American Journal of Food and Nutrition, 2019, Vol. 7, No. 2, 55-63
Available online at http://pubs.sciepub.com/ajfn/7/2/4
Published by Science and Education Publishing
DOI:10.12691/ajfn-7-2-4

Extraction and Quantification of Lycopene, β-Carotene and Total Phenolic Contents from Papaya (*Carica papaya*) and Formulation of Lycopene Enriched Fruit Drinks

M. Rayhan[1], B. Mumtaz[2], M. Motalab[2], M.A. Zubair[1], M. Z. Haque[2], B. K. Saha[2,*]

[1]Department of Food Technology and Nutritional Science, Mawlana Bhashani Science and Technology University, Tangail, Bangladesh
[2]Institute of Food Science and Technology, Bangladesh Council of Scientific and Industrial Research, Dhaka, Bangladesh
*Corresponding author: bksbcsir@yahoo.com

Received June 10, 2019; Revised July 10, 2019; Accepted July 19, 2019

Abstract The study was conducted on extraction and quantification of lycopene, β-carotene and total phenolic contents from edible parts of papaya fruits and formulation of two lycopene enriched fruit drinks. Different solvent extraction methods were used and solvent extracts were analyzed by spectrophotometer. Significant differences were observed among the lycopene, β-carotene and total phenolic contents of papaya fruits in different solvent extractions. Hexane:ethanol:acetone (2:1:1) solvent extract contain the highest quantity of lycopene, i.e. 1.02 mg/100g of fresh weight (F.W), which is significantly higher than ethyl acetate extract (0.03mg/100g of F.W). The β-carotene level was highest in acetone-petroleum ether extract (4.22 mg /100g of F.W) which is significantly more than ethyl acetate extract (0.17 mg/100g of F.W). Soxhlet extraction using ethyl acetate has a comparatively higher content of β-carotene (1.50mg/100g of F.W) than ethyl acetate with normal extraction (0.17mg/100g of F.W.). Soxhlet extraction has a comparatively higher content of total phenolic contents than normal solvent extraction. Soxhlet extraction with ethyl acetate has the highest content of total phenolic (5.67 mg/100g of F.W.) which is significantly more than ethyl acetate with normal extraction (1.64 mg/100g of F.W.). Two lycopene enriched fruit drinks have been formulated where tomato puree and watermelon pulp were added with papaya pulp as a natural lycopene source. Formulation of fruit drink -1 (Lycopene enriched papaya drink) tomato puree of 10 % added with 20% papaya pulp and formulation of fruit drink-2 (Lycopene enriched mixed fruit drink) 10% papaya, 10% tomato and 10 % watermelon were added. The total pulp content of the fruit drinks formulations was maintained at 30 %. Lycopene enriched mixed fruit drink has the highest lycopene content (3.9mg/100 ml) which is significantly more than lycopene enriched papaya drink (1.60 mg /100 ml). But in sensory evaluation lycopene enriched papaya drink is more acceptable than mixed fruit drink. Physicochemical and microbiological quality of the lycopene enriched fruit drinks indicated that the fruit drinks were acceptable during three months of storage at room temperature. No significant changes were found in total soluble solids, titrable acidity and pH during storage of lycopene enriched papaya drink. But reducing sugar and total sugar were increased and ascorbic acid content was decreased with increase in storage period.

Keywords: *Lycopene, β-carotene, total phenolic content, papaya, lycopene enriched fruit drinks, physicochemical and microbiological quality, sensory evaluation*

Cite This Article: M. Rayhan, B. Mumtaz, M. Motalab, M.A. Zubair, M. Z. Haque, and B. K. Saha, "Extraction and Quantification of Lycopene, β-Carotene and Total Phenolic Contents from Papaya (*Carica papaya*) and Formulation of Lycopene Enriched Fruit Drinks." *American Journal of Food and Nutrition*, vol. 7, no. 2 (2019): 55-63. doi: 10.12691/ajfn-7-2-4.

1. Introduction

Papaya (*Carica papaya* L.) belongs to the Caricaceae family [1,2]. It is a medium sized fruit commonly found in Bangladeshi market. Papaya is one of the indigenous fruits that is grown in many parts of Bangladesh. Fruits and vegetables are the rich sources of carotenoid. Among the carotenoids family β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin are the most abundant [3]. Lycopene is a carotenoid, found in different fruits and vegetables like tomatoes, papaya, mangoes, watermelon and processed vegetable juices. Lycopene and analogs are antioxidants, which help to prevent and repair damaged cells [4]. There are different types of papayas which are found in different parts of the world.

There are two majors colors for the papaya fruit flesh: The red and the yellow. These colors are basically controlled by a genetic locus where the red is dominated by the yellow [5]. The carotenoid contained in a fresh fruit largely determines the color of the flesh. A papaya fruit that has a red flesh is said to contain a bit high levels of lycopene unlike the yellow one [6]. Several epidemiological studies reported that lycopene-rich diets have beneficial effects on human health [7]. The antioxidant activity of lycopene can help protect against degenerative diseases by neutralizing free radicals in the body, thus preventing DNA damage in the cells and improving cell function. High levels of lycopene in the blood and fatty tissues are associated with reduced risk of cancer, heart disease and macular degeneration [8].

Fruits that are orange in color are rich in β-carotene. Such fruits include grapefruit (*Citrus paradise* Macfad.), persimmon (*Diospyros sp.*), guava (*Psidiumguajava*L.), papaya (*Carica papaya* L.), watermelon (*Citrulluslanatus*.) and mango (*Mangiferaindica*L.) [9]. β-Carotene is an antioxidant which helps to reduce the risk of some heart diseases, eye disorders and skin diseases [10]. β-Carotene is also known as a stimulant of the immune system, lowering serum cholesterol level and can be converted into vitamin A by the body [11].

