



Utilization of Breadfruit (*Artocarpus altilis*) Peel Waste and Blood Clam Shell Waste (*Anadara granosa*) as Raw Materials for Glycerol-Plasticized Degradable Bioplastic Production

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ABSTRACT

Nowadays, the world is in dire need of solutions to tackle the ever-growing plastic waste problem. Plastics cannot decay easily in the natural environment. Instead, it took conventional plastics such as PET – about 23 to 48 years to decay naturally in the environment. Therefore, it is urgently needed to find an alternative to these types of plastics, namely degradable plastics. One type of bioplastics, called starch-based bioplastic can be made using starchy materials from Breadfruit peel. This is in combination with Blood Clam shell-derived chitin nanowhiskers as a reinforcer and glycerol as the plasticizer. In this research, bioplastic is synthesized using the proposed formulation consisting of starch extracted from Breadfruit (*Artocarpus altilis*) peel waste, substituted by reinforcing agent chitin nanowhisiker made from Blood Clam (*Anadara granosa*) shell waste, and addition of glycerol as the plasticizer. Further, bioplastic was tested according to general standard plastic tests including Tensile Strength, Water Uptake, and Soil Burial test. Chitin nano whisker was made using the Acid Hydrolysis method in which HCl 0,1 M was used to hydrolyze blood clam shell chitin waste, while Breadfruit peel starch was extracted using the centrifugation method. Soil burial test results showed that in less than 15 days, the bioplastic was completely decomposed. Water uptake test results showed that the bioplastic made from breadfruit waste starch + Blood Cam-derived nano whisker chitin can achieve water uptake numbers as high as 94,077 %, this was lower than the control sample which was made without the addition of Blood Cam-derived nano whisker chitin. But this test result was still higher compared to conventional plastics such as clip plastic, having a water uptake percentage of 0,758 %, and to supermarket plastic bags having a water uptake percentage of zero. Tensile strength test showed that the bioplastic made from breadfruit waste starch + Blood Cam-derived nano whisker chitin was two times stronger than bioplastic made without the addition of Blood Clam derived nano whisker chitin and was also stronger than conventional plastics.

Keywords: *Anadara granosa*, *Artocarpus altilis*, degradable plastic, soil burial, tensile strength, water uptake.

INTRODUCTION

Nowadays, the plastic problem is faced by the whole world. One of the causes is PET (Polyethylene Terephthalate) which cannot be degraded naturally in a short time (Muller et al, 2001). One of the presented solutions is by using degradable plastics. Degradable

plastics are plastics that use products of living beings as their main ingredients, such as starch from fruits, which is safe and able to degrade naturally. Degradable plastics are composed of 3 main ingredients which are starch, reinforcer, and plasticizer. Starch is used as the base of the plastic which forms the main polymer, the reinforcer acts as

an additive ingredient that helps achieve certain goals for the plastic, such as better strength and flexibility, one such example is chitin nanowhisker. And the last main ingredient is a plasticizer which gave the plastic its "plastic" characteristics, such as its flexibility (Gironi & Piemonte, 2011). One example is glycerol.

On the other hand, degradable plastics still have their weaknesses. Which is the high cost of its ingredients compared to the conventional plastics such as PP and PE which is 3 – 4 times cheaper. One such example is PHA (polyhydroxyalkanoate) (Kourmentza et al, 2017). To combat these weaknesses, food waste and other wastes are utilized, one of the most prominent candidates is breadfruit peel waste. Indonesia produces around 115,000-ton breadfruits annually. Which contains 39,56 % starch in its total weight (Putri et al, 2017), these characteristics are why breadfruit peel waste can be utilized as the main ingredient of degradable bioplastic.

