardial infarction. Analyses using conditional logistic regression models controlling for age, body mass index, socioeconomic status, smoking, hypertension, and maternal and paternal history of disease showed lycopene to be protective. The effect of lycopene was the strongest in nonsmokers. The protective effect of lycopene increased at each increasing level of polyunsaturated fat and was significant in individuals whose fat tissue contained more

than 16.1% polyunsaturates. Levels of lycopene in the fatty tissue provide a better measure of long-term lycopene intake than do serum levels or dietary records.

Oxidation of LDL cholesterol was significantly reduced by the administration of lycopene in the diet of 19 healthy subjects not taking other drugs, when it was administered as tomato juice, spaghetti sauce, and tomato oleoresin [95]. The different sources of lycopene did not produce significantly different reductions. The study was randomized and crossover in design. A tendency toward lower oxidation in these groups was noted but the results were not statistically significant. Sutherland *et al.* [96] reported that the susceptibility of LDLs to oxidation was not changed by increased lycopene plasma concentrations caused by supplementation with tomato juice.

Vivekananthan *et al.* [97] performed a meta-analysis of randomized trials of the use of antioxidant vitamins for the prevention of CVD. Researchers at Kobe University in Japan tested tomato extracts for antithrombotic effects and identified varieties that have this effect. Tests were performed *in vitro* and evaluated *in vivo*, by testing laser-induced thrombosis in mice. One of the tomato varieties tested (KG99-4) showed a significant antithrombotic activity both *in vitro* and *in vivo*, inhibiting not only thrombus formation but also showing a thrombolytic effect [98]. The intake of tomato products is effective in preventing lipid peroxidation, a risk factor for atherosclerosis and CVD. Researchers at the University of Milan (Italy) evaluated the effects of tomato consumption on parameters of lipid oxidation in normal volunteers [99]. The oxidizability of LDL significantly decreased after tomato consumption, which is correlated with serum lycopene concentration. These results support the role of tomato products in the prevention of lipid peroxidation, a risk factor for atherosclerosis and CVD. The intake of tomato products has a beneficial effect by lowering LDL-cholesterol levels and increases resistance to oxidation, a preventive factor against the formation of atherosclerotic plaques. This study conducted by researchers at the University of Oulu (Finland) studied the effects of increased dietary intake of tomato products on plasma lipids and LDL oxidation [100]. Dietary intervention included two groups, one with a reference period of 3 weeks on a diet without tomato and the other on a diet of 3 weeks with a high intake of tomatoes (400 mL of tomato juice and tomato sauce 30 mg of tomato sauce daily). The study involved 21 healthy individuals. The results showed a significant decrease of 5.9% in total cholesterol and LDL cholesterol decreased by 12.9% in individuals who were on a diet with a high intake of tomato products. These changes were significantly correlated with serum levels of lycopene, β -carotene and γ -carotene. As a conclusion, it was found that a high intake of tomatoes and tomato products produces atheroprotective effects by significantly reducing LDL-cholesterol levels and increasing resistance to LDL oxidation in healthy normotensive cholesterolemics. These atheroprotective features are associated with changes in serum lycopene, β -carotene, and γ -carotene levels [100].

The oxidation of LDL in the vascular endothelium is considered to be important in the development of early atherosclerosis [101]. These authors performed a study with the aim of investigating the influence of fat-soluble vitamins and carotenoids in the concentration of conjugated dienes in LDL as an indicator of lipid peroxidation. The study sample included 124 women and 225 men. The levels of plasma lycopene and lutein were the major determinants of the inhibition of LDL-conjugated diene formation in women, while the β -carotene was the most important factor in men. The results suggested that lycopene, lutein, and β -carotene are potent antioxidants that may explain the inhibition of LDL oxidation *in vivo*.

The results reported by Thies *et al.* [102] indicated that a relatively high daily consumption of tomato-based products (equivalent to 32–50 mg lycopene/day) or lycopene supplements (10 mg/day) was ineffective in reducing conventional CVD risk markers in moderately overweight, healthy, middle-aged individuals. However, Burton-Freeman *et al.* [103] and Xaplanteris *et al.* [104] showed that consuming tomato products with a meal attenuated postprandial lipemia-induced oxidative stress and the associated inflammatory response. The relevance of oxidized LDL and inflammation to vascular injury suggests a potentially important protective role of tomato in reducing CVD risk. Furthermore, in a Ried and Fakler meta-analysis [90], it has been suggested that lycopene taken in doses of ≥ 25 mg daily is effective in reducing LDL cholesterol by about 10%, which is comparable to the effect of low doses of statins in patients with slightly elevated cholesterol levels.

Although many aspects of lycopene are yet to be clarified, supplementation of low doses of lycopene has already been suggested as a preventive measure, for contrasting and ameliorating many aspects of CVD, which is important for patients and clinicians [105].

Cancer

There are several human studies that find a statistically significant association between intake of lycopene from fresh tomatoes and tomato products and the prevention of cancers due to the antioxidant effect itself. Epidemiological studies demonstrated some benefits of lycopene in the treatment of cancer, and a few showed no effect. Several small uncontrolled trials have examined the efficacy of oral lycopene supplementation as cancer therapy with mixed results. However, observations of the well-being of the patients showed some improvement with a lycopene-rich diet. Although several mechanisms for the antineoplastic actions of lycopene have been studied, it is thought that the antioxidant properties of lycopene protect against molecular damage that is associated with carcinogenesis and/or that lycopene may modulate signal transduction in gene expression.

Lycopene's antineoplastic effect may also be related to the enhancement of immune cell activity, but some studies show no significant effect [106].

In another study, lymphocyte oxidative DNA damage was reduced in 10 healthy women who consumed a lycopene-containing tomato puree [107].

The half-maximal inhibitory concentration of lycopene (IC₅₀) for proliferation of endometrial (Ishikawa), mammary (MCF-7), and lung (NCI-H226) human cancer cell lines ranged from 1 to 2 μmol . When lycopene was added to these cell cultures, an inhibitory effect was noted within 24 h, which persisted for 2–3 days. In these test models, lycopene also suppressed insulin-like growth factor (IGF-I), which is a major autocrine/paracrine mammary and endometrial cancer cell growth regulator [33]. These results agree with those of Sharoni *et al.* [108] related to the influence of lycopene on IGF-I and the risk of prostate and breast cancers.

When tested for antioxidant properties in a multilamellar liposome system, lycopene was found to have the greatest antioxidant activity among carotenoids; ranked from greatest to least, they were lycopene, α -tocopherol, α -carotene, β -cryptoxanthin, zeaxanthin, β -carotene, and lutein [82]. Lycopene showed a dose-responsive ability to inhibit the mutagenicity of 2-amino-1-methyl-6-phenylimidazol(4-,5-b) pyridine (PhIP) with a 50% inhibitory dose (ID₅₀) of 271 μg of lycopene [109].

