The role of indigenous cellulolytic and amylolytic microbes in cassava pulp during the drying process

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Abstract. Cassava pulp is a solid waste resulted from the processing of cassava into tapioca. Cassava pulp is composed of cellulose and residual starch extract. In addition, cassava pulp contains undigested oligosaccharides. During the cassava pulp drying process, various indigenous microbes grow so that it affects the quality of dried cassava pulp. This study aimed to examine the role of indigenous cellulolytic and amylolytic microbes in cassava pulp during the drying process. Indigenous microbes were isolated from cassava pulp from 0 to 15 days using CMC and starch agar to obtain cellulolytic and amylolytic bacteria. Thirty-two types of cellulolytic bacteria and four types of amylolytic bacteria from cassava pulp were selected based on their cellulolytic and amylolytic index. The dominance of cellulolytic bacteria occurred during the drying process (15 days). The highest cellulolytic index obtained was 1.47 and the amylolytic index was 0.86. Selected isolates that had a high index value were then further tested for growth and cellulase enzyme activity. Cellulase produced by isolates of COC1 bacteria had the highest enzyme activity at the 15th hour, which was 0.06 U / mL. Cassava pulp which underwent a drying process for 15 days had crude fiber and carbohydrates changes.

Keywords: Microbes, enzyme activity, cellulolytic, cassava pulp, amylolytic.

1. Introduction
Cassava pulp is solid waste resulted from processing cassava into tapioca flour. According to the Central Bureau of Statistics, cassava production in Indonesia is 19,341,233 tons in 2018. Cassava is widely used as a source of raw materials for the food industry such as tapioca products. In addition, cassava has the potential as a raw material for the ethanol industry because it can able to produce as much as 2,000-7,000 ethanol l/ha/y ethanol [1]. High cassava production has an impact on the high byproducts produced such as solid waste cassava pulp. Agro-industrial solid waste can be used as a feed material for livestock because it does not compete with human needs and it’s cheap [2]. Also, cassava pulp is widely used as a thickening material by the food industry.

Cassava pulp contains low protein and high fiber. The crude protein content of cassava pulp is 1.6-2.5% and has a high energy content of 3000-3500 kcal/kg [3]. This agricultural solid waste also contains cellulose and starch residue from the extraction of cassava processing into tapioca flour. The cellulose component in cassava is 15.63% while starch is 60.10% [4]. Cassava pulp contains undigested oligosaccharides. Microbial in cassava pulp can be used to produce oligosaccharides which...
can be digested by the process of biodegradation and fermentation. The use of fermentation with selected microbial inoculums can increase the nutritional potential of cassava and its by-products both for humans and livestock consumption [5]. The addition of yeast inoculums, lactic acid bacteria, and cellulolytic bacteria as much as 30 mL in cassava fermentation can improve the quality of cassava flour [6]. The cyanide content found in cassava can be reduced by the drying process [7]. The drying process can cause a wet weight of the cassava pulp to decrease. Wet weight reduction can also be caused by the work of indigenous microbes. Indigenous microbes are microbes found naturally in the substrate. Microbial type in cassava pulp fermentation can increase the content of crude protein, dissolved protein, and available hemicellulose. In addition, microbes also play a role in reducing levels of crude fiber, cellulose, lignin, and silicates [3]. The presence of cellulose and starch content in cassava pulp can be a substrate for cellulolytic and amylolytic microbes. Cassava pulp is an ideal substrate for microbial production because of its rich organic properties, easy hydrolysis process, low price, and lack of competition with other industrial uses [4]. Cellulolytic microbes are microbes that can degrade cellulose on the substrate. Starches containing amylase and amylpectin can be degraded by amylolytic microbes. The process of degradation by microbes can increase the nutritional value of cassava pulp.

