

Extraction of Natural Food Color from Tomato, Carrot and Turmeric

ABSTRACT

The demand for natural and healthier food products has increased dramatically in recent years due to growing consumer awareness of the impact food has on health as well as evidence of adverse effects from various ingredients, including some additives. This study evaluates the extraction method, solvent effects, precipitation, identification, purification, and lycopene, carotene, and curcumin content from turmeric, carrot, and tomato. Along-side, tomato, carrot, and turmeric samples were subjected to two different extraction and purification processes: solvent extraction for lycopene and carotene, and alkalization for curcumin. The samples were extracted and purified at room temperature (30°C) and chilled (4°C) for a period of three weeks. Tomato extracts had lycopene contents ranging from 0.0153 to 0.0362 mg/100g. Carrot extracts had carotene contents ranging from 61.43 to 81.72 mg/100g. The alkalization process produced a curcumin concentration of 91.17 to 110.41 mg/100g. Comparing the ethyl acetate extraction technique to the anti-solvent method, a greater amount of lycopene (red) and carotene (orange) precipitation was obtained. The maximum amount of curcumin precipitation was obtained in lower pH solution. Lower pH is important to maintain the stability of curcumin precipitation. The experience of this research work suggested that lycopene from tomatoes, carotene from carrots, and curcumin from turmeric might be an excellent source to meet the increasing need for natural colorants.

Keywords: Natural color; lycopene; carotene; curcumin; tomato; carrot; turmeric; food colorin; food color.

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) [1]. 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" [2]. 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, give color to colorless products, or restore the original color that was lost as a result of exposure to light, temperature changes, moisture, and/or storage conditions [3].

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 [4,5].

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, to replace artificial colorings in food coloring, many nations have started extracting and using natural ingredients [6].

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 [7]. Strong antioxidants and carotenoids also offer anti-inflammatory, anticancer, and antiaging qualities that have been shown in pertinent studies [8,9]. 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); [10-12]. 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 those that are recognized as potent antioxidants. As an anti-inflammatory, a modulator of several neurotransmitters, and an inhibitor of monoamine oxidase enzymes, it also relieves depression. By raising insulin resistance and lowering leptin, resistin, and insulin levels, curcumin is an effective diabetes treatment [13]. Furthermore, studies conducted recently have shown that curcumin has strong antimicrobial qualities and may have anticancer potential [14,15].

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 tomatoes, carrots, and turmeric are available. Extraction of natural colors (lycopene, carotene, and curcumin) from fruits, vegetables, and spices can be more profitable and help to reduce the use of synthetic colors 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 a stainless-steel knife then pulp was made using a blender (PHILIPS HR1823, China) and then stored at 4°C until used for the 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 sulfate 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 sulfate hemihydrates were mixed and double the amount of water was added to form a paste. This paste was evenly distributed over the surface of the slide and kept aside to dry. Before 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 water from the pulp by hand pressed and dried at 60° C for 6 hrs. Then ethyl acetate added to the pulp and stirred the sample up to 1 hr. The lycopene extracts thus obtained were 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 an aqueous layer and the other was an 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 grades. Lycopene Extraction and purification method (a), extracted and purified sample (b) shown in Fig. 1.

2.4.2 Extraction of carotene (orange color)

Carrot roots were washed with water and roots were cut into small pieces. Then the pieces are ground in a grinder and water from the pulp by hand pressing and drying at 60° C for 6 hrs. Solvents and other reagents are saved for use. After that ethyl acetate is added to the pulp and stirred for 1 hr. The solvent containing carotene was filtered through a muslin cloth to remove coarse and 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 acetated 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 Fig. 2.

2.4.3 Extraction of curcumin (yellow color)

A Turmeric sample of proper morphotype was collected from the local market (Fulbaria, Mymensingh). Turmeric was sun-dried and torn into pieces and then extracted for curcumin by the 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 a 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 Fig. 3.



