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Characterization and determination of hydrolysis process parameters of superior sweet starch CIP-types enzymatically

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Abstract

Indonesia is rich in starch raw materials, but until now is still importing sugar and modified starch for food and non-food industrial raw materials. One of the reasons is the limited information regarding the feasibility of starches as industrial raw materials. Based on the whiteness and the high content of starch, the research institution of *Centro International de La Papa* (CIP) in Indonesia has produced five clones of superior sweet potatoes, which are CIP-1, CIP-2, CIP-3, CIP-4 and CIP-5. The feasibility of starch for industrial raw materials is not only seen from the physical aspect but also from the chemical aspect and process parameters. This research aims to study the physical and chemical properties of superior CIP-type and local sweet potato starch, as well as determine the best process parameter to be used in the enzymatic hydrolysis industry. Sweet potato starches that had been tested were five CIP starches and local sweet potato starches, using descriptive statistics method and Completely Randomized Design. The characteristics of physical and chemical properties under the study were degree of whiteness, granule size, amylose and amylopectin levels. The parameter processes tested were temperature and concentration of alpha amylase enzyme with observed variables comprising gelatinization time, reaction speed, dextrose equivalent (DE) and Unit Brabender (UB). According to the results of the research, it was found that starch types CIP-1, CIP-2 and CIP-5 were more qualified to be used for raw materials in the hydrolysis industry than CIP-3, CIP-4 and local sweet potato starch. Meanwhile, the best process parameter for hydrolysis was liquification at a temperature of 85°C and concentration of alpha amylase enzyme of 0.07%.

1. Introduction

The development of agricultural commodities can be carried out optimally if the results of upstream production activities can be utilized by various downstream production activities. Therefore, efforts to increase production, either through intensification or extensification, must be in line with efforts to increase its utilization, both for direct consumption and as a source of raw materials for various industries.

In Indonesia, sweet potato (*Ipomoea batatas* L) is one of the agricultural commodities producing starch in addition to corn, cassava or other starch sources. The initial attention to the development of this plant in the world was begun in 1971, with the establishment of *Centro International de La Papa* (CIP) in La Molina Peru. It has a main objective to increase the production and consumption of these commodities in Andes Mountain, South America. The institute has collected more than 3,500 cultivars and 1,500 wild species of potato, 6,000 cultivars and wild varieties of sweet potato plants and nearly 1,000 other varieties of tubers. In line with this, a branch of CIP was also established in Indonesia, located in Muara-Bogor and so far, it has collected 1,251 clones obtained from various regions. Of these clones, nine superior clones have been produced; superior in the production in which it has white color in its tuber flesh and superior in the percentage in which it has a high amount of dry matter and starch content. Therefore, it is expected to be able to answer the challenges of the starch-based industry. So far, starch used for various food and non-food industrial purposes in the world generally comes from corn, potatoes, cassava and wheat (Tjintokohadi, 2000). During 2020, the demand for starch for the industry in the world is predicted to reach more than 47.3 million tons per year.

Until 2017, there were five biggest countries

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producing sweet potato in the world, that is Nigeria, 47.94 million tons; Ghana, 7.97 million tons; Ivory Coast, 7.15 million tons; Benin, 3.13 million tons; and Ethiopia, 1.4 million tons. Before 1998, there were four countries utilizing sweet potatoes to produce starch, that is, Japan, Taiwan and South Korea. For the past twenty years, China, Philippines and Japan have used sweet potato starch to produce pasta, liquid sugar and modified starch and various other products. Up to date, its need as the main raw material continues to increase because of the development of various food and non-food industries (Oates, 1998).

Indonesia continues to import granulated sugar, this is because the domestic production of crystal sugar made from sugar cane and liquid sugar that uses sweet potato starch as raw material has not been able to meet the needs of consumers and industry. This picture shows that the rate of growth of Indonesia's sugar consumption continues to increase. The same thing happened to the import of modified starch which continued to increase from year to year.

In this regard, it is necessary to conduct various studies on the utilization of various starch sources in Indonesia to be used as raw materials for the food and non-food industries while reducing the need for imports.