Phenolics compounds are very important plant constituents exhibiting antioxidant activity by inactivating lipid free radicals or by preventing the decomposition of hydro peroxides into free radicals [12,13]. Honey is recognized as having different biological properties, including antioxidant effects. Phenolic acids and flavonoids are the main antioxidants in this apiary product [14,15]. Data on the antioxidant activity of honey from Malaysia suggest that the phenolics are responsible for free-radical scavenging and antioxidant activity [16].

The photo protective effects of synthetic lycopene were compared with tomato extract (Lyc-o-Mato R) and a beverage with lyco-o-Mato (Lyc-o-Guard-drink) by feeding volunteers with 10 mg lycopene per day [17]. Blended guava beverage contained ascorbic acid of 24.7 mg/100 g and carotene of 303.7 µg/100 g which was acceptable after six months of storage at room temperature [18]. Probiotic dairy beverages with acceptable flavors were reportedly developed with fermented buffalo milk. The fermented milk beverages fortified with carrot and pumpkin were found to be a good source of beta-carotene, whereas addition of tomato increased lycopene levels and addition of strawberries, black mulberries and red grapes increased anthocyanin levels [19]. It is evident from the study that pink guava beverage could be fortified with tomato puree for increasing the lycopene content and sensory quality of beverage. Guava fortified beverage developed, contained lycopene pigment as a source of natural color which improved the nutritional and sensory quality of the beverage [20].

The present study was conducted to provide idea about effective lycopene extraction method. Formulated lycopene enriched fruit drinks are rich source of micronutrients and lycopene, thus can be helpful to fight against micronutrient deficiency diseases in our country.

2. Materials and Methods

2.1. Sample Collection and Preparation

2.1.1. Sample Collection

The fresh, mature and ripe papayas were collected from the local market. The pulp from a single variety of papaya was used for Lycopene, β-carotene, Total Phenolic Content (TPC) extraction and others analysis. Chemicals (Analytical grade quality) and distilled deionized water were used from IFST, BCSIR Laboratory for the analysis.

For the preparation of Lycopene enriched fruit drinks, ripe tomato and watermelon were also collected from local market.

2.1.2. Sample Preparation

Papaya pulps were prepared from ripen papaya using a pestle mortar. Lycopene enriched fruit drinks samples were prepared from fruits pulps of ripen fruits.

2.1.3. Sample Preparation for Soxhlet and Methanol Extraction

After peeling, ripen papaya fruits were sliced. The papaya slices were placed in a freeze dryer system for 24 hours. After drying, samples were ground to a powder using grinder.

2.2. Physicochemical and Nutritional Analysis

The pH was determined with a digital pH-meter and titratable acidity was estimated with the visual acid-base method [21]. The moisture content was determined by the digital moisture analyzer (AnD MX-50). The total soluble solid (TSS) was determined with a hand refractometer (iTavah, COMINHKPR121676). Crude fiber, total fat was determined by the standard AOAC methods (978.10; 920.39) and the estimation of total protein was made by the method described -by Kirk and Sawyer [22]. The content of total carbohydrate and energy was determined by the method [23]. Ascorbic acid was determined by using the standard AOAC methods (967.21). Ash was determined by the process [21]. Minerals and heavy metals were determined by Atomic Absorption Spectrometric method described -by Kirk and Sawyer [22].

2.3. Microbiological Analysis

Enumeration of coliforms was done by the Most Probable Number (MPN) method. After incubation, the positive gas production tubes were recorded and results computed using MPN chart as total coliform number per milliliters [24]. The calculated results were expressed as colony forming unit (CFU) per milliliters of lycopene enriched fruit drinks [25]. Plates, which contained 30-300 developing yeast and mould colonies, were selected and counted. The arithmetic average of the two counts for every dilution was taken and multiplied by the respective dilution factor to get the result. The calculated result expressed as CFU per milliliters of lycopene enriched fruit drinks [25].

2.4. Extraction of Lycopene by Different Solvents

2.4.1. Acetone-petroleum Ether Extraction Method

Ten grams of sample was weighed and extracted repeatedly with acetone using pestle and mortar until the residue became colorless. The acetone extract was transferred to a separating funnel containing 10–15 ml of petroleum ether and mixed gently. Carotenoid pigments were collected in the petroleum ether fraction by diluting the acetone with water. The lower phase was transferred to another separating funnel and the petroleum ether extract containing pigments to an amber colored bottle. Extraction was repeated similarly with petroleum ether until it was colorless. To the petroleum ether extract, a small quantity of anhydrous Na_2So_4 was added and the volume was made up to 50 ml. The color was measured in a quartz cuvette at 503 nm in spectrophotometer (SP-UV500DB, UV-VIS Spectrophotometer) using petroleum ether as a blank. Lycopene content of the sample was calculated by using the relationship that optical density (OD) of 1.0-3.1206 μg of lycopene per ml [17].

$$Lycopene\ content\ (mg/100g) = \frac{3.1206 \times OD\ of\ sample \times volume\ made\ up\ to \times 100}{1 \times Weight\ of\ the\ sample \times 1000}$$

2.4.2. Ethyl acetate Extraction Method

Two hundred grams of papaya pulp was kept in a cloth for removing water. Then the paste was poured in a separating funnel and about 180 ml ethyl acetate was added. The mixture was shaken vigorously and kept for 24 hours. After 24 hours, the upper layer was separated in a beaker and was kept in a water bath for evaporation of the solvent. The beaker containing lycopene extracts were covered with aluminum foil and stored in the freezer until further analysis. Lycopene was determined from the ethyl acetate extract [26]. One hundred milligrams of extract were mixed with 10 ml of acetone–hexane mixture (4:6) for 1 min and filtered. The absorbance was recorded at three different wavelengths (453, 505 and 663 nm). The lycopene content was calculated by:

$$Lycopene\ (mg/100\ mL) = -0.0458 \times A663 + 0.372 \times A505 - 0.0806 \times A453.$$

The results were presented as mg of Lycopene /g of extract and mg of Lycopene /100 g raw pulp.