Fig. 1. Lycopene extraction and purification method (a), extracted and purified sample (b)



(a)



(b)

Fig. 2. Carotene extraction and purification method (a), extracted and purified sample (b)

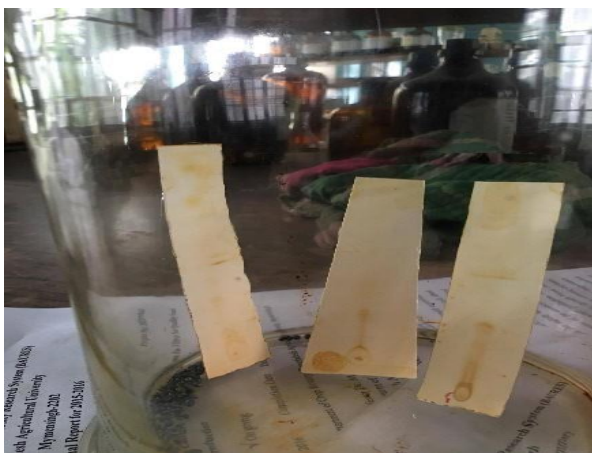


(a)

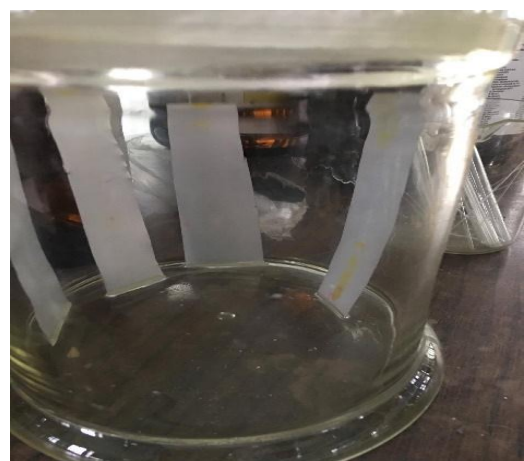


(b)

Fig. 3. Curcumin extraction and purification method (a), extracted and purified sample (b)



(a)



(b)

Fig. 4. Samples in solution chamber (a) and samples in iodine chamber (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. [16] with slight modifications. 50 g of benzene was added into a conical flask. Then the solution was vigorously stirred with a glass rod to avoid lumps. A TLC glass plate was sunk in a conical flask for a few seconds and removed to allow dry. Samples were spotted using a capillary tube. The plate was removed from the tank allowed to dry and placed in an iodine tank. The sample was viewed under light. Lycopene, carotene, and curcumin purity was calculated from retention values (Rf) which was values were similar to reference values. Samples in solution chamber (a) and samples in iodine chamber (b) are shown in Fig. 4.

2.6 RF value

The Rf 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.

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

Because the solvent front is always larger from the distance traveled by the solute. Rf values are always extreme between 0 – 1 where the solute remains fixed at its origin. Rf values do not have units since it is a ratio of distances.

2.7 Determination of Lycopene

The lycopene content in tomatoes was determined according to a slightly modified method described by Ali et al., [17] About 0.6 ml of extracted sample was taken in a falcon tube. Then 6 ml of pure acetone containing 0.05% butylated hydroxyl toluene (BHT), 5 ml of 95% ethanol, and 10 ml of hexane were added to it. After that falcon tubes were vortexed for 10 min. After that, 3 mL of distilled water was added and shaken again for 5 min. Finally, the mixture was left for phase separation. The absorbance of the 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:

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

Where X is the number of hexanes (ml), y is the weight of the sample (g), A503 is the absorbance at 503 nm and 3.12 is the extinction coefficient.

