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## **The Influence of Temperature to Rutin Concentration of Buckwheat Grains in Humid Tropic**

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### **Abstract**

Rutin concentration is influenced strongly by temperature. Temperature can increase rutin concentration of Buckwheat grains at high to the certain limit of temperature. The aim of the research was to identify the influence of temperature to rutin concentration of Buckwheat grains. This research was conducted on IPB Experimentation Field in Pasir Sarongge, Cianjur Regency, West Java Province (6°46'05.0" Northern Latitude and 107°02'57.0" Eastern Longitude with elevation of 1150 m asl) and Farmer Group Seeding Garden of Cijulang Asri, Kopo, Cisarua, Bogor Regency which has position of 6°39'31.3" Northern Latitude and 106°53'41.1" Eastern Longitude with elevation of 600 m asl. The study was design by using nested research design. The results of research indicated that temperature in high altitude was lower than that in low altitude so that it influenced the rutin concentration of Buckwheat grains that was higher in low altitude than in high altitude.

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This research showed that high temperature in low altitude increases 72.63% of rutin concentration which is more than in high altitude. Therefore, the rutin concentration was strongly influenced by temperature in which the higher temperature was the higher rutin concentration.

**Keywords:** Buckwheat grains, rutin concentration, temperature

## 1. Introduction

Rutin concentration is influenced by genetic and environment, especially temperature. Temperature is an indication of the amount of heat energy in a system so that the higher of heat contain is the higher of temperature. Temperature gradient explains the direction and speed of heat transpiration from one place to another place. Heat will flow from higher point to lower point with the speed that shows the difference between temperature and heat conductivity of something. According to [1], temperature influences the stability of enzyme system. At optimum temperature, enzyme system functions both well and unstable in a long time. At low temperature, enzyme system will keep stable but not function. While it influences strongly on biochemistry reaction and plant physiology and determines the stratification of plant functions such as absorption of nutrients and water. Photosynthesis will be slow at low temperature and as a result, the speed growth will be slow.

The average of optimum temperature for Buckwheat is 15°C and 25°C. The author in [2] Stated that the growth of Buckwheat from planting phase to flowering phase was function of temperature. Optimum temperature during flowering phase is 10°C, 14°C during flowering to maturation phase, and 18 to 19°C at maturation phase. Buckwheat is very sensitive to frost [3]. The result of research conducted by [4] in the chamber, the leaves will experience frost at the temperature of 2°C to -3°C.

## 2. Materials and Methods

This research was conducted on IPB Experimentation Station in Pasir Sarongge, Cipanas, Cianjur Regency, West Java Province (6°46'05.0" Northern Latitude and 107°02'57.0" Eastern Longitude with elevation of 1150 m asl) and Farmer Group Seeding Garden of Cijulang Asri, Kopo, Cisarua, Bogor Regency (6°39'31.3" Northern Latitude and 106°53'41.1" Eastern Longitude with elevation of 600 m asl) from February 2012 to April 2013.



**Figure 1:** Map of West Java

## 2.1. Experimental Design

Materials used in this research were buckwheat seed (*F. esculentum* Moench) Harunoibuki cultivar, organic fertilizer 10 ton ha<sup>-1</sup>, and rice-hull ash 2 ton ha<sup>-1</sup>. Research tools used were consisted of ground treatment equipment (hoe, hand hoe, dibble, stake), solarimeter tube, digital voltmeter, wet and dry bulb thermometer, oven, scales, paranet 55%, poles for supporting paranet shade (bamboo), ruler, stationary, and block millimeter. Rutin concentrations were measured using HPLC (High Performance Liquid Chromatography) Alliance 2695 (Waters) using detector of Photodiode Array 2996 (Waters), column type C18 size 5 µm x 4.6 mm x 150 mm (Waters), flow velocity 1 mL min<sup>-1</sup>, injection volume 10 µL in room temperature. Mobile phase chosen was H<sub>2</sub>O:0.1% formic acid: acetonitrile, used wavelength at 254 nm. Standardized rutin was obtained from Tokyo Chemistry Industry (TCI).

