CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journalhomepage:**http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

EXTRACTION OF CAROTENOIDS FROM VEGETABLE RESOURCES AND ITS UTILIZATION IN DAIRY PRODUCTS

Alak Jyoti Baishya[1],Subhajit Ray[2]✉

1.PG Scholar, Department of Food Engineering &Technology, Central Institute of Technology Kokrajhar, Kokrajhar, BTAD, Assam:783370, India
2.Associate Professor. Department of Food Engineering &Technology, Central Institute of Technology Kokrajhar, Kokrajhar, BTAD, Assam:783370, India
subhajit@cit.ac.in

https://doi.org/10.34302/crpjfst/2023.15.2.2

Article history:
Received:
16 February 2022
Accepted
1 April 2023

Keywords:
Extraction;
Total carotenoid;
Antioxidant activity;
Ascorbic acid,
Milk product processing,
Sensory quality.

ABSTRACT
Food colorants will be classified broadly into natural and synthetic colorants. Natural colorants are extracted mostly from plant material. Natural colorants such as lycopene, carotene, anthocyanin, etc. are used to provide colors in food. The aim of the study was to estimate the total amount of carotenoids from naturally available vegetables namely tomato (Lycopersicon esculentum), carrot (Daucus carota), broccoli (Brassica oleracea), beetroot (Beta vulgaricus) and to incorporate the extracted natural colors in processed dairy product e.g. sweetened syrupy cheese ball (rasgulla). Total carotenoid content in tomato, carrot, beetroot and broccoli were found to as 29.34 mg/100g, 37.13 mg/100g, 25.64 mg/100g, 23.33 mg/100g. Moreover, the ascorbic acid contents of tomato, carrot, beetroot and broccoli were found as 20.67 mg/100g, 8.05 mg/100g, 7.65 mg/100g and 15.12 mg/100g respectively. Similarly, the total phenolic contents of tomato, carrot, beetroot, and broccoli were 3.72 mg GAE/g, 2.02 mg GAE/g, 2.12 mg GAE/g and 1.87 mg GAE/g respectively. The antioxidant activity of tomato, carrot, beetroot and broccoli were obtained 40.24%, 38.46%, 56.52% and 42.23%. Sensory analysis data revealed that the rasgulla sample (S1) i.e. added natural colorant extracted from carrot showed better results in terms of color attributes. It was evident from the experimental results that naturally occurring vegetable resources contains significant health benefits as well as nutraceutical components and can suitably contribute the natural color during the preparation of processed food product.

1.Introduction

Fruits and vegetables e.g. carrot, beet root, broccoli, tomato, grapefruit, cabbage etc. are good sources of natural colorants and most often, mainly divided into 04 major groups: anthocyanins, betalains, carotenoids and chlorophyll(Rodriguez-Amaya,2019a).Carotenoids are a group of phytochemicals that are responsible for different colors of foods. All carotenoids are tetraterpenoids, meaning that they are produced from 8 isoprene molecules and contain 40 carbon atoms. Carotenoids partially or completely protect intact cells (e.g. human liver cell line HepG2) against oxidant-induced lipid peroxidation, and the protective effect is independent of provitamin A activity.The oxygenated carotenoids which are derivatives of these hydrocarbons known as xanthophylls, examples of these compounds are zeaxanthin and lutein (hydroxy), spirilloxanthin (methoxy), echinenone (oxo), and antheraxanthin (epoxy) (Meshram etal.,2013). Carotene is an orange

photosynthetic pigment important for photosynthesis. They are recognized as playing an important role in the prevention of human diseases and maintaining good health (Rebecca et al.,2014). β-carotene is the most widely studied carotenoid and present in human blood and tissues. Color varies from yellow to orange.Lycopene isan important carotenoid and principally responsible for the characteristics deep-red color of ripe tomato fruits and tomato products. Other sources of lycopene include watermelon, guava, papaya etc. Lycopene undergoes degradation via isomerization and oxidation. Lycopene is the principal hydrocarbon carotenoid in tomatoes with lesser amounts of α-carotene, β-carotene, ϒ-carotene and lutein. Lycopene is very sensitive to light, heat, oxygen and acids in degradation(Shi andMaguer,2000).Lycopene as an effective antioxidant quenches highly reactive singlet oxygen (O2) and traps peroxyl radicals(Shi andMaguer,2000). Lycopene has been shown to protect important bio-molecules, such as lipids, low-density lipoproteins, proteins, and DNA against oxidative damage, which is how lycopene contributes to the prevention of cancers, atherogenesis, and cell proliferation (Rao and Agarwal, 1999; Stahl etal.1998). Betalains are water-soluble nitrogen-containing pigments, which are synthesized from the amino acid tyrosine into two structural groups: the red-violetbetacyanins and the yellow-orange betaxanthins and provides these color shades respectively (Stintzingand Carle,2007). Betalains are natural dyes extracted from different fruits and vegetables. They are largely used as food colorants in food products like yogurts, ice cream and other products. Recent studies have shown that betanines have antioxidant, antimicrobial and antiviral activity (Ravichandran etal.,2013). Beet (*Beta vulgaricus*) is the main source of natural red dye, known as beetroot. The betalains are stable in the pH range 4-6 and their subject to degradation by thermal processing as in canning.Broccoli (Brassica oleracea) has the highest levels of carotenoids in the brassica family. It is particularly rich in lutein and also provides a modest amount of beta-carotene (ShadaksharaswamyandManay,2011). The use of natural colorants can provide technological and bioactive functionalities to those foods in which they are applied, delivering additional value-added properties (Rodriguez-Amaya,2016). Nowadays, single-phase coloring systems such as baking products (solid phase) or drinks (liquid phase) have been successfully assayed with natural colorants such as carotenoids or anthocyanins (Lin etal.,2018). Colorants can be added to food systems after a technological extraction or could be part of the colored raw material. However, as some of the natural bioactive compounds that chemically constitute these colorants can be lost due to the matrix storage and processing conditions, some of them can be encapsulated to take advantage of their technological and biological properties (Hidalgo etal.,2018). In addition, encapsulated colorants are easier to handle and often exhibit enhanced physicochemical properties such as better solubility, stability, and flow properties (Labuschagne,2018). Food colorants play a crucial role in food production, masking unpleasant attributes or enhancing the food products' natural properties (Nwoba etal.,2020). Therefore, based on their color, they can also be used for specific purposes. For instance, anthocyaninis highly common watersoluble flavonoids exhibiting pH-dependent colors from red to blue, and recognized by several bioactive properties such as antioxidant, anti-inflammatory, hypoglycemic, and chemo preventive effects (Nwoba etal.,2020). Carotenoids are highly appreciated for their red, orange, and yellow color, primarily fruits and vegetables, contributing to desirable flavors in food and beverages (Rodriguez-Amaya,2019b). Betalains are other type of colorants that have proven to be the most promissory candidates to replace Allura Red AC (Red 40), a synthetic colorant that contains benzidine, a potential human and animal carcinogen (Potera,2010).The aim of the study was to extract, analyze and purify the carotenoid content from various vegetable resources viz. tomato, carrot, broccoli and beetroot and finally

