

Extraction of Natural Food Color from Tomato, Carrot and Turmeric

Abstract

There is a growing attention in natural colorants as an alternative of synthetic colorants which have many biological activities and antioxidant potential on consumer health. The present study deals with the assessment of extraction process, effects of solvent, precipitation, identification, purification and determination of lycopene, carotene and curcumin content from tomato, carrot and turmeric. In this study, lycopene, carotene was extracted and purified using solvent extraction method and curcumin was extracted and purified using alkalization method at refrigerated temperature (4°C) and room temperature (30°C) for three weeks from tomato, carrot and turmeric. The purpose of this study was to develop a simple and effective method using conventional solvent extraction and anti-solvent precipitation for the isolation of natural colorant. Lycopene content extracted from tomato was obtained in the range between 0.0153 to 0.0362 mg/100g. Obtained carotene content followed by solvent extraction method in the range between 61.43 to 81.72 mg/100g. Curcumin content obtained by alkalization method was 91.17 to 110.41 mg/100g. Higher amount of lycopene (red) and carotene (orange) precipitation obtained in ethyl acetate extraction process compare to the anti-solvent method. The lycopene and carotene precipitation method developed in this study was more effective than the previous reported methods for the large-scale preparative precipitation of lycopene and carotene by anti-solvent method. Maximum amount of curcumin precipitation obtained in lower pH solution. Lower pH is important to maintain the stability of curcumin precipitation. Experience of this research work suggested that lycopene from tomato, carotene from carrot and curcumin from turmeric might be an excellent source to meet up the increasing need of natural colorants.

Keywords: Natural Color, Lycopene, Carotene, Curcumin, Tomato, Carrot and Turmeric

1. Introduction

One of the most important organoleptic properties of food products is color since it provides information to the consumer about the acceptability, composition, flavor, and freshness of the food (Sen et al., 2023; Singh et al., 2023 and Vega et al., 2023). According to the Food and

Drug Administration (FDA), a food colorant is “any dye, pigment, or substance which when added or applied to a food, drug or cosmetic, or the human body, is capable (alone or through reactions with other substances) of imparting color”(FDA 2022). Food coloring can be made with a wide variety of colorants that are available on the market. People's initial perception of food is shaped by its color. Food manufacturing companies investigated this as a vital marketing strategy. Food items may contain colorants to enhance their natural color or give color to products that are colorless, or to restore the original color that was lost as a result of exposure to light, temperature changes, moisture, and/or storage conditions (EFSA 2022).

When artificial colors are introduced to food goods, they can have a very appealing and dazzling impact, but they also frequently have distinct teratogenic and carcinogenic effects. Artificial food coloring may come from mineral compounds, petrochemicals, petroleum, and coal tar, all of which increase the risk of developing numerous dangerous conditions like brain tumors and attention deficit hyperactivity disorder (ADHD). As a result, the market for natural colors is growing twice as quickly as that for synthetic ones. There has been a noticeable shift in the previous ten to fifteen years toward natural ingredients, particularly in terms of flavors and colors (Bora et al.,2019 andNtrallou et al., 2020). Natural food coloring is inexpensive, simple to prepare, and doesn't give food an odd taste. Not only does using natural coloring enhance the food's appearance, but it also boosts its nutritious content. Additionally, some food colorings have pharmacological properties that include antioxidant, anti-tumor, anticonvulsant, nerve protection, anti-depressant, and memory enhancement. As a result, in order to replace artificial colorings in food coloring, many nations have started extracting and using natural ingredients (Durazzo et al., 2022).

A broad class of yellow-orange plant pigments known as carotenoids has several uses in food. The primary pigments found in plants are called carotenoids. Carotenoids are mostly found in fruits and vegetables in the diet of humans. Carrots, oranges, peppers, and green vegetables are rich in pigments called α - and β -carotene, which range in color from yellow to red. The primary pigment in tomatoes is called lycopene, or brilliant red, which also gives watermelon, papaya, and guava flesh its color (González-Peña et al., 2023). Strong antioxidants, carotenoids also offer anti-inflammatory, anticancer, and antiaging qualities that have been shown in pertinent studies (Pérez-Gálvez et al., 2020; Tanet al., 2019). Lycopene is one intriguing carotenoid pigment. Only 50–65% of the lycopene that is consumed is thought to originate directly from fruits and vegetables. Additionally, colored food products—primarily flavored milk, candies, and soft drinks—are a source of lycopene. Like other carotenoids, lycopene is well-known for having antioxidant qualities(Castro et al.,2023; Imran et al., 2020; Bin-Jumah et al 2022 and Khan et al.,2021).Curcumin, which is present in turmeric and has many health advantages, is why people respect this perennial plant in addition to the flavor and color it brings to food. Because it can

