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Extraction of Bioactive Compound from Some Fruits and Vegetables (Pomegranate Peel, Carrot and Tomato)

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Abstract The effects of different solvents, temperature conditions and solvent-solid ratios on solid-solvent extraction of the total phenolic and flavonoids herein also referred to as antioxidant from pomegranate marc peel (PMP) was studied. Water, methanol, ethanol and acetone extraction efficiencies at extraction times of 5 to 60 min, extraction temperatures of 25 to 95°C, ratios of solvent/solid of 5:1 to 50:1 and particle size of 40 mesh were evaluated. At 40 °C, solvent/solid ratio of 15:1, extraction time of 30 min and particle size of 40 mesh, methanol gave the highest content of the total phenolic and flavonoids (18.3, 2.8)%, followed by water (14.1, 2.1)%, ethanol (8.37, 2.55)%, and acetone (7.65,1.8)%, respectively. Beta-carotenes are extracted from carrot under different conditions involving different temperatures, treatment of samples, and solvents (ethanol, methanol). Carrot roots were tested for the extraction yields of carotenes at temperatures 20°C, 40°C, and 60°C, the samples having been examined after harvest, after cold storage (stored at 5°C), and after freezing (–5°C). The best extraction efficiency was achieved with the samples treated by freezing and using the extraction 60°C for 2–4 hours. Extraction of lycopene from tomato under different conditions involving different time and solvents (hexane, petroleum benzene and hexane: ethanol: petroleum benzene). Under the best conditions, ternary mixture gave the highest lycopene content (12.3 mg/100g), followed by hexane (9.4mg/100g), petroleum benzene (8.7mg/100g). At 20°C, the yield of carotenes from the fresh after-harvest sample was slightly affected by the time and temperature. After 5 h of extraction, the fresh sample showed 1.58 mg/100 g of carotene yield, the velocity of extraction being very slow. With the extraction at 40°C, the yield of carotenes (2.45 mg/100 g) was higher compared to that at 20°C while the highest extraction yield was found at 60°C. At 60°C, the extraction maximum was found in the second hour of extraction (4.28 mg/100 g). After this time, the extraction yield of β-carotene decreased. Compared to the third and fourth hours, the extraction was almost the same as the result of the degradation and loss of carotenes.

Keywords: *pomegranate peel, carrot, tomato, treatments, total phenol, flavonoids*

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1. Introduction

Fruits and vegetables are most popular due to their nutritional value worldwide and rich sources of beneficial anti-oxidants, minerals, vitamins and fibres [1,2]. The regular consumption of fresh fruits and vegetables may reduce the risk of cardiovascular diseases, stroke and certain cancers [1,3]. Usually fruits are processed into juice, beverage, squash and syrups. However by-products can be used as functional food ingredients such as phytochemicals, pharmaceuticals, food products, essential oils, seed oil, pectin and dietary fibers [4]. Therefore, fruits by-products not only good source of bioactive compounds but also could be used as several value-added products [5].

Phenolic compounds are secondary metabolites ubiquitous in plants and plant derived foods and beverages. They show a large diversity of structures including rather simple molecules. Act as an essential part of the human diet, and are of considerable interest due to their antioxidant properties. Phenolic compounds, including their subcategory Flavonoids, Anthocyanins, Catechins, Glucosinolates, Isoflavones, Lignans , Phenolic acids are present in all plants and have been studied extensively in legumes, cereals, olive oil, vegetables, nuts, fruits, tea and red wine. Many phenolic compounds have antioxidant properties. These compounds can be used as ingredients in cosmetics, pharmaceuticals, nutraceuticals and food. For

application in food, it can be used to prevent oxidation of food containing high amounts of lipid [6].

Usually, the use of synthetic antioxidants as Butylated Hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) in foods is discouraged due to their high levels of toxicity and carcinogenicity [5,7]. Therefore, natural antioxidants from fruits and its waste have attracted considerable attention due to their safety. In recent years, there has been a global trend toward the use of phytochemicals from natural resources such as vegetables, fruits, oilseeds and herbs, as antioxidants and functional ingredients [7]. Besides, this research will give new information about bioactive compounds of fruits and its waste in Bangladesh and develop a simple and rapid method for extraction of bioactive compounds. Utilization of pomegranate waste is rare in Bangladesh.

Tomato, Carrot, Red spinach grows abundantly in Bangladesh [1]. Many produce goes under the production loss and post-harvest loss due to improper handling, lacking of suitable storage condition or in the time of processing [8,9]. The extraction of bioactive compounds present in Pomegranate, tomato, carrot which are of economic importance in the national and international market and discusses some of their potential beneficial health effects, as well as their degradation during postharvest manipulation and storage. This work was conducted with some objectives as follow:

- To extract total phenolic content, flavonoid Content, evaluate antioxidant activities, β-carotene and lycopene from pomegranate peels (*Punica granatum L.),* tomato (*Lycopersicon esculentum L.),* and carrot *(Daucus carota)* respecttively.
- To study the influence of temperature, samples treatment, and solvents on the extraction yield of bioactive compound.
- To develop a simple and rapid method for extraction of bioactive compounds.

2. Materials and Methods

2.1. Extraction of Phenolic Compound from Pomegranate Waste

2.1.1. Materials

Pomegranate peel were collected from local market and kept at -5°C until used. Prior to experiments, the samples were thawed at 4°C followed by hot air oven drying at 40°C to a moisture content of about 8% (dry basis). The moisture content was determined by using oven drying at 105°C until constant weight was achieved. The dried peel was ground in mortar and pestle.

2.1.2. Methods

2.1.2.1. Extraction Procedures and Effects of Different Parameters on Total Phenolic

2.1.2.1.1. Effect of Solvents

The extraction yield of antioxidant compounds from plant materials is influenced mainly by the conditions under which the process of liquid-solid extraction is carried out to separate a soluble fraction from a permeable solid [10]. In this study, four solvents with different polarities were used to identify the most suitable one for the recovery of antioxidant components from pomegranate peel. The polarity of a solvent besides the dipole moment, polarizability and hydrogen bonding determines what type of compounds it is able to dissolve. The solvents which were used in this experiment are: (a) deionized (DI) water (polar solvent with a dielectric constant of 80); (b) ethanol (polar with a dielectric constant of 24); (c) methanol (polar with a dielectric constant of 33) and (d) acetone (polar with a dielectric constant of 21). All used chemicals were of analytical grade. For each solvent, dried and ground peel was extracted in a thermostatic water bath shaker with a 15:1 (w/w) ratio solvent/sample (dry weight) at 40°C for 30 min in a conical flask. The liquid extract was separated from solids by vacuum enhanced filtration through Whatman No. 1 filter paper.

The filtrates were air dried in hood at room temperature and residual moisture removed in a vacuum oven at 50±2°C. The dried extracts were weighted to analyze the total extract yield, the contents and yield of antioxidant compounds including total phenolic and flavonoids. The reported results, as illustrated in equations 1-3, include the total extract yield (%), the yield of total antioxidant (either phenolic or flavonoids) from the, PMP (%), and the content of antioxidant (either phenolic or flavonoids) (%) in extract respectively:

$$\text{Total extract yield } (\%) = \frac{\text{g dried extract}}{\text{100g PMP}} \times 100 \quad (1)$$

$$\text{Yield of antioxidant } (\%) = \frac{\text{g total of antioxidant}}{\text{100 g PMP}} \times 100 \quad (2)$$

$$\text{Content of antioxidant } (\%) = \frac{\text{g total of antioxidant}}{\text{100g dried extract}} \times 100 \quad (3)$$

All reported weights and percentages are dry basis unless specified otherwise.