Several epidemiological studies support the role of lycopene in prostate cancer prevention [110–112]. The World Cancer Research Fund (WCRF) reported that a high fruit and vegetable intake may be beneficial in reducing the risk of cancer, including a positive role of lycopene in prostate cancer prevention [113]. Specifically, in the last 10 years (2002–2012), 65 clinical trials in humans have assessed the impact of consumption of lycopene-rich products, showing a decrease in the incidence of different cancers, mostly prostate and breast cancer [89]. A statistically significant trend toward reduced prostate cancer risk was associated with self-reported intake of various fruits and vegetables containing lycopene [114]. The reduced risk of prostate cancer is associated only with lycopene in plasma and was related to a lowered risk of aggressive prostate cancers in 578 men who developed the disease over a 13-year period and a set of 1294 matched controls (age, marital status, and smoking) [115]. Other studies confirm the reduced risk of prostate cancer due to lycopene supplementation [116]. Some *in vitro* studies reveal the anti-proliferative effect on prostate cancer cell lines with the combination of lycopene and α -tocopherol while this effect was not seen with lycopene alone [117]. In this line, a study reported by Zhang *et al.* [118] revealed that lycopene as an antioxidant factor in the diet could significantly inhibit DNA synthesis following a dose-dependent pattern, as it could inhibit the activity and expression of the androgen receptor gene.

A meta-analysis of observational studies (including cohort, case-control, and nested-case control) indicates that tomato products may play a role in preventing the risk of prostate cancer [119]. Edinger and Koff [120] studied the effect of a daily dietary intake of 50 g of tomato paste for 10 weeks, and their results reflect a significant reduction in the mean plasma prostate-specific antigen (PSA) levels in patients with benign prostatic hyperplasia, probably

linked to the high lycopene content in tomato paste. However, these analyses examined only the relationship between diet and cancer risk and could not distinguish if the relationship was specific to dietary lycopene or related to other components in lycopene-rich food. Basu and Imrhan [9] summarized the findings from 20 clinical studies reviewing the effects of tomato product supplementation containing lycopene on biomarkers of oxidative stress and carcinogenesis in human clinical trials. Most of the clinical trials with tomato products suggest a synergistic action of lycopene with other nutrients in lowering biomarkers of oxidative stress and carcinogenesis. The authors concluded from their review of clinical trials that the consumption of processed tomato products containing lycopene provides significant health benefits and can be attributed to various naturally occurring nutrients in tomatoes; the specific role of lycopene *per se* remains to be investigated.

On the other hand, a recent Cochrane systematic review of three randomized controlled trials, investigating the role of lycopene in preventing prostate cancer in a total of 154 participants, concluded that there is insufficient evidence to either support or refute the use of lycopene for the prevention of benign prostate hyperplasia or prostate cancer [121]. Given the lack of randomized control trials on this topic, clinicians and consumers may refer to the 2004 meta-analysis observational studies that identified a 1% relative reduction in the risk of prostate cancer diagnosis in men consuming lycopene. So a well-designed, high-methodological-quality, randomized controlled trial to investigate the effectiveness of lycopene in the prevention of prostate cancer is required [86,122].

Various cohort epidemiological [123,124] and case-control studies [125] evaluated the relative risk of developing breast cancer. Sato *et al.* [126] performed a prospective study of carotenoids and risk of breast cancer. A study of women with a history of breast cancer concluded that a diet with increased vegetable and fruit intake was linked with a significantly reduced risk of cancer recurrence [127]. Results suggested by Aune *et al.* [128] indicated that blood concentrations of carotenoids are more strongly associated with reduced breast cancer risk, and recommended the use of certain biomarkers to clarify the inconsistent and weak association between dietary intake and breast cancer risk.

There are different human observational case-control studies addressing the protective effect of lycopene against lung or pleural cancer [129–133]. Low plasma lycopene concentration is associated with increased mortality in a cohort of patients with a history of oral, pharynx, or larynx cancers [134].

Studies related to lycopene and cancer have been conducted in animal models. Narisawa *et al.* [135] reported that tomato juice containing lycopene protects against the development of induced colon cancer. A meta-analysis of observational studies indicates the potential role of lycopene in the prevention of colorectal cancer [136]. A case-control epidemiological study of 728 Italian rectal cancer subjects and 1225 colon cancer patients confirmed a 20% reduction in the risk of colorectal cancer by the addition of one daily serving of vegetable (in the form of tomato) [137].

Other studies also suggest different effects of lycopene on several cancer types. Lycopene in combination with the drug piroxicam, or lycopene with β -carotene, produced a preventive effect against induced urinary bladder cancers in rats [138]. Leal *et al.* [139] showed that lycopene administration to chicks protected them against some of the hepatotoxic effects of T-2 mycotoxin. Some studies suggest a synergic relation between lycopene and vitamin D3 regarding antiproliferative effects, and an additive effect regarding cellular cycle progression. For this reason, the inclusion of lycopene in the diet has been recommended as a cancer prevention strategy [85].

The heterogeneity in the literature may be related to different additional factors as studies are based on intake (limited by the assessment of intake, food composition databases, and differences in bioavailability). Future studies should explore new markers of cancer risk and clarify the specific role and mechanisms of lycopene in cancer prevention.

Skin Damage

Ultraviolet radiation causes the accumulation of free radicals, which causes lipid oxidation at skin level and the formation of wrinkles and aging. Due to the liposoluble nature of carotenoids, they may accumulate preferentially on the stratum spinosum of the epidermis. Lycopene appears to be carried to the skin via sebaceous secretion, and it has a tendency to accumulate in specific regions rich in sweat glands such as the forehead, nose, chin, hand, and palms. In these areas, lycopene can provide an *in situ* prevention effect against skin oxidative damage.

Basavaraj *et al.* [140] reviewed various skin diseases caused by nutritional deficiencies, the role of diet in skin immunity, and the role of antioxidants and other supplements in maintaining skin health. In this review, the role of food in the prevention of various skin disorders and the photo-protective potential of antioxidants are evaluated. Several studies suggest that the intake of foods rich in carotenoids is a useful strategy to protect the skin from photoaging. Of the various carotenoids, lycopene may play a role in preventing oxidative damage to the skin caused by exposure to ultraviolet light. Epidemiological studies link lycopene with a protective effect against UV radiation and, therefore, the induced erythema. Thus, a study conducted on the volar forearm of 16 healthy Caucasian women exposed to three times their minimal erythema dose (determined previously) of ultraviolet light reported a 31–46% reduction in skin concentrations of lycopene [141].

According to the results obtained in a previous study, lycopene naturally present in tomatoes and tomato paste protects against skin damage caused by solar radiation [142]. These are also the conclusions of the recently published study by Terao *et al.* [143]. There is much evidence that lycopene protects the skin against sunburn (solar erythema) by increasing the basal defense against UV light-mediated damage [144].

LYCOPENE ANALYSIS

Lycopene differs from other carotenoids in its physicochemical properties such as solubility and instability. Therefore, it is necessary to carefully handle lycopene during its purification and food analysis stages to avoid its degradation and isomerization. Various methods for an efficient extraction of lycopene from food samples are reported in this review taking into account the importance of sample preparation, the selection of the extraction solvent, and the extraction procedure (with special emphasis on saponification as a possible way to improve the extraction).

The identification and quantification of lycopene can be done using different methods: high-performance liquid chromatography (HPLC) with an ultraviolet (UV) or photodiode array detector (HPLC–PDA), liquid chromatography–tandem nuclear magnetic resonance (LC–NMR), liquid chromatography tandem mass spectrometry (LC–MS/MS), UV/visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), and Raman resonance spectroscopy, including the separation and identification of *cis/trans* isomers of lycopene.

Mathematical models that have been used as useful tools to interpret spectroscopic and chromatographic data for the quantification of lycopene in complex mixtures are also considered.

Sampling and Sample Preparation

Lycopene is highly susceptible to degradation by heat, light, oxygen, and metal ions [57,145–149], which makes its preservation and analysis in complex samples such as foods difficult. It may undergo degradation, formation of stereoisomers, structural rearrangements, and other chemical reactions. The main mechanisms of lycopene losses are oxidation and isomerization of the all-*trans*-form to *cis*-isomers [150–153].

