Amylose Content and Physical Changes in Waxy Corn Starch Modification by Spontaneous Fermentation

Lisna Ahmad[1], *Yudi* Pranoto[2], *FMC*. *Sigit* Setyabudi[2] and *Djagal Wiseso* Marseno [2*]

[1] Departement of Food Science and Technology, Universitas Negeri Gorontalo. Kota Gorontalo,Provinsi Gorontalo Indonesia.
[2] Department of Food Science, Faculty of Agriculture Technology Universitas Gadjah Mada, Sleman, Provinsi DI Yogyakarta Indonesia

Abstract. This study aimed was to determine the changes in the ratio of amylose and amylopectin and the physical properties of spontaneously fermented waxy corn starch (WCS) granules. The results showed that the amylose content of WCS which was fermented spontaneously from 9 to 15 days experienced a significant change with native starch, namely, ± 86 – 117%. The hydrolyzed process is caused by lactic acid bacteria activity. The highest activity showed at 15th-day fermentation. Changes in starch composition also affect the swelling power, solubility, water absorption, and gelatinization profile of WCS. Starch that was fermented for 15th-days had the highest amylose percentage (24.92%) the otherwise, had the lowest swelling value compared to native starch. The percent solubility of fermented starch increased with increasing the amount of amylose and on the 15th day of fermentation, the highest value was (65.56%). Water absorption increased until the 6th-day of fermentation and then decreased due to an increase in the amount of amylose. The gelatinization profiles of native starch and fermented modified starch were of the same type. **Keywords**: starch, amylose, fermentation, waxy corn, Solubility

1 Introduction

Waxy corn is corn starch with dominant amylopectin composition. This starch composition made it difficult for WCS to be used as raw materials for food dough. The starch modification process can change the composition of starch so that the structure and properties of starch, both chemical and physical, will change.

The modification process using the fermentation method in previous studies was able to change the nature and composition of starch. The spontaneous fermentation process caused sweet potato starch to have low solubility, granule swelling ability, and higher paste viscosity [1]. It is also found that in African countries, the lactic acid fermentation process can improve the nutritional value and taste of fermented foods [2]. Another study showed that the fermentation process caused the percentage of short starch branch chains to decrease while the long starch chains decreased in sorghum starch [3]. Further, it was also found that the

* Corresponding author: djagal@ugm.ac.id

© The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (http://creativecommons.org/licenses/by/4.0/).

fermentation process of cassava flour caused air absorption and swelling contributed to the separation of the amylopectin to the amylose phase and the determination of crystallinity properties along with the amylose leaching process to the inter-granule space [4].

The starch fermentation process will be dominated by lactic acid bacteria which will produce enzymes capable of degrading starch granules so that the properties of starch or flour become modified [1] Therefore, this study was conducted to determine changes in amylose composition and physical properties of starch after undergoing the fermentation method

2 Materials and methods

2.1 Materials

The corn starch used was waxy corn starch (*waxy corn – Zea mays var L. Ceratina*) which was obtained from corn farmers in North Gorontalo Regency, Gorontalo province – Indonesia. Another additional material used for the starch extraction process is 0.1% NaOH.

2.2 Methods

2.2.1 Waxy Corn Starch (WCS) (4)

Dry shelled waxy corn was grounded using a hammer mill and disc mill to produce waxy corn flour. Furthermore, the starch extraction process was carried out by soaking cornflour in a 0.1% NaOH solution for the deproteination process with a ratio of 1: 3 (flour: aquadest). The immersion process was carried out for 24 hours at 4°C. The next process starch was centrifuged for 15 minutes and 3000 rpm. The starch precipitate was washed repeatedly until the washing water was clear. Next, the starch precipitate is dried.

2.2.2 Starch Fermentation (5)

Starch was made into a suspension with a ratio of 1: 3 with aquadest. Then put in a water bath with a controlled temperature of 25°C. Starch was left in an open condition and every 3 days the suspension was taken and then centrifuged to separate solids and water at 3000 RPM for 15 minutes. Further, the starch was dried to be analyzed for changes in properties consisting of pH, amylose content, and physical properties such as swelling power, solubility, water absorption, and gelatinization profile.

