Title: Lycopene - A review: Chemistry, Source, Health role, Extraction, Applications.

ABSTRACT

Lycopene is an unsaturated carotenoid pigment which is acyclic and open chain, of great dietary prominence obtained mainly from colored plant sources. It is a phytochemical which is found mainly in red amaranth, tomatoes, water melon and other plants and fruits mostly red colored which covered various antioxidant which attracted attentions due to its biological properties. Lycopene has critical role in the prevention of tumor and cancer. Various ways can be utilized to identify, extract and purify lycopene from various sources by efficient analytical support. Several analyses have been attended for the extraction and quantification of lycopene in various natural sources such as various red leaf plants and fruits. This review study highlights lycopene as a carotenoid pigment including auspicious nutraceutical implications, and counts the important plant and also microbial sources for its production, and methods to calculate its bioavailability and utilization.

Keywords: Lycopene; Chemistry; Extraction; Applications;

INTRODUCTION

Lycopene ($C_{40}H_{56}$) is an open chain, acyclic, fat-soluble, unsaturated, natural carotenoid pigment and mainly utilized in the coloring of different types and kinds of foodstuffs (Connell et al., 2007). 11-conjugated linearly arranged double bonds in lycopene structure make a unique characteristic which is easy to absorption in the human body and sort it bioavailable (Choudhary et al., 2009).

Previous study verified that lycopene has a free radical quenching ability which is several times more than β-carotene and α-tocopherol (Ishida and Chapman, 2009). It is also known as a powerful potent antioxidant which ability of trapping free hydroxyl radicals and also good potentiality of electron transference (Ishida and Chapman, 2009) (Basuny et al., 2009). Lycopene has several bioactivities and also has valuable effects against many diseases such as prevention of several cancers (prostate cancer, epithelial cancers) and digestive-tract tumor, ageing and

cataracts diseases, cardiovascular diseases (Liu et al., 2010; Palozza et al., 2010; Wawrzyniak et al., 2005).

For any diversified application of lycopene it is essential extraction and purification. The extraction and purification efficiency depends on various factor such as solvent types and its polarity. Normally polar and nonpolar solvent are mixed to utilize enhance extract ability and efficiency. Several studies shows lycopene could be extracted using various mixture of solvents including ethyl acetate (100%), mixtures of ethanol and hexane 1:1 ratio, acetone, ethanol and hexane in 1:1:2 ratio, ethyl acetate and hexane in 1:1, acetone and hexane 1:1 (Barba et al., 2006).

But hexane, ethanol and acetone different ratio extract highest amount of lycopene from tomatoes and tomatoes different products (Periago et al., 2004). Not only solvent extraction but various novel methods are utilize to extract lycopene from natural sources such as enzyme assisted extraction and supercritical fluid extraction (SFE), etc. But lycopene need to purify if we utilize this methods. Now-a-days pure lycopene can be acquired by crystallization and chromatographic method utilizing sophisticated equipment's such as column chromatography, thin layer chromatography (TLC), high performance liquid chromatography (HPLC) (Aghel et al., 2011). TLC and crystallization methods are popular due to simplicity, availability chemicals of extraction methods, low cost and higher efficiency of extraction and purification (Grady et al., 2007).

Lycopene has a great potentiality to utilize as a food additives, supplements, improving sensory quality as an important antioxidant, natural food coloring agent which can be imparts yellow to red color shades and alternative of synthetic color as in breakfast cereals, baked goods, bottled water, spreads, carbonated beverages, soybean beverages, fruit and vegetable juices, candy, salad dressings, soups, various dairy product such as general products, analogues, frozen dairy desserts, and other foods and beverages which is also inhibiting oxidation during processing and storage (Choksi and Joshi, 2007; EFSA, 2006; Kong et al., 2010).

CHEMISTRY

Lycopene is a contracts with forty carbon molecules and fifty six hydrogen molecules ($C_{40}H_{56}$) which is unsaturated acyclic tetraterpenic hydrocarbon shows in Figure 01. Usually it recognized as Ψ, Ψ-carotene, *all-trans*-carotene and (*all*-E) - lycopene. Lycopene contains thirteen double bonds among them eleven are conjugated and arranged in a linear array (Kumar et al., 2014). The chemical name of lycopene is 2, 6, 10, 14, 19, 23, 27, 31 – octamethyl - 2, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30 - dotriacontatridecaene ($C_{40}H_{56}$) (Olempska-Beer, 2006).

Lycopene is a major carotenoid which is fat-soluble natural plant pigment and mostly used as color agent in different kinds and types of foodstuffs. Due to various beneficial effects in specific diseases and numerous bioactivities, lycopene receives great attention as a popular antioxidant which can be exhibit physical quenching rate constant for singlet oxygen almost twice as high as that of β-carotene. Previous studies reported, cataracts, cancer, atheromatous plaque development and ageing diseases can be prevent by lycopene and it can be utilize as a treatment of digestive-tract tumors and prostate cancer (Pyke and Howells, 2002; Stacewicz-Sapuntzakis and Bowen, 2005; Palozza et al., 2010). Lycopene can be widely used as a natural colorant in cosmetics formulations, beverages and food industry for its extraordinary solubility in oils and fats.

