



## Effect of Soaking Duration of *Mucuna prurian* Seeds on Chemical Composition Changes

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**ABSTRACT:** This study is conducted to evaluate how different durations of soaking influence the levels of hydrogen cyanide (HCN), tannins, phenolic compounds, and soluble proteins present in fresh *Mucuna pruriens* seeds. The study was carried out on December 13, 2024 - December 27, 2024. This study involved four different treatment levels, each repeated four times: P0 (no immersion), P1 (submerged for 48 hours), P2 (submerged for 96 hours), and P3 (submerged for 144 hours). Then treated *Mucuna prurian* seeds were tested for the contents of cyanide, tannins, phenolics and soluble proteins. The data obtained were analyzed using analysis of variance (ANOVA) and continued with Duncan's Multiple Range Test if there was a significant effect. The results showed that soaking *Mucuna prurian* had a significant effect ( $P < 0,05$ ) on the content of cyanide, tannins, phenolics and soluble proteins. The conclusion of this research is soaking *Mucuna prurian* seed of 96 hours would reduce the chemical content recognized as antinutritional factors that inhibit digestibility of nutrients, so it might improve broiler if *Mucuna prurian* seed would be used as feed ingredient.

**KEYWORDS:** antinutrient, *Mucuna prurian* seeds, phenolic, soaking, soluble protein

### INTRODUCTION

*Mucuna prurian* seed are an innovation that can be developed by farmers as a nutritious food and feed useful for developing countries. *Mucuna prurian* is included in the group of multipurpose plants that have various roles such as fertilizer plants, ground cover, and as animal feed plants (Putri, et al., 2023). Beside contains high protein ( $>20\%$  crude protein) (Carmen, et al., 1999), but Utami and Lestari (2020) stated that this bean contains various antinutritive factors like saponins, phenols and fibers, and beneficial bioactive compounds such as niacin and isoflavones. Iyayi, et al. (1998) also reported that the antinutritive compounds HCN, tannin, Idopa and others. Yatno, et al. (2015) stated that the high HCN content in feed might cause growth reduction and even poisoning for livestock. In addition to having HCN content, Putra and Sjöfjan (2021) stated that the high tannin content in feed causes the formation of complex compounds with peptide bonds derived from protein, so that they cannot be dissolved in the digestive tract and excreted with feces which will affect the availability of protein, bind minerals and reduce the absorption of minerals that can inhibit digestion and absorption.

Technology has been implemented to reduce tannin and HCN content, ranging from washing, soaking, cooking, drying, and fermentation washing, soaking, cooking, drying, and fermentation (Arianto, et al. 2014). Such processing can be applied to reduce or eliminate HCN or cyanide content so that suitable for animal feed. Another method, reported by Sese, et al. (2013) indicated that boiling and heating Jack Bean seeds effectively reduces the antinutrient compounds contained. A simple method is proposed, namely by soaking with water for a duration of time. This research is evaluated the effect of duration of soaking *Mucuna prurian* seed on the contents of cyanide, tannins, phenolics and soluble proteins. It is then expected such a method would make *Mucuna prurian* seed becomes suitable for animal feed.

### MATERIALS AND METHODS

#### A. Research Materials 1) Research tools

The tools used in the first stage of the study included a UV-Vis spectrophotometer, analytical balance, volumetric pipette, measuring flask, beaker, micro burette 10 ml; g), erlenmeyer flask, distillation flask, Allihn condenser, electric heater,



vacuum pump, gas washing bottle, hotplate, rotary evaporator, aluminum foil, porcelain crucible, spatula, analytical balance, dropper pipette, stirring rod, micropipette, vortex mixer.

