

The Effect of Heating on Ascorbic Acid Levels of *Brassica oleracea* L. Determined by UV-Visible Spectrophotometry

Muzakkir Baits, Riski Mentari Jahri, Asriani Suhaenah, Aminah, Tadjuddin Naid, Muammar Fawwaz*

Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar 90231, Indonesia

* Corresponding Author. E-mail: muammar.fawwaz@umi.ac.id

Received: 23 July 2021 / Revised: 1 August 2021 / Accepted: 5 January 2022

ABSTRACT: Vitamin C can work as a coenzyme and in certain circumstances is a reducing agent and antioxidant. Vitamin C is one of the nutrients that act as antioxidants derived from fruits and green vegetables, such as the broccoli (*Brassica oleracea* L.). This study aims to warming influence on levels of vitamin C in broccoli (*Brassica oleracea* L.) with qualitative and quantitative analysis. Qualitative analysis was performed using specific reagents that ammonium molybdate, Fehling Fehling's A and B which have indicated a positive sample containing vitamin C, and vitamin C were analyzed quantitatively using UV- Vis spectrophotometry at a wavelength of 570 nm. The results showed levels of vitamin C in raw broccoli 0.62915 mg/g and steamed broccoli 0.45449 mg/g.

KEYWORDS: Broccoli; *Phaseolus vulgaris*; spectrophotometry; vitamin C.

1. INTRODUCTION

Broccoli belongs to the cabbage family and includes vegetables that are not resistant to hot air, therefore, broccoli is suitable for planting in humid highlands with low temperatures, which are above 700 m above sea level (above sea level). This vegetable cannot stand the constant rain. If this happens, the broccoli plant will turn yellow and if it rots it will have black spots. The leaves and growth properties of broccoli are almost the same as those of cabbage. The difference is, broccoli is green and has a longer growth period than flower cabbage. Broccoli is composed of small flowers that are green, but not as compact as cabbage. Compared to cabbage, after boiling broccoli the texture will feel softer (Dalimartha, 2000). Harvesting of broccoli flowers can be done after they reach 60-90 days after planting, before the flowers bloom, and while the leaves are still green. If the flowers bloom, the flower stalks will lengthen and yellow flowers will come out (Dalimartha, 2000).

Broccoli contains water, protein, fat, carbohydrates, fiber, calcium, iron, vitamins (A, C, E, thiamine, riboflavin, nicotinamide), calcium, beta carotene, and glutathione. In addition, broccoli contains compounds cyanohydroxybutene (CHB), sulforaphane, and isobutene which can stimulate the formation of glutathione. The content of useful substances, namely sulforaphane which can prevent cancer (Dalimartha, 2000).

Vitamin C (ascorbic acid) can work as a coenzyme and in certain circumstances is a reducing agent and antioxidant. This vitamin can directly or indirectly donate electrons to enzymes that require reduced metal ions, and work as a cofactor for the profile and lysis of hydroxylase in collagen biosynthesis (Ganiswarna, 2007). Ascorbic acid levels in various plants vary widely. The content of this vitamin is more in the skin than the flesh, and the part of the fruit that contains the least ascorbic acid is the seed. Also, in the same type of fruit, there were also differences in ascorbic acid levels due to differences in the environment in which they grew, the use of various types of fertilizers, the level of fruit maturity and others. Therefore, the difference in ascorbic acid levels contained in a fruit must be considered (Kumalaningsih, 2006).

2. EXPERIMENTAL SECTION

2.1. Chemicals

Distilled water, ammonium molybdate, 5% ascorbic acid (Merck), 0.4% oxalic acid (Merck), 5% sulfuric acid, fehling A and fehling B, and broccoli (*Brassica oleracea* L.). Fehling A reagent was prepared by dissolved 34.64 g of copper (II) sulfate and 0.5 mL of sulfuric acid with sufficient distilled water in a 500 mL volumetric flask and make up the volume with distilled water to the mark. Fehling B reagent was prepared by dissolved 176 g of potassium sodium tartrate and 77 g of sodium hydroxide with sufficient distilled water in a 500 mL volumetric flask and the volume is made up with distilled water to the mark. Oxalic acid was prepared by weighed 2 g of oxalic acid, put into a 500 mL volumetric flask dissolved in 50 mL of distilled water, shaken until dissolved and then made up to 500 mL in volume. Standard reference of ascorbic acid was prepared by dissolved 25 mg of pure ascorbic acid then put into a 25 mL volumetric flask and then dissolved with oxalic acid to the mark. Ascorbic acid solution of 1000 ppm was put into a 10 mL volumetric flask as much as 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL, 0.6 mL, and 0.7 mL, then added H₂SO₄ 4.0 mL, then added ammonium molybdate to the mark and homogenized to obtain concentrations of 20, 30, 40, 50, 60, and 70 ppm, then the absorption was measured at the maximum wavelength.