2. Materials and methods
2.1. Bacterial growth dynamics
Cassava pulp sample was taken from tapioca factory located in Sentul, Bogor. The sample was taken when cassava pulp aged was 0, 3, 6, 9, 12, 15 days and weighed as a source of isolates. Observation of the cassava pulp drying process was carried out on the 0th day and every 3 days for 15 days. Cassava sample was weighed as much as 1 g then added to 9 mL of physiological solution and homogenized using vortex. Then serial dilution was carried out until 10⁶ dilution and each dilution was taken as much as 0.1 mL of solution to be inoculated into solid Carboxymethyl cellulose (CMC) agar media (1 g CMC; 0.02 g MgSO₄·7H₂O; 0.075 g KNO₃; 0.05 g K₂HPO₄; 0.002 g FeSO₄·7H₂O; 0.004 g CaCl₂·2H₂O; 0.2 g yeast extract; 1.5 g bacto agar; and 0.1 g glucose) [8] and starch agar media (peptone 0.05%, KCl 0.01% (w/v); MgSO₄·7H₂O 0.05% (w/v); (NH₄)₂SO₄ 0.01% (w/v); NaH₂PO₄ 0.01%; 2% starch; and 0.8% agar) [9] to observe the amount of bacteria population.

2.2. Characterization and selection of bacterial isolates
The characterization of bacterial isolates was done by observing the morphological characters. Bacteria that had been grown on CMC agar and starch agar media were purified to obtain a single colony with the incubation time of each medium were 24 hours and 48 hours at room temperature. Purified bacterial isolates were observed for morphological characters including color, shape, edge, and elevation. Bacterial isolates were also characterized and selected using cellulase and amylase tests. All bacterial isolates were grown in CMC agar media slope and incubated for 24 hours while bacterial isolates grown in starch agar media slope were incubated for 48 hours. Bacteria that had been grown were taken as much as one ose and placed on agar media in the petri dish and incubated 24 hours and 48 hours severally. After the incubation process, bacteria that had grown on 1% solid CMC media were tested for cellulase activity using 0.1% congo red solution and incubated for 30 minutes at room temperature. The rinsing was carried out using 0.2 M NaCl then the clear zones formation was observed. Bacteria that had grown on 1% starch agar media were tested for amylase activity through the formation of clear zones using 0.02 M iodine solution then incubated for 15 minutes to 30 minutes and the clear zones formed around bacterial colonies was observed.

2.3. Growth curve and cellulase activity curve from selected isolate
The selected isolate that had the highest cellulolytic index value was observed for the growth rates and tested for the enzyme activity. The cellulolytic bacterial isolate was first rejuvenated in 1% CMC agar media. Furthermore, inoculum manufacturing was done by inoculating two ose of cellulolytic bacterial isolate into 50 mL of 1% CMC liquid media. The inoculum was incubated in a shaking incubator at a
speed of 120 rpm until the cell density reached OD 0.6-0.8 which required using a spectrophotometer ($\lambda$ 620 nm). Then as much as 10% of the inoculum was inoculated into 112.5 mL of the cellulase enzyme production media. The culture was incubated in a shaking incubator at 120 rpm at room temperature. Every 3 hours, 5 mL of culture was taken to measure the cell turbidity and enzyme activity.

The crude enzyme extract obtained by centrifuging the culture at 6000 rpm for 20 minutes. The supernatant was separated from its sediment for enzyme activity test. The supernatant as much as 0.5 mL mixed with 0.5 mL of 1% CMC substrate solution in a phosphate buffer pH 7 then incubated at room temperature for 10 minutes. The supernatant mixture with the substrate was reacted with 1 mL dinitro salicylic acid reagent (DNS) then heated in boiling water for 15 minutes. After cooling, the solution absorbance was measured with a spectrophotometer at a wavelength of 540 nm. One unit of activity is based on international standards determined by the amount of enzyme that produces 1 $\mu$molecule in one minute. Enzyme activity measurement was carried out simultaneously by monitoring the growth rate by taking 1 mL of culture and then cell density was measured using a spectrophotometer ($\lambda$ 620 nm). The number of bacterial colonies can be determined by using the method of spread / total plate count (TPC) at dilutions of $10^{-6}$ and $10^{-7}$.

2.4 The effect of the drying process on the characterization of cassava pulp

The nutritional changes in dried cassava pulp were observed by doing proximate analysis. Proximate analysis test consists of the analysis of water content, ash, protein, crude fat, crude fiber, and carbohydrates by difference. In addition to proximate analysis, changes in the morphological structure of starch and fiber during the drying process were observed using a SEM microscope.