MATERIALS AND METHODS

Materials

The materials that were used in this research were blood clam shell waste from Citraland Modern Market in Surabaya, breadfruit peel waste from Mojokerto, Pure chitin, pure starch, glycerol, hydrochloric acid, sodium hydroxide, distilled water common LDPE plastic (L), common clear LDPE (LC), commercial bioplastic (P) provided by the University of Surabaya where the research was conducted, humus soil from Lidah Kota Forest, Surabaya, Compost soil from a corn plantation near Lembah Harapan Residence, sand soil from a construction site within University of Surabaya. The equipment that was used was, varying sizes and types of glass ware, oven, fume hood, magnetic stirrer, analytical scale, Metrotex MBT 15 – 1000 P Bondign Tensile Tester, UV – vis Spectrophotometer, centrifuge, and freeze – dryer.

Methods

Chitin Extraction from Blood Clam Shell Waste

50g clam shells are washed and dried, then the shells were crushed and milled with the help of liquid nitrogen to form a fine powder. The powder was then subjected to the acid hydrolysis method to extract the contained chitin this method contains 2 main parts which start with deproteination. For the deproteination process, 500 mL of 4% NaOH (w/v)

is added to 50 g of shell powder and then homogenized at 80 °C for 1 hour. The resulting mixture is then settled at room temperature, following that, it was decanted using filtering paper with the addition of distilled water to neutralize the pH level^[9]. After a neutral pH level is achieved, the filtered particle is dried in the oven at 60 °C for 24 hours. Following that, the second part, demineralization is done by adding 500 mL of 1M HCl into the dried powder, the mixture is then homogenized at room temperature for an hour, then it was decanted and dried using the same method as before^[16].

CNW Production

The formed chitin was weighed and mixed with 0.1M HCl at 1:10 mass versus volume proportions^[29]. During the mixing process, the mixture is heated and stirred at 80 °C for 90 minutes, following that, the mixture is decanted through filter paper and the filtered particle is centrifuged with deionized water until neutral pH is achieved. The pellet formed after centrifugation is then freeze-dried using a freeze dryer for 48 hours at -50°C^[27]. After the production process, the resulting CNW (Chitin nanowhisker) is weighed and analyzed using an electron microscope at 5000 and 10000 magnifications.

CNW Microscopic Analysis

4,24 g of CNW is sent to Institut Teknologi Sepuluh Nopember's Department of Materials and Metallurgy Testing at ITS Sukolilo campus, Surabaya to be analyzed using an electron microscope at 5000 x and 10000 x magnification^[19].

Breadfruit Starch Extraction and Quantification

1500 g breadfruit peel waste is washed and blended. After blending, the mixture is then filtered using a Whatman™ filter paper. The water passed through the filter is centrifuged and the pellet is dried at 60 °C for 24 hours^[28]. The dried powder is then tested for starch levels using the iodine test method^[13]. The quantification of breadfruit starch extract is done by iodine – starch testing method, in which a standard curve consisting of 6 different concentrations (0.625, 1.25, 2.5, 5, 10, 20 mg/mL) of starch – iodine solutions, which is then used to determine sample starch concentration via UV – vis spectrophotometry running at 580nm wavelength^[10]. The sample was prepared by mixing 1 mL of sample and 1 mL of iodine^[1].

Degradable Plastic Formulation

0.125 g CNW, 0.625 g glycerol, 2.5 g starch and 50 ml distilled water were prepared. The ingredients were mixed at 80 °C for 20 minutes, and then molded using a silicone mold and cut into a rectangular shape (2.3 x 1.8 cm), and dried at 45 °C for 24 hours [32].

The same method is then repeated using pure starch and pure CNW for comparisons during the next tests [36]. The sample which contains CNW and breadfruit starch extract is abbreviated as BC, the sample which contains breadfruit starch extract without CNW is called B, the control which consists of pure CNW and pure starch is called CC, the control that consists of pure starch without CNW is called C, commonly found plastics is called L for LDPE and LC for clear LDPE and lastly commonly sold bioplastic brand used for comparison is designated as P.

