



CLUSTER ONE

MATERIAL & FOOD SCIENCE

The papaya structure symbolizes a commitment to exploring preservation methods, a focal point within the realm of food science. The mosaic-style depiction of the papaya is a nod to the investigation into innovative materials like bioplastics, drawing a connection to the study of eggshells. Additionally, the plastic encompassing the papaya signifies the exploration into the world of microplastics within this cluster. Each element of the logo seamlessly weaves together the diverse aspects of research pursuits, creating a visual representation of our multidisciplinary approach to scientific inquiry.

Material science is a multidisciplinary field that delves into understanding the properties and applications of materials. On the other hand, food science focuses on scrutinizing food production, processing, and preservation methods to ensure safety and elevate quality. Studies within this cluster further emphasize areas such as food quality assessment, bioplastics, and microplastics, illustrating the interdependence of the two scientific disciplines in contributing to advancements in materials and food technology.

Mechanical properties of bioplastics of various starch to eggshell reinforcement ratios

JOAQUIN J. CORDOVA, KAREN GRACE S. FARPARAN, and ELIZALDE MIGUEL S. FLORES

Philippine Science High School Western Visayas Campus - Department of Science and Technology (DOST-PSHS WVC), Brgy. Bito-on, Jaro, Iloilo City 5000, Philippines

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Abstract

Bioplastics are biodegradable polymers derived from the starch of natural and renewable sources such as tuber crops. While it is possible to create bioplastic with cassava starch, the resulting product has high moisture content and poor mechanical properties. In addition, the effects of varying the ratios of eggshell reinforcements on the mechanical properties of cassava-starch-based bioplastics have yet to be studied. The purpose of this study is to determine the optimal ratio of *Manihot esculenta* starch to eggshell reinforcement through comparison treatments and control groups. Unsuitable drying methods, improper storage, and handling caused multiple failed samples. This included the development of molds, samples not drying, and the inability to peel samples from the mold. Further development of better conditions in formulating plastic from cassava starch along with assessment of other properties should be investigated.

Introduction. - Bioplastics include all plastics that are derived from biodegradable resources. The importance of bioplastics lies in their biodegradability and source due to them being mainly composed of biological wastes. The study on bioplastics also has advantages for the environment due to their low carbon footprint and low energy cost in manufacturing [1]. While it is possible to create bioplastic with cassava starch, its unique chemical structure and processing behavior results in a product that has high moisture content and poor mechanical properties as stated by Xie et al. [2].

Existing research utilized cassava starch in bioplastic development with additional reinforcements to strengthen the mechanical properties of their plastics [3,4,5]. These reinforcements increased the tensile strength as well as the biodegradability of their bioplastics based on their results. Furthermore, other studies utilized eggshells for reinforcing their bioplastics from cornstarch and potato starch [6,7]. Results showed that reinforcing bioplastics with eggshells decreased the water absorption and increased tensile strength of the bioplastic. However, the cited studies only used a singular specific ratio during the conduct of comparative analysis between samples, the variation of the ratio of reinforcements and starch composition and the effect of these changes on the mechanical properties of cassava-starch-based bioplastics is yet to be investigated.

Based on Xie et al's [2] study, the creation of a starch-based bioplastic with the integration of a viable plasticizer and reinforcement ratio is necessary. Calcium carbonate or CaCO_3 reinforcements have properties that are

hydrophobic, this in turn causing the bioplastic sample to repel the water [4]. Eggshells contain approximately 95% calcium carbonate and 5% organic materials [19]. Therefore, the presence of CaCO_3 can increase the strength of the mechanical properties of the bioplastic. With the addition of reinforcements, the mechanical properties of the plastic would improve. Studies have stated that the integration of plasticizer and reinforcement is necessary; however, the amount used for these components in respect to the amount of cassava starch may negatively affect its tensile strength and water absorption. Thus, varying the ratios of reinforcements to the matrix is essential to determine how they would change the tensile strength and water absorption of the plastic.

The aim of the study was to determine the optimal ratio of *Manihot esculenta* (cassava) starch to eggshell reinforcement by comparing the water absorption and tensile strength. With this, the objectives of the study were:

- (i) To evaluate the tensile strength of the bioplastic treatment groups containing 10%, 15%, and 30% eggshell reinforcements in comparison to the control group (0% reinforcement);
- (ii) To evaluate the rate of water absorption among the bioplastic treatment groups, 10%, 15%, and 30% reinforcements;
- (iii) To evaluate the tensile strength and water absorption of the various treatment groups and analyze the data using ANOVA or Kruskal-Wallis test if the distribution of the data is not normal; and



(iv) To identify the functional groups present in the various ratios of bioplastic using Fourier-Transform Infrared Spectroscopy (FTIR) spectrometer and compare their spectra.

