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# Extraction, Separation and Determination of Carotenoid content in Vegetables and Fruits

**A. Mounika[1], A. Sai Padma[2]***

[1,2]Dept. of Biochemistry, Bhavan's Vivekananda College of Science, Humanities and Commerce, Sainikpuri, Secunderabad, India

*Corresponding author: saipadmabhavans@yahoo.co.in Tel: +91 9866351183*

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***Abstract*--**Carotenoids are a class of phytonutrients having important antioxidant, anti-cancer and anti-inflammatory benefits. Some carotenoids are converted by body to Vitamin A which is essential for vision, growth and proper development. Animals cannot synthesize carotenoids themselves and they have to get it in their diet through vegetables and fruits. The present study aims at providing public health awareness by measuring the levels of carotenoids in various fruits and vegetables and suggesting the best sources of these nutrients for their regular consumption. The isolation, estimation and separation of the compounds were based on solvent extraction, colorimetric and TLC methods. The experimental results here provide the amount of carotenes and xanthophylls (carotenoids) in the descending order as Carrots (*Daucus carota*) having 17.6mg/100g, Spinach (*Spinacia oleracea*) 4.4mg/100g, Orange (*Citrus sinenis*) 1.8mg/100g, Red capsicum ( *Capsicum annuum*) 1.44mg/100g, Yellow capsicum (Capsicum annuum) 1.32mg/100g, Muskmelon (*Cucumis memo*)1.32mg/100g, Papaya (*Carica papaya*) 1.08mg/100g and Sweet potato (*Ipomoea batatus*) 0.6mg/100g. The presented data indicates that Carrots are rich source and Sweet Potatoes are poor source of carotenoids from vegetables and fruits that were chosen for the study and provide useful information for human consumption.

***Key words: fruits and vegetables, carotenoids, solvent extraction, TLC.***

## I. INTRODUCTION

Most carotenoids are hydrocarbons containing 40 carbon atoms with two terminal rings. All photosynthetic organisms including plants, algae, cyanobacteria and some non-photosynthetic bacteria and fungi synthesize the carotenoids. Two classes of carotenoids are found in nature which includes the carotenes such as β-carotene, with linear hydrocarbons that are cyclized at one end or both ends of the molecule and xanthophylls, the oxygenated derivatives of carotenes such as lutein, violaxanthin, neoxanthin, and zeaxanthin. The carotenoids which are found in most fruits and vegetables, exert antioxidant and other effects [1, 2]. They have a range of functions in human health and humans cannot synthesize these carotenoids and must ingest them in food or via supplementation. Carotenoids primarily exert antioxidant effects, but individual carotenoids may also act through other mechanisms; for example, β-carotene has a pro vitamin-A function, while lutein/zeaxanthin constitute macular pigment in the eye [3]. There is evidence that carotenoids, in addition to beneficial effects on eye health, also produce improvements in cognitive function and cardiovascular health [4]. Recent studies have shown that consumption of vegetables reduces genetic damage in humans [5]. Though the carotenoids are available in supplement form, however, intervention trials with large doses of beta-carotene found an adverse effect on the incidence of lung cancer in smokers and workers exposed to asbestos [6]. Until the efficacy and safety of taking supplements containing these nutrients can be determined, current dietary recommendations of diets high in fruits and vegetables are advised.

The objective of the present study is to determine the concentration of carotenoids in some of the commonly available vegetables and fruits and provide useful information for daily consumption of carotenoid rich foods for maintaining good health.

## II. MATERIALS AND METHODS

The fruits and vegetables used for the study were bought fresh from local market and includes Carrot (*Daucus carota*), Spinach (*Spinacia oleracea*), Red capsicum (*Capsicum annuum*), Yellow Capsicum (*Capsicum annuum*), Papaya (*Carica papaya*), Muskmelon (*Cucumis memo*), Orange (*Citrus sinensis*) and Sweet Potato (*Ipomoea batatus*). A colorimeter was used to observe the absorbance at 630 nm. Solvent extraction was done using hexane: acetone (1:1). 10% NaCl solution was prepared in the laboratory which was used in the extraction procedure. Silica gel-G for TLC and double distilled water were used to prepare the thin layer chromatography (TLC) plates. The mobile phase consisted of a mixture of analytical grade hexane and acetone in the ratio of 3:2.

**Preparation of TLC plates**

Simple TLC plates were made using microscopic slides. A mixture of silica gel-G and water (12.5 ml of water for 5 grams of silica gel) were mixed together so as to form a paste. This paste was evenly distributed over the surface of the slide and kept aside to dry. Prior to use, slides were activated by baking in the oven at 120°C for 30-40 min.

**Extraction of carotenoids using solvents**

The extraction method followed was as described in the literature with slight modifications [7]. The fruits and vegetables were cut separately and 10g of each fruit and vegetable was weighed and kept separately. The same extraction procedure was followed for all the fruits and vegetables. 10g of the fruit or vegetable was placed in a mortar and crushed with a pestle. A mixture of hexane and acetone in the ratio of 1:1was added into the mortar and the sample was crushed. About 5 ml of acetone was added slowly at regular intervals. The solvents were collected separately and the process was repeated with the sample again for double extraction. The solvents containing carotenoids were filtered through a filter paper and then transferred into a separating funnel. 20ml of distilled water was added along with 20 ml of 10% NaCl solution. The mixture was shaken vigorously and kept aside for the layers to separate. The upper layer contained carotenoids and it was collected separately after the removal of the water and NaCl solution. The extract was collect in tubes. The extraction procedure was repeated thrice for reproducible values and by using a colorimeter, the absorbance of carotenoid was noted at 630 nm.