The solubility and instability of lycopene necessitates a very careful handling process and short analysis time to avoid degradation and isomerization. For this reason, the methods for lycopene analysis in vegetables are limited and the need for a reliable and rapid analytical method for this purpose is recognized [43,154]. Therefore, samples should be handled under dim or red light, the solvent evaporated with gas (nitrogen, argon, or helium), and stored in the dark at temperatures below $-20\text{ }^{\circ}\text{C}$ [155].

Knowledge of the sample matrix is critical for accurate quantification, as many factors regarding the food matrix must be considered for efficient carotenoid extraction (relative content of lipid to carotenoids, types and forms of the carotenoid present, etc.).

The sampling protocol must consider the variations among different fruits and the distribution of lycopene in each fruit. In order to ensure a complete extraction, samples should be ground or cut into small pieces.

Extraction Procedure

Solvent Extraction

As *carotenoids are lipophilic compounds*, they are usually extracted using a mixture of organic solvents [156]. Commonly used solvents for lycopene extraction are ethanol, hexane, acetone, ethyl acetate, chloroform dichloromethane, isopropanol, and petroleum ether [157–159]. As lycopene is insoluble in methanol (a solvent widely used for carotenoid analysis), it requires several extraction steps, which involves higher volumes and a more complex treatment of the extracts.

The use of *low-moisture samples (<10%)* usually simplifies the extraction process, and for this reason, freeze-drying or desiccation in a vacuum-oven is often carried out to reduce the water content in the samples with little damage to the carotenoids. According to Desobry *et al.* [160] and Gonzalez Castro *et al.* [161], freeze-drying does not cause significant changes in carotenoids, since it excludes O₂. Long-time storage of freeze-dried samples can cause carotenoid alterations in carotenoids [162]. However, some authors do not recommend a complete dehydration before extraction, since a small amount of water is often useful when a low-polarity solvent mixture is used in the extraction [163,164].

In the case of fruit and vegetables with a *high water content*, water-miscible solvents, such as acetone and tetrahydrofuran (THF), are needed for complete penetration of the sample [165].

Acetone, hexane, methanol, ethanol, ethyl ether, THF, dichloroethane, and their combinations have been used to extract carotenoids from foods [44,166–172]. After extraction, the pigment is transferred from the water-containing extract to the water-immiscible solvent by adding enough water or a saturated NaCl solution until the two layers separate [52,173].

The use of *nonpolar extraction solvents* such as hexane or THF enables a more efficient extraction and reduces the time and number of extractions. Partitioning, washing, and phase separation are sometimes applied to the extract, in order to change the solvent or eliminate undesirable compounds in the extracts. For example, partitioning with petroleum ether and methanol has been used to separate chlorophylls and carotenes in the organic phase and xanthophylls in the alcoholic phase from tomato seeds [151,163,174,175].

Chlorinated solvents could induce the formation of 9-*cis* and 13-*cis* isomers of carotenoids; however, THF or acetonitrile (ACN) does not have this effect [176]. THF rapidly dissolves carotenoid compounds and denatures proteins, avoiding the formation of emulsions, but it induces peroxide formation. To avoid this oxidation process, antioxidant agents, such as butyl-hydroxy-toluene (BHT), vitamin E, rosemary extract, or pyrogallol, is often added to THF [44,177,178]. Diethyl ether (Et₂O) must be freshly prepared (peroxide-free) before use [52]. Magnesium carbonate [159,166] or calcium carbonate

[168] is often added during the extraction process to neutralize organic acids that might be present in the sample [175,179,180].

The *influence of temperature* during the extraction procedure must be considered taking into account the stability of lycopene. Some authors recommend refrigeration conditions for carotenoid extraction. Konings *et al.* [179] and Tonucci *et al.* [181] performed the extraction of carotenoids from tomato products at 0 °C under gold fluorescent light.

On the other hand, due to the interference in carotenoid determination caused by the high sugar content, solubilization in a 100 g/L NaCl solution prior to the carotenoid extraction has been recommended [182]. In the method recommended by Craft, [180] methanol/THF (50:50, v/v) is applied until the extract is colorless (which usually can take more than 3 or 4 extraction steps in lycopene-rich samples); other authors have used combinations of hexane/acetone/ethanol (50/25/25) with very good efficiency of lycopene extraction from foods, involving only one extraction step [44,173].

The extraction should be repeated several times, until all the lycopene has been removed from the sample. Some authors [180,183] stop the extraction process when the sample is colorless and other authors [173] repeat the extraction process until no lycopene is found in the extracted sample.

Matrix solid-phase dispersion (MSPD) has been employed as a novel alternative to traditional solvent extraction methods. This technique combines sample preparation, extraction, and fractionation into one process, limiting the handling and transfer steps [184]. The sample is mechanically ground with a bounded-phase solid support material in a mortar and then packed into a chromatographic column to isolate the carotenoids of interest. In addition, MSPD has been found to require 95% less solvent and 90% less time than traditional liquid–liquid extraction methods [184]. While carotenoids are prone to oxidative degradation, several groups have suggested that MSPD precludes the formation of artifacts [185,186].

Saponification

Lycopene, as a hydrocarbon compound, does not form ester linkages and can be directly extracted by homogenization in the presence of lipophilic solvents, especially in samples with a low content of chlorophylls [180], but sometimes a saponification step has been reported to improve the accuracy of lycopene analysis [187]. Although saponification may be carried out directly with the homogenized matrix, it is frequently performed after organic extraction. In the determination of some carotenoids in plant foods (especially xanthophylls in chlorophyll-rich samples), saponification is an optimal method for removal of chlorophylls (which could otherwise sensitize photo-isomerization of carotenoids), unwanted lipids (as triglycerides in lipid-rich samples), and other interfering compounds, as well as the best way to hydrolyze carotenoid esters [187,188].

Different conditions have been used for the saponification: hydrolysis time, temperature, KOH concentration, and number and volume for partition and washing. Most of them use an alkaline treatment with 10–40% KOH/methanol over 5–60 min at 56–100 °C or 3–16 h at ambient temperature [167,179,180,182,189].

The disadvantage of this treatment is that certain saponification conditions may cause the degradation of carotenoids, and for this reason, the concentration of KOH, time, and temperature must be carefully assessed for the particular type of materials to avoid *trans*–*cis* isomerization and epoxidation [164,190]. These authors recommended saponification at room temperature in petroleum ether with 10% methanolic KOH, either under an N₂ atmosphere or in the presence of an antioxidant. For the determination of carotenoids in tomato and kale, this procedure was considered unnecessary.

Greater losses of lycopene (about 25%), as compared to other carotenoids in saponified extracts of vegetables and fruits, have been reported by Müller [189]. He found that the xanthophylls were the most resistant. On the contrary, Khachik *et al.* [191] and Scott [150] found greater losses of xanthophylls rather than carotenes after 3 h of saponification at ambient temperature. Shorter saponification times are thus desirable to minimize these losses. Wilberg and Rodriguez-Amaya [182] applied a 5–10 min saponification with 10% KOH and Granado *et al.* [187] developed a rapid saponification protocol (40% KOH/methanol, 5 min), which considerably improved the accuracy of lycopene HPLC determination in vegetables, with a negligible degree of lycopene isomerization due to shorter exposure of the sample to alkaline conditions.

