Original Research Article

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^{o}C) and chilled (4^{o}C) for a period of three weeks. Tomato extracts had lycopene contents ranging from 0.0153 to 0.0362 mg/100g. obtained carotene content, which was then extracted using a solvent in the range of 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 maximumamountofcurcumin precipitation obtained in lowerpH solution. LowerpH is importantto maintain the stability of curcumin precipitation. The experience of this researchwork suggested that lycopene from tomatoes, carotene from carrots, and curcuminfrom turmeric might be an excellent source to meet the increasing need fornaturalcolorants.

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

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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; 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 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 (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.,2019Ntrallou 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, 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 and 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 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 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(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;Ciuca and Racovita 2023).

Scientists are becoming more and more interested in the studies on the extraction of natural food

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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. Extractionofnaturalcolors(lycopene,carotene,andcurcumin)fromfruits,vegetables,andspicesca nbemoreprofitableandhelptoreducethe useofsyntheticcolorsinfoodproduction. 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 Samplecollectionsandpreparation

Thefreshtomato(*Solanumlycopersicum*L.),Carrot(*Daucuscarota*L.),andturmeric(*Curcumalonga*L.)werepurchasedfromthelocalmarketofMymensingh Bangladesh.Sampleswerewashedwithwatertoremoveadherences,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) andthenstoredat4^{o}Cuntilusedforthe experiment.

2.2 Chemicalsand reagents

Ethanol, methanol, acetone, n-hexane, chloroform, ethyl acetate, diluted HCL,NaOH,silicagel(TLCgrade),benzene,iodine, butylated hydroxytoluene(BHT),calciumsulfatehemihydrateswere usedinthestudy.

2.3 PreparationofTLCplates

Simple TLC plates were prepared using TLC glass slides. A mixture of silica gel(finelypowdered)andcalciumsulfatehemihydrateswеremixedanddouble the amount of water was added to form a paste. This paste wasevenly distributed over the surface of the slide and kept aside to dry. Beforeusage,slideswereactivatedbybakingintheovenat120° Cfor30-40min.

2.4 Extractionprocess

2.4.1 Extractionoflycopene(redcolor)

Fresh Tomato was thoroughly washed with potable water and the fruits werethenchoppedintopieces.Thechoppedtomatowasgroundinagrinderand water from the pulp by hand pressed and dried at 60^{o} C for 6 hrs.Then ethyl acetate added to the pulp and stirred the sample up to 1 hr. Thelycopene extracts thus obtained were filtrated through a muslin cloth to removecoarse particles. After that filtrated extract was transferred into a separatingfunnel. The mixture was shaken vigorously and kept aside for the layers

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toseparate. Two layers were separated, one was an aqueous layer and the other wasan ethyl acetate layer with lycopene. The lower layer containing ethyl acetate withlycopene was collected in a conical flask and the upper layer was discarded in abeaker.Solventsandotherreagentswereanalyticalgrades.LycopeneExtractionand purification method (a), extracted and purified sample (b) shown in Figure1.

(a) (b)

Figure1: Lycopene extraction and purification method (a), extracted andpurifiedsample(b)

2.4.2 Extractionofcarotene(orangecolor)

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 handpressing and drying at 60^{o} C for 6 hrs. Solvents and other reagents are saved foruse. After that ethyl acetate is added to the pulp and stirred for 1 hr. The solventcontainingcarotenewasfilteredthroughamuslinclothtoremovecoarseandparticles then transferred into a separating funnel. The mixture was shakenvigorously and kept aside for the layers to separate. The lower layer was anaqueous layer which was discarded from the separating funnel and collected ina beaker. The upper layer contained ethyl acetated with carotene and it wascollected separately after the removal of the aqueous layer. The extract wascollectedinaconicalflask.Caroteneextractionandpurificationmethod(a),extractedandpurifiedsample(b)showninFigure2.

(a) (b)

Figure 2:Caroteneextractionandpurificationmethod(a),extractedandpurifiedsample(b)

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2.4.3 Extractionofcurcumin(yellowcolor)

A Turmericsampleofpropermorphotypewascollectedfromt h e localmarket(Fulbaria, Mymensingh). Turmeric was sun-dried and torn into pieces and then extracted forcurcuminbythe Alkalizationmethod**(**theactofmakingasubstancealkaline,asthroughtheadditionofabaseNaOH).Thee xtractedcurcuminwasprecipitatedatlowpH(dilutedHCL)assoonaspossiblebyproperinstrumentalarr angements.Thecurcuminprecipitatewascoagulatedandfilteredundera vacuum.Thecurcuminpowderthusobtainedwastreatedbypropersolvent extraction method (n-hexane) for removal of gum, oil, resin, etc. The final step isdrying the extract and grinding to obtain powder form. Curcumin extractionand purification method (a), extracted and purified sample (b) shown in Figure3.