decrease mitochondrial oxidative stress by enhancing the actions of glutathione, catalase, and superoxide dismutase, curcumin is among these that are recognized as potent antioxidants. As an anti-inflammatory, modulator of several neurotransmitters, and inhibitor of monoamine oxidase enzymes, it also relieves depression. By raising insulin resistance and lowering leptin, resistin, and insulin levels, curcumin is an effective treatment for diabetes (Marton et al 2021). Furthermore, studies conducted recently have shown that curcumin has strong antimicrobial qualities and may have anticancer potential (Hussain et al. 2022 and Ciuca and Racovita 2023).

Scientists are becoming more and more interested in the studies on the extraction of natural food colorings these days because it is becoming harder to monitor the source of food and because natural products are generally used worldwide. The difficulty lies in the fact that synthetic colorants, when applied in realistic conditions like temperature, light, pH, and oxygen, are more stable than natural colorants. As such, research on the extraction of natural food coloring has generated a great deal of interest. In Bangladesh tomato, carrot and turmeric are available. Therefore,

extraction of natural colors (lycopene, carotene and curcumin) from those fruits, vegetables and spices can be more profitable and help to reduce use of synthetic color in food production. Therefore, the goals of this study were to evaluate the effects of various solvents on the extraction processes of lycopene, carotene, and curcumin from tomatoes, carrots, and turmeric, respectively, and to design the most effective extraction method.

2. Methods and materials

2.1 Sample collections and preparation

The fresh tomato (*Solanum lycopersicum* L.), Carrot (*Daucus carota* L.) and turmeric (*Curcuma longa* L.) were purchased from the local market of Mymensingh Bangladesh. Samples were washed with water to remove adherences, dirt and other surface impurities. After that it was cut into small pieces with stainless steel knife then pulp was made using blender (PHILIPS HR1823, China) and then stored at 4°C until use for experiment.

2.2 Chemicals and reagents

Ethanol, methanol, acetone, n-hexane, chloroform, ethyl acetate, diluted HCL, NaOH, silica gel (TLC grade), benzene, iodine, butylated hydroxytoluene (BHT), calcium sulphate hemihydrates were used in the study.

2.3 Preparation of TLC plates

Simple TLC plates were prepared using TLC glass slides. A mixture of silica gel (finely powdered) and calcium sulphate hemihydrates were mixed together and double the amount of water was added so as to form a paste. This paste was evenly distributed over the surface of the slide and kept aside to dry. Prior to usage, slides were activated by baking in the oven at 120°C for 30-40 min.

2.4 Extraction process

2.4.1 Extraction of lycopene (red color)

Fresh Tomato was thoroughly washed with potable water and the fruits were then chopped into pieces. The chopped tomato was ground in a grinder and remove water from the pulp by hand pressing and drying at 60^o C for 6 hrs. Then ethyl acetate added to the pulp and stirred the sample up to 1 hr. The lycopene extracts thus obtained was filtrated through a muslin cloth to remove coarse particles. After that filtrated extract was transferred into a separating funnel. The mixture was shaken vigorously and kept aside for the layers to separate. Two layers were separated, one was aqueous layer and other was ethyl acetate layer with lycopene. The lower layer containing ethyl acetate with lycopene was collected in a conical flask and the upper layer was discarded in a beaker. Solvents and other reagents were analytical grade. Lycopene Extraction and purification method (a), extracted and purified sample (b) shown in Figure 1.