2.4.3. Hexane: Ethanol: Acetone Extraction Method

Lycopene was extracted and quantified following the established method with some modifications [27]. Started with papaya juice, using a 1000 µL micropipette was used to take the sample. The sample was dispensed into a 15 ml centrifuge tube and 8.0 ml of hexane: ethanol: acetone (2:1:1) was added. The tube was caped, and the centrifugation was performed immediately followed by incubation. After at least 10 minutes or as long as several hours later, 1.0 ml water was added to each sample and centrifugation was carried out again. The samples were left for 10 minutes to allow phases to separate and all air bubbles to disappear. The absorbance of the upper phase was determined by the spectrophotometer at 503nm wavelength. Lycopene level in the hexane extracts was calculated according to:

$$Lycopene\ (mg/kg\ fresh\ wt.) = A503\ x\ 171.7/W$$

Where W is the exact weight of samples pulp added, in grams.

2.5. Extraction of β-carotene and Total Phenolic Content (TPC) by Different Solvents Extraction Method

2.5.1. Ethyl acetate Extraction Method

Two hundred grams paste of papaya fruit were kept in a cloth for removing water. Then the paste poured in a separating funnel and about 180 ml ethyl acetate was added. The mixture was shaked vigorously and kept still for 24 hours. Then upper layer was separated in a beaker and kept in a water bath for evaporation of solvent.

2.5.2. Benzene Extraction Method

One hundred grams of papaya pulp were taken in a 250 ml beaker and about 30 ml of warm benzene was added to it. The solution was well-stirred and the benzene layer was decanted. Again, 30 ml warm benzene was added stirred and decanted the benzene. This was done about five times. Then benzene was distilled off and residue of β-carotene and total phenolic content was obtained. The residue was recrystallized by ether and weighed [28].

2.5.3. Acetone-petroleum Ether Extraction Method

Sample (1.0-1.5 g powder) was extracted with 10 mL acetone-petroleum ether (50% v/v). The upper β-carotene and total phenolic content containing organic layer were removed using a pipette and collected in a test tube. Extraction was repeated. The extracts were combined, washed with 15 mL saturated aqueous sodium chloride (NaCl) and the aqueous wash was removed with a micropipette. The extract was washed with 10mL of 10% aqueous potassium carbonate (K2CO3) and the aqueous wash was removed. The β-carotene and total phenolic content containing organic layer were dried with calcium chloride. The excess solvent was allowed to evaporate at room temperature for a few minutes in the dark. The tubes containing β-carotene and total phenolic content extracts were covered with aluminum foil and stored in the freezer until further analysis [29].

2.5.4. Methanol Extraction Method

Five grams of papaya powder was dehydrated by adding 65 ml methanol and shaken vigorously. After 2 hr, the thick suspension was filtered; the dark red cake was shaken for another 15 min with 75 ml mixture of an equal volume of methanol and carbon tetrachloride and separated by filtration. The carbon tetrachloride phase was transferred to a separating funnel with one volume of water and was shaken well. After phase separation, the carbon tetrachloride phase was evaporated and the residue was diluted with about 2ml of benzene. The crystals were washed ten times using benzene and boiling methanol [30].

2.5.5. Soxhlet Extraction

β-Carotene and Total Phenolics were extracted from dried papaya paste powder by Soxhlet (solvent) extraction. Duplicate, accurately weighed samples of dried papaya powder (approx. 5 g) were placed in cellulose extraction thimbles. The thimbles were placed in the Soxhlet extraction unit and extracted for 20 hours with about 150 ml of solvents. Ethyl acetate, petroleum ether, n-Hexane solvents were used.

2.6. Determination of β-carotene

β-carotene was determined from the four different solvent extract and three Soxhlet solvent extract according to Kumara et al. [26]. One hundred milligrams of extract were mixed with 10 ml of acetone–hexane mixture (4:6) for 1 min and filtered. The absorbance was recorded at three different wavelengths (453, 505 and 663 nm). The β-carotene content was calculated by:

$$\beta - carotene\ (mg/100\ mL)$$
$$= 0.216 \times A663 - 0.304 \times A505 + 0.452 \times A453.$$

The results were presented as mg of β-carotene/g of extract and mg of β-carotene /100 g pulp.

2.7. Determination of Total Phenolic Content (TPC)

Total phenolic content was determined from the four different solvent extract and three Soxhlet solvent extract following the established method described by Chanda *et al*. with some modifications [31]. 0.5 ml of extract (concentration of extract is 1.072 mg/ml) and 0.5 ml of Folin-Ciocalteu reagent (0.5 N) were mixed and incubated at room temperature for 5 min. Then 2.0 ml saturated sodium carbonate was added and further incubated for 30 min at room temperature and absorbance was measured at 765 nm. Gallic acid [32] was used as a positive control. TPC was extrapolated by using the linear equation y=98.42x-0.744 (R square is 0.986) where x is absorbance and y is a concentration of Gallic acid (µg/mL).