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(a) (b)

Figure 3:Curcuminextractionandpurificationmethod(a),extractedandpurifiedsample(b)

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

TLC was done according to the method reported by Edwards etal.(2003)with slight modifications. 50 g of benzene was added into a conical flask.Then the solutionwasvigorously stirred witha glass rod to avoid lumps. A TLCglass 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 thetank allowed to dry and placed in an iodine tank. The sample was viewedunderlight.Lycopene,carotene,andcurcuminpuritywascalculatedfromretentionvalues(Rf)whichwasvaluesweresimilartoreferencevalues.Samplesinsolutionchamber(a)andsamplesi niodinechamber(b)are showninFigure4.

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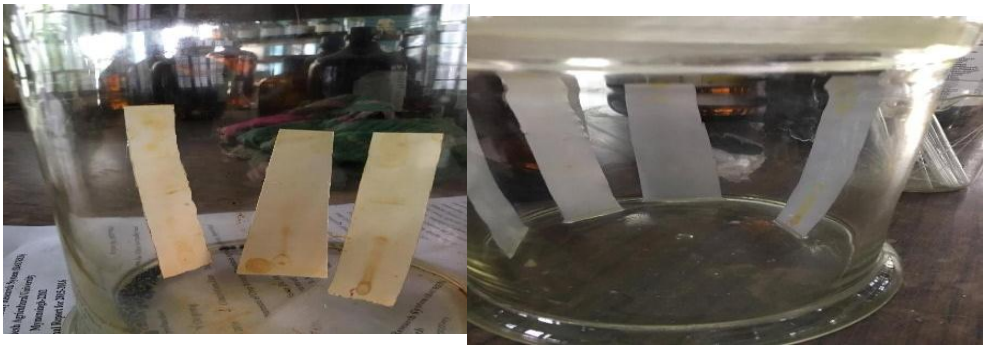

(a) (b)

Figure 4:Samplesinsolutionchamber(a)andsamplesiniodinechamber(b)

2.6 Rfvalue

The Rf valueis defined as theratio of the distance moved by thesolute (i.e., thedyeorpigmentundertest)andthedistancemovedbythesolvent(knownasthe solvent front) along the paper, where both distances are measured from thecommonorigin.

$$\text{RfValue} = \frac{\text{DistancetravelledbySolute}}{\text{DistancetraveledbySolvent(SolventFront)}}$$

Becausethesolventfrontisalwayslargerfromthedistancetraveledby the solute. Rf values are always extreme between 0 – 1 where the solute remainsfixedatitsorigin.Rfvaluesdonothaveunitssinceitisaratioofdistances.

2.7 Determinationoflycopene

The lycopene content in tomatoes was determined according to a slightly modifiedmethod described by Ali et al., (2013). About 0.6 ml of extracted sample wastaken in a falcon tube. Then 6 ml of pure acetone containing 0.05% butylatedhydroxyl toluene (BHT), 5 ml of 95% ethanol, and 10 ml of hexane were addedtoit.Afterthatfalcontubeswerevortexedfor10min.Afterthat,3mLofdistilledwaterwasaddedandshakenagainfor5min.Finally,themixturewasleftfor phase separation. Theabsorbance ofthe upper hexane layerwasmonitoredat 503nmusingaUV-VISSpectrophotometer(T60U,PGinstrument, UnitedKingdom)withhexaneastheblank.Theamountoflycopenewascalculatedusingthefollowingequation:

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

Where X is the number of hexanes (ml), y is the weight of the sample (g), A503is theabsorbanceat 503nm and3.12istheextinction coefficient.

2.8 Determinationofcarotene

Carvalho et al. (2014) method was used with slight modifications to determine carotene content.Rootswerecutintoslices.Theextractionyieldofcaroteneswasobservedat60°Cusingethanol(96%,Reachem,SlovakRepublic)and2-propanol.Initially,25gofcut carrot samples were added to 100 g of 96% ethanol. Carrot slices wereextracted in a water bath, shaken every 10 min, and after every hour ofextraction,a 5 ml sample was taken and mixed with n-hexane (20 ml). Water wasaddedfortheseparationofphases,andaftertheseparation,thehexane-carotenoidphasewasmadeuptothevolumeof50ml.Thecontentofβ-caroteneinthehexane(Reachem,SlovakRepublic)extractwasdeterminedspectrophotometrically,theabsorbencywasmeasuredatthewavelengthof454mmusingaUV-VISSpectrophotometer(T60U,PGinstrument,UnitedKingdom(Jenway,GreatBritain).Theconcentrationof carotenesexpressedasβ-carotene(mg/100ml)wascalculatedusingtheresponsefactorsasfollows:

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

Where:A–absorbent,d–dilution, E1%–coefficientofabsorbent, w–the weightofthe sample(g), V–volume(ml)

2.9 Determinationofcurcumin

The curcumin content in turmeric was determined according to a slightly modifiedmethod described by(Jayaprakasha et al., 2002).Inconventionalapparatus,turmericpowderwasaccuratelyweighedandextractedwithethanolfor24h.Thecurcumincontentoftheextractwasdetermined using UV-Vis spectroscopy. Batch extraction was carried out for allextraction experiments. The stirring speed was set at the appropriate level, to ensure the suspension of solid in the liquid phase and a good contactbetween them. In a closed flask, 2 g of turmeric powder was carefully weighedand 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 upto 100 ml. 2 ml of this was taken and made up to 50 ml. Extractions wereperformedinthetemperaturerangeof25–60°C.Allexperimentswereconducted to find out the extraction conditions that correspond to

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themaximumcurcumincontentoftheextracts. Turmericoleoresinscommonlycontain30–45%curcuminoidpigments.Consequently,thecurcumincontentintheextractswasusedtoevaluatetheeextractionefficiency.Curcuminwasdeterminedbymeasuringtheabsorbanceof an ethanol curcumin solution, at a fixed wavelength at 421 nm using a UV-VISSpectrophotometer(T60U,PGinstrument,UnitedKingdom,Jenway,GreatBritain).

2.10 Storageconditions

Isolatedandpurifiedlycopeneandcarotenewerekeptinamberbottlesatambienttemperature(30±2^{O}C)andrefrigeratedtemperature(4^{O}C)forthedeterminationoflycopenecarotenecontentandcolor.Curcuminpowderwaspreservedinanamberbottleatroomtemperature.

2.11 Statisticalanalysis

Eachexperimentincludedtworeplications.Datawereanalyzedusingstatisticalsoftware R (windows version 2.13.1). A single-factor analysis of variance wascarried out. Significant differences were estimated using Duncan Multiple RangeTests(DMRT).Differenceswereconsideredtobesignificantat$P \leq 0.05$.

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*etal.*,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

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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 studyfound that tomato puree contains 1.18 mg/100g lycopene (Pandya et al.,2017).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 (Halim et al., 2022 and Rahman et al., 2024).All samples showed significantly higher levels of lycopene over the storage period when kept at room temperature as compared to refrigeration.However,therewerenosignificantdifferencesinlycopenecontentbetweenzerodaysatrefrigeratedtemperatureandroomtemperature.However,significantdifferenceswerefoundinlycopenecontentbetween h e 7^{th},14^{th},and21^{th}daysatroomtemperatureandrefrigeratedtemperature.Degradationoflycopeneatrefrigeratedtemperaturewasinducedbyhighermoisturecontent.Highermoisturecontentwasfoundatrefrigeratedtemperaturethan at roomtemperature.Lycopenelosswasobservedafter7thdays ofstorageat4^0C.Lycopenedegradationmightbeinfluencedduringacidicconditions and at all conditions might be due to oxidation which depends on temperature and moisture(Singh et al., 2020; 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 (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).

To identify the precipitated lycopene, TLC was performed. Judgingfromapreviousreport(Britton,2008),thesingleredspot-onTLCcanbeidentifiedaslycopene,astheprecipitatedandauthenticlycopenehavethesameRfvalue.Rfvalueconfirmedthepurityoflycopenecontent.T h e Rfvalueobtainedfromthisexperimentwas0.65 (Figure 5)whichwassimilartotheresults(redRf=0.63) reportedbyMyong-Kyunetal.(2013).

Theconventionalsolventextractionand anti-solventprocessesarefeasiblemethodstorapidlyextractlycopenefromtomatoes.Whenusingthe anti-solventmethod to precipitate lycopene, various solventsare used to extract lycopene

