

Identification of Phenolic and Carotenoids Compounds in Carica Papaya L. 'Maradol' Using Liquid Chromatography – Mass Spectrometry: A Review

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ABSTRACT

Recent studies have demonstrated that vitamin C, phenols, and carotenoids are bioactive compounds that protect the body from oxidative stress, reducing the risk of cardiovascular diseases and some types of cancer. Qualitative and quantitative analysis of the major phytochemicals found in papaya fruit flesh and skin (*Carica papaya* L., cv Maradol) was conducted in four stages of ripeness, using high-performance liquid chromatography mass spectrometry. Phenolic compounds identified in the fruit skin tended to decrease with ripening. The use of organic compounds has low impact on the environment compared to conventional agriculture. Biofertilizers has many types and they are liquid bioferment, compost, vermicompost, vermicomposting leachate, and green manure. Pest and disease controllers such as predators, antagonistic organisms, and extracts or infusions of various plants. Trap plants such as lucerne, roselle, and sunflowers, among others can be used into organic agriculture.

Keywords: Natural anti-oxidants, Bioactive compounds, Conjugated phenolics, Papaya fruit, LC-MS

I. INTRODUCTION:

In recent years, the consumption of fruits and vegetables has increased considerably because of their benefits for good health. These benefits have been attributed, at least in part to the amount of antioxidant compounds present in these foods, which reduce the oxidative stress produced by free radicals, cellular damage. In Carotenoids the lipophilic compounds are present that they have been formed by 8 isoprenoid units. They play a very important role in human health. Based on

structure, they divided in two groups they are carotenes and xanthophylls.



The papaya contains high rich antioxidants such as Polyphenols, Vitamin C & carotenoids. Polyphenols as known to act as antioxidants because of their ability to donate hydrogen or electrons and also for their stable radical instruments. The consumption of antioxidant phenolic compounds is to prevent such chronic diseases such as cancer, diabetes, cardiovascular diseases. Since ancient times the plants and their products has been used to prevent diseases. Natural products shown major advantages compared with synthetic drugs such as cost effective, negligible side effects.

LIQUID CHROMATOGRAPHY:

The column liquid chromatography, because the liquid cell segment passes via the column, additives within the cell segment have interaction with various levels with the strong desk bound segment, additionally called the chromatography media or resin. Molecules of

hobby withinside the cell segment are separated primarily based totally on their differing physicochemical interactions with the stationary phase and mobile phases. These interactions may be primarily based totally on molecular length (length exclusion chromatography), charge (ion change chromatography), hydrophobicity (hydrophobic interplay chromatography), particular binding interactions (affinity chromatography), or a mixture of these (multimodal or mixed-mode chromatography). The composition of the cell mobile phase is commonly modified in the course of a separation run in order to modify the strengths of the interactions of the compounds of hobby, that is, to extrade the segment partitioning of every compound among the stationary phase and cell phases. Each compound then elutes from the column in a specific order relying at the relative strengths of its interplay with the resin and the mobile phase. As the mobile phase maintains to waft thru the column, the column effluent, or eluate, is commonly gathered in fractions while monitoring the concentrations of the compounds eluted from the column through the years to yield an elution curve, or chromatogram. The mode of detection varies with the analyte to be detected. For protein separations through column chromatography, protein awareness may be monitored manually the use of a dye-primarily based totally protein assay consisting of the Bradford assay; however, such guide tracking is hard work intensive.

MASS SPECTROMETRY:

Mass spectrometry (MS) is an analytical approach this is used to degree the mass-to-rate ratio of ions. The outcomes are offered as a mass spectrum, a plot of depth as a feature of the mass-to-rate ratio. Mass spectrometry is used in lots of

extraordinary fields and is implemented to natural samples in addition to complicated mixtures. A mass spectrum is a kind of plot of the ion sign as a feature of the mass-to-rate ratio. These spectra are used to decide the fundamental or isotopic signature of a sample, the loads of debris and of molecules, and to explain the chemical identification or shape of molecules and different chemical compounds.