2.2.3 pH (AACC,2000)

The pH of fermented starch was measured using a pH meter. Starch (5 g) was put in a beaker glass, then mixed with 20 ml of distilled water. Then the suspension was homogenized with a stirrer for 5 minutes. Then the suspension was measured using a pH meter

2.2.4 Amylose Content (6)

The calculation of the amount of amylose produced from the treatment during fermentation was carried out using [6]. Analysis of amylose content begins with making a standard curve. A total of 0.04 g of pure amylose was put into a test tube and added 1 ml of 95% ethanol and 9 ml of 1 N NaOH. The mixture was then heated for 10 minutes. The standard solution that has been heated is then put into a 100 ml volumetric flask which is diluted with distilled water to the mark. For variations in concentration, 1, 2, 3, 4, and 5 ml of the standard solution were taken into a 100 ml volumetric flask. In each flask, 0.2 N of 1 N acetic acid was added; 0.4; 0.6; 0.8 and 1 ml. Next, 2 ml of iodine solution and distilled water were added to the mark. After that, it was allowed to stand for 20 minutes and the absorbance was measured at a wavelength of 625 nm. The absorbance results were entered into a standard amylose curve. Determination of amylose content, 0.1g starch sample was put into a test tube and then added 1 ml of 95% ethanol and 9 ml of 1N NaOH. The sample mixture and other solutions were then heated for 10 minutes. The heated sample was put into a 100 ml volumetric flask and added 1 ml of acetic acid and 2 ml of iodine solution. Furthermore, distilled water is added to the mark. Before measuring the absorbance at a wavelength of 625 nm, the sample was allowed to stand for 20 minutes. Calculation of amylose content is carried out by the formula

$$\%\ amylose = \frac{A\ X\ DF}{S\ X\ W}\ x\ 100 \quad (1)$$

Exp : A = absorbance ; DF = Dilution Factor ; S = Slope ; W = Weight

2.2.5 Scanning Electron Microscopy (7)

WCS pellets were mounted on a tray and coated with gold to detect micrographs. The morphology of native and waxy corn fermented samples was observed under a Phenom Pro X Desktop Scanning Electron Microscope SEM with Energy Dispersive X-Ray (EDX). The samples were viewed at an accelerating voltage of 10 kV, and representative micrographs from all the samples were selected for illustration.

2.2.6 Swelling Power and Solubility (8)

The analysis of swelling power and solubility was carried out using the method proposed by Parwiyanti et al [8]. A 0.1g starch sample was put into a centrifugation tube then added 10 ml and vortexed for ± 10 seconds. The suspension was then heated at 85°C for 30 minutes and periodically vortexed every 5 minutes for 10 seconds. Before being centrifuged at 2000 rpm for 30 minutes, the suspension was cooled. The filtrate formed was poured into a porcelain dish with a known weight (Initial Weight of the plate) and then dried at a temperature of 105°C until the weight was constant (Final weight of plate). The weighing data will be used for solubility data. The remaining precipitate in the centrifuge tube was weighed for swelling power. The data obtained is then entered in the following formula:

$$swelling\ power = \frac{sediment\ weight\ (g)}{Initial\ Weight\ (g)} \quad (2)$$

$$\%\ Solubility = \frac{Final\ weight\ of\ the\ plate - initial\ weight\ of\ plate}{initial\ weight}\ x\ 100\% \quad (3)$$

2.2.7 Water Absorption Capacity (WAC)

Analysis of water absorption was carried out by weighing 1 g of starch in a centrifuge tube and then adding 10 ml of water (10% suspension). Then it was vortexed for 15 minutes and followed by centrifugation at 3500 rpm for ± 10 minutes. The precipitate formed is then separated from the filtrate and then weighed. The formula used to calculate the % water absorption is as follows