The experiment was consisted of four factors, plant population, shade, season, and area elevation. Plant population were comprised two standard factors, namely population 200 plant m<sup>-2</sup> (2.5 cm x 20 cm) (J1) and population 50 plant m<sup>-2</sup> (10 cm x 20 cm) (J2). Shade was consisted of two standard factors, without shading (N0) and using shading (N1). Plant season was divided into two standard factors, namely dry season (M1) and wet season (M2). Area elevation was comprised of two factors, namely Cisarua low altitude (600 m asl) (T1) and Pasir Sarongge high altitude (1150 m asl) (T2). Experiment was designed using nested design method in integrated analysis. Data from observation result were analyzed using variance analysis and Duncan advanced test (DMRT) with  $\alpha = 0.05$ .

## 2.2. Analysis of Rutin

The Extraction procedure modified [5]. Dried buckwheat grains are separated between the outer skin (dark brown-black color) with the inside (flour and epidermis). Then, the inside was smoothed by using a mortar. A total of 0.25 grams of the sample inside coupled with 3 ml of solvent mixture of methanol-water 1: 1, then extracted with a horizontal shaker at room temperature and at 300 rpm for 30 minutes. After it is centrifuged for 5 minutes at a speed of 5,000 rpm. Liquid and solid phases are separated. Solid phase coupled with 1 ml of solvent and then vortex, then separated by centrifugation, the aqueous phase are combined. Then into the solid phase, it is added with another solvent of 1 ml solvent and divortex. All liquid phases are combined, the total volume of 5 ml. A total of 1 ml of liquid phase extraction results centrifuged for 10 minutes at a speed of 13,000 rpm and then separated from the solids to be analyzed using HPLC.

Standard calibration procedure of rutin. A total of 1 mg rutin tri-hydrate (molecular weight of 664.51 g/mol) was dissolved in 1 ml of methanol, in order to obtain a solution of 1000 ppm tri-hydrate rutin. Then, it is diluted in proportion of 1:9 to get 100 ppm solution. Then from a stock solution of 100 ppm standard solution prepared with regular levels tri-hydrate 5, 10, 15, 20 and 25 ppm, equivalent to regular levels of 4.59, 9.19, 13.78 and 22.97 ppm. Furthermore, blank solution and the 4 standard solutions mentioned above are injected into the HPLC. Having obtained the results of HPLC (chromatogram), a standard curve is created using Microsoft Excel software.

