DIGESTIBILITY IN VITRO OF STARCH AND PROTEIN ON ANALOG RICE BY FORMULATION OF NAGARA BEAN FLOUR MODIFIED \textit{L. plantarum} AND SAGO STARCH WITH CONCENTRATION OF GLYCEROL MONOSTEARATE

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Abstract. Limited of rice stock as a source of carbohydrates and protein deficiencies in some communities in Indonesia is also still commonly found. Therefore, the strategy used one of them is food diversification to substitute or replace the rice needs by other carbohydrate sources with the use of analog rice based nagara bean \textit{(Vigna unguiculata ssp Cylindrica)}. Nagara bean flour fermented by \textit{Lactobacillus plantarum} has better characteristics, namely high protein content, starch digestibility and better protein digestibility. Nagara bean flour modified \textit{L.plantarum} was formulated with sago starch and glycerol monostearate to produce analog rice. The analog rice obtained has the characteristics of high rehydration rate, in the proportion of nagara bean flour and sago starch 50:50 with glycerol monostearate of 1% had starch digestibility vitro of 81.76% db ± 0.12, and protein digestibility in vitro was 75.96% ± 2.30.

Keywords: nagara bean flour, \textit{L. plantarum}, starch digestibility vitro, protein digestibility in vitro

1. Introduction

Analog rice is a food product shaped like rice with carbohydrate content approaching or exceeding rice which can be made from local flour or rice. Analog rice is artificial rice made from starches other than rice and flour [1]. Analog rice can increase diversification of staple foods without changing people's eating habits. Analog rice is an effort to diversify the pattern of carbohydrate food sources by means of mind set manipulation as if it consumes like rice from rice.

Analog rice is generally processed from cereals or tubers with a predominant content of carbohydrates, but on the other hand there are actually some dominant legumes that contain carbohydrates, one of which is the nagara bean, type of sub optimal land agricultural product in swamp areas.

Nagara beans contain protein of 22.7-27% and carbohydrates of 62%, the characteristics of flour in nagara beans can be modified through soaking and fermentation, this treatment was also able to reduce and eliminate antinutrient components [2,3]. [4] showed that fermented beans using \textit{L. plantarum} of 24 hours had in vitro protein digestibility of 80.87%, increase about 37.07% compared to unfermented beans.

Analog rice made from nagara bean flour which has been fermented by \textit{Lactobacillus plantarum}, other than as source of carbohydrate in the future, can also supply protein needs. Generally carbohydrate
products have a low protein content and are limited to the amino acid content, therefore by utilizing nagara bean two uses of nutritional sources can also be obtained. It is known that nagara bean flour has amylose content ranging from 23-25% [4], analog rice formulation based on Nagara bean flour fermented by L. plantarum which composed with sago flour as a binding material and also as a balancer to amylose and amylopectin content to obtain the best rehydration power and the texture approaches rice. Amylose amylopectin composition is related to the characteristics of analog rice gelatinization and digestibility. Rice is actually a digestible source of carbohydrates, but the glycemic response depends on rice varieties and preparation techniques. According to [5] amylose content affects digestibility and glycemic response this is due to the tendency of amylose to maintain its crystalline structure after cooking which can reduce accessibility to enzymes so that it is slowly digested.

This research was aimed to examine the digestibility in vitro of starch and protein of analog rice is resulted formulation of nagara bean flour fermented by L. plantarum and sago flour by with several concentrations of glycerol monostearate.

2. Material And Methods

Nagara bean flour was obtained from Nagara Hulu Sungai Selatan South Kalimantan, glycerol monostearate, L. plantarum ENCC-0127, Agar MRS, Broth MRS, pepsin from gastric mucosa 250 units / mg, α amylase from porcine pancreas Type VI-B 10 units/mg, amyloglucosidase from Aspergillus niger 30-60 units/mg, 3,5-Dinitrosalisyclic acid, NaOH, Na₂CO₃, Na K Tartrat, CuSO₄.5H₂O, ethanol, trichloroacetic acid, potassium iodide, and iodine.