$$\% WAC = \frac{Sediment\ weight}{Initial\ weight}\ x\ 100\% \qquad (4)$$

2.2.8 Pasting Properties (9)

Measurement of pasting properties is carried out by adopting the method used by [9]. Measurement of pasting properties using the RVA (Rapid Visco Analyzer) instrument with the following steps: The first step before using the RVA instrument is to measure the water content of each sample. After the water content of the sample is known, then start with the use of the RVA instrument. The sample is dissolved into aquadest according to the number that appears in the software after entering the sample moisture content data. The starch the ratio between the difference between the final viscosity and the heat viscosity and the heat viscosity (in percent)suspension is then placed into the sample container on a device that will stir the sample at 160 rpm. In the first minute, preheating is carried out until the temperature reaches 50°C. Next, the heating temperature was increased to 95°C at 8.5 minutes and kept constant at 95°C for 5 minutes. Then the temperature was lowered back to 50°C (minute 21) and maintained at 50°C for 2 minutes (minute 23). The relative reverse viscosity is known from

3 Results and Discussion

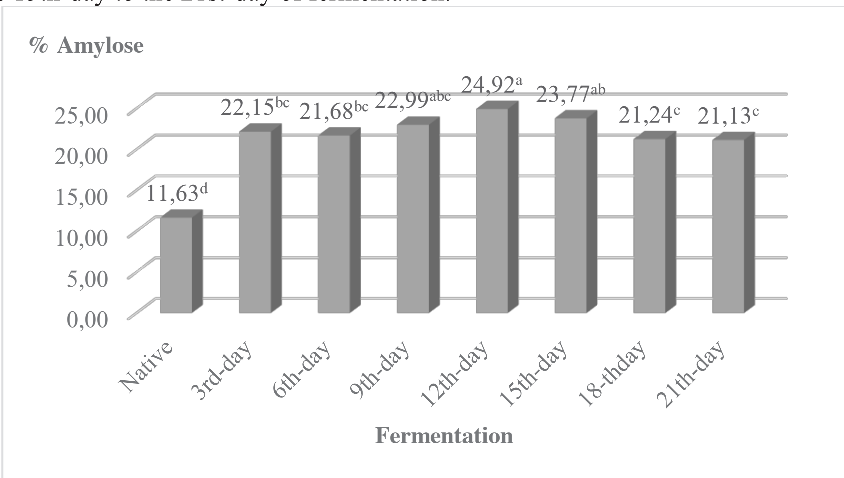
3.1 Amylose Content

Spontaneously fermented WCS showed a significant increase in the percentage of amylose content compared to native starch. A native of WCS has a lower amylose component than fermented starch The fermentation process causes a significant change in the ratio of amylose to amylopectin, especially with native WCS, which is around 86 – 117%. Amylose content of waxy starch increased after fermentation, where the highest percentage of amylose was obtained in waxy corn starch fermented for 12th-days although statistically, this increase was not significantly different from starch fermented for 9th and 15th-days. This happens because the fermentation process causes the growth of microorganisms on the soaked starch substrate, especially lactic acid bacteria so that the substrate conditions become acidic. This condition is very beneficial if this starch product is used as a thickener or filler in food products.

This is evidenced by the analysis of the growth of lactic acid bacteria (LAB) shown in Figure 2 below. The formation of acid in the substrate causes the starch to undergo a hydrolysis process which disrupts the structure of the starch bond. The spontaneous fermentation process causes a hydrolysis process by acids and enzymes produced by microorganisms during fermentation [10]. Data on starch amylose content during the 21st-day fermentation process can be seen in Figure 1 below:

The increase in the percentage of amylose indicated that the fermentation conditions were able to cut the α-1,6 bonds in the amylopectin branched chains to form straight chains of α-1,4 amylose. The maximum hydrolysis process is possible on the 12^{th}-day of fermentation, as evidenced by the percentage of amylose produced. After that, it started to decrease because of the possibility of further fermenting starch to form simple sugars.

