Biological Forum – An International Journal **14(2): 582-586(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Extraction of Carotenoids from Carrot and Pumpkin using different Solvent Proportions

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(Received 04 February 2022, Accepted 19 April, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The study aimed to extract carotenoids from carrot (*Daucus carota*) and pumpkin (*Cucurbita moschata*) by using different proportion of solvents such as ethanol, acetone and hexane. Different proportions of solvents (ethanol, acetone, hexane, ethanol: hexane in the proportion (1:1, 2:1.5, 4:3), ethanol: acetone in the proportions (:1, 2:1.5, 4:3) and acetone : hexane (1:1, 2:1.5, 4:3) were used to optimize the extraction procedure. The total carotenoids were identified using UV spectrophotometer method. The detected carotenoids were high in carrots when compared with pumpkins in all the solvent proportions. Furthermore, results obtained from the total carotenoid content and yield showed that ethanol, acetone, hexane, ethanol: acetone in the proportions (:1, 2:1.5, 4:3) had low yield and total carotenoid content and this differed from that with acetone : hexane in the proportion (4:3)(24.96 %, 30.06 mg/g) for carrots and (22.41 %, 16.83mg/g) for pumpkins. Meanwhile the same extracted with ethanol: hexane (4:3) had presented comparatively high yield (15.90 %, 27.55 mg/g) for carrot and (16.08 %, 12.08 mg/g) for pumpkins. Nevertheless, extraction of carotenoids from carrots and pumpkin was found to be optimized in the solvent proportion acetone: hexane (4:3).

Keywords: carotenoids, carrot, pumpkin, ethanol, acetone, hexane, yield, total carotenoid content.

INTRODUCTION

Carotenoids are a group of natural colours produced by plants, algae, fungus, and bacteria. There are over 1100 identified carotenoids. Yellow/red fruits and vegetables contain a lot of carotene. Lutein, β-carotene, α-carotene, zeaxanthin, β-cryptoxanthin, and lycopene are the most common carotenoids found in food (Conboy *et al*., 2021). In the human food supply, beta-carotene is an important functional ingredient in the carotenoid family, providing vitamin A activity from vegetable sources (Hamade *et al*., 2019). Carotenoids, in addition to their provitamin A activity, can be used as natural antioxidants to extend the shelf life of food products and as bioactive components in functional foods to protect against oxidative stress-related diseases. The main carotenoid molecules, such as lycopene and -carotene, on the other hand, are natural colour pigments that have been employed as natural dyes and food colourants (Amr and Hussein, 2013).

Plants and a variety of microorganisms produce carotenoids in nature. Animals are able to digest them in a specific way, but they cannot synthesise them. Carotenoids are terpenoids that are made from isopentyldiphosphate, a basic C5-terpenoid precursor. Geranyl-geranyldiphosphate is formed from this chemical (XVIII). Its dimerization produces phytoene, which undergoes stepwise dehydrogenation to produce lycopene via phytofluene (X), zeta-carotene (XXI), and neyrosporene (XXII) . The other naturally occurring carotenoids are formed as a result of subsequent cyclisations, dehydrogenations, and oxidation processes. It was discovered that carotenoid absorption is improved by light cooking and the addition of dietary lipids. This is likely owing to the release of carotenoids from plant cellular components when heated, as well as the creation of carotenoid-containing micelles from dietary fat, which aid carotenoid absorption in the intestine (Kiokias *et al*., 2002).

Carotenoids reacted most efficiently with peroxyl radicals among the many radicals generated in the organism under oxidative conditions. They were produced during the lipid peroxidation process, and scavenging them disrupts the chemical sequence, resulting in damage to lipophilic compartments. Carotenoids were hypothesised to play a significant role in the preservation of cellular membranes and lipoproteins against oxidative damage due to their lipophilicity and unique ability to scavenge peroxyl radicals (Stahl and Sies, 2003).