Tensile Strength Test

Each sample (B, BC, C, CC, L, LC, and P) was cut into 2.3 x 1.8 cm area [21] and is subjected to the Tensile Strength Test. The test was done using Metrotex MBT 15 – 1000 P Bonding – Tensile Tester which is done at PT. Grand Premier Plaspak, Desa Krikilan RT 005 RW 002 Krikilan, Driyorejo, Gresik. The resulting tensile strength number was calculated in g/Cm². The numbers were then processed using MiniTab 18 software to be tested using two sample T-test method with error levels of 5% (P<0,005) and was followed by an ad hoc test to analyse each means significant difference [41].

Soil Burial Test

Each sample (B, BC, C, CC, L, LC, and P) was weighed before being subjected to the soil burial tests. Afterwards, the samples were then buried in 3 different kinds of soils: Compost, humus, and sand soil for 15 days at 29°C and 50% humidity. And was analyzed for morphological changes such as cracks and weight changes [30]. The measurement for sample weigh changes was carried out every single day for the total 15 days period. For the last measurement for each sample which was carried out on the last day, it was compared directly to the initial measurement values by using the formula (initial values – final value) x 100% and divided by the initial value to find the weight loss of each sample [7].

Water Uptake Test

Each sample (B, BC, C, CC, L, LC, and P) was cut into 2.3 x 1.8 Cm size and weighed before being subjected to the water uptake test. Each sample were then completely submerged into distilled water for 1 minute. After one minute of submersion, the samples were lifted and dried using tissue paper. After dried, the samples were physically analysed and weighed again to find the weight difference to calculate its water uptake percentage using the following equation [31]. % water uptake = (final sample weight – initial weight) x 100% divided by its initial weight.

RESULTS

Chitin Extraction from blood clam (*Anadara granosa*) shell waste

From the starting 100 g of blood clam shell waste, 22.412 g of chitin was obtained from the acid hydrolysis chitin extraction method, with a yield value of 22.412 %, and from that 22.412 g of chitin, 7.311 g of CNW was produced which had the yield value of 32,62 %. The extracted chitin is physically seen as a white fine powder and was around 100 mesh in size, after undergoing formation into CNW, the resulting powder retains its white color but is reported to form small chunks of spike-like substance as shown in Figure 1.



Figure 1. Chitin Powder extracted from blood clam shell (left) and blood clam shell chitin nanowhisker (right)

CNW microscopic analysis

The resulting CNW was analyzed using an electron microscope at 5000- and 10000-times magnifications, from which the results, shown in Figure 2 were obtained. The picture shows that under

such magnifications, some CNW was found in sheet-like form shown in Figure 2 (a), and some in crystal-like form shown in Figure 2 (b).

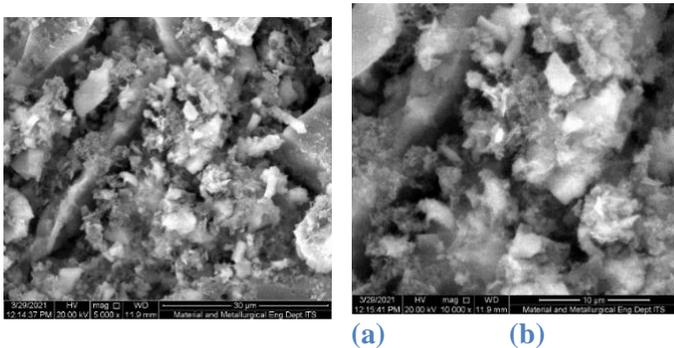


Figure 2. Chitin Nanowhisker samples viewed from an electron microscope, (a) 5000 x, (b) 10000 x.

Breadfruit peels waste starch extraction.

The starch extraction process yields 721.22 g of starch from 2000 g of breadfruit peel waste, which is 36.061%. The yield number is close to the theoretical breadfruit peel starch yield that was included in earlier studies of 39.56% the possible reason that caused the decrease in starch yield was because of the waste status that the breadfruit peel had at the time of the research. The starch extract was physically observed as a fine powder and off-white as shown in Figure 3.



Figure 3. Breadfruit peel waste extracted starch.

Breadfruit peel waste starch content quantification

The starch content quantification uses the starch-iodine test method. 721.22 g of starch was extracted from 2 kg of breadfruit peel waste. The starch quantification shows that the sample contains 28.9 mg/mL of starch. The measurement is done using the linear regression method as shown in Figure 4 which came from the standard curve that was made beforehand.