2.1.2.1.2. Effect of Extraction Time and Temperature

To study the effect of extraction time, samples of 3 g PMP powder (40mesh) were mixed with 45 g DI water and extracted at 25, 60, and 95 °C for 5, 10, 15, 20,30,40,50 and 60 min. The liquid extract was separated from solids by vacuum enhanced filtration through Whatman No.1 filter paper. The filtrate was transferred to a 50 ml flask after filtration, and DI water was added to make the finally volume to 50 ml. After the filtrate volume adjustment, the total phenolic concentration was measured. To determine the effect of extraction temperature on the recovery of phenolic, temperatures of 20, 40, 60, 80, 95°C were tested during a 2 min extraction. Samples (40 mesh) of 5, 3, 1.8 g were mixed with 45 ml DI water to achieve the following ratios: 9, 15, 25(w/w).

2.1.2.1.3. Solvent-Solid Effect

The effect of solvent-solid ratio on the total phenolic extraction was studied. Samples (40mesh) were mixed with 45 g DI water at ratio of solvent-solid from 5 to 50, and extraction performed at temperature of 60°C for 2 min. The phenolic yields were determined.

2.1.2.2. Analysis Assay

2.1.2.2.1. Total Phenolic Content

The total phenolic content in the extract was determined by the Folin-Ciocalteu method [7,11]. The 0.5 g of dried

extracts was dissolved in 5 ml methanol or the filtrate made up to 50 ml were used directly. Aliquots of 1 ml of samples were mixed with 2.5 ml of 2N Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The total volume of the mixture was adjusted to 25 ml using DI water and allowed to stand for 30 min at room temperature before the absorbance was measured at 760 nm using a spectrophotometer (TVT- 300XPH, Sweden, Perten Instrument). The total phenolic content in the extract was calculated and expressed as tannic acid equivalents (TCE; g/100 g dry mass) using a tannic acid (0–0.004 mg/ml) standard curve.

2.1.2.2.2. Flavonoid Content

The flavonoids content was measured using a modified colorimetric [7,11]. A quantity of 0.5 g of dried extracts was dissolved in 5 ml methanol or the filtrates made up to 50 ml were used directly. A volume of 0.4 ml of the solution was transferred to a 25ml flask containing 5 ml of 30% ethanol and mixed with 0.75 ml of 5% sodium nitrite for 5 min. Then, 0.75 ml of 10% aluminum nitrate was added. After 6 min, the reaction was stopped by adding 5 ml of 1 M sodium hydroxide. The mixture was further diluted with 30% ethanol up to 25 ml. The absorbance of the mixture was immediately measured at 510 nm. The flavonoids content was calculated and expressed as rutin equivalents (RE, g/100 g dry mass) using a rutin (0~0.03 mg/ml) standard curve.

2.1.2.2.3. Storage Temperature

Three different storage temperatures were used to identify the rate of degradation of polyphenols and flavonoids in the aqueous extracts in order to choose the most suitable one for the storage of extracts. The storage temperatures used in this experiment were: laboratory temperature (+20°C), refrigerator temperature (+4°C), and (-5°C) freezer temperature.

2.2. Extraction of Beta-Carotene from Carrot

2.2.1. Materials

A good variety of carrot (*Daucus carota L.*) used for the extraction of beta-carotene. The roots of carrots were analyzed after harvest, after storage in cold room (4–5°C), and after freezing (–5°C) for one month. The content of carrot moisture was determined gravimetrically- moisture analysis of the samples (10 g) was performed in hot air oven at 105°C. The content of carrot moisture was determined gravimetrically according to the method given in Slovak Technical Standard 56 0053 [12].

2.2.2. Methods

The most popular methods for carotenes extraction are those using organic solvents. Most of them are toxic and expensive [13].

2.2.2.1. Extraction Conditions

Roots were cut into small pieces with the help of sharp knife. The extraction yield of carotenes was observed at different temperatures (20°C, 40°C, and 60°C) using ethanol and methanol. Initially, 25 g of cut carrot samples were added to 100 g of ethanol. Carrot slices were extracted in water bath (20°C 40°C, 60°C), shaken after every 10 min, and after every hour of extraction 5 ml sample was taken and mixed with petroleum ether (20 ml). Water was added for the separation of phases, and after the separation the petroleum-ether-carotenoid phase was made up to the volume of 50 ml.

2.2.2.2. Determination of Beta-Carotenes

The content of β-carotene in the petroleum-ether extract was determined spectrophotometric ally; the absorbency was measured at the wavelength of 450 mm using the spectrophotometer. The concentration of carotenes expressed as β-carotene (g/100 ml) was calculated using the response factors as follows:

$$\beta - \text{carotene} = \frac{A \times V \times D}{E^{1\%}1\text{cm} \times W}$$

Where:
A – Absorbancy
d – Dilution
$E^{1\%}$1cm – coefficient of absorbancy (2592 for petroleum-ether)
w – Weight of sample (g)
V – Volume (ml).

2.2.2.3. Dry Matter Determination

(a) Gravimetrically – moisture analysis of the samples (10 g) was per-formed in hot air oven at 105°C;

(b) Refractometry – with laboratory refractometer, to get the information about dry matter content in the extraction solvents as obtained during the technology of the carotenoid concentrate production. The results were expressed as g/100 g.

2.2.2.4. Condensation of the Extract

The extraction product was condensed in the rotary evaporator. The pressure of 2–3 k Pa and the temperature of 40°C were used until the solution is condensed. Under a higher pressure and the temperature of 50°C, methanol and water were removed. The product of this step – the concentrate was analyzed for the dry matter (21.887%) and carotenes 82.51mg/100 g contents

2.3. Extraction of Lycopene from Tomato

2.3.1. Materials

Winter variety of fresh ripe tomatoes were purchased from a local market and stored at 4 °C for a maximum of 2 days before use. This particular variety is not generally marketed for direct consumption, but is used primarily in the production of this lycopene extract. Then the tomato paste was extracted with hexane, petroleum benzene and ethanol. The final product is obtained after solvent removal by evaporation under rotary evaporator at 40-60°C.

2.3.2. Extraction Yield and Determination of Lycopene

A mashed sample of 1.0-1.5 g of tomato paste was placed in a flask. To the flask, 10ml of hexane, ethanol or the ternary mixture hexane: petroleum benzene: ethanol (50:25:25) was added into the flask. After the 50:25:25 additions the flask was capped and shaken. After the settling of the sediments, the liquid portion was pipetted in a 50ml flask. The process of separating the liquid from the solid was repeated for a total of four times. To the

extracted liquid, 5ml of NaCl was added and put into a separatory funnel to remove the water layer. 5ml of 10% K_2CO_3 was added to the extracted organic layer, and then placed into the separatory funnel for the removal of the water layer. NaCl was then again added to the organic layer, and put through the separatory funnel to again remove more of the water layer. After the water removal using the separatory funnel took place, $MgSO_4$ was added until clumping subsided. This addition was done to remove any access water that the previous processes did not remove. Lycopene concentration if the extracting solvents were determined by spectrophotometric measurement at room temperature in the wave length range 503nm.

The concentration of carotenes expressed as β-carotene (g/100 ml) was calculated using the response factors as follows:

$$\text{O.D of 1.0} = 3.1206\ \mu\text{g of Lycopene / ml}$$

$$\text{Lycopene } (\text{mg}/100\text{g}) = \frac{\left(\begin{array}{l}3.1206 \times \text{O.D of sample} \\ \times \text{volume made up} \times \text{dilution} \times 100\end{array}\right)}{1\times \text{ wt. of sample} \times\ 1000}$$

Dry the lycopene by evaporating solvent with rotary evaporator at 40-60°C for 4 h.

