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# Enzymatic liquification pattern of superior sweet potato starch of CIP-type

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**Abstract**

The liquification pattern of each type of starch at the best temperature and enzyme concentration varies, and the physical and chemical properties influence it. The whiter the starch, the smaller and more uniform the size of the granule and the higher amylose level and more amorphous regions, thus it results in a better liquification pattern and feasibility of starch for industrial raw materials. This research aimed to study the best changes in saccharide pattern during the liquification process of superior Centro International de La Papa (CIP)-type sweet potato starch at a temperature of 85°C and the concentration of alpha-amylase enzyme (0.07%) obtained from preliminary research. Hence, the advantages of the resulting hydrolyzate products were compared. The sweet potato starches tested were CIP-1, CIP-2, and CIP-5, tested using the Completely Randomized Design method with two repetitions. The variables observed were DE, dextrin, changes in amylase and amylopectin levels, high saccharide levels, maltotriose, maltose, glucose, and viscosity. The results showed that liquification of CIP-1 sweet potato starch for 225 mins produced better hydrolysate (30.52% DE, 16.28% maltose, 18.70% maltotriose, 1.06% glucose). Meanwhile, 20 mins of liquification resulted in higher dextrin (84.86%). However, the viscosity of CIP-1 was slightly thicker (22 cPs) than CIP-2 (15 cPs) and CIP -5 (15 cPs). The results showed that three types of starch were qualified for raw materials in the advanced hydrolysis industry (DE value was in the range of 20 - 45%), such as saccharification, with the order that CIP-1 was better than CIP-2 and CP-2 was better than CIP-5.

## 1. Introduction

Indonesia is one of the world's countries with diverse plants producing starch potentials, including starches from root crops, cereals, bananas and palms (such as sago). Due to various challenges, the genetic and production potentials that are sufficiently available and can be developed to meet food and non-food needs have not been utilized optimally. One of the challenges is the limited information regarding the feasibility of the hydrolyzate products to be used as industrial raw materials. To date, Indonesia still imports crystal sugar, liquid sugar, and various modified starches, such as dextrin, maltodextrin and gelatin. Indonesia imports more than 3.4 million tons per year of gelatin for Marshmallow products raw materials (Ainezzahira *et al*., 2019; BPS, 2020).

From the results of the preliminary research focusing on the study of the characterization of physical and chemical properties, as well as the parameters determination of the hydrolysis process of superior CIP (Centro International de La Papa) type sweet potato starch, it was observed that sweet potato starch CIP-1, CIP-2, and CIP-5, at a temperature of 85°C and concentration of 0.07% alpha-amylase enzyme is the best to be used in hydrolysis industry, both for food and non-food industries. Based on these findings, this research focuses on the "Enzymatic Liquification Pattern of Superior Sweet Potato Starch from the CIP Type" and uses the alpha-amylase enzyme. Alpha-amylase was used in this study because it is widely used in hydrolysis. Its hydrolysis results are impeccable (not partial), and the final product is generally glucose (Whitaker, 1984; Oktaviyani, 2012; Ainezzahira *et al*., 2019).

During the liquification process, the saccharide changes pattern of each type of CIP starch tested was studied. The changes in the starch structure consisting of amylose and amylopectin fractions were profoundly observed because straight chains or branched ones will

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break up into shorter chains. These changes indicate the formation of chains containing simple sugars that compose the starch. It is necessary to observe these changes based on the increase in the concentration of reducing sugars and the decrease in the degree of polymerization. The product in the form of starch hydrolysate is a starch derivative that can be utilized in various products, depending on the degree of polymerization, dextrose equivalent, and hydrolysis. By understanding the changes pattern of saccharides at each liquification time, the time to produce a hydrolyzed starch, including functional starch, can be determined effectively and efficiently (Van Beynum *et al*., 1985; Ainezzahira *et al.,* 2019).

It is necessary to conduct a hydrolysis study at the liquification phase of superior sweet potato starch type CIP-1, CIP-2, and CIP-5 in Indonesia. This study can obtain important information, such as the levels of Dextrose Equivalent (DE), dextrin, changes in amylose and amylopectin, maltose, maltotriose, glucose, high saccharides, and the resulting viscosity. This information is needed to utilize superior sweet potato starch for further hydrolyses, such as saccharification. High production of CIP sweet potato reaching 16 – 21 tons per ha and dry matter content of 32 – 38.6% with superior hydrolysate products is predicted to have the potential to become a source of carbohydrates in the future, both as raw material for food and non-food industries (Oates, 1998; Tjintokohadi, 2000).