The results of the CIP Indonesia study found 9 superior sweet potato clones, 5 of which were CIP-1, CIP-2, CIP-3, CIP-4 and CIP-5 could be used as a source of starch in the food and non-food industries because of their high starch content and their white starch color (Tjintokohadi, 2000). As described by Aboubacar *et al*. (1999), Tang *et al*. (2002), Siswati *et al*. (2007) and Singh *et al*. (2010) that in addition to the starch content and the white flesh color, other important physical properties in the hydrolysis process, such as liquification and saccharification for the production of various food and industrial products, are starch which is easy to dissolve and has a high amylose content, small granules, low temperature required for gel formation as well as low viscosity and content of β-carotene, as well as for the production of modified starch. To date, the properties of starch from various sweet potato varieties in Indonesia are not widely known so its use as an alternative industrial raw material in producing modified starch, liquid sugar, and other products is still finite. Van Beynum and Roels (1985) reported that corn, cassava, and wheat starch which are widely used for the production of liquid sugar have an average amylose content of 28%.

Hydrolysis of starch, such as liquification, saccharification, and manufacture of modified starch can be carried out using enzyme and acid catalysts. The use of enzymes tends to not have an environmental impact and enzymes have a specific way of working, while the use of acids tends to have the opposite effect, the way they work is random and produces saccharides with certain spectra (Ainezzahira *et al.,* 2019). However, the use of an acid catalyst in the process is easier because it is not affected by various factors, such as substrate conditions, high temperatures, and acidic pH. The hydrolysis rate will be faster if the starch granule size is small and uniform and the amorphous part is more dominant than the crystalline part.

Based on these problems, this study was conducted by focusing on the observation of the physical and chemical properties of CIP superior sweet potato starch and the determination of the parameters of the hydrolysis process using an enzyme catalyst.

2. Materials and methods

2.1 Materials and tools

This study was carried out at the Agricultural Product Technology Laboratory, Faculty of Agriculture, Universitas Pattimura, Ambon, Inter-University Central Bioprocess Engineering Laboratory, IPB University and the Biochemistry Laboratory of the Bogor Food Crops Biotechnology Research Institute, from 2018 - 2019. The materials for sweet potato clones of CIP-1, CIP-2, CIP-3, CIP-4 and CIP-5 were obtained from Centro International de La Papa (CIP) Indonesia in Muara Bogor, while local sweet potatoes were obtained from the Bogor market. The sweet potatoes were then processed into starch.

The enzyme used was Alpha Amylase (Termamil LC). The chemicals used in this study were hydrochloric acid (HCl), calcium chloride ($CaCl_2$) and sodium hydroxide (NaOH) as well as other chemicals used for the reaction mixture and analysis were sodium carbonate, sodium bicarbonate, anhydrous sodium sulfate, rochelle salt, cuprum sulfate, ammonium molybdate, sulfuric acid, Antrone reagent, Pb acetate, Pb oxide, sodium hydrogen phosphate, calcium carbonate, sodium oxalate, dinitrosalicylate, sodium potassium tartarate, ethanol, iodine, standard glucose, potassium sulfate, alcohol, methylene blue, methyl red, hydrogen barium oxide, diethyl ether, acetone, ptalein phenol, filter paper number 40 and 41 and various other auxiliary materials.

Tools used in this study were grater, hydraulic press, cabinet drayer, 80 mesh filter cloth, bucket, oven, thermoshaker, UV-Vis Recording Spectrophotometer (Shimadsu Model UV-VIS 200 S), Kjeldahl equipment, Soxhlet, Millipore filter, desiccator, refrigerator, Erlenmeyer, Eppendorf pipette, vacuum pump, centrifuge, vortex, analytical balance, pH meter, microscope (Polarizing Microscope, Olympus BH-2 model PM-10ADS, Japan), kiln, hot plate, burette, condenser, glasses and other auxiliaries.

2.2 Characterization of CIP and local superior sweet potato starch

Characterization of the chemical and physical properties of CIP and local varieties of sweet potato was carried out to determine the feasibility as raw materials for the modified starch or liquid sugar industry by analyzing the amylose content, amylopectin, size-shape and uniformity of starch granules, temperature and time of gelatinization, viscosity of the starch solution and starch proximate analysis. The statistical method used was descriptive.