(a)

(b)

Figure 1: Lycopene extraction and purification method (a), extracted and purified sample (b)

2.4.2 Extraction of carotene (orange color)

Carrot roots were washed with water and roots were cut into small pieces. Then the pieces were ground in a grinder and remove water from the pulp by hand pressing and drying at 60^o C for 6 hrs. Solvents and other reagents are saved for use. After that ethyl acetate added to the pulp and stirred for 1 hr. The solvent containing carotene was filtered through a muslin cloth to remove coarse particles then transferred into a separating funnel. The mixture was shaken vigorously and kept aside for the layers to separate. The lower layer was an aqueous layer which was discarded from the separating funnel and collected in a beaker. The upper layer contained ethyl acetate with carotene and it was collected separately after the removal of the aqueous layer. The extract was collected in a conical flask. Carotene extraction and purification method (a), extracted and purified sample (b) shown in Figure 2.



(a)



(b)

Figure 2: Carotene extraction and purification method (a), extracted and purified sample (b)

2.4.3 Extraction of curcumin (yellow color)

Turmeric sample of proper morphotype (Fulbaria) was collected from local market. Turmeric was sun dried and torn into pieces and then was extracted for curcumin by Alkalization method (the act of making a substance alkaline, as through the addition of a base NaOH). The extracted curcumin was precipitated at low pH (diluted HCL) as soon as possible by proper instrumental arrangements. The curcumin precipitate was coagulated and filtered under vacuum. The curcumin powder thus obtained

was treated by proper solvent extraction method (n-hexane) for removal of gum, oil, resin etc. the final step is drying the extract and grinding to obtain powder form. Curcumin extraction and purification method (a), extracted and purified sample (b) shown in Figure 3



(a)



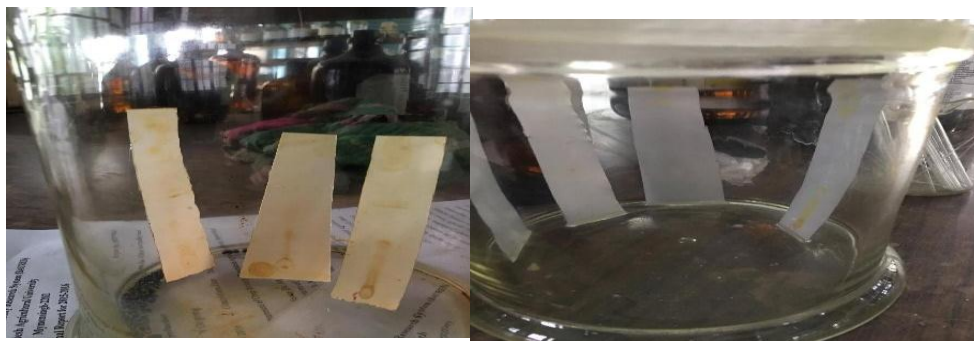
(b)

Figure 3: Curcumin extraction and purification method (a), extracted and purified sample (b)

2.5 Purity of lycopene, carotene and curcumin confirmed by thin layer chromatography (TLC)

TLC was done according to the method reported by Edwards et al. (2003) with slight modification. 50 g of benzene was added into a conical flask. Then solution was vigorously stirred with glass rod to avoid lumps. A TLC glass plate was sunk in conical flask for few seconds and removed to allow dry. Samples were spotted using capillary tube. The plate was removed from the tank and allowed to dry and placed in iodine tank. The sample was

viewed under light. Lycopene, carotene and curcumin purity was calculated from retention values (R_f) which was values were similar to reference value. Samples in solution chamber (a) and samples in iodine chamber (b) showed in Figure 4.



(a)

(b)

Figure 4: Samples in solution chamber (a) and samples in iodine chamber (b)

2.6 R_f value

The R_f value is defined as the ratio of the distance moved by the solute (i.e., the dye or pigment under test) and the distance moved by the solvent (known as the solvent front) along the paper, where both distances are measured from the common origin.

$$R_f \text{ Value} = \frac{\text{Distance travelled by Solute}}{\text{Distance travelled by Solvent (Solvent Front)}}$$

Due to the fact that the solvent front is always larger from the distance travelled by the solute. R_f values are always extreme between 0 – 1 where solute remains fixed at its origin. R_f values do not have units since it is a ratio of distances.