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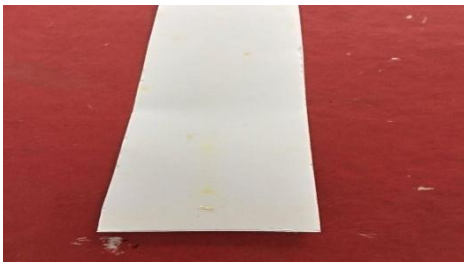
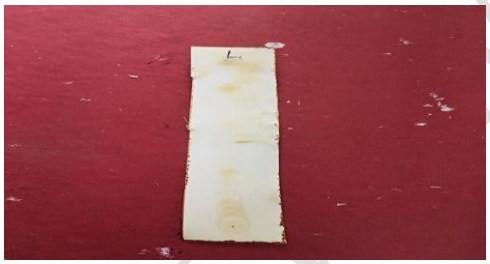
fromtomatoes. However, in the conventional solvent extraction method, only ethyl acetate is usedtoextractandprecipitatelycopene.Ethyl acetate is the greatest option financially because it is far less expensive than other solvents. Using chloroform, n-hexane, and methanol is more costly than usingsingle solvent ethyl acetate. When using ethyl acetate, less time is required toprecipitate lycopene. The use of the anti-solvent methanol method the extractionis not satisfactory in terms of profitability. To achieve the highest solubility oflycopenethechoiceofethylacetateasthesolventwasthemostreasonable.



(a)



(b)

Figure 5:TLCplatesofcarotene developedinbenzenesolution(a),andiodinechamber(b)

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

		Extraction Interval (Days)			
Temperature	**Samples**	**0th**	**7th**	**14th**	**21th**
4° C	L1	$^{C}0.0185\pm1.32^{c}$	$^{D}0.0153\pm2.02^{d}$	$^{C}0.0287\pm3.29^{a}$	$^{D}0.0252\pm3.14^{b}$
	L2	$^{B}0.0298\pm3.42^{b}$	$^{C}0.0183\pm1.30^{c}$	$^{C}0.0286\pm3.42^{b}$	$^{B}0.0331\pm3.42^{a}$
	L3	$^{A}0.0325\pm2.36^{b}$	$^{B}0.0251\pm3.42^{d}$	$^{A}0.0351\pm3.47^{a}$	$^{C}0.0285\pm5.78^{c}$
30° C	L1	$^{C}0.0185\pm1.74^{b}$	$^{C}0.0171\pm1.47^{b}$	$^{D}0.0250\pm1.32^{a}$	$^{D}0.0258\pm2.56^{a}$
	L2	$^{B}0.0298\pm1.32^{b}$	$^{B}0.0257\pm1.69^{c}$	$^{B}0.0314\pm3.29^{a}$	$^{C}0.0287\pm1.45^{b}$

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,valuesinparenthesesrepresentthepercentageofretentionascomparedto0-daystartingtomatolycopene. a-dmeansfollowedbydifferentsuperscriptalphabetsineachrowaresignificantlydifferent(P≤0.05)amongextractionintervals. A-Dmeansfollowedbydifferentsuperscriptalphabetsineachcolumnaresignificantlydifferent(P≤0.05)amongdifferenttemperatures.

3.2 Effectsofsolvent,extractiontime,extractionandpurificationmethods,andtemperatureoncarotenecontent

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 (Rasdianaand Syukri, 2022).Carotenoidsareinsolubleinwaterandsolubleinpolarsolvents,includingediblefatsandoils.Becausecarotenoidsareliposoluble,theyareusuallyextractedfromplantsourceswithorganicsolventssuchaschloroform,hexane,acetone,and petroleumether (Regal et al.,2019; Cheng et al.,2019; Shankar et al 2022; Varaprasad et al.,2019, and ValcareggiMorcelli, et al 2022).Thesamplescancontainlargeamountsofwater;water-miscibleorganicsolventssuchasethylacetatearealsoused.Oneoftheproblemsistheeliminationoftheresidual solventstoobtainasafeextract;thiscanbeavoidedbyusingfood-gradesolventssuchasethylacetate,and ethanol (**Mezzomo and Ferrerira 2016**,Calvoetal., 2007).The extraction procedure using solvents proved to be highly efficient. Ethanolis 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 lowconcentration) to human choices of solvents (Capello, 2007) but exhibits poorsolubility (**Huaman-Castilla et al.,2019**) When using ethanol as an extracted solvent,heatingishelpfultoincreasetheextractionrate.Heatingtheextractat60^{o}Cfora few minutes and then filtrated the extract through a muslin cloth to remove thecoarse partials. After a few hours later precipitate was observed in the solution.However,theamountofprecipitationwasverylowandunstableinthesolution.Ethylacetatewaschosenastheextractivesolutioninourlaboratoryconditions.Precipitation was observed in the solution within a few minutes and the amount ofprecipitation was

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higher compared to ethanol extraction. Carotene precipitationobservedinthisprocess wasmorestable.

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 (Ikram et al., 2024). 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 (Singh et al., 2013).Thebestcaroteneswerefoundat60°C, and heattreatmentssuchasblanching,cooking, andstreaminghelptoreleasethecarotenoidsboundbyproteinandrenderthemeasilyextractable **(González-Peña et al 2023)**.Hence,thecarotenecontentincreasedfrom61.43mg/100gto81.72mg/100gat30°Cin21thday(Table2).

