

Quantitative Determination of Ascorbic Acid Content in Two Varieties of Bird's Eye Chili (*Capsicum frutescens* L.) using UV-Vis Spectrophotometry

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ABSTRACT

This study quantified and compared the ascorbic acid content in green and red bird's eye chili (*Capsicum frutescens*) using UV-Vis spectrophotometry. The method was optimized by determining the maximum wavelength of ascorbic acid in an acid-stabilized solution, found to be 231 nm. A calibration curve was constructed with standard solutions (25-100 ppm), yielding a linear equation $y = 0.0514x + 0.6032$ ($R^2 = 0.9361$). Sample extracts were prepared and analysed in triplicate at this wavelength. The results demonstrated a significant difference in ascorbic acid concentration between the two varieties. The red chili exhibited a higher concentration of 34.39 ppm in the measured solution, corresponding to 0.90% (b/b) of the fresh weight. In contrast, the green chili contained 23.95 ppm (0.60% b/b). This indicates a 50% higher ascorbic acid content in the mature red fruit. The study concludes that ripening significantly enhances the vitamin C content in bird's eye chili, and the red variety is a nutritionally superior source. The validated method proved effective for the quantitative analysis of ascorbic acid in complex food matrices.

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Introduction

Chili peppers (*Capsicum* spp.) are widely consumed around the world, not only for their distinctive taste and aroma but also for their rich content of bioactive compounds, including vitamins, carotenoids, and capsaicinoids [1]. Recent studies have emphasized their potential as a significant source of vitamin C, in some cases surpassing the levels found in citrus fruits [2]. As such, quantifying vitamin C in chili peppers is essential for nutritional assessments and the development of dietary recommendations[3].

Beyond their nutritional value, chili peppers hold considerable economic and cultural importance, especially in countries like Indonesia, where they are a key component of daily cuisine. Although not a staple ingredient,

chili peppers, especially bird eye chili varieties are almost indispensable in Indonesian meals due to their ability to enhance flavour and stimulate appetite with their spiciness. Consumed both fresh and in various processed forms, chili peppers appeal to a wide range of consumers and offer multiple health benefits due to their natural chemical compounds and vitamin content.

Vitamins are essential organic compounds required in small amounts for normal physiological functions, including metabolism, immunity, and cellular repair. Vitamins can be found in food, like fruits, vegetables, and food supplement [4]. Vitamin C or ascorbic acid is one of the vitamins that can be found in chili pepper. Vitamin C (**Figure 1**) is a vital water-soluble antioxidant that plays a crucial role in

immune function, collagen synthesis, and protection against oxidative stress [5]. As humans cannot synthesize vitamin C endogenously, dietary intake from fruits and vegetables is essential to prevent deficiency-related disorders such as scurvy [6]. Chili peppers, known for their pungency and nutritional value, are a significant dietary source of vitamin C, particularly in regions where they are a staple food [2]

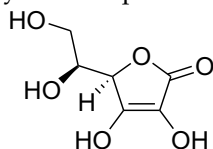


Figure 1. Chemical Structure of Ascorbic Acid

Vitamin C is highly susceptible to degradation due to heat, light, and oxidation, making its accurate quantification challenging [7]. Traditional methods such as titration (e.g., iodometric or DCPIP) are time-consuming and less precise compared to modern spectroscopic techniques. UV-Vis spectrophotometry offers a rapid, cost-effective, and reliable alternative for vitamin C determination in plant matrices [8].

UV-Vis spectrophotometry measures the absorption of light by a compound at specific wavelengths, allowing for quantitative analysis [9]). For vitamin C, the maximum absorbance typically occurs around 265 nm, where ascorbic acid exhibits strong UV absorption ([10]). This method is advantageous due to its simplicity, high sensitivity, and minimal sample preparation requirements ([11]).

Several studies have reported varying vitamin C concentrations in chili peppers depending on cultivar, maturity, and post-harvest handling [12], [13], [14]). For instance, red ripe peppers generally exhibit higher vitamin C content than green ones due to continued biosynthesis during ripening [15]. However, standardized methods for extraction and quantification are still needed to ensure comparability across studies [16].