3. Results

3.1 Bacterial growth dynamics

The dynamics of bacterial growth during the cassava pulp drying process can be determined by calculating the number of microbes using the spread method. Reduction of moisture content in cassava pulp occurred during 15 days of cassava pulp drying process. Moisture content on wet day 0 was obtained 77.42% and on the 15th day was 27.26% (figure 1).

![Figure 1. Moisture content in wet cassava pulp for 15 days of drying.](image)

Based on figure 2, it can be seen that the number of cellulolytic and amylolytic bacteria on the 15th day was 5.233 log cells and 4.330 log cells while on the 0th day was 2.003 log cells and 1.181 log cells. The results of this study also showed that there were more cellulolytic bacteria compared to amylolytic bacteria.
3.2 Characterization and selection of bacterial isolates

Morphological characters can be used as a distinguishing identity between a bacterium with other bacteria. Morphological characters observed were color, shape, edge, and elevation. Cellulolytic isolates were obtained; amounting to 32 isolates while amylolytic isolates amounted to 4 isolates with different morphological characters (table 1 and table 2). It can be seen that the dominant color found in cellulolytic bacteria and amylolytic bacteria was white and yellowish-white. Other morphological characters such as shapes, edges, and elevations were found to be varied.

Bacterial isolates were further tested qualitatively by observing the clear zones formed. The cellulolytic and amylolytic index can be known from the comparison of colony diameter and clear zone diameters. The number of cellulolytic isolates that have the ability to degrading cellulose was 22 isolates. Amylolytic isolates that can able to degrade starch were 2 isolates. The isolate which has the highest cellulolytic index value was then tested quantitatively by measuring its enzyme activity. Cellulolytic bacterial isolate which had the highest index value was COC1 of 1.47 while the highest amylolytic index value of 0.86 from COS1 isolate (table 3). COC1 cellulolytic isolate and COS1 amylolytic isolate formed clear zones (figure 3).

3.3 Growth curve and cellulase activity curve from selected isolate

The quantitative test was carried out by measuring the cellulase activity of selected isolates. Observation of cellulolytic bacterial growth was carried out to determine the harvest time of the enzymes produced. Measurements of growth rate and enzyme activity were carried out simultaneously for 27 hours with an interval of 3 hours. The growth curve of the COC1 isolate was presented along with the enzyme activity curve (figure 4). The cellulase enzyme activity of the COC1 isolate reached its optimum point at the 15th hour of 0.06 U / mL.

3.4 The effect of the drying process on the characterization of cassava pulp

The characteristics of cassava pulp during the drying process were known by analyzing the proximate components and observations using an SEM microscope. The proximate analysis tested consisted of analyzing moisture content, ash, protein, crude fat, crude fiber, and carbohydrates by the by difference method. It can be seen that cassava pulp before drying and after drying for 15 days there were changes in the value of the composition of ash, fat, protein, crude fiber, and carbohydrates (table 4). Carbohydrates contained in cassava before being dried and after being dried for 15 days was 89.93% and 88.66%. Cassava pulp contained 7.55% crude fiber before drying and 7.82% after drying.
Table 1. Morphological characters of cellulolytic bacteria.