2.8. Lycopene Enriched Fruit Drinks Formulations

Papaya, watermelon and tomato were purchased from the local fruit and vegetable shop of Dhaka city. Ready for consumption and were transported to the laboratory. Samples were washed and then crushed in a blender. A paste was made separately for each of the three fruits. Tomato puree and watermelon pulp were added with papaya pulp as a natural lycopene source. Fruit drink -1 (lycopene enriched papaya drink) was formulated by adding 10% tomato puree to 20% papaya pulp and fruit drink-2 (lycopene enriched mixed fruit drink) was formulated by adding 10% tomato and 10 % watermelon to 10% papaya.eThe total pulp content of the fruit drinks formulations was maintained at 30 %. Sugar syrup was prepared by weighing cane sugar, citric acid and water. Papaya pulp alone or in combination with tomato puree or watermelon pulp were weighed according to the formulation and added to the syrup. The fruit drinks formulation was blended in a homogenizer, hot filled into plastic bottles of 250 ml capacity and sealed with a cork.

2.9. Sensory Evaluation of Fruit Drinks

Sensory analysis of formulated fruit drinks were carried out using Quantitative difference tests—'Numerical scoring test'. The samples were assessed for color, flavor, texture and overall quality by a six-member trained panel, on a 9 point scale, where 9=Like extremely, 8=Like very much, 7=Like moderately, 6=Like slightly, 5=Neither like nor dislike, 4=Dislike slightly, 3=Dislike moderately, 2=Dislike very much and 1=Dislike extremely[33]. Samples receiving an overall quality score of 7 or above were considered acceptable and those receiving below seven were considered unacceptable.

2.10. Statistical Analysis

Statistical analyses were carried out using Python programming language (version 3.6.6). The results obtained from the experiment are reported as mean values (obtained from the three replications) ± standard deviation (SD). The significant differences among the samples were analyzed using p values obtained from two-tailed t-tests with significance level of $p<0.05$.

3. Results and Discussion

3.1. Physico-chemical and Nutritional Properties

Physicochemical characteristics of papaya pulp and lycopene enriched fruit drinks were shown in Table 1. Papaya pulp had total soluble solids 10±0.04%, titrable acidity 0.19±0.08% and moisture content of 86.13±0.07%. It is evident that both drinks show significant difference at different parameters such as in lycopene enriched papaya fruit drink moisture, ash, total soluble solid, total sugar were 83.34±0.03%, 0.23±0.02%, 13.00±0.02% and 12.80±0.31% respectively, whereas in lycopene enriched mixed fruit drinks moisture, ash, total soluble solid, total sugar were 76.42±0.02%, 0.36±0.03%, 21.00±0.03% and 20.00±0.23% respectively.

The results of the nutritional analysis are given in Table 2. Papaya pulp had carbohydrate, protein and crude fiber of 10.19±0.39%, 1.32±0.20% and 1.60±0.12% respectively. Carbohydrate, protein and fiber yield 7.2 %, 0.6 % and 0.8 % respectively were earlier reported in ripe papaya [34]. It is evident that the physicochemical parameters of papaya varieties differed from one another which are supposed to be due to different genetic make-up, the variety and also because of the difference in their total fruit development and ripening period [35].There was a significant difference between two fruit drinks for energy and carbohydrate content. In lycopene enriched papaya drink energy and carbohydrate content were 65.93±0.36kcal and 16.09±0.27% respectively, whereas in lycopene enriched mixed fruit drinks energy and carbohydrate content were 92.97±0.21kcal and 22.78±0.35% respectively.

3.1.1. Trace Elements, Minerals and Heavy Metals

Important trace element and mineral contents were shown in Table 3. An essential trace element is a dietary element that is needed in very minute quantities for the proper growth, development and physiology of the organism. The trace elements that were found in selected samples are iron, manganese and zinc but copper was not found. These papaya pulp and lycopene enriched fruit drinks were enriched with minerals like sodium, potassium, iron, zinc. Minerals play an important role in maintaining proper function and good health in the human body.

Heavy metals are harmful and become toxic to health if they are taken above the limit of daily dietary allowance recommended. Heavy metals such as cadmium (Cd), arsenic (As) and lead (Pb) are highly poisonous. In ready-to-drink beverages permissible limits are zinc 5.0 ppm, copper 2.0 ppm and lead 2.0 ppm [36]. Results of Table 4 showed that concentration of lead (Pb) in lycopene enriched fruit drinks were below the detection limit and arsenic (As) and cadmium (Cd) were not present, so they are safe for consumption.

Table 1. Physicochemical Composition of papaya fruits and Lycopene enriched fruit drinks

Parameter	papaya pulp (Per 100g)	Lycopene enriched papaya fruit drink(Per 100 ml)	Lycopene enriched mixed fruit drinks(Per 100 ml)
Moisture (%)	86.13±0.07 [A]	83.34±0.03 [B]	76.42±0.02 [C]
Ash (%)	0.52±0.08 [A]	0.23±0.02 [B]	0.36±0.03 [A]
Total soluble solid (ºBrix)	10.00±0.04 [A]	13.00±0.02 [B]	21.00±0.03 [C]
Total sugar (%)	9.02±0.51 [A]	12.80±0.31 [B]	20.00±0.23 [C]
Reducing sugar (%)	4.20±0.14 [A]	4.16±0.18 [A]	6.68±0.26 [C]
pH	4.60±0.02 [A]	3.90±0.03 [B]	4.00±0.01 [C]
Acidity (%)	0.19±0.08 [A]	0.18±0.05 [A]	0.14±0.06 [A]
Vitamin-C (mg/100 g)	30.00±0.09 [A]	20.00±0.07 [B]	20.00±0.05 [B]

Note: Result were expressed as mean values ± standard deviation. Row-wise same capital letter ([A], [B], [C]) denote similar samples and different letter denote significantly different samples based on p values ($p<0.05$).

