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DETERMINATION AND QUANTIFICATION OF TOTAL CAROTENOIDS IN PERSIMMON BY USING COLORIMETRIC METHOD

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ABSTRACT

The present study was carried out to determine and quantify the total carotenoid content in persimmon fruit (Diospyros kaki) using a colorimetric method. Fresh ripe fruits were collected, shade-dried, powdered, and extracted using acetone and ethanol by maceration. Qualitative tests and Thin Layer Chromatography confirmed the presence of carotenoids such as β-carotene, lutein, and zeaxanthin, with an Rf value of 0.96. Quantitative estimation was performed using β-carotene as a standard. The maximum absorbance (λ_{max}) was observed at 470 nm. A calibration curve was prepared at different concentrations, and the absorbance of the fruit extract at 470 nm was found to be 1.14. The total carotenoid content in 10 g of persimmon fruit dry powder was calculated to be 0.00188 g, corresponding to 0.018%. The study demonstrates that persimmon fruit contains measurable amounts of carotenoids, and the colorimetric method employed is simple, economical, and suitable for routine analysis and quality control applications.

KEYWORDS: Persimmon, Carotenoids, Colorimetry, β-carotene, Spectrophotometry, Phytochemical analysis.

1. INTRODUCTION

One of the first fruit trees that humans have cultivated is the Persimmon (Diospyros kaki), which belongs to the Ebenaceae family. The tree has great cultural value; in Japan, it is regarded as the "Tree of Peace" and the "Food of the Gods." It originated in China and eventually flourished throughout the Far East in Korea and Japan. Although it made its way to Europe and the Americas in the middle of the 19th century, it has established a stronghold in Italy, particularly in the regions of Campania, Emilia Romagna, and Sicily, where variations like the Sicilian Misilmeri and the Loto di Romagna are now well-known exports. the persimmon tree, which is distinguished by its massive, glossy

leaves and towering height, yields a vivid orange berry that is usually picked between October and November. This is traditionally handled by a post-harvest ripening process in which ethylene, which is frequently obtained by keeping the fruit with apples or pears, transforms tannins into sugars, producing a pulp that is sweet and creamy.

Fig. 1: Persimmon Fruit (Diospyros Kaki).

In order to eliminate astringency while maintaining the fruit's hard texture and nutritional integrity, sophisticated industrial processes utilizing inert gases like carbon dioxide or nitrogen have been developed more recently. The Persimmon has a chemical makeup with enormous biological and medicinal significance, despite being labeled a "minor fruit" in some markets. It is becoming more well-known for both its calorie contribution and its concentration of bioactive compounds that can help prevent a number of diseases. Understanding Diospyros kaki's ripening processes and nutritional preservation is crucial for its continued integration into the global functional food market, given its potential for export and short shelf life. The fruit, which is native to China, Korea, and Japan, has long been used for its therapeutic and nutritional qualities. It is a major crop on a global scale; as of 2014, 5.191 million tons were produced worldwide, with China controlling more than 73% of the market.

Early in the 20th century, European settlers brought Persimmons to India. Nowadays, "Hachiya" is the most popular type, and it is grown in temperate areas like Himachal Pradesh, Jammu & Kashmir, Uttarakhand, and Tamil Nadu. The Indian market is still small despite its potential because to a lack of structured agricultural technologies and low consumer awareness. There is a shortage of supply in mainstream commercial marketplaces because the majority of production is still limited to small-scale orchards.

Persimmon quality and marketability rely greatly on accurate harvesting and handling procedures. Harvested in the autumn, specifically in October, the fruit must be carefully cut using secateurs to keep the calyx and stem intact, as snapping the fruit by hand increases the danger of physical harm and subsequent decomposition. While a mature tree can produce between 150 and 200 kilograms per year, the fruit is quite susceptible to seasonal changes. This research investigates the problems of persimmon farming and the need of enhanced post-harvest management in transforming this "minor fruit" into a more major agricultural commodity in India.

2. MATERIALS AND METHODS

2.1 Materials

Persimmon fruits, Acetone, Ethanol.

Apparatus

Whatman filter paper, Spatula, Funnel, Tripod stand, Measuring cylinder, Filter paper, Beaker, Volumetric flask, Glass rod, Pipette, Test tubes, Cuvettes.

Instruments

Digital balance, Colorimeter.