Based on these problems, this study focuses on the liquification pattern of superior sweet potato starch type CIP-1, CIP-2 and CIP-5 using alpha-amylase enzyme catalysts with the best process parameters from the preliminary research.

To study the pattern of saccharide changes during the liquification process of superior sweet potato starch type CIP-1, CIP-2, and CIP-5 at 85$^{o}$C and with the best alpha-amylase enzyme concentration (0.07%) obtained from the results of the preliminary research. To identify yield advantages and utilization opportunities for the production of various hydrolysate products, such as modified starch and liquid sugar.

## 2. Materials and methods

### *2.1 Materials*

This study was carried out at the Agricultural Products Technology Laboratory, Faculty of Agriculture, Pattimura University, Ambon, Inter-University Central Bioprocess Engineering Laboratory, IPB University, and Biochemistry Laboratory of Food Crops Biotechnology Research Institute, Bogor in 2019. The superior sweet potato used was type CIP-1, CIP-2, and CIP-5 obtained from Centro International de La Papa (CIP) Indonesia in Muara Bogor. The sweet potatoes were then processed into starch. The enzyme used was Alpha-Amylase (Termamil LC). The chemicals used were hydrochloric acid (HCl), calcium chloride ($CaCl_2$), sodium hydroxide (NaOH), and other chemicals for reaction mixture and analysis, consisting of sodium carbonate, sodium bicarbonate, anhydrous sodium sulfate, Rochelle salt, cuprum sulfate, ammonium molybdate, sulfuric acid, Anthrone reagent, Pb acetate, Pb oxide, sodium hydrogen phosphate, calcium carbonate, sodium oxalate, dinitrosalicylate sodium potassium tartrate, ethanol, iodine, standard glucose, standard maltose (from Zigma), standard maltotriose (from Zigma), potassium sulfate, alcohol, methylene blue, methyl red, hydrogen barium oxide, diethyl ether, acetone, phenolphthalein, filter paper number 40 and 41 and other supporting materials.

### *2.2 Tools*

Tools used were grater, hydraulic press, cabinet dryer, 80 mesh filter cloth, bucket, oven, Thermo shaker, UV-Vis Recording Spectrophotometer (Shimadzu Model UV-VIS 200 S), HPLC by ICI brand, Kjeldahl equipment, Soxhlet, Millipore filter, desiccator, refrigerator, Erlenmeyer, Eppendorf pipettes, vacuum pumps, centrifuges, vortex, analytical balances, pH meters, furnace, hot plate, burette, back cooler, glassware, and other supporting tools.

### *2.3 Methods*

The statistical method used in this research was the Completely Randomized Design, with two repetitions. The best process parameters to study the liquification pattern of 3 types of superior sweet potato starch of CIP-1, CIP-2, and CIP-3 were gelatinization at a temperature of 85$^{o}$C, a concentration of 0.07% alpha amylase enzyme, a substrate of 30%, pH 5.4, $CaCl_2$ concentration of 5 ppm and agitation of 200 rpm. The analyzed variable was the hydrolysis reaction rate and compared to the requirements of raw materials, such as the value of DE (Dextrose Equivalent) liquification results that must be within the range of 20 - 45%. If the DE yield obtained at a normal reaction time (approximately 3 hours) is less than 20%, then the starch is classified as unfit to be used for the saccharification process. The liquification process was carried out for 255 mins, and sampling was carried out every 15 mins. The enzyme inactivated each sample by lowering the pH to 3 then heated at 90$^{o}$C for 5 mins. Subsequently, the pH of the sample was raised again to 6 (neutral pH). Prior to the analysis of reducing sugars, the samples were stored in a refrigerator. The variables observed were reducing sugars to determine the value of DE, dextrin levels, amylose, amylopectin, maltose, maltotriose, glucose, high saccharides (polysaccharides)

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and the viscosity of the solution.