For these reasons, saponification is recommended for chlorophyll-rich samples, and the need for this procedure should be assayed depending on the food matrix, the analytical method, and the particular aim of the analysis [188]. In the case of saponification, when alkaline and heat conditions are used, BHT should be avoided in the solvents because in these conditions, it can form polymers that absorb light in the visible range and may coelute with some carotenoids in HPLC analysis [180].

In order to know the magnitude of the possible loss of carotenoids during the analytical procedures, internal standards, such as ethyl- β -apo-8'-carotenoate, echinenone, or retinyl palmitate, are sometimes added to the samples [163,179,192].

Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) has been used as an alternative method to extract carotenoids from food samples. This is an advanced separation technique based on the enhanced solvating power of gasses above their critical point. The preferred gas is CO₂, because of its lower critical temperature and its nontoxic, nonflammable nature, its low cost, and its high purity. It can be used at temperatures of 40–80 °C and pressures of 35–70 MPa [193].

SFE performs selective isolation of carotenoids in one step, avoiding elevated processing temperatures. This makes it favorable for the extraction of heat-sensitive carotenoids. Furthermore, the elimination of organic solvents offers the advantage that the extracts obtained are free of chemical residues [194]. SFE is compatible with supercritical fluid chromatography since both techniques can share the same mobile phase and devices, favoring the development of extraction and separation methodologies. This technique has been applied to successfully separate lycopene from other carotenoids in tomato fruits [43,195–199].

The extraction efficiency of carotenoids with CO₂ has been shown to increase with temperature and pressure. Among the various parameters tested, temperature has been observed to be the most important factor affecting lycopene yield in SFE extraction. Increasing the temperature increases the solubility of the carotenoids, which results in higher yields, but could also contribute to greater carotenoid degradation. The highest temperature used for the extraction gave the maximal extraction yield [200]. However, accounting for lycopene losses, lycopene yields at 60 °C extraction temperature were 14% greater than those obtained at 70 °C [201]. The extraction yield of lycopene from freeze-dried tomato pomace (peels and seeds) with CO₂ was determined at different technical parameters, such as temperature (40, 50, 60 °C) and pressure (35, 40, 45 MPa), using statistical analysis. The highest concentration of lycopene was obtained at over 35 MPa, while that of β-carotene and lutein was obtained at below 30 MPa. The product obtained by supercritical CO₂ extraction at 40 MPa and 60 °C contains the highest lycopene concentration of 32.52 ± 1.02 g/100 g dry material [202]. In another study carried out at almost similar conditions, the maximum recovery of lycopene (54%) and β-carotene (50%) was found at 300 bar with 5% ethanol (2 h with a 4 kg/h flow rate) [200], while 61% of lycopene was recovered at 86 °C, 34.47 MPa, and 500 mL of CO₂ at a flow rate of 2.5 mL/min [197].

Isomer separation of 9-*cis* and all-*trans* β-carotene extracted from algae using SFE at different dissolution rates in CO₂ has been reported [203]. The effect of CO₂ density on extraction of all-*trans*-lycopene from tomatoes was studied and a greater yield was found at the highest density (0.90 g/mL) [198].

Extraction of pigments (chlorophylls and carotenoids) from marjoram (*Origanum majorana* L.) with supercritical CO₂ was investigated and compared with Soxhlet extraction. The results showed that the amounts of lutein and β-carotene recovered using SFE were similar to those obtained from a traditional Soxhlet extraction [193].

Lycopene Extraction Without Solvent

Recently, advances have been made to devise new methods for the extraction of lycopene from food samples. These methods are based on the *action of enzymes* on lysed cells, using a combination of pH changes and hydrolytic

treatments. The lycopene-containing chromoplasts are then precipitated by lowering the pH and isolated through a centrifugation step. At this stage, the lycopene content of the isolated chromoplasts showed a 10-fold increase (3–5%, w/w, dry basis) with respect to untreated tomato peels [204]. A further improvement in lycopene concentration was obtained by a second enzymatic treatment using a protease cocktail as shown in Fig. 3. This catalytic step eliminated unwanted proteins bound to the chromoplasts, but not essential for their stability. The final extract showed a lycopene content of around 8–10% (w/w, dry basis), which represented a 30-fold increase with respect to the lycopene concentration of the untreated peels [204].

A coupling of ultrasonication and biocatalysis has been reported to improve the recovery of lycopene from tomato peel. Only cellulase treatment resulted in an increment of about 225% of lycopene (260 $\mu\text{g/g}$) over the untreated sample. 5% (w/w) of the biocatalyst and an incubation period of 20 min were found optimal for maximal lycopene extraction from the samples [205].

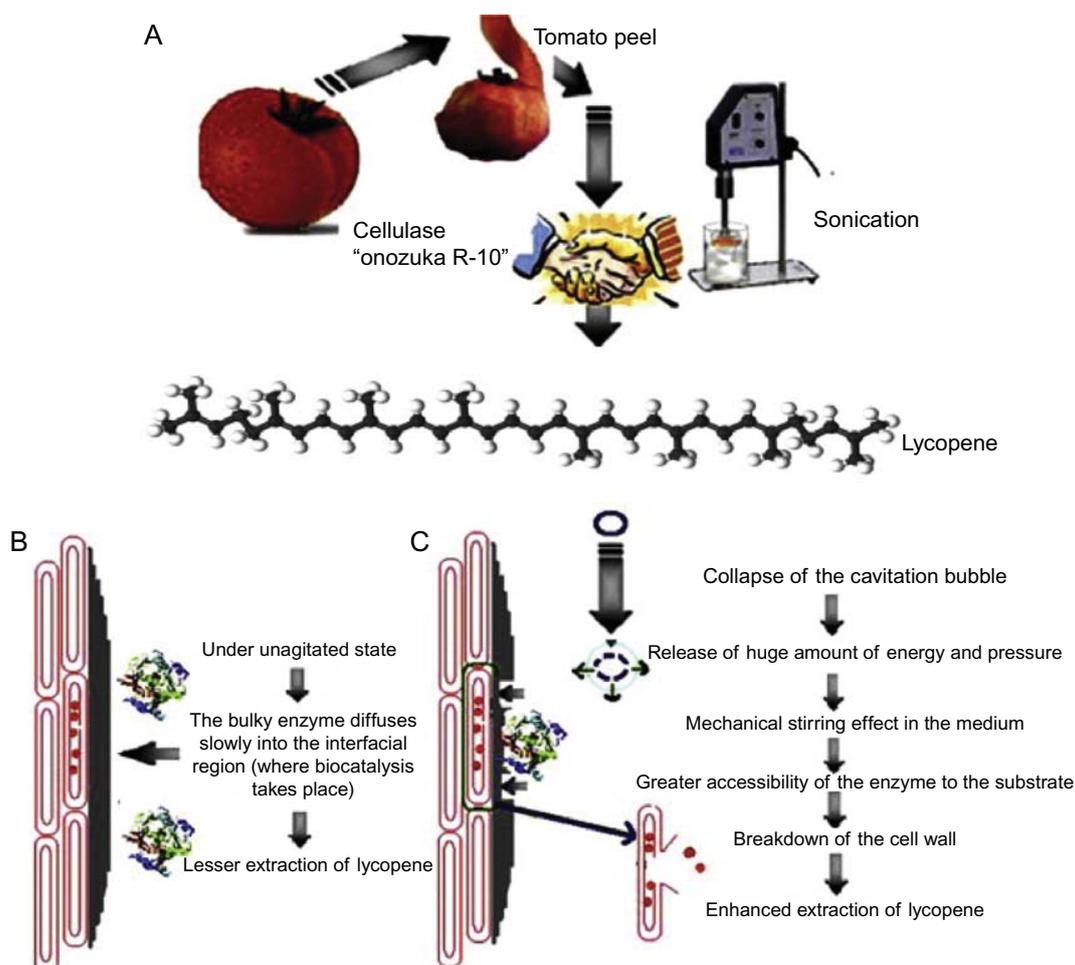


FIGURE 3 (A) Schematic representation of the coupled action of sonication and biocatalysis in the extraction of lycopene, (B) and (C) the proposed mechanism of enhanced extraction of lycopene under the concomitant exploitation of ultrasonication and biocatalysis. *Reprinted with permission from Ref. [205], published by Elsevier.*

This method works in accordance with green chemistry as it does not involve the use of organic solvents for the extraction of lycopene. Such methodology is expected to represent an economic alternative to the CO₂ extraction of lycopene to be used especially for food supplement analysis.