Table 2. Nutritional composition of papaya pulp and Lycopene enriched fruit drinks

Components	Papaya pulp (Per 100g)	Lycopene enriched papaya fruit drink (Per 100 ml)	Lycopene enriched mixed fruit drink (Per 100 ml)
Energy(kcal)	48.20±0.67[A]	65.93±0.36[B]	92.97±0.21[C]
Carbohydrate (%)	10.19±0.39[A]	16.09±0.27[B]	22.78±0.35[C]
Protein (%)	1.32±0.20A]	0.18±0.07[B]	0.26±0.09[B]
Fat (%)	0.24±0.03[A]	0.09±0.03[B]	0.09±0.02[B]
Crude Fiber (%)	1.60±0.12[A]	0.07±0.03[B]	0.08±0.02[B]
Total Mineral (%)	0.52±0.05[A]	0.23±0.02[B]	0.36±0.04[C]
Lycopene(mg)	1.02±0.17[A]	1.60±0.31[A]	3.90±0.23[C]

Note: Result were expressed as mean values ± standard deviation. Row-wise same capital letter ([A], [B], [C]) denote similar samples and different letter denote significantly different samples based on p values ($p<0.05$).

Table 3. Mineral content in papaya pulp and Lycopene enriched fruit drinks

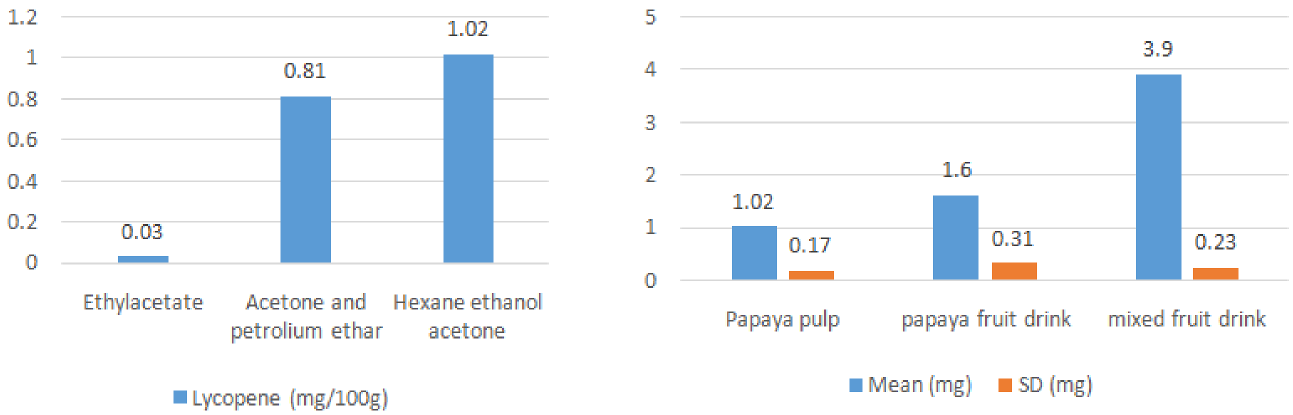
Constituents	Papaya pulp (mg Per 100 g) (Mean±SD)	Lycopene enriched papaya fruit drink (mg Per 100 ml) (Mean±SD)	Lycopene enriched mixed fruit drink (mg Per 100 ml) (Mean±SD)
Iron(Fe)	0.73±0.09[A]	0.19±0.07[B]	0.36±0.06[B]
Sodium(Na)	31.06±0.15[A]	43.78±0.19[B]	20.37±0.12[C]
Potassium(K)	88.14±0.21[A]	17.72±0.11[B]	2.9±0.13[C]
Copper(Cu)	ND*	ND	ND
Zinc(Zn)	0.25±0.07[A]	0.42±0.05[A]	0.07±0.03[C]*
Manganese(Mn)	0.15±0.09[A]	0.05 ±0.02[A]	0.05±0.01[A]

Note: Result were expressed as mean values ± standard deviation. Row-wise same capital letter ([A], [B], [C]) denote similar samples and different letter denote significantly different samples based on p values ($p<0.05$).
* ND= Not Detected
[C]* - zinc in Mixed fruit drinks is significantly different from papaya fruit drinks ($p<0.05$) but is similar in papaya pulp ($p>0.05$)

Table 4. Heavy metal test of Lycopene enriched fruit drinks

Name of fruit drinks	Lead(Pb) (Mean±SD)	Arsenic(As) (Mean±SD)	Cadmium(Cd) (Mean±SD)
Lycopene enriched Papaya drinks (ppm)	0.045±0.004	ND*	ND
Lycopene enriched Mixed fruit drinks (ppm)	0.052±0.005	ND	ND

*ND= Not Detected.



(A) Lycopene content in papaya fruits by using different solvent extraction method.

(B) Lycopene content in papaya pulp and fruits drinks

Figure 1. Spectrophotometric result of Lycopene in papaya fruits and Lycopene enriched fruit drinks

3.2. Lycopene Content in Papaya Fruits and Lycopene Enriched Fruit Drinks

Figure 1(A) shows the spectrophotometric result of lycopene extraction with different solvent extraction from yellow-fleshed papaya pulp. Hexane:ethanol:acetone extract has the highest lycopene content i.e. 1.02 mg /100 g papaya paste which is significantly more than ethyl acetate extract. Acetone petroleum ether and ethyl acetate extract have 0.81mg/100g and 0.03 mg/100g lycopene content respectively, again both have a significantly different value. Neelu Malviya [31] reported that lycopene content in papaya paste 1.14 mg /100 g. A papaya fruit that has a red flesh is said to contain a bit high levels of lycopene unlike the yellow one and lycopene content especially the red-fleshed which has about 4.10 mg lycopene per 100g [6]. Lycopene enriched mixed fruit drinks has the highest lycopene content i.e. 3.9 mg /100 ml, which is significantly more than lycopene enriched papaya drink i.e. 1.60 mg /100 ml (Figure 1B). Lycopene content of tomato and watermelon increased the color intensity of fruit drinks. Fortification of lycopene from tomato puree resulted in a significant increase in the lycopene content of pink guava beverage and also improved the color and appearance of the fortified beverage [17].