2.4. Statistical Analysis

These experiments carried out in triplicate of each sample. The results were statistically analyzed (SPSS for windows version 17.0) and expressed as mean ± standard deviation. One–way analysis of variance was performed using ANOVA procedures. Mean comparisons were performed using Duncan's multiple range tests for significant effect at $P < 0.05$.

3. Results and Discussion

3.1. Phenolic Compound Extraction from Pomegranate Waste

3.1.1. Effect of Extraction Procedures and Different Parameters

3.1.1.1. Influence of Solvents

Results for the total extract yields reported as percentage of g of extract per 100g pomegranate peel on dry basis indicated that the pomegranate peel extracted with methanol gave the highest total extract yield (44.66±0.1), followed by water (42.33±2.10), ethanol (16.66±0.30), acetone (3.33±0.1) when the extractions were done with the ratio of solvent/sample of 15:1 (w/w) at 40°C for 30 min. The solubility of the solute into the solvent is different because of polarity differences between solvents. Water, methanol and ethanol are polar protic solvents of dielectric constants of 80, 33 and 24 respectively, while acetone is polar aprotic and non-polar solvents of dielectric constants of 21 and 6 respectively. It has been reported that pomegranate peel extract yield (%, w/w) were 9.38, 7.53 for methanol, water respectively under the following experimental conditions: peel powder (25 g) extraction by mixing using a magnetic stirrer with 100 mL of the corresponding solvents at 30°C for 1 h, filtration through Whatman No.41, residue re-extraction with the same solvent, extract pooling and concentration under vacuum at 40°C [10].

The findings agree in terms of solubility trend but differ in the extracted yield. The effect of different solvents on the yield of total phenolic, and flavonoids from the pomegranate peels are shown in Figure 1.

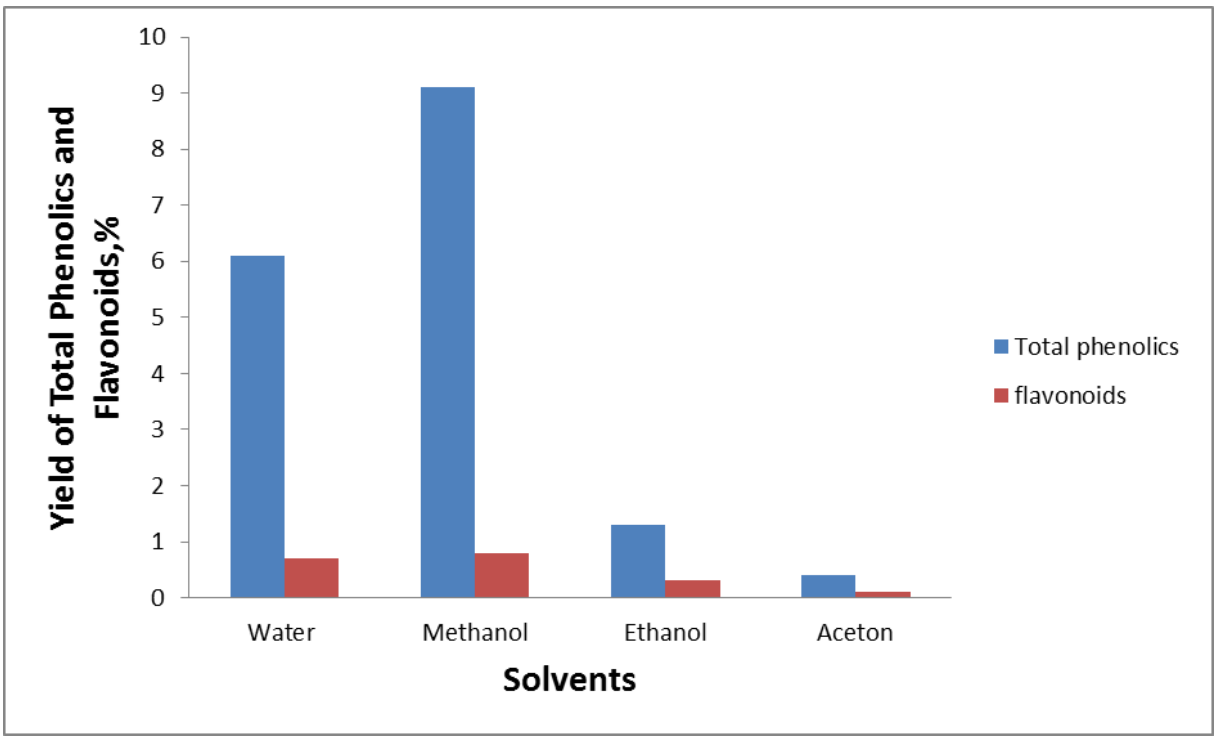

Figure 1. The effect of different solvents on the yield of total phenolics and flavonoids from the marc of pomegranate peels. Extraction was conducted at a temperature of 40°C, a solvent/solid ratio of 15:1, a particle size of 40 mesh and an extraction time of 30 min

Methanol and water gave the top two yields of all two antioxidants components, which indicate that they are more effective than ethanol, acetone for the antioxidants' extraction from the pomegranate peel. Particularly for the phenolic content, results which are found different from the result reported elsewhere in literature [9] that the phenolic content from water extraction was the lowest among ethyl acetate, acetone, methanol and water. In the preceding results, the phenolic contents of ethyl acetate, acetone, MeOH and water extracts were found to be 16.5,52, 46.2 and 4.8% respectively. That withstanding, the value for the total phenolic yield obtained using MeOH is comparable to that reported by other researchers [10]. This deviation particularly in the values is likely to be due to the difference in extraction and phenolic content determination procedures [14]. For instance, the powder from pomegranate peel was extracted with a Soxhlet extractor for 4h [14], filtered, concentrated under vacuum at 40°C [10] and then dissolved in methanol: water (6:4 v/v) (1 mg/ml) for evaluation of antioxidant capacity; the concentration of phenolic in the extracts was determined [10] and results were expressed as (+) catechin equivalents. The results were expressed as tannic acid equivalents (TCE; g/100 g dry mass).

The total phenolic content was higher in methanol extract (18.3%) than in water extract (14.1%) and comparatively lower in ethanol extract (8.37%) shown in (Figure 2). It is reported [7] that pomegranate phenolic content was 44% with methanol, 3.0% with water. The results, however, show that the total phenolic content in the water extract and the MeOH extract was nearly the same: 14.1% and 18.3% respectively. Factors that have been attributed to bringing variation include the method of extraction [14], mixture of different solvents [3,11] and use of different materials [15] among others.