An extensive table summarizing the different extraction and analytical techniques can be found in the review by Rao [12].

Identification and Quantification

Chromatographic Methods

Chromatographic methods are specific and allow the separation of different compounds. However, gas chromatography is not a suitable technique for carotenoid analysis, since these compounds decompose when exposed to the high temperatures used in this technique.

The first method applied for separating carotenoids and chlorophylls by *open column chromatography (OCC)* using solid-phase CaCO₃ and petroleum ether as liquid phase was developed as early as 1906 [206]. OCC has been used especially for preparative purposes, with adsorbents, such as alumina or silica gel, MgO, CaCO₃, sugar or cellulose, and eluents of increasing polarity (starting with hexane) to obtain different fractions of pigments depending on its polarity [207]. These fractions can then be analyzed by spectrophotometry [175]. OCC has the advantage of being more economical to perform but is more time consuming.

Thin layer chromatography (TLC) has been successfully applied in carotenoid separation and purification from tomato, either alone or in combination with OCC. By OCC, the carotenoids can first be separated into fractions of different polarity, which are then further separated into individual compounds by TLC, using MgO, diatomaceous earth, and cellulose as the absorbent, and with solvent systems consisting of hexane, isopropanol, and methanol [52]. A chromato-scanner can be used as the detection system or a high-performance TLC (HPTLC) system can be applied to accurately quantify the compounds detected [208,209].

Numerous papers have been published on *HPLC* analysis of carotenoids including lycopene from food samples [210]. For the last few decades, HPLC has been the preferred method to separate, identify, and quantify carotenoids in food and biological samples, allowing us to distinguish between the geometrical structures of carotenoids, including some mono- and di-*cis*-isomers [211,212].

Stewart and Wheaton [213] developed the first HPLC method applied to carotenoid analysis, which was normal-phase using a laboratory-packed MgO column with a linear gradient of hexane/acetone containing 10% benzene. This method could separate several carotenoids in tomato samples, including lycopene. After this, several methods have been developed to analyze lycopene in food matrices by HPLC, especially reverse-phase (RP) ones.

Niizu *et al.* [214] used a monomeric C18 column to achieve carotenoid separation from salad vegetables; however, Jinno and Lin [215] recommended the use of a polymeric octadecylsilica (ODS) stationary phase for its better selectivity than monomeric ODS columns, taking into account their molecular shape and size recognition, as well as the better separation obtained using them. Most authors use C18 RP-columns for lycopene analysis [178,187,189] as they can provide a good resolution for lycopene and other carotenoids. The use of metal-free columns (such as titanium ones) is desirable, so as to avoid damages to the carotenoids during the analysis [150].

C30 RP-columns were specifically developed for the separation of carotenoids [216]. This stationary phase, with a very high efficiency, is preferred when the interest is focused on the separation of different isomers of carotenoids, including the positional and geometrical ones. Yeum *et al.* [217] separated five isomers of lycopene by using a C30 column and a gradient solvent system of methanol/methyl-ter-butyl-ether (MTBE)/water. Lee and Chen [211] compared two types of columns (C18 and C30) and various solvent systems for separation. All-*trans*-lycopene and 9-*cis* isomers (5-*cis*, 9-*cis*, 13-*cis*, 15-*cis*, and 4-di-*cis*-lycopene) were resolved by employing a C30 column with a mobile phase of n-butanol-ACN-dichloromethane (30:70:10, v/v/v) within 35 min [211]. Fröhlich *et al.* [218] also used a C30 column to distinguish between lycopene isomers. Extensive reviews of the development of C30 stationary phases for carotenoid analysis can be found elsewhere [219].

A *guard column* with a stationary phase similar to the column is sometimes used to increase the life of the column and improve the resolution of the peaks [167,187,220]. The control of *column temperature* is an additionally important factor in reducing analytical variation of the results, especially with C30 stationary phases [221], and it can also be useful to speed up the analysis. Temperatures should not be higher than 30 °C (to avoid isomerization); however, the effect of temperature should be assessed for each system, and conditions should be optimized for each case [215,222].

Regarding the *mobile phases*, they may include solvents such as ACN, methanol, 1-butanol, 2-propanol, ethyl acetate, THF, water, MTBE, or halogenated hydrocarbons (as dichloromethane or chloroform) [12]. Despite the poor solubility of lycopene in methanol, this solvent can be included in some proportion in the mobile phase, taking care that all the solutions injected do not have a high concentration of lycopene to avoid precipitation in the column [180]. The use of gradients is sometimes applied to achieve a better separation of the compounds.

Some extraction solvents can interfere with the HPLC mobile phases used for the separation of carotenoids. They can produce chromatographic artifacts, that is, broaden, or deform the chromatographic peaks. This interaction is usually produced by nonpolar extraction solvents (hexane or THF) and more polar HPLC solvents (methanol or ACN) [44]. For this reason, evaporation (if possible under N₂) is sometimes necessary, followed by a redissolution

in the mobile phase or in another noninterfering solvent. These practices usually involve a compromise between the solvent's compatibility with the mobile phase and the good solubility of lycopene (which can, in these cases, usually be restricted to 3–6 $\mu\text{g}/\text{mL}$ maximum) [44,179].

The addition of a solvent modifier such as *n*-decanol, *N,N*-diisopropylethylamine, or triethylamine (TEA) to the mobile phase, in quantities ranging between 0.05% and 0.1%, has been shown to prevent nonspecific adsorption and oxidation and, thus, improve recovery of carotenoids; it may also reduce retention times with no compromise in resolution [167,180]. A mobile phase consisting of 90/10 methanol/ACN and 0.1% TEA has shown good results in lycopene separation from other carotenoids in several food samples [44,180].

The simplest *HPLC detection systems* for lycopene are UV–visible; usually λ of 475 nm is used, because it allows the quantification of other carotenoids present in the sample. The photodiode array (PDA) is one of the most commonly used detectors for HPLC carotenoid analysis, although other detectors, such as electrochemical detectors (ECDs), fluorescence, mass spectrometers, and nuclear magnetic resonance (NMR) can be used. PDA is useful to identify and quantify lycopene and other carotenoids in food samples, following the simultaneous elution in the full UV–visible range, which guarantees the identification of each pigment by its spectrum and its quantification at its absorbance maximum.

