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*Research Article*

# A Study on the Antioxidant Activity of $\alpha$-tocopherol Extracted from Seven Indigenous Rice Varieties

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***Keywords:***
*α-tocopherol*
*Indigenous rice*
*Northern Thailand*
*Antioxidant*
*HPLC analysis*

***Abstract:*** *This study aimed to determine the α-tocopherol content in seven Thai indigenous rice varieties from northern Thailand and compare their antioxidant potential. The rice varieties studied were Doi, Khanhi, Hengor Leothin, Riceberry, Khao Niew Kam, Man Pu, and Khao Chao Mali. α-tocopherol was extracted using 95% ethanol, n-heptane, and sodium sulfate. The results showed that Khao Niew Kam had the highest α-tocopherol content (0.25 mg/100 g), followed by Hengor Leothin and Man Pu (0.13 mg/100 g). The lowest content (0.01 mg/100 g) was found in Doi, Riceberry, Khao Chao Mali, and Khanhi rice.*



## 1. INTRODUCTION

Environmental pollution in the modern world has increasingly raised public awareness of health and well-being. As a result, people have become more concerned about consuming nutritious foods and protecting their bodies from the harmful effects of free radicals. Free radicals are unstable molecules that can damage cells and contribute to cellular degeneration and aging. Consequently, there is growing interest in health-promoting products that possess antioxidant properties. Vitamin E is an important antioxidant that the human body cannot synthesize on its own and therefore must be obtained from dietary sources such as plants and animals. Rice has long been a staple food for Thai

people and has been widely consumed for generations. In addition to serving as a major source of energy, rice also contains antioxidant compounds. Thailand is home to a wide variety of rice cultivars, including numerous indigenous rice varieties found in the upper northern region. These traditional rice varieties may contain antioxidant compounds similar to those found in other rice types. Therefore, this study aims to investigate the vitamin E content and its antioxidant potential in seven rice varieties. The findings of this research may help promote the value of indigenous rice varieties and provide useful information for the development of health food products or cosmetic products in the future (Noppamas, 2002).

## 2. LITERATURE REVIEW

The rice grain (Cereal grain) possesses a complex structure consisting of various layers that provide different nutritional values. The outermost layer is the husk, composed primarily of cellulose and hemicellulose. Once dehusked, the resulting brown rice contains essential components: the rice bran and the germ (embryo), both of which are rich in proteins and lipids. In contrast, the endosperm serves as the primary storage for carbohydrates in the form of starch, comprising both amylose and amylopectin (Pimpen & Nitiya, n.d.). Specifically, the rice germ and bran are vital sources of Vitamin E ($\alpha$-tocopherol), a crucial micronutrient and fat-soluble antioxidant (Nitiya, 2005).

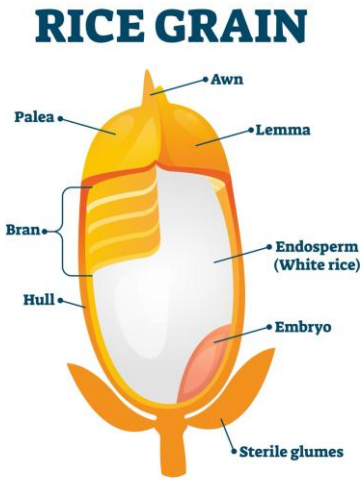



**Figure 1**. Rice grain

Vitamin E consists of several derivatives; however, $\alpha$-tocopherol is recognized as the derivative with the highest biological activity compared to others (Cheong et al., 2008). It plays a significant role in inhibiting lipid peroxidation within the human body. Therefore, analyzing the concentration of this compound in various rice varieties is highly important. The most widely used and highly accurate technique for this analysis is High Performance Liquid Chromatography (HPLC). This method operates on the principle of separating compounds between a stationary phase and a mobile phase, producing a chromatogram that clearly identifies both the type and quantity of the substance based on Retention Time ($t_R$) and peak area (Mendes et al., 2005).