## 3. Results and discussion

Figure 1 shows that the DE value reached its maximum at the liquification time of 225 mins. Sweet potato starch CIP-1 resulted in a higher yield (30.53%), followed by CIP-2 (27.78%) and CIP-5 (25.90%). Based on the analysis of variance followed by Duncan's difference test (data in Figure 1), it was found that the DE value of CIP-1 provided a significant difference between CIP-2 and CIP-5. Meanwhile, CIP-2 was significantly different from CIP-5. Furthermore, the changing pattern of starch into dextrin was quadratic and maximum when the liquification time reached 30 mins, with the highest value obtained by CIP-5 was 85.51%, followed by CIP-2 at 84.86% and CIP-2 of 70.71%.

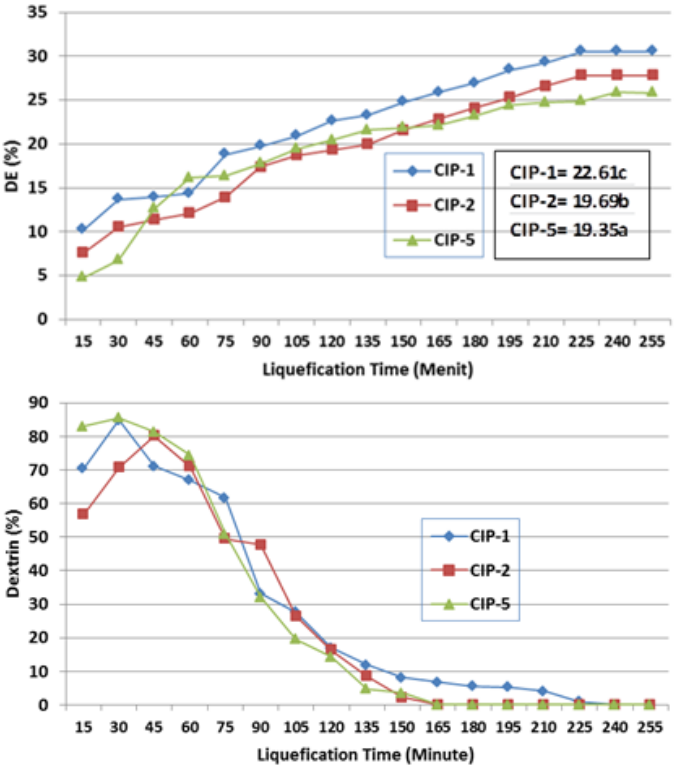


Figure 1. The pattern of changes in DE values and dextrin during liquification of superior CIP-type sweet potato starch using alpha-amylase enzyme

The high DE and dextrin values of CIP-1 and CIP-5 depicted the rate of the initial hydrolysis reaction of the alpha-amylase enzyme, which was higher than that of CIP-2. Based on the preliminary research, CIP-1 had higher amylose content (32.92%) with a smaller and more uniform granule size on average (36.67 µm). Even though CIP-5 had lower amylose content (25.84%) than CIP-2 (28.04%), it had a smaller (37.50 µm) and more uniform granule size (average granule size of CIP-2: 42.50 µm). However, the DE values obtained from these three types of starch meet the requirements as raw materials for the saccharification process, as reported by Johnson (1979) and Whistler *et al.* (1984) of 20 – 45%. The data on the relationship between time and pattern of dextrin changes in the liquification process using alpha-amylase enzyme shows a pattern that can be used to produce dextrin according to the desired types of dextrin; amylodextrin (20 – 30 mins), erythrodextrin (120 – 135 mins) and acrodextrin (hydrolysis time is longer than 135 mins).

During the liquification process, the results of color change measurement through the Lugol test to observe the pattern of changes in dextrin are similar to the results of the observation of the changes in amylose and amylopectin patterns. Figure 2 shows that the amylose and amylopectin levels of sweet potato starch CIP-1, CIP -2, and CIP-5 decreased drastically when the liquification time reached approximately 120 mins. Furthermore, when reaching 195 mins, the hydrolysis of the three starches can be said to reach the maximum point. In the process of enzymatic liquification and saccharification, not all starch can be completely hydrolyzed into saccharides (Whistler *et al*., 1984; Asare *et al*., 2011).

