

## Quantification of Pyridoxine Level in Banana (*Musa paradisiaca*) Fruit with Processing Method Variation

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### ABSTRACT

Traditionally, a banana (*Musa paradisiaca*) fruit is used to treat emesis on pregnant individual. Earlier, some research presented that the effect is probably caused by the pyridoxine content in the fruit, which is widely utilized to treat nausea and vomiting in pregnancy. It is important to acknowledge a better way consuming the fruit to achieve the best anti-emesis effect for a pregnant woman. This research aims to determine the level of pyridoxine in banana fruits with four different processing process. The study compares the pyridoxine composition in the mature fruit with and without any further heating treatment. The level was measured using HPLC method with the mixture of methanol and water for a mobile phase. The result shows that there is not a significant change of pyridoxine level in the sample before and after boiling. It was obtained that the content of pyridoxine in a before boiling mature banana fruit and in the fruit after boiling for 20, 30 and 40 minutes was 0.2530 and 0.2860; 0.3060; and 0.3646 mg/g, respectively. In conclusion, even though there is a slight increase, a differentiation of boiling time does not affect the level of pyridoxine in banana fruits significantly.

**Keywords:** Banana, heating, processing method, *Musa paradisiaca*, pyridoxine.

## 1. INTRODUCTION

Vitamins play an important role in human health, though in a small amount [1]. Moreover, vitamins also can be used to treat some diseases or heal a disease's symptoms [2]. One of vitamins that is essential for human is pyridoxine (Sami et al, 2014). Besides its function in amino acid transamination [3], it also can delight nauseous and vomiting condition in a pregnant individual [4].

Nausea and vomiting happens in first trimester of pregnancy and occurs up to 80% of pregnancies. Combination of doxylamine and pyridoxine is currently the first line pharmacological therapy for nausea and vomiting of pregnancy. However, in some countries, the pyridoxine alone is recommended for the first line therapy the syndrome in pregnancies, such as in the United States and Australia [5]. Some studies report that the usage of pyridoxine alone has a significant result in minimizing nausea and vomiting symptoms compared to placebo in a double-blind randomized trial [6]. Furthermore, there is no evidence of adverse outcome in pyridoxine monotherapy in pregnancies [7].

In Indonesia, traditionally, a pregnant individual is suggested to consume banana fruit to overcome nausea and vomiting condition. It is consumed with or without boiling process of the raw or edible fruit. The effect, possibly, is caused by pyridoxine content in it [8]. However, the level of pyridoxine content in banana could be affected by ways of the fruit consumption. It is obtained that pyridoxine is an unstable compound and can be degraded by light, heat, acids, alkali and oxidizing agents [9] [10]. Moreover, the substance is a water-soluble [11], so a boiling process probably can reduce its concentration in samples because of an extraction mechanism.

It is important to analyze the effect of boiling process on pyridoxine content in banana fruit to obtain the best way of banana fruit consumption in nausea and vomiting treatment in pregnancy. The level of pyridoxine in samples can be quantified using high-performance liquid chromatography (HPLC) [12]. About 40% higher vitamin B contents can be detected on an average than that by using classical microbiological methods [13].

## **2. METHODOLOGY**

### **Materials**

HPLC-grade solvents were used for analysis and obtained from manufacturer. Deionized water was used in all procedures and was carried out by means of a Millipore deionizer. The banana fruit was obtained from Pekanbaru, Indonesia.

### **Methods**

#### **Sample Preparation**

Edible banana fruits, with its peel, were boiled in water (100°C) for 20; 30 and 40 minutes. The pyridoxine level was detected before and after boiling process.

#### **Determination of Pyridoxine Level**

The pyridoxine was extracted using buffer solution (a mixture of sodium salt of hexane sulfonic acid). The process was prepared at room temperature. Prior to sample injection the solution was filtered through a Milipore filter to remove any undissolved particle. Twenty microliters of the filtrate were injected into HPLC system. Quantification of pyridoxine level was accomplished by comparison to pyridoxine standards. Standard stock solution and calibration curve were prepared as reported by Anyakora et al (2008) [2]. Chromatographic separation was achieved on a reversed phase HPLC (Shimadzu) through the isocratic mobile phase (20-70 of methanol and the buffer) at a flow rate 0.75 ml/minute. Ultraviolet absorbance was recorded at 255 nm at room temperature.