the extracted natural colorant especially from carrot and beetroot was added during processing to develop a popular dairy product (sweetened syrupy cheese ball) rasgulla for enhancing nutraceutical and sensorial qualities. Therefore, the functional aspect of natural colorant will be successfully exploited in milk processing industry to develop the value added dairy products for consumption.

2.Material and Methodology

2.1. Collection of raw materials and reagents

The vegetables used for the experiment are tomato(*Lycopersicon esculentum*), carrot(*Daucus carota*),broccoli (*Brassica oleracea*) and beetroot (*Beta vulgaricus*) respectively. The fresh vegetables were brought from the localmarket of Kokrajhar, Assam, India adjacent to the institute and stored in the refrigerator.Chemicals or reagents used for the purpose of extraction and analysis are acetone/hexane/ethanol,Nacl,methanol/acetonitrile, starch solution and iodine solution (Ascorbic acid determination), Folin-Ciocalteau reagent, sodiumcarbonate (Total phenolic content) and 2,2-diphenyl-1-picrylhydrazyl/ethanol(Antioxidant activity) respectively.

2.2. Extraction of carotenoids

The vegetables used for the study were Tomato (*Lycopersicon esculentum*), Carrot (*Daucus carota*), Broccoli (*Brassica oleracea*), Beetroot (*Beta vulgaris*). The vegetables used were bought fresh from the market and preserved in the refrigerator. A spectrophotometer was used to observe the absorbance at 450 nm. Solvent extraction was done using hexane: acetone (1:1) along withethanol, 10% NaCl solution was also prepared in the laboratory which was used in the extraction (Rebecca *et al.,*2014).

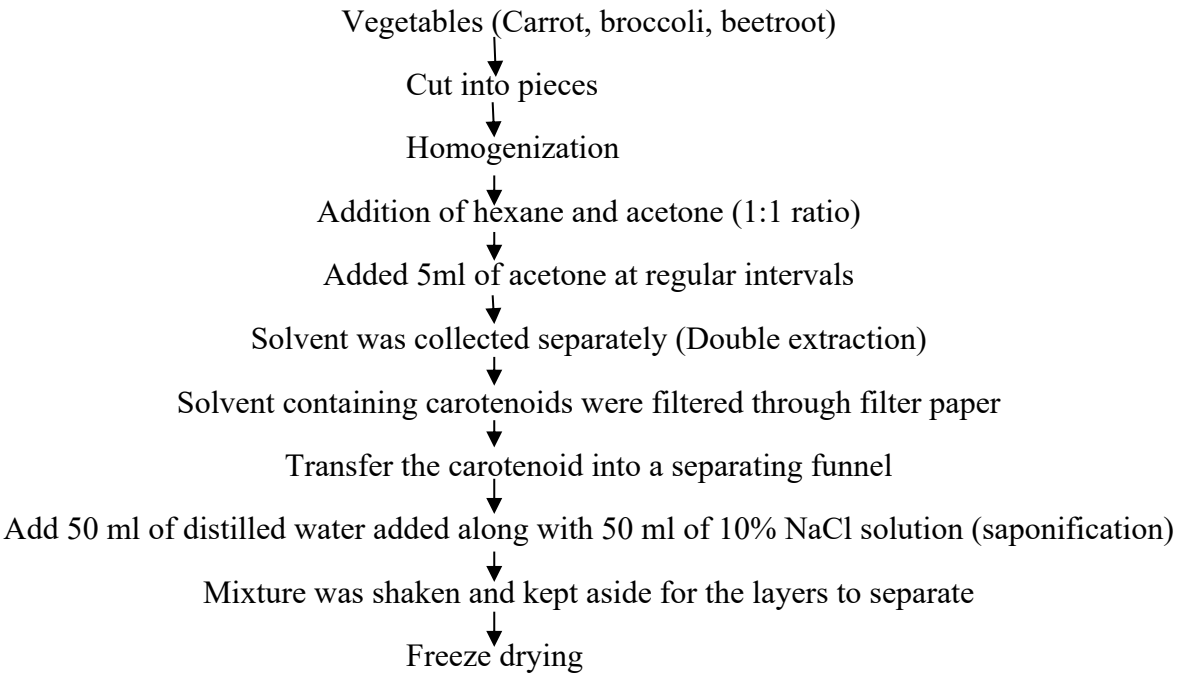

Figure1. Extraction of carotenoid from vegetable resources

2.2.1. Extraction of carotenoids using solvent

Vegetables viz. tomato, carrot, broccoli and beetroot were sliced separately and 100 g of each vegetable was weighed and kept separately. The same extraction procedure was followed for all the vegetables. 100 g of the vegetable was placed in a mortar and crushed with a pestle. A mixture of hexane and acetone in the ratio of 1:1 was added into the mortar and the sample was crushed. About 5ml of acetone was added slowly at regular intervals. The solvents were collected separately and the

process was repeated with the sample again for double extraction. The solvents containing carotenoids were filtered through a filter paper and then transferred into a separating funnel. 50ml of distilled water was added with 50 ml of 10% NaCl solution. The mixture was shaken properly and kept for the layers to separate. The upper layer contained carotenoids and it was collected separately after the removal of the water and NaCl solution. The extract was collected in tubes. The absorbance of the carotenoid was noted at 450 nm. The amount of carotenoid present in 100g of each food sample was calculated (Rebecca *et al.,*2014). The carotenoid extraction process from different vegetable resources will be represented in figure 1

2.3. Extraction of Lycopene from Tomato

Fresh tomatoes were first to cut into pieces and seeds were separated. The material was homogenized to form a paste. The paste was extracted with n- hexane (1:1 w/w) for two hours with constant stirring at 40-45 ºC in reaction assembly. The n-hexane layer was separated by using a separating funnel and further saponified for lycopene isolation (Roldan-Gutierrez *et al.,* 2007).