2.3 Determination of the parameters of the liquification process

The purpose of this stage was to study and find the best hydrolysis process parameters from selected high-yielding sweet potatoes to be used in studying liquification and saccharification patterns or the production process of modified starch, liquid sugar, and other hydrolysis products. The process parameters tested were hydrolysis temperature and alpha amylase concentration with the following treatment levels:

Temperature treatments at 75°C, 80°C, 85°C, 90°C and 95°C. These five temperature treatments were tested to hydrolyze the three best types of sweet potato starch CIP, that is CIP-1, CIP-2 and CIP-5 using an alpha amylase enzyme concentration of 0.03% and a starch substrate concentration of 30%. The hydrolysis process of each treatment lasted for 90 minutes and every 15 minutes a sample was taken to observe the value of Dextrose Equivalent (DE).

Treat the concentration of alpha amylase enzyme, that is 0.03%, 0.05%, 0.07%, 0.09% and 0.11%. Testing the hydrolysis process of the five levels of enzyme treatment using the best CIP sweet potato starch substrate concentration, namely CIP-1, CIP-2 and CIP-5 by 30%. The hydrolysis process of each treatment lasted for 90 minutes and every 15 mins a sample was taken to observe the value of Dextrose Equivalent (DE).

Both treatments were tested using 30% sweet potato starch substrate, pH 5.4, 200 rpm agitation and 5 ppm calcium chloride. The temperature testing stage was first carried out and then the finding of the best temperature was used to test the enzyme concentration. The mathematical model used was a Completely Randomized Single Factor Design with the following model:

$$Y_{ij} = \mu + P_i + \varepsilon_{ij}$$

Where Y_{ij}: the value of the observation on the i-th treatment and j-th repetition, μ: the mean treatment value, P_i: the effect of the i-th temperature treatment or the i-th enzyme concentration on the hydrolysis reaction rate, and ε_{ij}: random effect on the i-th treatment and j-th repetition.

3. Results and discussion

3.1 Characterization of sweet potato starch

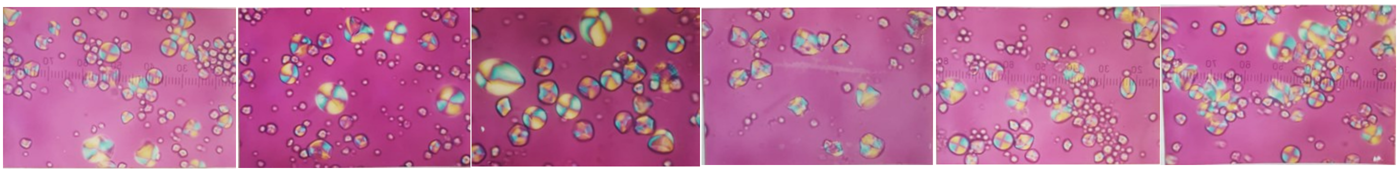
Based on the study of characterization of 5 types of CIP and local superior sweet potato starch, it was concluded that of all physical and chemical properties obtained, the results meeting the requirements as industrial raw materials were CIP-1, CIP-2 and CIP-5.

The results of the complete chemical analysis of CIP and local sweet potato tubers can be seen in Table 1. The data in the table shows that CIP-5 has the highest carbohydrate content (27.51%), followed by CIP-4 (26.94%), CIP -2 (21.30 %), CIP-3 (20.59 %), CIP-1 (15.62%) and local sweet potato (15.06 %). In general, it can be said that the higher the carbohydrate content, the better it is to be used as raw material for hydrolysis products (Singh *et al*., 2010). Amylose and amylopectin levels (%), that is CIP-1 was 32.92/66.08, CIP-2 was 28.04/71.96, CIP-3 was 27.31/72.69, CIP-4 was 28.04/71.96, CIP-5 was 25.84/74.16, and local was 24.37/75.63. The higher the amylose content the better for raw materials for the food industry, such as the production of liquid sugar and modified starch. (Patindol *et al*., 2006; Singh *et al*., 2010).