2.7 Determination of lycopene

The lycopene content in tomato was determined according to a slight modified method described by [Ali et al., \(2013\)](#). About 0.6 ml of extracted sample was taken in a falcon tube. Then 6 ml of pure acetone containing 0.05% of butylated hydroxyl toluene (BHT), 5 ml of 95% ethanol and 10 ml of hexane were added to it. After that falcon tube were vortexed for 10 min. After that, 3 mL of distilled water was added and shake again for 5 min. Finally, the mixture was left for phase separation. The absorbance of upper

hexane layer was monitored at 503 nm using a UV-VIS spectrophotometer (T60U, PG instrument, United Kingdom) with hexane as the blank. The amount of lycopene was calculated using the following equation:

$$\text{Lycopene} = \frac{X \times A \times 3.12}{y}$$

Where,

X is the number of hexanes (ml), y the weight of sample (g), A 503 the absorbance at 503 nm and 3.12 is the extinction coefficient.

2.8 Determination of carotene

Carvalho et al. (2014) method was used with slight modifications to determine carotene content. Roots were cut into slices. The extraction yield of carotenoids was observed at 60°C using ethanol (96%, Reagent, Slovak Republic) and 2-propanol. Initially, 25 g of cut carrot samples were added to 100 g of 96% ethanol. Carrot slices were extracted in water bath, shaken after every 10 min, and after every hour of extraction 5 ml sample was taken and mixed with n-hexane (20 ml). Water was added for the separation of phases, and after the separation the hexane-carotenoid phase was made up to the volume of 50 ml. The content of β -carotene in the hexane (Reagent, Slovak Republic) extract was determined spectrophotometrically, the absorbency was measured at the wavelength of 454 nm using a UV-VIS spectrophotometer (T60U, PG instrument, United Kingdom (Jenway, Great Britain)). The concentration of β -carotene expressed as β -carotene (mg/100 ml) was calculated using the response factors as follows:

$$\beta\text{-carotene} = \frac{A \times d \times V}{E1\% \times W}$$

Where: A – absorbance, d – dilution, E1% – coefficient of absorbance, w – the weight of the sample (g), V – volume (ml)

2.9 Determination of curcumin

The curcumin content in turmeric was determined according to a slight modified method described by Jayaprakasha et al., (2002). In conventional apparatus, turmeric powder was accurately weighed and extracted with ethanol for 24 h. The curcumin content of the extract was determined using UV-Vis spectroscopy. Batch extraction was carried out for all extraction experiments. The stirring speed was set at the appropriate level, in order to ensure the suspension of solid in the liquid phase and a good contact between them. In a closed flask 2 g of turmeric powder was carefully weighed and contacted under stirring with different volumes of ethanol and n-hexane. The sample was

filtered in a 100-ml measuring flask. The volume was made up to 100 ml. 2 ml of this was taken and made up to 50 ml. Extractions were performed in the temperature range of 25–60°C. All experiments were conducted in order to find out the extraction conditions which correspond to a maximum curcumin content of the extracts. Turmeric oleoresins commonly contain 30–45% curcuminoid pigments. Consequently, the curcumin content in the extracts was used to evaluate the extraction efficiency. Curcumin was determined by measuring the absorbance of an ethanol curcumin solution, at fixed wavelength at 421 nm using a UV-VIS spectrophotometer (T60U, PG instrument, United Kingdom, Jenway, Great Britain).

2.10 Storage conditions

Isolated and purified lycopene and carotene were kept in amber bottles at ambient temperature ($30 \pm 2^\circ\text{C}$) and refrigerated temperature (4°C) for the determination of lycopene carotene content and color. Curcumin powder was preserved in an amber bottle at room temperature.

2.11 Statistical analysis

Each experiment included two replications. Data were analyzed using statistical software R (windows version 2.13.1). A single-factor analysis of variance was carried out. Significant differences were estimated using Duncan Multiple Range Tests (DMRT). Differences were considered to be significant at $P \leq 0.05$.