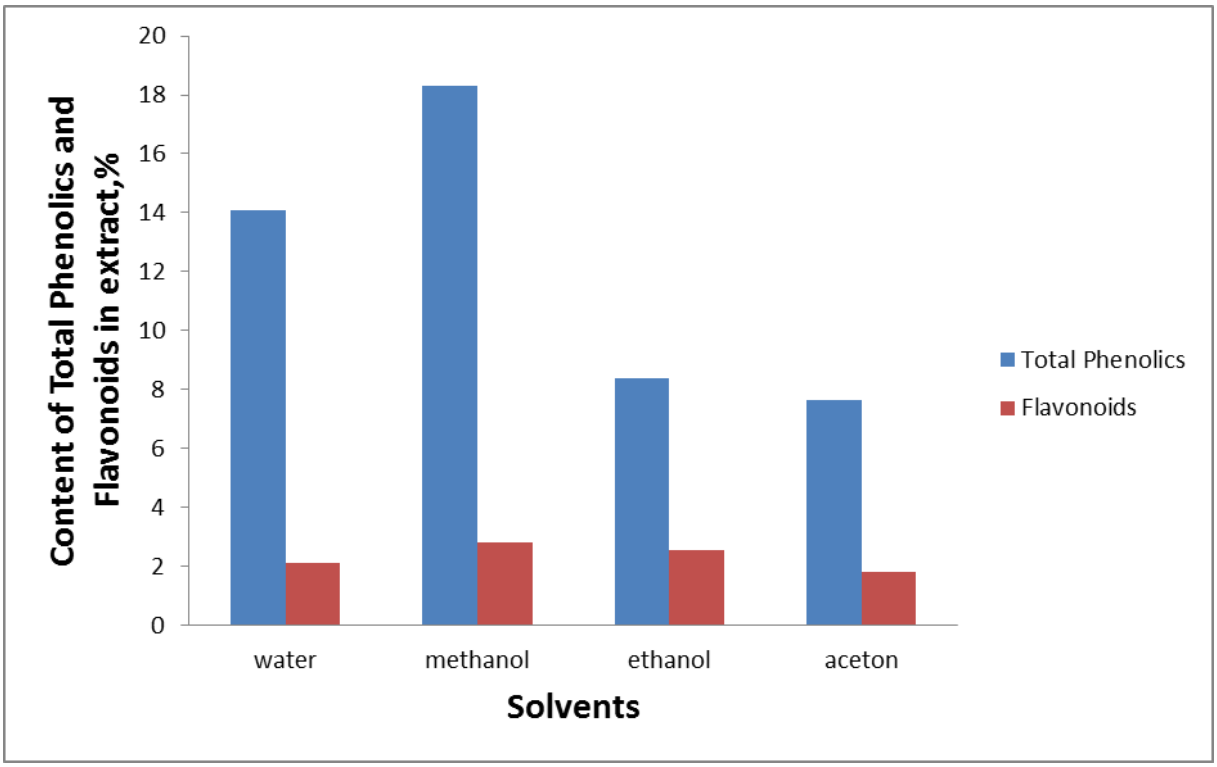

Figure 2. The effect of different solvents on the content of total phenolic and flavonoids from the marc of pomegranate peels (PMP) in the extract (g dried extract/100 g PMP). Extraction was conducted at a temperature of 40°C, a solvent/solid ratio of 15:1, a particle size of 40 meshes and an extraction time of 30 min

Table 1. Analysis of Variance for total phenolic and flavonoid extraction– Main Effects

Source of variation	Sum of squares	Degree of Freedom	Mean square	F-value	Pr(>F)
Temperature of extraction	5.43	6	0.89	0.036	1
Time of extraction	2.55	7	0.364	0.013	1

Table 1 shows that there is no significant difference for different time and temperature to extract total phenolic and flavonoid .

3.1.1.2. Influence of Extraction of Time and Temperature

Conventional extraction and concentration of phenol is typically conducted at temperatures ranging from 20 to 40°C [16] because temperatures above 70°C have been shown to cause rapid polyphenol degradation [17]. For example, in order to extract flavanones from orange juice, added methanol and heats the mixture to 55°C for 15 min [18]. Also, Spigno *et al.*, [19] extracted phenolic from grape marc at 60°C and Pinelo *et al.*, [20] extracted antioxidant phenolic from pine sawdust at 50 °C for 90 min. The effect of extraction temperature on the total phenols and flavonoids yield in PMP extract and results are shown in Figure 3.

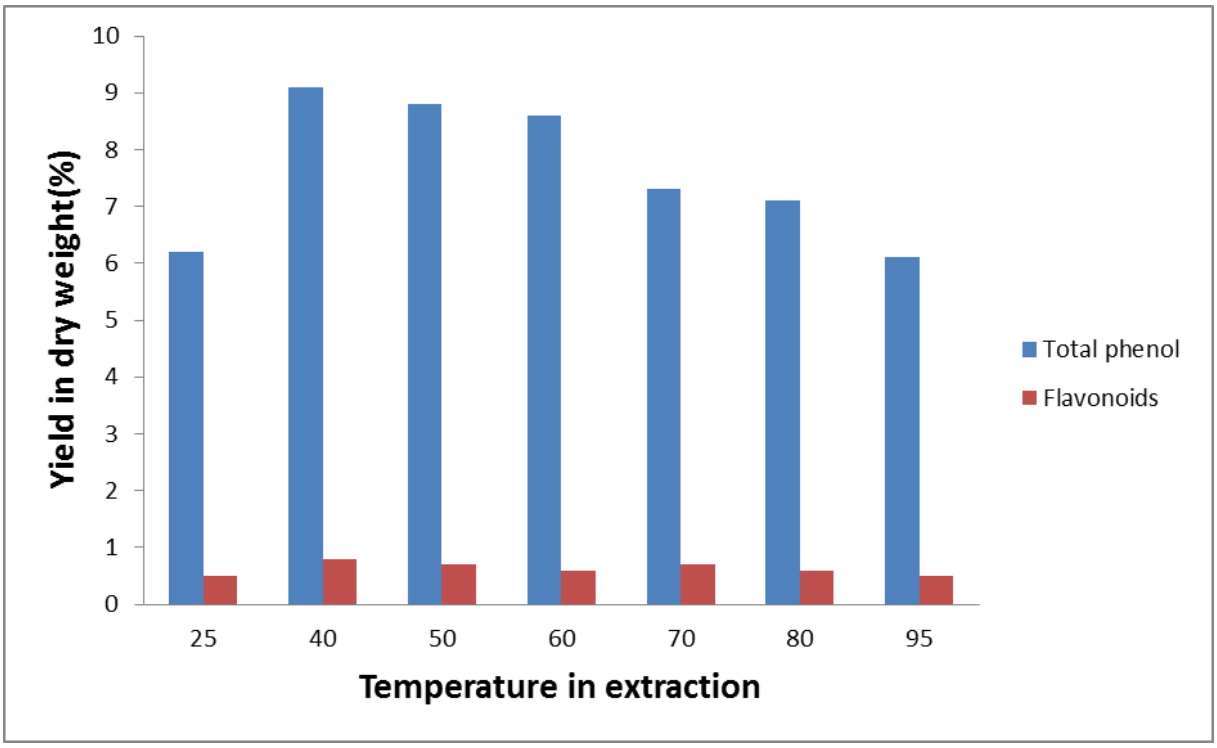

Figure 3. Effect of extraction temperatures on total phenols and flavonoids yield

An increase in temperature increases the efficiency of the extraction since heat render the cell walls permeable, increase solubility and diffusion coefficients of the compounds to be extracted and decreases the viscosity of the solvent, thus facilitating its passage through the solid substrate mass. However, the use of temperatures higher than 40°C decreases the total polyphenols and flavonoids yield which is probably due to their degradation.

According to Figure 4 as extraction time increased (5 to 30 minutes), the yield of phenols and flavonoids also increased. Times longer than 40 min have been shown to cause rapid phenols and flavonoids degradation.