Changes in the amount of amylose were caused by the process of acid action produced by microorganisms that we're able to cut polysaccharide chains both in straight chains α-1,4 and in branched chains α-1,6. Acidic conditions in starch suspension can cut starch chains into smaller molecules or shorter chains. The acid produces H^+ ions and binds with H_2O to form $H3O^+$ which will break the glycosidic bonds in amylose and amylopectin to form simple monomers [11]. This is evidenced by changes in pH during fermentation. The initial pH of the starch suspension before fermentation was 6 and began to show a decrease after 3rd-days of fermentation and so on until the 12nd-day of fermentation. pH did not show any change after the 15th-day to the 21st-day of fermentation.



exp: Different notation shows significance at =5%

Fig 1. Amylose Content of WCS with Spontaneous Fermentation

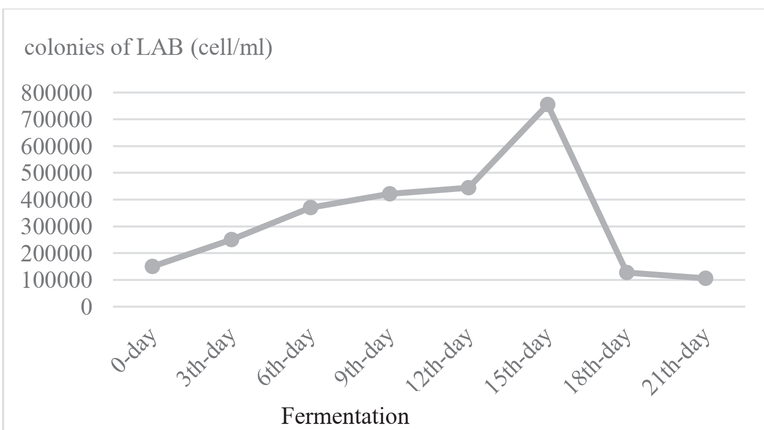


Fig 2. Lactic Acid Bacteria Growth Data During Fermentation

Fermentation on 18th-day and 21st-day, amylose content decreased significantly but was still higher than native corn starch. This is due to decreased microbial growth which may have experienced a death phase so that it no longer produces acids that can hydrolyze starch chains. In addition, the possibility of the fermentation process continuing is no longer changing the previously hydrolyzed starch monomers but turning into simple sugars and alcohol.

3.2 Starch Granule Morphology

WCS naturally has an oval shape with varying starch sizes. The size of WCS granules has a diameter in the range of 2 - 20 m in diameter [12]. In addition, it is oval with a smooth surface.

The size and shape of the corn starch granules of native glutinous rice and fermented corn starch can be seen in Figure 3.