Normally red color is responsible for lycopene in vegetables and fruits which is a lipophilic compound which is soluble in fat and insoluble in water. The lycopene pigments belongs to the largest group of carotenoids which is mainly utilized as coloring agent of different kinds and types of foods. Normally tail to tail bond joins with two tetraterpenes formed as a carotenoids which consist of one or two benzene ring, sometimes contain extra carbon atom as well as hydrogen and oxygen atoms too. But lycopene is free from ring which is consist of 40 carbon atoms with one open chain. Lycopene consist of eleven (11) conjugated and two (2) non conjugated double bonds organized hydrocarbon by a liner way which is shown in figure 01 (Britton, 1995; Rao and Agarwal, 1999).

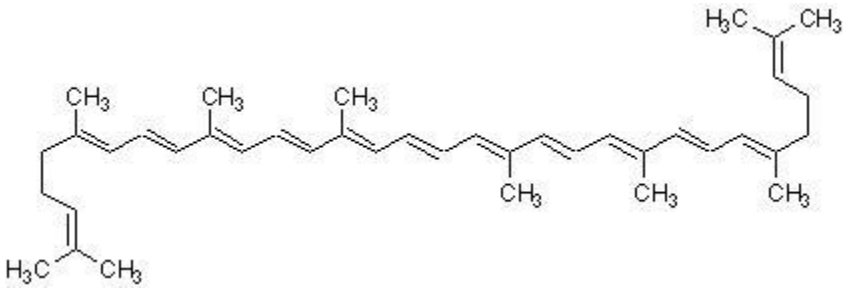

Figure 01: General structure of Lycopene

Due to different chemical reactions or other environmental effect such as light, temperature lycopene's bond can be sustain by isomerization such as *trans*- isomer to mono or *trans*- isomer to poly–*cis* isomers. Lycopene has 72 *trans*- isomeric forms and six –*cis* isomeric forms which are 5-*cis*, 9-*cis*, 13-*cis*, 15-*cis.* Lack of β-ionone, lycopene are free from pro-vitamin A. The molecular expressed as $C_{40}H_{56}$ and 536.85 Dalton is molecular weight and it also absorbs light during visible range (Rao and Agarwal, 1999).

Lycopene occurs in the all-*trans* and various *cis* configurations. In previous records, all *trans*-lycopene is referred to as all-E-lycopene and *cis* isomers are referred to as isomers. Previous study reported, from human plasma roughly 50% E-lycopene and 50% Z-lycopene mixture found (Olempska-Beer, 2006). All-*trans*-lycopene are commonly exist in nature such as 94 to 96 percent of all *trans*- lycopene presence in tomato (Schierle et al., 1997). All-*trans*-lycopene are more forms crystals then *cis* form which is more bioavailable then *trans*-lycopene and it also affect solubility (Kun et al., 2006). Due to presence of the double bonds, theoretically there are a lot of geometrical isomers can be possble. Only a small number of the possible total *cis* isomers are found because there is steric hindrance between side groups in most of the configurations. The favorable isomers are all-*trans* lycopene, where none of the double bonds are in the *cis* conformation, as well as 5-*cis*, 9-*cis*, 13-*cis* and 5-*cis* are shown in Figure 02.