## **3. RESULT AND DISCUSSION**

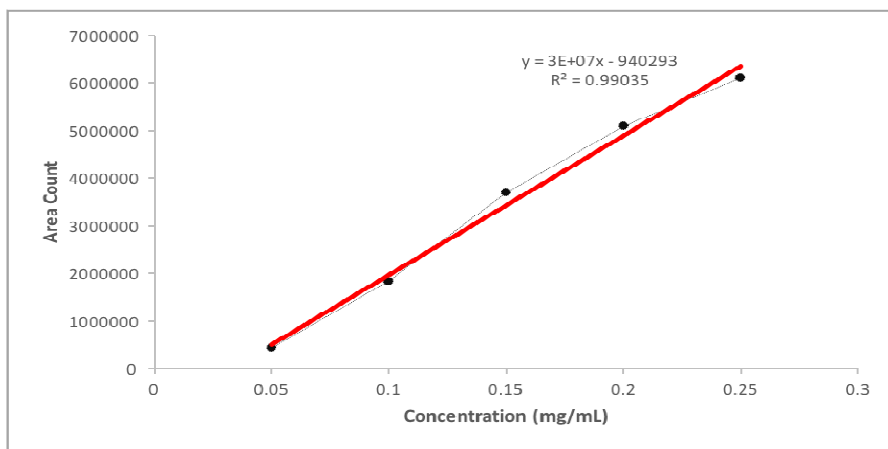
### **Extraction**

The pyridoxine from banana fruit sample was extracted in buffer solution from the mixture of sodium salt, hexane, sulphonic acid, glacial acetic acid and deionized water. Mixing sample in the buffer

was done by a vigorous shaking to make sure the homogeneity of the mixture. Hexane sulphonic acid in the buffer utilized for removing the bulky proteins in the food, hence fostering the proper dissolution of the vitamin [2]. The filtering process was done to to remove solid material before analyzing. The vitamin target is a compound that is soluble in the buffer.

**Calibration curve of pyridoxine standard**

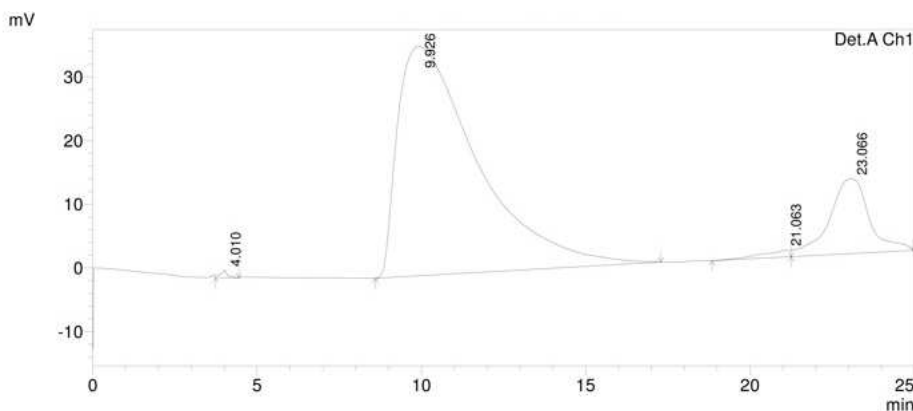
The regression equation was achieved trough a calibration curve of standard with the concentration of 50; 100; 150; 200 and 250 ppm. It obtained a linear curve with regression coefficients value of 0.992. Figures 1 shows the calibration curve for pyridoxine standard. The regression equation was used to quantify pyridoxine concentration in sample.



