Research Article

Colorimetric method for ascorbic acid analysis in fruit and processed products of pineapple (*Ananas comosus* (L.) Merr) var. Madu Subang

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ABSTRACT

Ascorbic acid is widely found in fruits, including pineapple (*Ananas comosus* (L) Merr). Fruits are rotten easily after harvested, so preservations are required. Pineapple fruits were processed into chips, jam, syrup, and dodol so can be stored in a long time. This study was aimed to analyze the ascorbic acid in fruit and processed products of pineapple var. MaduSubang which collected from Subang district, West Java, Indonesia. Colorimetric method was used based on the ability of ascorbic acid to reduce methylene blue color. Ascorbic acid content was 0.229 ± 0.005 mg/g in fruits, 0.057 ± 0.005 mg/g in chips, 0.076 ± 0.004 mg/g in jam, 0.086 ± 0.005 mg/g in syrup, and 0.0004 ± 0.00005 mg/g in dodol. Ascorbic acid content in processed products was decreased by 75.2% in chips, 66.9% in jam, 66.1% in syrup, and 99.8% in dodol. Reduction in ascorbic acid content depends on the type of food processing.

KEY WORDS: Decreased ascorbid acid content, Methylene blue, Reduction, Subang district

INTRODUCTION

Ascorbic acid consumption restains degenerative diseases.[1] This is due to antioxidant activities which prevent diseases associated with oxidative stress with a terminate radical chain reaction.[2] Ascorbic acid is essential nutrient which can be obtained from vegetables and fruits. Its content depends on fruits type, sun exposure, and growth condition.[3]

Ascorbic acid is the main content of pineapple fruits.[4] Pineapple (*Ananas comosus*, Bromeliaceae) fruit is non-climacteric category fruit based on the pattern through ripening.[5] In 2014, the production of pineapple was 1.84 million tons in Indonesia.[6] Subang district is one of pineapple producing centers in Indonesia.[7] Ascorbic acid decreases because of rotten fruits due to short storage life of fresh fruits, so preservations are required. Sundanese people in West Java, Indonesia, process pineapple into chips, jam, syrup, and dodol, so processed products can be stored in a long time. This study was aimed to analyze the ascorbic acid in fruit and processed product of pineapple var. MaduSubang which collected from Subang district, West Java, Indonesia.

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MATERIALS AND METHODS

Materials

Pineapple fruits were collected from Subang district, West Java, Indonesia, in July 2016. Fruits were identified by the Department of Biology, Universitas Padjadjaran with No. 452/HB/08/2016. All analytical grades of chemical reagents were purchased from Merck (Germany), i.e., ascorbic acid, citric acid, methylene blue, and calcium hydroxide.

Preparation of Processed Product

a. Chips: Pineapple fruits were peeled and cut with a thickness of 1 cm and then soaked in 1% calcium hydroxide for 3 min. Pineapple pieces were dried under the sunlight until half dry, then put in a vacuum for 180 min.
b. Jam: Pineapple pulp was fried and then added sugar with a ratio of 2:1. The mixture was stirred until brownish-yellow.
c. Syrup: Pineapple juice was added to sugar with a ratio of 1: 1 and cooked until boil for 3 min.

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d. Dodol: Pineapple jam was added sugar and rice flour with a ratio of 5: 3: 1. The mixture was cooked with roasted coconut until stiff.

Analytical Method Validation

Mixture of 50 ppm of methylene blue solution and aquadest (1:1) was incubated for 10 min. Absorbance was measured at 500–700 nm with citrate buffer pH 4.2 as blank. The analytical method was validated for linearity, accuracy, precision, detection limit, and quantification limit. Calibration graph was constructed from five concentrations of ascorbic acid which reacted with 50 ppm of methylene blue solution. All measurements were prepared in triplicates. The absorbance of methylene blue was plotted against the ascorbic acid concentration to obtain the calibration graph.[8]

Ascorbic Acid Extraction

Fruits and its processed products each added citrate buffer pH 4.2 with a ratio of 1:2, then mashed and filtered into a 50 mL of volumetric flask, and fulfilled with citrate buffer pH 4.2. Each extract was centrifuged at 3000 rpm for 10 min, and the supernatant was collected.

