# NUTRITIONAL VALUE OF FERMENTED SAGO DREGS (METROXYLON SAGU ROTTB.) BY VARIOUS CONSORTIUMS OF PROBIOTICS FOR FEEDSTUFF

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#### Abstract

Sago dregs is rarely used and pollutes the environment but has the potential to enhance nutrition value for cattle feed. A consortium of probiotics such as Bacillus, Lactobacillus, and Aspergillus, which inoculated fermented sago dregs, can improve nutritional content. This study aims to evaluate the nutritional value of fermented sago dregs by a consortium of Bacillus, Lactobacillus and Aspergillus. Material and Method: The experimental method used in this study was a completely randomized design with two factors and three replications. The first t factor was the type of consortia were consortium of B. licheniformis, L. acidophilus, A. niger, consortium of B. subtilis, L. plantarum, A. oryzae and consortium B. licheniformis, B. subtilis, L. acidophilus, L. plantarum, A. niger, A. oryzae. The second factor was the duration of fermentation were 0, 4, and 8 days. Parameters measured include the nutrient content: crude fibre, crude protein, level of amino acids, and vitamin B2. Data were analysed using ANOVA followed by Duncan's Multiple Distance Test. Results: The results showed that the fermenting dregs sago with various consortia of Bacillus, Lactobacillus, Aspergillus decreasing crude fibre, increasing the crude protein and some essential amino acids; however, it was unable to raise the vitamin B2. A consortium of B. subtilis, L. plantarum, A. oryzae in four days of fermentation effectively increased crude protein content by 12.63% and several types of essential amino acids. Meanwhile, the consortium B. licheniformis, L. acidophilus, A. niger in 8 days of fermentation effectively reduced crude fibre content by 10.88%. Conclusions: Fermentation of sago dreg by consortium B. subtilis, L. plantarum, A. oryzae for four days decreased crude fibre, increased protein by 12.63%, and increased the amino acid content of phenylalanine (48.75%), valine (21.8%), threonine (20.88%), isoleucine (37.59%), leucine (32.95%) and lysine (74.4%).

Key words: Sago dregs, fermentation, probiotic consortium, nutritional value

#### INTRODUCTION

Indonesia is an agrarian country that has an abundance of dregs agricultural products, including sago. Processing of the sago tree will produce sago starch and sago dregs. Muhsafaat et al., [16] state that the production of sago dregs is relatively abundant, with a ratio of starch and dregs is 1:6. Sago dregs can cause pollution of the environment. One of the utilization of sago dregs as material feed livestock cattle.

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Nutrients needed by livestock include carbohydrates, fats, proteins, vitamins, water and inorganic elements and minerals Balitbangtan Riau [3] reported that the nutritional content of dry matter sago dregs is 47.20%, crude protein is 0.83%, crude fibre is 11.44%, crude fat is 0.99%, ash is 1.80%, and carbohydrates are 65.7%. Sago dregs can be an alternative source of energy because it contains a high Nitrogen-Free Extract (NFE), which is 76.51%, but processing must be carried out because sago pulp has a high crude fibre content and low crude protein content. High crude fibre causes less

digestibility. Crude protein and crude fibre content of sago dregs have not met the requirements for cattle feed concentrate according to SNI standards. Cattle feed concentrate according to the Indonesian National Standard (SNI 3148.2:2009) must have a maximum moisture content of 14%, ash 12%, crude fibre 12-17%, crude fat 7%, crude protein min 13%, Ca 0.8-1%, P 0.6-0.8%, neutral detergent fibre (NDF) max 35%, undegraded dietary protein (UDP) min 5.2%, aflatoxin max 20 g/Kg, and total digestible nutrient (TDN) min 70%.