2.8 Determination of Carotene

Carvalho et al. [18] method was used with slight modifications to determine carotene content. Roots were cut into slices. The extraction yield of carotenes was observed at 60°C using ethanol (96%, Reachem, 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 a water bath, shaken every 10 min, and after every hour of extraction, a 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 (Reachem, 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 carotenes expressed as β -carotene (mg/100 ml) was calculated using the response factors as follows:

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

Where: A – absorbent, d – dilution, E1% – coefficient of absorbent, 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 slightly modified method described by Jayaprakasha et al. [19]. 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, 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 to find out the extraction conditions that correspond to the 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 a 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. RESULTS 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) [20]. 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 [21]. 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. In contrast, the previous study found that tomato puree contains 1.18 mg/100g lycopene [22]. The disparity between the contemporary study with other studies could be attributable to several variables, including both abiotic and biotic components, cultivation region, climate, cultivar type, harvest time, fruit maturation stage, and extraction technique, which affect the phytochemicals [23,24]. All samples showed significantly higher levels of lycopene over the storage period when kept at room temperature as compared to refrigeration. However, 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 the 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 [25] Tola & Ramaswary, 2013). The production of fragments such as acetone, methyl-heptane, laevulinic aldehyde, and glyoxal may be another way that low temperatures quench lycopene (Kaur et al., 2006). In a study [26], 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 [27].

To identify the precipitated lycopene, TLC was performed. Judging from a previous report (Britton, 2008), the single red spot-on TLC can be identified as lycopene, as the precipitated and authentic lycopene have the same Rf value. Rf value confirmed the purity of

lycopene content. The Rf value obtained from this experiment was 0.65 (Fig. 5) which was similar to the results (red Rf = 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 tomatoes. When using the anti-solvent method to precipitate lycopene, various solvents are used to extract lycopene from tomatoes. However, in the conventional solvent extraction method, only ethyl acetate is used to extract and precipitate lycopene. Ethyl acetate is the greatest option financially because it is far less expensive than other solvents. Using chloroform, n-hexane, and methanol is more costly than using single solvent ethyl acetate. When using ethyl acetate, less time is required to precipitate lycopene. The use of the 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.



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



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

Table 1. Total lycopene content (mg/100g) of tomato obtained from various samples 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
30° C	L1	^C 0.0185±1.74 ^b	^C 0.0171±1.47 ^b	^D 0.0250±1.32 ^a	^D 0.0258±2.56 ^a
	L2	^B 0.0298±1.32 ^b	^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	^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 tomato lycopene. a-d means followed by different superscript alphabets in each row are significantly different ($P \leq 0.05$) among extraction intervals. A-D means followed by different superscript alphabets in each column are significantly different ($P \leq 0.05$) among different temperatures

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 to extract the carotenes. Water should not have been present in the plant sample when the carotenoid extraction process took place [28]. Carotenoids are insoluble in water and soluble in polar solvents, including edible fats and oils. Because carotenoids are liposoluble, they are usually extracted from plant sources with organic solvents such as chloroform, hexane, acetone, and petroleum ether (Regal et al., 2019 and Valcareggi Morcelli, et al 2022) [29-31]. 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, and ethanol [32,33]. The extraction procedure using solvents proved to be highly efficient. Ethanol is also used to extract carotene from carrots which is also a food-grade solvent. Ethanol is environmentally friendly and thereby often healthier (at least at low concentration) to human choices of solvents [34] but exhibits poor solubility [35] When using ethanol as an extracted solvent, heating is helpful to increase the extraction rate. Heating the extract at 60° C for a few minutes and then filtrated the extract through a muslin cloth to remove the coarse partials. After a few hours later precipitate was observed in the solution. However, the amount of precipitation was very low and unstable in

the solution. Ethyl acetate was chosen as the extractive solution in our laboratory conditions. Precipitation was 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.

The total carotene concentration of several carrot samples at various storage times is displayed in Table 2. Total carotene levels, after extraction and purification, varied between 61.45 and 76.04 mg/g at 4 °C and 62.07 and 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 [36]. The disparity between the contemporary study with other studies could be attributable to variations in materials variety and environmental circumstances. 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 [37]. The best carotenes were found at 60°C, and heat treatments such as blanching, cooking, and streaming help to release the carotenoids bound by protein and render them easily extractable [7]. 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 changes following the solvent used, pigment concentration, experiment

results, and calculation. The Rf value obtained from this experiment was 0.67 which was similar to the result (orange Rf = 0.6) reported by Myong-Kyun et al. [38]. 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.

