ISSN 2347-2375

UNIQUE JOURNAL OF AYURVEDIC AND HERBAL MEDICINES

Available online: www.ujconline.net

Research Article

BETA CAROTENE ANALYSIS FROM VEGETABLES USING FOURIER TRANSFORM INFRARED SPECTROSCOPY

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Received 24-08-2016; Revised 22-09-2016; Accepted 20-10-2016

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ABSTRACT

Natural products have been the basis of treatment of human diseases for a long period of time. Modern medicine or allopathy has gradually developed over the years due to the scientific and observational efforts of scientists. However the basis for its development remains in the roots of traditional medicine and therapies. Herbal medicinal preparations and their proprietary products are also being used more and more widely throughout the world. Plants show enormous versatility in synthesizing complex materials, which have no immediate obvious growth or Metabolic functions. These complex materials are referred to as secondary Metabolites. Secondary Metabolites present in plants are biologically active and these bioactive compounds are believed to be effective in combating or preventing disease due to their antioxidant effect which is due to their capability to quench lipid peroxidation, prevent DNA oxidative damage and scavenge reactive oxygen species. FTIR is a technique particularly useful for the identification of organic molecular groups and compounds due to the range of functional groups, side chains and cross-links involved. Vegetables can be eaten either raw or cooked and play an important role in human nutrition, being mostly low in fat and carbohydrates, but high in vitamins, minerals and fiber. Particularly important are the antioxidant vitamins A, C and E. When vegetables are included in the diet, there is found to be a reduction in the incidence of cancer, stroke, cardiovascular disease and other chronic ailments. The present study is a pioneer work in analyzing the Beta carotene from selected Vegetables using FTIR Technique.

Keywords: Natural products, Allopathy, Herbal Medicine, FTIR, Vegetables.

INTRODUCTION

Traditional medicine has a long history of serving people all over the world. India is without doubt a herbal hub. Medicinal plants that are native to India and their use in various traditional systems of medicine are indeed awe-inspiring. The Ethnobotany of ubiquitous plants provides a rich resource for natural drug research and development[1]. The World Health Organization (WHO) defines traditional medicine as "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness"[2].

The well-documented health benefit of a diet high in fruits and vegetables has led to a growing interest in so-called "functional foods" and their application in health and disease. In recent years, the root vegetable *Beta vulgaris*, otherwise known as red beetroot (herein referred to as beetroot) has attracted much attention as a health promoting functional food. While scientific interest in beetroot has only gained momentum in the past few decades, reports of its use as a natural medicine date back to Roman times[3].

VEGETABLES AND THEIR HEALTH BENEFITS

Research has shown that compared with individuals who eat less than three servings of fruits and vegetables each day, those that eat more than five servings have an approximately twenty percent lower risk of developing coronary heart disease or stroke (**Figure 1**). Vegetables contain a great variety of other phytochemicals (bioactive non-nutrient plant compounds), some of which have been claimed to have antioxidant, antibacterial, antifungal, antiviral and anticarcinogenic properties[4].

Current research suggests that carrots may possess anti-cancer properties, as well as benefits for people with high blood pressure and cardiovascular disease. Beta-carotene is converted by the body into vitamin A and is a powerful antioxidant, protecting the body from free radicals and maintaining healthy skin and eyes[5]. Carrot is a root vegetable with carotenoids, flavonoids, polyacetylenes, vitamins, and

minerals, all of which possess numerous nutritional and health benefits[6]. Besides lending truth to the old adage that carrots are good for eyes, carotenoids, polyphenols and vitamins present in carrot act as antioxidants, anticarcinogens, and immunoenhancers[7]. Anti-diabetic, cholesterol and cardiovascular disease lowering, anti-hypertensive, hepatoprotective, renoprotective, and wound healing benefits of carrot have also been reported. The cardio and hepatoprotective, anti-bacterial, anti-fungal, anti-inflammatory, and analgesic effects of carrot seed extracts are also noteworthy[8].

In recent years there has been a growing interest in the biological activity of red beetroot (*Beta vulgaris*) and its potential utility as a health promoting and disease preventing functional food. As a source of nitrate, beetroot ingestion provides a natural means of increasing *in vivo* nitric oxide (NO) availability and has emerged as a potential strategy to prevent and manage pathologies associated with diminished NO bioavailability, notably hypertension and endothelial function. Beetroot is also being considered as a promising therapeutic treatment in a range of clinical pathologies associated with oxidative stress and inflammation. Its constituents, most notably the betalain pigments, display potent antioxidant, anti-inflammatory and chemo-preventive activity *in vitro* and *in vivo*[9]. The recent interest in beetroot has been primarily driven by the discovery that sources of dietary nitrate may have important implications for managing cardiovascular health[10]. However, beetroot is rich in several other bioactive compounds that may provide health benefits, particularly for disorders characterised by chronic inflammation. Recent studies have also provided compelling evidence that beetroot ingestion offers beneficial physiological effects that may translate to improved clinical outcomes for several pathologies, such as; hypertension, atherosclerosis, type 2 diabetes and dementia[11].