The nutritional quality of sago dregs can enriched through fermentation cellulolytic, amylolytic microorganisms to increase crude protein, degrade crude fibre, remove lignin and antinutrients and increase levels of essential amino acids and vitamins so that sago dregs are suitable as feedstuff. Probiotics including the genera Bacillus, Lactobacillus, and saccharomyces such as B. coagulans, B. licheniformis, B. megaterium, subtilis, A. oryzae, Bifidobacterium various Lactobacillus, and Saccharomyces can be used as agents for producing cellulase, amylase, proteases, lipases and to synthesize varieties of bioactive peptides [7]. FAO and WHO define probiotics as microorganisms living if given the amount that sufficiently provides health benefits on its host. Fermentation by a consortium of probiotics is often used because it is synergistic between these microorganisms [13] (Manin et al., 2012). B. licheniformis and B. subtilis is a probiotic that is safe to use and can survive in high temperatures, so it is often used in the industrial manufacture of feed livestock [7]. B. licheniformis and B. subtilis can degrade crude fibre on fermentation cake kernel oil [12] and produce vitamins B2 on fermented beans [1]. L. acidophilus and L. plantarum are lactic acid bacteria (LAB) that are environmentally friendly because they are not pathogenic and are able to inhibit the growth of pathogenic bacteria so that they are used as probiotics [10]. L. acidophilus and L. plantarum can increase crude protein and acidic amino free [17], [23]. Probosari [20] stated cattle require acidic amino essential for methionine, growth, such as lysine,

isoleucine, threonine, and leucine. Deficiency of one of the essential amino acids can slow growth and reproductive maturity [5]. A. niger and A. oryzae are probiotic fungi that do not produce mycotoxins and are safe to use, can increase crude protein and decrease crude fibre, increasing essential and nonessential amino acid content [5]. Crude fibre is an essential component of feed. Crude fibre content affects the digestibility of feed in ruminants. The lower the crude fibre, the higher the digestibility of the ration. In this study, sago dregs fermentation will be carried the Bacillus, Lactobacillus, Aspergillus consortium to evaluate the potential of probiotics in the consortium to increase the nutritional value and essential amino acids of sago dregs as raw material for cattle feed.

#### MATERIAL AND METHOD

# Microorganism preparation B. licheniformis and B. subtilis

The isolate suspension was prepared in nutrient broth (Oxoid) NB and added as much as 10% into sterile NB and incubated at 37°C until growth reached turbidity McFarland 4  $26.55 \times 10^{13} \, \text{CFU/mL};$ (B.licheniformis: B. subtilis: 22.49×10<sup>13</sup> CFU/mL).

# L. acidophilus and L. plantarum.

The pure isolate was suspended in sterile MRS medium based on the formulation of deMan, Rogosa, and Sharpe, then incubated at 37°C until the turbidity growth reached McFarland 4 (L. acidophilus: 3.95 x 10<sup>10</sup> CFU/mL; L. plantarum: 30.46 x 10<sup>11</sup> CFU/mL).

Aspergillus niger and Aspergillus oryzae grew in potatoes dextrose agar, and then the spore was suspended in sterile physiological NaCl and inoculated into sterilized sago dregs. Furthermore, it fermented for 48-72 hours at 25°C, the number of cell counts using the TPC technique (cell count A. niger: 2.52 x 10<sup>4</sup> CFU/g; A. oryzae:  $2.4 \times 10^3$  CFU/g).

### Sago dregs fermentation

The sterilized sago dregs were mixed into the starter culture. Prepared of consortium 1, 10% starter cultures of B. licheniformis, L. acidophilus and A. niger, Consortium 2

added 10% starter culture of B. subtilis. L. Plantarum and A. oryzae, and consortium 3 added 10% starter cultures licheniformis, B. subtilis, L. acidophilus, L. plantarum, A. niger and A. oryzae and each were added to sago dregs. The sago dregs that have been inoculated are stirred, then fermented for ten days at room temperaturethe growth rate of bacteria and fungi counted during fermentation.

### Nutritional analysis

Analysis of nutritional value includes crude protein content, crude fibre, amino acid content, and content analysed by HPLC, vitamin B2 by LC-MS/MS method.

It is recommended to include in the paper those data that were achieved using methods which comply with all biological, analytical and statistical techniques. If the data are issued from the usage of some new methods or from others that supposed modifications of the standard procedures, they must be mentioned. The biological material presentation must include the following elements: species, breed, gender, body weight, age or age category. There will be also specified the conditions existing during data sampling, surgical techniques, measurements and statistical models, all clearly and briefly described, in accordance with the reference literature conventions.

## RESULTS AND DISCUSSIONS

# Growth of bacteria and fungi consortium during sago dregs fermentation.

The success strongly influences the success of the fermentation process in optimizing the desired microbial growth factors. Growth in each consortium showed that the growth of the genus Bacillus was higher than that of the genus Lactobacillus and fungi and reached the peak of the logarithmic phase from the fifth (Figures 1, 2 & 3).