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

Temperature	Samples	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°	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 intervals. A-D means followed by different superscript alphabets in each column are significantly different ($P \leq 0.05$) among different temperatures

Table 3. Total curcumin content (mg/100g) of turmeric obtained from various samples 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±9.87 ^{ab}	^C 98.5±7.45 ^c	^C 103.21±7.45 ^a	^B 101.76±6.87 ^b
	Cu3	^A 108.63±7.45 ^b	^B 103.33±6.08 ^d	^A 109.54±9.87 ^a	^A 107.47±5.00 ^c
30°	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±5.00 ^{bc}	^B 103.95±7.45 ^b	^B 105.32±9.87 ^a	^B 100.76±7.45 ^c
	Cu3	^A 108.63±6.08 ^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 intervals. A-F means followed by different superscript alphabets in each column are significantly different ($P \leq 0.05$) among different temperatures.



(a)



(b)

Fig. 7. TLC plates of curcumin developed in benzene solution (a), and iodinechamber (b)

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 [39]. 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 [40-44] (Fernandez-Marin et al., 2022) 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. The range of values for the total curcumin concentration of turmeric is 62–90 mg/100 g and 90–136 mg/100 g, according to the quantitative results of this study [45,46]. Nonetheless, the discrepancies in the current study's results could be due to changes in the extraction techniques or solvents used in each study. We measured the curcumin concentration at room temperature and across various storage durations.

However, there were no appreciable variations in the amount of curcumin between ambient temperature and zero days at refrigeration. Significant variations in curcumin concentration were observed on 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 at 4° C on the seventh day. Lower pH and higher

moisture content caused curcumin to degrade at chilled temperatures. According to Bekle and Ramaswary [47], curcumin degradation may be affected by acidic conditions. Significant variations in turmeric's curcumin content were observed at different pH levels [47-49].

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) (Fig. 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. [38,50]. 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 [50-52].