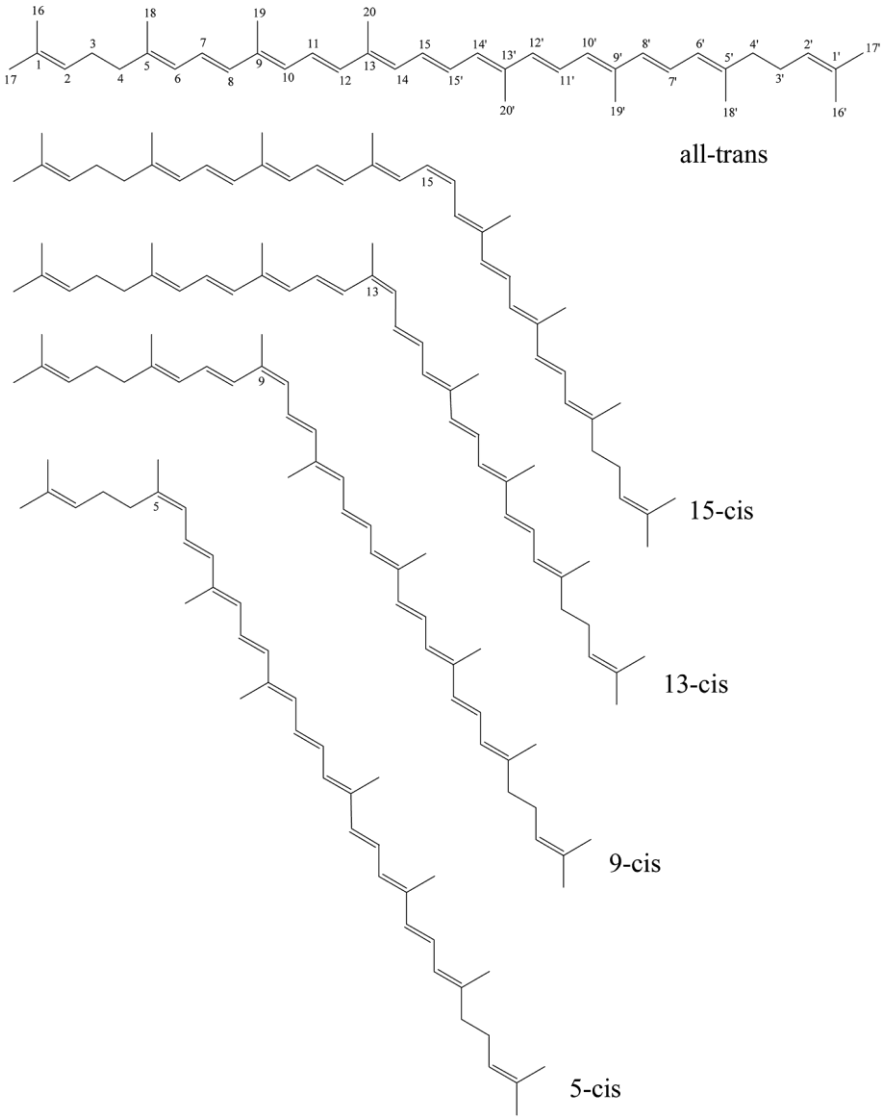

Figure 2: Molecular structures of lycopene isomers (Kong et al., 2010)

PHYSICAL PROPERTIES

Chemical formula of lycopene: $C_{40}H_{56}$

Molecular weight of lycopene: 536.89 Da

Melting point of lycopene: 172–175°C

Crystal form of lycopene: A mixture of carbon disulphide and ethanol as like long red needles

Powder form of lycopene: Dark reddish - brown

Solubility of lycopene: Soluble in benzene, hexane, chloroform, acetone, carbon disulphide, petroleum ether.

Sensitivity of lycopene: Light, oxygen, high temperature and acids (Naz et al., 2014)

SOURCES OF LYCOPENE

Natural sources such as plants and chemical synthesis in laboratory and industry can utilize as lycopene source which can be used as soft gel form, capsules and tablets. The colors of plant parts such as flowers, fruits, and stems are due the fat soluble pigments of lycopene which is tightly bonded with vegetable fibre (Yamaguchi, 2010; Ishida et al., 2004). There are numerous sources appropriate for extracting lycopene, including blood oranges, watermelon, red amaranth, tomato, papaya, mango, raspberries, red beans, strawberries, and grapes (Rao and Rao, 2007). Table 01 shows some vegetables, fruits and products which contain lycopene.

Table 01: Amount of lycopene in general foodstuffs

Lycopene source	Amount (µg/g wet weight)	Lycopene source of common tomato based productss	Amount (µg/g wet weight)
Tomato	8.8 to 42	Cooked tomatoes	37
Papaya	20 to 53	Sauce	62
Pomegranate	9.16	Paste	54
Pumpkin	23.58	Soup (condensed)	79.9
Rosehip puree	7.8	Juice	50 to 116
Red amaranth	27.13	Pizza Sauce	127.1
		Ketchup	99 to 134.4

Source: Summarized from Kalpana and Manisha, 2015; Rao and Rao, 2007; Rao and Agarwal 1999.

From Table 01, we can observed that tomatoes and red amaranth is a potential natural resource to extract lycopene. However, with new sources of natural color agent being mandatory by industry, food waste is being inspected as a potential novel source to extract, lycopene such as tomato paste and pulp (Baysal and Starmans, 2000; Chiu et al., 2007), apple, strawberry, red beet (Peschelet al., 2006) etc.

Chemical synthesis of lycopene

Lycopene can be extract from natural raw materials and chemically synthesize in laboratory by utilize synthetic reagents and chemical solvents which includes chain chemical reactions. Small amount of chemical solvents, impurities and reaction by-products may present in the end product after extraction which could be toxic for these reasons cannot be used in food. There is an unfavorable environmental impact due to utilization of huge amount of chemical solvents for industrially produced lycopene (EFSA, 2006). High concentration and ninety to ninety five percent purity can be achieved if lycopene synthesize in laboratory and industry using various solvents which is not appropriate for human consumption but utilize as coloring agent in cosmetics, creams and different types of soaps. This chemically synthesize lycopene is low bioavailability, stable at light, temperature and oxygen (Haroon, 2014).

Biological sources of lycopene

Plant and microbial sources are normally utilize as biological representative of lycopene.