**Separation of Carotenoids using thin layer chromatography method**

A thin line was drawn on the activated TLC plate about 1.5 cm above the bottom. With the help of capillary tube, a spot of the extract was applied on the line and allowed to dry. This was followed by a repeated addition of the extract on the same spot. The developing chamber was a beaker containing a mixture of hexane and acetone in the ratio of 3:2.The TLC plate was placed inside the developing chamber and the top was covered. The solvents were allowed to rise on the plate till it reached 1.5 cm close to the top. It was then taken out and the Rf value was calculated. The same procedure was repeated for all the extracts.

## III. RESULTS

**Extraction of Carotenoids:** The extraction of carotenoid from the vegetable and fruit samples using solvent extraction method was done using a separating funnel. The different samples were collected in test tubes for further analysis of carotenoids. (Fig:1)

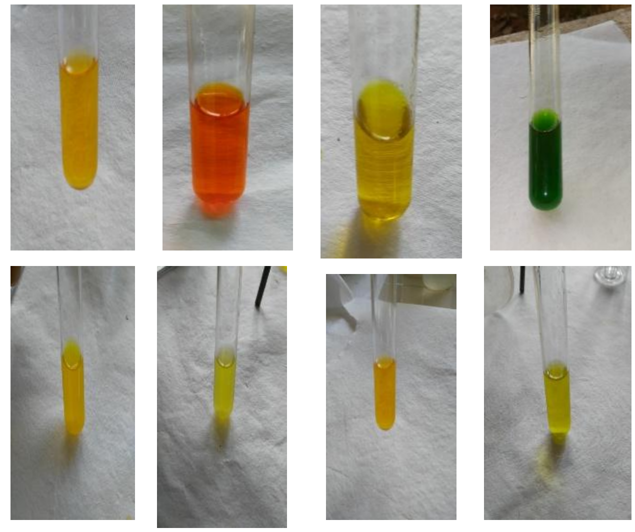

**Figure. 1 Extraction of carotenoids from vegetables and fruits:** (from top left to bottom left, in clockwise direction)**:** Carrot, Red capsicum, Yellow capsicum, Spinach, Muskmelon, Papaya, Sweet Potato, Orange.

**Separation of carotenoids on TLC plates:** The extracted carotenoids were separated on TLC plates on which a spot of extract was placed with repeated applications and kept in a developing chamber with mobile phase of hexane: acetone in the ratio of 3:2. The developed TLC plates with different bands is shown in Fig.2.

**Figure. 2. Separation of carotenoids from vegetables and fruits:** (from top left to bottom left, in clockwise direction): Carrot, Red capsicum, Yellow capsicum, Spinach, Muskmelon, Papaya, Sweet Potato, Orange

**Estimation of Carotenoids:** The absorbance of the carotenoid extracts derived from each vegetable and fruit was noted at

 

630 nm and the formula was used to calculate the amount in each vegetable and fruit. The results obtained is tabulated in **Table 1.** From the data, it can be inferred that per 100g of the sample of vegetables, Carrots yields the highest amount of carotenoids and Sweet potato the least.

**Formula for calculating the concentration of carotenoids in samples:**

Amount of carotenoids in 100mg = 4 x OD value x Total volume of sample / Weight of fresh fruit or vegetable.

**Identification of carotenes and xanthophylls using Rf values:** The extracts were separated using Thin Layer Chromatography method and the Retention factor (Rf) was calculated and the data is presented in **Table.2**.

**Table 1: carotenoids content in vegetables and fruits**

| Fruits and vegetables | Carotenoid content in mg/100g of the sample (±standard deviation) |
|---|---|
| Carrot | 17.6 (±0.084) |
| Spinach | 4.4 (±0.014) |
| Orange | 1.8 (±0.014) |
| Red capsicum | 1.44 (±0.021) |
| Yellow capsicum | 1.32 (±0.028) |
| Muskmelon | 1.32 (±0.014) |
| Papaya | 1.08 (±0.014) |
| Sweet potato | 0.6 (±0.014) |

**Table 2: Rf values of carotenes and xanthophylls**

| Fruits and Vegetables | Rf values | |
|---|---|---|
| | Carotene | Xanthophyll |
| Papaya | 0.94 | 0.42 |
| Carrot | 0.94 | 0.30 |
| Yellow capsicum | 0.94 | 0.38 |
| Red capsicum | 0.88 | 0.42 |
| Muskmelon | 0.88 | 0.48 |
| Orange | 0.86 | 0.46 |
| Spinach | 0.82 | 0.44 |
| Sweet potato | 0.80 | 0.53 |

## IV. DISCUSSION

The procedure of separation of carotenoids by TLC using silica gel-G coated slides is a simple, convenient and reproducible method. However, the absorbance values and Rf values depend on the solvents used, carotenoids concentration and experimental protocols. Our reported values of Rf are in accordance with the typical values, for Carotenes-0.95 and for Xanthophylls 0.35 and were very close to the earlier reported studies [7]. The pigments separated on TLC plates have shown good separation and the experimental results are reproducible. The maximum concentration of carotenoids was found in Carrots (17.6mg/100g) and the minimum in Sweet potato (0.6mg/100g). The present study gives overall report of presence and concentration of carotenoids in different food groups to consume at required levels. Though carotenoids benefit human health in many ways, higher levels of β-carotene above 15-20 mg/d are rather discouraged, especially for smokers and intake in the range of 2-7 mg/d is recommended [8]. There is a large inter individual variability regarding carotenoid response in relation to individual differences in digestion, absorption, cleavage and bio distribution [9].

## V. CONCLUSIONS

The results presented here indicate that Carrots are rich source of carotenoids followed by Spinach, Orange, Red capsicum, Yellow capsicum, Muskmelon, Papaya and the poor source was Sweet potato from the vegetables and fruits that were chosen for the study and provide useful information for appropriate consumption by humans.

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