Identificationandpurificationusingthin-layerchromatographywasveryeffectiveforcarotene.TheextractwasidentifiedusingThinLayer Chromatography method and the retardation factor was calculated. The TLCplateonwhichaspotoftheextractwasplacedandkeptinadevelopingchamber to separate into different bands is shown in Figure 6 and the developedTLCplatewiththeorangespotrepresentscarotene.Studiesstatethatabsorbance changesfollowing the solvent used, pigment concentration,experimentresults,andcalculation.The Rfvalueobtainedfromthisexperimentwas0.67 which was similar to the result (orange Rf = 0.6) reported by Myong-Kyunetal.(2013).In terms of time the best choice ethyl acetate, considerably less time requiredthan other solvents. In this study, the use of ethanol is not good enough. Theuse of ethanol in the extraction is not satisfactory in terms of profitability. Toachieve the highest solubility of β-carotene the choice of ethyl acetate as thesolventwasthemostreasonable.

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(a) (b)

Figure 6:TLCplatesofcarotene developedinbenzenesolution(a),andiodinechamber(b)

Table2:Totalcarotenecontent(mg/100g)ofcarrotobtainedfromvarioussamplesbysolvent -extractedandpurifiedmethodsatdifferentExtractionPeriods

Temperature	Samples	Extraction Interval (Days)			
		0th	**7th**	**12th**	**21th**
4°C	C1	$^{C}68.91\pm5.00^{a}$	$^{E}64.29\pm6.39^{b}$	$^{D}65.71\pm5.69^{b}$	$^{F}61.43\pm6.08^{c}$
	C2	$^{B}72.12\pm5.42^{b}$	$^{D}66.94\pm1.32^{c}$	$^{C}73.53\pm7.10^{b}$	$^{B}76.04\pm5.78^{a}$
	C3	$^{A}80.13\pm1.32^{b}$	$^{A}78.32\pm5.78^{c}$	$^{A}80.90\pm5.00^{a}$	$^{C}73.61\pm6.90^{d}$
30° C	C1	$^{C}68.91\pm5.78^{a}$	$^{E}64.44\pm6.08^{b}$	$^{E}62.85\pm6.44^{c}$	$^{E}62.07\pm6.08^{c}$
	C2	$^{B}72.12\pm4.71^{b}$	$^{C}69.42\pm6.39^{c}$	$^{B}76.34\pm5.78^{a}$	$^{D}70.27\pm5.47^{c}$
	C3	$^{A}80.13\pm5.00^{a}$	$^{B}75.05\pm5.00^{b}$	$^{C}73.63\pm6.21^{c}$	$^{A}81.72\pm6.40^{a}$

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≤0.05) among extraction intervals.A-D means followed by different superscript alphabets in each column are significantly different (P≤0.05) among different temperatures.

3.3 Effectsofsolvent,extractiontimeextractionandpurificationmethods,andtemperatureoncurcumincontent

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

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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) 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 (Meng F-C et al., 2018 and Thangavel and Dhivya, 2019). 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^{o} 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 Rf 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^{o} C and 30^{o} C.

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(a) (b)

Figure 7:TLCplatesofcurcumindevelopedinbenzenesolution(a),andiodinechamber(b)

Table3:Totalcurcumincontent(mg/100g)ofturmericobtainedfromvarioussamplesextractedbyalkalizationmethodatdifferentExtractionPeriods

Temperature	Samples	Extraction Interval (Days)			
		0th	**7th**	**12th**	**21th**
4°C	Cu1	$^{C}96.39\pm1.32^{a}$	$^{D}93.61\pm6.08^{b}$	$^{E}94.95\pm7.87^{ab}$	$^{C}91.17\pm5.00^{c}$
	Cu2	$^{B}102.39\pm9.87^{ab}$	$^{C}98.5\pm7.45^{c}$	$^{C}103.21\pm7.45^{a}$	$^{B}101.76\pm6.87^{b}$
	Cu3	$^{A}108.63\pm7.45^{b}$	$^{B}103.33\pm6.08^{d}$	$^{A}109.54\pm9.87^{a}$	$^{A}107.47\pm5.00^{c}$
30°C	Cu1	$^{C}96.39\pm5.78^{a}$	$^{D}94.01\pm1.32^{b}$	$^{F}92.83\pm5.78^{d}$	$^{C}92.75\pm6.08^{c}$
	Cu2	$^{B}102.39\pm5.00^{bc}$	$^{B}103.95\pm7.45^{b}$	$^{B}105.32\pm9.87^{a}$	$^{B}100.76\pm7.45^{c}$
	Cu3	$^{A}108.63\pm6.08^{b}$	$^{A}105.06\pm9.87^{c}$	$^{D}102.53\pm6.08^{d}$	$^{A}110.41\pm9.87^{a}$

Here, valuesinparenthesesrepresentthepercentageofretentionascomparedto0-daystartingturmericcurcumin. a-dmeansfollowedbydifferentsuperscriptalphabetsineachrowaresignificantlydifferent(P≤0.05)amongextractionintervals. A-Fmeansfollowedbydifferentsuperscriptalphabetsineachcolumnaresignificantlydifferent(P≤0.05)amongdifferenttemperatures.