Sun *et al.* (2009) reported that chlorogenic acid made up 52.4 percent of the total phenolic components. Carotenoids did not add to total antioxidant capacity, although they did correlate with hydrophobic extract antioxidant capacity. Carrots had total phenolic levels ranging from 114 to 306 mg catechin/kg FW (Koca and Karadeniz, 2011). β-carotene is a source of provitamin A, along with many other carotenoids. Vitamin A, a fat-soluble vitamin, was essential for eyesight and the prevention of nyctolapia (night blindness) and xeropthalmia (Handelman, 2001)

Muntean (2005) concluded that pumpkin juice could be used as a source of carotenoids and as a food colour. The juice added a beautiful yellow to orange colour to a variety of meals without adding any unpleasant flavours. It has the ability to turn cheese orange and to give milk beverages a creamy yellow hue. The advantage of utilising such colourants is that they give value to a food product by contributing to a natural picture. Besides serving as precursors of vitamin A, carotenoids possess antioxidant capacity and are thought to offer protection against atherosclerosis and age- related macular degeneration. The concentration of carotenoids in the extracted juice is 12.45 µg/ g, the major carotenoid being β-carotene (5.80 µg/g), followed by lutein (1.52 µg/g).

Karnjanawipagul *et al*. (2010) reported carrots are a popular vegetable that contain a lot of beta-carotene. β-carotene belongs to the carotene class, which is one of the most abundant found in the diet and is used as food colorants. One of the most important physiological functions of carotenoids in human nutrition is to act as pro-vitamin A. Pumpkins belong to the family of *Cucurbitaceae* (Xanthopoulou *et al*. 2009). Norshazila *et al*. (2014) reported that carotenoids, such as α-carotene, β-carotene and lutein, are present in pumpkin pulp. Pumpkin is an excellent source of carotenoids for food and pharmaceutical industries.

Carotenoids are a class of hydrophobic compounds that have an extremely low aqueous solubility and function in the cell's hydrophobic regions (Jomova and Valko, 2013). According to Cheng *et al.* (2020), the solvent extraction technique served as a physical carrier for moving key compounds across diverse phases such as solid, liquid, and vapour, making it the most widely utilised and practical method. The most common solvents used to extract carotenoids from plant materials were hexane, acetone, and ethanol/hexane. Due to its significant affinity for carotenoids, hexane is one of the most widely used solvents in the industry for extracting β–carotene. Semi-polar carotenoids such as lutein are extracted with semi-polar solvents such as acetone, ethanol, or ethyl acetate.

This study aims to choose the best proportion of solvents to extract carotenoids from carrot and pumpkin.

MATERIALS AND METHODS

Materials

Sources of raw materials

Carrot

Fresh orange color, well matured and unblemished *Daucus carota* were obtained from the local market, Chennai.

Pumpkin

Fresh yellow coloured *Cucurbita moschata* were obtained from the local market, Chennai.

Chemicals and glassware

The media, reagents and chemicals used were obtained from Hi-Media, Mumbai, India. All the glassware (Borosil Pvt. Ltd.) used for this study were thoroughly washed, air dried and sterilized in hot air oven at 160-180 ℃ for 2 hrs prior to use.

Equipment

The list of several laboratory equipment used in the study include Hot air oven, Weighing balance, Rotary evaporator, UV spectrophotometer.

METHODS

Selection of rawmaterials

Carrots (*Daucus carota*) and pumpkins(*Cucurbita moschata*) of good quality were selected for the extraction of carotenoids from pumpkin.

Carrot paste

Fresh carrots (*Daucus carota*) were cleaned with knife and then washed with potable water. Carrots were blanched using hot water and ground to fine paste in mixer. The ground carrot was kept in freezer for about 30 minutes. The frozen ground carrot paste was used for the extraction of carotenoids.

Pumpkin paste

The outer layer of fresh pumpkin (*Cucurbita moschata*) were peeled and thoroughly washed with potable water. Pumpkins were blanched using hot water and made into paste with the help of mixer. The pumpkin paste was kept in freezer for 30 minutes. The frozen ground pumpkin paste was used for the extraction of carotenoids.