Olives Barba *et al.* [44] optimized and compared an HPLC method with the spectrophotometric standard method mentioned earlier for the determination of lycopene and β -carotene in vegetables. They used extraction of different fruits and vegetables with hexane/acetone/ethanol (50:25:25, v/v/v), evaporation of the hexane layer, redissolution in THF/ACN/methanol (15:30:55, v/v/v), and injection in a C18 column with methanol/ACN (90:10, v/v) + TEA 9 μM as mobile phase (a flow rate of 0.9 mL/min) and detection at 475 nm. The HPLC method was comparable to the standard spectrophotometric method in precision, accuracy, and sensitivity, involving a simple preparation of the samples (one-step direct extraction) and short run times (10 min) for the quantification of lycopene in fruit and vegetable samples.

Other detection systems include a coulometric ECD which is recommended when very low levels of carotenoids need to be quantified [168,180], or mass spectrometry, which should be performed under 100 °C because of the heat instability of carotenoids. Mass spectrometry allows the identification of lycopene and other carotenoids on the basis of the structural information obtained from the fragmentation of the molecules provided by classical ionization methods (such as electron impact and chemical ionization), or by soft ionization techniques (such as fast atom bombardment, matrix-assisted laser desorption/ionization, electrospray ionization, and atmospheric pressure chemical ionization), which have facilitated the molecular weight determination of carotenoids by minimizing fragmentation. The differentiation of structural

isomers (such as carotene and lycopene) can be carried out with the aid of collision-induced dissociation and tandem mass spectrometry, which augment fragmentation and obtain structurally significant fragment ions. For example, the ion of $[M-69]^{+*}$, indicating the presence of a terminal acyclic isoprene unit, is observed in the tandem mass spectra of lycopene, neurosporene and γ -carotene, but not of α -carotene, β -carotene, lutein, or zeaxanthin [223].

However, the structural elucidation of carotenoid stereoisomers can be accomplished only by the use of NMR spectroscopy. HPLC–NMR on-line coupling has been shown to be particularly advantageous as it allows the direct identification of carotenoid stereoisomers in food as well as in physiological samples [185].

Ultra high-performance liquid chromatography (UHPLC) has been used to monitor carotenoids, but mostly in conjunction with other fat-soluble vitamins [224,225]. UHPLC methods for the quantification of a wide range of carotenoids are uncommon. The column temperature should be controlled and maintained above 20 °C to promote a consistent separation and to prevent the carotenoids from crystallizing out of the solution [221,222].

Calibration of HPLC equipment with pure standards is often necessary, although standards for many carotenoids are not commercially available. The accuracy of the results would depend on the purity of these standards. In the case of lycopene, commercial standards of all-*trans*-lycopene can be used for identification and quantification purposes. *Cis*-lycopenes can be tentatively identified based on their spectral properties: they have smaller extinction coefficients than the all-*trans* forms. The mono-*cis* isomers result in a hypsochromic shift of about 4 nm when compared to the all-*trans* form. The central *cis*-isomers have a strong peak at about 340 nm and the di-*cis* isomers may be shifted to shorter wavelengths than the mono-*cis* isomers [211,226–228].

Commercial standards have the disadvantage of being expensive and unstable. For this reason, they should be verified using a spectrophotometer to avoid errors in quantitation due to possible impurities and to the fact that carotenoids dissolve slowly in many solvents. Accuracy of the analysis can be improved by calculating the real concentration of the standard solution from the absorbance and the extinction coefficient. The value assigned can be further refined by correcting for peak purity, which is obtained from the spectrum of each standard. Once the standard solution concentrations have been established, the individual standard solutions can be mixed to form the final calibration solutions [180].

All the standards should be prepared daily and used immediately. The use of stock solutions is not desirable because of the risk of degradation. Although lycopene solutions can be stable over one week at –20 °C if protected from light and oxygen [180], if standard stock solutions are to be used, they should be evaluated daily for purity by measurements of absorbance or chromatographic peak area [179]. The precipitation of lycopene can also take place in a standard solution when it is placed at freeze temperatures; in this

case, the standard must be redissolved by agitation in an ultrasonic bath, or filtered and its concentration reassigned.

Due to the problems of availability and instability of commercial standards of lycopene, some authors prefer to prepare their own standards by means of extraction from tomato samples, purification, and evaluation of the concentration. For this purpose, the usual organic solvent for carotenoid extraction can be used, followed by concentration in a rotatory evaporator. OCC has also been used for purification purposes in an MgO:Hyflosupercel column [214]. Rodriguez [164] also recommended the purification of carotenoids using crystallization of fractions derived from a preparatory chromatographic technique such as TLC or OCC.

Interlaboratory studies on HPLC procedures for carotenoid analysis on foods have shown relative uncertainties, attributed to the effect of the chromatographic system and standardization of the carotenoid extract, while the preparation of the carotenoid extract (protocol and efficiency) may account for more than half of the total variance [229,230]. To evaluate the magnitude of these uncertainties, some certified standard reference materials (CRM), available from different organisms, can be used [187], which are essential to the development and harmonization of methods. Scott *et al.* [229] developed a CRM consisting of a mixture of freeze-dried vegetables, including tomato, which maintained a 97% retention after 36 months of being stored at -18°C under nitrogen and out of the incidence of light. There are some commercially available CRM for carotenoid analysis, consisting of vegetable mixtures and baby food composites; however, the lycopene content in these materials is usually given not as a certified value but as a reference one, because of the instability of this pigment.

UV/Visible Spectroscopy

Spectrophotometric methods are simple, but exhibit a lack of specificity for carotenoid analysis. However, they have been shown to be reliable methods to identify lycopene as the major pigment present in a mixture. A compilation of all spectra of the known carotenoids can be found in the *Carotenoids Handbook* [155]. As an example, a UV/visible spectrum of lycopene is shown in Fig. 4. The differences in the absorption spectrum between lycopene and other major carotenoids in foods such as α -carotene, β -carotene, or lutein make the quantification of lycopene easy at its characteristic wavelength maximum of 503 nm, with no interference of other compounds, in lycopene-rich samples such as tomato or watermelon. Fish *et al.* [231] stated that in samples where lycopene is at least 70% of the constituent carotenoids, the contribution of carotenoids other than lycopene to absorbance at 503 nm is less than 2% for watermelon, 4% for tomato, and 6% for pink grapefruit.

A large number of lycopene studies have been published that report the use of this method [173,232–234] as it allows for a quick routine analysis in the previously mentioned samples. Fish *et al.* [231] have also reported a

