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Research Article

ANALYSIS OF BETA CAROTENE FROM FRUITS USING FOURIER TRANSFORM INFRARED SPECTROSCOPY

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ABSTRACT

Citrus fruits, which belong to the family of Rutaceae are one of the main fruit tree crops grown throughout the world. Citrus fruits are well endowed with a variety of phytonutrients. Phytonutrients are vital in both; health promotion and disease prevention. Phytonutrients are mainly natural bioactive compounds from plants with general benefits to human health. The secondary metabolities of plants provide humans with numerous biologically active products, which have been used extensively as food additives, flavours, colors, insecticides, drugs, fragrances and other chemicals. These plant secondary metabolites include several classes such as terpenoids, flavonoids and phenolics compounds having diverse chemical structures and biological activities and exist widely in citrus fruits. The dietetic and therapeutic properties of all citrus fruits are similar due to their phytonutrient contents. Thus Fruits help to maintain optimum health due to the health promoting phytochemicals it contains – many of which are still being identified. One to 2-1/2 cups of fruits are recommended each day for a healthy living. The present study is a pioneer work in analyzing the Beta carotene from selected Fruits using FTIR Technique.

Keywords: Citrus fruits, Phytonutrients, Phytochemicals, Beta Carotene and FTIR.

INTRODUCTION

Fruits have been recognized as good sources of vitamins and minerals, and for their role in preventing vitamin C and vitamin A deficiencies. Fruits are important sources of many nutrients, including potassium, fiber, vitamin C and folate (folic acid). People who eat fruit as part of an overall healthy diet generally have a reduced risk of chronic diseases. United States Department of Agriculture (USDA's) My Plate organization encourages making half your plate with fruits and vegetables for healthy eating. They insist on incorporating fruits rich in vitamin C which contain phytochemicals for added health benefits (**Figure 1**).

Figure 1: Dried fruit samples

Citrus plants synthesize and accumulate in their cells a great variety of phytochemicals including low molecular phenolic hydroxy benzoic and hydroxycinnamic acids, acetophenones, terpenoids, flavonoids, stilbenes and condensed tannins. There are about 40 limonoids in citrus with limonin and nomilin being the principal ones. These compounds, which occur in high concentration in orange juice (*Citrus sinensis* L.Osbeck) partly, provide the bitter taste in citrus. Limonoids possess the ability to inhibit tumor formation by stimulating the enzyme glutathione S-transferase (GST). Orange and lemon oil contains substantial amounts of GST that also possesses anti-cancer activity. Citrus pulp and the albedo (the white of the orange) are rich in glucarates. These substances are being studied extensively for their potentials in preventing breast

cancer and lower the risk and symptoms of premenstrual syndrome[1].

Another group of phytochemicals found in citrus are carotenoids. Pink grapefruit have a high content of β-carotene while other citrus fruits such as tangerines and oranges contain high levels of other carotenoids (lutein, zeaxanthin, cryptoxanthin) that have significant anti-oxidant activity. These carotenoids are associated with a lower incidence of age-related macular degeneration, the leading cause of blindness in human after the age sixty five[2].

All parts of the banana plant have medicinal applications[3]. Antifungal and antibiotic principles are found in the peel and pulp of fully ripe bananas[4]. The antibiotic acts against Mycobacteria[5]. A fungicide in the peel and pulp of green fruits is active against a fungus disease of tomato plants[6]. Norepinephrine, dopamine, and serotonin are also present in the ripe peel and pulp[7]. Some of the specific diseases known to be cured by banana are Anaemia as they are believed to be rich in iron to stimulate the production of haemoglobin in the blood and so help in cases of anaemia. Banana is extremely high in potassium yet low in salt, making it the perfect food for helping to beat blood pressure[8].

Bananas can also help people trying to give up smoking, as the high levels of Vitamin C, A1, B6, B12 they contain, as well as the potassium and magnesium found in them, help the body to recover from the effects of nicotine withdrawal[9]. Potassium is a vital mineral, which helps normalize the heartbeat, sends oxygen to the brain and regulates the body's water-balance[10].