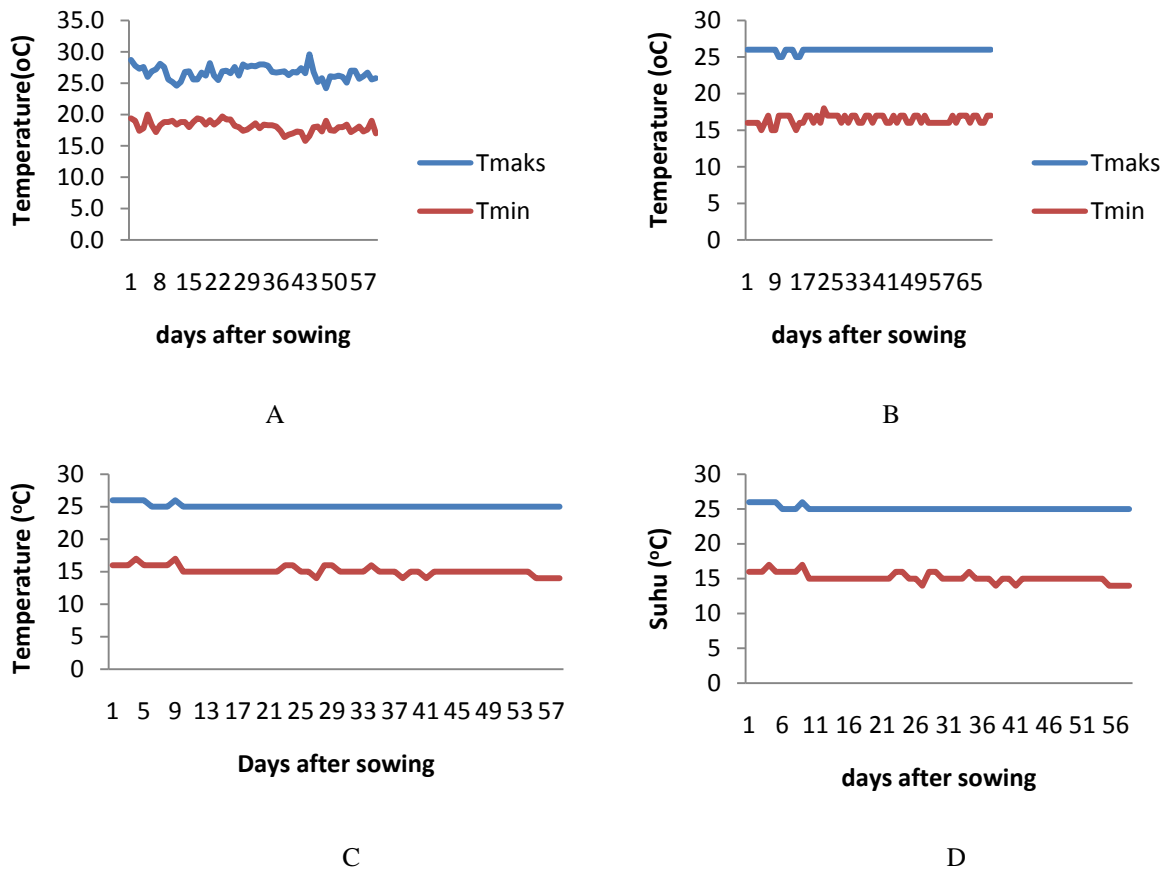
### 2.3. Statistical Analysis

In experimental design, data were analyzed using the Statistical Analysis System (SAS) Software 9.1, SAS Institute Ltd., USA. Mean comparisons were made using the Duncan's multiple range tests at the 0.05 level of probability based on the analysis of variance [6].

## 3. Results and Discussion

### 3.1. Temperature

The field measurement result on temperature was performed in figure 2. The graph shows that difference of elevation will have the difference of air temperature. At elevation 600 m asl, air temperature average extended from 27.0 to 29.3 °C. On the other hand, at elevation 1150 m asl, the air temperature average was from 21.7 to 23.8 °C.

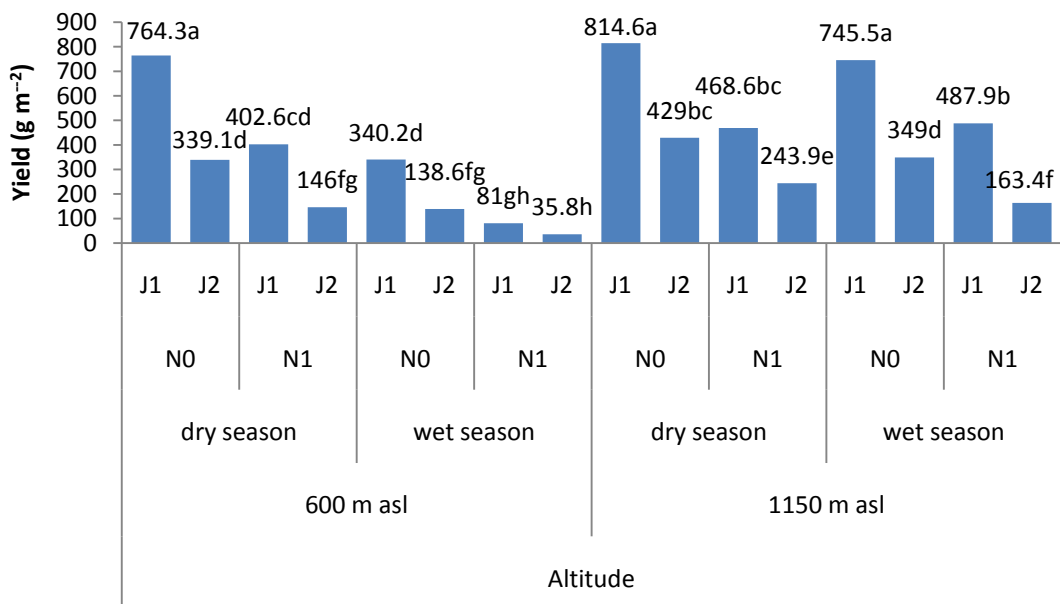


**Figure 2.** Temperature at some locations: (A) Cisarua, dry season, (B) Pasir sarongge, dry season, (C) Cisarua, wet season, and (D) Pasir sarongge, wet season

Air temperature at low altitude was higher than that at high altitude. The air temperature was influenced by elevation factor in un-linearity. The decrease of air temperature related significantly to the increase of elevation due to the thin of air layer at high altitude and the low of gas concentration so that the heat absorption tended to decrease. According to [7], the decrease average of air temperature in Indonesia extends from 5 to 6 °C in every increase of 1000 m.