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

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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.

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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Reference

Ali, I., Saleem, K., Wesselinova, D., & Haque, A. (2013). Synthesis, DNA binding, hemolytic, and anti cancer assays of curcumin I-based ligands and their ruthenium (III) complexes. Medicinal chemistry research, 22, 1386-1398.

Barba, A. O., Hurtado, M. C., Mata, M. S., Ruiz, V. F., & De Tejada, M. L. S. (2006). Application of a UV–vis detection-HPLC method for a rapid determination of lycopene and β-carotene in vegetables. *Food chemistry*, *95*(2), 328-336.

Bekele, Y., & Ramaswamy, H. S. (2013). Study on extraction and storage stability of watermelon pulp powder as food ingredient. Ethiopian Journal of Applied Science and Technology, (1), 121-128.

Bin-Jumah, M. N., Nadeem, M. S., Gilani, S. J., Mubeen, B., Ullah, I., Alzarea, S. I. & Kazmi, I. (2022). Lycopene: a natural arsenal in the war against oxidative stress and cardiovascular diseases. Antioxidants, 11(2), 232.

Bora, P., Das, P., Bhattacharyya, R., & Barooah, M. S. (2019). Biocolour: The natural way of

colouring food. Journal of Pharmacognosy and Phytochemistry, 8(3), 3663-3668.

Calvo, M. M., Dado, D., & Santa-María, G. (2007). 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, 224, 567-571.

Capello, C., Fischer, U., &Hungerbühler, K. (2007). What is a green solvent? A comprehensive framework for the environmental assessment of solvents. Green Chemistry, 9(9), 927-934.

Carvalho, L. M. J. D., Smiderle, L. D. A. S. M., Carvalho, J. L. V. D., Cardoso, F. D. S. N., & Koblitz, M. G. B. (2014). Assessment of carotenoids in pumpkins after different home cooking conditions. *Food Science and Technology*, *34*, 365-370.

Castro, T. A., Leite, B. S., Assunção, L. S., de Jesus Freitas, T., Colauto, N. B., Linde, G. A., ... & Ferreira Ribeiro, C. D. (2021). Red tomato products as an alternative to reduce synthetic dyes in the food industry: A review. Molecules, 26(23), 7125.

Chandra, A., Rekhi, H., Dharmender, Gautam, A. K., & Arya, R. K. (2022). Enzyme-assisted turmeric oil extraction from turmeric rhizomes. Chemical and Process Engineering, 43(2). 183–191.

Cheng, S.-H., Khoo, H.E.; Kong, K.W., Nagendra Prasad, K., Galanakis, C.M. (2019). Extraction of Carotenoids and Applications. In Carotenoids: Properties, Processing and Applications; Academic Press: Cambridge, MA, USA, 259–288.

Ciuca, M. D., &Racovita, R. C. (2023). Curcumin: Overview of Extraction Methods, Health Benefits, and Encapsulation and Delivery Using Microemulsions and Nanoemulsions. International Journal of Molecular Sciences, 24(10), 8874.

Durazzo, A., Carocho, M., Heleno, S., Barros, L., Souto, E. B., Santini, A., & Lucarini, M. (2022). Food dyes and health: Literature quantitative research analysis. Measurement: Food, 7, 100050.

Edwards, A. J., Vinyard, B. T., Wiley, E. R., Brown, E. D., Collins, J. K., Perkins-Veazie, P., ... & Clevidence, B. A. (2003). Consumption of watermelon juice increases plasma concentrations of lycopene and β-carotene in humans. *The Journal of nutrition*, *133*(4), 1043-1050.

European Food Safety Authority (EFSA). Available online: **https://www.efsa.europa.eu/en/topics/topic/food-colours** (accessed on 3 May 2022).

Fernández-Marín, R., Fernandes, S. C., Andrés, M. A., & Labidi, J. (2021). Microwave-assisted extraction of curcuma longa l. Oil: Optimization, chemical structure and

composition, antioxidant activity and comparison with conventional soxhlet extraction. Molecules, 26(6), 1516.