| Isolate   | Color          | Morphological characters            | Edge    | Elevation   |
|-----------|----------------|-------------------------------------|---------|-------------|
| CP113     | White          | Round                              | Entire  | Convex      |
| CK332     | Yellow         | Round with raised edge             | Lobate  | Raised      |
| CP312     | White          | Round with raised edge             | Entire  | Raised      |
| CP632     | White          | Irregular                          | Lobate  | Raised      |
| COC1      | White          | Curled                             | Erose   | Raised      |
| CP522     | White          | Curled                             | Wavy    | Raised      |
| CP525     | White          | Curled                             | Wavy    | Umbonate    |
| CPK521    | Yellowish-white| Curled                             | Wavy    | Flat        |
| CP435     | White          | Wrinkle                            | Lobate  | Umbonate    |
| CP813     | White          | L shape                            | Entire  | Convex      |
| CK522     | Yellow         | Curled                             | Wavy    | Raised      |
| CP541     | White          | Curled                             | Erose   | Flat        |
| CP641     | White          | Irregular                          | Erose   | Flat        |
| CPK522    | Yellowish-white| Curled                             | Wavy    | Raised      |
| CPB113    | Clear white    | Round                              | Entire  | Convex      |
| CP842     | White          | L shape                            | Erose   | Raised      |
| CPB813    | Clear white    | L shape                            | Entire  | Convex      |
| CK525     | Yellow         | Curled                             | Wavy    | Umbonate    |
| CP413     | White          | Wrinkle                            | Entire  | Convex      |
| CP872     | White          | L shape                            | Like wool| Raised      |
| CK312     | Yellow         | Round with raised edge             | Entire  | Raised      |
| CPK113    | Yellowish-white| Round with a coral edge            | Entire  | Convex      |
| CP273     | White          | Round with raised edge             | Like wool| Convex      |
| CP822     | White          | L shape                            | Wavy    | Raised      |
| CP475     | White          | Wrinkle                            | Like wool| Umbonate    |
| CP825     | White          | L shape                            | Wavy    | Umbonate    |
| CP345     | White          | Round with raised edge             | Erose   | Umbonate    |
| CP118     | White          | Round                              | Entire  | Crateriform |
| CP313     | White          | Round with raised edge             | Entire  | Convex      |
| CK531     | Yellow         | Curled                             | Lobate  | Flat        |
| CK113     | Yellow         | Round                              | Entire  | Convex      |
| CP885     | White          | L shape                            | Like yarn| Umbonate    |
Table 2. Morphological characters of amylolytic bacteria.

| Isolate | Color       | Shape  | Edge  | Elevation |
|---------|-------------|--------|-------|-----------|
| SP113   | White       | Round  | Entire| Convex    |
| COS1    | White       | Curled | Lobate| Raised    |
| SPK113  | Yellowish-white | Round | Entire| Convex    |
| SP855   | White       | L shape| Siliat| Umbonate  |

Table 3. Cellulolytic and amylolytic indexes of various isolates.

| Isolate | Cellulolytic index | Amylolytic index |
|---------|--------------------|------------------|
| CP312   | 1.34               | -                |
| CPK332  | 0.92               | -                |
| COC1    | 1.47               | -                |
| CP632   | 1.40               | -                |
| CP522   | 0.64               | -                |
| CP435   | 0.32               | -                |
| CP541   | 0.89               | -                |
| CP641   | 0.13               | -                |
| CPK522  | 0.80               | -                |
| CP842   | 0.86               | -                |
| CK525   | 0.14               | -                |
| CPB813  | 0.22               | -                |
| CP413   | 1.04               | -                |
| CP872   | 0.39               | -                |
| CK113   | 0.13               | -                |
| CK312   | 0.88               | -                |
| CP273   | 0.24               | -                |
| CP475   | 0.24               | -                |
| CP825   | 0.30               | -                |
| CP118   | 0.07               | -                |
| CK531   | 0.06               | -                |
| CP885   | 0.39               | -                |
| COS1    | -                  | 0.86             |
| SP855   | -                  | 0.30             |

Figure 3. Formation a clear zone of (a) COC1 cellulolytic bacteria on 1% CMC media and (b) COS1 amylolytic bacteria on 1% starch media.
Protein in cassava pulp was quite low at 1.55% before drying and after drying there was an increase reaching 2.32%. Besides protein, cassava pulp also contained crude fat of 0.12% before drying and 0.47% after drying. Ash content in cassava pulp after the drying process decreased to 0.73%. Changes in the morphological structure of starch granules and fiber in cassava pulp on days 0, 6, and 15 were observed under the SEM microscope (figure 5). Starch on day 0 had a round, smooth morphology and large size, while starch on the 6th and 15th days had many holes on its surface and smaller size. The morphology of fiber on day 0 was a fibrous and smooth surface, while fiber on days 6 and 15 had a perforated surface.