### 3.2. Grains Production of Buckwheat

Low grain weight was harvested in the rainy season at 600 m altitude due to the presence of cloud so that the intensity of the radiation was blocked to the plant canopy. The results of the plant canopy photosynthesis were determined by the efficiency of leaf photosynthesis and light reception. Light reception capacity depends on the amount of radiation and the efficiency of acceptance [8]. In other words, the accumulation of dry matter depends on the amount of light radiation and the ability of plants capture, absorb, and utilize light. So the shade will cause the production of buckwheat grain fall down because the radiation availability to the plant is reduced. The high rainfall intensity in the afternoon caused plants treated with shade. Seasons, varieties, and high different places produces different buckwheat crop productivity. Productivity in the spring was around 1.76-1.97 tons ha<sup>-1</sup> and in fall around 0.65-1.48 tons ha<sup>-1</sup>. At a elevation of 70 m and 600 m respectively, the productivity was around 1.76-1.97 tons ha<sup>-1</sup> and 1.65-1.71 ton ha<sup>-1</sup> in the spring. In autumn, it was obtained around 0.65-1.48 tons ha<sup>-1</sup> and 0.68-0.93 tons ha<sup>-1</sup> [9].



**Figure 3.** Interaction effects of altitude, season, shading, and plant population toward buckwheat production

### 3.3. Rutin Concentration

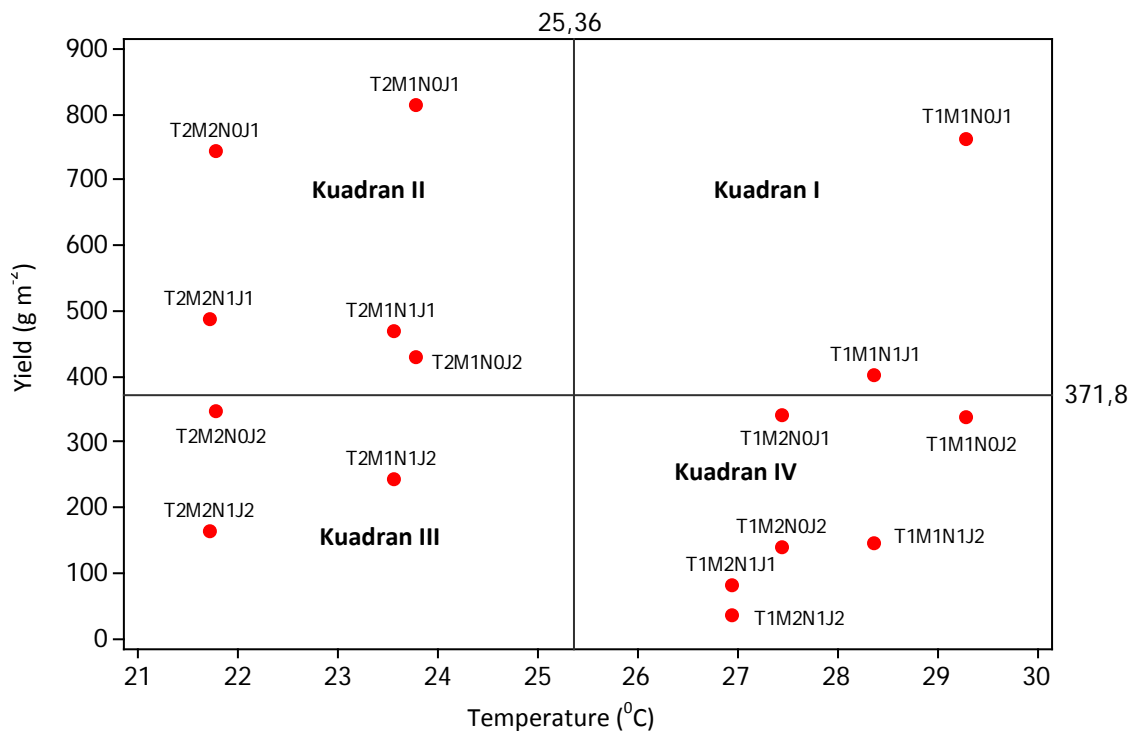
The test results of overall observation temperature conditions influence on rutin concentration of buckwheat grains can be seen in Table 1. In table 1 above shows that the highest concentration of rutin obtained on the interaction of altitude of 600 m above sea level, in the dry season, without shade, with a population of 50 plants m<sup>-2</sup>. At an altitude of 600 meters above sea level, shade and plant population affect the rutin concentration of buckwheat seed which can be seen on the results of statistical tests with  $\alpha = 0.05$ . Value of rutin concentration ranged from 0.27 to 0.39 mg g<sup>-1</sup>. While at an altitude of 1150 m above sea level season, shade and different populations were not real. At an altitude of 600 m above sea level, the rutin concentration obtained was higher than that at an altitude of 1150 m above sea level.