1) Plant sources for lycopene

As ingredients in dietary supplements antioxidants are widely used and also used for health purposes such preventing cancer and heart disease. Lycopene, a carotenoid that have good antioxidant activity and found in variety of plant especially vegetables and fruits (Table 01).

2) Microbial sources for lycopene

Not only mammalian cells such as lymphocytes, epithelial **cells**, fibroblasts, and macrophages but also bacteria and fungi utilize to enhance lycopene content by genetic manipulation which is named as bio-engineering. Previous study reported that producing carotenoids such as lycopene, α & β carotene is an easy process in biological entity by Deoxyribonucleic acid (DNA) modification (Olempska-Beer, 2006). Lycopene and β-carotene can commercially produce from *Blakeslea trispora*, a fungus which is known also fungal plant pathogen (Vereschagina et. al, 2010). Producing Lycopene which are mainly all-*trans*-lycopene and β-carotene from *Blakeslea trispora* can be utilize as food additives and dietary supplements (Vereschagina et. al, 2010). At 2006, Olempska-Beer also reported not only all-*trans*-lycopene are produce from *Blakeslea trispora* but also some 13-*cis*-lycopene and γ-carotene. Different studies utilize mated

fermentation to plus and minus strains of *Blakeslea trispora* to achieve high yield of lycopene (López-Nieto e al., 2004). *Blakeslea trispora* including Blakeslea group has a great industrial interest because it is an excellent source of lycopene and β-carotene and presence of intracellular lipids named triacylglycerols which escalate lycopene solubility (Mantzouridou and Tsimidou, 2008). From these studies we can suggest microbial sources are can be an alternate of industrial sources of lycopene.

BIOLOGICAL ROLE OF LYCOPENE

Bioactive compounds such as carotenoids group, flavonoid group shows various health benefits when consumption of fruits and vegetables enriched by them. Lycopene like carotenoids has a lot of health benefits which can be identified in different conditions in different environments which are describe below.

Role of lycopene in the human body

Previous study reported not only the risk of cardiovascular and neurodegenerative diseases and cancer can be reduce by taking lycopene in diet regularly but also it decrease the symptoms of urinary tract such as expansion of the prostate and benign prostatic hyperplasia (BPH). Type-II diabetes is related to cardiovascular risk and symptoms of urinary tract related to prostate cancers (Ranveer et al., 2013). Taking lycopene in everyday diet reducing the risk of various chronic disease regulators such as various genetic functions, inter cellular communications improvements, controlling and modifications in immune system and hormones, regulation of metabolism (Agarwal and Rao, 2000; Camara et. al., 2013; Edward, 2002). Previous studies reported that high intake of rich source of lycopene, shows protective effects in human bodies such as fifty percent reduce the rates of mortality an elderly United States population by cancers. Some case-control reported that high amount lycopene consumption (fourteen times minimum per month) had reduce the risk of prostate cancer in about 30% than low amount lycopene consumes (three times minimum per month) (Xi, 2006; Cho, 2013). Over 220 case study in 1976 to 1979 in U.S. state Minnesota, under favorable condition and environment control epidemiologic studies examining lycopene intake reported that, prostate cancer risk decreases up to 70% of tomato consumption (>14 times per month vs <03 times per month) (Agarwal and Rao, 2000; Edward, 2002). Again another study in U.S. state Hawaii indicate no relation of lycopene consumptions and prostate cancer in over 450 case studies (Edward, 2002).

Lycopene as antioxidant

Most of the carotenoids considered as antioxidant, lycopene is one of them which has singlet-oxygen quenching ability. This ability is β-carotene is half as lycopene and α-tocopherol is one tenth (Liu et al., 2010). Reactive oxygen species capturing, oxidative stress and reduce oxidative damage of proteins (enzymes and hormones), Deoxyribonucleic acid (DNA), fats and oils such as membrane lipids and lipoproteins, are major work of antioxidant (Naz et al., 2014). This major work normally reduced any kinds and types of cancer and cardiovascular heart disease (Naz et al., 2014). Previous study reported, lycopene also increase the levels of blood and quality of sperm improved in human body as well as other antioxidant work (Mangiagalli, 2012; Edward, 2002).

Reducing prostate cancer

Studies reveal that slow the progression of prostate cancer resulting taking high doses of lycopene. From the clinical data shown that lycopene protects against prostate cancer (Shahzad et al., 2014). Consuming about ten or more servings of carotenoid containing fruits and vegetable like watermelon, tomato and their relative products per week decreased the threat about thirty five percent of advanced or aggressive prostate cancer. Previous studies represented, utilization of prediagnostic serum carotenoids reduced the threat regarding prostate cancer (Agarwal and Rao, 2000; Edward, 2002).