3.3. β-carotene and Total Phenolic Content (TPC) in Papaya Fruits

Table 5 showed that higher amount of β-carotene (4.22±0.14 mg/100g FW) was found in acetone petroleum ether extraction while lower was found in ethyl acetate extraction. β-carotene content in methanol, n-Hexane and petroleum benzene extraction were 1.13±0.03, 1.85±0.05, 0.79±0.07 mg/100g FW respectively. Using Soxhlet extraction method higher amount of β-carotene (1.50±0.11mg/100g FW) was found in ethyl acetate extraction than ethyl acetate solvent extraction .β-carotene contents vary from 866 – 7,807 μg/100 g dry matter in ripe papaya fruits [37]. It is as a result of using different methods of analysis that has contributed to these variations of the content of the beta-carotene with relation to the other carotenoids [38].

Yield of TPC was higher with Soxhlet extraction than normal solvent extraction. The highest amount of TPC was found in ethyl acetate Soxhlet extraction (5.67±0.09 mg/100g FW) which is significantly more than ethyl acetate solvent extraction i.e. 1.64±0.09 mg/100g FW. The earlier study reported that the concentration of TPC in the analyzed papaya pulp varied significantly from135.2 to 39.10 (mg/g, dry weight), as it can be seen from the result different solvent has different extraction efficiency in which the concentration of total polyphenol also varies significantly with change in solvent [39].

3.4. Storage Studies of lycopene Enriched Fruit Drinks

Lycopene enriched papaya drink was stored at room temperature and analyzed periodically. No significant changes were found in total soluble solids, titrable acidity and pH during storage. But reducing sugar and total sugar were increased and ascorbic acid content was decreased. Reducing sugars of the beverage increased with increase in storage period, which could be due to the inversion of sucrose at acidic pH and storage at room temperature [17]. Increase in reducing sugar content of untreated guava pulp and guava pulp treated with pectinolytic enzymes [40]. The increase of reducing sugars was associated with the hydrolysis of non-reducing sugars, which was very high in acidic medium at high temperature [41]. The ascorbic acid content of the beverage formulation decreased during the storage of 6 months which could be due to the oxidation of ascorbic acid. The loss of ascorbic acid was 35–40 % in guava beverage stored at 20–36 °C for 12 months [42].

3.5. Microbiological Study of Lycopene Enriched Fruit Drinks

Microbiological test results of Lycopene enriched fruit drinks at initial period and during storage was given in Table 7 and Table 8.The permissible limit for Total viable count, Yeast & mold and coliform are $5*10^2$, 10^2 and 5 per ml [43]. Thus from tables it is evident that the microbiological qualities of lycopene enriched fruit drinks were within the acceptable limits of the Gulf standards for fruit juices [43].

Table 5. Different solvent extraction results of β-carotene and Total Phenolic Content

Solvent extract	β-carotene (mg/100g Fresh wt.) (Mean±SD)	TPC (mg/100g Fresh wt.) (Mean±SD)
Ethyl acetate	0.17± 0.05	1.64±0.09
Petroleum benzene	0.79±0.07	1.16±0.11
Acetone & Petroleum ether	4.22±0.14	4.64±0.08
Methanol	1.13±0.03	3.08±0.05
Soxhlet solvent extraction results of β-carotene and TPC		
Ethyl acetate	1.50±0.11	5.67±0.09
Petroleum ether	1.05±0.03	2.62±0.12
n-Hexane	1.85±0.05	3.98±0.07

Table 6. The effect of storage on the composition of Lycopene enriched Papaya drinks (Results are expressed as per 100 ml of juice)

Parameters	**Initial (Mean±SD)**	**30 days (Mean±SD)**	**60 days (Mean±SD)**	**90 days (Mean±SD)**
Total soluble solid (Brix)	13.00±0.03[A]	15.00±0.05[B]	15.00±0.01[B]	15.00±0.02[B]
Total sugar (%)	12.80±0.13[A]	13.30±0.10[B]	14.70±0.11[C]	14.79±0.09[C]
Reducing sugar (%)	4.16±0.08[A]	6.28±0.06[B]	6.90±0.05[C]	7.97±0.07[D]
pH	3.90±0.03[A]	3.80±0.02[B]	4.00±0.03[C]*	3.90±0.02[A]
Acidity (%)	0.18±0.03[A]	0.18±0.05[A]	0.19±0.08[A]	0.22±0.05[A]
Ascorbic Acid (mg/100 ml)	20.00±0.13[A]	15.00±0.11[B]	15.00±0.09[B]	10.00±0.08[D]

Note: Result were expressed as mean values ± standard deviation. Row-wise same capital letter ([A], [B], [C]) denote similar samples and different letter denote significantly different samples based on p values ($p<0.05$). **[C]*** - PH after 60 days is similar to PH at the start ($p>0.05$) but significantly different from PH after 90 days ($p<0.05$).

Table 7. Microbial load in Lycopene enriched fruit drinks at the initial period

Name of fruit drinks	TVC (cfu/ml)	Yeast/Mold (cfu/ml)	Total Coliform (cfu/ml)
Lycopene enriched Papaya drinks	<10*	ND**	ND
Lycopene enriched Mixed fruit drinks	156	ND	ND

*<10 indicate the absence of test organism in 1ml of sample, ****** ND= Not Detected.