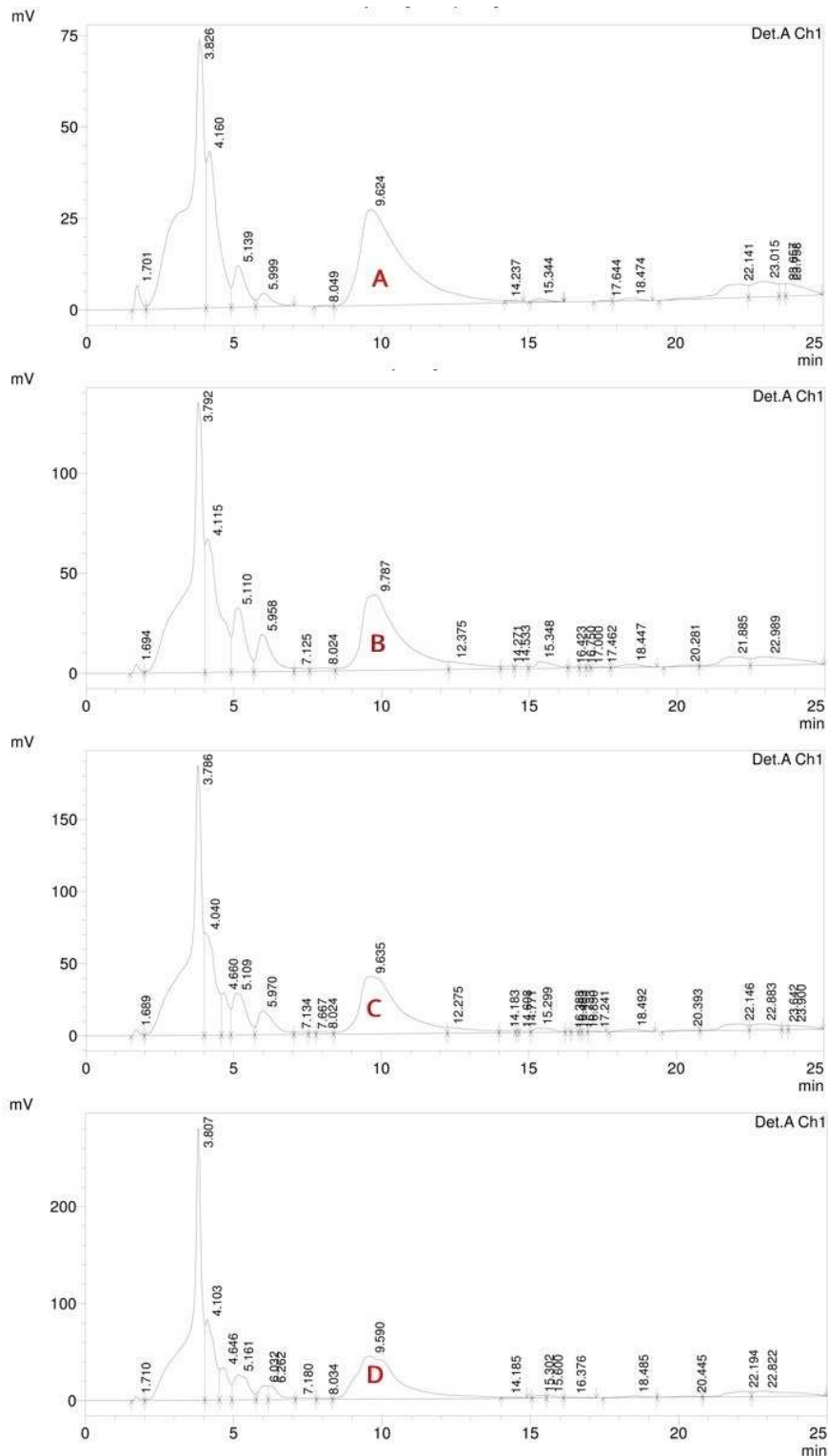
**Figure 1.** Calibration curve of pyridoxine.

**Effect of boiling process on the level of pyridoxine**

RP-HPLC can be used to analyzed the level of pyridoxine in banana fruit sample. Identification of the compound was done by comparing the retention time of sample and pyridoxine standard. In this research, the standard gave three peaks at 255 nm detection. The pyridoxine showed a peak at 9.926 minutes (250 ppm) (Figure 2).



**Figure 2.** Chromatogram of pyridoxine standard concentration 250 ppm at 255 nm



**Figure 3.** Identification of pyridoxine content in sample (A) before heating (B) after boiling 20 min (C) after boiling 30 min (D) after boiling 40 min.

All of sample contain pyridoxine with retention time of from 9.590 to 9.787 (Figure 3). It is obtained that there was no a significant change of pyridoxine level in banana fruit sample after boiling in water for 20 to 40 minute. The pyridoxine level in sample before boiling process and after boiling for 20,

30 and 40 minute was 0.2530; 0.2860; 0.3060; and 0.3646 mg/g, respectively. It can be seen that the level of pyridoxine after boiling increased slightly compared to the level before boiling. However, an increase of pyridoxine level did not significant statistically. Moreover, the concentration experienced a gradual increase after boiling process for 20, 30 and 40 minutes. Concentration of pyridoxine in the sample would increase with an increase of boiling time, but did not significant (Table 1). The heating process possibly could affect the level of pyridoxine in boiled banana fruit, but boiling the fruit without peeling the skin could help preventing the degradation process. Furthermore, the skin also prevented an extraction mechanism of pyridoxine in the sample into water.

**Table 1.** The level of pyridoxine in the sample.

Variable	AUC	Retention time	Concentration of pyridoxine (mg/g)
Without heating	2871602	9.624	0.2530
Boiling 20 min	3379615	9.787	0.2860
Boiling 30 min	3680188	9.635	0.3060
Boiling 40 min	4612640	9.590	0.3646

#### 4. CONCLUSION

It is true that there is a slight increase of pyridoxine content by the boiling time prolongation, though the number is not significant. Moreover, boiling banana fruits without peeling its skin possibly could prevent the degradation process of pyridoxine content in it.

#### 5. ACKNOWLEDGEMENT

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