2.3.1. Saponification of n-hexane extract

The oleoresin was mixed with saponification mixture containing 60% propylene glycol, 20% KOH prepared in 45% methanol and 20% water and kept at 65ºC under gentle stirring for 30 minutes followed by n-hexane addition. The mixture was washed with warm water to remove saponified matter and excess propylene glycol and KOH. The lycopene crystals formed were filtered through Whatman filter paper no 1 and dried under vacuum or freeze drier.The lycopene extraction process from tomatoes will be represented in figure 2.

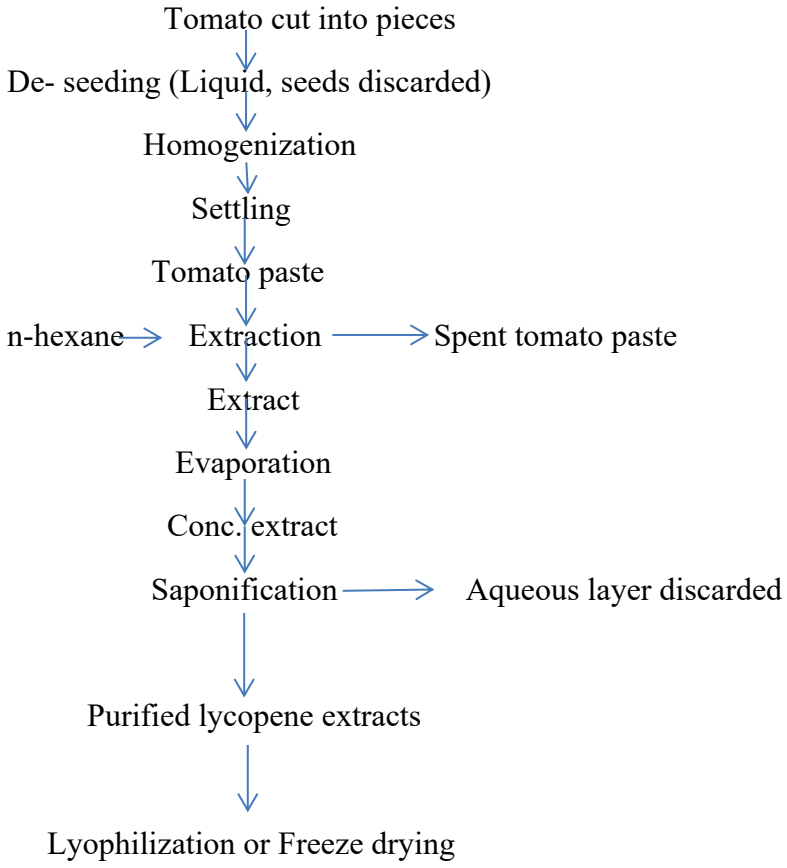

Figure 2. Extraction of lycopene (carotenoid) from tomato

2.4. Determination of Ascorbic acid

Ascorbic acid was estimated experimentally according to **(**Kaur and Goswami,2018**).** In this technique,20 mL of sample solution was pipetted into a 250 mL conical flask and add about 150 mL of distilled water and 1 mL of starch indicator solution. Titrate the sample with 0. 005 mol L iodine solution. The end point of the titration is identified as the first permanent trace of a dark blue-black colour due to the starch-iodine complex. Repeat the titration with further aliquots of sample solution until obtain the results**.**

2.4.1. Preparation of iodine solution

Weigh 2 g of potassium iodide into a 100 ml beaker. Weigh 1.3 g of iodine and add it into the same beaker. Add a few mL of distilled water and swirl for a few minutes until iodine is dissolved. Transfer iodine solution to a 1L volumetric flask, making sure to rinse all traces of solution into the volumetric flask using distilled water. Make the solution up to the 1 L mark with distilled water.

2.4.2. Preparation of starch solution

Starch indicator solution: (0.5%). Weigh 0.25 g of soluble starch and add it to 50 mL of near boiling water in a 100 mL conical flask. Stir to dissolve and cool before using.

2.5.Determination of total phenolic content(TPC)

Total phenolic content was determined according to (Annisworthand Gillespie, 2007). The reaction mixture was prepared by mixing 0.2 ml of natural juice separately mixed with .6 ml of distilled water. After addition of .25 ml of Folin- Ciocalteau reagent, 1 ml of saturated sodium carbonate (8% W/V) and 3 ml of distilled water were added. The mixture was then incubated for 30 min at 37 ºC and the absorbance was recorded at 765 nm using an UV-Spectrophotometer. The measurement was compared to calibration curve prepared using the standard gallic acid solution. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per 100 ml of juice.

2.6. Determination of antioxidant activity

The antioxidant activity of the extracts was determined using the modified method of(Sharma and Bhat,2009). In this method, 0.001g of DPPH (2,2-diphenyl-1-picrylhydrazyl) in ethanol was prepared and 1ml of this solution was added to a test sample. The reaction mixture was shaken well and incubated for 30 min at 37ºC. The absorbance was read at 517 nm using a UV-Spectrophotometer against the ethanol blank. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of (Yenand Duh, 1994).

$$IP = [(AC\ (0)- AA(t)/\ AC\ (0))] \times 100 \quad (1)$$

Where AC (0) is the absorbance of the control at t = 0 min; and AA(t) is the absorbance of the Anti-oxidants at t = 16 min.

2.7. HPLC analysis of carotenoids

HPLC analysis was carried out by the following experimental procedures and the materials are taken such as tomato, carrot, beetroot and broccoli.