2.2 Collection ofFruit

Fresh, ripe persimmon fruits were collected and selected based on uniform bright orange colour, as beta- carotene content is highest in well-ripened fruits. The fruits were washed thoroughly with distilled water, cut into thin uniform slices, and spread evenly on clean lined trays. The slices were shade-dried, protected from direct sunlight, until they became completely dry and brittle.

2.3 Method of Extraction

The dried Persimmon fruit slices were grinded. The powdered sample was transferred into a clean dry conical flask. Suitable solvent such as acetone, ethanol sufficient quantity and it is added to sample. The flask was tightly closed to prevent solvent evaporation. The mixture allowed stand at room temperature for one week with shaking which enhance the extraction beta carotene.

Table 1: Qualitative Tests for Carotenoids.

TEST	PROCEDURE	OBSERVATION
Sulphuric acid test	Take 2 ml extract and add few drops of concentrated sulphuric acid.	A green colour was appeared. It indicates the presence of carotenoids.
Nitric acid test	Take 2ml extract and add few drops of concentrated nitric acid.	A green colour was appeared. It indicates the presence of carotenoids.
Ferric chloride test	Take 2ml extract and add few drops of ferric chloride.	A green colour was appeared. It indicates the presence carotenoids.

2.4 Thin Layer Chromatography (TLC)

Thin Layer Chromatography was performed using a silica gel TLC sheet as the stationary phase and hexane: acetone (8:2) as the mobile phase. The extract was spotted on the TLC plate and developed in a saturated chamber. After development, colored bands were observed directly. Orange, yellow bands and yellow-green bands indicated the presence of carotenoids such as β-carotene, lutein, and zeaxanthin. The Rf values were calculated using the formula: Rf = distance traveled by spot / distance traveled by solvent front, confirming the separation and identification of carotenoids in persimmon fruit. Rf value for carotenoids is between 0.89 -0.98.

$$Rf = \frac{distance\ travelled\ by\ spot}{distance\ travelled\ by\ solvent}$$

=4.9/5.1

=0.96

Fig. 2: TLC of Carotenoids.

2.5 DETERMINATION OF TOTAL CAROTENOIDS CONTENT

2.5.1 Preparation of Beta carotene solution:

- 100 mg of beta-carotene, precisely weighed, was added to a 100 ml volumetric flask.
- 50 ml of a solvent (acetone: ethanol) was added to a flask, and the solvent was dissolved to fill the remaining capacity to 100 ml.

2.5.2 Selection of wavelength

- Take standard beta-carotene solution.
- At various wavelengths, measure the produced beta-carotene solution's absorbance.
- The wavelengths are 450, 470, 510, 520, 540, 570, 600, and 670 nm.
- At 470 nm, the standard solution shows maximum absorbance.
- Therefore, the λ_{max} is 470nm.

Table 2: Wavelength and absorbance values of Carotenoids.

S.NO	WAVELENGTH	ABSORBANCE
1	450nm	0.44
2	470nm	0.45
3	510nm	0.26
4	520nm	0.08
5	540nm	0.02
6	570nm	0.01
7	600nm	0.02
8	670nm	0.01

2.5.3 Preparation of calibration curve

- Seven beta carotene solution concentrations were made. 0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml, 3 ml, and 3.5 ml are available.
- The solvent in each solution adds up to 10 ml.
- The absorbance was measured at 470 nm and absorbances were 0.28, 0.49, 0.7, 0.91, 1.22, 1.49 and 1.72.

2.5.6 Procedure for determination of extract Absorbance

- Take 5ml of persimmon fruit extract into a 10ml volumetric flask.
- Add solvent up to 10 ml to make up the volume.
- Filter the mixture with a Whatman filter.
- The absorbance measured at 470 nm was 1.14.

3. RESULTS AND DISCUSSION

Table 3: Calibration Results of carotenoids.