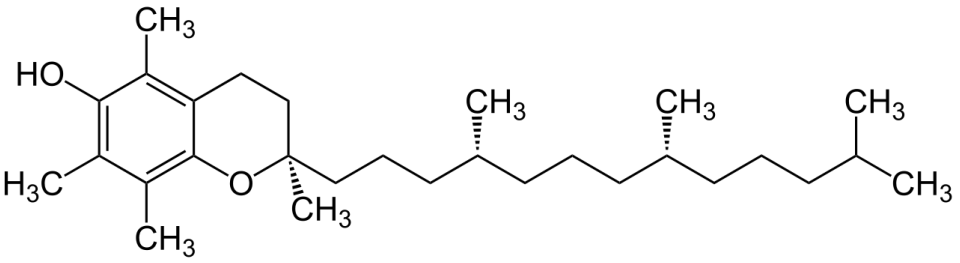



**Figure 2**. Chemical structure vitamin e (α-tocopherol)

Regarding sample preparation for analysis, the extraction of Vitamin E can be achieved through several methods, such as low-temperature solvent extraction or alkaline hydrolysis (Saponification) to separate the lipid fraction, thereby increasing the purity of Vitamin E before HPLC analysis (Kim, 2005). This is consistent with various research findings indicating that extraction conditions, such as the use of ethanol or hexane, directly affect the efficiency of recovering antioxidants from rice bran (Proctor et al., 1994; Thidarat, 2007). Studying northern Thai indigenous rice varieties is thus a crucial pathway for identifying food sources with high nutraceutical value for future consumption and export.

## 3. METHODOLOGY

*3.1 Extraction of Vitamin E*

The extraction process begins by taking 2 grams of finely ground rice and extracting it with 20 ml of 90% ethanol in a beaker. The beaker is then tightly sealed with aluminium foil to prevent evaporation and placed in a water bath at 85°C for 30 minutes, with occasional shaking to ensure thorough mixing. Afterward, 20 ml of N-heptane is added and stirred for 5 minutes, followed by the addition of 10 ml of 1.25% sodium sulfate and further stirring for 3 minutes. The resulting mixture is then transferred to a separatory funnel and allowed to stand until the layers are completely separated. Finally, the bottom layer of the sample is drained out and collected for use in the subsequent analysis steps.

*3.2 Separation of Vitamin E Using the Slight Saponification Method*

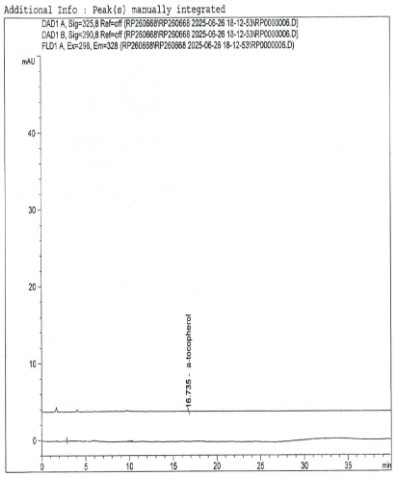
In the subsequent step, 7 mL of the sample is pipetted into a test tube, followed by the addition of 5 mL of 5% L-ascorbic acid. Potassium hydroxide in ethanol is then added to facilitate the saponification process. The mixture is shaken for 2.5 minutes and allowed to stand until the layers are completely separated. Then, 5 mL of the upper layer solution is pipetted out and mixed with 10 mL of 80% ethanol, followed by 1 minute of shaking for further purification. Finally, the resulting upper layer solution is collected for use in the next analytical stage.

*3.2 Analysis of α-tocopherol concentration*

Begins by injecting 20.0 µl of the prepared extract into the High-Performance Liquid Chromatography (HPLC) system. A Fluorescence Detector (FLD) is employed, with the excitation wavelength set at 298 nm and the emission wavelength at 328 nm to ensure maximum sensitivity and specificity for Vitamin E compounds. During the separation process within the column, α-tocopherol is identified by its specific retention time, which typically appears as a peak between 16.4 and 17.1 minutes based on the test results. Finally, the peak area obtained from the chromatogram is calculated and compared against a calibration curve to determine the exact concentration in milligrams per sample for each rice variety.