3. Result and Discussion

3.1 Effects of solvent, extraction time, extraction and purification methods, and temperature on lycopene content

Utilizing various selective solvents, lycopene was extracted from tomatoes. The rate of extraction was very high when lycopene was extracted from tomatoes using chloroform. Following extraction, the lycopene solution dissolves in n-hexane, and methanol is added as an anti-solvent to the extracted solution, causing lycopene to precipitate. The precipitate was then filtered out of the mixture. Lycopene was present in extremely minimal quantities. Lycopene was extracted from tomatoes using anti-solvent precipitation and standard solvent extraction. Various organic solvents, including dichloromethane, hexane, ethanol, acetone, chloroform, ethyl acetate, petroleum ether, and mixtures of polar or nonpolar solvents in varying ratios, like acetone-chloroform (1: 2) and hexane-acetone-ethanol (2: 1: 1), have been tested for lycopene extraction because lycopene is lipid-soluble (fat soluble) (Barba *et al.*, 2006). The antisolvent precipitation procedure was used to further isolate lycopene. Lycopene can dissolve in methanol, which is used as an extraction solvent. Ethyl acetate was chosen as an anti-solvent concerning the

solubility of lycopene. The crystalline solid form of lycopene that was not dissolved in methanol and ethyl acetate was recovered using precipitation (Vasapollo et al., 2004). We decided to use ethyl acetate as the extraction solution in our lab. A few minutes later, precipitation was seen in the solution, and it was more abundant than when anti-solubilizer methanol was used. These findings suggested that precipitating lycopene using ethyl acetate is considerably more effective than using methanol as an anti-solvent.

The total lycopene content of various tomatoes during storage is displayed in Table 1. Total lycopene concentration, after extraction and purification, varied between 0.0153 and 0.0331 mg/100g at storage temperature and 0.0171 to 0.0362 mg/100g at ambient temperature. The study's quantification results aligned with the total lycopene concentration values, which range from 0.0112- 0.0484 mg/100g in tomato puree. All samples showed significantly higher levels of lycopene over the storage period when kept at room temperature as compared to refrigeration.

Although, there were no significant differences in lycopene content between zero days at refrigerated temperature and room temperature. However, significant differences were found in lycopene content between 7th, 14th, and 21th days at room temperature and refrigerated temperature. Degradation of lycopene at refrigerated temperature was induced by higher moisture content. Higher moisture content was found at refrigerated temperature than at room temperature. Lycopene loss was observed after 7th days of storage at 4°C. Lycopene degradation might be influenced during acidic conditions and at all conditions might be due to oxidation which depends on temperature and moisture (Singh et al., 2020; Tola & Ramaswamy, 2013). The production of fragments such as acetone, methyl-heptenone, laevulinic aldehyde, and glyoxal may be another way that low temperatures quench lycopene (Kaur et al., 2006). In a study (Gil et al., 2006), watermelon that had undergone minimum processing was kept at 5°C for up to 9 days, during which time the amount of lycopene decreased slightly. When tomato paste, pulp, and puree were tested once more over three months at 25°C, 30°C, and 40°C, the researchers found that the lycopene level of every sample remained constant (Pathak et al., 2023). Furthermore, it is thought that several variables, including both abiotic and biotic components, cultivation region, climate, cultivar type, harvest time, fruit maturation stage, and extraction technique, affect the phytochemicals (Halim et al., 2022 and Rahman et al., 2024).

To identify the precipitated lycopene, TLC was performed. Judging from a previous report (Britton, 2008), the singlered spot on TLC can be identified as lycopene, as the precipitated and authentic lycopene have the same R_f value. R_f value confirmed the purity of lycopene content. R_f value obtained from this experiment was 0.65 (Figure 5) which was similar to the results (red R_f=0.63) reported by Myong-Kyun et al. (2013).

The conventional solvent extraction and anti-solvent processes are feasible methods to rapidly extract lycopene from tomato. When using anti-solvent method to precipitate lycopene, various solvents used to extract lycopene from tomato. But in conventional solvent extraction method, only ethyl acetate used to extract and precipitated lycopene. Ethyl acetate is the greatest option financially because it is far less expensive than other solvents. Using chloroform, n-hexane and methanol are costly than using single solvent ethyl acetate. When using ethyl acetate, less time required to precipitate lycopene. The use of anti-solvent methanol method the extraction is not satisfactory in terms of profitability. To achieve the highest solubility of lycopene the choice of ethyl acetate as the solvent was the most reasonable.