The results of the analysis of physical properties, such as the whiteness of starch showed that the color of CIP-2 (93.03 %) starch was whiter than CIP-4 (90.44 %), CIP-1 (90.24 %), CIP-5 (87.59 %), CIP- 3 (86.72%) and

Table 1. Proximate analysis of CIP and local superior sweet potato tubers

Varieties	Content (%)					
	Water	Fat	Protein	Ash	Fiber	Carbohydrate
CIP-1	68.00	2.93	0.93	0.67	11.85	15.62
CIP-2	62.00	2.03	0.65	1.22	12.80	21.30
CIP-3	67.00	1.36	0.53	0.97	9.55	20.59
CIP-4	62.30	2.25	0.69	0.74	7.08	26.94
CIP-5	61.40	1.07	1.23	0.89	7.90	27.51
Local Sweet Potato	68.50	0.70	1.80	0.99	12.95	15.06



CIP-1 Granules CIP-2 Granules CIP-3 Granules CIP-4 Granules CIP-5 Granules Local Starch

Figure 1. The shape and size of the superior sweet potato starch granules of CIP and local types (study results)

Table 2. Temperature and time of gelatinization of CIP and local superior sweet potato starch

Plant variety	Initial Temperature (°C)	Initial Time (mins)	Max Temperature (°C)	Max Time (mins)	Brabender Unit (UB)	Carbohydrate
CIP-1	77.0	38.0	95.0	50.0	1,330	15.62
CIP-2	75.0	37.0	94.0	49.0	1,145	21.30
CIP-3	74.0	35.5	88.5	45.0	1,220	20.59
CIP-4	76.5	38.5	87.0	45.5	1,320	26.94
CIP-5	73.5	34.0	85.5	42.0	1,230	27.51
Localization	77.0	42.0	96.0	56.0	1,180	15.06

Note: The initial time is the time when the starch begins to gelatinize, the end time is the time when all starch has undergone gelatinization.

local sweet potato starch (78.01%). The higher the whiteness value of starch, the better it is to be used as industrial raw materials.

The results of the analysis of the average granule size (µm) and standard deviation (S) showed that sweet potato starch CIP-1 (36.67 µm/20.83) was smaller than CIP-5 (37.50 µm/20.85), CIP-4 (38.33 µm/22.18), CIP-2 (42.50 µm/23.94) and CIP-3 (48.33 µm/24.19) (See Figure 1). The smaller the granule size and the smaller the standard deviation value, the better the starch is to be used as raw material for the hydrolysis industry (Tang *et al*., 2002; Cornejo-Ramirez *et al*., 2018).

The results of observations and analysis of temperature and gelatinization time (Table 2) show that CIP-5 sweet potato starch has a lower initial temperature and maximum temperature, and shorter gelatinization time compared to CIP-3, CIP-2, CIP-4 starch and CIP-1. One of the causes is that the granule size of each type of starch is different, and CIP-5 starch has a smaller size and standard deviation so that its development requires less energy.

Hodge and Osman, (1976) and Langenaeken *et al.* (2019) reported that gelatinization of a type of starch has a range of temperature and time. This is due to the differences in starch not only in shape and size but also in terms of the energy required for granule development. The difference in energy occurs because of the different degrees of association in the amorphous part for each type of starch. CIP-2 showed a lower Brabender Unit (UB) (1.145 UB) so the level of viscosity of the solution was low (diluted).

Whistler *et al.* (1984) and Zheng *et al.* (1999) mentioned that the high viscosity of the starch solution is closely related to its molecular weight. CIP-1 starch had a heavier molecular weight because it contained more amylose. The molecular weight of amylose was 50,000–200,000 daltons higher than amylopectin which was only 50,000–100,000 daltons.

Based on the characterization of the physical and chemical properties that had been carried out, it was concluded that the sweet potato starch was more qualified to be used in the test phase to determine the gelatinization temperature and the concentration of the alpha amylase enzyme in the hydrolysis process were CIP-1, CIP-2, and CIP-5.

3.2 Determination of hydrolysis process parameters

3.2.1 Determination of gelatinization temperature for liquification process

From the test results, gelatinization temperature of 85^{o}C tended to give the highest initial reaction rate (Vo) and higher Dextrose Equivalent (DE) compared to other temperature treatments (Figures 2 and 3, Tables 3 and 4).