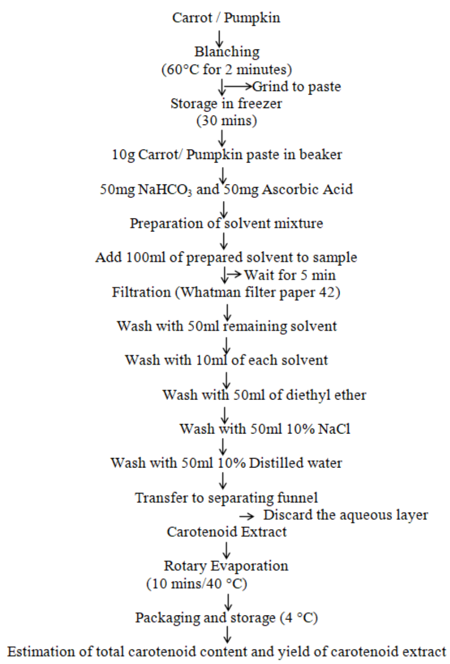
Various proportions for the solvents acetone, ethanol and hexane was given in the following table

Treatments	**Solvents**	**Ratios**
T1	Hexane	-
T2	Acetone	-
T3	Ethanol	-
T4	Ethanol : Hexane	1:1
T5	Ethanol : Hexane	2:1.5
T6	Ethanol : Hexane	4:3
T7	Ethanol : Acetone	1:1
T8	Ethanol : Acetone	2:1.5
T9	Ethanol : Acetone	4:3
T10	Acetone : Hexane	1:1
T11	Acetone : Hexane	2:1.5
T12	Acetone : Hexane	4:3

Extraction of carotenoids from carrot and pumpkin using different solvent proportions

Fresh carrots and pumpkins were thoroughly rinsed with tap water to remove external contaminants before being peeled by hand with a stainless steel knife. Carrots and pumpkins were peeled and cut into slices. To aid the extraction of carotenoids, these slices were blanched in water at 60°C for 2 minutes. To decrease the heat produced during grinding, blanched carrots and pumpkin were ground to a paste and placed in the freezer for 30 minutes. 10 g of frozen carrot/pumpkin samples were extracted using various solvents in varied quantities such as 1:1, 2:1.5, and 4:3. (hexane, ethanol. acetone, ethanol: hexane, ethanol: acetone, acetone: hexane). It should be macerated for 5 minutes before being filtered through Whatman filter paper 42 and rinsed again with 50ml of solvent combination. After washing the sample with 10ml of each solvent, 50ml of diethyl ether is added. Finally, the sample was washed in 50ml sodium chloride and distilled water until it was colourless. A separating funnel was used to collect the filtered extract. The carotenoid layer on top was collected, while the watery layer was discarded. Rotary evaporation at 40°C for 10 minutes was used to remove the solvent. For further investigation, the carotenoid extracts were collected and kept at 4°C. The flow chart for extracting carotenoids from carrots and pumpkin with various solvent proportions is shown below.

Flow chart for the extraction of carotenoids from vegetable sources



Extraction method, (Taungbodhitham *et al*., 1998)

Characterisation of bioactive components from crude extracts

Estimation of Total carotenoid content

The estimation of total carotenoid content was carried by UV-Visible spectrophotometer as per the method described by (Dauqan*et al*., 2011) with some modification. The sample was homogenized and weighed 5 mg of extract in a 250ml volumetric flask. The sample was diluted with n-hexane and made upto mark. Transfer the diluted extract to 1cm quartz cuvette and the absorbance of the sample was measured at 450nm.

Calculation

$$\text{Total carotenoid content}(\mu g/g) = \frac{\text{A (Total)} \times \text{ Volme (ml)} \times 10^4}{\text{A X Sample weight (g)}}$$

Where, A(Total) = absorbance value of extract at 450nm

A= Extinction coefficient of carotenoids (383)

Estimation of Yield of Extract(%)

Yield of the extract was estimated as per the procedure described by (Chen *et al.* 2011).