4. CONCLUSION AND FUTURE PERSPECTIVES

It has become clear in recent years how important it is to investigate and employ natural colorants more frequently in creative and appealing food matrices. Food products that contain pigments like anthocyanins, carotenoids, and chlorophylls not only have visually appealing colors, but also offer consumers health benefits like anti-inflammatory, anti-cancer, and antibacterial properties. However, for natural food colorants to become widely used in the food industry, several obstacles need to be overcome, including their high cost and low stability as well as the stringent guidelines, requirements, and drawn-out toxicological assessments conducted by the FDA and the EU. Although it still has many controversial features, the recovery of pigments from food wastes and byproducts using clean technology appears to be an irreversible trend and the best approach to make their production sustainable. Lastly, to address the challenges of cost and stability, future research should expand our understanding of the biochemical characteristics of natural pigments. This will help us explore their potential as nutraceuticals and functional food additives.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Vega EN, Ciudad-Mulero M, Fernández-Ruiz V, Barros L, Morales P. Natural sources of food colorants as potential substitutes for artificial additives. *Foods*. 2023;12(22):4102.
2. United States Food and Drug Administration (FDA). Available: <https://www.fda.gov/industry/color-additives/color-additives-history> Accessed on: 3 May 2022.
3. European Food Safety Authority (EFSA). Available: <https://www.efsa.europa.eu/en/topics/topic/food-colours> Accessed on: 3 May 2022.
4. Bora P, Das P, Bhattacharyya R, Barooah MS. Biocolour: The natural way of colouring food. *Journal of Pharmacognosy and Phytochemistry*. 2019;8(3):3663-3668.
5. Ntrallou K, Gika H, Tsochatzis E. Analytical and sample preparation techniques for the determination of food colorants in food matrices. *Foods*. 2020;9(1):58.
6. Durazzo A, Carochio M, Heleno S, Barros L, Souto EB, Santini A, Lucarini M. Food dyes and health: Literature quantitative research analysis. *Measurement: Food*. 2022;7:100050.
7. González-Peña MA, Ortega-Regules AE, Anaya de Parrodi C, Lozada-Ramírez JD. Chemistry, Occurrence, Properties, Applications, and Encapsulation of Carotenoids—A Review. *Plants*. 2023;12(2):313.
8. Pérez-Gálvez A, Viera I, Roca M. Carotenoids and chlorophylls as antioxidants. *Antioxidants*. 2020;9(6):505.
9. Tan BL, Norhaizan ME. Carotenoids: How effective are they to prevent age-related diseases? *Molecules*. 2019;24(9):1801.
10. Imran M, Ghorat F, Ul-Haq I, Ur-Rehman H, Aslam F, Heydari M, Rebezov M. Lycopene as a natural antioxidant used to prevent human health disorders. *Antioxidants*. 2020;9(8):706.
11. Bin-Jumah MN, Nadeem MS, Gilani SJ, Mubeen B, Ullah I, Alzarea SI, Kazmi I. Lycopene: A natural arsenal in the war against oxidative stress and cardiovascular diseases. *Antioxidants*. 2022;11(2):232.
12. Khan UM, Sevindik M, Zarrabi A, Nami M, Ozdemir B, Kaplan DN, Sharifi-Rad J. Lycopene: Food sources, biological activities, and human health benefits. *Oxidative Medicine and Cellular Longevity*; 2021.
13. Marton LT, Pescinini-e-Salzedas LM, Camargo MEC, Barbalho SM, Haber JFDS, Sinatora RV, Cincotto dos Santos Bueno P. The effects of curcumin on diabetes mellitus: A systematic review. *Frontiers in Endocrinology*. 2021;12:669448.
14. Hussain Y, Alam W, Ullah H, Dacrema M, Daglia M, Khan H, Arciola CR. Antimicrobial potential of curcumin: Therapeutic potential and challenges to clinical applications. *Antibiotics*. 2022;11(3):322.
15. Ciuca MD, Racovita RC. Curcumin: Overview of extraction methods, health benefits, and encapsulation and delivery using microemulsions and nanoemulsions. *International Journal of Molecular Sciences*. 2023;24(10):8874.
16. Edwards AJ, Vinyard BT, Wiley ER, Brown ED, Collins JK, Perkins-Veazie P, Clevidence BA. Consumption of watermelon juice increases plasma concentrations of lycopene and β -carotene in humans. *The Journal of Nutrition*. 2003;133(4):1043-1050.
17. Ali I, Saleem K, Wesselinova D, Haque A. Synthesis, DNA binding, hemolytic, and anti cancer assays of curcumin I-based ligands and their ruthenium (III) complexes. *Medicinal Chemistry Research*. 2013;22:1386-1398.
18. Carvalho LMJD, Smiderle LDASM, Carvalho JLVD, Cardoso FDSN, Koblitz MGB. Assessment of carotenoids in pumpkins after different home cooking conditions. *Food Science and Technology*. 2014;34:365-370.
19. Jayaprakasha GK, Jagan Mohan Rao L, Sakariah KK. Improved HPLC method for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. *Journal of Agricultural and Food Chemistry*. 2002;50(13):3668-3672.
20. Barba AO, Hurtado MC, Mata MS, Ruiz VF, De Tejada MLS. Application of a UV-vis detection-HPLC method for a rapid determination of lycopene and β -carotene in vegetables. *Food Chemistry*. 2006;95(2):328-336.
21. Vasapollo G, Longo L, Rescio L, Ciurlia L. Innovative supercritical CO₂ extraction of lycopene from tomato in the presence of