Data from Tables 3 and 4 also show that CIP-1 and CIP-5 sweet potato starches were more easily hydrolyzed (higher DE) than CIP-2. This was possible because both starches had smaller granule sizes and higher amylose content. In addition to supporting enzyme activity to be optimum, a temperature of 85^{o}C also provides a sufficient amount of energy to the starch molecule so that it can reach the transition level at the peak of the energy limit and then it can be ready to react with the alpha amylase enzyme.

Table 3. The rate of the liquification reaction of the alpha amylase enzyme at various temperatures of the process

Temperature of the Process	Initial Reaction Rate, Vo (DE/Min)		
	CIP-1	CIP-2	CIP-5
75^{o}C	0.522	0.396	0.419
80^{o}C	0.601	0.436	0.503
85^{o}C	0.645	0.481	0.622
90^{o}C	0.612	0.476	0.622
95^{o}C	0.615	0.466	0.628

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Whitaker (1984) and Blazek and Gilbert (2010) reported that the rate of each chemical reaction is proportional to the concentration of the compound in the transition state. The higher the transition concentration, the higher the chemical reaction is.

Table 4. Duncan's different test of average DE (%) results of determination of temperature parameters in the liquification process of selected CIP sweet potato starch

Temperature of the Process	Average DE Value (%)		
	CIP-1	CIP-2	CIP-5
75^{o}C	15.71^{a}	13.77^{a}	15.01^{a}
80^{o}C	16.27^{b}	14.60^{b}	15.75^{b}
85^{o}C	16.94^{d}	15.07^{c}	16.53^{c}
90^{o}C	16.50^{c}	14.90^{bc}	16.38^{c}
95^{o}C	16.48b^{c}	15.04^{c}	16.31^{c}

Values with different superscripts within the same column are significantly different at α = 0.05% level.

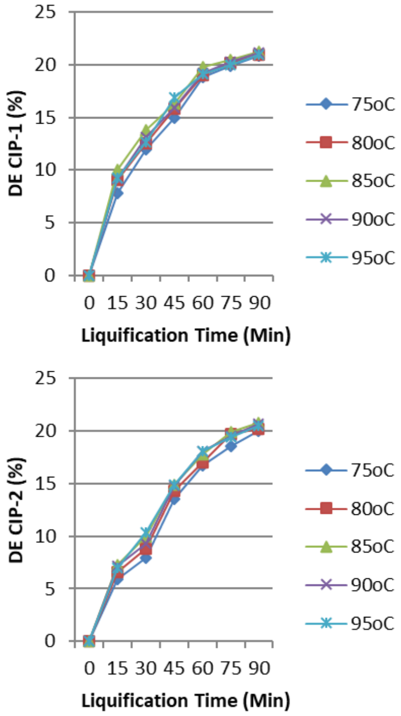

Figure 2. Graph of the relationship of DE value and liquification time of superior sweet potato starch of CIP-1 and CIP-2 with alpha amylase enzyme at various levels of temperature treatment (°C)

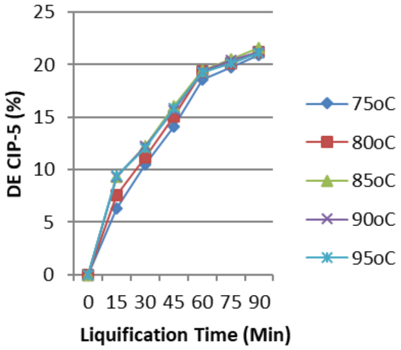

Figure 3. Graph of the relationship of DE value and liquification time of superior sweet potato starch of CIP-5 with alpha amylase enzyme at various levels of temperature treatment (°C)

3.2.2 Determination of alpha amylase enzyme concentration for liquification process

The results of the test using the best gelatinization temperature of 85°C (the results of the study of temperature treatment) with a substrate concentration of 30%, indicated that the best enzyme concentration is 0.07%. Below this concentration, the number of enzymes becomes the limiting factor. The concentration exceeding 0.07% tends to no longer increase the reaction rate because the liquification has reached the equilibrium limit point or the reaction has reached its maximum rate (Figures 4 and 5 and Tables 5 and 6).

