

# Effect of Different Storage Time of Petai (*Parkia speciosa*) on Amino Acid Score and Protein Digestibility (*In Vitro*)

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**Abstract.** Stink bean, locally known as petai, is widely consumed fresh as meals' side dish. Petai often stored at room temperature for a relatively long time. This study aims to determine differences in amino acid scores and protein digestibility (*in vitro*) in petai with differences in storage time. Petai was kept at room temperature for 0, 4 and 8 days. Amino acid analysis uses HACCP and compared with PAAP (Provisional Amino Acid Pattern) to get the Amino Acid Score (AAS). Protein digestion is carried out *in vitro* with the addition of enzymes pepsin and pancreatin. The amino acid methionine was not detected at 0, 4, and 8 days storage. So, methionine as a limiting amino acid in the petai. Petai protein digestibility is between 66.37% - 70.83%. Consumption of petai as a side dish requires the substitution of other types of protein. Storage has no effect on the protein digestibility.

**Keywords:** decubitus; DC Motor; Microcontroller; Button; Bed Position.

## 1. Introduction

Petai (*Parkia speciosa*) is a legume which is commonly found in Indonesia. Some people like a petai as companion to eat rice, either raw as fresh vegetables or processed first. Protein inside petai is quite high, so it is often used as a side dish.

Protein is needed for growth and repair of body cells. The function of growth and improvements can be made if high-quality protein is shown from a high protein digestibility values in a material. Digestibility value can be calculated using the technique of *in vivo* and *in vitro*. One technique that is often used is the calculation of the digestibility of protein *in vitro* is done by adding multienzyme. Extra multienzyme in the analysis of protein digestibility will reduce the effects of endogenous inhibitors that are specific to a particular enzyme (Hahn, *et al.*, 1982).

Petai belonged to the legume is one source of protein commonly consumed by people of Indonesia. However, the digestibility of protein in legumes is lower than the digestibility of

the casein or sources other animal protein, due to factors intrinsic to the chemical structure of the protein legume as antinutritive factor. Examples of protease inhibitors in legumes can inhibit serine protease pancreatic work and lower the value of protein digestibility (Lingsberger-Martin, *et al.*, 2013).

Rule tasteless food is tasty, nutritious and safe for consumption. According Sutanti (2016), the protein content of the petai range at 22.85 to 36.03 g / 100 g. Good protein is ready to digest and contains essential amino acids in quantity in accordance with human needs (FAO, 1991 in Mokrane, *et al.*, 2010).

Petai is usually used as secondary crops grown in villages. In the distribution of up to cities in Indonesia takes time and storage. However, during storage, changes the physical and chemical properties. Physical changes such as wilting, shriveled seeds, and loss of fluids (Sari, *et al.*, 2016). Based on Sutanti, *et al.* (2016) an increase in levels of protein in the petai that has been stored in cold temperatures.

No studies have reported the digestibility of protein in the petai value. Therefore, the researchers looked at the need to examine the changes in amino acid score (AAS) and protein digestibility petai to long storage time.

## **2. Instruments and Methods**

### **INSTRUMENT**

The research material in the form of Petai obtained from Krasak Village, District Salaman, Magelang. The used chemicals were: aqua pro-injection, aqua bidestilata, HCl, NaOH, buffer phosphate, acid trichloro acetate, o-phthalaldehyde (OPA), methanol HPLC grade, standard mix of 17 amino acids (Sigma-Aldrich), pepsin enzyme digestion and pankreatin of pork (Sigma).

### **STORAGE**

Petai stored at room temperature (28°C) by means of overlaid. Sampling was done on days 0, 4, and 8. The samples were stored in a freezer (-20°C) until analysis.

### **ANALYSIS OF AMINO ACID-FREE**

Total amino acid analysis based research Jork, *et al* (1990) in Sulvi, *et al.* (2013). Petai powder (600 mg) was added to 4 ml of 6N HCl, refluxed for 24 hours at a temperature of 120°C, neutralized with 10 ml of 6N NaOH (pH7), and filtered through Wattman paper 0,2µm. A total of 50 mL sample was added a solution of 300 mL OPA.

Amino acid analysis was done by HPLC SHIMIDZU LC 10 column LiChrospher 100 RP-C18 (125 x 4.0 mm). The mobile phase consisted of eluent A (50 mM sodium acetate: THF: methanol = 96: 2: 2) and eluent B (65% methanol with a flow rate of 1 ml / min. Elution gradient of 100% for solution A for 0.1 min, 100 % of solution B for 45 minutes and terminated at the 50th minute.

### **Amino Acid Score (AAS)**

Amino acid score was calculated based on Almasyhuri *et al.* (1999). Essential amino acids in the material compared to respectively the same amino acids in PAAP (Provisional Amino Acid Pattern).

$$AAS = \frac{mg \text{ amino acid in } 1 \text{ g protein tested}}{mg \text{ amino acid in } 1 \text{ g standart protein}}$$