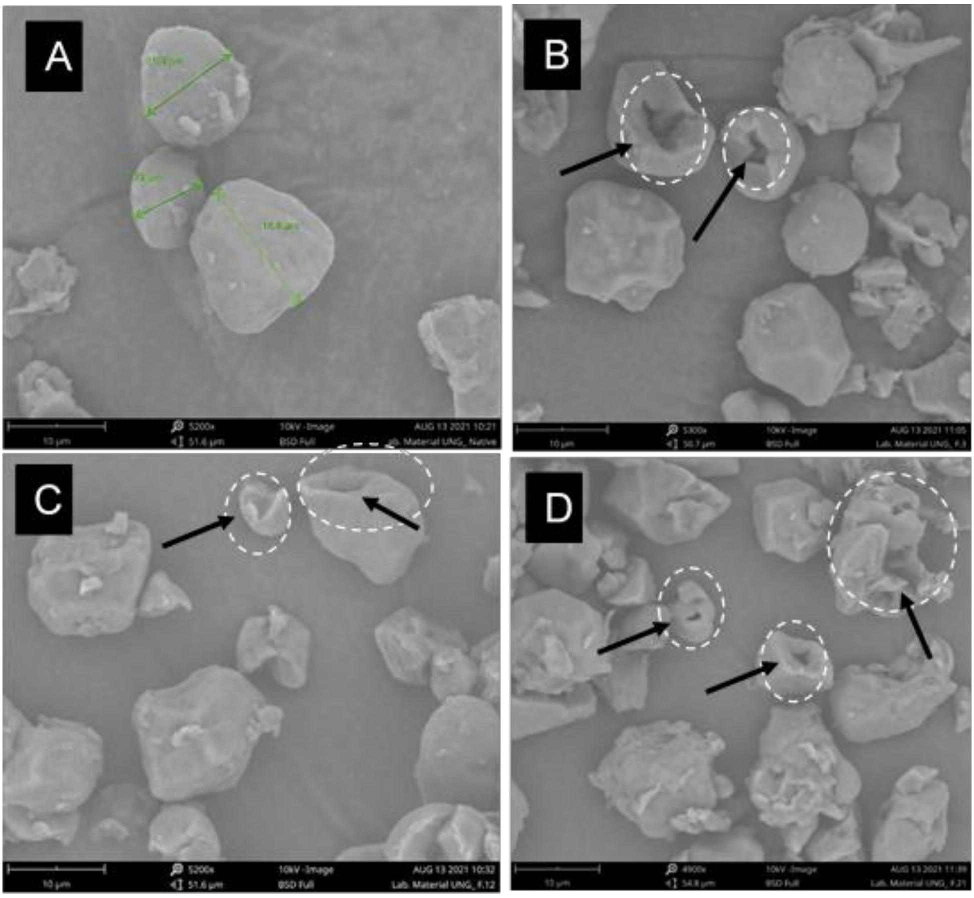


Fig. 3. Morphology of native WCS (A); WCS after 3th-days of Fermentation (B); WCS after 12th-days of Fermentation (C); WCS after 21th-days of Fermentation (D).

The spontaneous fermentation process of waxy corn suspension may result in enzymatic activity produced by microorganisms that grow in it. Evers noted that this enzymatic activity has five attack patterns on the starch surface, namely: pin-holes, sponge-like erosion, numerous medium-sized holes, distinct loci leading to single holes in individual granules, and surface erosion[13]. The holes formed in the starch granules after fermentation are caused by the lysis of amylose that occurs due to hydrolysis. Hydrolysis occurred due to changes in the acidity of the substrate (pH = 4, data not shown). Hydrolysis occurs mainly in more amorphous zones whereas crystalline lamellae are resistant to enzymatic action [13],[14].

The hydrolysis process on the surface of starch granules according to (13) moves radially, forming pores that prevent the free diffusion of enzymes into the granules. This causes the formation of channels until it reaches the centre point of the granule. Furthermore, the enzyme molecules will be trapped in the granules and will hydrolyze the granules in a certain diffusion range according to the availability of the substrate. Therefore, until the 21st-day of fermentation (Figure 3.D) the surface of the granules still looks hollow even though the size of the starch granules is smaller than the starch granules on the 3rd and 12th-days of fermentation

3.3 Solubility dan Swelling Power

The starch fermentation process affects the physical properties of starch such as solubility and swelling power. Changes in the solubility of glutinous corn starch after spontaneous fermentation can be seen in Fig 4. This change is influenced by the size of the granule[15]. The fermentation process causes the size of the starch granules to decrease because the hydrolysis process causes the starch structure to change so that it affects the size of the granules. Small granule sizes will have a high solubility value. The longer the fermentation process, the higher the percentage of solubility. The results of this analysis are relevant to the results of the microstructure analysis of starch granules using SEM (Figure 4).