2.2. Sample preparation

The broccoli (*Brassicas oleracea* L.) which will be analyzed is cleaned first and then cut into small pieces and then weighed as much as 100 g. Steamed for 3 to 5 minutes, then put into a blender and then added 100 mL of 0.4% oxalic

acid solution, blended until smooth and then filtered to separate the residue and the filtrate. The filtrate was taken and put in a 100 mL volumetric flask and the volume was made up with 0.4% oxalic acid solution to the mark. The same is done for the samples that are not steamed.

2.3. Ascorbic acid analysis

The sample solution to be analyzed was prepared in advance, each sample filtrate was pipetted 5 mL and 1 mL of 10% TCA was added. Then the sample was centrifuged, and the supernatant was taken to make it easier to read. The supernatant in the sample was taken and diluted by taking 1 mL of the sample supernatant diluted into a 10 mL volumetric flask. Then 4.0 mL of H₂SO₄ was added, and ammonium molybdate was added until the mark was further homogenized, then the absorption was measured at the maximum wavelength.

2.4. Data analysis

Calculation of ascorbic acid levels was carried out by extrapolating ascorbic acid absorption data in a linear regression equation from the standard curve of ascorbic acid. The best fit of the line curve was calculated by equation of line. Linearity was evaluated through the correlation coefficient (R²). The correlation coefficient, intercept and slope of calibration curve were calculated. The best fit of data was determined by linear regression using the following equation:

$Y = bx + a$, where:

- Y = Absorbance
- b = Slope
- x = Concentration
- a = Intercept

3. RESULTS AND DISCUSSION

Ascorbic acid is needed to help form connective tissue or intracellular material, into which body cells are embedded. This vitamin is also needed for the formation of red blood cells (Febry, 2013). This study was conducted with the aim of analyzing and determining the levels of ascorbic acid contained in broccoli before and after heating using UV-Vis spectrophotometry method. The heating process used is steaming. Before the research was carried out, observations and samples were taken of broccoli from the Makassar City supermarket, South Sulawesi Province. Broccoli that must be taken is broccoli that has good quality, namely broccoli that is still fresh and has not bloomed.

In this study, qualitative and quantitative tests were carried out, first the preparation of a sample solution was carried out. Broccoli which has been washed, steamed and then blended with 0.4% oxalic acid to extract broccoli juice, oxalic acid is added to avoid oxidation of ascorbic acid, then filtered to separate the residue and the filtrate. The filtrate was taken and put in a volumetric flask, and the volume was made up with 0.4% oxalic acid to the mark. The same is done with broccoli that is not steamed.

Qualitative analysis was carried out using several specific reagents to determine the presence or absence of ascorbic acid in broccoli before and after heating. The specific reagents used in this study were Fehling A and Fehling B, and ammonium molybdate. The results obtained in this study were all positive according to the literature, in the broccoli sample which Fehling A and Fehling B added to form a brick red precipitate, Fehling is a solution of cupric (III) sulfate in an alkaline solution will be blue, in the presence of an aldehyde group in ascorbic acid, Copper metal easily oxidizes ascorbic acid through the 2nd and 3rd carbon atom groups which easily release hydrogen atoms into dehydroascorbic acid under the influence of heating, and with bases the lactone ring opens to form 2,3 diketogulonate. Spontaneously Cupric ions (Cu⁺⁺) become Cupro (Cu⁺) which precipitate and are brick red in color, while the sample with the addition of ammonium molybdate produces a complex color, namely molybdene blue which is formed between the ascorbic acid contained in the sample.