Gil, M. I., Aguayo, E., & Kader, A. A. (2006). Quality changes and nutrient retention in fresh-cut versus whole fruits during storage. Journal of Agricultural and Food chemistry, 54(12), 4284-4296.

González-Peña, M. A., Ortega-Regules, A. E., Anaya de Parrodi, C., & Lozada-Ramírez, J. D. (2023). Chemistry, Occurrence, Properties, Applications, and Encapsulation of Carotenoids—A Review. Plants, 12(2), 313.

González-Peña, M. A., Ortega-Regules, A. E., Anaya de Parrodi, C., & Lozada-Ramírez, J. D. (2023). Chemistry, Occurrence, Properties, Applications, and Encapsulation of Carotenoids—A Review. Plants, 12(2), 313.

Halim, M. A., Kanan, K. A., Nahar, T., Rahman, M. J., Ahmed, K. S., Hossain, H., ... & Ahmed, M. (2022). Metabolic profiling of phenolics of the extracts from the various parts of blackberry plant (Syzygiumcumini L.) and their antioxidant activities. LWT, 167, 113813.

Huaman-Castilla, N. L., Martínez-Cifuentes, M., Camilo, C., Pedreschi, F., Mariotti-Celis, M., & Pérez-Correa, J. R. (2019). The impact of temperature and ethanol concentration on the global recovery of specific polyphenols in an integrated HPLE/RP process on Carménère pomace extracts. Molecules, 24(17), 3145.

Hussain, Y., Alam, W., Ullah, H., Dacrema, M., Daglia, M., Khan, H., & Arciola, C. R. (2022). Antimicrobial potential of curcumin: therapeutic potential and challenges to clinical applications. Antibiotics, 11(3), 322.

Ikram, A., Rasheed, A., Ahmad Khan, A., Khan, R., Ahmad, M., Bashir, R., & Hassan Mohamed, M. (2024). Exploring the health benefits and utility of carrots and carrot pomace: a systematic review. International Journal of Food Properties, 27(1), 180-193.

Imran, M., Ghorat, F., Ul-Haq, I., Ur-Rehman, H., Aslam, F., Heydari, M., ... &Rebezov, M. (2020). Lycopene as a natural antioxidant used to prevent human health disorders. Antioxidants, 9(8), 706.

Insuan, W., Hansupalak, N., &Chahomchuen, T. (2022). Extraction of curcumin from turmeric by ultrasonic-assisted extraction, identification, and evaluation of the biological activity. Journal of Herbmed Pharmacology, 11(2), 188-196.

Jayaprakasha, G. K., Jagan Mohan Rao, L., &Sakariah, K. K. (2002). Improved HPLC method for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. *Journal of agricultural and food chemistry*, *50*(13), 3668-3672.

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Khan, U. M., Sevindik, M., Zarrabi, A., Nami, M., Ozdemir, B., Kaplan, D. N. & Sharifi-Rad, J. (2021). Lycopene: Food sources, biological activities, and human health benefits. Oxidative medicine and cellular longevity, 2021.

Lateh, L., Yuenyongsawad, S., Chen, H., &Panichayupakaranant, P. (2019). A green method for preparation of curcuminoid-rich Curcuma longa extract and evaluation of its anticancer activity. Pharmacognosy Magazine, 15(65), 730-735.

Le-Tan, H., Fauster, T., Haas, K., & Jaeger, H. (2022). Aqueous extraction of curcuminoids from Curcuma longa: effect of cell disintegration pre-treatment and extraction condition. Food and Bioprocess Technology, 15(6), 1359-1373.

Marton, L. T., Pescinini-e-Salzedas, L. M., Camargo, M. E. C., Barbalho, S. M., Haber, J. F. D. S., Sinatora, R. V.&Cincotto dos Santos Bueno, P. (2021). The effects of curcumin on diabetes mellitus: a systematic review. Frontiers in endocrinology, 12, 669448.

Meng, F. C., Zhou, Y. Q., Ren, D., Wang, R., Wang, C., Lin, L. G. & Zhang, Q. W. (2018). Turmeric: A review of its chemical composition, quality control, bioactivity, and pharmaceutical application. Natural and artificial flavoring agents and food dyes, 299-350.

Mezzomo, N., & Ferreira, S. R. (2016). Carotenoids functionality, sources, and processing by supercritical technology: a review. Journal of chemistry, 2016,3164312.

Myong-Kyun, R., Min-Hee, J., Jin-Nam, M., Woi-Sook, M., Sun-Mee, P., & Jae-Suk, C. (2013). A simple method for the isolation of lycopene from Lycopersicon esculentum. Botanical Sciences, 91(2), 187-192.