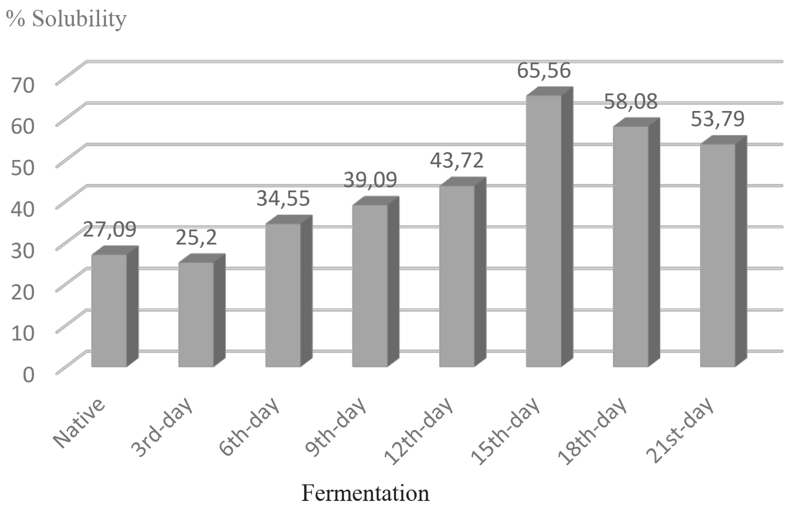


Fig 4. Solubility Data of WCS During Fermentation

The percent solubility value indicates the number of dissolved starch granules. The results of the analysis showed that fermented starch tended to increase in solubility due to the fermentation process. The hydrolysis process that occurs during fermentation causes the glycosidic bonds of starch to be easily broken and then easily soluble in water. This process occurs until the next 15th-days of fermentation decreases again due to the possibility of further hydrolysis of starch into organic acids that are more difficult to dissolve. These organic acids are less soluble than simple sugars (the result of further fermentation of starch). The formation of acid during the fermentation process is evidenced by the decrease in pH levels until the 15^{th}-day from the native starch pH = 6 then decreases to pH = 4.

This less soluble property is due to its lower dielectric constant than sugar. The dielectric constant of sucrose sugar is 63.88 and glucose has a dielectric constant of 60.70. While the dielectric constants of organic acid molecules such as acetic acid and formic acid are 6.20 and 58[16]. Water has a dielectric constant of 80. Based on the data above, it can be seen that simple sugars are more soluble in water than organic acids because they have a smaller dielectric constant than water. Starch which has high solubility properties can be used to make dough for bakery products.

The solubility of starch is inversely proportional to the swelling property. Starch with low amylose will produce high swelling. Native starch has a lower amylose content than fermented starch so the swelling value is higher. The graph of the swelling power of starch can be seen in Figure 5.

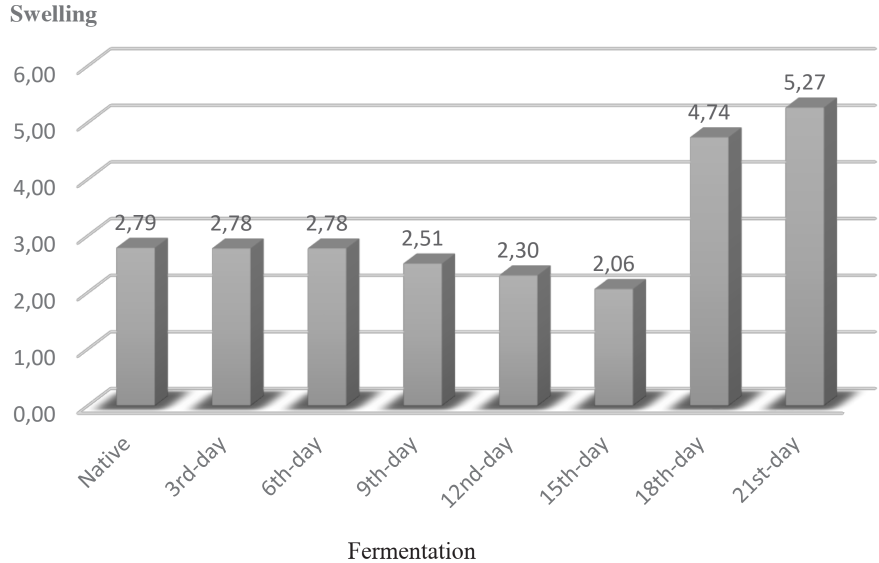


Fig 5. Swelling Power Data of WCS During Fermentation

The 12nd-day and 15th-day fermentation treatments showed high amylose content so the swelling value in this treatment was lower than other starches. However, after the 18th-day and 21st-days of fermentation, the swelling power value of starch began to increase again because the previously formed amylose content was likely to experience leaching so that the water around the starch was easier to bind to the starch, in this case, amylose so that the starch experienced maximum swelling again. The longer the corn fermentation process, the lower the swelling power value of modified cornflour, this is amylose is an inhibitor of starch swelling, therefore when the amount of amylose increases the value of swelling power decreases [5].