- vegetable oil as co-solvent. *The Journal of Supercritical Fluids*. 2004;29(1-2):87-96.
22. Pandya D, Akbari S, Bhatt H, Joshi DC, Darji V. Standardization of solvent extraction process for Lycopene extraction from tomato pomace. *J. Appl. Biotechnol. Bioeng.* 2017;2(1):12-16.
 23. Halim MA, Kanan KA, Nahar T, Rahman MJ, Ahmed KS, Hossain H, Ahmed M. Metabolic profiling of phenolics of the extracts from the various parts of blackberry plant (*Syzygium cumini* L.) and their antioxidant activities. *LWT*. 2022;167:113813.
 24. Rahman MT, Halim MA, Mozumder NR, Ove TA, Khatun AA. Phytochemicals and antioxidant properties of bael (*Aegle marmelos* L.) pulp powder and its products. *Journal of Agriculture and Food Research*. 2024;15:100971.
 25. Singh S, Singh B, Singh AK, Singh K, Pandey VK. Preparation and effect of different temperature and time on nutritional quality of tomato paste. *Int. J. Curr. Microbiol. App. Sci.* 2020;9(11):1144-1151.
 26. Gil MI, Aguayo E, Kader AA. Quality changes and nutrient retention in fresh-cut versus whole fruits during storage. *Journal of Agricultural and Food chemistry*. 2006;54(12):4284-4296.
 27. Pathak M, Sagar NA. Influence of thermal treatments, extraction methods, and storage conditions on lycopene content of foods. *Journal of Food Quality*; 2023.
 28. Rasdiana FZ, Syukri D. Extraction technique for maximum yield of carotene from crops (carrot). In *IOP Conference Series: Earth and Environmental Science*. IOP Publishing. 2022;1059(1):012044.
 29. Cheng SH, Khoo HE, Kong KW, Nagendra Prasad K, Galanakis CM. Extraction of Carotenoids and Applications. In *Carotenoids: Properties, Processing and Applications*; Academic Press: Cambridge, MA, USA. 2019;259–288.
 30. Shankar U, Lenka SK, Ackland ML, Callahan DL. Comparison of different solvent extraction compositions for the analysis of phytoplankton pigments and lipids using a rapid and sensitive lc-ms method. *Res. Sq.*, preprint; 2022.
 31. Varaprasad D, Ragasudha N, Parveen SN, Chandrasekhar T. Effect of various solvents on chlorophyll and carotenoid extraction in green algae: *Chlamydomonas reinhardtii* and *Chlorella vulgaris*. *Annals of Plant and Soil Research*. 2019;21(4):341-345.
 32. Mezzomo N, Ferreira SR. Carotenoids functionality, sources, and processing by supercritical technology: A review. *Journal of Chemistry*. 2016;31 64312.
 33. Calvo MM, Dado D, Santa-María G. Influence of extraction with ethanol or ethyl acetate on the yield of lycopene, β -carotene, phytoene and phytofluene from tomato peel powder. *European Food Research and Technology*. 2007;224:567-571.
 34. Capello C, Fischer U, Hungerbühler K. What is a green solvent? A comprehensive framework for the environmental assessment of solvents. *Green Chemistry*. 2007;9(9):927-934.
 35. Huaman-Castilla NL, Martínez-Cifuentes M, Camilo C, Pedreschi F, Mariotti-Celis M, Pérez-Correa JR. The impact of temperature and ethanol concentration on the global recovery of specific polyphenols in an integrated HPLC/RP process on Carménère pomace extracts. *Molecules*. 2019;24(17):3145.
 36. Ikram A, Rasheed A, Ahmad Khan A, Khan R, Ahmad M, Bashir R, Hassan Mohamed M. Exploring the health benefits and utility of carrots and carrot pomace: A systematic review. *International Journal of Food Properties*. 2024;27(1):180-193.
 37. Singh P, Kulshrestha K, Kumar S. Effect of storage on β -carotene content and microbial quality of dehydrated carrot products. *Food Bioscience*. 2013;2:39-45.
 38. Myong-Kyun R, Min-Hee J, Jin-Nam M, Woi-Sook M, Sun-Mee P, Jae-Suk C. A simple method for the isolation of lycopene from *Lycopersicon esculentum*. *Botanical Sciences*. 2013;91(2):187-192.
 39. Le-Tan H, Fauster T, Haas K, Jaeger H. Aqueous extraction of curcuminoids from *Curcuma longa*: Effect of cell disintegration pre-treatment and extraction condition. *Food and Bioprocess Technology*. 2022;15(6):1359-1373.
 40. Shirsath SR, Sable SS, Gaikwad SG, Gogate PR. Ultrasound assisted curcumin recovery from *Curcuma aromatica*: Understanding the effect of different operating parameters. *Chemical Engineering and Processing-Process Intensification*. 2021;169:108604.
 41. Insuan W, Hansupalak N, Chahomchuen T. Extraction of curcumin from turmeric by ultrasonic-assisted extraction, identification, and evaluation of the