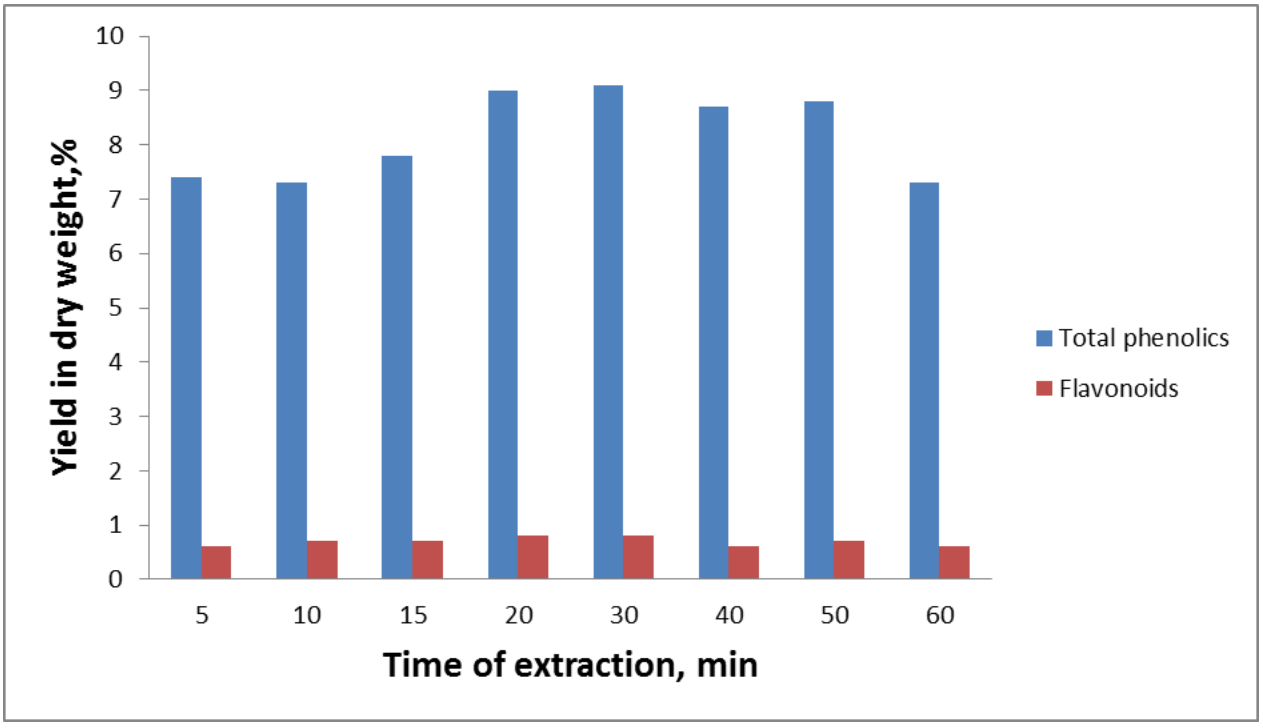

Figure 4. Effect of extraction time on total phenols and flavonoids yield

3.1.1.3. Influence of Number of Extraction

Two sequential extractions appear sufficient; the first extract contain more than 80% of total extractable phenols and flavonoids and the second one contain about 10%. Only small amount of these compounds are found in the third and the fourth extract as shown in Figure 5. While flavonoids are not detectable in the fifth extract.

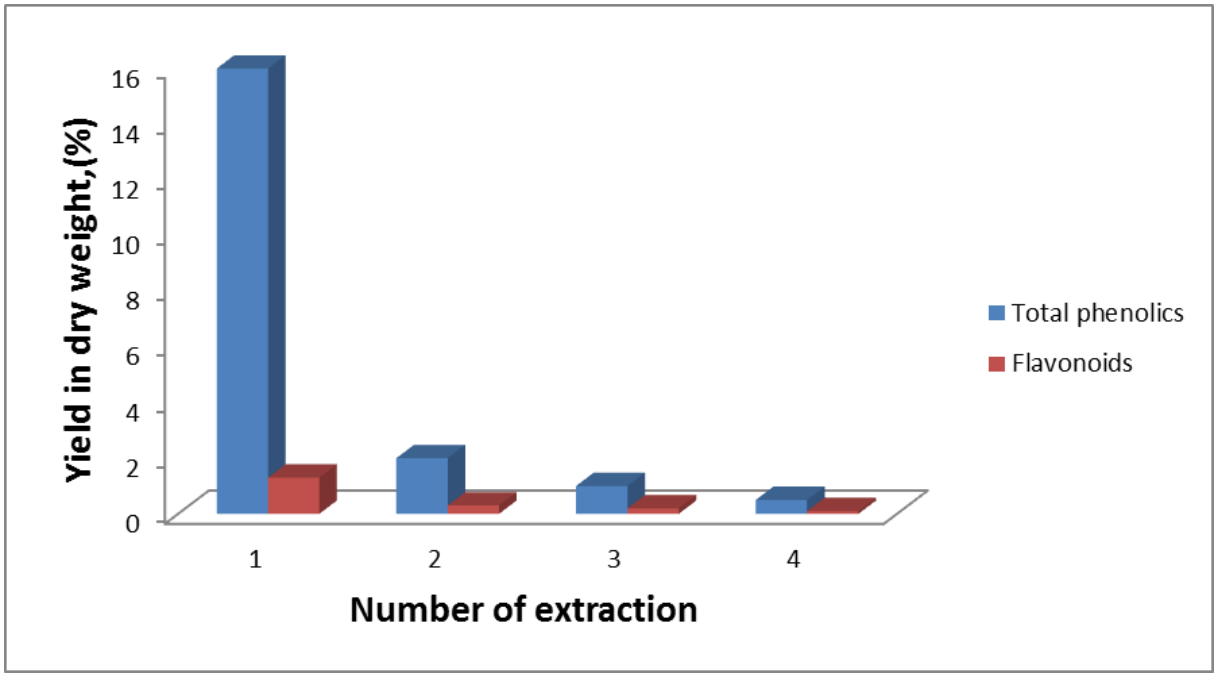

Figure 5. Total extractable phenols and flavonoids in four sequential extractions

3.1.1.4. Influence of Storage Temperature

The total polyphenols degrade quickly during the initial period of storage and then it will slow down. The total phenols degrade quickly during the initial period of storage slow down when stored at laboratory temperature (+20°C) and are more stable when stored at refrigerator temperature (+4°C), and at freezer temperature (-5°C) as shown in Figure 6.

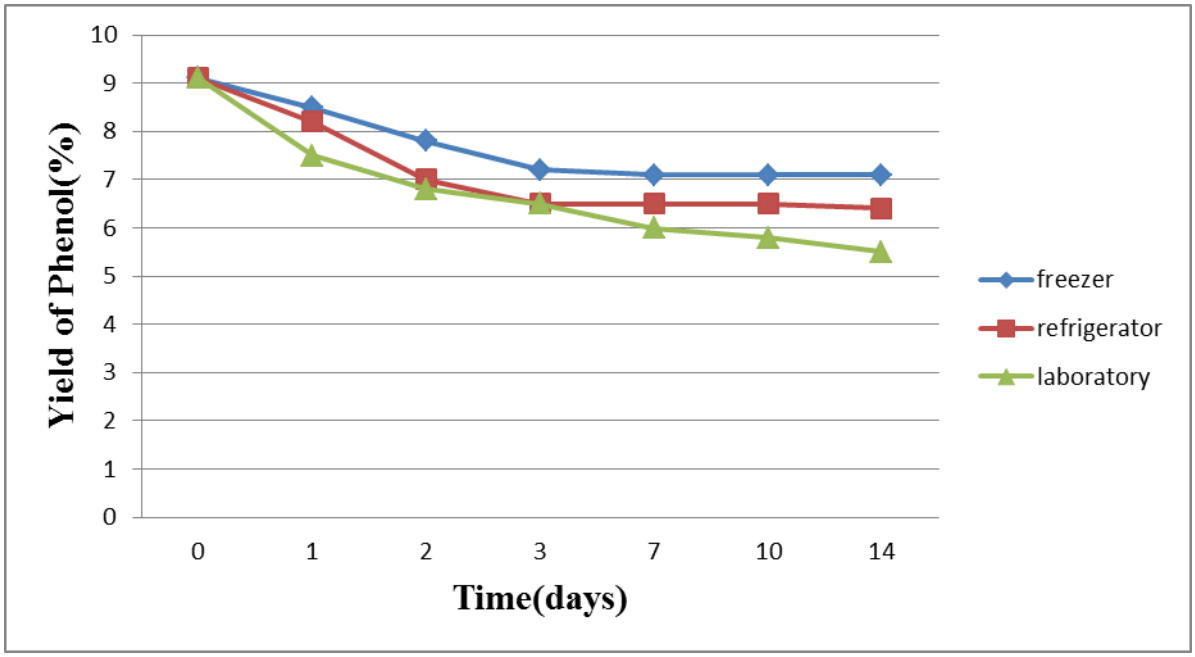

Figure 6. Yield of total phenols in different storage temperatures for 14 days

3.2. Beta-Carotene Extraction from Carrot

3.2.1. Extraction Yield of β-carotene From Fresh Samples by The Influence of Time, Treatment, and Temperature

The processing of foods involves changes in the structural integrity of the matrix which produces both negative (loss of carotenoids due to oxidation) and positive (increased bioavailability) effects [21]. Light, heat etc. promote isomerization of carotenoids. Oxidative degradation, the principal cause of extensive losses of carotenoids, depends on the availability of oxygen and is stimulated by factors such as light.