3.4 Water Absorption Capacity

Water absorption indicates the amount of water (in weight) bound to the dry where this binding process depends on hydrophilic groups that can bind water and gelling capacity in macromolecules [17]. The results of the water absorption analysis of glutinous corn starch during the fermentation process are shown in Fig 6.

The results of the statistical analysis of the fermentation time affect the water absorption of the modified fermented starch. The ability of the modified waxy corn starch to absorb water began to increase from its native starch until the 6th-day of fermentation, but after that its ability to absorb water began to decrease. When the fermentation took place a lot of starch bonds were hydrolyzed so that it gave a great opportunity for water to enter the starch granules, but after 9th-days of fermentation, the ability of starch to absorb water began to decrease this was probably due to the fermentation process causing the amount of amylose to increase (fig 1). Amylose is a starch fraction that inhibits the entry of water into the starch granules. In addition, previous research revealed that amylose content is negatively correlated with the water absorption ability of traditional Sri Lanka rice [16].

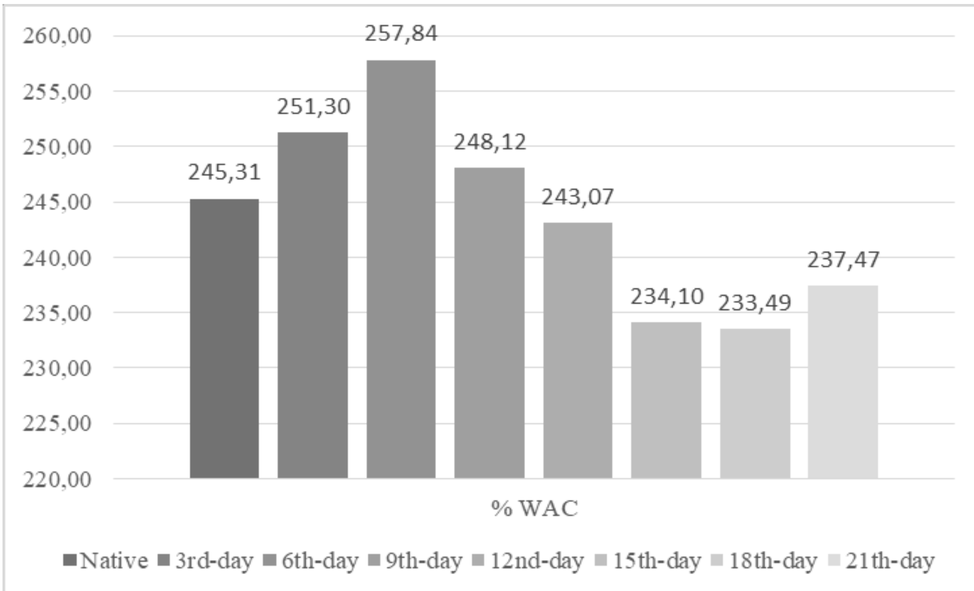


Fig 6. Water Absorption Capacity of WCS during Fermentation

The water absorption value is in line with the swelling power value of fermented corn starch (fig 5). The longer the fermentation process, the ability of glutinous corn starch to absorb water decreases. Studies have found that in starch granules, amylose fraction formed a crystalline structure while amylopectin had an amorphous structure [19] [20]. The crystalline structure is more rigid, making it more difficult for water to penetrate.

3.5 Pasting Properties

The gelatinization profile of WCS, both native and fermented starch, was almost the same as the gelatinization profile of ordinary corn starch. The results of the analysis of the gelatinization profile of starch using the Rapid Visco Analyzer (RVA) method are shown in Table 1.

Table 1. Pasting Properties of WCS during Fermentation

Fermentation	Peak viscosity (PV)	Through (T)	Breakdown (BDV)	Final Visc (FV)	Setback (SV)	Pasting Temp (SV)
Native	2879	1340	1539	2050	710	75,35
3rd-day	3037	1145	1892	1511	366	75,05
6th-day	3168	1188	1980	1521	333	75,05
9th-day	3000	1049	1951	1318	269	75,35
12th-day	3227	1197	2030	1570	373	75,4
15th-day	3438	1281	2157	1674	393	75,05
18th-day	3401	1177	2224	1616	439	75,05
21st-day	3594	1209	2385	1566	357	75

Starch which has viscosity characteristics like this is very suitable to be applied in noodle products. Fermentation on 9th-day has the lowest setback value so as to produce a dough texture that is not hard on the final product.