Inhibiting cancer cells by lycopene

Most of the carotenoids especially lycopene shows defensive effect against various types of cancers such as stomach, colon, lung and skin cancers in the body. DNA and proteins can be damage by free radicals in the tissues and cells of the human bodies. This damage can be cause inflammation which resulting cancer (Palozza et al., 2010). Normally antioxidant activity by lycopene can be removing free radicals resulting prevention of cancers threat (Hussain et al., 2003). Previous studies proved lycopene have ability reduce endometrial, lung and breast cancers more than α and β-carotene. Slowing the cell cycle progression from one growth phase to the next which resulting tumor cells can be inhibited and prevented by all carotenoids especially lycopene, α and β-carotene (Singh and Goyal, 2008). Lycopene also plays a role in modulating intercellular communication by regulating irregular pathways which results associated with

cancer (Singh and Goyal, 2008). Previous studies reported, Consumption of vegetables, tomatoes and tomato-based products which resulting higher quantity of lycopene in human body, reduced the threat of developing breast, cervical and digestive tract cancer such as pharyngeal, rectal, gastric, colon, oral and esophageal (Agarwal and Rao, 2000).

Reducing atherosclerosis and heart disease

Preventing oxidation caused by lycopene can be reduced by low density lipoprotein (LDL) cholesterol. Lycopene also reduced the threat of arteries becoming thickened and blocked. Most of the previous studies reported tomato and tomato juice utilize as a treatment for reducing by low density lipoprotein (LDL) cholesterol and arteries. Research recommended minimizing LDL cholesterol by drinking approximately 600 milliliters of processed juice which contain minimum 40 milligrams lycopene (Ghavipour et al., 2013; Haroon, 2014). Studies reported that serum lycopene can be increased by taking dietary supplements of lycopene consumed by tomato juice, spaghetti sauce and tomato oleoresin once a day per week each. Lycopene rich fruits and vegetables consumption results expressively decreased LDL oxidation and lipid peroxidation. The risk of cardiovascular diseases especially in women decreased by increase the plasma level of lycopene (Sesso et al., 2004; Li et al., 2015).

Reducing osteoporosis and prevent skin damage

Previous studies reported that, postmenopausal women often suffers oxidative stress, resulting osteoporosis (Rao et al., 2006). Lycopene reduces this stress by playing an important roles in bone health. Dietary alternative provided by lycopene also have therapeutic value who are at the risk of osteoporosis (Kim et al., 2003; Rao et al., 2006). Reported evidence indicates that Reactive Oxygen Species (ROS) may induced the pathogenesis of osteoporosis related to oxidative stress. Previous studies reported, antioxidants and carotenoids pigments such as lycopene, vitamin C, E and β-carotene plays an important roles to reduce the risk of osteoporosis (Rao, et al., 2003). Inflammation can be minimize by carotenoids such as lycopene which can be protect body skin from UV sun exposure damaging. For this reason lycopene and others carotenoids are general compounds in various cosmetic items such as lotions and anti-aging creams. But lycopene degradation by light and temperature, so that containers must be properly closed between uses (Haroon, 2014).

Lycopene bioavailability

Various unit operations during food processing can be damaging cell walls. As a result lycopene can be released from cell matrix because unit operation such as heat transfer induced isomerization results *trans* to *cis* which develops lycopene bioavailability (Su et al., 2002). Lycopene in fresh tomatoes one quarter bioavailable less than tomato paste. Lycopene in tomato products increased due to physical disruption in the cell structure which increased bioavailability more than fresh tomatoes. Even, lycopene bioavailability also increased by reduction of physical size of the fruits and vegetables by chopping and pureeing (Hadley et al., 2002). Mechanical operations and processing by heat facilitate to escape lycopene from the fruits and vegetables matrix. Thermal weakening and disruption by mechanical operations and processing accelerate rupturing of cell walls, protein complexes and dispersion of crystalline carotenoid combinations (Kun et al., 2006).

DETERMINATION AND EXTRACTION METHOD OF LYCOPENE

Lycopene assay methods

Lycopene can be determine and quantify from vegetable and fruits samples utilizing various analytical methods which includes thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), ultraviolet-visible (UV/VIS) spectrophotometry, and liquid chromatography (LC) (Davis et al., 2003; Ishida and Chapman, 2009). Now-a-days as like as chromatography many others high-speed determination methods such as infrared spectroscopy and fibre optic visible reflectance spectroscopy also developed (Baldermann et al., 2008; Choudhary et al., 2009; De Nardo et al., 2009). But infrared spectroscopy and fibre optic visible reflectance spectroscopy need more than 20 minutes to separate all-E-lycopene with its Z isomers. Usually to achieve large quantity of samples in short times and economical, we should utilize UV-VIS spectrophotometry because HPLC is more expensive and slow because it needs strenuous and skilled technicians. But if we want to determine very little amount of lycopene we need to utilize HPLC because UV-VIS spectrophotometry unable to detect lycopene less than one microgram. HPLC has ability to detect any pigments in very small quantity by utilize isomers separation (Hyman et al., 2004).