2.7.1. Sample preparation

The solvent mixture used for the HPLC method is generally methanol/Acetonitrile (90:10 v/v). A sample of 5 grams of vegetables (carrot, tomato, beetroot and broccoli) was placed in a vessel, protected from light, and mixed with 100 ml of extraction solvent. The mixture was magnetically stirred during 30 minutes. The extracts were centrifugated to separate the supernatant, and these operations were repeated until the pulp was completely colorless. After that saponification was done with BHT/Methanol (40:60 v/v) (Barba et al.,2006).

2.7.2. Standard carotenoid preparation

Individual stock standard solutions were freshly prepared every day adding a suitable volume of hexane to the vial containing the carotenoid standard and mixing until complete dissolution; then the solutions were transferred to a volumetric flask and the concentration was determined spectrophotometrically. Individual working standard solutions of around 0.5–9.5

µM were freshly prepared every day from individual stock standard solutions by diluting in hexane.

2.7.3. Chromatographic conditions

Several mobile phases were assayed. Methanol/ ACN (90:10 v/v) and different mixtures of methanol/THF/ water. The mobile phase was filtered through a 0.45 µm membrane, and degassed ultrasonically prior to use. The mobile phase flow rate was 0.9 ml/min. The column temperature was 30 ºC and the absorbance was read at 455 nm and for Lycopene the absorbance was read at 503. The injected volume was 50 µL. The efficiency of the separation was evaluated by the calculation of the number of plates (N), using the width of the peak at half its maximum height. The identification of the peaks was carried out by comparing the retention times with those obtained with a mixed standard solution of all-trans lycopene, b-carotene. The quantification was performed using calibration curves made with different injected amounts of all-trans-lycopene and b-carotene, in a similar proportion as in the samples (Barba et al.,2006).

2.7.4. Analysis of ß- carotene

Determination of ß – carotene was made according to the formula,

$$C\ (\mu g/g) = \frac{A_x \times C_s \times V}{A_s \times P} \quad (2)$$

Where, A_x= Carotenoid peak area, C_s= Standard concentration, A_s = Standard area, V = Total extract volume, P = Sample weight

2.8. Utilization of extracted natural colorant in dairy product preparation viz. Rasgulla

In this study, rasgulla preparation was performed by the addition of extracted and purified colour. Mainly the color obtained from the carrot (beta carotene) and beetroot (beta lain) under the class of carotenoid was considered for the development of the product.

2.8.1. Preparation of rasgulla

The processed dairy product viz. rasgulla preparation will be represented by figure 3, where ingredients are considered as milk, lemon/citric acid, refined sugar, water and colors (from carrot, and beetroot).

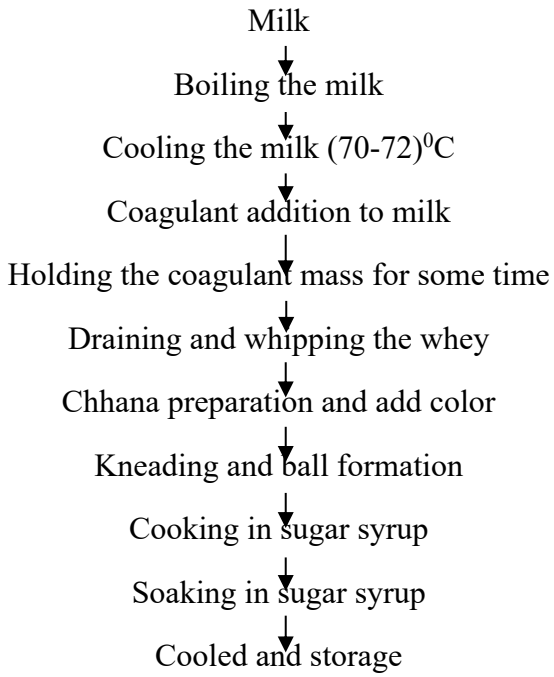

Figure 3. Preparation of rasgulla

2.9. Sensory evaluation of rasgulla (sweetened syrupy cheese ball)

A total of twenty two panelists including faculty members and students of Central Institute of Technology Kokrajhar, Kokrajhar, Assam, India rated the prepared rasgulla added with extracted natural color for preference of color, appearance, flavor, taste, texture and overall acceptability of the products. Evaluation of organoleptic properties was done by nine point hedonic rating scale from like extremely to dislike extremely according to (Ihekeronye and Ngoddy, 1985).

3. Results and Discussions

3.1.Extraction of carotenoids as natural colorant

In this study, different vegetables viz. carrot, beetroot, broccoli and tomato were considered for the extraction of natural colors viz. carotenoids e.g. beta carotene, lycopene, betalain etc. by using a mixture of acetone and hexane (1:1) for the first three and hexane only for remaining one respectively. It was suitably represented by table 1. From the experimental investigation, it was found that carrot and broccoli show maximum and minimum extraction efficiency respectively. This is due to the fact of the ability of interaction and dispersibility. The extraction efficiency of either beta carotene or lycopene by using different solvents e.g. hexane, ethanol, acetone etc. depends upon interaction effect and method of extraction (Vieira et al., 2020). Carotenoid extraction from *Bixa orellana L* was shown a lower value by using acetone as solvent (Cruz *et al.,* 2008).Extraction of carotenoids by using a combination of different solvents was shown comparatively better result than that of use of an individual solvent (Amr and Hussein,2013).

Table 1. Estimation of extraction of carotenoids from different vegetables and their yield

Sample	Weight of sample(g)	Extracted sample (ml)	Yield (%) w/v
Tomato	195.22	85	43.40±0.42
Carrot	211.10	95	45.68±0.34
Beetroot	200.65	88	42.36±0.64
Broccoli	190.55	82	41.15±0.76

3.2.Carotenoid content of different vegetables

Carotenoid is a class of natural color contains different carotenes (alpha carotene, beta carotene, Lutein, cryptoxanthin, lycopene etc.). Now different vegetables as indicated in table 2 were considered for carotenoid content (mg/100g). it was observed that the carotenoid content was found to be highest in carrot and conventionally the major fraction is beta-carotene which is the precursor of vitamin A whereas for broccoli it was minimum. Experimental investigation revealed that lycopene content in tomato was varied from 55 to 181 mg/kg (Malviya,2014), 4.31 to 5.97 mg/100 g fw (Shahzad *et al.,*2014).The amounts of major carotenoids of carrots, β-carotene and α-carotene, ranged from 29 to 130 mg/kg and from 9 to 66 mg/kg, respectively (Kocaand Karadeniz,2008; Alasavar*et al.,*2001; Hart and Scott,1995;Kidmose *et al.,*2004; Bushway,1986; Bureau and Bushway,1986; Heinonen *et al.,*1989; Konings and Romans,1997; Niizuand Rodriguez-Amaya,2005).