Table 4. The composition of cassava pulp before drying and after drying for 15 days.

| Component                  | Day 0 Wet basis (%) | Day 0 Dry basis (%) | Day 15 Wet basis (%) | Day 15 Dry basis (%) |
|----------------------------|---------------------|---------------------|----------------------|----------------------|
| Moisture                   | 77.42               | -                   | 27.26                | -                    |
| Ash                        | 0.19                | 0.83                | 0.53                 | 0.73                 |
| Fat                        | 0.03                | 0.12                | 0.34                 | 0.47                 |
| Protein                    | 0.35                | 1.55                | 1.69                 | 2.33                 |
| Crude fiber                | 1.79                | 7.55                | 5.79                 | 7.82                 |
| Carbohydrates by difference| 20.22               | 89.93               | 64.39                | 88.66                |

4. Discussion

The 15-day drying process of cassava pulp caused a reduction in the wet weight of the cassava pulp, specifically a reduction in moisture content. The moisture content in wet cassava pulp can facilitate the process of degradation of cassava pulp by microorganisms [10]. The decreased water content during the drying process caused the solid cassava pulp to become heavier. This affects the number of cellulolytic and amylolytic bacteria in cassava pulp. The number of cellulolytic and amylolytic bacteria on the 15th day of the drying process was higher than before the drying process. Nutrient content in cassava pulp is a substrate for the growth of cellulolytic and amylolytic bacteria. Cellulolytic and amylolytic bacteria utilize cellulose and starch in cassava as a carbon source. The heavier solid cassava pulp then the number of bacteria contained in it was higher.

Bacteria that have been isolated can be classified based on observations of colony, genetic, biochemical, and other characteristics. Bacteria exhibit the type of growth characteristics in agar media under suitable conditions [11]. Various microorganisms require special media to be able to grow and multiply [12]. Cellulolytic bacteria need a cellulose component in their growth media and amylolytic bacteria can grow on media that contain starch in them. A similar result was formerly reported that bacterial isolates which were isolated from the majority of cassava pulp had a rounded...
colony, smooth-edged, smooth surface, button-like edge, and had a diameter of 0.1-0.3 cm [13]. In addition, the cell morphology classified as Gram-positive and Gram-negative bacteria.

A qualitative test through the formation of clear zones was carried out as a selection process to obtain bacterial isolates that can produce cellulase and amylase enzymes. The formation of clear zones on CMC media after adding a congo red solution indicates that COC1 isolate can degrade cellulose contained in the media. The use of congo red as an indicator of the degradation of β-D-glucans on agar media provides rapid testing and sensitive selection for cellulolytic bacteria [14]. In starch media that has been grown by amylolytic bacteria, clear zones were formed when stained with iodine solution. This showed that COS1 isolate was able to degrade starch contained in selective agar media. Bacteria secrete extracellular amylase enzymes to degrade starch in the media into simple sugar compounds that do not show a color reaction with iodine [15]. Isolates which do not form clear zones on CMC media or starch media can live by the presence of yeast and peptone extracts that bacteria can use for growth.

![Figure 5](image-url)

**Figure 5.** Starch morphology structure on day 0 (a), day 6 (b), day 15 (c) and fiber on day 0 (d), day 6 (e), day 15 (f) during the drying process.

Cellulolytic bacteria were grown in 1% CMC media which functioned as inducer components. Bacterial isolates that can grow on cellulose media prove that these isolates can use cellulose as a source of nutrients [16]. The growth curve is made by plotting the incubation time with optical density. The higher absorbance value indicates a higher amount of bacteria in the media. The growth of cellulolytic bacteria marked by the increased density value or media turbidity that competes with the increased incubation time [17]. COC1 cellulolytic isolate through the lag phase, the logarithmic/exponential phase, and the stationary phase. The COC1 isolate went through the lag phase from 0 to 3 hours. The lag phase is the phase of bacterial adaptation to the growth media.

The logarithmic phase or exponential phase occurred at the 3rd hour to the 15th hour of incubation. In this phase, bacteria underwent a very rapid cell division marked by a rapid increase in the number
of bacteria. This showed that bacteria have been able to adjust themselves with media. In addition, bacteria also synthesize cellulase enzymes to break down cellulose into glucose. Bacteria will utilize the carbon cellulose source by synthesizing the cellulase enzyme after the glucose in the growing medium was depleted. An enzyme activity test was carried out by measuring the level of reducing sugars formed in the ingredients. One of the reducing sugars is glucose. The higher glucose level indicates the higher cellulase activity produced by bacteria.