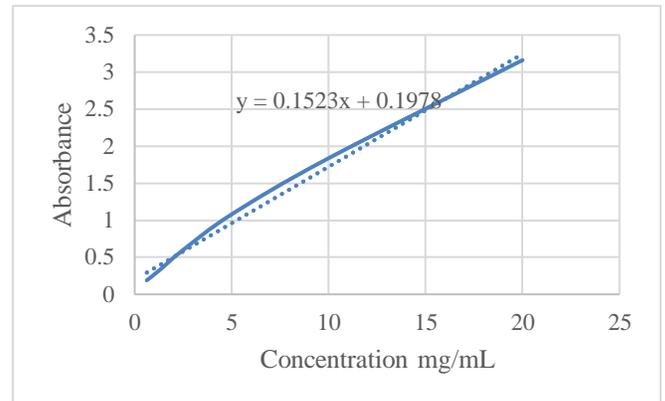


Figure 4. Linear regression of the standard curve.

Degradable bioplastic formulation

The degradable bioplastic made from breadfruit peel waste starch extract and CNW as an additive is light brown and had a relatively smooth surface as shown in Figure 5. The bioplastic forms a smooth surface in contact with the mold during drying. On the other hand, the side not in contact with the mold is slightly rougher in texture.



Figure 5. Breadfruit peel waste degradable bioplastic reinforced with CNW.

Tensile strength test

The result from the tensile strength test was directly compared to ASTM D882 – 12, which stated that the standard tensile strength for thin layer of LDPE plastic is 0.024 MPa. Shown in Table 1, is the results from the tensile testing process. Compared to the ASTM D882 – 12 standards, B and BC samples yield stronger tensile strength than the standard value for LDPE. Other than that, the result from two sample T – test ($P < 0.05$) shows a significant difference between B and BC, B and C, BC and C, and C and CC.

Table 1. Tensile strength test results

Sample	Replications			Tensile strength value (MPa)
	I	II	III	
B	0.058676	0.058673	0.058694	$0.0587^a \pm 1.14 \times 10^{-5}$
BC	0.099407	0.099402	0.099403	$0.0994^b \pm 0.24 \times 10^{-5}$
C	0.030924	0.030924	0.030943	$0.0309^c \pm 1.12 \times 10^{-5}$
CC	0.060409	0.060411	0.060400	$0.0604^a \pm 0.6 \times 10^{-5}$
L	0.043313	0.043303	0.042108	$0.0604^a \pm 6.92 \times 10^{-4}$
LC	0.043410	0.043211	0.042876	$0.0432^c \pm 2.6 \times 10^{-4}$
P	0.062141	0.060133	0.060177	$0.0608^a \pm 1.14 \times 10^{-3}$

Water uptake test

The water uptake test results that were obtained previously were directly compared to the standard that is ASTM D570 – 98, which states that the standard water uptake percentage for all type of plastics were 0.01 %, which both BC and CC didn't surpass.

Table 2. Water uptake test results

Sample	Replications			Water Uptake (%)
	I	II	III	
BC	94.077	90.211	93.854	92.714 ± 2.17
CC	260.134	242.065	211.451	237.883 ± 24.6
L	0	0	0	0 ± 0
LC	0.758	0.801	0.786	0.781 ± 0.021
P	36.15	36.021	34.099	35.423 ± 1.148

Soil burial test

The weight change of the samples that were subjected to the soil burial test was directly compared to the standard values of PLA and PCL plastic film's soil degradation which were included in the ASTM 5336 standard for said plastic films, the standard weight loss for those plastic films in 60 days were 100 %.

Table 3. Sample weight loss after being subjected to the soil burial test.