Figure 5: TLC plates of carotene developed in benzene solution (a), and iodine chamber (b)

Table 1: Total lycopene content (mg/100g) of tomato obtained of various sample by solvent extracted and purified methods at different Extraction Periods

Temperature	Samples	Extraction Interval (Days)			
		0 th	7 th	14 th	21 th
4° C	L1	^C 0.0185±1.32 _c	^D 0.0153±2.02 ^d	^C 0.0287±3.29 ^a	^D 0.0252±3.14 ^b
	L2	^B 0.0298±3.42 _b	^C 0.0183±1.30 ^c	^C 0.0286±3.42 ^b	^B 0.0331±3.42 ^a
	L3	^A 0.0325±2.36 _b	^B 0.0251±3.42 ^d	^A 0.0351±3.47 ^a	^C 0.0285±5.78 ^c
	L1	^C 0.0185±1.74	^C 0.0171±1.47 ^b	^D 0.0250±1.32 ^a	^D 0.0258±2.56 ^a

30° C

L2	^b 0.0298±1.32	^B 0.0257±1.69 ^c	^B 0.0314±3.29 ^a	^C 0.0287±1.45 ^b
L3	^A 0.0325±5.78	^A 0.0294±3.42 ^c	^C 0.0280±3.42 ^d	^A 0.0362±3.42 ^a

Here, values in parentheses represent the percentage of retention as compared to 0-day starting to tomato lycopen.

^{a-d} means followed by different superscript alphabets in each row are significantly different ($P \leq 0.05$) among extraction interval.

^{A-D} means followed by different superscript alphabets in each column are significantly different ($P \leq 0.05$) among different temperature.

3.2

Effects of solvent, extraction time, extraction and purification methods, and temperature on carotene content

Carotenes are a type of secondary metabolite found in plants that have a low polarity. Consequently, using a non-polar solvent is the most effective technique to extract carotenoids. Because the plant matrix is polar, it is necessary to take into account the presence of water in the plant or sample in order to extract the carotenes. Water should not have been present in the plant sample when the carotenoid extraction process took place (Rasdiana and Syukri, 2022). Carotenoids are insoluble in water and soluble in polar solvents, including edible fats and oils. Because carotenoids are lipophilic, they are usually extracted from the plant sources with organic solvents such as chloroform, hexane, acetone, petroleum ether (Regal et al., 2019; Cheng et al., 2019; Shankar et al., 2022; Varaprasad et al., 2019, and Valcareggi Morcelli, et al., 2022). The samples can contain large amounts of water; water-miscible organic solvents such as ethyl acetate are also used. One of the problems is the elimination of the residual

solvents to obtain a safe extract; this can be avoided by using food grade solvents such as ethyl acetate, ethanol (Mezzomo and Ferrerira 2016, Calvo et al., 2007). The extraction procedure using solvents proved to be highly efficient. Ethanol also used to extract carotene from carrots which is also food grade solvent. Ethanol is environmentally friendly and thereby often healthier (at least at low concentration) to human choices of solvents (Capello, 2007) but exhibit poor solubility (Huaman-Castilla et al., 2019) When using ethanol as an extracted solvent, heating is helpful to increase the extraction rate. Heating the extract at 60°C for few minutes and then filtrated the extract through a muslin cloth to remove the coarse particles. After few hours later precipitated was observed in the

solution. But the amount of the precipitation was very low and unstable to the solution. Ethyl acetate was chosen as the extractive solution in our laboratory conditions. Precipitation observed in the solution within a few minutes and the amount of precipitation was higher compared to ethanol extraction. Carotene precipitation observed in this process was more stable.

Table 2 showed the total carotene content of different carrot samples during different storage periods. The extracted and purified total carotene contents ranged from 61.45 to 76.04 mg/g at 4°C and 62.07 to 81.72 mg/g at 30°C. The study's quantification results aligned with the range of values for the total carotene content in carrot puree, which is between 61 and 180 mg/100 g (Ikram et al., 2024). The amount of beta-carotene was determined both during and after various periods of room temperature storage. On the other hand, there was no discernible difference in the carotene concentration between zero days at room temperature and chilled temperature. However, there was a noticeable difference in the amounts of carotene at room temperature and chilled temperature on the 7th, 14th, and 21st days. The lowest value at cooled temperature on the 21st day has been obtained. Oxidation is the primary cause of the breakdown of carotenoids in diet. The complicated oxidation process in food that has been processed is influenced by a variety of variables, including lipids, temperature, pro-oxidants, and antioxidants (Singh et al., 2013). The best carotenes were found at 60°C, the heat treatment according to such as blanching, cooking, and steaming help to release the carotenoids bound by protein and render them easily extractable (González-Peña et al., 2023). Hence, the carotene content increased from 61.43 mg/100 g to 81.72 mg/100 g at 30°C in 21th day (Table 2).