This study aims to (1) optimize a UV-Vis spectrophotometric method for vitamin C determination in bird eye chili peppers, (2) evaluate the vitamin C content in 2 different bird eye chili varieties found in Tondano local

market, namely green bird eye chili, and red bird eye chili Malita FM (**Figure 2**). The findings will contribute to the nutritional profiling of chili peppers and support food industry applications [17].



Figure 2. a. green bird's eye chilli; b. red bird's eye chili

Materials and Method

Materials

Chemical used are green bird's eye chili pepper, red bird's eye chili pepper Malita FM, Vitamin C (Merck), and distilled water.

Instrumentation

The apparatus and instruments used are analytical balance (Shimadzu), volumetric flask 25 mL, 50 mL, and 100 mL, mortar and pestle, Whatman No.2 filter paper, and UV-Vis (Perkin Elmer Lambda 25).

Procedure

1. Preparation of Chili Sample Extract:

Fresh bird's eye chili fruits were washed. A 100 mg portion was weighed, homogenized into a pulp using a mortar and pestle with a small volume of distilled water, and then filtered. The resulting filtrate was transferred into a 25 mL volumetric flask and diluted to the mark with distilled water.

2. Preparation of Ascorbic Acid Standard Solutions:

A primary stock solution of ascorbic acid (1000 ppm) was prepared by dissolving 100 mg of pure ascorbic acid in distilled water within a 100 mL volumetric flask and diluting to volume. This stock solution was subsequently diluted with distilled water to prepare a series of working standard solutions with concentrations of 25, 50, 75, and 100 ppm.

3. Determination of Maximum Wavelength (λ_{max}):

A diluted solution for wavelength scanning was prepared by transferring 5 mL of the 1000 ppm stock solution into a 50 mL volumetric flask and diluting to the mark. The absorbance

of this solution was scanned across the 200-400 nm wavelength range using a UV-Vis spectrophotometer. The wavelength corresponding to the highest absorbance value was identified and selected as the maximum wavelength (λ_{max}) for all subsequent measurements.

4. Construction of the Calibration Curve:

The absorbance of each working standard solution (25, 50, 75, and 100 ppm) was measured in triplicate at the predetermined λ_{max} . A calibration curve was constructed by plotting the average absorbance values against their respective concentrations. The equation of the resulting linear regression line was calculated.

5. Quantification of Ascorbic Acid in Chili Samples:

The initial dense extract was diluted for analysis by weighing approximately 1 g into a 100 mL volumetric flask and diluting to volume with distilled water. A further dilution was performed by pipetting 2 mL of this solution into a 50 mL volumetric flask and again diluting to the mark. The absorbance of this final diluted sample solution was measured in triplicate at the λ_{max} . The replication conducted only as technical replicates. The average absorbance value was interpolated into the linear regression equation from the calibration curve to calculate the concentration of ascorbic acid in the sample, expressed in parts per million (ppm).

Result and Discussion

The maximum wavelength determination

The maximum wavelength (λ_{max}) for ascorbic acid quantification was determined empirically using UV-Vis spectrophotometry. An aliquot of a standard ascorbic acid solution was appropriately diluted and its absorbance was scanned across the ultraviolet spectrum from 200 to 400 nm. The resulting spectrum exhibited a distinct absorption peak, with the maximum absorbance occurring precisely at 231 nm. This characteristic peak corresponds to the electronic transitions within the ascorbic acid molecule. This value is a bit lower than the usual wavelength of ascorbic acid. This might happen due to ascorbic acid is highly unstable in neutral or basic aqueous solutions and rapidly oxidizes. However, Consequently, 231 nm was identified and selected as the optimal

analytical wavelength for all subsequent measurements to ensure maximum sensitivity and accuracy in the quantitative determination, as measurements at a compound's λ_{max} provide the strongest signal and minimize the potential impact of instrumental noise.