BIOLOGICAL SOURCE:

Kingdom : Plantae
Sub kingdom : Tracheobionta
Class: Magnoliopsda
Subclass : Dilleniidae
Division : Magnoliophyta
Subdivision : Spermatophyta
Phylum : Steptophyta
Order : Brassicales
Family : Caricaceae
Genus : Carica
botanical name : Carica papaya Linn.

CHEMICAL CONSTITUENTS:

Caffeic acid, protocatechuic acid-hexoside, sinapic acid, quercetin-3-O-rutinoside, ferulic acid, myricetin, quercetin, isorhamnetin, kaempferol, caffeoylhexose-deoxyhexose, caffeoylhexoside and gallic acid-deoxyhexoside phenolics had been determined in peel extracts at the same time as caffeic acid, protocatechuic acid and gallic acid hexosides had been gift withinside the flesh. Carotenoids had been recognized withinside the flesh through LC/DAD coupled to atmospheric stress ionization mass spectrometry (LC/DAD-MS-APCI)

NUTRITIONAL COMPONENTS:

NUTRITIONAL AND PHYTO-THERAPEUTIC POTENTIAL OF PAPAYA

Papaya fruit nutritional value (per 100 g edible portion).

Nutrient	Value—USDA nutrient database	Nutrient	Value—USDA nutrient database
Water	88.83 g	Calcium	24 mg
Energy	163 kJ/39 kcal	Magnesium	10 mg
Protein	0.61 g	Iron	0.1 mg
Fat	0.14 g	Zinc	0.07 mg
Carbohydrate (total)	9.81 g	Beta-carotene	276 µg
Carbohydrate (sugar)	5.9 g	Thiamin	0.027 mg
Dietary fiber	1.8 g	Riboflavin	0.032 mg
Cholesterol	Nil	Niacin	0.338 mg
Sodium	3 mg	Vitamin C	61.8 mg
Potassium	257 mg	Vitamin A Eq	150 µg

Source: USDA national nutrient database for reference (2006).

DATA COLLECTION:

Papaya is a plant this is local to the tropics of Mexico and Central America .It has excessive medicinal and dietary cost oxidant properties. Papaya flora develop in 3 sexes: male, woman, and hermaphrodite. The male produces handiest pollen, in no way fruit. The woman produces small, inedible end result until pollinated. The hermaphrodite can self-pollinate due to the fact that its plants incorporate each male stamens and woman ovaries. Almost all business papaya orchards incorporate handiest hermaphrodites.Originally from southern Mexico (particularly Chiapas and Veracruz), Central America, northern South America, and southern Florida the papaya is now cultivated in maximum tropical countries. In cultivation, it grows rapidly, fruiting inside three years. It is, however, rather frost-sensitive, proscribing its manufacturing to tropical climates. Temperatures below -2 °C (29 °F) are significantly dangerous if now no longer fatal. In Florida, California, and Texas, increase is usually constrained to southern components of these states. It prefers sandy, well-tired soil, as status water can kill the plant inside 24 hours.

SOLVENTS USED:

Formic acid , acetronitrile , actone, n-hexane , dichloromethane , methanol Na₂ S₂ O₂ [Sodium thiosulfate] and anhydrous granular sodium sulfar were purchased from mexico. Diethyl ether , methyl tertiary- butyl ether lycopon (purity less than 90 %) , Beta- carotene (purity = 95 %)

obtained from carrots . Solvents used for chromatography were HPLC grade Water [Didistilled water],This HPLC grade water is purified by Millipore corp . , Bedford , MA.,

II. MATERIALS AND METHODS:

Plant Material:

Maradol papayas (MP) have been received from a nearby marketplace in Querétaro, México. Fruits have been decided on for uniform size, mature-inexperienced ripening level and unfastened from defects, and have been saved at 25±1°C and 60% relative humidity for as much as 12 days. Sampling became finished every different day. Exocarp tissue of uniform thickness (0.three cm) became eliminated with a pointy chrome steel potato peeler. Mesocarp tissue became chopped with a chrome steel knife into 3x3 cm cubes. Both exocarp and mesocarp tissue have been straight away saved at -70°C for 24h earlier than freeze-drying at -49°C and 0.05 mBar. Freeze-dried samples have been floor finely in a porcelain mortar beneathneath dark, and saved at -20°C till extraction.