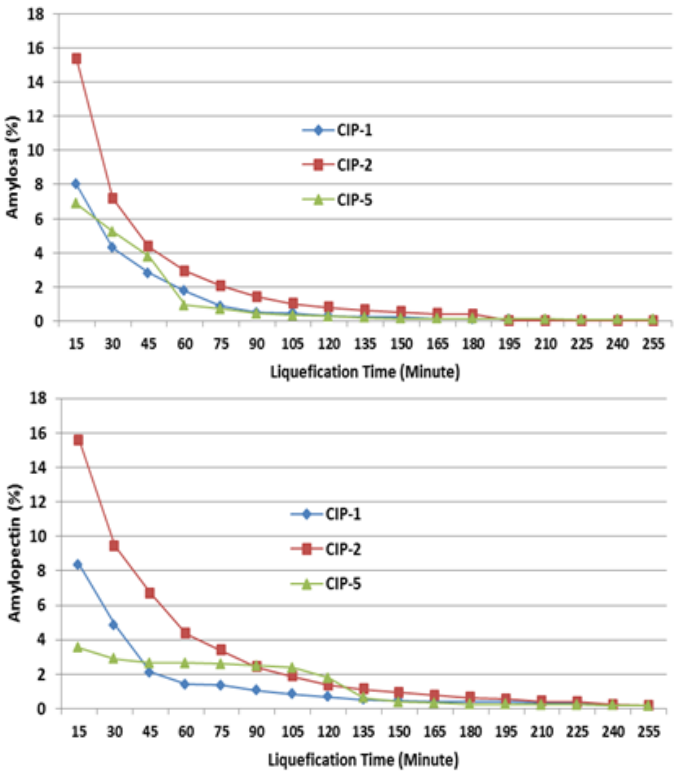


Figure 2. A pattern of changes in amylose and amylopectin of superior CIP-type sweet potato starch during liquification using alpha-amylase enzyme

Hydrolysis of alpha-amylase to amylose can occur in 3 ways (De Wit *et al.,* 1993; Whitaker, 1994): Single change attack: when alpha-amylase attacks one polymer, then degrades it completely and then attacks other polymers, Multi change attack: when amylase attacks one polymer, then degrade it and releases the first product, then attacks the second polymer and releases the second product and continues the same pattern of

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attacking other polymers,

Multiple attacks: when alpha-amylase attacks one polymer and then breaks down the first degradation product several times, then attacks other polymers, and this process continues.

During liquification, the changes pattern of high saccharides (Figure 3) shows an inverse (quadratic) pattern with changes in amylose and amylopectin. It was found that high saccharides were formed maximally when the liquification time was 30-60 mins, then subsequently, gradually decreased. It explains that the peak of high saccharides formation, such as maltotetraose, maltopentose, maltohexose, maltoheptose occurs when the liquification time is 30-60 mins. Then it slowly degrades back into low saccharides, especially maltotriose and maltose. The total high saccharide content reached within the time range was CIP-1: 82.21%, CIP-2: 83.86% and CIP-5: 87.75%.

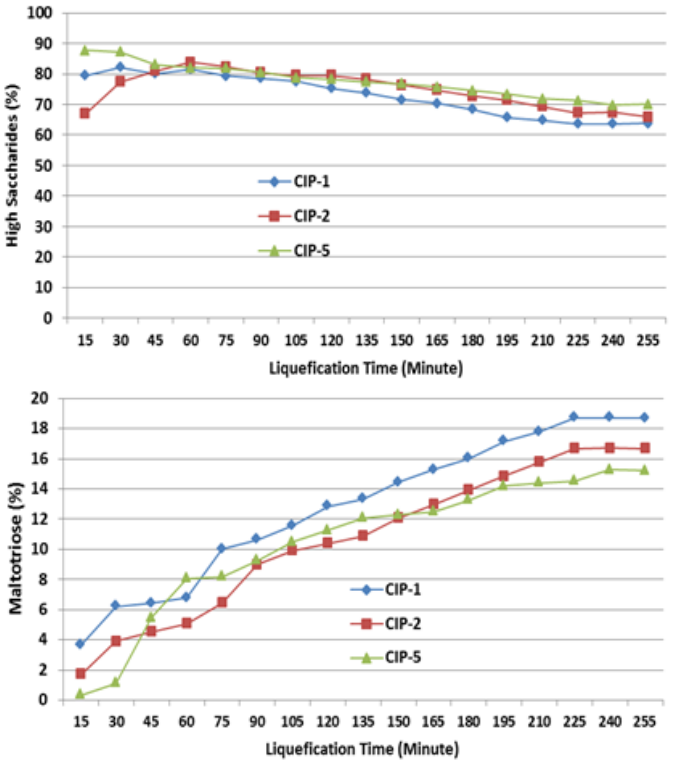


Figure 3. The pattern of changes in high saccharides and maltotriose of superior CIP-type sweet potato starch during liquification using alpha-amylase enzyme