Sample	Weight loss (%)		
	Humus	Sand	Compost
BC	97.467 ± 1.774	37.896 ± 1.836	97.545 ± 1.643
CC	76.063 ± 1.452	10.841 ± 2.001	89.054 ± 3.21
L	53.846 ± 3.444	12.881 ± 2.545	48.088 ± 2.897
LC	43.137 ± 2.912	6.497 ± 2.129	30.617 ± 1.218
P	76.063 ± 2.041	28.291 ± 3.001	46.344 ± 2.318

DISCUSSIONS

Chitin extraction was done using deproteination followed by demineralization method. The deproteination method was done to separate the protein contained in the blood clam shell

waste from the rest of its composition, by doing so it helps with chitin purification. During the deproteination process, 4% NaOH was used to hydrolyse the amino acids in the blood clam shell waste sample^[13]. This process was followed by demineralization process which was done using 1M

HCl to dissolve the metal ions contained in the shell waste, as a byproduct CO_2 was produced, which was observable by the fizzing phenomenon that happened during the process. The chitin yield of 32.62 % doesn't differ much from its theoretical value of 35 % [27] even with the clam shell's status as a waste, because it is relatively hard to decompose quickly because of its calcium carbonate content. After the chitin extraction, the chitin powder was subjected to the acid hydrolysis method to form CNW with the help of 0.1 M HCl. HCl was used to hydrolyse the amorphous chitin, leaving alone the crystalline chitin. Despite its downsides of high amount of acidic wastewater and some of the chitin being deacylated [19], this method is still desired because the needed ingredients are relatively cheaper and easier to obtain.

As shown in Figure 3, the sheet – like CNW will be formed when CNW samples were introduced to extremely low temperatures such as -80°C which was used to freeze the chitin sample before freeze-drying. The crystalline form on the other hand, was its original form. Also shown in Figure 3, was the individual crystal structure packing tightly together caused by the freeze – drying process, which then forms those spiky structures [19]. As the CNW was intended to be used as strength reinforcement for the degradable bioplastic, it needs to be more easily dispersed throughout the bioplastic's surface area, as such the crystalline form that CNW has was profiting [5].

The starch extraction process was done using physical method, in which it had achieved the yield % of 36.061, the obtained yield percentage was less than what was found at a literature [24]. This was due to how the breadfruit peel waste was already been in the waste bin for around 2-4 hours, which within those hours degradation process would have started and the starch component would have been starting to be degraded as it made up around 1/3 of the whole peel waste and would then be the main source of carbon for the microorganism trying to devour it. The quantification is done using the iodine – starch test. The test was done on room temperature ($293 - 303 \text{ K}$) and the measurement using spectroscopy methods was carried out instantly after the color starts to show to avoid color loss and inaccuracy [6]. The absorbance results were then substituted to the regression equation shown at Figure 5. Linear regression was used because the relation between absorbance and the concentration of starch was linear [32].

The brown color of the dried bioplastic, as shown in Figure 6 was the result of heating the bioplastic mould mixture which contained starch, glycerol, distilled water and CNW. During the heating process, the mixture was subjected to heating using an oven at 45°C for 24 hours, and during the time, Maillard's reaction occurs between the reducing sugars still present on the starch extract and the amine groups of proteins still contained in the mixture [18]. Also observable in the bioplastic from Figure 6 was that it formed a convexed form, this happened because during moulding and drying the starch content of the mixture gelatinized because of the heating at 80°C that was done prior to moulding [37]. The smooth but uneven surface of the produced bioplastic was caused by the water bubbles popping during moulding and drying process which leaves uneven but smooth surface.