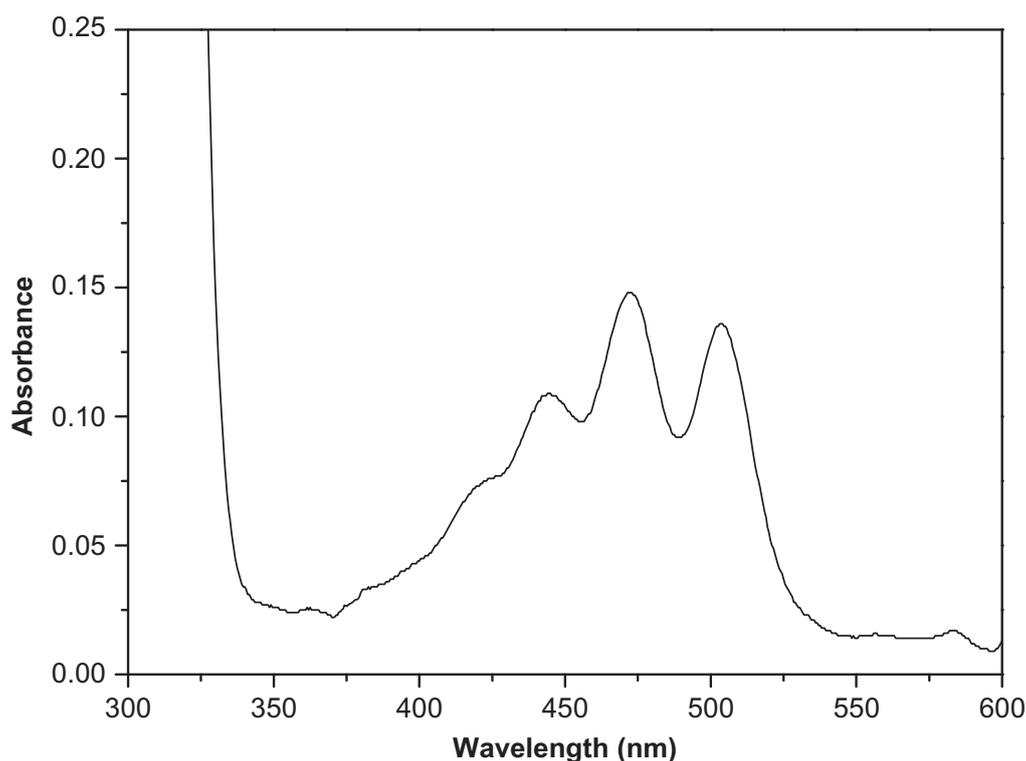


FIGURE 4 UV/visible spectrum of lycopene.

spectrophotometric method providing the advantage of using reduced volumes of organic solvents. For quantification purposes, the molar extinction coefficient of lycopene can be used [13], avoiding the problems of instability and availability of commercial standards.

Biehler *et al.* [235] have compared different spectrophotometric and HPLC methods for the rapid quantification of carotenoids in fruits and vegetables (with and without chlorophylls). A significant correlation was found between the results obtained by the newly developed method (based on the average molar absorption coefficient and wavelength) and standard HPLC.

New computerized approaches and linear models (LMs) to solve the UV/visible spectroscopy interference effects of β -carotene with lycopene analysis by neural networks (NNs) have been reported in recent years [236,237]. The data (absorbance values) obtained by UV/visible spectrophotometry were transferred to an NN-trained computer for modeling and prediction of output. Such an integrated NN/UV/visible spectroscopy approach is capable of determining β -carotene and lycopene concentrations with a mean prediction error 50 times lower than that calculated by LM/UV/visible spectroscopy.

Raman and Infrared Spectroscopy

The spectra in Et_2O solutions of α -carotene, β -carotene, leaf xanthophyll, lycopene, and β -ionone were determined as early as 1932 [238]. The nondestructive character of *Raman spectroscopy* in the analysis of organic pigments and dyes was also reported by B. Guineau [239]. Other studies have shown

that due to the strong electron–phonon coupling that occurs in carotenoids, two bands in the 1100–1200 and 1400–1600 cm^{-1} regions of the resonance Raman spectrum are strongly enhanced. This indicates that resonance Raman spectroscopy can be usefully applied to the investigation of carotenoids for analytical applications [240].

The use of spatially offset Raman spectroscopy (SORS) for nondestructive evaluation of the internal maturity of tomatoes has been assessed [241]. A Raman system using a 785-nm laser was developed to collect spatially offset spectra in the wavenumber range of 200–2500 cm^{-1} . Two peaks appeared consistently at 1001 and 1151 cm^{-1} , and the third peak was gradually shifted from 1525 cm^{-1} (lutein at the mature green stage) to 1513 cm^{-1} (lycopene at the red stage) owing to the loss of lutein and β -carotene and the accumulation of lycopene during tomato ripening. The Raman peak changes were evaluated by spectral information divergence (SID) with pure lycopene as the reference. The SID values decreased as the tomatoes ripened and so, these values can be used to evaluate the internal ripeness of tomatoes.

Near-infrared (NIR) excited Fourier transform (FT) Raman spectroscopy has been applied for *in situ* analysis of carotenoids in living plant samples. The *Pelargonium x hortorum* leaf has been mapped using a Raman mapping technique to illustrate the heterogeneous distribution of carotenoids (Fig. 5)

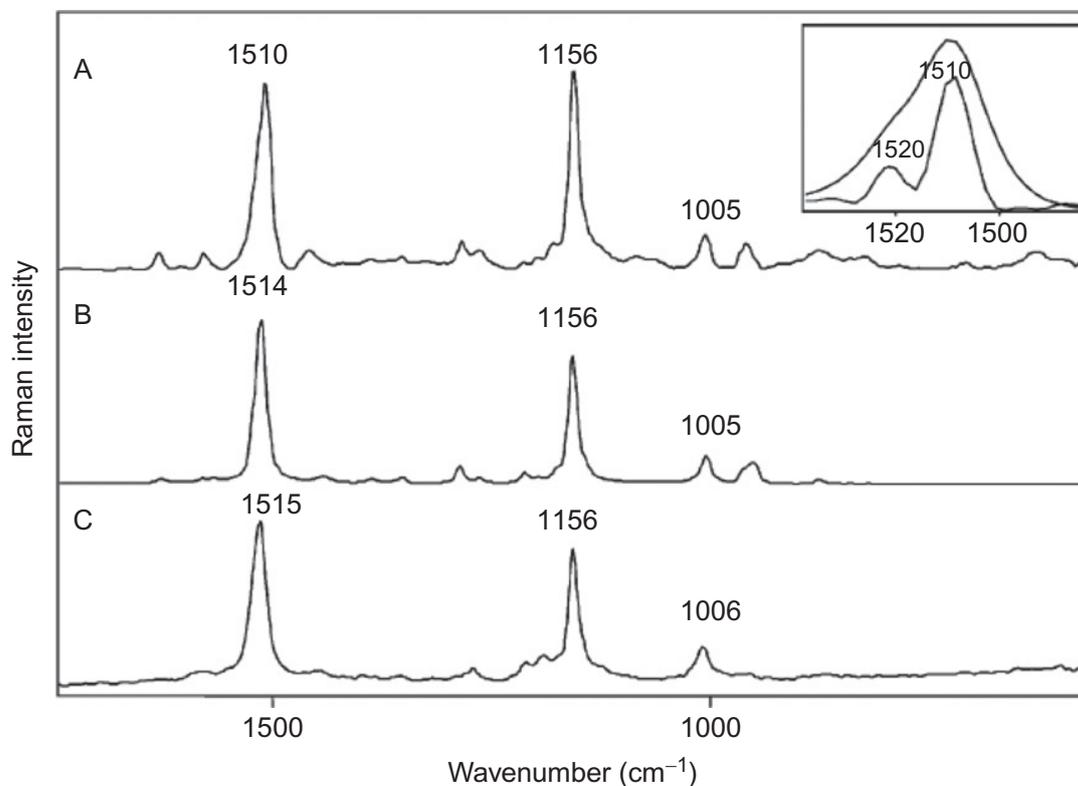


FIGURE 5 FT-Raman spectra of tomato puree (A), lycopene (B), and α -carotene (C). The inset presents a deconvoluted band of tomato puree. Reprinted with permission from Ref. [243], published by American Chemical Society.

[242]. NIR-FT-Raman spectroscopy has been shown to be a very useful technique for nondestructive analysis of carotenoids in various living plant tissues.