Lycopene extraction

Now-a-days, by-products from different food processing are utilizing as a source of cognitive food constituents. Previous studies represented, utilizing mild heat treatment in tomatoes puree and matrices stable lycopene. But lycopene can be rapidly degrade and isomerize when it dissolved in organic solvents, fats as well as utilizing intense and extreme processing can degrade too (Colle et al., 2010). Different types of extraction methods describing in this section.

Lycopene quantification method

Generally, to extract and quantify lycopene from plant cells using organic solvents by utilize HPLC assays and other conventional spectrophotometric equipment. HPLC assays and other conventional spectrophotometric are reliable because it needs considerable amount of time and effort, heavy machinery and equipment and huge amounts of solvents (Colle et al., 2010). Utilization of organic solvents for separate pigments and bioactive compounds from natural source was an excellent methods in food processing industries. As most tomato carotenoids are lipid soluble, common organic solvents such as dichloromethane, hexane, ethanol, acetone, ethyl acetate, petroleum ether, and mixtures of polar or nonpolar solvents in different ratios such as acetone chloroform (1:2) and hexane-acetone-ethanol (2:1:1) have been tested for carotenoid extraction (Barba et al., 2006). Some of them solvent extraction method describe below.

A) Chloroform extraction method

According to Barba et al. (2006), Sabio et al. (2003), Calvo et al. (2008), Rozzi et al. (2002) lycopene can be extracted by chloroform from various fruits and fruits by products. Rozzi et al. (2002) described that 2 g dried sample placed into an extraction tube with 20 ml chloroform. Sonicate 30 min and centrifuged by centrifuged machine for 15 min at 2000 RPM. Removed the aliquot for analysis of lycopene content determines by high performance liquid chromatography (HPLC).

B) Acetone, n-hexane and ethanol extraction method

Wee and Wai (2012), Roberto and Antonio (2008), Kalpana and Kulsange (2015) described acetone, n-hexane, ethanol extraction method. Acetone, n-hexane and ethanol taken on the

extraction tube in a ratio 1:2:1. Then add sample and rested for 30 minutes. Then absorbance was identified at 510 NM using a spectrophotometer.

Lycopene content was calculated on the basis of the following equation.

$$\text{Lycopene content (mg/gm of fresh weight)} = \frac{A \times M \times S \times V}{w \times m} - - - - - - (1)$$

Where,

A= Absorbance of 510 NM spectrum

M= Molecular weight of lycopene ($C_{40}H_{56}$, 537 g/mol)

S= The volume of mixed solvent (8 ml)

V= The volume ratio of the upper layer to the mixed solvents (0.55)

w= Sample weight (0.10 g)

m= the molar extraction co-efficient (172 mM^{-1})

C) Acetone and petroleum ether extraction method

Rocktotpal et al. (2011), Zahra et al. (2015), Emmanouil et al. (2016), Rosaria et al. (2016) described lycopene extraction from various fruits and vegetables and its byproducts using acetone and petroleum ether. Utilizing acetone-petroleum ether (50% v/v) mixture of 10 ml to extract from one gram of products sample. Lycopene store in the upper layer which was collected in a test tube. This extraction process was repeated several times for better result. Running the procedure in several times and all collected extracts in a conical flask. Wash the extracts by saturated aqueous sodium chloride (Minimum 15 milliliters) and remove all the aqueous washing. Again wash the extract by ten percent aqueous potassium carbonate and remove all the aqueous washing. The organic layer of the lycopene containing was dried by calcium chloride and evaporate the solvents. The absorbance value of 503 NM was used for the determination where hexane was use as blank and the results was calculated on the basis of the following equation.

$$\text{Lycopene content (mg/kg)} = \frac{A \times M \times S}{w \times m} - - - - - - (1)$$

Where,

A= Absorbance of 503 NM spectrum

M= Molecular weight of lycopene ($C_{40}H_{56}$, 537 g/mol)

S= The volume of mixed solvent (10 ml)

w= Sample weight (1 g)

m= the molar extraction co-efficient (172 mM^{-1})

D) N-hexane extraction method

Lu et al. (2008), Nancy et al. (2010), Ann and John (2011) described lycopene extraction from various fruits and vegetables and its byproducts using N – hexane. Lu et al. (2008) described that 3 g Sample was weighed in a centrifuge tube and 4-12 mL ethanol with butylated hydroxytoluene solution (0.1%, w/v) added. Vortex this tube thoroughly at level 8 and homogenized at level 7 for 120 second. Then saponified by 2 ml of KOH solution immersed in a water bath (60°C). 2 ml distilled water and 9 ml n-hexane wad added again vortex in level 8 and centrifuge for 10 minutes at refrigerated temperature (4°C). After that hexane layer is collected and lycopene determined.