Calculation

$$\text{Yield}(\%) = \frac{\text{weight of extract (g)}}{\text{weight of the sample taken}(g)} \times 100$$

RESULTS AND DISCUSSION

Effect of solvents on the extraction of total carotenoid and its yield from carrot and pumpkin

Data pertaining to the yield and total carotenoid content of carotenoid extracts from carrot and pumpkin using hexane, acetone and ethanol are presented in Table 1. The yield and total carotenoid content of carrot and pumpkin had highly significant difference (P<0.05) between T1, T2 and T3. The findings in the study correlated with Norshazila *et al*. (2017) who reported that extraction of carotenoids from pumpkin using ethanol had less carotenoid content. In this study, using of individual solvents hasn't improved the extraction yield and TCC.

Table 1: Effect of solvents on the extraction of total carotenoid and its yield from carrot and pumpkin (Mean ± SE)@

Solvents	Carrot		Pumpkin	
	Yield (%)	Total carotenoid content mg /g	Yield (%)	Total carotenoid content mg /g
Hexane	1.26^{c}±0.19	2.38^{b}±0.13	35.20^{b}±0.18	3.23^{b}±0.13
Acetone	5.15^{b}±0.16	1.98^{a}±0.11	4.28^{a}±0.16	2.01^{a}±0.15
Ethanol	4.18^{a}±0.14	1.68^{a}±0.14	4.08^{a}±0.09	1.80^{a}±0.16
F value	15115.12**	7.184**	13898.19**	25.809**

@- Average of six trials (Different superscripts in a column differ significantly)

**- Highly significant (P≤0.01); *-Significant (0.01<P≤0.05); NS-Non-significant (P>0.05)

Yield and TCC of carotenoid extracts from carrot and pumpkin using ethanol : hexane are presented in Table 2. The yield and TCC obtained from carrot and pumpkin were highly significant (P<0.05) between T4, T5 and T6. Among the various proportions T6 provided higher TTC and yield for carrot when compared with pumpkin. The mean±SE values of yield and TTC for carrot were 15.90±0.220 and 27.55±0.218, whereas the pumpkin had yield and TTC of16.08±0.2495 and 12.08±0.260, respectively.

Table 2: Effects of various proportions of ethanol and hexane on the extraction of total carotenoid and its yield from carrot and pumpkin.

Solvents	Carrot		Pumpkin	
	Yield (%)	Total carotenoid content mg /g	Yield (%)	Total carotenoid content mg /g
Ethanol : Hexane (1 : 1)	2.70^{a}±0.1238	0.56^{a}±0.105	2.71^{a}±0.116	0.56^{a}±0.088
Ethanol : Hexane (2 : 1.5)	11.63^{b}±0.236	24.80^{b}±0.186	9.78^{b}±0.227	9.70^{b}±0.225
Ethanol : Hexane (4 : 3)	15.90^{c}±0.220	27.55^{c}±0.218	16.08^{c}±0.2495	12.08^{c}±0.260
F value	1136.475**	7066.027**	1052.139**	879.500**

@- Average of six trials (Different superscripts in a column differ significantly)
**- Highly significant (P≤0.01); *-Significant (0.01<P≤0.05); NS-Non-significant (P>0.05)

Yield and TCC of carotenoid extracts from carrot and pumpkin using ethanol: acetone is presented in table 3. The yield and TCC obtained from carrot and pumpkin had highly significant difference (P<0.05) between T7, T8, T9. This proportion provided with low yield and TCC. Luengo *et al*. (2015) reported that the use of a mixture of polar and non-polar solvents results in the highest extraction yield of carotenoids. In this present study acetone and ethanol were used to extract carotenoids. Acetone and hexane were in polar nature and carotenoids were in non-polar nature, hence the total recovery of carotenoid was not possible.