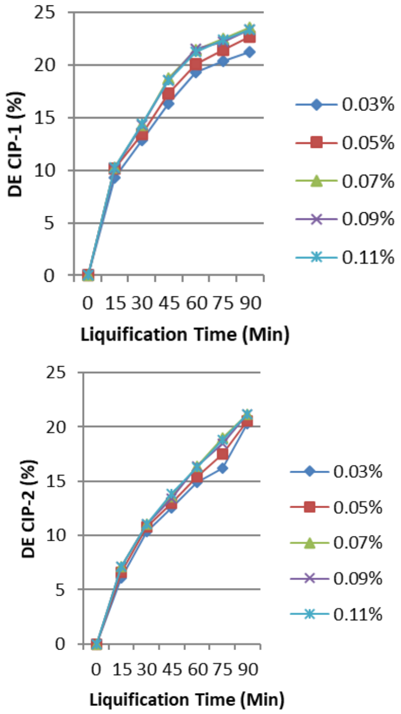

Figure 4. Graph of relationship of DE value and level of alpha amylase enzyme concentration during CIP-1 and CIP-2 superior sweet potato starch liquification process

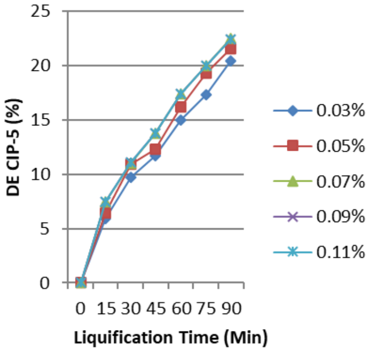

Figure 5. Graph of relationship of DE value and level of alpha amylase enzyme concentration during CIP-5 superior sweet potato starch liquification process

The data indicated that CIP-1 sweet potato starch was easier to hydrolyze than CIP-5 and CIP-2 starch, as well as CIP-5 starch to CIP-2.

In the hydrolysis process, the determination of the

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enzyme concentration is one of the critical stages of the process. This is because the use of low enzyme concentrations will result in low production (under conversion). On the other hand, the use of high enzyme concentrations will result in repolymerization in the product, such as the formation of other polymers or colored compounds such as 5-hydroxymethylfurfural or levulinic acid and a bitter taste, this condition is called over conversion (Johnson, 1979; Whitaker, 1994; Oktaviyani, 2012; Almeida *et al*., 2019; Das and Kayastha, 2019).

Table 5. The rate of liquification reaction of superior sweet potato starch with CIP type at various levels of alpha amylase enzyme concentration

Enzyme Concentration (%)	Initial Reaction Rate, Vo (DE/Min)		
	CIP-1	CIP-2	CIP-5
0.03	0.618	0.407	0.392
0.05	0.677	0.438	0.427
0.07	0.689	0.477	0.498
0.09	0.676	0.474	0.498
0.11	0.685	0.477	0.498

Table 6. Duncan's different test of average DE (%) results of determination of temperature parameters in the liquification process of selected CIP sweet potato starch

Enzyme Concentration (%)	Average DE Value (%)		
	CIP-1	CIP-2	CIP-5
0.03	16.56^{a}	13.40^{a}	13.33^{a}
0.05	17.53^{b}	13.99^{b}	14.46^{b}
0.07	18.48^{c}	14.72^{d}	15.35^{c}
0.09	18.37^{c}	14.61^{c}	15.35^{c}
0.11	18.38^{c}	14.72^{d}	15.36^{c}

Values with different superscripts within the same column are significantly different at α = 0.05% level.

4. Conclusion

Superior sweet potato starch types CIP-1, CIP-2 and CIP-5, based on the results of the analysis of physical and chemical properties, that is degree of starch whiteness, amylose and amylopectin levels, average granule size and standard deviation, temperature and time of gelatinization and Brabender unit were more qualified to be used as raw materials for the hydrolysis industry compared to CIP-3, CIP-4 and local sweet potato starch. The best process parameters in the hydrolysis of starch substrates with 30% concentration of CIP-1, CIP-2, CIP-3, CIP-4 and CIP-5 were temperature of 85^{0}C and concentration of alpha-amylase enzyme 0.07 %. In the treatment of these process parameters, starches CIP-1, CIP-2 and CIP-5 gave better hydrolysis results, such as higher DE values.

Conflict of interest

The authors declare no conflict of interest.

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