Nondestructive calibration methods have been established using FT-Raman, attenuated total reflection (ATR)-IR, and NIR spectroscopy for quantification of lycopene and β -carotene content in tomato fruits and related products (Fig. 6) [242,243]. It has been found that *FT-Raman spectroscopy* can be successfully applied for the identification of carotenoids directly in the plant tissue and food products without any preliminary sample preparation. Compared with the very intense carotenoid signals, the spectral impact of the surrounding biological matrix is weak and, therefore, it does not contribute significantly to the results obtained. On the other hand, ATR/FT-IR and NIR measurements mainly show strong water signals, and no characteristic bands for carotenoids are observed. However, calibration models for determination of lycopene and β -carotene contents in tomato samples obtained on the basis of the three applied vibrational methods show the best statistics when IR spectroscopy is used.

Calibration models were developed for the quantitative measurement of lycopene with the use of mathematical models. These methods simplified the use of the IR technique for the measurement of carotenoids in tomatoes [244–246].

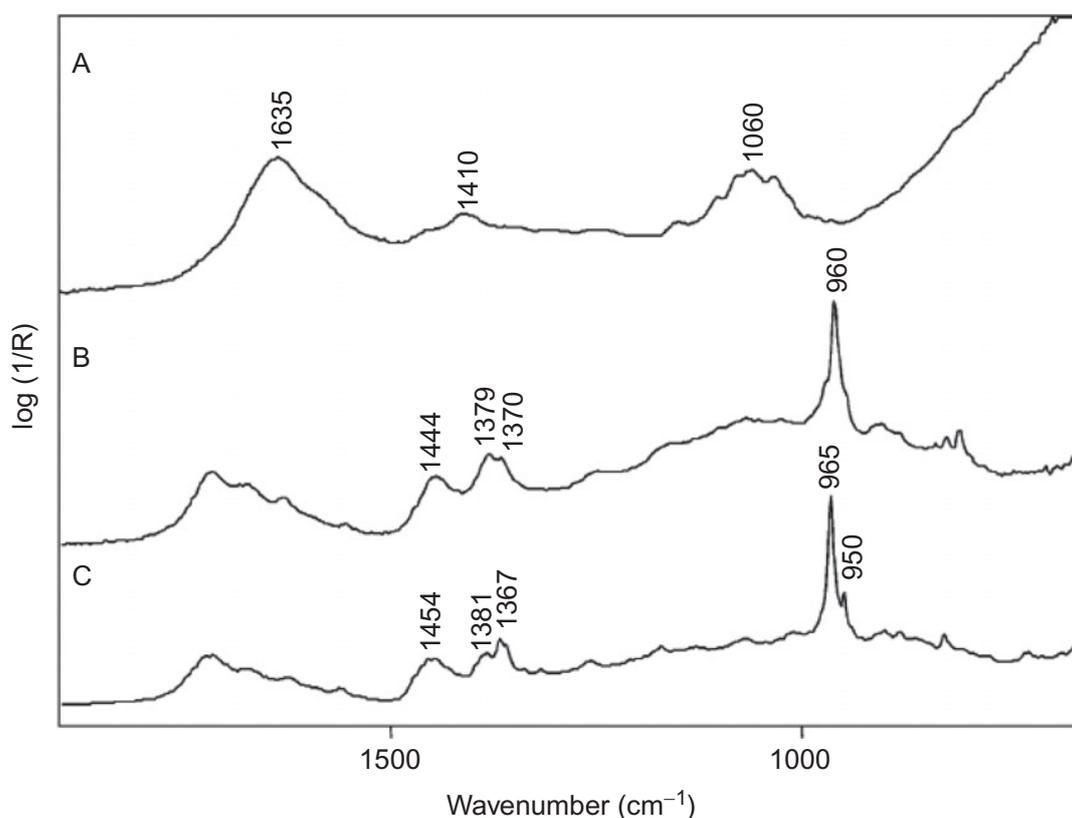


FIGURE 6 ATR-IR spectra of tomato puree (A), lycopene (B), and α -carotene (C). Reprinted with permission from Ref. [243], published by American Chemical Society.

CONCLUSIONS AND FUTURE PROSPECTS

The direction of an arrow is only known by its previous path, so the trajectory in research on lycopene shows a clear direction, and a significant growth in this field is anticipated in the coming years.

Scientific evidence strongly supports an association between a healthy diet and the prevention of chronic diseases. In recent years, there has been increasing interest in the role of lycopene, an antioxidant carotenoid present in many fruits and vegetables, in human health. Research activities on lycopene have proliferated exponentially in the past decade. The increasing number of scientific reports on the role of lycopene in the prevention of human diseases has led the food, pharmaceutical, and cosmetic industries to innovate, by including lycopene in their products, such as functional foods, supplements, and cosmetic products. However, the dosing of dietary supplements is highly dependent on a variety of factors such as quality of raw materials, manufacturing process, and packaging. To date, no official standards have been established in Europe to regulate the production of dietary supplements, so dosage ranges must be employed as guidelines only. There is an increasing involvement of consumers in health care, resulting in lifestyle modification and incorporation of complementary and alternative medicines into their dietary routine to maintain their health and prevent disease. In this regard, lycopene may help support prostate function and it is often used as an adjunctive therapy in treating prostate cancer.

To have a better understanding of the role of lycopene *in vivo*, well-controlled clinical and dietary intervention studies investigating its role in the different phases of chronic diseases should be conducted, focused on specific and standardized outcome measures of each specific disease.

Progress in the analytical techniques has brought about an improvement in the analysis of carotenoids in the last years. The accuracy of parent carotenoid as well as carotenoid metabolite identification and quantitation can be achieved by using sample preparation methods that allow a quick and efficient separation of the carotenoid content from its matrix, without any loss or degradation of the carotenoids.

Production of tomato varieties with an increased level of lycopene is desired, which requires the analysis of a greater number of samples. Therefore, analytical techniques that offer fast, simple, and accurate analysis are required. The studies conducted have shown that techniques such as UHPLC and various other spectroscopic methods such as IR and Raman spectroscopy provide a simple and fast protocol for the accurate, reproducible, and sensitive quantification of lycopene in tomatoes and tomato-based products. Raman chemical imaging has proved to be a promising spectroscopic technique for mapping constituents of interest in complex food matrices. The progress in UHPLC by the development of C30 columns, which can resist ultra-high pressures, would also increase the usefulness of this technique in performing such analyses.

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ABBREVIATIONS

ACN	acetonitrile
ATR	attenuated total reflection
BHT	butyl-hydroxide-toluene
cav-1	caveolin 1
CHD	coronary heart disease
COX-2	cyclooxygenase-2
CRM	certified reference materials
CVD	cardiovascular disease
DNA	deoxyribonucleic acid
ECDs	electrochemical detectors
Et₂O	diethyl ether
GJC	gap junction communication
HMG-CoA	3-hydroxy-3-methyl glutaryl-coenzyme A
HPLC	high-performance liquid chromatography
IGF-I	insulin-like growth factor
IR	infrared
LDL	low-density lipoprotein
LMs	linear models
MAPK	mitogen-activated protein kinase
mRNA	messenger ribonucleic acid
MS	mass spectrometers
MSPD	matrix solid-phase dispersion
MTBE	methyl-ter-butyl-ether
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
NF-$\kappa$$\beta$	nuclear factor κ β
NIR	near-infrared
NMR	nuclear magnetic resonance
NNs	neural networks
OCC	open column chromatography
ODS	octadecylsilica
PDA	photodiode array
RNA	ribonucleic acid
ROS	reactive oxygen species
RP	reverse-phase

SFE	supercritical fluid extraction
SID	spectral information divergence
TEA	triethylamine
THF	tetrahydrofuran
TLC	thin layer chromatography
UHPLC	ultra high-performance liquid chromatography

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