The growth curve was used to determine the rate of cell growth and the effect of the fermentation substrate on the growth rate. In consortium 1, the number of cells at the peak of the logarithmic phase in Bacillus was  $34.27 \times 10^{19} \text{ CFU/ml}$ , Lactobacillus 15.97 x 10<sup>15</sup> CFU/ml, and in Aspergillus 32.33 x 10<sup>13</sup> CFU/g.

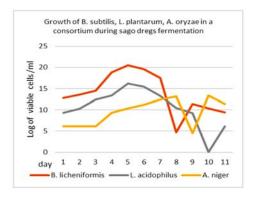


Fig. 1 Growth curve of B. licheniformis, L. acidophilus, A. niger in consortium on sago dregs fermentation

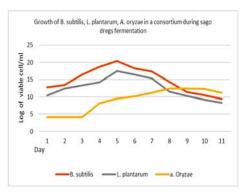


Fig. 2 Growth curve of B. subtilis, L. plantarum, A. oryzae in consortium on sago dregs fermentation

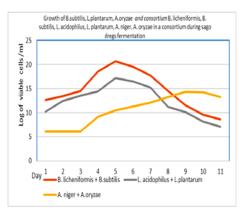


Fig. 3 Growth curve of B. licheniformis, Lacidophilus, A. niger, B. subtilis, L. plantarum, A. oryzae in consortium on sago dregs fermentation



In consortium 2, the number of cells at Bacillus 23.94 x 10<sup>19</sup> CFU/ml, Lactobacillus 32.85 x 1016 CFU/ml, and Aspergillus 29.56 x 10<sup>11</sup> CFU/g. In consortium 3, the number of cells in Bacillus was 51.14 x 10<sup>18</sup> CFU/ml, Lactobacillus 17.18 x 1016 CFU/ml, and Aspergillus 22.84 x 10<sup>13</sup> CFU/g.

The high cell growth during fermentation of all isolates in sago, especially Bacillus subtilis and Bacillus licheniformis, indicated that the strain could use sago dregs as nutrition and produce high cellulase and amylase enzymes.

B. licheniformis and B. subtilis strains produced several enzymes that can mediate several activities, degrade several substrates, and thrive under diverse conditions [21]. In addition, B. subtilis and B. licheniformis exhibits strong cellulolytic and proteolytic activities, which confer them the ability to strongly degrade food wastes [15]. On the curve, Bacillus and Lactobacillus reach the logarithmic phase within four days. Harris et al. [8] stated that the growth of starter cultures B. subtilis and B. licheniformis reached a logarithmic phase on day 4. Likewise, according to Usmiati and Juniawati curd [23], milk fermentation by L. acidophilus and L. plantarum experienced a phase logarithmic on day 4. In contrast, Aspergillus takes seven days to reach the logarithmic phase. According to Augustine [2], A. niger and A. oryzae were at the peak of the logarithmic phase on the 8th day of wheat bran fermentation. In the process of fermentation, the chemical composition of the medium changes because nutrients will be consumed and metabolites will be produced, so that the environment is in a steady state. Cells will divide at a constant rate, cell mass increases and cell growth is in a balanced state in the logarithmic phase.

#### Crude protein

Protein is a macronutrient that plays a role important in forming biomolecules from the source energy, but the protein acts as a source of energy when needed.

Protein is one of the macronutrient groups, where protein plays an important role in forming biomolecules rather than an energy source. Protein can also act as an

energy source when needed. Crude protein content during fermentation can be seen in table 1.

Results of Analysis of (ANOVA) and Duncan's Multiple Range Test (MRT) fermented Sago dregs by all consortium could increase crude protein content for 4 and 8 days compared to protein content without fermentation. Many studies have shown that fermentation increased crude protein content and decreased crude fibre content. According to Hilakore et al. [9], increased protein in the fermentation process is due to an increase in cell mass, which contributes to an increase in protein donated by cells due to their growth. The increased protein also may be attributed to the higher biomass of all species as single cell protein.