Table 2. Carotenoid content in vegetables

Vegetables	Carotenoid content(mg/100g)
Tomato	29.34±0.46
Carrot	37.13±0.32
Beetroot	25.64±0.74
Broccoli	23.33±0.86

3.3. Estimation of Vitamin C

Determination of vitamin C or ascorbic acid is so important in this study and represented by figure 4. The experimental result revealed that tomato and beetroot was shown the maximum i.e. 17.67mg/100g and minimumi.e.7.65mg/100gvitamin C content respectively compared to others. This is due to the inherent characteristics of these two vegetables.

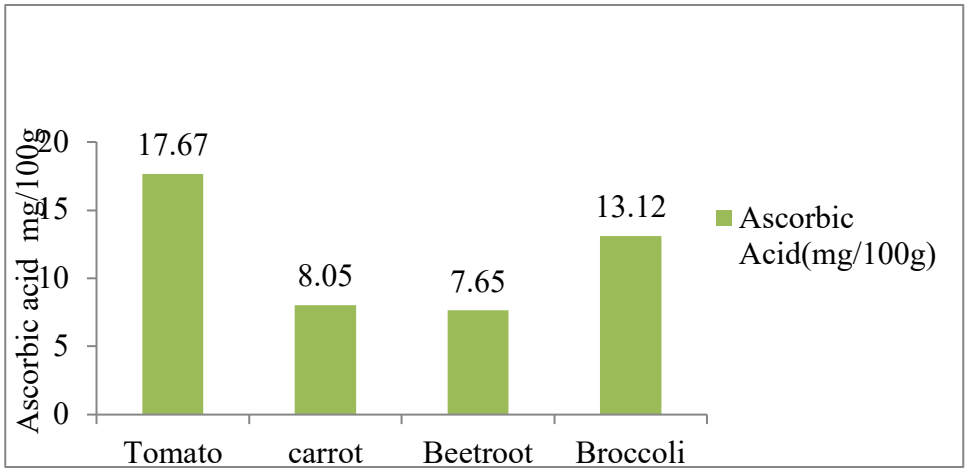

Figure 4. Graphical representation of different vegetables vs ascorbic acid

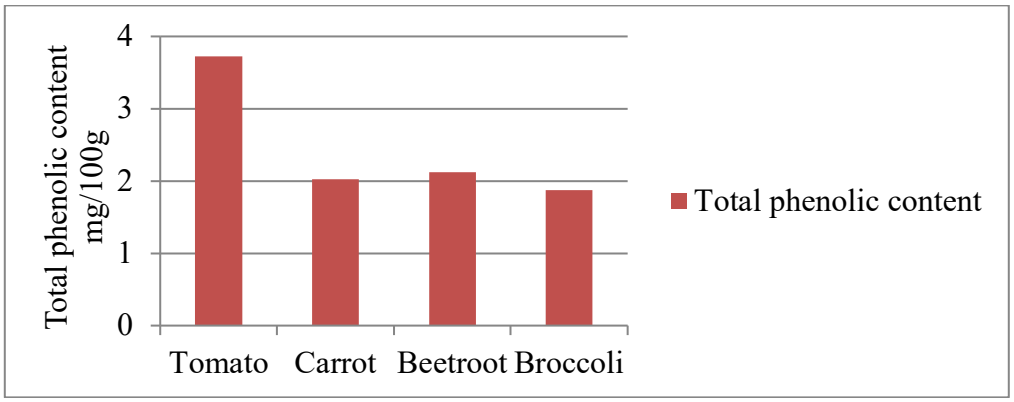

Figure 5. Graphical representation of different vegetables vs total phenolic content

3.4. Estimation of total phenolic content (TPC)

Determination of total phenolic content (mg GAE/100g) is considered to be a potential marker or indicator for antioxidant content. It acts as an indicator of health benefit. Therefore, it is represented by figure 5. Experimental investigation revealed that tomato and broccoli was shown the maximum i.e. 3.72mg/100g and minimum i.e.1.87mg/100g total phenolic content respectively compared to others. According to (Gebczynski, 2006), the total

polyphenols in fresh carrot was 20.9 mg of chlorogenic acid/100 g. However, (Vinson *et al.,*1998) found 46.4 mg of catechin/100 g FW. On the other hand, the range for total phenolics was also reported by (Kendall, 2006) as 1.1–1.6 mg of gallic acid/g for carrot samples.

3.5. Estimation of antioxidant activity by DPPH radical scavenging assay

DPPH reagent is very much useful for free radical scavenging assay of compounds viz. naturally occurring colorants extracted from vegetable resources. The nutraceutical potential of natural colorants is exhibited by the antioxidant activity. The experimental result was shown by figure 6. It was observed that carrot and beetroot were shown maximum(56.46%)and minimum (36.52%) values respectively compared to others. (Ou*et al.,* 2002) reported that the antioxidant activity varied considerably from variety to variety in carrots.(Kendall, 2006) found that antioxidant activities against ABTS cation radicals in carrots varied between 3.1 and 7.2 µmol TE/g DW.