2) *Research materials*

The materials used in the first stage of the study included bengkok bean flour, NaOH, potassium chromate indicator (K<sub>2</sub>CrO<sub>4</sub>), silver nitrate solution (AgNO<sub>3</sub>), barbituric acid-pyridine, chloramine-T solution, acetate buffer, potassium cyanide solution (KCN), hydrochloric acid (HCl), magnesium chloride (MgCl<sub>2</sub>·6H<sub>2</sub>O), sulfamic acid, acetone solution, pdimethylamino benzal-rhodanin, ethanol solution, distilled water, 96% ethanol, gallic acid, folin ciocalteu, 1% FeCl<sub>3</sub> solution, 15% Na<sub>2</sub>CO<sub>3</sub> solution, 1% gelatin solution, Bradford reagent, extraction buffer and protein standard solution.

B. *Research Methods*

A Completely Randomized Design (CRD) was applied, involving four soaking durations and five repetitions per treatment.

The treatments plan is :

P0 : No Soaking applied

P1 : Soaking for 48 Hours

P2 : Soaking for 96 Hours

P3 : Soaking for 144 Hours

During soaking process, it was ensured that all the *Mucuna prurian* seed surface was covered by the water. After the treatment the seed was then dried, ground and brought to the laboratory for chemical analysis.

C. *Research Variables*

- 1) Hydrogen cyanide (HCN) content in *Mucuna prurian* flour
- 2) Tannin content in *Mucuna prurian* flour
- 3) Phenolics in *Mucuna prurian* flour
- 4) Soluble protein in *Mucuna prurian* flour

D. *Statistic Analysis*

A completely randomized design (CRD) consisting of four treatments with four replications each was implemented in this study. The data to be obtained include the content of HCN, tannin, phenol and soluble protein in *Mucuna prurian* seeds. The collected data were organized and analyzed using analysis of variance (ANOVA) with the aid of Microsoft Excel. If significant differences were detected, the analysis was followed by Duncan’s Multiple Range Test (DMRT) for further comparison. The summary results of the treatments are presented in Table 1.

**Table 1. The effect of treatment on the content (HCN, Tannin, Phenolic and Soluble Protein).**

Parameter	Treatment			
	P0	P1	P2	P3
HCN	43,86±1,89 <sup>d</sup>	33,21±0,51 <sup>c</sup>	21,83±1,63 <sup>b</sup>	17,85±0,79 <sup>a</sup>
Tannin	9,24±0,15 <sup>d</sup>	8,15±0,06 <sup>c</sup>	5,67±0,31 <sup>b</sup>	3,02±0,21 <sup>a</sup>
Phenolics	8,12±0,70 <sup>a</sup>	12,50±0,39 <sup>b</sup>	16,64±0,65 <sup>c</sup>	7,24±0,62 <sup>a</sup>
Soluble proteins	62,90±3,71 <sup>d</sup>	45,40±0,54 <sup>c</sup>	32,95±1,83 <sup>b</sup>	13,73±0,67 <sup>a</sup>

\*Different superscripts in the same row indicate a significant difference (P<0.05).

A. *Effect of Soaking Duration on HCN Content*

The analysis of the research data indicated that soaking treatments applied to *Mucuna prurien* seeds had a highly significant influence (P <0.05) on the content of HCN with the lowest to highest values, namely P3 (17.85 ± 0.79<sup>a</sup>), P2 (21.83 ± 1.63<sup>b</sup>), P1 (33.21 ± 0.51<sup>c</sup>) and P0 (43.86 ± 1.89<sup>d</sup>). These data show that the longer the soaking, the lower the HCN content. These findings



align with those reported by Handajani et al. (2001), who observed that soaking *Mucuna pruriens* seeds for 24 hours led to a 29% reduction in HCN content, while 48- and 72-hour treatments lowered cyanide levels by 62% and 77%, respectively. Similarly, Nwaoguikpe et al. (2011) demonstrated that combining soaking and boiling methods reduced HCN levels by 71.43%. Supporting this, Arianto et al. (2014) found that extended soaking durations were effective in decreasing cyanide concentrations in *Mucuna pruriens* seeds. Furthermore, Fauzi and Zannah (2024) explained that this reduction occurs because HCN is water-soluble and can be removed through hydrolysis and diffusion during the soaking process