2.1. Fermentation Process

The fermentation process used ratio of nagara bean : soaked water = 1: 4. L. plantarum which is inoculated into treatment of 1% (v/w based of nagara bean weight). Nagara bean were fermented for 48 hours, then cleaned from the skin and washed, then dried at a temperature of 60 ºC for 48 hours, and powdered and sieved 80 mesh.

2.2. Processing methods of analog rice [1]

The mixing process was carried out in two stages, namely mixing dry ingredients (nagara bean flour and sago flour) with a ratio of 70: 30, 60: 40, 50: 50, 40: 60, 30: 70 and adding glycerol monostearate concentration, 1%, 2% and 3%, mix for 5 minutes, then add water with temperature of 60 °C as much as 10% and the mixing process is continued for 5 minutes. The dough is put into analog rice extruder with a cold extrusion system. Extrudate analog rice pregelatinized for 10 minutes, then dried at 60 °C to reach moisture content of less than 14%.

2.3. Parameter of analysis

Analog rice obtained was chemically tested including starch content (Luff schroll method), protein content (kjeldahl method), starch digestibility in vitro [6] and protein digestibility in vitro [7].

2.4. Analisis data

Data of chemical analysis and rehydration power were analyzed using analysis of variance (ANOVA), if there was a significant effect of treatment then continued with a significant difference test of Duncan Multiple Rate Test (DMRT).

3. Result And Discussion

3.1. Starch content

Nagara bean is one of the legumes that have high carbohydrate and protein content compared to other legumes, Starch content of Nagara bean fermented with L. plantarum is 74.73% db, whereas the sago starch has a starch content of 77.00 % db. Analog rice produced from nagara bean flour formulated with sago starch and glycerol monostearate have starch content ranging from 55.59 to 65.10% db.
Analysis of variance showed that the proportions of nagara bean flour and sago starch with GMS concentration had no significant effect on starch content, while the GMS concentration significantly affected the analog rice starch content (p ≤ 0.05). Duncan's test showed that the highest starch content was obtained in GMS of 1% (66.55% db) but not different from starch content in GMS of 2% (59.10% db). The increasing concentration of GMS tends to reduce starch content in analog rice, sago starch tends to predominantly contribute to starch content in analog rice formulations.

| GMS   | Starch content (% db) |
|-------|-----------------------|
| 1%    | 66.55%                |
| 2%    | 59.10%                |

![Figure 1. Starch content of analog rice](image)

Figure 1 showed that the starch content increases by the proportion of nagara bean flour to 40%, which is further due to the increasing proportion of nagara bean flour then the starch content in analog rice tends to decrease.

3.2. Protein content

Protein content of fermented nagara bean flour about 7.44% - 14.87%. The results of the variance analysis showed that the proportion of nagara bean flour to sago starch and GMS concentrations had a significant effect on the protein content of analog rice protein produced. Protein content data are presented in Figure 2.

| GMS  | Protein content (% db) |
|------|------------------------|
| 1%   | 20.00                  |
| 2%   | 15.00                  |
| 3%   | 10.00                  |

![Figure 2. Protein content of analog rice](image)

Figure 2 showed that increasing the proportion of fermented nagara bean flour to sago starch, protein content is increasing whereas analog rice produced from GMS concentrations of 2% and 3% relatively have higher protein content than analog rice produced from GMS with a concentration of 1%.
**Table 1** Duncan test of protein content in analog rice by formulation of fermented nagara bean flour to sago starch and GMS concentration

| Factors                      | Levels  | Protein content (%) |
|------------------------------|---------|---------------------|
| Proportion nagara bean flour | 30 : 70 | 9.05 ±1.28          |
| sago starch                  | 40 : 60 | 9.81 ±1.04          |
|                              | 50 : 50 | 11.78 ±1.50         |
|                              | 60 : 40 | 12.87 ±1.00         |
|                              | 70 : 30 | 13.77 ±1.44         |
| GMS concentration            | 1%      | 10.06 ±2.05         |
|                              | 2%      | 12.06 ±1.83         |
|                              | 3%      | 12.25 ±1.18         |

Duncan test results (Table 1) showed that the highest protein content in analog rice form the proportion of nagara bean flour to sago starch 70 : 30 is 13.77% and not different from the proportion of 60: 40 (12.87%), while the GMS concentration of 2% gave protein content of analog rice not different from GMS 3%.