In recent times, Banana peel has been utilized for various industrial applications including bio-fuel production, bio-sorbents, pulp and paper, cosmetics, energy related activities, organic fertilizer, environmental cleanup and biotechnology related processes[11, 12, 13]. It has been observed that antimicrobial activity of the plants is associated with the presence of some chemical components such as phenols, tannis, saponins, alkaloids, steroids, flavonoids and carbohydrates[14].

Pineapples are a funny-looking fruit with a serious impact on health, and their health and medicinal benefits include their ability to improve respiratory health, cure coughs and colds, improve digestion, help lose weight, strengthen bones, improve oral health, boost eye health, reduce inflammation, prevent cancer, increase heart health, fight off infections and parasites, improve the immune system and increases circulation. A single serving of pineapple has more than 130% of the daily requirement of vitamin-C for human beings, making it one of the richest and most delicious sources of ascorbic acid. Vitamin C is mainly associated with reducing illness and boosting the immune system by stimulating the activity of white blood cells and acting as an antioxidant to defend against the harmful effects of free radicals. Free radicals are dangerous byproducts of cellular metabolism that can damage various organ systems and disrupt function, as well as cause healthy cells to mutate into cancerous ones. The vitamin C content of pineapples defends against this. High vitamin C content helps to heal wounds and injuries to the body quickly, along with defending against infections and illness. In addition to the antioxidant potential of vitamin C in the battle against cancer, pineapples are also rich in various other antioxidants, including vitamin A, beta carotene, bromelain, various flavonoid compounds, and high levels of manganese, which is an important co-factor of superoxide dismutase, an extremely potent free radical scavenger that has been associated with a number of different cancers. Pineapple has directly been related to preventing cancers of the mouth, throat, and breast[15]. Pineapple contains a lot of ascorbic acid which is a water-soluble vitamin that has a number of biological functions. Acting as an antioxidant, ascorbic acid important function is to protect Low-density Lipoprotein (LDL) cholesterol from oxidative damage.

CAROTENOIDS

Carotenoids are an abundant group of naturally occurring pigments. They occur ubiquitously in all organism of conducting photosynthesis**.** Citrus carotenoid was demonstrated to have significant reductions in the risk of developing neovascular ARMD as a function of plasma levels of α- carotene, β-carotene, cryptoxanthin, lutein and zeaxanthin. Based on epidemiological data, it can be assumed that diets rich in carotenoid containing fruits are associated with significant decreased risks for a variety of degenerative diseases. Several epidemiological studies have supported the observation that a high content of blood carotenoids decrease the risk of cataract formation. The ability of carotenoids, to act as antioxidant has been reported**.**

Carotenoids are known to suppress the growths of tumors in in vitro (test tube) and in vivo (animal) studies[16]. The various carotenoids such as lycopene, β-carotene, α-carotene, lutein and canthaxanthin can decrease malignant transformation of cells. There have been positive reports on dietary carotenoids improving fertility or reproduction capacity in a number of animals[17]. Carotenoids besides the anticancerous effect, showed a strong antioxidant character, which plays an important role in the prevention and treatment of cardiovascular, ophthalmological, dermatological diseases and prevents the oxidative damages that are specific to ageing phenomena and also prevents the immunological disorders. Due to carotenoids great sensitivity to light, heat, oxygen, acids, their isolation from different raw materials must be accomplished choosing the optimal work conditions to gum up their degradation[18].

The present study is aimed at isolating carotenoid pigments from various **Fruits** such as Orange, Lemon, Pineapple and Banana which are rich in vitamin A, vitamin C and beta carotene and to evaluate their applications in various fields of medical sciences.

MATERIALS AND METHODOLOGY

SAMPLES USED IN THE PRESENT STUDY ARE AS FOLLOWS

Orange (*Citrus reticulate* Blanco)
Lemon (*Citrus limon* (L.)Brum.f.)
Pineapple (*Ananas comosus* (L.)Merr.)
Banana (*Musa acuminate* Colla.)

PREPARATION OF EXTRACTS

The FRUITS 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 were further subjected to Thin Layer Chromatography and FTIR studies to confirm the presence of Beta Carotenes.

RESULTS AND DISCUSSION

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.