Ntrallou, K., Gika, H., &Tsochatzis, E. (2020). Analytical and sample preparation techniques for the determination of food colorants in food matrices. Foods, 9(1), 58.

Pathak, M., & Sagar, N. A. (2023). Influence of Thermal Treatments, Extraction Methods, and Storage Conditions on Lycopene Content of Foods. Journal of Food Quality, 2023.

Pérez-Gálvez, A., Viera, I., & Roca, M. (2020). Carotenoids and chlorophylls as antioxidants. Antioxidants, 9(6), 505.

Pandya, D., Akbari, S., Bhatt, H., Joshi, D. C., &Darji, V. (2017). Standardization of solvent extraction process for Lycopene extraction from tomato pomace. *J. Appl. Biotechnol. Bioeng*, *2*(1), 12-16.

Rahman, M. T., Halim, M. A., Mozumder, N. R., Ove, T. A., & Khatun, A. A. (2024). Phytochemicals and antioxidant properties of bael (Aegle marmelos L.) pulp powder and its products. Journal of Agriculture and Food Research, 15, 100971.

Rasdiana, F. Z., &Syukri, D. (2022, July). Extraction technique for maximum yield of carotene from crops (carrot). In IOP Conference Series: Earth and Environmental Science (Vol. 1059, No. 1, p. 012044). IOP Publishing.

Regal, P., Lamas, A., Fente, C. A., Franco, C. M., & Cepeda, A. (2020). Analysis and metabolomics of carotenoids. In Carotenoids: Properties, Processing and Applications (pp. 189-222). Academic Press.

Sen, T., Barrow, C. J., & Deshmukh, S. K. (2019). Microbial pigments in the food industry—challenges and the way forward. Frontiers in nutrition, 6, 7.

Shankar, U.; Lenka, S.K.; Ackland, M.L.; Callahan, D.L.(**2022)** 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.

Shirsath, S. R., Sable, S. S., Gaikwad, S. G., & Gogate, P. R. (2021). Ultrasound assisted curcumin recovery from Curcuma aromatica: Understanding the effect of different operating parameters. Chemical Engineering and Processing-Process Intensification, 169, 108604.

Singh, P., Kulshrestha, K., & Kumar, S. (2013). Effect of storage on β-carotene content and microbial quality of dehydrated carrot products. Food Bioscience, 2, 39-45.

Singh, S., Singh, B., Singh, A. K., Singh, K., & Pandey, V. K. (2020). Preparation and Effect of Different Temperature and Time on Nutritional Quality of Tomato Paste. Int. J. Curr. Microbiol. App. Sci, 9(11), 1144-1151.

Tan, B. L., &Norhaizan, M. E. (2019). Carotenoids: How effective are they to prevent age-related diseases?. Molecules, 24(9), 1801.

Thangavel, K., & Dhivya, K. (2019). Determination of curcumin, starch and moisture content in turmeric by Fourier transform near infrared spectroscopy (FT-NIR). Engineering in Agriculture, Environment and Food, 12(2), 264-269.

United States Food and Drug Administration (FDA). Available online: **https://www.fda.gov/industry/color-additives/color-additives-history** (accessed on 3 May 2022).

Valcareggi Morcelli, A., da Silva Andrade, W., Frankenberg, C. L. C., Rech, R., & Marcílio, N. R. (2021). Extraction of Chlorophylls and Carotenoids from Microalgae: COSMO- SAC- Assisted Solvent Screening. Chemical Engineering & Technology, 44(7), 1227-1232.

Varaprasad, D., Ragasudha, N., Parveen, S. N., & Chandrasekhar, T. (2019). Effect of various solvents on chlorophyll and carotenoid extraction in green algae: Chlamydomonas reinhardtii and Chlorella vulgaris. Annals of Plant and Soil Research, 21(4), 341-345.

Vasapollo, G., Longo, L., Rescio, L., &Ciurlia, L. (2004). Innovative supercritical CO2 extraction of lycopene from tomato in the presence of vegetable oil as co-solvent. *The Journal of Supercritical Fluids*, *29*(1-2), 87-96.

Vega, E. N., Ciudad-Mulero, M., Fernández-Ruiz, V., Barros, L., & Morales, P. (2023). Natural

Sources of Food Colorants as Potential Substitutes for Artificial Additives. Foods, 12(22), 4102.

Widmann, A. K., Wahl, M. A., Kammerer, D. R., & Daniels, R. (2022). Supercritical Fluid Extraction with CO2 of Curcuma longa L. in Comparison to Conventional Solvent Extraction. Pharmaceutics, 14(9), 1943.

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