Separation and Identification of Phenolic Compounds:

1.Extraction:

Pulverized exocarp mesocarp tissue (0.5g) was extracted twice with 20 ml of 80 % methanol and sonicated for 30 min at 30°C in a 2510 model ultrasonic bath (Branson, Whetersfield, CT) with a constant sonic power of 40kHz. Extracts were cent

rifuged at 12000g for 15 min at 5°C in a Hermle centrifuge model Z323K (Labor Technik Technologies, Germany), and then filtered through number 1 Whatman paper. 2 ml of the filtrate were filtered again with a 0.45 µm pore size nylon membrane prior to LC-MS analysis of soluble free phenolics. Alkaline hydrolysis was performed by adding 10 ml of 4 M NaOH, incubated for 4 h at room temperature and protected from light. After incubation, extracts were acidified to pH 2.0 with 4 M HCl. Acidified solution was then extracted twice with 20 ml ethyl acetate. Organic phases were combined and evaporated to dryness at 35°C at reduced pressure. Samples were re-suspended in 10 ml of 80% methanol for exocarp tissue extracts and in 5 ml for mesocarp tissue extracts, and finally passed through a Millipore 0.45 µm nylon membrane for LC-MS analysis of conjugated phenolics.

Separation and Identification of Carotenoids:

Extraction: Extraction turned into done consistent with Ornelas-Paz et al. (2007) with a few modifications. Freeze dried and pulverized mesocarp tissue (0.5 g) turned into homogenized with 10 ml of hexane-dichloromethane (1:1, v/v). After 1 min vortex stirring, the pattern turned into centrifuged for 10 min at 9000 ×g at 5°C and natural section turned into separated. The technique turned into repeated and natural levels had been mixed, earlier than saponification with 2 ml of 40% (w/v) methanolic KOH for 1 h at 50°C at a stirring tub set at a hundred rpm. The extract turned into then washed with water and one quantity of 10% sodium sulfate answer earlier than natural section separation and evaporation at 30°C in a Buchi low strain evaporator. The pattern turned into re-suspended in 2 ml acetone and filtered via nylon membrane of 0.45 µm of pore size (Millipore Corp., Bedford, MA) for injection into LC-MS system.

OBJECTIVE OF THE STUDY:

The main objective of the present study was to investigate the potential of *C. papaya* leaf extract against dengue fever. Secondary metabolism of plant origin produces a vast repository of compounds with a wide range of biological activities. There are several reports that higher plant extracts have relatively good antiviral potency (Van den Berke, 1978). Several plant extracts, including *Spilanthes calva*, *Sterculia guttata*, *Balanites aegyptiaca*, *Vitex negundo*, *Solanum xanthocarpum*, *Artemisia annua*, *Fagonia indica*, *Nerium indicum*, *Trigonella foenum*, in

various solvents, have been reported against exudative activity. *C. Papaya* contains two important biologically active compounds: chymopapain and papain which are widely used for digestive disorders. It showed that papain, carrigin, chymopapain and glycine endopeptidase derived from papaya can improve acid pH conditions and pepsin degradation. Other active compounds of *C. papaya* are lipase or CPL, a hydrolase, which binds tightly to the water-insoluble portion of crude papain, thus making it considered a “naturally immobilized” biochemical. According to folk medicine, papaya latex cures dyspepsia and applies to external burns and scarring. Seeds and fruits are excellent anthelmintic and anti-amoebic. Dried and powdered leaves are sold to make a tea, and a leaf decoction is administered to horses as a purgative and used to treat genitourinary system.

