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Research Article

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Extraction and purification of carotenoids from vegetables

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ABSTRACT

A diet rich in vegetables is recommended along with fruits and whole grains.An epidemiological study found that a diet of this composition has a negative association with the risk of chronic diseases. Carotenoids in vegetables are of the significant importance, besides other vitamins, minerals, flavonoids and phytochemicals, which have been reported to contribute to health. A precursor to Vitamin A, β-carotene is commonly found in many vegetables. β-carotene is also converted to Vitamin-A in the intestinal wall and stored in the liver. This makes β-carotene an important natural product of organic chemistry. This study was conducted to estimate the total amount of carotenes and its counterpart, xanthophylls in vegetables, namely: Red capsicum (Capsicum annuum), Yellow capsicum (Capsicum annuum), Red spinach (Amaranthus dubius), Carrot (Daucus carota), Broccoli (Brassica oleracea), Beetroot (Beta vulgaris). The experiment yielded appreciable results and the vegetables were graded in the order of their respective carotene content. The most notable amount of carotenoid was obtained from carrots.

Keywords: carotenoids, β-carotene, carrots, beetroot, capsicum, xanthophylls

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INTRODUCTION

Oxidative stress is an important contributor to the risk of chronic diseases. Dietary guidelines recommend increased consumption of fruits and vegetables to combat the incidence of human diseases such as cancer, cardiovascular disease, osteoporosis and diabetes. Fruits and vegetables are good sources of antioxidant phytochemicals that mitigate the damaging effect of oxidative stress. Carotenoids are a group of phytochemicals that are responsible for different colours of the foods [1, 2, 3]. They are recognized as playing an important role in the prevention of human diseases and maintaining good health. The term carotene (also carotin, from the Latin *carota*, or carrot) is used for several related unsaturated hydrocarbon substances having the formula $C_{40}H_x$, which are synthesized by plants but cannot be made by animals. Carotene is an orange photosynthetic pigment important for photosynthesis. Carotenes are all coloured to the human eye. Carotenes are valuable preventive medicines, too. Research shows that people, who eat a lot of foods rich in beta-carotene-the carotenoid with the greatest vitamin A value, are less likely to develop lung cancer. They are metabolized by hydroxylation, epoxidation, isomerization, oxidation-reduction and degradation. In addition to being potent antioxidants some carotenoids also contribute to dietary vitamin A. They are all synthesised by higher plants, algae and bacteria and are widely distributed in animals, which acquire them via their diet. In the plant carotenoids act as photosynthetic accessory pigments and also play a protective function as scavengers of oxygen radicals released from chloroplasts during photosynthesis, thus protecting cellular constituents such as DNA from free radical damage [1, 2, 3]. Carotenoids generally cannot be manufactured by species in the animal kingdom so animals obtain carotenoids in their diets, and may employ them in various ways in metabolism. They are split into two classes, xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons, and contain no oxygen). All carotenoids are tetraterpenoids, meaning that they are produced from 8 isoprene molecules and contain 40 carbon atoms. Carotenoids in general absorb blue light. They serve two key roles in plants and algae: they absorb light energy for use in photosynthesis, and they protect chlorophyll from photodamage. In humans, three carotenoids (beta-carotene, alpha-carotene, and beta-cryptoxanthin) have vitamin A activity (meaning they can be converted to retinal), and these and other carotenoids can also act as antioxidants. In

the eye, certain other carotenoids (lutein, astaxanthin and zeaxanthin) apparently act directly to absorb damaging blue and near-ultraviolet light, in order to protect the macula of the retina, the part of the eye with the sharpest vision. Food coloring carotenoids include beta-carotene, paprika, lycopene, lutein, carrot oil and saffron. Each contains different types and ratios of carotenoids.

Xanthophylls (originally phylloxanthins) are yellow pigments that form one of two major divisions of the carotenoid group. The name is from Greek *xanthos* (yellow) and *phyllon* (leaf), due to their formation of the yellow band seen in early chromatography of leaf pigments. Their molecular structure is similar to carotenes, which form the other major carotenoid group division, but xanthophylls contain oxygen atoms, while *carotenes* are purely hydrocarbons with no oxygen. Xanthophylls contain their oxygen either as hydroxyl groups and/or as pairs of hydrogen atoms that are substituted by oxygen atoms acting as a bridge (epoxide) [4]. Like other carotenoids, xanthophylls are found in highest quantity in the leaves of most green plants, where they act to modulate light energy and perhaps serve as a non-photochemical quenching agent to deal with triplet chlorophyll (an excited form of chlorophyll), which is overproduced at high light levels in photosynthesis [5, 6, 7].

The objective of the study is not only to estimate the amount of carotenoids but to also compare the estimated amouts with the other samples present in the study. The primary objective of this study is to estimate the concentration of carotenoids (carotenes and xanthophylls) using simple solvent extraction ant thin layer chromatography in the following vegetables: Red capsicum (*Capsicum annuum),* Yellow capsicum (*Capsicum annuum*), Red spinach (*Amaranthus dubius*), Carrot (*Daucus carota*) and Broccoli (*Brassica oleracea*).