The protein content in analog rice is proportional with the protein content of the raw material used where the protein content in *L. plantarum* modified nagara bean flour ranges from 19 - 22% while according to [8] the starch content of sago starch is 0.53%, so that the protein content in analog rice is more dominantly obtained from nagara bean flour, where with the greater proportion of nagara bean flour used, the protein content increases. While the concentration of monostearate glycerol of 1% has a protein content lower than 2 and 3%, it is thought that the concentration of monostearate glycerol at 2 and 3% gives better surface activity of peptides and increases the amount of dissolved nitrogen.

### 3.3 Starch digestibility in vitro

Starch digestibility is influenced by several factors including starch source, granule size, amylose/amylopectin ratio, crystallinity degree and form of crystalline polymorphic. [11] stated that digestibility was negatively correlated with starch granule size, granule size was related to surface area and starch volume so that substrate and enzyme contact would decrease if granule size increased. Reducing the size of nagara bean to flour tends to have higher starch digestibility. According to [9, 10] large particles will have a smaller surface area so they will be digested more slowly.

GMS concentration significantly affected starch digestibility in vitro (p ≤0.05) of analog rice by formulation of nagara bean flour and sago starch. Duncan's test showed that the highest digestibility was obtained from analog rice produced at GMS concentration of 1% (81.73), and this was different in GMS of 2% (75.38%) and GMS of 3% (74.42%) The increasing GMS concentration up to 3% caused starch digestibility in vitro of analog rice decreased.
3.3. Starch digestibility in vitro

The proportion of nagara bean flour to sago starch (\%) of the analog rice with variations in GMS concentration is shown in Figure 3.

Figure 3 showed that the pattern of increase in starch digestibility in vitro was not too significant with an increase in the proportion of nagara bean flour to sago starch, it's relatively constant. However, it was seen that starch digestibility in vitro of analog rice at GMS concentration of 1% higher than starch digestibility in vitro in GMS of 2% and 3%. Amylose can bind to lipids and proteins which will complicate the enzyme so that the digestibility of starch decreases. GMS is an emulsifier with one of stearate fatty acid structure, this fatty acid can bind to amylose to form a matrix which can cause a decrease in water absorption into the matrix, the gelatinization process also does not occur optimally. This will correlate with the difficulty of the enzyme to break down the starch into a simpler structure so that the starch digestibility is lower.

3.4. Protein digestibility in vitro

Protein nutritional quality, besides concern to the composition of amino acids, is also considered based on the easily of digestibility so that it can be used by the body. Protein digestibility in analog rice formulated from nagara bean flour and sago starch ranges from 73.29% - 77.54%. The proportion of nagara bean flour to sago starch and GMS concentration did not have a significant effect (p = 0.05) on the protein digestibility in vitro of analog rice produced.

Figure 4 showed that in analog rice by GMS concentration of 1%, protein digestibility in vitro of analog rice still increased up to the proportion of nagara bean flour of 60%, while in GMS concentration of 3% was relatively stable and tended to be lower than GMS concentration of 1% and
2%. This is allegedly due to the increase in GMS concentration will give matrix complexity in analog rice so that the accessibility of enzymes will be difficult.

4. Conclusion

Analog rice formulation based on nagara bean flour modified *L. plantarum* and sago starch at 50 : 50 has starch content of 59.55% db ± 4.61, protein content 11.78% ± 1.50, starch digestibility in vitro of analog rice by GMS concentration of 1% produced higher starch digestibility while protein digestibility in vitro of analog rice in proportion of nagara bean flour of 50% and GMS concentration of 2% was relatively not different from the proportion nagara bean flour of 60% and GMS concentration of 1%. This showed the proportion of nagara bean flour up to 50% and GMS concentration of 2% can be developed into analog rice based on digestibility of starch and protein in vitro.

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