SI.NO	CONCENTRATION (mg)	ABSORBANCE
1	0.05mg	0.28
2	0.1mg	0.49
3	0.15mg	0.7
4	0.2mg	0.91
5	0.25mg	1.22
6	0.3mg	1.49
7	0.35mg	1.72
8	Sample	1.14

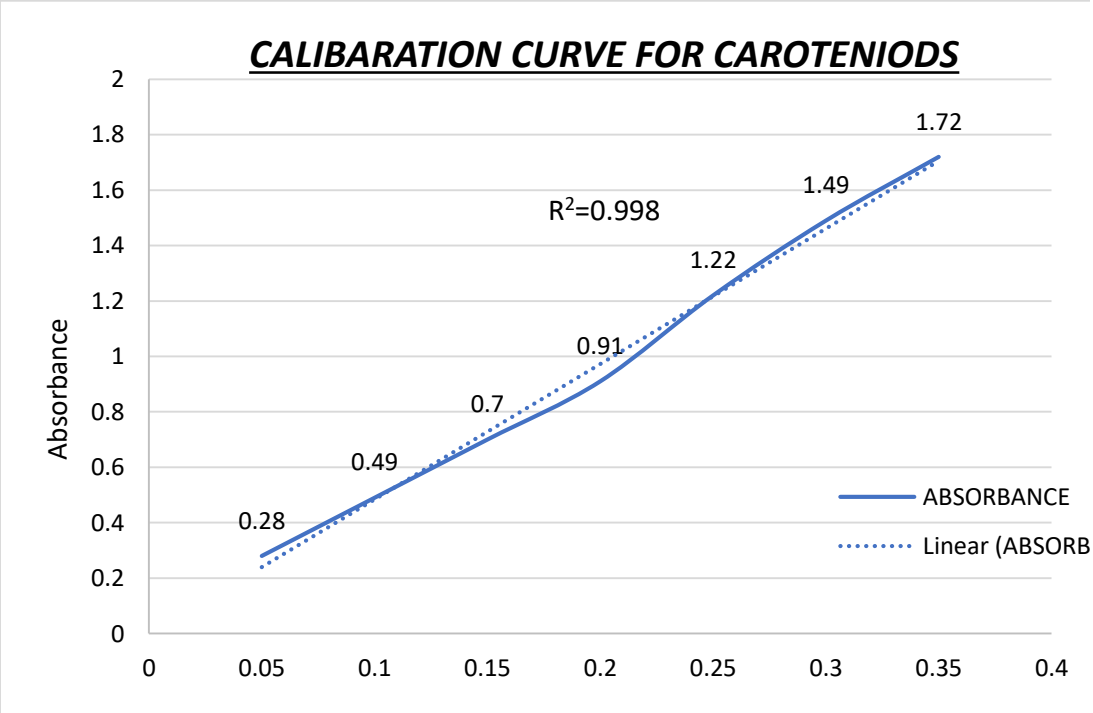

Fig. 3: Calibration curve of Carotenoids.

CALCULATION

Concentration of carotenoids content in 10gms of Persimmon fruit.

FORMULA

Total carotenoids content = $\frac{Concentration\ found \times volume\ of\ final\ extract}{Quantity\ of\ extract\ taken}$

$= \frac{0.235 \times 40}{5} = \frac{9.4}{5}$

= 1.88mg

10 gm of Persimmon fruit dry powder contains 0.00188 gm of carotenoids.

PERCENTAGE OF CAROTENOIDS

% of carotenoids in 10 gm = $\frac{Concentration\ in\ 10gm}{weight\ of\ powder} \times 100$

$= \frac{0.00188}{10} \times 100$

= 0.018%

The total carotenoids content inPersimmon fruit was found to be 0.018%.

4. SUMMARY

This study was carried out to determine and quantify the carotenoid content present in persimmon fruit using a colorimetric technique. Carotenoids, which are well known for their therapeutic properties, were extracted from the persimmon fruit material by the maceration method. Quantitative analysis was based on the formation of a colored carotenoid-reagent complex, and the absorbance of this complex was measured calorimetrically at 470nm and was 1.14. A calibration curve prepared using standard carotenoid solutions was employed to calculate the carotenoid concentration in the fruit extract. The findings indicated a considerable amount of carotenoids in persimmon fruit, highlighting their potential pharmaceutical value. Overall, the method proved to be simple, economical, and dependable, making it suitable for future pharmacological studies.

5. CONCLUSION

The colorimetric method used in this study successfully determined and quantified the carotenoids content in the Persimmon fruit. The extraction process followed by colorimetric analysis provided a reliable, simple, and cost-effective approach for carotenoid estimation. The results confirmed a significant presence of carotenoids are 0.018% highlighting their nutritional and anti-oxidant potential. This method can serve as a valuable tool for quality control in herbal formulations and food products. Further studies may focus on improving sensitivity, validating the method, and exploring the biological and therapeutic activities of the quantified carotenoids.

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