Identification and purification using thin layer chromatography was very effective for carotene. The extract was identified using Thin Layer Chromatography method and the retardation factor was calculated. The TLC plate on which a spot of the extract was placed and kept in a developing chamber to separate into different bands is shown in Figure 6 and the developed TLC plate with the orange spot represents carotene. Studies state that absorbance change in accordance to the solvent used, pigment concentration, experiment results and calculation. R_f value obtained from this experiment was 0.67 which was similar to the result (orange R_f = 0.6) reported by Myong-Kyun et al. (2013). In terms of time the best choice ethyl acetate, considerably less time required than other solvents. In this study, the use of ethanol is not good enough. The use of ethanol in the extraction is not satisfactory in terms of profitability. To achieve the highest solubility of β-carotene the choice of ethyl acetate as the solvent was the most reasonable.



Figure 6: TLC plates of carotene developed in benzene solution (a), and iodine chamber (b)

Table 2: Total carotene content (mg/100g) of carrot obtained of various sample by solvent extracted and purified methods at different Extraction Periods

Temperature	Sample	Extraction Interval (Days)			
		0 th	7 th	12 th	21 th
4°C	C1	^C 68.91±5.00 ^a	^E 64.29±6.39 ^b	^D 65.71±5.69 ^b	^F 61.43±6.08 ^c
	C2	^B 72.12±5.42 ^b	^D 66.94±1.32 ^c	^C 73.53±7.10 ^b	^B 76.04±5.78 ^a
	C3	^A 80.13±1.32 ^b	^A 78.32±5.78 ^c	^A 80.90±5.00 ^a	^C 73.61±6.90 ^d
30°C	C1	^C 68.91±5.78 ^a	^E 64.44±6.08 ^b	^E 62.85±6.44 ^c	^E 62.07±6.08 ^c
	C2	^B 72.12±4.71 ^b	^C 69.42±6.39 ^c	^B 76.34±5.78 ^a	^D 70.27±5.47 ^c
	C3	^A 80.13±5.00 ^a	^B 75.05±5.00 ^b	^C 73.63±6.21 ^c	^A 81.72±6.40 ^a

Here, values in parentheses represent the percentage of retention as compared to 0-day starting carrot Carotene. a-d means followed by different superscript alphabets in each row are significantly different ($P \leq 0.05$) among extraction interval. A-D means followed by different superscript alphabets in each column are significantly different ($P \leq 0.05$) among different temperature.

3.3

Effects of solvent, extraction time, extraction and purification methods, and temperature on curcumin content

As alkaline pH conditions replace acidic pH conditions, curcumin becomes more soluble, which improves its diffusion under weakly alkaline pH conditions. Remarkably, curcumin decreases bioavailability and stability at alkaline pH levels despite being more soluble in this environment (Le- Tan et al., 2022). Through these experiments, an appropriate technique was developed to yield greater concentrations of curcumin. Using an n-hexane solvent treatment, the hazardous gum, oil, and resin found in curcumin were eliminated.

According to literature reviews on the subject, numerous approaches can be used to extract

curcuminoids from turmeric. Extractions must be performed using nonpolar solvents since curcumin is not soluble in water. Previous investigations (Shirsath et al., 2021; Insuan et al., 2022; Widmann et al., 2022; Fernandez-Marin et al., 2022, Lateh, 2019 and Chandra et al., 2022) they have extracted the curcumin analogs using hexane, acetone, ethylene dichloride, and other alcohols.

Table 3 depicts the total curcumin concentration of various samples of turmeric at varying storage times. At 4°C and 30°C, the extracted and purified total curcumin concentrations varied from 91.17 to 109.54 mg/g and 92.75 to 110.41 mg/g, respectively. According to the quantitative outcomes of this study, the range of values for the total curcumin content of turmeric is 62–90 mg/100 g and 90–136 mg/100 g (Meng F-C et al., 2018 and Thangavel and Dhivya, 2019). However, the differences between the results of the present study could be the consequence of variations in the types of the extraction method utilized in each study, or the solvent employed.