Buckwheat plants should be planted in the lowlands, in the dry season, without shade, with a population of 50 plants m<sup>-2</sup>. However, if the highest seed production is desired, then the buckwheat plants should be grown in the highlands, in the dry season or rainy season without shade, with a population of 200 plants m<sup>-2</sup>. To achieve the production of grains and high

**Table1.** Interaction effect of altitude, season, shade, and plant population toward buckwheat rutin concentration

<b>Treatment</b>	<b>Rutin Concentration (mg g<sup>-1</sup>)</b>
Low altitude, dry season, without shading, population of 200 plant m <sup>-2</sup>	0.391a
Low altitude, dry season, without shading, population of 50 plant m <sup>-2</sup>	0.404a
Low altitude, dry season, using shading, population of 200 plant m <sup>-2</sup>	0.312bc
Low altitude, dry season, using shading, population of 50 plant m <sup>-2</sup>	0.351ab
Low altitude, wet season, without shading, population of 200 plant m <sup>-2</sup>	0.301bc
Low altitude, wet season, using shading, population of 50 tanm <sup>-2</sup>	0.319bc
Low altitude, wet season, using shading, population of 200 plant m <sup>-2</sup>	0.273cd
Low altitude, wet season, using shading, population of 50 plant m <sup>-2</sup>	0.276cd
High altitude, dry season, without shading, population of 200 plant m <sup>-2</sup>	0.203 e
High altitude, dry season, without shading, population of 50 plant m <sup>-2</sup>	0.223de
High altitude, dry season, using shading, population of 200 plant m <sup>-2</sup>	0.176e
High altitude, dry season, using shading, population of 50 plant m <sup>-2</sup>	0.186e
High altitude, wet season, without shading, population of 200 plant m <sup>-2</sup>	0.186e
High altitude, wet season, without shading, population of 50 plant m <sup>-2</sup>	0.196e
High altitude, wet season, using shading, population of 200 plant m <sup>-2</sup>	0.170e
High altitude, wet season, using shading, population of 50 plant m <sup>-2</sup>	0.186e

Notes.:Numbers which are followed by identic letter on same column are insignificantly different according to DMRT test with  $\alpha = 0.05$ .



**Figure 4.** Relationship between production and temperature

Based on average plot between production and temperature at figure 4 which are gained from four quadrants, the quadrants are divided based on average value for each variable of production and temperature. Average of production was in amount of 371.8 and average of temperature was in amount of 25.36. The interaction which has the highest of production and temperature was T1M1N0J1, T1M1N1J1 (quadrant I). The interaction which has high production and low temperature was T2M1N0J1, T2M1N0J2, T2M1N1J1, T2M2N1J1, and T2M2N0J2 (quadrant II). Meanwhile, the interaction which has low production and temperature was T2M2N0J2, T2M2N1J2, and T2M1N1J2 (quadrant III). The interaction which has low production and high temperature was T1M1N0J2, T1M1N1J2, T1M2N0J1, T1M2N0J2, T1M2N1J1 and T1M2N1J2 (quadrant IV).

Based on figure 5, the average plot between rutin concentration and temperature was gained four quadrants which were divided into quadrant I to IV based on average value for each variable of rutin concentration and temperature. Average of rutin concentration was in amount of 0.256 and average of temperature was in amount of 25.36. The interaction which has the highest rutin concentration and temperature was T1MIN0J1, T1MIN0J2, T1M1N1J1, T1M1N1J2, T1M2N0J1, T1M2N0J2, T1M2N1J1, and T1M2N1J2 (Quadrant I). The interaction which has low rutin concentration and temperature was T2MIN0J1, T2MIN0J2, T2M1N1J1, T2M1N1J2, T2M2N0J1, T2M2N0J2, T2M2N1J1, and T2M2N1J2 (quadrant III).

Concentration of rutin, the buckwheat plants should be planted in the lowlands, in the dry season, without shade with a population of 200 plants m<sup>-2</sup>. It was predicted that the best rutin concentration of buckwheat plants grown in the lowlands, in the dry season, and without shade with a population of 50 plants m<sup>-2</sup> was influenced by

several dominant factors, such as temperature, radiation, shade, precipitation, humidity, and plant populations. Rutin concentration of buckwheat plants was influenced by genetic and environmental factors.