A process for the extraction of carotenoid dyes from pre dried natural starting materials is described using com pressed gases such as propane and/or butane in which organic entrainlng agents can be additionally added in order to facilitate and complete the extraction process. With the aid of this process highly concentrated carotenoid dyes are obtained in high yield [8,9]. The methods of TLC and UV were used for the separation and identification of carotenoid pigment extracted from carrot through the application of different organic solvent. Brassica oleracea vegetables, such as broccoli (B. oleracea L. var. italica) and cauliflower (B. oleracea L. var. botrytis), are known to contain bioactive compounds associated with health, including three classes of photosynthetic lipid-soluble compounds: carotenoids, chlorophylls, and tocopherols. Carotenoids and chlorophylls are photosynthetic pigments. Tocopherols have vitamin E activity. Due to genetic and environmental variables, the amounts present in vegetables are not constant. To aid breeders in the development of Brassica cultivars with higher provitamin A and vitamin E contents and antioxidant activity, a more efficient method was developed to quantitate carotenoids, chlorophylls, and tocopherols in the edible portions of broccoli and cauliflower. The novel UPLC method separated five carotenoids, two chlorophylls, and two tocopherols in a single 30 min run, reducing the run time by half compared to previously published protocols. The objective of the study was to develop a faster, more effective extraction and quantitation methodology to screen large populations of Brassica germplasm, thus aiding breeders in producing superior vegetables with enhanced phytonutrient profiles [10, 11].

EXPERIMENTAL SECTION

The vegetables used for the study are Red capsicum (*Capsicum annuum*), Yellow capsicum (*Capsicum annuum*), Red spinach (*Amaranthus dubius*), Carrot (*Daucus carota*), Broccoli (*Brassica oleracea*), Beetroot (*Beta vulgaris*). The vegetables used were bought fresh from the market and preserved in the refrigerator. A calorimeter was used to observe the absorbance at 630 nm.

Solvent extraction was done using hexane: acetone (1:1) along with ethanol, 10% NaCl solution was also prepared in the laboratory which was used in the extraction. Silica gel and calcium sulphate hemihydrates were used in the ratio of 4:1 along with double the amount of water to prepare the thin layer chromatography (TLC) plates. The mobile phase consisted of a mixture of 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 (finely powdered) and calcium sulphate hemihydrates were mixed together and double the amount of water was added so as to form a paste. This paste was evenly distributed over the surface of the slide and kept aside to dry. Prior to usage, slides were activated by baking in the oven at 120°C for 30-40 min.

Extraction of carotenoids using solvents

The vegetables were cut separately and 2g of each vegetable was weighed and kept separately. The same extraction procedure was followed for all the vegetables. 2g of the vegetable was placed in a mortar and crushed with a pestle. A mixture of hexane and acetone in the ratio of 1:1 was added into the mortar and the sample was crushed. About

5ml of acetone was added slowly at regular intervals. The solvents were collected separately and the process was repeated with the sample again for double extraction. The solvents containing carotenoids were filtered through a filter paper and then transferred into a separating funnel. 50ml of distilled water was added along with 50 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. Using a calorimeter, the absorbance of carotenoid was noted at 630 nm. The amount of carotenoid present in 100g of each food sample was calculated.

Purification using thin layer chromatography method

A thin line was drawn on the activated TLC plate about 1.5 cm above the bottom. A spot of the extract was placed 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 was calculated.

RESULTS AND DISCUSSION

The procedure for this experiment proved to be quite interesting although a few changes were required in certain aspects. The extraction procedure using solvents proved to be highly efficient. Purification using thin layer chromatography was not very effective for certain samples. The TLC showed the orange band at a lower R_f value than the yellow band.

Fig 1. Extraction of pigments from carrot

Fig. 2 Collection of extracted pigments

The extraction of carotenoid from the vegetable samples using solvent extraction method in a separating funnel is shown in Fig 1. The different samples were collected in test tubes for further analysis (Fig. 2). The TLC plate on which a spot of the extract was placed and kept in a developing chamber to separate into different bands is shown in Fig 3 and the developed TLC plate with the orange spot is shown in Fig 4, respectively.

Fig.3 TLC plate plate inside developing chamber **Fig. 4 TLC plate**

The absorbance of the extract derived from each vegetable at 630 nm was noted down and the value was used to calculate the carotenoid content in each vegetable. The result is tabulated in Table 1. It can be inferred that per 100g of the sample of vegetables, carrot yields the most amount of carotenoids and broccoli yields the least.

Table 1: Beta-carotene content in vegetable sample

Vegetable	Carotenoid content (mg/100g of the sample)
Carrot	18.3
Spinach	5.6
Red capsicum	2.4
Yellow capsicum	2.4
Beetroot	1.9
Broccoli	1.3

The extract was purified using Thin Layer Chromatography method and the retardation factor was calculated (Table 2).

Table 2: Results of Thin layer chromatography

Vegetable sample	Rf factor	
	Carotene	Xanthophyll
Carrot	0.95	0.31
Spinach	0.77	0.44
Red capsicum	0.94	0.36
Yellow capsicum	0.88	0.38
Beetroot	0.84	0.42
Broccoli	0.81	0.52

The results thus obtained were either same or marginally different for all of the vegetables when compared to a few similar studies that were undertaken based on carotenoids. Studies state that absorbance change in accordance to the solvent used, pigment concentration, experiment results and calculation. However, the results should be similar to the following typical value: Carotene – 0.95, Xanthophylls – 0.35. Using the works of Tomkins in the paper " a rapid extraction and fast separation of leaf pigments using thin layer chromatography", published in 1994, comparisons were made between the results of both the studies and they were found to be in accordance with one another.

CONCLUSION

Based on the results, it can be noted that carrots contained the highest concentration of carotenoids followed by spinach, red capsicum, yellow capsicum and beetroot. Broccoli was reported to have the least amount of carotenoids of the six vegetables that were chosen for the study.

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