At room temperature and throughout different storage times, the curcumin concentration was recorded. However, there were no appreciable variations in the amount of curcumin between ambient temperature and zero day at refrigeration. Significant variations in curcumin concentration were observed at the 7th, 14th, and 21st days at room temperature and refrigerated temperature. Due to a higher moisture content in the storage temperature, a lower amount of curcumin was discovered in 4° C on the seventh day. Lower pH and higher moisture content caused curcumin to degrade at chilled temperatures. According to Bekle and Ramaswary (2013), curcumin degradation may be affected by acidic conditions. Significant variations in turmeric's curcumin content were observed at different pH levels (Bekele and Ramaswary, 2013).

The results of the thin layer chromatography experiment indicated that the curcumin that was extracted, separated, and purified was extremely pure. A picture of purified curcumin illuminated by light reveals yellow dots on TLC (benzene) (Figure 7). The compound's R_f value of 0.71 was comparable to our reference value of 0.74, as reported by Myong-Kyun et al. (2013). Table 3 shows that 30°C room temperature and lower pH levels yielded the highest amount of curcumin. The amount of curcumin rose from 91.17 to 110.41 mg/gm between 4° C and 30° C.



(a)

(b)

Figure 7: TLC plates of curcumin developed in benzene solution (a), and iodine chamber (b)**Table 3:** Total curcumin content (mg/100g) of turmeric obtained of various sample extracted by alkalization method at different Extraction Periods

Temperature	Samples	Extraction Interval (Days)			
		0 th	7 th	12 th	21 th
4°C	Cu1	^C 96.39±1.32 _a	^D 93.61±6.08 ^b	^E 94.95±7.87 ^{ab}	^C 91.17±5.00 ^c
	Cu2	^B 102.39±9.8 _{7^{ab}}	^C 98.5±7.45 ^c	^C 103.21±7.45 ^a	^B 101.76±6.87 _b
	Cu3	^A 108.63±7.4 _{5^b}	^B 103.33±6.08 ^d	^A 109.54±9.87 ^a	^A 107.47±5.00 _c
30°C	Cu1	^C 96.39±5.78 _a	^D 94.01±1.32 ^b	^F 92.83±5.78 ^d	^C 92.75±6.08 ^c
	Cu2	^B 102.39±5.0 _{0^{bc}}	^B 103.95±7.45 ^b	^B 105.32±9.87 ^a	^B 100.76±7.45 _c
	Cu3	^A 108.63±6.0 _{8^b}	^A 105.06±9.87 ^c	^D 102.53±6.08 ^d	^A 110.41±9.87 _a

Here, values in parentheses represent the percentage of retention as compared to 0-day starting turmeric curcumin. a-d means followed by different superscript alphabets in each row are significantly different ($P \leq 0.05$) among extraction interval. A-F means followed by different superscript alphabets in each column are significantly different ($P \leq 0.05$) among different temperature.

Conclusion

In the present study, solvent extraction method was used to extract and purified of lycopene from tomato, carotene from carrot and curcumin from turmeric. This study also provided necessary information regarding the lycopene, carotene and curcumin content under refrigerated temperature and room temperature during storage. The result showed that higher lycopene degradation was found in refrigerated temperature as compared to ambient temperature throughout storage periods. This research shows that,

curcumin was fruitfully extracted as well as the effect of various conditions (pH, extraction media and purification) from turmeric. However, extraction of lycopene and carotene were not extracted as much as curcumin. Thin layer chromatography method helped to identify lycopene, carotene and curcumin color. The purity of these pigments was also confirmed by the R_f values, which was obtained by thin layer chromatography method. Therefore, our study revealed that solvent extraction method could be employed to extract lycopene and carotene from tomato, carrot and alkalization method could be employed to extract curcumin from turmeric might be great impact on socioeconomic in terms of natural colorant. It could be concluded that this study would hold the potential use of lycopene, carotene and curcumin as a source of natural colorants for the industry to avoid the carcinogenic effect of synthetic colorants.

Data Availability

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Consent to participate

All authors have expressed their authorization to engage in this publication.

Ethical Statement

This is to inform you that in this study, we have not been involved in any animal and human studies.

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