The highest protein increase of 12.63% with a percentage increase of 278.14% was produced by consortium 2 (B. subtilis, L. plantarum, A. oryzae) within four days of fermentation. L. plantarum, L. acidophilus, A. niger, A. oryzae in the consortium are proteolytic microbes that can increase crude protein. Mumpuni [17] stated that L. acidophilus and L. plantarum could increase protein because of protease enzymes that can break down proteins into amino acids and simple peptides. A. oryzae was superior in increasing crude protein compared to A. niger in palm kernel cake fermentation [19]. The increase in protein content makes agricultural waste a more desirable ingredient for food or feed applications. Sago dregs contain 3.34 percent protein. It also means that sago dregs are low in amino acid content, so they do not qualify as feedstuff and need improvement in their nutritional quality and an increase in crude protein low in crude fibre. The well-growth mould is expected to produce plenty of cellulose enzymes to degrade and decrease crude fibre. Aspergillus niger can produce cellulase, xylanase, glucanase, and protease activity in neutral and acidic conditions. A. niger and A. oryzae are two filamentous fungi widely used in industry to produce various enzymes (e.g., pectinases, amylases) and metabolites (e.g., citric acid) [4]. Fermentation is the process of breaking down organic compounds into

simple ones involving microorganisms. The fermentation process can increase food substances such as protein and energy can also increase the nutritional value of lowquality feed, function in preserving feed and one way to remove antinutrients or toxins contained in feed.

Table 1 Crude protein levels in Consortium 1, Consortium 2, and Consortium 3 during fermentation

Parameter	Consortium type	Days to-		
		0(CN)	4	8
Crude	Consortium 1	3.34ª	10.61°	9.61 <sup>b</sup>
Protein (%)	B. licheniformis, L. acidophilus, A. niger	A	A	A
	Consortium 2	3.34ª	12.63°	11.65 <sup>b</sup>
	B. subtilis, L. plantarum, A. oryzae	Α	С	С
	Consortium 3			
	B. licheniformis, B. subtilis,	3.34ª	11.61°	10.6 <sup>b</sup>
	L. acidophilus, L. plantarum, A. niger,	Α	В	В
	A. oryzae			

Description: The average value of crude protein content followed by the same ABC letter (vertical direction) and the same ABC letter (horizontal direction) showed no different values

### Crude fibre content in sago dregs fermentation

Crude fibre is part of the carbohydrates separated from the nitrogen-free extract (NFE), consisting of cellulose, hemicellulose, and lignin. Crude fibre is an important

component of feed. Crude fibre content affects the digestibility of feed in ruminants. The lower the crude fibre, the higher the digestibility of the ration.

Table 2 Crude fibre levels in Consortium 1, Consortium 2, and Consortium 3 during fermentation

Parameter	Consortium type	Days to-		
		0(CN)	4	8
Crude Fibre (%)	Consortium 1 B. licheniformis, L. acidophilus,	42.08 <sup>b</sup>	14.39ª	10.88 <sup>b</sup>
(70)	A. niger	Α	Α	Α
	Consortium 2	42.08 <sup>b</sup>	16.23ª	12.3ª
	B. subtilis, L. plantarum, A. oryzae	Α	С	С
	Consortium 3 B. licheniformis, B. subtilis,	42.08 <sup>b</sup>	15.74ª	11.75ª
	L. acidophilus, L. plantarum, A. niger, A. oryzae	Α	В	В

Description: The average value of crude protein content followed by the same ABC letter (vertical direction) and the same ABC letter (horizontal direction) showed no different values

Table 3 The percentage decrease in crude fibre content in fermentation for 4 days and 8 days

Consolitium tumo	Decrease in crude fibre (%)		
Consortium type	4th day	8th day	
Consortium 1  B. licheniformis, L. acidophilus, A. niger	65.8%	74.14%	
Consortium 2 B. subtilis, L. plantarum, A. oryzae	61.43%	70.77%	
Consortium 3 B. licheniformis, B. subtilis, L. acidophilus, L. plantarum, A. niger, A. oryzae	62.5%	72.08%	