## 4. FINDINGS

### *4.1 Vitamin E (α -Tocopherol) Content in Seven Thai Indigenous Rice Varieties*

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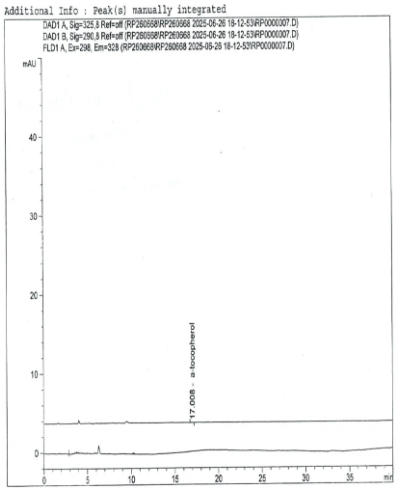


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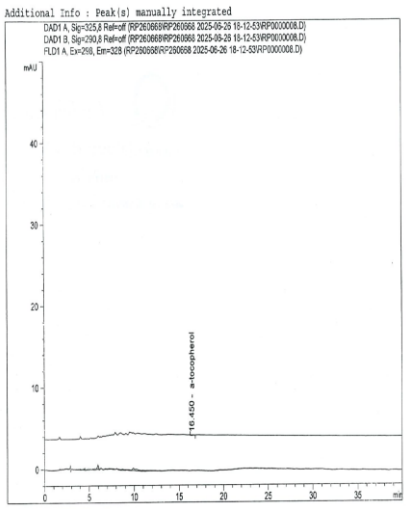


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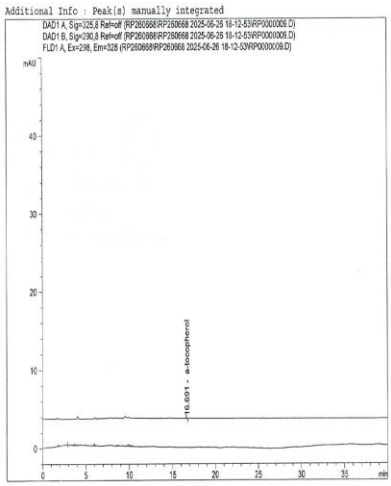


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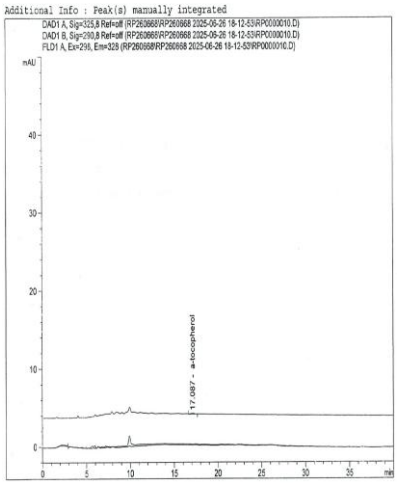


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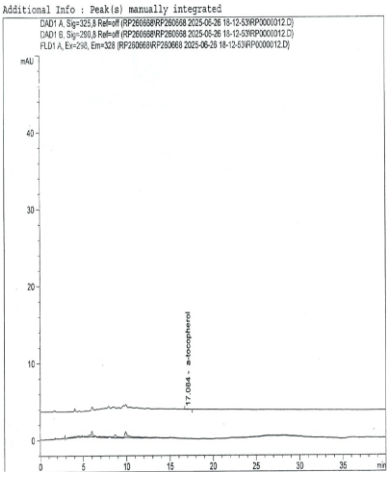


**(G)**

**Figure 3**. HPLC chromatograms of samples analyzed for $\alpha$-tocopherol content: Profiles of rice extract (A) Doi, (B) Khanhi, (C) Hengor Leothin, (D) Riceberry, (E) Khao Niew Kam, (F) Man Pu, and (G) Khao Chao Mali were obtained using DAD detector at 290 nm and FLD detector at Ex 298 nm/Em 328 nm. The peak of $\alpha$-tocopherol was identified at a retention time($t_R$) of approximately 16.4 – 17.1 minutes.