Hydrostatic pressure processing for lycopene extraction

The efficiency of lycopene yield increases utilizing high hydrostatic pressure processing (HPP) without heating from waste materials such as tomato products wastes. Normally HPP methods has more efficiency to extract lycopene from solvents methods as well as less time consuming (Xi, 2006). Utilizing pressurized extraction equipment to the tomato by-products achieved high grade lycopene which is named “Extractor Naviglio”. Tap water is utilize in this extraction method with minimal organic solvent.

Enzymatic treatment for lycopene extraction

Utilizing enzymes such as pectinase and cellulose, we can extraet lycopene from different waste materials such as tomato waste from tomato product manufacturing (Choudhari and Ananthanarayan, 2007). Lycopene yield is increased up to twenty times higher than other method through using optimal enzyme concentration and process time (Kong et al., 2010).

Supercritical fluid extraction (SFE) with CO_2 for lycopene extraction

Previous studies represented, utilizing liquid carbon dioxide (CO_2) in the supercritical fluid extraction methods one of the best output for lycopene extraction from vegetables, fruits and various by-products from different industries (Choksi and Joshi, 2007). Normally liquid CO_2 is utilized as a solvents and mix with products with desired processing conditions such as heated or pressurized. The mixture matrix placed in the extractor cell. Controlling valve the extract separated after the extraction (Tzia and Liadakis, 2003). Due to several factors, Supercritical carbon dioxide is best solvent from among others to extract and separate pigments and compounds from foodstuffs. The factors are its economic, non-flammable, safe to handle and non-toxic (Rozzi and Singh, 2002).

Again CO_2 has less polarity and specific solvent-solute interactions can be achieved by adding little quantity of polar solvents. Hexane, water, methylene chloride or ethanol can be utilize to achieve better results to extract lycopene which is very sensitive on acids, lights, temperatures and oxygen (Shi et al., 2008; Shi et al., 2009). Shi et al. (2009) also observed that at pressure 200-400 bars and temperature at 50–80°C were the suitable condition for carotenoid extraction. Utilizing supercritical fluid extraction we can achieved highest yield form tomato skin (Vagi et al., 2007).

Ultrasonic extraction for lycopene extraction

We can utilize ultrasonic extraction method for separate lycopene from any organic source (Kumcuoglu et al., 2014). Utilizing this method we can achieve 75.93% for trans-lycopene in compare to other conventional extraction methods as well as we can minimize degradation and isomerization of lycopene (Ranveer, 2018; Eh and Teoh, 2012). This method is less time and energy consuming, economic for extraction solvents. Several steps on operation can be improved this method for lycopene extraction (Eh and Teoh, 2012).

Soxhlet extraction method for lycopene

One of the simple, famous and economic extraction methods is Soxhlet extraction. Utilizing this semi-continuous method we can extract the compounds or pigments which has low solubility in

any mixture mainly solid mixture by extraction of fats and oils from foodstuffs (Zhang et al., 2018). To extract lycopene we can utilize soxhlet methods due economic, simple, easily handled, solvent can be recycled for multiple extraction, easily operate the whole system under vacuum condition, temperature and condensation control system separate from the system and easily maintainable (Soxhlet, 1879). In soxlet extraction eight types of solvent can be used for lycopene extraction from plants and vegetables. Utilizing these excellent features we can easily extract lycopene from plant samples such as watermelon, tomato paste in controlled laboratory conditions (Kyun et al., 2013; Tambun et al., 2017). Dried product powders can be utilize to extract lycopene from plants and vegetables. Minimum five gram dried sample was placed in cellulose extraction thimbles and covered with wool and placed in the soxlet apparatus. After the placing, extraction was running and utilizing twenty hours and below two hundred milliliters of any kinds of solvents from the eight we can extract lycopene. After the extraction process the solvent evaporated by a vacuum rotating evaporator and extracted lycopene can be collect concentrate dried state and weighed (Haroon, 2014).

Purification of lycopene

Purification and stabilization of impure lycopene from natural source is an essential work after extraction. To utilize lycopene in cosmetics industry, food industry, pharmaceuticals processing and supplements, purification is essential. Nowadays, different methods of purification and stabilizations were introduced in manufacturing industries. Now-a-days lycopene purifications done by High-performance liquid chromatography (HPLC), Thin-layer chromatography (TLC) and solid phase extraction in lycopene purification (Aghel et al., 2011). Crystallization which is considered as very useful and economical, is another purifications methods which did not utilize solvents for isolation of pure lycopene (Grady et al., 2007). MARs which also named by macro porous adsorption resins another well-known methods to separate and purify organic and natural compounds and pigments from different plant parts such as flower, fruits, leaves, stems, roots and nodes (Liu et al., 2010; Sun et al., 2009). This methods well-known because it is easy to operate, economical, user friendly, high efficient rate, easier regeneration and zero environment waste (Liu et al., 2010; Sun et al., 2009). Various research examined, Sophisticated chromatographic equipment utilization to obtained pure lycopene by different chromatographic techniques such as liquid-liquid extractions (LLE), Solid-phase extraction (SPE), medium

pressure liquid chromatography (MPLC) and High-performance liquid chromatography (HPLC) but this sophisticated chromatographic equipments are not economical or not available in everywhere (Díaz et al., 2010; Schwarz 2003; Tzouganaki et al., 2002; Wybraniec et al., 2009).