The tensile strength test results shown in Table 1, was affected by certain factors which were the type and amount of additives used to strengthen or to create an overall better bioplastic, the aspect ratio of the bioplastic, the treatment process of the reinforcement material, the physical treatment introduced to the polymer forming ingredients, chemical treatments, and biological treatments. Milling and blending were introduced to the breadfruit peel waste, these processes reduce the starch's crystallinity which usually ranges from 15 to 45 % [6]. The reduced crystallinity had a positive effect on the formed bioplastic's hydrolysis rate and its mass transfer characteristics [5], it also helps the starch particle to distribute more evenly because of its smaller particle size [43]. Other than the aforementioned physical treatment, chemical treatment was also done during CNW production process. In which acid hydrolysis was used to hydrolyse the amorphous chitin, only leaving crystalline formed chitin. Other than those physical and chemical treatments, the CNW as a strength reinforcer and glycerol as a plasticizer also plays a main role in determining the finished bioplastic's tensile strength. The amount of CNW added to the moulding mixture represents the strength reinforcement the CNW gave to the formed bioplastic, the 0.125 g of CNW added was done so by keeping in mind the finished bioplastic's plasticity, because too much strength reinforcement will create a stiff and brittle bioplastic [6]. Other important factor that affect the tensile strength of the bioplastic is the presence of glycerol as its plasticizer

and CNW as the reinforcer. Glycerol as plasticizer works by forming hydrogen bonds with hydroxyl groups found on the starch molecule, which then in turn destroys the bond between hydroxyl groups that was already present^[35] this gave the formed bioplastic its elasticity^[39]. The reason why glycerol was chosen as plasticizer, was because of its high plasticizing capacity and stability across a broad range of temperature^[4]. Meanwhile, CNW works by creating a network of CNWs across the surface of polymers such as the degradable bioplastic^[8], which strengthen the overall mechanical properties of the degradable bioplastic.

The water uptake test results shown in Table 2 shows that the samples coded B for breadfruit and CNW bioplastic and C for pure starch and pure CNW bioplastic were not able to surpass the international standard for plastics with 1mm thickness. This was caused by the ingredients that formed the degradable bioplastic. The breadfruit peel waste starch extract contains starch which was highly hydrophilic^[13]. This characteristic made starch-based bioplastics easier to dispose in wet areas, such as bodies of water and soil. Being the reason of high-water absorption percentage, using starch as the main ingredient for the degradable bioplastic does come with some advantage such as ease of raw material collection and ease of bioplastic disposal^[33]. The comparison was done against ASTM D570 – 98 which was the international standard for plastics water uptake percentage this shows that other than its ingredients, the other factor that leads to the degradable bioplastic not being able to surpass said standard was because the standard was measured for common plastics such as PETs and LDPEs, which were fully synthesized to have very low water sensitivity^[24].

The soil burial test results were directly compared to ASTM 5336, the international standard for PLA and PCL plastic films, in which was stated that during 60 days of soil burial, the samples should be 100% gone. Shown in Table 3, sample B and C were almost gone by the fifteenth day of soil burial which leads to the conclusion that those bioplastic samples were soil – degradable. There are factors that affect the results of this test. Such as, soil temperature, pH, humidity, and the bioplastic's ingredients^[23]. The degradable bioplastic samples were made using 100 % natural ingredients which can be degraded naturally, this includes starch, CNW and glycerol which is the 3 main ingredients of the degradable bioplastic^[3]. Other than that, another

notable phenomenon was how after the initial day of burial, the weight of B and C samples across all soil platforms shot up rather than going down, this happened because the bioplastic samples were absorbing water from the surrounding soil because of the starch's high water absorbing capacity^[10].

CONCLUSION

Based on the achievement and results obtained from this research, a conclusion was able to be reached, which was starch extracted from breadfruit peel waste, chitin nanowhisker made from blood clam shell waste chitin and glycerol as the plasticizer has a potential to be used as the main ingredient of a degradable bioplastic. The tensile strength of the degradable bioplastic produced during the research of 0.0587 MPa was able to surpass the international standard of 0.024 Mpa. It can also be used as proof that the presence of CNW as a strength reinforcer may nearly double the tensile strength of degradable bioplastic to 0.0994 MPa, another characteristic to note is that the produced bioplastic still has a relatively low water resistance which was proven during the water uptake testing process in which the sample (BC) reached 92.714% water uptake. The produced degradable bioplastic was able to degrade naturally during the soil burial test for as long as 15 days, with 97.467% weight loss in humus soil, 37.896 % in sand soil, and 97.545 % in compost soil, which has proven that the compost soil is the best choice to naturally degrade the degradable bioplastic.

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