Construction of Calibration Curve

A calibration curve for the quantitative determination of ascorbic acid was constructed at the maximum wavelength of 231 nm (Figure 3). Standard solutions with known concentrations of 25, 50, 75, and 100 ppm were prepared and analysed. To ensure precision and account for any measurement variability, the absorbance of each standard solution was measured in triplicate. The average absorbance value for each concentration was then calculated and plotted against its respective concentration. The relationship between absorbance (y-axis) and concentration (x-axis, in ppm) was found to be linear, as confirmed by linear regression analysis. The equation of the best-fit line was determined to be $y = 0.0514x + 0.6032$, where 'y' represents the absorbance and 'x' represents the ascorbic acid concentration in ppm. The coefficient of determination, $R^2 = 0.9361$, indicates a reasonably strong linear relationship between vitamin C concentration and absorbance. However, this value falls below the ideal threshold ($R^2 \geq 0.99$) for precise quantitative analysis. This may reduce the accuracy of concentration measurements, but can be acceptable to determine the unknown sample concentrations within this range. This fulfilled the Lambert-Beer law[18].

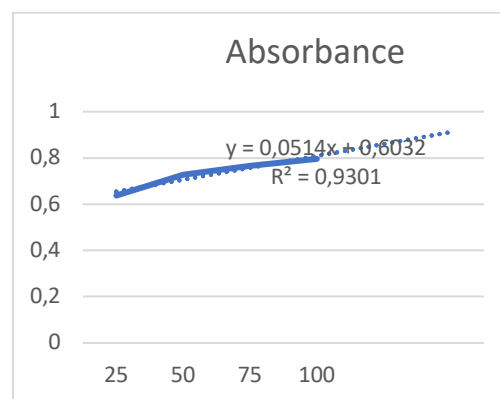


Figure 3. Ascorbic Acid Calibration Curve

Determination of Ascorbic Acid in Chili

The quantification of ascorbic acid levels in green and red bird's eye chili varieties was performed using UV-Vis spectrophotometry at the analytical wavelength of 231 nm. The measured absorbance values of the sample solutions were interpolated into the previously established linear calibration curve to determine their concentrations. The analysis revealed a clear difference between the two varieties (**Table 1**). The green bird's eye chili extract showed an absorbance of 1.834, corresponding to a concentration of 23.95 ppm in the measured solution. In contrast, the red bird's eye chili extract exhibited a significantly higher absorbance of 2.371, which calculated to a concentration of 34.39 ppm.

Table 1. Ascorbic Acid Concentration in 2 Bird's Eye Chili varieties

Sample	Absorbance	Concentration (ppm)	Value (% b/b)
Green bird's eye chili	1.834	23.95	0.60
Red bird's eye chili	2.371	34.39	0.90

To express these results in a more practical context relevant to the original food sample, the concentrations were converted from parts per million (ppm) in the solution to a percentage by weight (% b/b) in the fresh chili. **Equation 1** is used to convert the concentration (ppm) to % (b/b). This conversion accounts for the exact mass of chili used and the dilutions performed during the extraction process. The calculated ascorbic acid content for the green bird's eye chili was 0.60% (b/b), meaning that 100 grams of fresh green chili contains 0.60 grams of ascorbic acid. The red bird's eye chili contained a higher level of 0.90% (b/b), equating to 0.90 grams of vitamin C per 100 grams of fresh fruit.

Equation 1

$$\% \text{ value} = \frac{\text{mg ascorbic acid}}{\text{mg sample}} \times 100\%$$

The results demonstrate a significant quantitative difference in the ascorbic acid content between the two maturation stages of bird's eye chili. The red, mature chili contained 50% more vitamin C (0.90%) than its green counterpart (0.60%). Previous study displayed that vitamin C in red chili was around 0.92% [18]. This increase is consistent with known biochemical processes in many fruits, where the synthesis and accumulation of certain vitamins and antioxidants often continue or are enhanced during the ripening process. Therefore, based on this spectrophotometric analysis, the red variety of bird's eye chili provides a superior dietary source of ascorbic acid.

Conclusion

In conclusion, this study successfully utilized UV-Vis spectrophotometry at 231 nm to quantify and compare the ascorbic acid content in green and red varieties of bird's eye chili. The analysis revealed a significant difference, with the red chili exhibiting a substantially higher concentration of **0.90% (b/b)** compared to **0.60% (b/b)** in the green chili. This 50% increase in vitamin C content in the mature red fruit underscores the significant influence of ripening on nutrient biosynthesis. Therefore, the red bird's eye chili represents a nutritionally superior source of dietary ascorbic acid, a finding valuable for nutritional science and consumer dietary choices. Further investigation of bioactive compounds in bird's eye chili from Indonesia would be beneficial to develop more comprehend information.

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