Table 8. The effect of storage on the microbial load of Lycopene enriched Papaya drinks

Parameters	**Initial**	**30 days**	**60 days**
TVC (cfu/ml)	<10*	<10	178
Yeast/Mold (cfu/ml)	ND**	ND	ND
Coliform (cfu/ml)	ND	ND	ND

*<10 indicate the absence of test organism in 1ml of sample, ** ND= Not Detected
The permissible limit for TVC**,** Yeast & mold and coliform are 5*10, 10^2 and 5 per ml (Gulf Standards, 2000).

Table 9. Sensory result of lycopene enriched fruit drinks

Sensory attributes	**Papaya drinks**	**Mixed fruit drinks**
Color	7.0	7.5
Texture	7.0	6.5
Flavor	6.5	6.0
Taste	8.0	5.5
Mouth feel	7.5	6.0
Overall acceptance	7.5	7.0

The hedonic scale used: 9 = Like extremely, 8 = Like very much, 7 = Like moderately, 6 = Like Slightly, 5 = neither like nor dislike, 4 = Dislike slightly, 3 = Dislike moderately, 2= Dislike very much, 1 = Dislike extremely.

3.6. Sensory Evaluation of Lycopene Enriched Fruit Drinks

From Sensory analysis, it was found that the lycopene enriched fruit drinks were acceptable during the storage of three months and lycopene enriched papaya drink is more acceptable than mixed fruit drink. It is evident from the study that pink guava beverage could be fortified with tomato puree for increasing the lycopene content and sensory quality of the beverage [17]. Enrichment of lycopene; improved the nutritional and sensory quality of the fruit drinks.

4. Conclusion

Results obtained from this study revealed that the highest lycopene content (1.02 mg/100g fresh wt.) in papaya pulp was found in hexane: ethanol: acetone (2:1:1) solvent extraction method. This method is more efficient for lycopene extraction. The highest amount of β-Carotene (4.22 mg /100 g fresh wt.) was found in acetone-petroleum ether extraction method and total phenolic contents (5.67 mg/100g fresh wt.) was in Soxhlet extraction method. Physicochemical quality characteristics of the lycopene enriched fruit drinks indicated that the fruit drinks were acceptable during three months of storage at room temperature. Enrichment of lycopene significantly improved the color and appearance of the lycopene enriched fruit drinks, which is in accordance with the growing consumer preference for natural additives in processed foods.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

Acknowledgements

This study was carried out under the research and development (R&D) project entitle “Isolation of Lycopene from tomatoes and development of various value added products by using it” of IFST, BCSIR. The authors are indebted to Chairman, Bangladesh Council of Scientific and Industrial Research (BCSIR) and Director, IFST, BCSIR, Dhaka for providing instrumental facilities to carry out this research work. Authors are also grateful to Mr. Aquib Ahmed for helping in extensive statistical analysis using Python programming language and all other lab colleagues of fruit technology research section for helping to carry out this study.

References

[1] Samson, J.A., Tropical fruits (2nd Ed), *Longman Scientific and Technical*, New York, 256-269. 1986.

[2] Nakasone, H.Y. and Paull, R.E., Papaya. In: Tropical fruits, *CAB international*, Wallingford. Oxon. UK., 239-269. 1998.

[3] Agata, W., Aneta, M. and Justyna, R., “Lycopene content of selected foods available on the polish market and estimation of its intake,” *Polish Journal of Food Nutrition Sciences*, 14/55, 2: 195-200. 2005.

[4] Muthui, G.G., “Quantification of lycopene and its analogues in different varieties of papaya grown in Tharaka Nithi County”, *School of Pure and Applied Sciences*, Kenyatta University. 2015.

[5] Storey, W.B. “Segreggation of sex type in solo papaya and their application to the selection of seeds”, *American Society for Horticultural Science*, 35: 83-85. 1969.

[6] Yamamoto, H.,“Differences in carotenoid composition between red and yellow-fleshed papayas”, *Nature*, 201, 1049-1050. 1964.

[7] Borguini R.G. and Da Silva Torres E.A.F., “Tomatoes and tomato products as dietary sources of antioxidants”, *Food Rev Int*, 25(4): 313-325. 2009.

[8] Wang, C.Y. and Chen, B.H., “Tomato pulp as source for the production of lycopene powder containing high proportion of cis-isomers”, *European Food Research and Technology, 222*(3-4), 347-353. 2006.

[9] Bhaskarachary, K., Rao, D.S.S., Deosthale, Y.G. and Reddy V., “Carotene content of some common and less familiar foods of plant origin”, *Journal on Food Chemistry, 54*: 189-193. 1995.

[10] Krinsky, N., Mayne, S. and Sies, T., “Carotenoids in health and diseases”, 1 st Edition, *Marcel Dekker, Inc*., NY. iii-vii, 1-31. 2004.

[11] Bowden, J., “The most effective natural cures on earth”. *Fairwinds press*, 20-27. 2008.

[12] Maisuthisakul, P., Suttajit, M. and Pongsawatmanit, R. “Assesment of phenolic content and radical scavenging capacity of some Thai indigenous plants”, *Food Chem*., 100(4):1409-1418. 2007.

[13] Nasapon, P., Ampai, P, Maltree, S., Chaiyasutt, C.C. and Leelapornpisid, P., “Phenolic content and in vitro inhibitory effects on oxidation and protein glycation of some thahi medicinal plants”, *Pak. J. Pharm. Sci*, 23(4), 403-408. 2010.

[14] Bertoncelj, J., Doberšek, U., Korošec, M. and Golob, T., “Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey”, *Food Chemistry*, 105(2): 822-828. 2007.

[15] Socha, R., Juszczak, L., Pietrzyk, S. and Fortuna, T., “Antioxidant activity and phenolic composition of herbhoneys”, *Food Chemistry*, 113(2): 568-574. 2009.