**Table 1**. This table displays the laboratory test results for Vitamin E ($\alpha$ -Tocopherol) content in various types of rice extracts.

| Test Items ($\alpha$-tocopherol) | Test results (mg/100g) | LOD | Reference Method |
|---|---|---|---|
| Doi | 0.01 | - | In-house method based on Asean Food Journal 15(1).P89-96(2008) and J. of Food Composition and Analysis 18(2005).Detected by HPCL |
| Khanhi | 0.01 | - | |
| Hengor Leothin | 0.13 | - | |
| Riceberry | 0.01 | - | |
| Khao Niew Kam | 0.25 | - | |
| Man Pu | 0.01 | - | |
| Khao Chao Mali | 0.13 | - | |

The quantification of Vitamin E ($\alpha$-tocopherol) in seven Thai indigenous rice varieties was performed using High-Performance Liquid Chromatography (HPLC). The chromatographic analysis yielded a major peak with a consistent retention time ($t_R$) between 16.4 and 17.1 minutes, accurately corresponding to the $\alpha$-tocopherol standard. Detection via the Fluorescence Detector (FLD) at Ex 298 nm / Em 328 nm provided superior sensitivity and specificity, characterized by a stable baseline that confirmed the precision of the chromatographic separation. Following manual integration to calculate the peak areas, the results revealed significant variations in Vitamin E content among the different rice varieties.

Khao Niew Dam was found to contain the highest Vitamin E concentration at 0.25 mg/100 g, suggesting it is a superior natural source of this nutrient compared to the other varieties examined. This was followed by Khao Klawng Hengor-Lertin and Khao Mun Pu, both of which contained a moderate level of 0.13 mg/100 g. In contrast, the lowest Vitamin E levels were observed in Khao Doi, Khao Khani, Khao Riceberry, and Khao Chao Mali, each yielding only 0.01 mg/100 g. These findings demonstrate that Vitamin E concentration is highly dependent on the specific rice variety, with Khao Niew Dam standing out as the richest source of $\alpha$-tocopherol among the indigenous Thai rice samples in this study.

## 5. DISCUSSION

The analysis of seven indigenous Thai rice varieties revealed significant variations in vitamin E ($\alpha$-tocopherol) content, likely attributed to genetic characteristics and the unique composition of the rice grain, particularly within the bran layer where lipid-soluble compounds are concentrated. Khao Niew Dam exhibited the highest vitamin E content at 0.25 mg/100 g, followed by Khao Klawng Hengor-Lertin and Khao Mun Pu at 0.13 mg/100 g, while Khao Doi, Khao Khani, Khao Riceberry, and Khao Chao Mali showed the lowest levels at 0.01 mg/100 g. Given that vitamin E is a well-documented natural antioxidant capable of protecting cells from oxidative damage, varieties with higher concentrations—most notably Khao Niew Dam—represent a potentially superior dietary source for antioxidant intake. However, as this study focused exclusively on quantifying vitamin E levels, the discussion of its antioxidant significance is based on established scientific literature rather than direct bioactivity assays. Consequently, while these findings highlight the nutritional diversity of indigenous rice, further research is required to directly evaluate and confirm the biological antioxidant effectiveness of these specific varieties.

## 6. CONCLUSION

This study successfully identified and quantified $\alpha$-tocopherol in seven Thai indigenous rice varieties using HPLC (FLD/DAD), with peaks confirmed at 16.4–17.1 minutes. The results showed that Khao Niew Dam contained the highest vitamin E content (0.25 mg/100 g), while Khao Doi, Khao Khani, Khao Riceberry, and Khao Chao Mali exhibited the lowest levels (0.01 mg/100 g). These variations are likely due to genetic factors and grain composition. Although higher vitamin E levels suggest potential antioxidant benefits, further research is needed to directly evaluate their biological effectiveness.

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