The results of the measurement of crude fibre content (Table 2.) showed that all types of the consortium could reduce the crude fibre content of sago dregs with a decrease ranging from 61-65% for four days and reached 74% for eight days. In a consortium consisting of B. licheniformis, L. acidophilus, A. niger, the decrease reached 74 percent with a crude fibre 10.88%. content of In comparison, fermentation by consortium 2 consisting of B. subtilis, L. plantarum, and A. oryzae decreased 61% with a crude fibre content of 16.23%. Consortium 2 has almost fulfilled the nutritional content as feedstuff, namely crude fibre between 12.17% and crude protein at least 13%, as it is known that the crude fibre content is crucial for the digestibility requirements of feed ingredients. Fermentation of sago dregs by probiotic microorganisms and mix culture is crucial to accelerate the decrease in crude fibre content and increase in crude protein content. According to Gelber et al. [4], co-cultivation both of A. niger and A. orvzae showed an equal distribution of the two strains forming mixed colonies with a broad carbohydrate-active range of enzymes produced. A. niger and A.oryzae produce enzymes, including amylase, proteases, lipases, and cellulases. While the genus Bacillus produces many enzymes such as amylase, proteinase, mannase, catalase, cellulose. Also acknowledged, Bacillus spp. is fast-growing bacterium with strong extracellular enzymes that show different effects on hydrolysing organic compounds and decomposing of the food wastes facilitated by the degradative enzyme activity [22].

## Production of essential amino acids in sago dregs during fermentation

Amino acids are the main components of protein, classified into two categories, namely essential and non-essential amino acids. Essential amino acids are amino acids that cannot be synthesized by animals and must be provided through feed intake. Essential amino acids include phenylalanine (Phe), valine (Val), threonine (Tre), tryptophan (Trp), isoleucine (Iso), methionine (Met), histidine (His), arginine (Arg), leucine (Leu) and lysine (Lys). Non-essential amino acids are amino acids that can be synthesized in the body [9]. essential amino acid components important for cattle feed are methionine, lysine, isoleucine, threonine, and leucine. The

deficiency of any of these amino acids results in growth retardation and delayed reproductive maturity [5]. Table 4. shows that the fermentation of sago dregs by various probiotic species in the all consortium can increase the amino acid content phenylalanine, valine, threonine, isoleucine, leucine, and lysine on day four and day eight fermentation compared to amino acid content in without fermentation. Bacillus subtilis, B. licheniformis, L. acidophilus, L. plantarum, A. niger and A. orvzae are cellulolytic. proteolytic, amylolytic, and lipolytic microorganisms capable of using sago dregs as nutritional ingredients for cell growth, energy, and the formation of various amino other bioactive compounds. acids and perform Microorganisms catabolic anabolic functions, break down complex compounds, and synthesize various amino acid complex vitamins and other growth factors.

Fermentation of sago dregs with a fermentation time of 4 days by a consortium of Bacillus subtilis, L. plantarum, A. oryzae can amino acid levels increase the phenylalanine, valine, threonine, isoleucine, leucine and lysine. According to Kumitch et al. [11] A. oryzae was superior in increasing amino acids than A. niger in Pea Protein-Enriched Flour (PPEF) fermentation.

On the 8th day for all consortiums, content amino acids phenylalanine, valine. threonine, isoleucine, leucine and lysine decreased compared to the 4th day. This decrease was in line with the growth of bacteria that entered the stationary phase and the death phase. The reduction in amino acid levels is because amino acids are essential and are needed by microbes to support their growth and synthesis of various other bioactive peptide.

Vitamin B2 content

Cattle need vitamin B2 (riboflavin) to efficiently use nutrients, antioxidant systems, growth, and reproduction. Vitamin B2 deficiency in cattle can cause poor growth, short bones, dermatitis, lesions at the corners of the mouth, photophobia, cataracts, anaemia, foetal death, reduced fertility, and diarrhoea [6]. In the fermentation of sago dreg by the consortium, vitamin B2 (riboflavin) was not detected either on the sago substrate or during fermentation.