Dark leafy greens like spinach are important for skin, hair and bone health by providing protein, iron, vitamins and minerals. The possible health benefits of consuming spinach include improving blood glucose control in diabetics, lowering the risk of cancer, lowering blood pressure, improving bone health, lowering the risk of developing asthma and more. Spinach is also one of the best sources of dietary magnesium, which is necessary for energy metabolism, maintaining muscle and nerve function, heart rhythm, a healthy immune system and maintaining blood pressure. Magnesium also plays a major part in many biochemical reactions that occur in the body. Those with digestive disorders, alcoholic, older adults and individuals taking medications such as antibiotics and diuretics are more likely to have a magnesium deficiency and should consume more leafy greens. Spinach also contains vitamin K, fiber, phosphorus and thiamine[12].

Figure 1: Dried Vegetable samples

CAROTENOIDS

Carotenoids play a very important role in human health. They are known to be very efficient physical and chemical quenchers of singlet oxygen (O2), as well as potent scavengers of other reactive oxygen species (ROS), thus acting as very important natural antioxidants. This it is of special significance, because the uncontrolled generation and concomitant increase of ROS level in the body results in "oxidative stress", an essential contributor to the pathogenic processes of many diseases[13]. Some carotenoids, such as lycopene, zeaxanthin, lutein, capsanthin, and canthaxanthin are not converted into vitamin A in the body. But again, they are powerful cancer fighters, prevalent in fruits and vegetables. There is abundant evidence that lycopene in particular helps reduce the risk for prostate cancer[14].

Carotenoids are also suggested to participate in: (i) the stimulation of the immune system; (ii) the modulation of intracellular signaling pathways (gap junction communication); (iii) the regulation of the cell cycle and apoptosis; (iv) the modulation of growth factors; (v) cell differentiation; and (vi) the modulation of various types of receptors or adhesion molecules and many other physiologically significant processes. Thus carotenoids are pigments that are found to have multimedicinal value[15].

The present study is aimed at isolating the carotenoid pigments from various **Vegetables** such as Carrot, Beet root, Red spinach and Green spinach which are rich in beta carotene and analyze them using FTIR Technique.

MATERIALS AND METHODOLOGY

SAMPLES USED IN THE PRESENT STUDY ARE AS FOLLOWS

Green spinach (*Sauropus androgynus* (L.)Merr.)
Beetroot (*Beta vulgaris* L.)
Red spinach (*Amaranthus dubius* Mart.ex Thell.)
Carrot (*Daucus carota* L.)

PREPARATION OF EXTRACTS

The VEGETABLES were collected and dried in shade for few weeks. The dried samples were ground into powder. 5gm of the dried sample powder was weighed and immersed in 50 ml of the solvents – Ethanol, Ethyl acetate and Chloroform for 48 hours. After 48 hours, the extracts were filtered. The

carotenoid pigments were isolated using Column Chromatography and was quantified using the formula

Total carotenoid content (µg/g) = A x V (ml) x 10^4 / $A^{1\%}$cm x W (g)

Where A is the absorbance of the carotenoid pigment at 450 nm, V is the total extract volume, $A^{1\%}$cm is the absorption coefficient of β carotene in hexane (2600), W is the sample weight. The samples were further subjected to Thin Layer Chromatography and FTIR studies to confirm the presence of Beta Carotenes.

RESULTS AND DISCUSSIONS

ISOLATION OF CAROTENOID PIGMENTS BY COLUMN CHROMATOGRAPHY

Carotenoid pigments were effectively separated from the sample extracts separately in a silica gel column with 100% hexane. The yellow colour band which gets separated when eluted with 100% hexane is identified to be carotenoid pigments **(Figure 2)**.The carotenoid pigments eluted with hexane was collected and stored in vials at -20ºC.