Ascorbic Acid Analysis

Mixture of 50 ppm of methylene blue solution and supernatant (1:1) was incubated for 10 min. Absorbance was measured at maximum wavelength with citrate buffer pH 4.2 as blank.

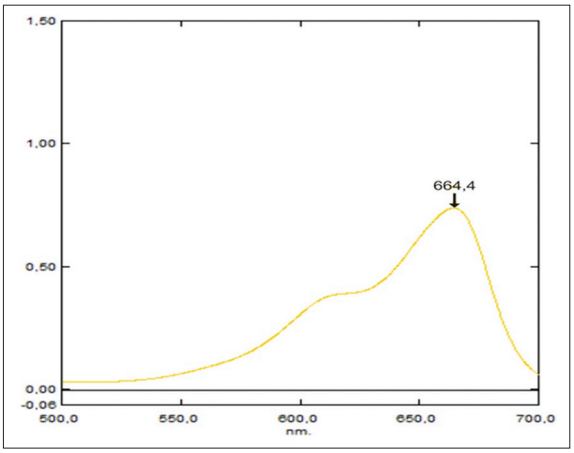

Figure 1: Methylene blue spectrum

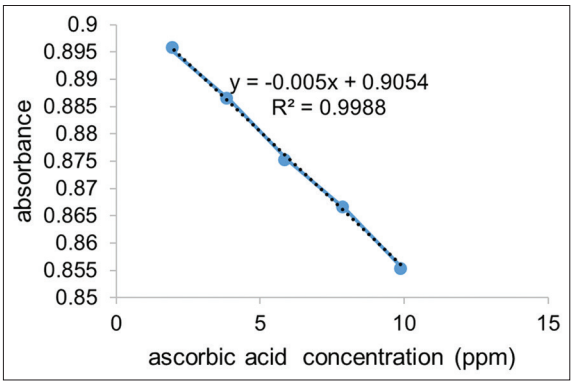

Figure 2: Calibration curve of methylene blue

RESULTS AND DISCUSSION

Pineapple fruits were collected in July, because of its non-climacteric category fruit. July was the coolest month in Subang district while January to March are the rainy season.[9] This condition is in accordance to pineapple which needs to grow.[10] Subang altitude is 1500 m above sea level[7] which, accordance with pineapple, needs to medium altitude, i.e., 1350 m.[10] Fruit of pineapple var. MaduSubang is large with pale yellow, fibreless, and juicy flesh.

Highest absorbance methylene blue was measured on 664.4 nm [Figure 1]. Analytical method validation was conducted to determine the method suitability for its intended purpose.[8] Ascorbic acid is a reducing agent due to electron donor to oxidizing agent.[12] Ascorbic acid will reduce methylene blue to colorless leucomethylene blue,[11] so absorbance will decrease with increased ascorbic acid concentration [Figure 2]. The validation result met the criteria [Table 1], so this method can be used to measure ascorbic acid based on methylene blue reduction.

Citrate buffer pH 4.2 maintains the ascorbic acid stability during extraction and measurement. All extracts were turbid so centrifuge to obtain clear supernatants for colorimetric measurement. Ascorbic acid content was lower in processed products compared to fresh fruit [Table 2]. Type of food processing, heating, and cutting method was affected the ascorbic acid content. The longer the heating time, the lower the ascorbic acid content [Table 2]. Heating time in a processed product, from the shortest to the longest, was syrup, jam, chips, and dodol. This finding was in accordance with the cooking process or prolonged storage will reduce ascorbic acid content in food.[13]

Table 1: Analytical validation method

Parameter	Result	Reference (ICH, 1996)
Linearity	y=−0.005x+0.9054 R^2=0.9988	0.99
Accuracy	99.0–101.8%	>98%
Precision	1.503%	1–2%
Detection limit	0.574 ppm	-
Quantification limit	1.914 ppm	-

Table 2: Ascorbic acid content in processed product

Sample	Ascorbic acid content (mg/g)[a]	Reduced ascorbic acid (%)
Fruit	0.229±0.005	0
Chips	0.057±0.005	75.2
Jam	0.076±0.004	66.9
Syrup	0.086±0.005	66.1
Dodol	0.0004±0.00005	99.8

[a]Mean±SD, *n*=3. SD: Standard deviation

CONCLUSION

Reduction in ascorbic acid content depends on the type of food processing.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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