4 Conclusion

The fermentation process of waxy corn starch (WCS) causes a change in the ratio of amylose and amylopectin. Amylose levels increased by 117% on the 12th day of fermentation treatment. The morphological structure of the surface of starch forms holes and the condition of the starch suspension causes the growth of microorganisms such as lactic acid bacteria (LAB). The properties of swelling and solubility also change compared to native. The ability of fermented starch in absorbing water initially increases but decreases again after 9th-day fermentation due to increased amylose which inhibits the entry of water into the granules. Fermented WCS can be applied in noodle products.

5 References

1. N. Yuliana, N. Siti, S. Ribut, A. Microbiol Indones ;**8**, 1, 1–8 (2014).
2. S. A.I, M-Guyot J, Guyot Jp. Int J Food Microbiol;**72**, 1–2, 53–62 (2002).
3. G. Yunfei, W. Weihao, S. Meng, K. Ziyue, W. Juan, Q. Zhigang, X. Jinling, Z. Shuting, L. Dezhi, C. Longkui. J Chem. **2020**; (2020).
4. H. Parichat, I. Kamolwan, K. Nopmanee, S. Tanaboon. Characteristics Of Thai Yam (Dioscorea Alata L .) And Spherulitic Structure In Starch Film. The 52nd Kasetsart University Annual Conference, Bangkok, Thailand (2014)
5. Y. Mei-lan, L. Zhan-hui, C. Yong-qiang, L. Li-te. J Food Eng **85**, 12–7 (2008).
6. B.O. Juliano. *Amylose Analysis in Rice*. IRRI. Los Banos. (1979).
7. T. Han, Y. Juan, Z. Jianwei, T. Yaoqi, J. Zhengyu, X. Xueming. Plos One. **10**, 5, 1–11 (2015).
8. P.F. Pratama, W.A., Malahayati, N. Prosiding Seminar Hasil Penelitian Tanam Aneka Kacang Dan Umbi 692–9 (2015)
9. E. Syamsir, P,Hariyadi, D,Fardiaz, N.Andarwulan. J Teknol dan Ind Pangan **23**, 1, 100–6 (2012).
10. Z. Tong, L. Xiaoping, Z. Ruizhen, M. Zhen, L. Liu, W. Xiaolong, H. Xinzhong. Carbohydr Polym **218**, April,163–9 (2019).
11. M. Balat, H. Balat, Öz C. Prog Energy Combust Sci **34**, 5, 551–73 (2008).
12. K.Danupol, S.Bhalang, T.Ratchada, J.Jay-lin, L.Kamol.. J Food Sci Technol **52**, October, 6529–37 (2015).
13. M. Sujka and J.Jamroz. Int Agrophysics **21**, 1, 107–13 (2007).
14. W.Helbert, M. Schülein, B. Henrissat. Int J Biol Macromol **19** ,3, 165–9 (1996).
15. M.C.Nun˜ez-Santiagoa, L.A.Bello-Pe´reza, A. Tecanteba Centro. Carbohydr Polym **56**, 1, 65–75 (2004).
16. R.Widyasaputra, and S.S.Yuwono. J Pangan dan Agroindustr**1**, 1, 78–89 (2013).
17. E.K.Asare, S.Sefa-Dedeh, E.O.Afoakwa, E.Sakyi-Dawson, A.S.Budu. Int J Food Eng **6**, 4, (2010).
18. TG.C. Thilakarathnaa, S.B. Navarathneb, I. Wickramasinghec. Int J Adv Eng Res Sci **4**, 12, 186–94 (2017).

19. R.F.Tester, J.Karkalas, X.Qi. J Cereal Sci **39**, 2, 151–65 (2004).
20. C.Wariyah, C.Anwar, M.Astuti, Supriyadi. Agritech, **27**, 3, 112–7 (2007).