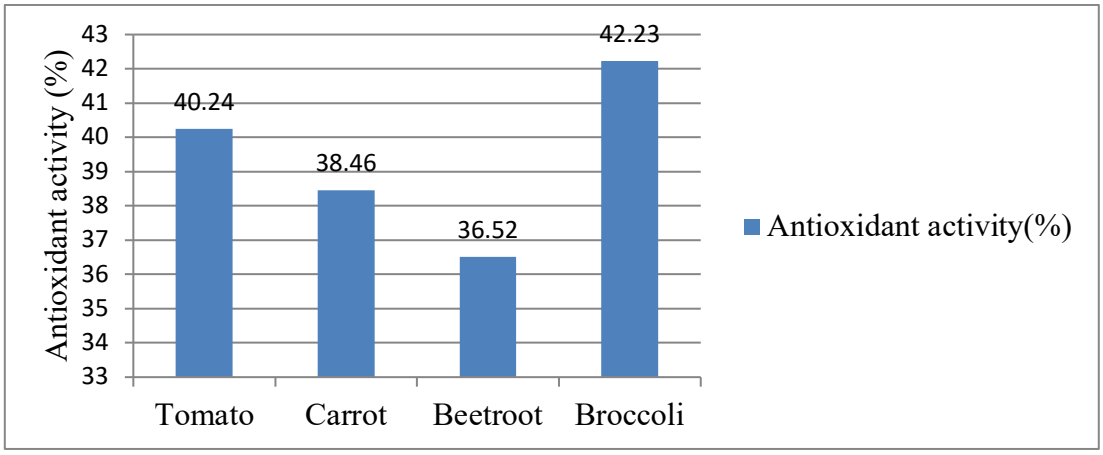

Figure 6. Graphical representation of different vegetables vs antioxidant activity (%)

3.6. HPLC analysis of extracted carotenoids from carrot

This experiment was considered to be important for the purpose of purification of extracted carotenoid.Enhancement of nutraceutical potential was occurred.Here,experimental investigation was carried out for purification of carotenoid from carrot and represented by figure 7.

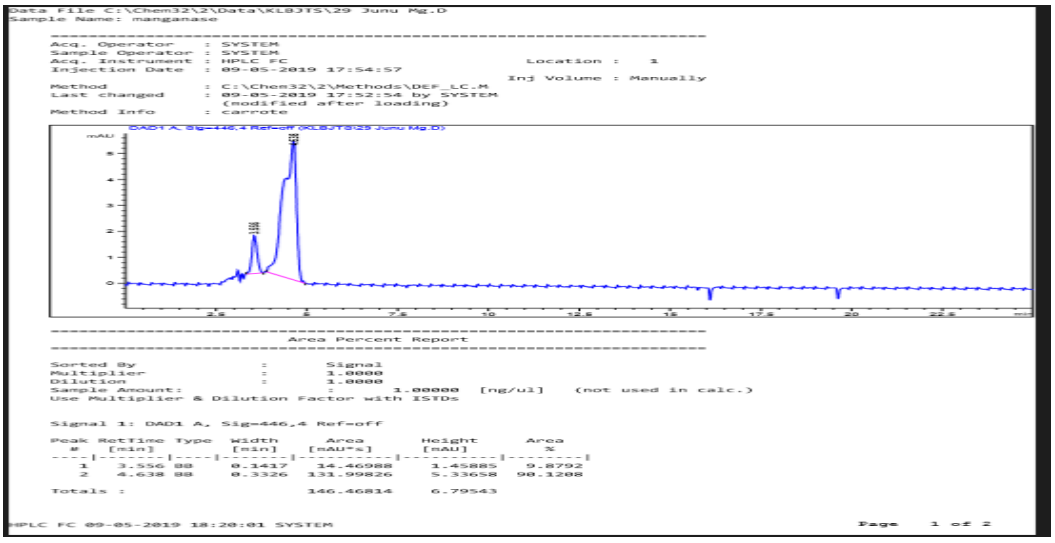

Figure 7. HPLC analysis of carotenoid (carrot)

3.7. HPLC analysis of lycopene from tomato

This experiment was considered to be important for the purpose of purification of extracted lycopene. Enhancement of nutraceutical potential was occurred. In this study, experimental investigation was carried out for purification of lycopene from tomato and represented by figure 8.

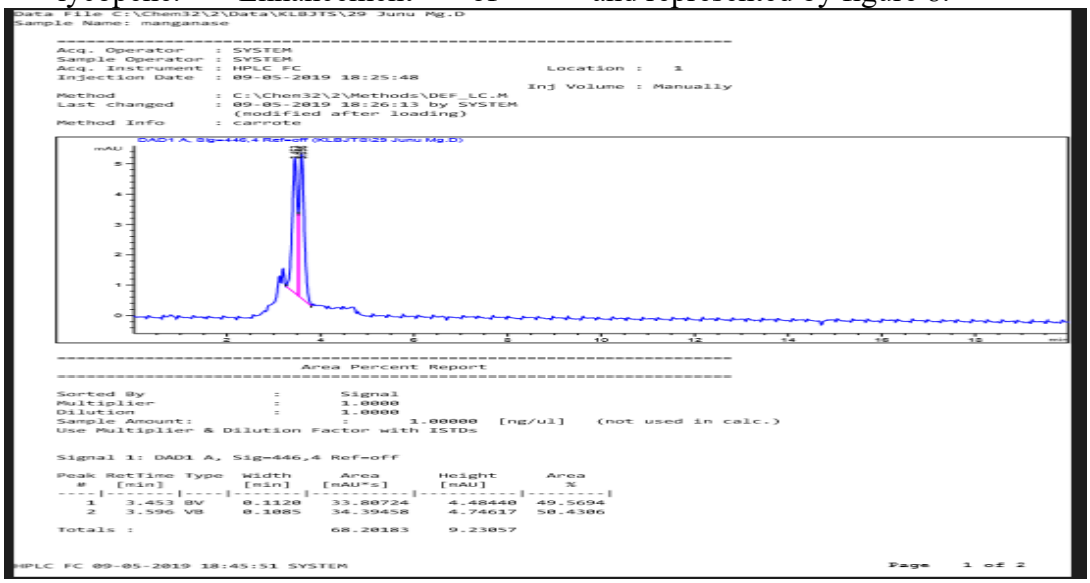

Figure 8. HPLC analysis of lycopene (Tomato)

3.8. Evaluation of sensory quality of rasgulla

The sensory analysis of rasgulla was done by 9 point Hedonic Rating Test with the help of twenty two panelists and represented by table 3. During this test natural color extracted from carrot i.e. beta-carotene and natural color extracted from beetroot i.e. betalain were added in rasgulla sample (S1) and rasgulla sample (S2) respectively**.**

Table 3. Sensory characteristics of rasgulla sample (S1) and (S2)

Parameters	**Sample (S1)**	**Sample(S2)**
Color	7.25±0.43	6.5±0.5
Appearance	6.5±0.5	6.75±0.43
Flavor	6.25±0.82	6.25±0.43
Taste	6.25±0.82	5.75±0.82
Texture	7±0.70	7±0.70
Overall acceptability	6.75±0.43	6.25±0.43

From table 3. it was evident that there was no such significant variation of overall acceptability of both the two kinds of rasgulla samples i.e. S1 and S2. However, the color ofthe rasgulla sample (S1) i.e.beta carotene added was more attractive according to hedonic rating score in comparison to the color of rasgulla sample (S2) i.e. betalain added.