QUANTIFICATION OF CAROTENOIDS

The total carotenoid content quantified are as follows

Total carotenoid content in carrot = 0.252x10x10^4 / 2600x10 = **0.96 µg/g.**

Total carotenoid content in red spinach = 0.231x10x10^4 / 2600x10 = **0.88 µg/g.**

Total carotenoid content in green spinach = 0.252x10x10^4 / 2600x10 = **0.96 µg/g.**

Total carotenoid content in beet root = 0.145x10x10^4 / 2600x10 = **0.56 µg/g.**

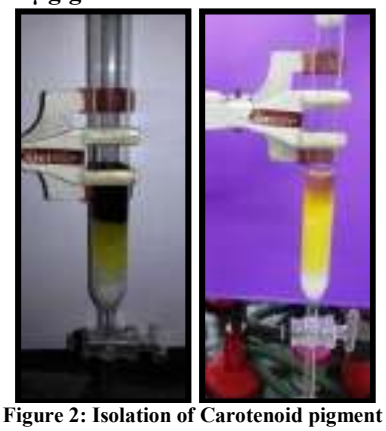


Figure 2: Isolation of Carotenoid pigment

THIN LAYER CHROMATOGRAPHY

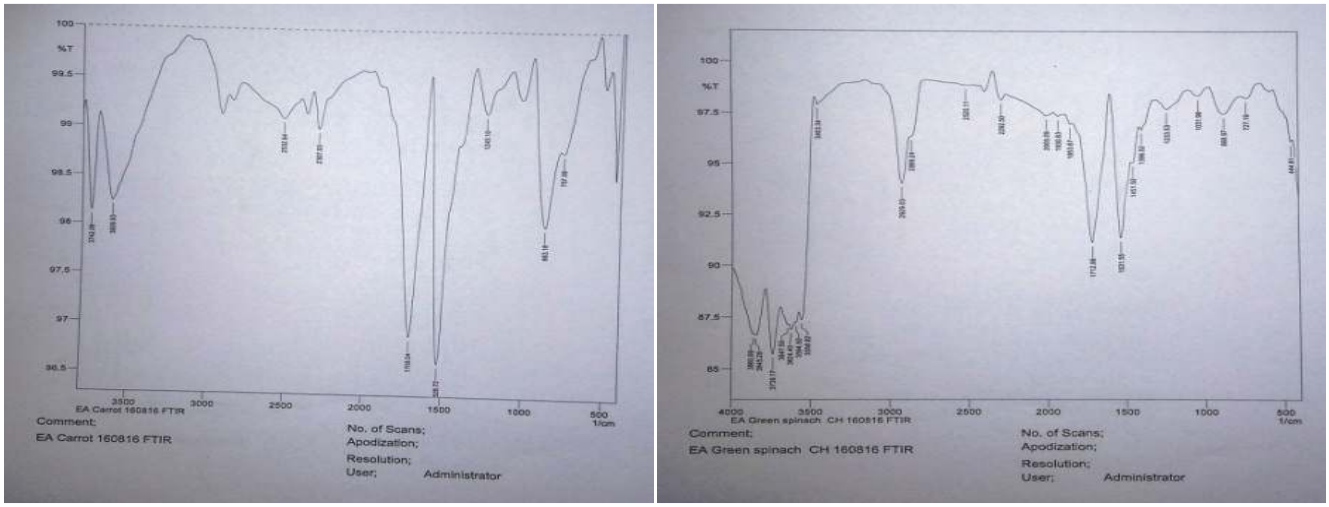
The crude extracts and the purified carotenoid pigments and the standard were subjected to thin layer chromatography. The standard used was beta carotene. The mobile phase used was hexane and acetone in the ratio 6:4. The respective Rf values for the Vegetables (Carrot, Beet root, Red spinach and Green spinach) were calculated **(Table 1).**

Table 1 : R_f Values of Crude Extract and Carotenoid

Sample	Ethanol Crude	Ethyl Acetate Crude	Chloroform Crude	Carotenoide Pigment
Carrot	0.94	0.91	0.94	0.94
Red Spinach	0.95	0.95	0.94	0.94
Green Spinach	0.95	0.95	0.94	0.94
Beet Root	0.92	0.91	0.94	0.92

FOURIER TRANSFORM INFRARED SPECTROSCOPY

The FTIR spectrum of β-carotene spectrum was recorded in the range of 4000-500 cm^{-1} respectively. In the spectrum of β-carotene the infrared band at between 1250 cm^{-1} and 740cm^{-1} are characteristic of 7-cis configuration and the infrared band at 780 cm^{-1} is characteristic of 15-cis configuration isomers being present. All isomers specifically cis-isomer of β-carotene give two characteristic coupled C==C—C stretchings at 1720 cm^{-1} and 1680 cm^{-1} in the infrared spectrum. Thus in the FTIR analysis of samples, Carrot and Green spinach, the presence of beta carotene was confirmed **(Figures 3 – 4).**



Figures 3 - 4: FTIR result of Vegetables

CONCLUSION

The carotenoids were extracted from the Vegetables **(Carrot, Beetroot, Red Spinach and Green spinach)** by Column Chromatography and were subjected to Thin Layer Chromatography. The pigments were further analysed by Fourier Transform Infrared Spectroscopy to find the carotenoid pigment. It was found that beta carotene is the extracted carotenoid pigment present in the **Carrot and Green Spinach** samples.

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Source of support: Nil, Conflict of interest: None Declared