At 20°C, the yield of carotenes from the fresh after-harvest sample was slightly affected by the time and temperature. After 5 h of extraction, the fresh sample showed 1.58 mg/100 g of carotene yield, the velocity of extraction being very slow.

With the extraction at 40°C, the yield of carotenes (2.45 mg/100 g) was higher compared to that at 20°C while the highest extraction yield was found at 60°C (Figure 7 and Figure 8). At 60°C, the extraction yield of carotenes was found to be high already after 10 min of extraction, which can be explained by a good release of carotenes from the disturbed texture of the carrot at 60°C. At 60°C, the extraction maximum was found in the second hour of extraction (4.28 mg/100 g). After this time, the extraction yield of β-carotene decreased (Table 2).Compared to the third and fourth hours, the extraction was almost the same as the result of the degradation and loss of carotenes.

Naturally, β-carotene exists in the all-trans form. After processing, some part of all-trans form is converted into its different cis-isomers [22]. Calvo *et al*., [23] (2007) state, that in the extractions performed with ethanol at 60°C, the yield of lycopene and its isomers was lower than at 50°C, that could indicate an extensive isomerization at the high temperature with ethanol, but the oxidative degradation being the predominant reaction.

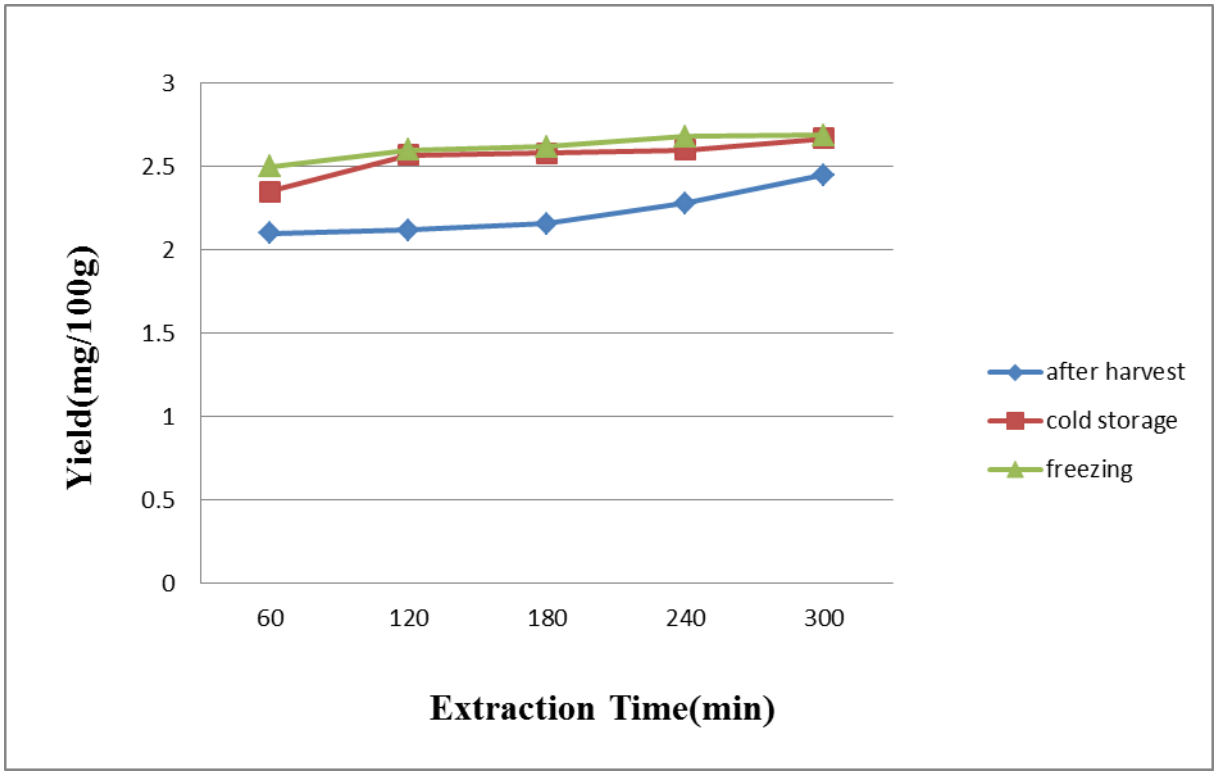

Figure 7. β- carotene yield obtained during extraction of differently treated samples of carrot with ethanol at 40°C.

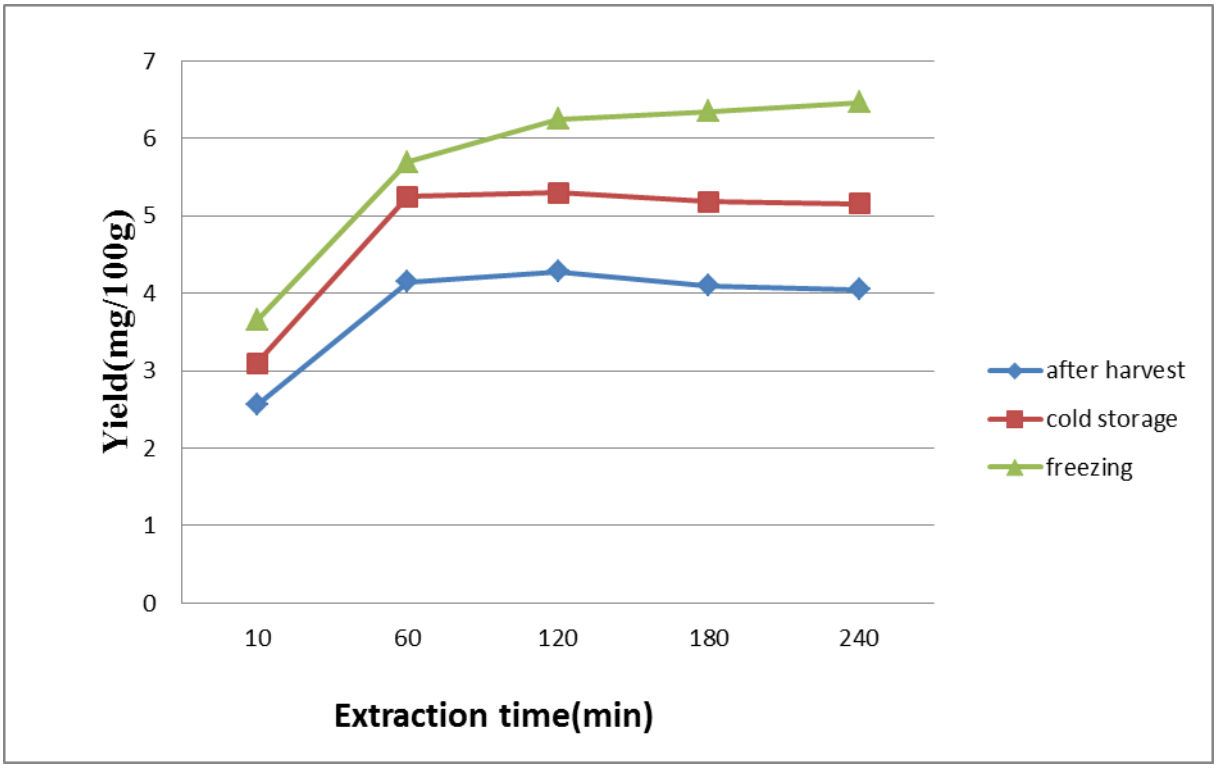

Figure 8. β- carotene yield obtained during extraction of differently treated samples of carrot with ethanol at 60°C.