The optimum time for enzyme production was at the 15th hour with cellulase activity of 0.06 U/mL. In seaweed waste, it has been reported that cellulolytic bacterial isolates had the highest cellulase activity on the third day of incubation time of 0.074 U/mL [18]. The difference in the number of cellulase enzymes produced depends on the type of bacteria, genes owned, and the carbon source used. The optimum time of enzyme production is used as the time to harvest enzymes to degrade agricultural substrate [8]. Bacteria underwent a stationary phase at the 18th hour to the 24th hour of incubation. In this phase, the bacteria has decreased the rate of the division [19]. That was because the nutrients contained in the CMC media have been reduced.

Cellulase is a hydrolase enzyme consisting of 1,4-β-d-glucan glucanohydrolase (endoglucanase), 1,4-β-d-glucan cellobiohydrolase (exoglucanase), and β-d-glucoside glucohydrolase (β-glucosidase). Endoglucanase cut β-1,4 cellulose randomly in the form of soluble and amorphous substrates. Furthermore, exoglucanase releases cellobiose from the end of non-reducing cellulose [20]. This cellobiose is broken down into transition by the catalytic action of β-glucosidase [21]. There were several peaks in the result of the enzyme activity measurement that can be caused by the presence of isoenzymes or different enzymes. Isoenzymes are enzymes that have different amino acid sequences but can catalyze the same reaction. In the β-glucosidase activity test, there were two peak proteins found as isoenzyme A and isoenzyme B [22].

The growth of indigenous microbes during the drying process caused changes in the produced cassava flour. This research shows that cassava contains high carbohydrates. Carbohydrates can be in the form of polysaccharides such as starch. In cassava pulp, it has been reported that starch contained in cassava pulp dry weight is 65.6% [23]. The high starch components in the waste reflect the efficiency level of the starch extraction process from the tapioca factory. Cassava pulp that was analysed contained many starches and fibrous materials such as cellulose and hemicellulose which were the source of sugar fermentation [4].

Crude fiber is residue from food ingredients that contains boiling acid or alkaline [24]. The acid compound used for the analysis of crude fiber is sulfuric acid while the alkaline compound used is sodium hydroxide. Crude fiber consists of cellulose, hemicellulose, lignin, and other components. The value of crude fiber was not significantly different between before drying and after drying process. This indicates that indigenous microbes in cassava pulp more utilizing a simpler compound than fiber. In cassava pulp, it has been reported that fiber content in cassava is 20.1% and cellulose is 8.1% [23]. The difference in fiber obtained in the study can be caused by the influence of cassava cultivars used in processing cassava into tapioca flour.

Low protein content and high fiber make cassava pulp less suitable for animal feed except if mixed with other raw materials that are rich in protein [10]. An increase in protein in cassava pulp after drying for 15 days can be related to the presence of indigenous microbes. The high population of microbes caused high protein because microbes also contain protein [25]. This high protein content can be related to the ability of microorganisms to secrete several extracellular enzymes such as amylase, linamarase, and cellulose into cassava pulp for their metabolic activities [26]. Increased levels of fat and protein in cassava can be caused by the decrease in other components. Fat content in cassava is 0.2% [23]. Ash content was analyzed to determine the percentage of minerals in cassava.

It has been proven that during the drying process, cellulolytic and amylolytic bacteria caused changes in the chemical components of cassava pulp. Besides, changes in the morphological structure of cassava pulp also occurred due to microbial work. Starch and fibers undergo morphological structure changes during the 15 days of the cassava drying process. That showed during the drying process there was a degradation process by cellulolytic and amylolytic bacteria.
5. Conclusion
The cassava pulp drying process for 15 days affects the number of cellulolytic and amylolytic bacteria that play an important role especially in degrading cassava pulp. COC1 isolate can degrade cellulose to glucose which characterized by high cellulose activity in the logarithmic phase. There were changes in the content of ash, fat, protein, crude fiber, and carbohydrates in cassava pulp which underwent a drying process for 15 days. Besides, there were changes in the morphological structure of fiber and starch during the 15 days of the cassava drying process.

Acknowledgments
We thank Mr. Edi and Mr. Arif (tapioca flour factory owner) for allowing us to take cassava pulp samples. We would also like to show our gratitude to the Center for Biological Resources and Biotechnology Research (PPSHB) for allowing us to be able to use laboratories for research purposes. We thank Mrs. Dewi (laboratory assistance in PPSHB) for helping with the technical implementation of the research.

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