Previous studies mentioned earlier, normally organic acids, carbohydrates and amino acids considered as non-phenolic substances extraction methods may not be selected for lycopene. Lycopene needs a noble separation and purification processes which are mandatory. Previous studies mentioned earlier utilization of HPLC with photodiode array (PDA) detectors or UV-VIS mostly utilized to separate lycopene though it is not economical (Choksi et al., 2007; Castanedaovando et al., 2009). Though many researcher tried to separate and purify lycopene from plant source such as vegetables and fruits, but very few industry utilize it in their manufacturing and the production scale is very poor because several factors are responsible for that. The factors are low efficacy rate for lycopene extraction and purifications, low market value and practical utilization and suitability for utilizations in cosmetic, pharmacuticals and food industry (Castanedaovando et al., 2009; Choksi et al., 2007).

APPLICATION

Considering lycopene benefits, it draws attention on utilizing it in different processing such as food processing, cosmetic manufacturing and pharmaceuticals use. Now-a-days synthetic lycopene is utilized as food additive such as antioxidant and coloring agent (Chatterjee, 2016). Lycopene would be used include breakfast cereals, baked goods, bottled water, spreads, carbonated beverages, soybean beverages, fruit and vegetable juices, candy, salad dressings, soups, various dairy product such as general products, analogues, frozen dairy desserts, and other foods and beverages (EFSA, 2006). Carotenoid's such as lycopene as an organic and natural foodstuff colorant which reducing the adverse properties of artificial dyeing agents. In pharmaceuticals lycopene can be utilized to manufacture various supplementary tablets and capsules such as vitamin and minerals supplements which can be vary from two milligram per liter to two hundred milligram per liter in bottled drinking water and ready-to-eat depends on human diet, age, body structure and intended use (Chatterjee, 2016). Normally lycopene utilized in food additives as low as possible such as two milligram of lycopene utilized in beverage industry (Chatterjee, 2016; Rath, 2009). Studies found according to food scientists, several

industry utilize by-products as food components which are lycopene-rich. Tomato and tomato by products such as dried tomato peel are mixed with dry fermented sausage during mixing operations in sausage production. Tomato puree and pomace mix with other ingredients during extrusion processing of snacks (Altan et al., 2008). For various food processing and pharmaceutical manufacturing companies utilize industrially manufactured lycopene from vegetables and fruits such as tomatoes (Berra, 2012).

Consumers recognize their desired taste and flavor by the color of the food product. Several indicators utilize as identification of food colorants quality, such as ensuring health benefits, reducing health hazards, original or natural appearance and uniformity of the foods (Giusti and Wrolstad, 2003). For this reason color is a most important issue for the food manufacturing factories. An alarming issue for manufacturer to keep the food products as natural. Various factors can be alter fruits, vegetables and their products color during harvesting, processing and storage such as cultivation time or season, cultivation site, soil compositions, fertilizer, postharvest handling, metal ions, light, oxygen, endogenous enzymes, microorganisms, humidity, temperature and heats Et cetera (Stintzing and Carle, 2004).

Nowadays, consumers are more concern and careful about their health and they reject synthetic colorants often used in the food manufacturing industry to make the food product attractive to the ultimate consumer. Because synthetic colorants are often harmful and carcinogenic for the consumer health. Therefore, these synthetic colorants are now being replaced with natural colorants. Many researches have been done on the extraction of lycopene from various sources to use lycopene as a natural food colorant that has positive health effects for the consumer. For example, since 1977 in China, purple corn and red rice is utilized as a food coloring agent for bread, soft drinks, ice cream and other liquors (Aoki et al., 2002; Sugiyama Chemical Institute, 1977; Yoshinaga, 1986).

Lycopene can be utilize as natural coloring agent almost any kinds of foods and beverages. Some novel food applications are added below:

1. Pharmaceutics (soft gel capsules)
2. Lycopene drink
3. Confectionery and ice cream

4. Lycopene based bread
5. Lycopene based cakes
6. Lycopene based jam, jelly, squash and marmalades etc. (Stahl et al., 2006)

CONCLUSION

Tumor cells and several cancers such as breast cancer and prostate cancer are prevented by the consumptions of lycopene by various fruits and vegetables. Previous studies and analysis concludes red colored fruit and vegetables are very good source of lycopene. It is also reported that different noble extraction and purification methods are available for lycopene from various natural source. But solvent extraction is easy and economical but supercritical fluid extraction by carbon di-oxide gives excellent outcome other then all methods. For human consumptions, utilizations, drives lycopene should be one of the important carotenoids because of its various antioxidant properties and nutraceutical, epidemiological, and pharmaceutical importance.

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