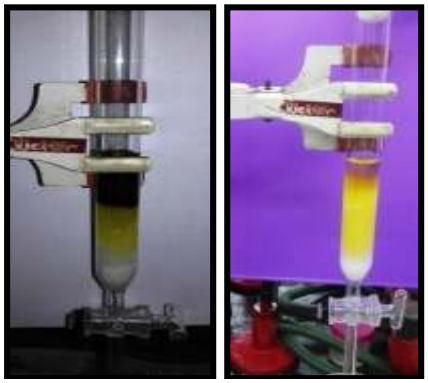


Figure 2: Isolation of Carotenoid pigment

QUANTIFICATION OF CAROTENOIDS

The total carotenoid content was calculated and the absorbance of the extracted carotenoids was measured at 450nm. The extracted carotenoids were quantified by the following 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.

Total carotenoid content in orange = 0.245x10x10^4 / 2600x10 **= 0.94 µg/g.**

Total carotenoid content in lemon = 0.220x10x10^4 / 2600x10 **= 0.84 µg/g.**

Total carotenoid content in pineapple = 0.251x10x10^4 / 2600x10 = **0.96 µg/g.**

Total carotenoid content in banana = 0.254x10x10^4 / 2600x10 **= 0.97 µg/g.**

THIN LAYER CHROMATOGRAPHY

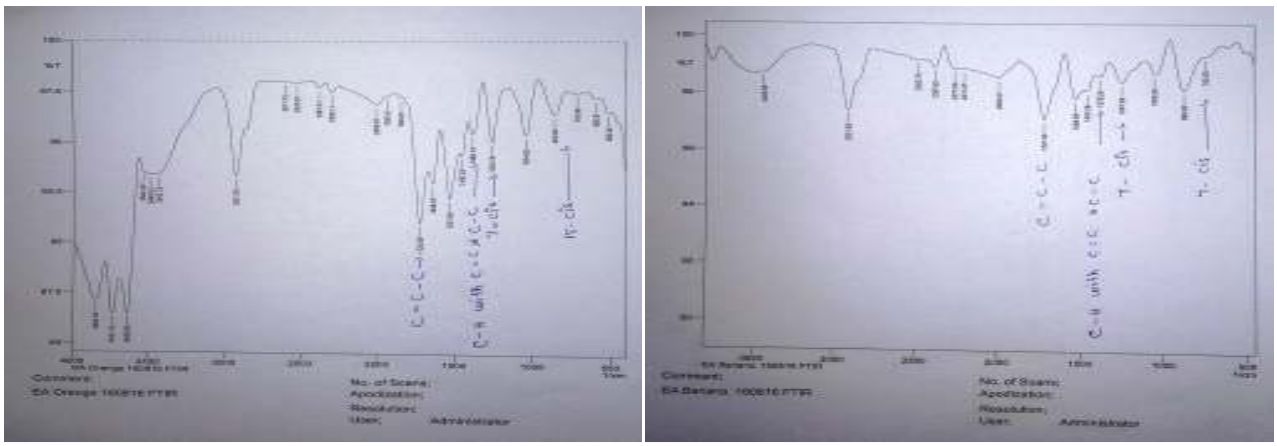
The crude extracts and the purified carotenoid pigments and the standard were subjected to thin layer chromatography. The standard used was beta carotene. The mobile phase used was hexane and acetone in the ratio 6:4. The respective Rf values for the fruits (Orange, Lemon, Pineapple and Banana) were calculated (**Table 1).**

Table 1: R_f Values of Crude Extract and Carotenoid

Sample	Ethanol Crude	Ethyl Acetate Crude	Chloroform Crude	Carotenoide Pigment
Orange	0.95	0.94	0.94	0.94
Lemon	0.92	0.92	0.92	0.92
Pineapple	0.92	0.92	0.92	0.92
Banana	0.94	0.91	0.95	0.94

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 of Orange and Banana the presence of beta carotene was confirmed **(Figures 3 - 4).**



Figures 3 – 4: FTIR result of Fruits

CONCLUSION

The carotenoids were extracted from the fruits **(Orange, Lemon, Pineapple and Banana)** 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. Beta Carotene, the extracted carotenoid pigment was present in the **Orange and Banana** samples.

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