Table 3: Effects of various proportion of ethanol and acetone on the extraction of total carotenoid and its yield from carrot and pumpkin(Mean ± SE)[@]

Solvents	Carrot		Pumpkin	
	Yield (%)	Total carotenoid content mg /g	Yield (%)	Total carotenoid content mg /g
Acetone : Hexane (1 : 1)	2.51^{a}±0.107	0.783^{a}±0.1137	2.56^{a}±0.120	0.566^{a}±0.114
Acetone : Hexane (2: 1.5)	14.08^{b}±0.153	24.81^{b}±0.166	14.10^{b}±0.159	10.56^{b}±0.170
Acetone : Hexane (4 : 3)	24.96^{c}±0.192	30.06^{c}±0.264	22.41^{c}±1.134	16.83^{c}±0.247
F value	5227.446**	6628.447**	224.579**	1954.237**

**- Highly significant (P≤0.01); *-Significant (0.01<P≤0.05); NS-Non-significant (P>0.05)
@- Average of six trials (Different superscripts in a column differ significantly)

Table 4: Effects of various proportion of acetone and hexane on the extraction of total carotenoid and its yield from carrot and pumpkin(Mean ± SE)[@]

Solvents	Carrot		Pumpkin	
	Yield (%)	Total carotenoid content mg /g	Yield (%)	Total carotenoid content mg /g
Ethanol : Acetone (1 : 1)	2.10^{b}±0.057	0.66^{a}±0.057	2.00^{c}±0.057	0.60^{a}±0.057
Ethanol : Acetone (2 : 1.5)	1.80^{a}±0.057	1.00^{c}±0.0057	1.60^{a}±0.057	1.00^{b}±0.057
Ethanol : Acetone (4 : 3)	2.00^{b}±0.057	0.83^{b}±0.053	1.80^{b}±0.057	0.60^{a}±0.057
F value	7.00*	9.81*	12.0**	0.004**

@- Average of six trials (Different superscripts in a column differ significantly)
**- Highly significant (P≤0.01); *-Significant (0.01<P≤0.05); NS-Non-significant (P>0.05)

Effects of various proportions of acetone and hexane on the extraction of total carotenoid and its yield from carrot and pumpkin are presented in table 4. The yield and TCC from carrot and pumpkin were highly significant between T10, T11 and T12. T12 provided high yield and TTC in carrot (24.96±0.192 and 30.06±0.264) and in pumpkin (22.41±1.134 and 16.83±0.247).Hexane was one of the most widely used solvents in the industry to extract β-carotene, due to its high affinity for carotenoids. Semi-polar solvents such as acetone, ethanol, and ethyl acetate are used to extract those semi-polar carotenoids like lutein (Saini and Keum, 2017). In this present study, T12 yielded high total carotenoid content during extraction, Because polar and nonpolar nature of acetone and hexane improved the extraction efficiency. The findings in the study correlated with Mezzomo *et al*. 2013 who observed that highest yield of total carotenoids was obtained by maceration using acetone, followed by Soxhlet extraction with hexane.

CONCLUSION

According to the findings of this investigation, T12 was the optimal solvent proportion for extracting carotenoids from carrots and pumpkin. As a result, it produced more yield and total carotenoid content. In T12, carrots had a higher carotenoid content than pumpkin. As a result, carotenoids may be extracted from carrot and pumpkin using acetone and hexane (4:3).

FUTURE SCOPE

In future, we would like to encapsulate the carotenoid extracts from carrot and pumpkin and incorporate in various dairy products. Thus it can be an innovative solution as a value added functional product to all groups of people especially, old age people and children who suffer from deficiency of vitamin A. Because the encapsulated carotenoids significantly increased the antioxidant activity of the dairy products, they can be employed to make fortified dairy products with high nutritional value.

Acknowledgement. The authors are grateful to College of Food Dairy Technology, TANUVAS, Chennai for providing financial support to undertake this research work.

Conflict of Interest. None.

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How to cite this article: Athira Surendran, K. Sudha, B. Murugan and Rita Narayanan (2022). Extraction of Carotenoids from Carrot and Pumpkin using different Solvent Proportions. *Biological Forum – An International Journal*, *14*(2): 582-586.