Table 4 Types and levels of essential amino acids in the fermentation of Sago dregs by Probiotics

Amino Acid (mg/kg)	Consortium Type	Days to-		
		0	4	8
Phenylalanine (Phe)	Consortium 1 B. licheniformis, L. acidophilus, A. niger	236.18 <sup>a</sup>	314.59°	306.55 <sup>b</sup>
	Consortium 2 B. subtilis, L. plantarum, A. oryzae	236.18 <sup>a</sup>	<b>351.33</b> °	329.96 <sup>b</sup>
	Consortium 3 B. licheniformis, B. subtilis, L. acidophilus, L. plantarum, A. niger, A. oryzae	236.18ª	335.55°	313.65 <sup>b</sup>
Valine (Val)	Consortium 1 B. licheniformis, L. acidophilus, A. niger	386.73 <sup>a</sup>	433.44 <sup>b</sup>	399.99ª A
	Consortium 2 B. subtilis, L. plantarum, A. oryzae	386.73 <sup>a</sup>	<b>471.05</b> °	415.88 <sup>b</sup>
	Consortium 3  B. licheniformis, B. subtilis, L. acidophilus, L. plantarum, A. niger, A. oryzae	386.73 <sup>a</sup>	456.9°	408.59 <sup>b</sup>
Threonine (Tre)	Consortium 1  B. licheniformis, L. acidophilus, A. niger	388.85 <sup>a</sup>	413.85°	403.98 <sup>b</sup>
()	Consortium 2 B. subtilis, L. plantarum, A. oryzae	388.85 <sup>a</sup>	470.07°	456.18 <sup>b</sup>
	Consortium 3  B. licheniformis, B. subtilis, L. acidophilus, L. plantarum, A. niger, A. oryzae	388.85 <sup>a</sup>	448.04° B	428.41 <sup>b</sup>
Isoleucine (Ile)	Consortium 1 B. licheniformis, L. acidophilus, A. niger	262.44ª	337.05°	311.35 <sup>b</sup>
	Consortium 2 B. subtilis, L. plantarum, A. oryzae	262.44 <sup>a</sup>	<b>361.11</b> °	338.42 <sup>b</sup>
	Consortium 3  B. licheniformis, B. subtilis, L. acidophilus, L. plantarum, A. niger, A. oryzae	262.44ª	317.93°	294.13 <sup>b</sup>
Arginine (Arg)	Consortium 1  B. licheniformis, L. acidophilus, A. niger  Consortium 2	263.86 <sup>b</sup>	249.95 <sup>a</sup>	250.51 <sup>a</sup>
	B. subtilis, L. plantarum, A. oryzae	263.86 <sup>a</sup>	261.58 <sup>a</sup> c	259.55° B
	Consortium 3 B. licheniformis, B. subtilis, L. acidophilus, L. plantarum, A. niger, A. oryzae	263.86ª	254.05 <sup>a</sup>	257.84ª B
Leucine (Leu)	Consortium 1 B. licheniformis, L. acidophilus, A. niger	466,06 <sup>a</sup>	584,02°	535,87 <sup>b</sup>
	Consortium 2 B. subtilis, L. plantarum, A. oryzae	466,06 <sup>a</sup>	619,64°	557,04 <sup>b</sup> c
	Consortium 3 B. licheniformis, B. subtilis, L. acidophilus, L. plantarum, A. niger, A. oryzae	466,06ª A	599,42°	520,87 <sup>b</sup>
Lysine (Lys)	Consortium 1 B. licheniformis, L. acidophilus, A. niger	450,84 <sup>a</sup>	708,39° A	620,21 <sup>b</sup>
	Consortium 2 B. subtilis, L. plantarum, A. oryzae	450,84ª A	<b>786,62</b> °	692,18 <sup>b</sup>
	Consortium 3 B. licheniformis, B. subtilis, L. acidophilus, L. plantarum, A. niger, A. oryzae	450,84 <sup>a</sup>	758,64° <sub>B</sub>	648,17 <sup>b</sup>
Histidine (Hys)	Consortium 1 B. licheniformis, L. acidophilus, A. niger	ND	ND	ND
	Consortium 2 B. subtilis, L. plantarum, A. oryzae	ND	ND	ND
	Consortium 3 B. licheniformis, B. subtilis, L. acidophilus, L. plantarum, A. niger, A. oryzae	ND	ND	ND

#### CONCLUSIONS

Fermentation of sago dreg by consortium B. subtilis, L. plantarum, A. oryzae for four days decreased crude fibre, increased protein by 12.63%, and increased the amino acid content of Phenylalanine (48.75%), Valine (21.8%), Threonine (20.88%), Isoleucine (37.59%), Leucine (32.95%), and Lysine (74.4%).

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