### Protein Digestibility Analyzis (*In vitro*)

Analysis of *In vitro* Protein Digest based method Almeida, *et al* (2015) with modifications. 250 mg samples of Petai powder or 0.25 mL akuabides (as a blank) were suspended in 15 mL of 0.1N HCl containing 1.5 mg / mL pepsin, incubated for 2 hours in a water bath temperature of 37 ° C. Pepsin hydrolysis was stopped by adding 7.5 mL of NaOH 0,5N. Hydrolysis followed by using 10 mg pankreatin that has been dissolved in 10 mL of 0.2 M phosphate buffer (pH 8) and incubated for 3 hours in a water bath temperature of 37 ° C. Hydrolysis was stopped with 1 ml trichloro acetic acid 10 g / 100 ml and centrifuged (530 g for 20 minutes). The protein content in the supernatant was determined as total nitrogen use kjeldahl method. Value of *In vitro* Protein Digestibility (IVPD) is calculated by the following formula:

$$\%IVPD = \frac{(Ns-Nb)}{Ns} \times 100\%$$

Ns and Nb represent the content of nitrogen in the sample and blank

## 3. Results and Discussion

**Table 1 Essential Amino Acid Content Variation Petai with Storage**

| Essential Amino Acids    | The content of Essential Amino Acids (mg / g db) |      |      |
|--------------------------|--|------|------|
|                          | K  | 4    | 8    |
| isoleucine               | 1:03   | 0.82 | 2:14 |
| leucine                  | 2.64   | 1:45 | 4.60 |
| lysine                   | 3.67   | 4:38 | 5:58 |
| Methionine + cysteine    | ND   | ND   | ND   |
| Phenylalanine + tyrosine | 2:04   | 3:59 | 7.85 |
| threonine                | 1:20   | 0:31 | 2:42 |
| Valin                    | 1:56   | 1:29 | 2.72 |

Description: Nd = not detected

The content of essential amino acids in a petai can be seen in Table 1. The amino acid methionine, cysteine and tryptophan were not detected in this study. Legumes often contain high amino acids lysine and methionine amino acids are low, as in *Pisum sativum* varieties Xantos and Svir which has the highest content of the amino acid lysine (6.4 g / 16 g N) and methionine in the lowest levels (1.1 g / 16g N) (Amarakoon, 2009). So we can say that the

limiting amino acid in legumes, including petai, is methionine. When a group of nuts consumed in a single, then proteins that enter the body are of low quality.

The quality of protein in food one of which can be seen from the presence of amino acids essential by calculating the amino acids compared reverensinya, the pattern of amino acids that have been determined by FAO / WHO to evaluate the adequacy of protein to grow normally later called PAAP (Provisional Amino Acid Pattern ). The proposed pattern is expressed as mg of amino acids per gram of protein. The value of the amino acids in the food must meet or higher than a predetermined pattern to become food with good protein quality (Harper, 1981). Scores of amino acids compared with PAAP petai can be seen in Table 2.

**Table 2. Amino Acid Score compared PAAP  
(Provisional Amino Acid Pattern)**

| Essential Amino Acids    | PAAP * | Amino Acid Score |      |       |
|--------------------------|--------|------------------|------|-------|
|                          |        | 0                | 4    | 8     |
| isoleucine               | 40.00  | 2:58             | 2:06 | 5:36  |
| leucine                  | 70.00  | 3.77             | 2:07 | 6:57  |
| lysine                   | 55.00  | 6.68             | 7.97 | 10:15 |
| Met + cysteine           | 35.00  | ND               | ND   | ND    |
| Phenylalanine + tyrosine | 60.00  | 3:39             | 5.99 | 13:09 |
| threonine                | 40.00  | 3:00             | 0.77 | 6:05  |
| Valin                    | 50.00  | 3.12             | 2:58 | 5:44  |

\* Source: Harper (1981)

Description: ND = not detected

Amino acids methionine and cysteine when compared with the same amino acids at PAAP, showed the lowest score among the amino acids to another. The content of the amino acid methionine was not detected on the storage day 0 until the 8th. Therefore it can be said that the amino acids methionine and cysteine is a limiting amino acid in a petai.

Based on the results of the analysis (Table 3) the protein content petai protein digestibility value on a petai before administration of the enzyme, the increase during. Protein levels on day 4 was 24.16% and 29.91% on the 8th day. Values *in vitro* protein digestibility with room temperature storage of an increase of 66.37% on the 4th day of storage becomes 70.83% on the 8th day. According to Hejazi (2016), an increase amarath seed protein digestibility value during seed maturation.

**Table 3 Changes in protein digestibility in Petai Varying Storage**

| samples | Protein levels<br>Petai Initial (%<br>db) | After administration<br>of Enzyme Protein<br>Levels<br>(% Db) | <i>In vitro</i><br>Protein<br>Digest (%) |
|---------|---|---|--|
|---------|---|---|--|

|   |         |        |          |
|---|---------|--------|----------|
| 0 | 23.62 a | 4:46 b | 68.71 ab |
| 4 | 24.16 a | 4:18 a | 66.37 a  |
| 8 | 29.91 c | 4.85 c | 70.83 b  |

Petai protein digestibility values similar to bean seed *Glycine max* 74.9%, *Lupinus albus* 66.0%, and 65.6% *Faba vulgaris* (Amarakoon, 2009). However, when compared with soybean, pigeon pea (*Cajanus cajan*) and cowpea (*Vigna unguiculata*) which is almost 100%, petai protein digestibility is low. According Akporhonor (2006), the protein digestibility of soybean by 94.49%, 94.12% pigeon pea and cowpea 94.55%. Antinutrisi, such as tannins, trypsin inhibitors and hemagglutinin is one of the factors that affect the digestibility of protein legumes (Mohammed, *et al.*, 1987).

#### 4. Conclusions

Petai as well as nuts in general, has a limiting amino acid in the amino acid methionine. Therefore, there needs to be a substitution in their consumption of other foods to complement the needs of amino acids. Storage did not affect protein digestibility petai.

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