### B. Effect of Soaking Duration on Tannin Content

The analysis of the experimental data revealed that the application of soaking treatments had a notable impact on the observed parameters of *Mucuna prurian* beans had a very significant effect ( $P < 0.05$ ) on tannin content with the lowest to highest values, namely P3 ( $3.02 \pm 0.21^a$ ), P2 ( $5.67 \pm 0.31^b$ ), P1 ( $8.15 \pm 0.06^c$ ) and P0 ( $9.24 \pm 0.15^d$ ). Rahmah, et al. (2019) stated that *Mucuna prurian* processing which includes soaking, boiling, de-husking and heating is reported to be able to reduce tannin and HCN levels. Kurnianingtyas, et al. (2013) stated that in the soaking process, tannin which is also soluble in water will be hydrolyzed by the tannase enzyme which is naturally active due to soaking.

### C. Effect of Soaking Duration on Phenolics Content

The results of the research data analysis showed that the soaking treatment of *Mucuna prurian* beans had a very significant effect ( $P < 0.05$ ) on the phenolic content with the lowest to highest values, namely P3 ( $7.24 \pm 0.62^a$ ), P0 ( $8.12 \pm 0.70^a$ ), P1 ( $12.50 \pm 0.39^b$ ) and P2 ( $16.64 \pm 0.65^c$ ). These data indicate that the treatment effectively increases phenolic compounds in *Mucuna prurian* beans. Retnaningsih, et al. (2013) stated that processing processes such as fermentation or boiling and soaking of *Mucuna prurian* beans can increase antioxidant compounds such as phenolics and others. Pramita, et al. (2008) stated that the soaking process can activate the hydrolase enzyme which plays a role in breaking down complex compounds into free phenolic forms so that the available phenolic compounds increase. The research data also showed that the soaking treatment at P3 (144 hours soaking) showed a lower phenolic content compared to other treatments. Pramita, et al. (2008) stated that the duration of boiling and soaking of *Mucuna prurian* seeds is a factor that needs to be considered because the increased water content in *Mucuna prurian* seeds due to soaking can affect the physical quality of *Mucuna prurian* seeds which has an impact on reducing the nutritional content and other compounds contained therein. This is supported by the statement of Astawan, et al. (2023) in his research which stated that the process of soaking *Mucuna prurian* seeds for too long causes some phenolic compounds to be washed away, resulting in a decrease in the total final phenolic content.

### D. Effect of Soaking Duration on Soluble proteins

The results of the research data analysis showed that the soaking treatment of *Mucuna prurian* beans had a very significant effect ( $P < 0.05$ ) on the levels of soluble protein with the lowest to highest values, namely P3 ( $13.73 \pm 0.67^a$ ), P2 ( $32.95 \pm 1.83^b$ ), P1 ( $45.40 \pm 0.54^c$ ) and P0 ( $62.90 \pm 3.71^d$ ). This is in line with the results of research by Djaafar, et al. (2012) which stated that in their research the soaking treatment could reduce the levels of soluble protein in the material. Ahenkora, et al. (1999) also stated that in their research the longest soaking time of *Mucuna prurian* beans showed the lowest levels of soluble protein. Sitohang, et al. (2024) stated that in the soaking process, the protein contained in *Mucuna prurian* seeds undergoes denaturation, resulting in changes in structure and reducing its solubility in *Mucuna prurian* seeds. This statement is reinforced by Prihatiningsih, et al. (2021) who stated that soluble protein is a protein component that belongs to the oligopeptide group which consists of less than ten amino acid chains that are soluble in water. With this statement, it can be concluded that soaking koro benguk seeds can reduce the content of soluble protein which is basically soluble in water..

## CONCLUSION

Different soaking duration treatments on *Mucuna prurian* beans affect the content of cyanide acid (HCN), tannins, phenolic compounds and soluble proteins contained. The best soaking treatment was shown in P2 with a soaking treatment for 96 hours.

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