Consuming unripe and semi-ripe papaya fruits or applying them to the uterus causes abortion. However, consumption of unripe and semi-ripe papaya fruits may be unsafe during pregnancy, but consumption of ripe fruits during pregnancy poses no risk. Plant extracts of *Quercus lusitanica* are reported to have maximum activity against DENV-2 replication of dengue virus. Methyl gallate of plant origin interacts with herpes simplex virus proteins and alters virion uptake and penetration. *Gastrodia elata* B1 influences the replication cycle of dengue virus serotype 2. Two compounds, 1-beta-D-ribofuranosyl-3-ethynyl-[1,2,4]triazole (ETAR) and 1-beta-tribofuranosyl-4-ethynyl[1,3]imidazole (IM18), have been reported to be present in significant amounts. Reduced replication of dengue virus serotype 2 (DENV-2) in cultured Vero cells and a novel trypsin Cazale-type inhibitor from *Aedes aegypti* has thrombin inhibitory activity. Dengue attacks in Pakistan begin in late summer and end in early winter. Most of the affected patients are between 30-45 years of age. Dengue fever reoccurs every year and causes many deaths. An increase in PLT count from 55×10³/µL to 168×10³/µL at present indicated the activity of *C. papaya* leaf extract. However, this is a preliminary work and further work is needed to isolate active compounds from this valuable species, which may help control such infectious diseases.

USES:

Papaya has a wide range of uses, various parts of the papaya plant are used for a variety of medical conditions. Papaya pulp is used for treating wounds and burns because of its wound healing property. The uses of unripe fruit of papaya

includes: Expectorant, carminative, skin diseases, ulcer, Diuretic. The green leaves is the source of many essential nutrients. The fruit part of the papaya plant has immuno stimulating property and it also has anti oxidant property.

III. BACKGROUND OF THE STUDY

Tropical fruits are wealthy in phenolic and carotenoid compounds, and those are related to cultivar, pre- and postharvest managing factors. The purpose of this paintings changed into to pick out foremost phenolics and carotenoids in 'Maradol' papaya fruit and to research their reaction to garage temperature. The papaya fruit is native to the forests in Southern Mexico and Central America.

The papaya is grown today in most countries of the inter-tropical area of the world. The plant has a limbless trunk with a height of between 2 and 10 m. with branches having palm-shaped foliage. The flowers are developed in the leaf axils. The fruit is oblong in shape, can be yellow, orange or pink colored and weighs up to 12 kg. However, the market trend has led breeders to seek smaller papayas, which often do not weigh more than 500 or 600 g from small, highly productive plants, generally destined for exportation (OECD, 2003). Another byproduct of the plant is the sap extract that has a milky consistency containing papaina, a very useful enzyme employed as meat tenderizer. The species is dioecious in wild populations, meaning that there are plants that produce only male flowers and plants that produce only female flowers; however, hermaphroditic plants exist in cultivated varieties, where there are flowers with both sexes. This being a desirable characteristic in a commercial plantation, since all plants are therefore productive. Papaya plants always having flowers and fruits at the same time are considered one of the most productive plants in relation to their size. To obtain seeds for a 100-hectare crop, approximately 25 papaya trees are required, which will yield approximately 871 grams of seeds; the human effort needed is equivalent to 475 man-hours. This human resource of about 475 hours includes planting, pollination, maintenance and care, harvesting, and processing. Also, at least four-1200 m² of greenhouse area are required for the production of female plants, and two greenhouses for the production of male plants, a spraying pump, seed extraction equipment, irrigation machinery, a seed dryer and grader, a bagger, as well as other different agricultural material.

IV. CONCLUSION:

The presence of phenolic compounds with antioxidant activity identified in peel, as well as carotenoids with provitamin A and radical quencher activities in flesh of 'Maradol' papaya, could contribute to the high antioxidant properties and the beneficial effects of this fruit to human health. This fruit being very important in the Mexican diet and other countries, information presented in this study could justify its inclusion to form part of a healthy diet. Future epidemiological studies following the sustained consumption of papaya are needed, in times where dietary strategies are necessary to overcome chronic diseases. Phenolics and carotenoids of 'Maradol' papaya were influenced by postharvest storage temperature with exception of β -carotene and rutin. Ripe papaya stored at 25 °C had more carotenoids than those stored at 1 °C. Low (chilling) temperature (1 °C) negatively affected the content of major carotenoids, except β -carotene, but preserved or increased ferulic and caffeic acids levels, as compared to high (safe) temperature (25 °C).

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