Optimal time for the best extraction of carotenes seems to be 2 to 4 h, depending on the treatment of the sample; at lower temperatures (40°C) and with fresh samples, a longer time (min 5 h) is needed for the extraction Calvo *et al*., [23] (2007) found the highest β-carotene concentration from lyophilized and milled skin of tomatoes at 25°C while the times assayed did not have a great influence on the β-carotene yield. Rafajlovska *et al*., [24] showed that the increase of temperature positively influences the mass transfer processes. The increased color yields also result from the changes in the cellular structure of the biological matrix. On the other hand, the increase of the extraction temperature may cause raw material browning. Due to this circumstance, it is required to establish the optimal extraction temperature.

3.2.2. Extraction Yield of β-Carotene from Treated Samples by The Influence of Time, Temperature and Treatment

Increased carotenes yields due to storing and freezing of the samples were shown as compared to the after-harvest samples (Table 2). The best solubility of carotenes was found at 60°C, the heat treatment according to [25] Dutta *et al*., (2005) such as blanching, cooking, and streaming help to release the carotenoids bound by protein and render them easily extractable. Hence, the carotenes content increased from 4.15 mg/100 g fresh sample to 5.7 mg/100 g frozen sample already after the first hour of extraction at 60°C (Table 2), meaning an increase of 37%. After the fourth hour of extraction at 60°C, the increase caused by freezing was from 4.05 mg/100 g in the fresh sample to 6.47 mg/100 g in the frozen sample, which means 62% higher yield of carotene.

Table 2. β-Carotene Yield (mg/100 g) at Temperatures of 40°C and 60°C from Different Treated Samples of Carrot

Time of extraction (min)	Treatment of samples at 40°C			Time of extraction (min)	Treatment of samples at 60°C		
	after harvest	cold storage (5°C)	freezing (-5°C)		after harvest	cold storage (5°C)	freezing (-5°C)
60	2.10	2.35	2.50	10	2.56	3.1	3.65
120	2.12	2.57	2.60	60	4.15	5.25	5.7
180	2.16	2.58	2.62	120	4.28	5.30	6.25
240	2.28	2.60	2.68	180	4.10	5.18	6.35
300	2.45	2.67	2.69	240	4.05	5.16	6.47
CV*	3.73	4.65	2.87^{+}	CV	2.38	1.23^{+}	5.49

*CV = coefficient of variations (%) – measuring after extractions 1st, 2nd, 3rd, 4th hours.

Storage of the samples increased the extraction yield of carotenes, but a statistically significant effect of cold storage was confirmed only at 60°C. Comparing the fresh after-harvest carrots and the stored and frozen samples, the samples treated by freezing showed the best and the highest release of carotene, the influence of freezing having been statistically significant at 40°C and 60°C as well. According to [26] Van Het Hof *et al*., (2000), the processing such as the mechanical homogenization or heat treatment has the potential to enhance the bioavailability of carotenoids from vegetables (from 18% to a six fold increase). The better resorption of carotenes from the heat-treated carrot is explained by the disruption of the cells, the nutrients being better utilized in the body. Enzymes can also affect the cells disruption.

Table 3. Multifactor Analysis of Variance (extraction at 40°C and 60°C) – Main Effects

Temperature (°C)	Source of variation	Sum of squares	Degree of Freedom	Mean square	F-ratio	Significance level (5%)
40	samples treatment	0.458	2	0.229	16.343	**
	time of extraction	0.135	4	0.031	0.705	Not significant
60	samples treatment	8.617	2	4.308	4.620	**
	time of extraction	10.519	4	2.624	2.831	Not significant

From this study, it was statistically confirmed that with increasing time, the extraction yield of β-carotene is higher (Table 3). The statistical evaluation of the influence of the extraction time showed a significant difference between, the first hour and the fourth hour of extraction at 40°C. In the case of 60°C, a significant difference was found between, the extraction yields after 10 min and the first hour of extraction, then between 10 min and the second hour, and the third hour and the fifth hour of extraction as well.

3.2.3. Role of Solvent

Carotenoids are soluble in apolar solvents, including edible fats and oils. Because carotenoids are liposoluble, they are usually extracted from the plant sources with organic solvents such as chloroform, hexane, acetone, petroleum ether, etc. [27]. The samples can contain large amounts of water; water-miscible organic solvents such as ethanol are also used. One of the problems is the elimination of the residual solvents to obtain a safe extract; this can be avoided by using food grade solvents such as ethanol. [23] Calvo *et al*., (2007) found that the yield of each carotene from peel powder of tomatoes was noticeably higher with extraction performed with ethanol than with that using ethyl acetate. Ethanol was chosen as the extractive solution in laboratory conditions. Another solution for the model production of carotenoid concentrate tested was methanol; the extraction of β-carotene into this solution was lower as compared to ethanol (Figure 9). Therefore, to obtain the carotenoid concentrate in semi-manufacturing conditions, ethanol was used as solvent.