[16] Kishore, R. K., Halim, A. S., Syazana, M. S. and Sirajudeen, K. N., “Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources”, *Nutrition Research*, 31(4), 322-325. 2011.

[17] Vijayanand, P. and Kulkarni, S.G., “Lycopene fortification on the quality characteristics of beverage formulations developed from pink flesh guava (*Psidiumguajava* L.)”, *J Food Sci Technol*, 51(12): 4126-31. 2013.

[18] Tiwari, R.B., “Studies on blending of guava and papaya pulp for RTS beverage”, *Indian Food Pack* 54:68–72. 2000.

[19] Salem, A.S., Gafour, W.A. and Essawy, E.A.B., “Probiotic milk beverage fortified with antioxidants as functional ingredients”, *Egypt J Dairy Sci* 34: 23-32. 2006.

[20] Vijayanand, P., Kulkarni, S.G. and Prathibha, G.V., “Effect of pectinase treatment and concentration of litchi juice on quality characteristics of litchi juice”, *J Food Sci Technol* 47(2): 235-239. 2010.

[21] Ranganna, S. Handbook of Analysis and Quality Control for Fruit and Vegetable Products, *Tata McGraw-Hill Publishing company Lt*. New Delhi, Page: 7-88.1986.

[22] Kirk, R,S, and Sawyer, R., “Pearson’s Composition and Analysis of Foods”, Ninth edition (student edition), Page: 33, 35-36. 1991.

[23] Osborne, D.R. and P. Voogt, “(Eds) calculation of calorific value in: The analysis of Nutrients in Foods” *Academic Press*, New York. 1978.

[24] American Public Health Association (APHA), “Standard methods for the examination of water and waste water” 20th edition, Washington. 1998.

[25] Collins C.H. and Lyne M.P., “Microbiological Methods (5th edn.)”, *Butterworth Co. (Publishers) Ltd.*, London. pp. 56-113. 1984.

[26] Kumari, D., Reddy, M.S. and Upadhyay, R.C., “Antioxidant Activity of three Species of Wild Mushroom Genus Cantharellus Collected from North-Western Himalaya, India”, *Int J Agric & Biol* 13(3): 415-418. 2011.

[27] Nair, V. and Lilwani, S. “Extraction and Isolation of Lycopene from Solanum Lycopersicum and Citrullus Lanatus for Bioplastic Colouring”, *International journal of scientific & technology research* volume 5, issue 05, 2016.

[28] Malviya, N. “Isolation and Quantification of Lycopene from Watermelon, Tomato and Papaya”, *Research Journal of Recent Sciences*, Vol. 3(IVC-2014), 68-70. 2014.

[29] Shahzad, T., Ahmad, I., Choudhry, S. Saeed, M.K. and Khan, M.N., “DPPH free radical scavenging activity of tomato, cherry tomato and watermelon: lycopene extraction, purification and quantification.”, *International Journal of Pharmacy and Pharmaceutical Sciences* , Vol 6, Suppl 2, . 2014.

[30] Aghel, N.,Ramezani, Z. and Amirfakhrian, S., “Isolation and quantification of lycopene from tomato cultivated in dezfoul, Iran”, *Jundishapur Journal of Natural Pharmaceutical Products;* 6(1): 9-15. 2007.

[31] Chanda, S. and Dave, R. “In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview”, Afr. J. Microbiol. Res., 3: 981-996. 2009.

[32] Wolfe, K.,Wu, X. and Liu, R.H. "Antioxidant activity of apple peels", *Journal of Agricultural and Food Chemistry* 51(3): 609-614. 2003.

[33] Askar, A. and Treptow, H., "Quality Assurance in Tropical Fruit Processing", *Springer Laboratory* 238 p. 1993.

[34] Krishna, K.L., Paridhavi, M., Jagruti Patel, A., "Review on nutritional, medicinal and pharmacological properties of papaya (*Carica papaya* Linn)" *Nat prod Rad,* 7(4):364-373. 2008.

[35] Selvaraj, Y., Subramanyam, M. D. and Iyer, C. P. A. "Changes in the chemical composition of four cultivars of papaya (*Carica Papaya* L.) During growth and development", *J. Hort. Sci***.** London. 57(1): 135-143. 1982.

[36] FAO, "Manuals of Food Quality Control. 2. Additives, Contaminants, Techniques", *Food & Agri. Org*., Rome, Italy. 1980.

[37] Saxholt, E., Christensen, A.T., Moller, A., Hartkopp, H.B., Hess Ygil, K. and Hels, O.H., "Danish Food Composition Databank, revision 7, Department of Nutrition", *National Food Institute*, Technical University of Denmark. 2008.

[38] Rodriguez-Amaya, D.B., Kimura, M., Godoy, H.T. and Amaya-Farfan, J., "Updated Brazilian Database on Food Carotenoids: Factors Affecting Carotenoid Composition", *Journal of Analytical Food Composition*, 21(6): 445-463. 2008.

[39] Terefe, T.B., "Spectrophotometric quantification of total polyphenols in commercially available fruits", *Journal of Scientific and Innovative Research*; 4(4): 187-190.2015.

[40] Isabella, M.B., Geraldo, A.M. amd Raimundo, W.F., "Physical-chemical changes during extraction and clarification of guava juice", *J Food Chem* 54: 383-386. 1995.

[41] Hernandez, T.M., Villegas, M.J., "Effect of freezing storage on the quality of some tropical fruit pulps-preliminary study". *Technology Chemistry* 7: 33-37. 1986.

[42] Kalra, S.K., Tandon, D.K., Lohani, H.C., "Prevention of discoloration in guava beverage during storage", *Indian Fd Pakr* 41: 21-25. 1987.

[43] Gulf Standards, "Microbiological Criteria for Foodstuffs", Part-1, GCC, Riyadh. 2000.