Figures 4 and 5 data also show that maltotriose and maltose formation reached their maximum at a liquification time of 225 mins. The formation tended to stagnate and began to decline afterward. At 225 mins, the highest maltotriose levels were CIP-1: 18.70%, CIP-2: 16.66% and CIP-5: 14.53%, while the highest maltose levels were obtained by CIP-1: 16.28%, CIP-2: 14.73% and CIP-5: 13.02%.

Based on the saccharide composition resulting from the liquification of superior sweet potato starch CIP-1, CIP-2 and CIP-5 by the alpha amylase enzyme, the level of maltotriose and maltose formed tended to be more dominant. According to Whistler *et al.* (1984) and Johnson (1979), it is suitable because the alpha-amylase enzyme in the liquification process functions to break down starch into maltotriose and maltose (Figure 1 of DE value is greater than 25-30%). It can be concluded that the results tend to be higher than that of corn starch.

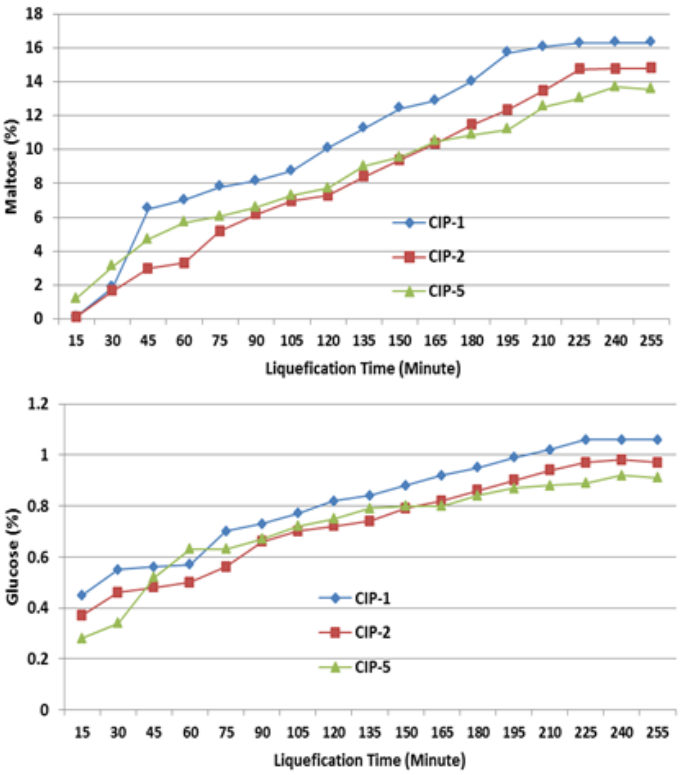


Figure 4. The pattern of changes in maltose and glucose of superior CIP-type sweet potato starch during liquification using alpha-amylase enzyme

In contrast to the pattern of changes in maltotriose and maltose, from the beginning until the liquification for 255 mins, the pattern of changes in glucose showed a very small degree of increase (Figure 4). The increase in the first 15 mins was 0.45%, and after 255 mins, the level only increased by 1.06%. As shown by the research data, the pattern of changes in glucose in the liquification process using an alpha-amylase enzyme is a general pattern, as this enzyme works by cutting the bonds of starch molecules in the form of 2 molecules. Therefore, the most formed products are maltose and maltotriose. Then it was followed by other types of high saccharides, such as maltotetrose, maltopentose, maltoheptose and maltohexos.