4. Conclusions

The vegetables from where the colors were extracted are the source of ascorbic acid, total phenolic content and possess of significant of antioxidant activity. The antioxidant activity was found to be 40.24%, 56.46%, 36.52%, 42.23%respectively for tomato, carrot, beetroot and broccoli. Similarly, the total phenolic content was 3.72 mg GAE/g for tomato, 2.02 mg GAE/g for carrot, 2.12 mg GAE/g for beetroot and 1.87 mg GAE/g for broccoli respectively. Again, the ascorbic acid content was found to be 20.67 mg/100g, 8.05 mg/100g, 7.65 mg/100g and 15.12 mg/100g respectively for tomato, carrot, beetroot and broccoli. From this study the amount of carotenoid content in fresh tomato, carrot, beetroot and broccoli were

found as 29.34 mg/100g, 37.13 mg/100g, 25.64mg/100g and 23.33mg/100g respectively. Carotenoids extracted from carrot i.e. beta-carotene and betalain from beetroot were utilized on processed foods i.e. rasgulla preparation and sensory analysis data revealed that beta carotene added rasgulla sample (S1)was found to be satisfactory.

5. References

Annisworth, E.A., Gillespie, K. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteau reagent. *Nature protocols,* 2(4),875-877. ttps://doi.org/10.1038/nprot.2007.102.

Amr, A. S., Hussein, D. S. (2013). Tomato pomace pigment: extraction and use as food colorant. *Jordan Journal of Agricultural Sciences,* 9(1),72-85. http:// dx.doi.org/ 10.12816/0001093.

Alasalvar, C., Grigor, J.M., Zhang, D., Quantick, P.C., Shahidi, F. (2001). Comparison of volatiles, phenolics, sugars, antioxidant vitamins, and sensory quality of different colored carrot varieties. *Journal of Agricultural and Food Chemistry,* 49, 1410–1416.https://doi.org/10.1021/ jf000595h.

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. doi: 10.1016/j. foodchem.2005.02.028.

Bushway, R.J. (1986). Determination of α- and β-carotene in some raw fruits and vegetables by high performance liquid chromatography. *Journal of Agricultural and Food Chemistry,* 34, 409–412.https:// doi.org/ 10.1021/ jf00069a006.

Bureau, J., Bushway, R.J. (1986). HPLC determination of carotenoids in fruits and vegetables in the United States. *Journal of Food Science,51,*128–130.https:// doi.org/ 10.1111/j.1365-2621. 1986.tb10851. x.

Cruz, A. C. F., Santos, R. P., Iarema, L., Fernandes, K. R. G., Kuki, K. N., Araújo, R. F.,Otoni, W. C. (2008). Métodoscomparativosnaextração de pigmentosfoliares de trêshíbridos de Bixa orellana L. *RevistaBrasileira de Biociências,* 5(2), 777-779.

Gebczynski, P. (2006). Content of selected antioxidative compounds in raw carrot and in frozen product prepared for consumption. *Electronic Journal of Polish Agricultural Universities,*9(3),art 03

Hidalgo, A., Brandolini, A., Canadanovi´c B. J., Cetkovi´c, G..,TumbasŠ.V. (2018). Microencapsulates and extracts from red ´ beetroot pomace modify antioxidant capacity, heat damage and colour of pseudocereals-enriched einkorn water biscuits. *Food Chemistry,* 268, 40–48. https://doi.org/10.1016/j. foodchem.2018.06.062.

Hart, D.J.,Scott, K.J. (1995). Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. *Food Chemistry,* 54, 101–111. https://doi.org/10.1016/0308-8146(95)92669-B.

Heinonen, M.I., Ollilainen, V., Linkola, E.K., Varo, P.T., Koivistoinen, P.E. (1989). Carotenoids in Finnish foods: Vegetables, fruits, and berries. *Journal of Agricultural and Food Chemistry,* 37, 655–659. https://doi.org/10.1021/jf00087a017.

Ihekeronye, A.I.,& Ngoddy, P. O. (1985).Integrated Food Science and Technology for the Tropics. London: McMillan.p.165-193.

Kidmose, U., Hansen, S.L., Christensen, L.P., Edelenbos, M., Larsen, E., Nørbæk, R. (2004). Effects of genotype, root size, storage and processing on bioactive compounds in organically grown carrots (*Daucus carota L.*). *Journal of Food Science,* 69,388–394.https:// doi.org/ 10.1111/ j.1365-2621.2004.tb09955.

Koca, N., Karadeniz, F. (2008). Changes of bioactive compounds and antioxidant activity during cold storage of carrots. *International Journal of Food Science and*

*Technology,*43, 2019–2025. http://dx.doi.org/10.1111/j.1365-2621.2008.01811. x.

Kendall, P.A. (2006). Enhancing the market competitiveness of Colorado vegetables. http://www.colostate.edu/Dept/AES/projs/637.html.

Kaur, G., Goswami, T. K. (2018). Effect of defatted soy flour and concentration of stevia on physico-chemical and sensory characteristics of rasgulla. *Asian Journal of Dairy & Food Research,* 37(3),187-191. DOI: 10.18805/ajdfr. DR-1358.

Konings, E.J.M., Romans, H.H.S. (1997). Evaluation and validation of an LC method for the analysis of carotenoids in vegetables and fruit. *Food Chemistry,* 59, 599–603. https://doi.org/ 10.1016/S0308-8146(96)00343-3.