- biological activity. *Journal of Herbmед Pharmacology*. 2022;11(2):188-196.
42. Widmann AK, Wahl MA, Kammerer DR, Daniels R. Supercritical Fluid Extraction with CO₂ of *Curcuma longa* L. in Comparison to Conventional Solvent Extraction. *Pharmaceutics*. 2022;14(9):1943.
 43. Lateh L, Yuenyongsawad S, Chen H, Panichayupakaranant P. A green method for preparation of curcuminoid-rich *Curcuma longa* extract and evaluation of its anticancer activity. *Pharmacognosy Magazine*. 2019;15(65):730-735.
 44. Chandra A, Rekhil H, Dharmender, Gautam AK, Arya RK. Enzyme-assisted turmeric oil extraction from turmeric rhizomes. *Chemical and Process Engineering*. 2022;43(2):183–191.
 45. Meng FC, Zhou YQ, Ren D, Wang R, Wang C, Lin LG, Zhang QW. Turmeric: A review of its chemical composition, quality control, bioactivity, and pharmaceutical application. *Natural and Artificial Flavoring Agents and Food Dyes*. 2018;299-350.
 46. Thangavel K, Dhivya K. Determination of curcumin, starch and moisture content in turmeric by Fourier transform near infrared spectroscopy (FT-NIR). *Engineering in Agriculture, Environment and Food*. 2019;12(2):264-269.
 47. Bekele Y, Ramaswamy HS. Study on extraction and storage stability of watermelon pulp powder as food ingredient. *Ethiopian Journal of Applied Science and Technology*. 2013;(1):121-128.
 48. Castro TA, Leite BS, Assunção LS, De Jesus Freitas T, Colauto NB, Linde GA, Ferreira Ribeiro CD. Red tomato products as an alternative to reduce synthetic dyes in the food industry: A review. *Molecules*. 2021;26(23):7125.
 49. Fernández-Marín R, Fernandes SC, Andrés MA, Labidi J. Microwave- assisted extraction of curcuma longa l. Oil: Optimization, chemical structure and composition, antioxidant activity and comparison with conventional soxhlet extraction. *Molecules*. 2021;26(6):1516.
 50. Regal P, Lamas A, Fente CA, Franco CM, Cepeda A. Analysis and metabolomics of carotenoids. In *Carotenoids: Properties, Processing and Applications*. Academic Press. 2020;189-222.
 51. Sen T, Barrow CJ, Deshmukh SK. Microbial pigments in the food industry— challenges and the way forward. *Frontiers in Nutrition*. 2019;6:7.
 52. Valcareggi Morcelli A, Da Silva Andrade W, Frankenberg CLC, Rech R, Marcílio NR. Extraction of chlorophylls and carotenoids from microalgae: Cosmo-sac-assisted solvent screening. *Chemical Engineering and Technology*. 2021;44(7):1227-1232.