The data in Figure 5 shows the viscosity change of the starch substrate solution during the liquification process. During the gelatinization and hydrolysis processes, the starch will lose its birefringent properties, and there will be a drastic change in the viscosity of the solution. The faster the gelatinization of a type of starch

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occurs and the faster it undergoes hydrolysis with a high reaction rate, the more dilute the starch solution is. Figure 5 shows that the change in viscosity of the three superior sweet potato starch solutions was divided into three patterns as follows:

a) The steep negative linear pattern in CIP-1 occurred from the beginning until liquification lasted for 45 mins. CIP-2 lasted up to 120 mins, and CIP-5 lasted up to 90 mins. The viscosity value achieved in this pattern was CIP-1: 27 – 23 cPs, CIP-2: 43.5 – 17 cPs, and CIP-5: 40.5 – 15.5 cPs.
b) Slope negative linear pattern in CIP-1 occurred between the liquification time of 45 – 195 mins. For CIP-2, it was 120 – 135 mins, and for CIP-5, 90 – 105 mins. The viscosity value for this pattern was CIP-1: 23 – 22.05 cPs, CIP-2: 17 – 16 cPs, and CIP-5: 15.5 – 15 cPs.
c) The stationary pattern (flat) in CIP-1 occurred after liquification for longer than 210 mins, CIP-2 for longer than 135 mins, and CIP-5 for longer than 105 mins.

In general, it was found that the superior sweet potato starch of CIP-2 and CIP-5 has a viscosity that tends to be similar and lower (thinner) than CIP-1. CIP-1 starch has a higher amylose content; thus, the molecular weight will be heavier, the glucose content will be higher, and the solution's viscosity will be thicker. The pattern of changes in viscosity as found here shows a difference in the hydrolysis rate over time (Tang *et al.* 2002). According to Satin (2004) and Whistler *et al.* (1984), there are two stages of the mechanism of action of alpha-amylase in starch hydrolysis, that is: (1) rapid degradation stage, the amylose molecule is hydrolyzed to maltose and maltotriose at this stage, which occurs randomly. Meanwhile, in amylopectin, the degradation produces glucose, maltose α-limit dextrin. At this stage, the viscosity of the starch solution decreases rapidly; and (2) slow degradation stage, at this stage, maltotriose and other saccharide molecules are hydrolyzed to maltose and glucose at a very slow rate, and the process is not random.

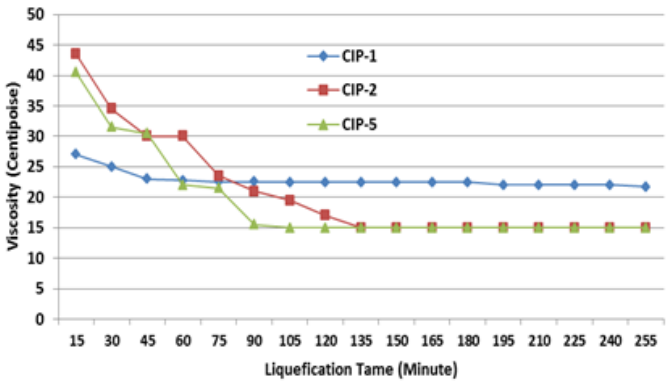


Figure 5. The pattern of viscosity changes of superior sweet potato starch type CIP during liquification using alpha-amylase enzyme

Based on these two reasons, a stationary pattern of changes in viscosity can occur because, during the liquification process, which enters the slow degradation stage, changes in the composition of saccharides tend to decrease over time. Therefore, it also does not significantly affect changes in the viscosity of the solution (Zheng *et al*. 1999; Singh *et al*. 2010 ).

## 4. Conclusion

Data from the findings and discussion concluded that the liquification of superior sweet potato starch type CIP-1 used the best process parameters. The parameter was the concentration of alpha-amylase enzyme of 0.07% and at a temperature of 85°C when the hydrolysis reached 225 mins. This parameter resulted in important variables such as DE (30.52%), maltose (16.28%), maltotriose (18.70%), and acrodextrin, which were higher than CIP-2 and CIP-5. However, the viscosity value of CIP-1 was thicker (22 cPs) than CIP-2 (15 cPs) and CIP-5 (15 cPs).

The variables obtained from the liquification process of the three superior sweet potato starches are overall qualified as industrial raw materials for advanced hydrolysis processes (DE value: 20 – 45%), such as saccharification. The best order is CIP-1, followed by CIP-2, and the last is CIP-5. It requires more advanced study to determine the parameters of the saccharification process.

## Conflict of interest

The authors declare no conflict of interest.

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