Labuschagne, P.(2018). Impact of wall material physicochemical characteristics on the stability of encapsulated phytochemicals: A review. *Food Research International,* 107,227–247. DOI: 10.1016/j.foodres.2018.02.026.

Lin, W.S., He, P.H., Chau, C.F., Liou, B.K., Li, S., Pan, M.H.(2018). The feasibility study of natural pigments as food colorants and seasonings pigments safety on dried tofu coloring. *Food Science and Human Wellness,* 7, 220–228.https:// doi.org/ 10.1016/j. fshw.2018.09.002.

Meshram, M. S., Itankar, P. R., Patil, A. T.(2013). To Study Antidiabetic Activity of Stem Bark of Bauhinia purpurea. *Journal of Pharmacognosy and Phytochemistry,* 2(1),171-175.

Malviya, N.(2014). Isolation and Quantification of Lycopene from Watermelon, Tomato and Papaya. *Research Journal of Recent Sciences,* 3,68-70.

Niizu, P.Y., Rodriguez-Amaya, D.B. (2005). New data on the carotenoid composition of raw salad vegetables. *Journal of Food Composition and Analysis,* 18, 739–749. https://doi.org/ 10.1016/j.jfca.2004.09.001.

Nwoba, E.G., Ogbonna, C.N., Ishika, T., Vadiveloo, A. (2020). Microalgal Pigments: A Source of Natural Food Colors. In Microalgae Biotechnology for Food, Health and High Value Products; AsrafulAlam, M., Xu, J.-L., Wang, Z.(ed). Springer: Singapore.p. 81–123. https://doi.org/10.1007/978-981-15-0169-2_3.

Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J.A.,Deemer, E.K. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. *Journal of Agricultural and Food Chemistry,*50, 3122–3128. https://doi.org/10.1021/jf0116606.

Potera, C. (2010). Diet and nutrition: The Artificial Food Dye Blues. *Environmental Health Perspective,* 118(10), 428. http://dx.doi.org/10.1289/ehp.118-a428.

Rebecca, L. J., Sharmila, S., Das, M. P., Seshiah, C. (2014). Extraction and purification of carotenoids from vegetables. *Journal of Chemical and PharmaceuticalResearch,*6(4), 594-598.

Rodriguez-Amaya, D.B. (2016). Natural food pigments and colorants. *Current Opinion in Food Science,* 7, 20–26. https:// doi.org/ 10.1016/j.cofs.2015.08.004.

Rodriguez-Amaya, D.B. (2019a). Natural food pigments and colorants. In: Merillon, J.M.& Ramawat K.G. (Ed.), Bioactive molecules in food. Reference Series in Photochemistry. (pp.1-35), Springer International Publishing: Switzerland.

Rodriguez-Amaya, D.B. (2019b). Natural Food Pigments and Colorants. In: Bioactive Molecules in Food; Mérillon, J.M.& Ramawat, K.G. (Ed.),Reference Series in Phytochemistry. (pp.867-901), Springer International Publishing: Switzerland.

Roldan-Gutierrez, J.M., Luque de Castro, M.D. (2007). Lycopene: The need for better methods for characterization and determination. *Trends in Analytical Chemistry,* 26, 163–170.http://dx.doi.org/ 10.1016/j.trac.2006.11.013.

Rao, A.V.&.Agarwal, S.(1999). Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases. A review.*Nutrition Research,* 19(2), 305-323.https:// doi.org/ 10.1016/S0271-5317(98)00193-6.

Ravichandran, K., Thaw Saw, N.M.M., Mohdlay, A.A., Smetanska, I. (2013). Impact of processing of red beet on betalain content and antioxidant activity,*FoodResearch International,*50(2),670–675. https: // doi.org/10.101 6 / j. foodres. 2011.07.002.

Shi, J., Maguer, M. L. (2000). Lycopene in tomatoes: chemical and physical properties affected by food processing. *Critical reviews in food science and nutrition,* 40(1), 1-42. doi: 10.1080/10408690091189275.

Shadaksharaswamy, M., & Manay, N. S. (2011). Food, facts and principles. (2nd ed). New Delhi: New Age international publisher,520p.

Stintzing, F.C., Carle, R. (2007). Betalains – emerging prospects for food scientists. *Trends in Food Science and Technology,*18(10),514–525. http://dx.doi.org/10.1016/ j. tifs.2007.04.012.

Sharma, O. P., Bhat, T.K. (2009). DPPH antioxidant assay revisited. *Food Chemistry,* 113(4), 1202-1205. https://doi.org/10.1016/ j. foodchem.2008.08.008.

Shahzad, T., Ahmad, I., Choudhry, S., Saeed, M.K., Khan, M.N. (2014). DPPH free radical scavenging activity of tomato, cherry tomato and watermelon: lycopene extraction, purification and quantification. International *Journal of Pharmacy and Pharmaceutical Sciences,* 6(2), 223-228.

Stahl, W., Junghans, A., De Boer, B., Driomina, E.S., Briviba, K., Sies, H.(1998). Carotenoid mixtures protect multilamellar liposomes against oxidative damage: synergistic effects of lycopene and lutein. *FEBS Letter,* 427(2), 305-308. doi: 10.1016/s0014-5793(98)00434-7.

Vinson, J.A., Hao, Y., Su, X.,Zubik, L. (1998). Phenol antioxidant quantity and quality in foods: vegetables. Journal of Agricultural and *Food Chemistry,* 46(9),3630–3634.http:// dx.doi.org/10.1021/jf980295o.

Vieira, D.A.P., Caliari, M., DeSouza, E.R.B.D.,Junior, M.S.S. (2020). Methods for and pigments extraction and determination of color in tomato for processing cultivars. Food Science and Technology. *Food Science and Technology Campinas,40(1),*11-17. DOI: https://doi.org/10.1590/fst.42217.

Yen, G.C., Duh, P.D. (1994). Scavenging effect of methanolic extract of peanut hulls on free radical and active oxygen species. *Journal of Agricultural and Food Chemistry, 42(3),* 629-632.https://doi.org/10.1021/jf00039a005.

Acknowledgment

Authors are grateful to the Department of Food Engineering&Technology, Central Institute of TechnologyKokrajhar, Kokrajhar, Assam, India for providing the necessary laboratory facilities to carry out this research work.