In the quantitative analysis of ascorbic acid was determined by UV-Vis spectrophotometry with a wavelength of 570 nm as the maximum wavelength. Samples were measured on UV-Vis spectrophotometry because ascorbic acid has a chromophore group, namely a group that has a conjugated double bond, ammonium molybdate reagent is used to form a colored compound that produces a blue molybden color so that it can be observed at visible wavelengths. In this study, the supernatant was diluted by taking 1 mL of the supernatant for each sample and put into a 10 mL volumetric flask. Then 4.0 mL of 5% sulfuric acid was added, the addition of this solution aims to provide an acidic atmosphere during the color formation reaction and 5% ammonium molybdate was added to the mark, then the absorption was measured at a wavelength of 570 nm.

The color intensity of the reaction of ammonium molybdate with ascorbic acid is very time dependent and will affect the absorbance value and determination of ascorbic acid levels, according to previous studies that there were no significant differences at 20 minutes, 25 minutes, and 30 minutes. So, it can be explained that the color formed is stable in a time span of 20-30 minutes (Sudjarwo, 2010).

In this study, the regression $y = 0.0103x + 0.0017$ was obtained. $r = 0.9984$ based on these data it can be concluded that there is a linear relationship between concentration and absorption according to Lambert-beert's law. Based on the results of research that has been carried out on broccoli before and after heating by UV-Vis spectrophotometry, the average level of ascorbic acid in broccoli that has not gone through the heating process is 0.63 mg/g, while the heating process obtained an average level of 0.45 mg/g.

From the results of sample analysis, it was found that there were differences in ascorbic acid levels between broccoli before and after heating, with the percent decrease in ascorbic acid levels in broccoli after heating, which was 72.23% due to the effect of heating on ascorbic acid levels in broccoli.

Table 1. Quantitative analysis of ascorbic acid in broccoli by UV-Vis spectrophotometry

| | Replication | Absorbance (570 nm) | Ascorbic acid level (mg/g) | Average (mg/g) |
|------------------|-------------|---------------------|----------------------------|----------------|
| Fresh broccoli | 1 | 0.671 | 0.64 | 0.63 |
| | 2 | 0.652 | 0.62 | |
| | 3 | 0.631 | 0.61 | |
| Steamed broccoli | 1 | 0.487 | 0.47 | 0.45 |
| | 2 | 0.455 | 0.43 | |
| | 3 | 0.475 | 0.45 | |

4. CONCLUSION

Ascorbic acid levels in broccoli dropped dramatically after heating when compared to without heating. Thus, to maximize the potential of ascorbic acid in broccoli, low heating with a time that is not too long is highly recommended.

Acknowledgements: The authors are grateful to The Head of Pharmaceutical Chemistry Laboratory, Universitas Muslim Indonesia. The authors are thankful to The Dean of Faculty of Pharmacy Universitas Muslim Indonesia for the space to do this research.

Author contributions: Concept – M.P., T.N.; Design – M.P., Z.A.; Supervision –M.B., M.P., S., T.N; Materials – R.M.J., M.B.; Data Collection and/or Processing – R.M.J., A.S.; Analysis and/or Interpretation – M.P., M.B., R.M.J., A.S; Literature Search – M.P., M.B., R.M.J., A.S.; Writing – M.P., M.B., A; Critical Reviews – M.P., M.B., A.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

- Dalimartha, S., 2000, *Atlas Tumbuhan Obat Indonesia*, Jilid 2, Trubus Agriwidya, Jakarta.
- Febry, B.A., Pujiastuti, N., & Fajar, I., 2013, *Ilmu Gizi Praktis Kesehatan*, Penerbit PT. Graha Ilmu, Jakarta.
- Ganiswarna, G.S., 2007, *Farmakologi dan Terapi*, Edisi V, Universitas Indonesia, Jakarta.
- Kumalaningsih, S., and Suprayogi, 2006, *Tamarillo (Terung Belanda), Tanaman Berkhasiat Penyedia Antioksidan Alami*, Malang.
- Sudjarwo dan Badriah, 2010, *Optimization and Validation of Visible-Spectrofotometric Method for Determination Ascorbid Acid in Jeruk Bali (Citrus maxima) Fruit*, Airlangga University

This is an open access article which is publicly available on our journal's website under Institutional Repository at <https://www.umi.ac.id/id/>