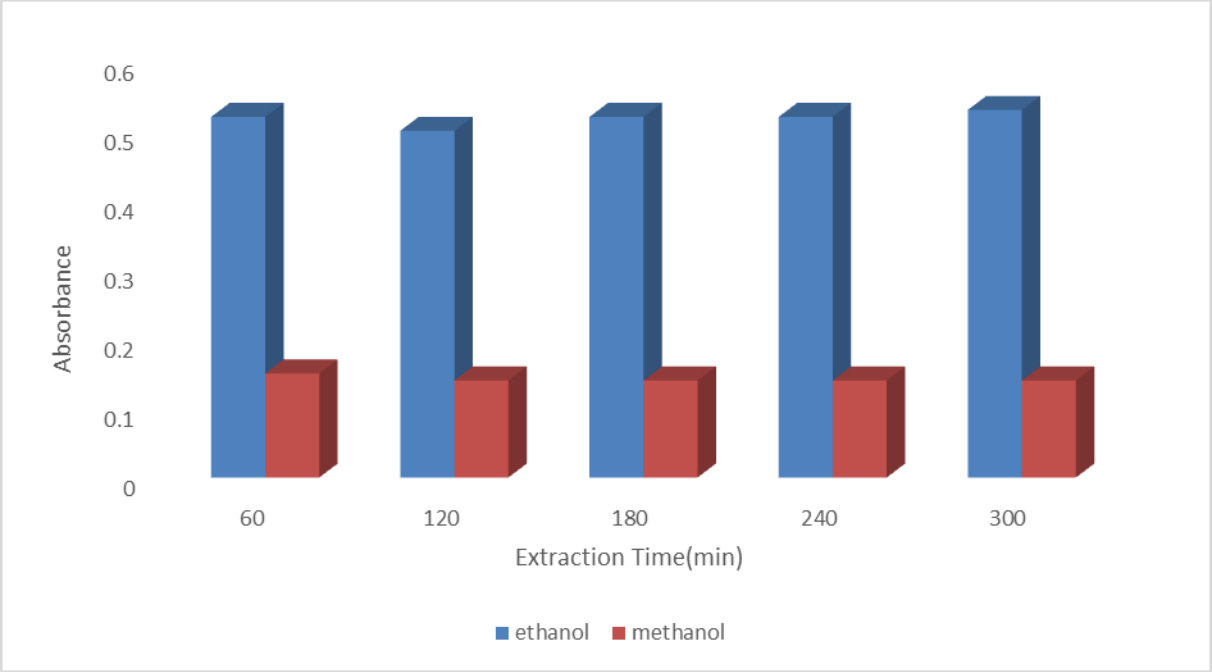

Figure 9. Comparison of different solvents for carotenes extraction from carrot

3.3. Lycopene Extraction from Tomato

3.3.1. Influence of Solvent

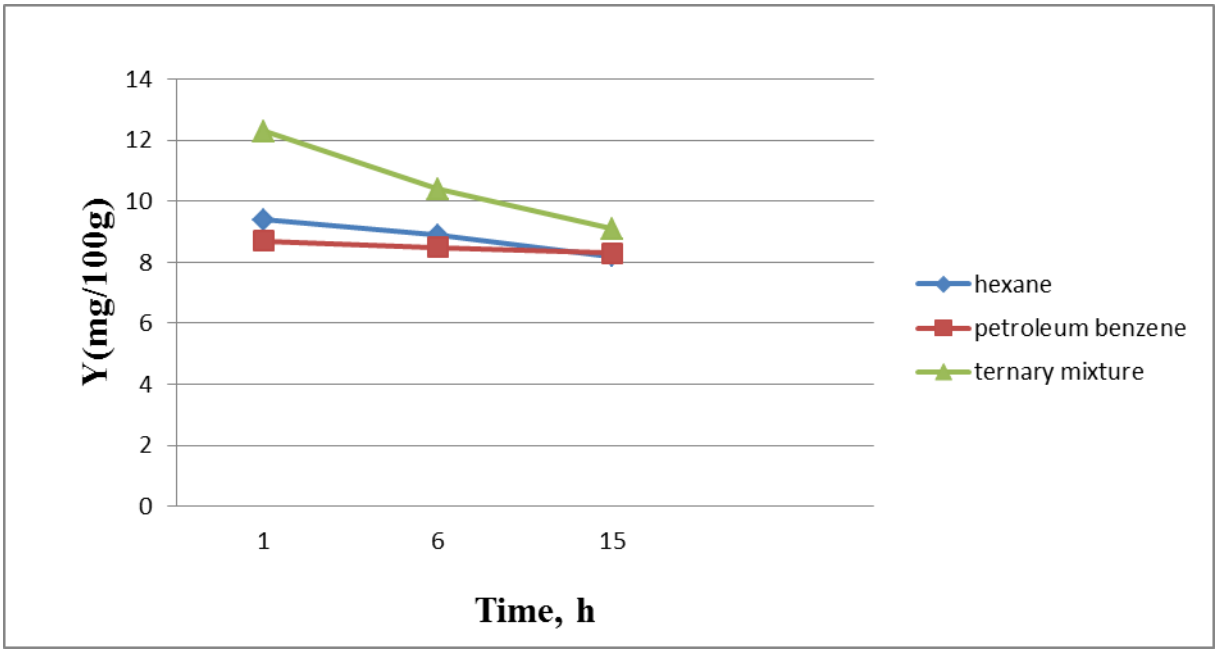

Figure 10. Influence of extraction time on lycopene recovery (Y, mg/100 g) from tomato paste using different extraction solvents (*** Ternary mixture:** Hexane/ethanol/ petroleum benzene (50: 25:25) v/v)

Extraction yields were expressed as mg of lycopene per 100 g of tomato paste. Differences in the lycopene content due to the different ripeness of the tomatoes used and/or the storage conditions of the material.

3.3.2. Study of the Influence of Extraction Conditions on Yields

Figure 10 shows the influence of extraction conditions on the recovery of lycopene from tomato with the best performance.

For all solvents, the highest recovery was achieved with an extraction time of 1h. Under these conditions, lycopene extraction yields in hexane, petroleum benzene and the ternary mixture (hexane: petroleum benzene: ethanol) 50:25:25 were 9.4, 8.7, and 12.3 mg/100g respectively. Increasing the extraction time resulted in a progressive reduction in yields. This suggests that the enzymatic degradation of cell wall components is very fast and occurs within the first hour of extraction. Therefore, the vast majority of lycopene molecules contained in the plant tissue is likely to be rapidly released from the protective

chromoplast structures and exposed to the conditions of the external environment. Because of their high reactivity, the released lycopene molecules can undergo rapid oxidative degradation [28].

Although the underlying mechanisms are only partially elucidated, it has been shown that lycopene oxidation leads to the formation of several cleavage products, including apo-lycopenals/ones and apo-carotendials, whose spectra are shifted to shorter wavelengths compared to that of lycopene [29]. So, the reduction in extraction yields observed at prolonged extraction times could be a reflection of the progressive lycopene loss due to oxidation. As regards the influence of solvent type, it is noted that using hexane or petroleum benzene did not produce significant differences in extraction efficiency. In contrast, the mixture hexane/petroleum benzene/ethanol 50:25:25 appeared to be much more effective. Since hexane is the only component of the mixture with a high affinity for lycopene, it follows that petroleum benzene and ethanol must play some auxiliary role in the overall extraction process. A possible explanation is that the two polar compounds, due to their small molar volume, high hydrogen bonding capability and large basicity, could cause the swelling of the plant tissue [30,31], thus facilitating solvent penetration.

From Figure 10 it is noted that beneficial effects associated with the ternary mixture are more evident when the structural integrity of the tomato pulp is preserved. Considering that the tomato pulp used had a lycopene content of 12.3 mg per 100 g, recoveries at 1h with the mixture hexane/ petroleum benzene /ethanol 50:25:25 as the solvent.

Table 4. Analysis of Variance for extraction of lycopene– Main Effects

Source of variation	Sum of squares	Degree of Freedom	Mean square	F value	Pr(>F)
Time of extraction	6.22	5	1.244	0.908	0.507

From Table 4, no significant difference shows for various time for the extraction of lycopene.

4. Conclusion

The research showed that the pomegranate peel, carrot and tomato are a potential resource for phenolic, beta carotene and lycopene. The pomegranate peel extracted with methanol gave the highest total extract yield, followed by water, ethanol and acetone when the extractions were done with the ratio of solvent/sample of 15:1 (w/w) at 40 °C for 30 min. Comparing methanol with water as the solvent in pomegranate antioxidant extraction, the total extract yield were 42.33% and 44.66%, the yield of total phenolic were 6.1% and 9.1%, the content of phenolic were 14.1% and 18.3%. Shorter extraction time was needed with higher extraction temperature and smaller particle size. High yield was attainable with increased ratio of solvent/solid and was also affected by the extraction temperature. β-carotene extraction from carrot under different experimental conditions was performed. The influence of different extraction conditions was also studied. Ethanol is a more suitable solvent in terms of the carotenes yield as compared to methanol. The extraction yield increases with the increase of the solvent/solid ratio and with temperature up to 60°C. A positive effect was also shown by freezing or cold storage of carrot, the time (approximately 2–4 h) needed for the extraction depending on the treatment of the sample and temperature used. Besides carotenoids concentrate, another interesting by-product gained is dietary fiber, with excellent water binding capacity, which can be therefore used for functional foods production (as bread or pastry supplement). Lycopene extraction from tomato under different solvents and time were performed. Ternary mixture is a more suitable solvent in terms of the lycopene yield as compared to hexane and petroleum benzene. The highest extraction yield was found after 1 h with ternary mixture.

Overall, the above points strongly support the possibility of using different solvents to obtain phenolic compound, beta carotene and lycopene from the pomegranate peel, carrot and tomato. The utilization of this material could add significant value to improve economic performance and decrease disposal problems.

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