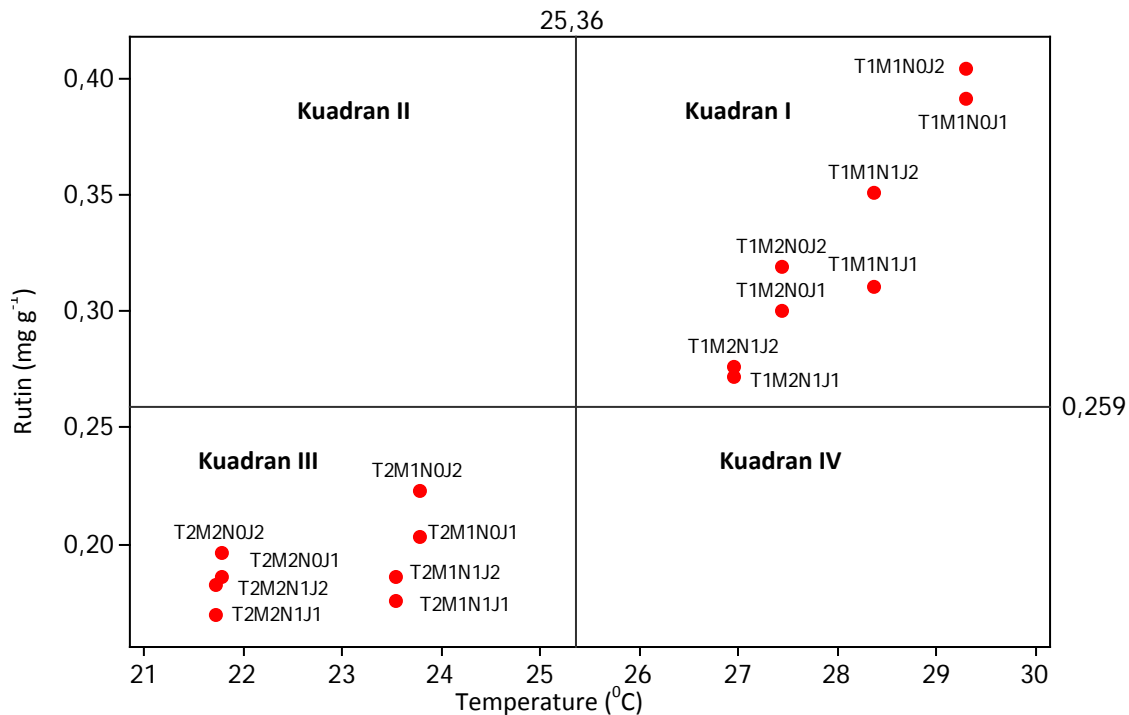


Figure 5. Relationship between rutin concentration and temperature

Rutin concentration was positively correlated with temperature, duration of drought, and solar radiation. [10] reported that the concentration of rutin was influenced by a variety of environmental factors. The same result was reported by [11] that the rutin concentration differed between varieties of buckwheat seed. *F. esculentum*, *F. tataricum* and *F. homotropicum* have 0.02, 0.101 and 1.669 percent. [12] reported that rutin concentration at different plant organs in *F. esculentum* such as roots, stems, leaves, flowers, bark, grains without skin, flour, and whole grains were respectively 3.6, 0.5, 69.9, 83.6, 0.1, 0.1, 0.1, 0.6 mg g<sup>-1</sup>. [13] also reported that rutin concentration in skinless seed was 0.2 mg g<sup>-1</sup>, dark flour 0.2 mg g<sup>-1</sup>, and bright flour 0.1 mg g<sup>-1</sup>. Furthermore [5] reported that environmental conditions affect the concentration of rutin, planting Buckwheat conducted in 2005, 2006, and 2007 resulted in different concentration of rutin 0.094 mg g<sup>-1</sup>, 0.057 mg g<sup>-1</sup>, and 0.079 mg g<sup>-1</sup>.

#### 4. Conclusion

The difference of temperature in high altitude and low altitude influences rutin concentration of buckwheat grains in humid tropic. High temperature in low altitude increases 72.63% of rutin concentration more than in high altitude. Therefore, the rutin concentration was strongly influenced by temperature in which the higher temperature was the higher rutin concentration.



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