Methods. - The methods of the study were done in seven steps. These steps consisted of: preparation of materials, formulation of the samples, casting and drying, tensile strength test, water absorption test, and Fourier-transform infrared spectroscopy. The methodology of the study included several changes during the different trials in the formulation of the plastics. All the methods and changes were stated in each step.

Preparation of materials. Medium sized (21 oz.) chicken eggs were utilized as the source for 120 grams of eggshells. The shells were thoroughly washed with distilled water and then dried in a preheated forced-air oven at 60°C for 10 minutes. Once no excess water was observed in the eggshells, these were powderized inside an industrial blender (2.0L Drink Machine; 220 V) for 3 minutes.. Subsequently, the resulting eggshell powder was initially sieved using a mesh size 60 sieve (<0.250 mm), followed by a 30 µm sieve to ensure a uniform and fine particle size.

For the preparation of the cassava starch, the starch was initially sieved using a mesh size 60 sieve before oven drying. The starch was oven-dried for 30 minutes at 60°C using a Biobase BOV-V65F oven of the PSHS-WVC laboratory before it was used for the formulation of the samples.

Formulation of the bioplastic. The conduct of the study involved the following treatment groups:

Control Group = 100: 0 with 5 g starch and 0 g eggshell reinforcement

Treatment 1 = 90: 10 with 4.5 g starch and 0.5 g eggshell reinforcements

Treatment 2 = 85: 15 with 4.25 g starch and 0.75 g eggshell reinforcements

Treatment 3 = 70: 30 with 3.5 g starch and 1.5 g eggshell reinforcements

Starch and eggshell reinforcement were mixed with 70 mL of distilled water. The mixture was then stirred starting from 350 RPM for 2 to 3 minutes until the starch had been dissolved by the water. Five (5) mL of glycerol and 1.5 mL of 0.1 M acetic acid were then added to the same mixture. In a separate beaker, 1.25 g of polyvinyl alcohol (PVA) was placed in a separate beaker and dissolved with distilled water in a 1:24 mass-to-volume ratio. The PVA-water solution was stirred with a magnetic stirrer at 350 RPM until the polyvinyl alcohol was visually observed to be visually observed to be dissolved in water. The contents of the two separate beakers were then combined in a singular 400 mL beaker. The mixture was then heated on a hot plate at an internal temperature range of 60 to 70°C. The content after all the components have been added was stirred

gradually with a magnetic stirrer for 30 minutes. The formulation involving the diluted PVA solution was only used for trials 1 to 4. The rest of the trials followed the same formulation without the polyvinyl alcohol. The solutions formulated during trials 4 to 9 were also subjected to degassing using a Bransonic ultrasonic bath.

Casting and drying of samples. After the mixture was stirred and heated, the mixture was poured into a 15 cm x 8 cm aluminum pan. The initial trial followed this procedure however, further development prompted the researchers to change the container to a glass Petri dish with a diameter of 9 cm. This method was followed for trials 2 and 3. For the succeeding trials, 4 to 9, a plastic petri dish with similar dimensions was utilized in the casting of the bioplastic mixture. Once the mixture was casted into the Petri dish, the samples were subjected to oven drying for 30 minutes at 60°C. After the oven-drying process, the Petri dishes were transferred to a fume hood to be air-dried for a period of 48 hours. Trials 4 to 9 were also sprayed with 99% ethanol to eliminate surface bubbles.

Tensile strength test. After the bioplastic samples had fully dried in their respective molds, a minimum of 100 mm length of sample was required to be tested using the Ametek Brookfield Texture Analyzer for food-grade and low-density samples. It was then cut and peeled from the casting molds. The samples were contained and sealed in a container which was transported to the University of the Philippines Visayas Regional Research Center Food Laboratory for further testing.

Water absorption test. ASTM-D570 was used to measure the percent of water absorption [4]. Samples with dimensions of 1 cm x 3 cm were cut from the bioplastic sheets peeled from the casting mold (i.e., petri dish). These samples were created using a centimeter ruler and were cut from the bioplastic sheet using a scalpel from a dissecting kit. The initial weight of the sample was measured using an analytical balance (KERN ABJ-NM). Then, it was placed inside a 250 mL beaker containing 100 mL of distilled water for 24 hours. Once the immersion period had ended, the sample was cloth-dried until no water could be retrieved from its outer surface. The final weight was recorded using an analytical balance. Similarly, the percentage of water absorption was solved through this formula:

$$WA(\%) = \frac{(FW - IW)}{IW} \times 100\%$$

Where:

WA = Water Absorption

FW = Final Weight of the Sample Post-Immersion

IW = Initial Weight of the Sample Pre-Immersion



FT-IR Spectroscopy. The samples were subjected to FT-IR analysis to determine the presence of bonds and functional groups present. A 1 cm x 1 cm sample was cut from the prepared bioplastic sheets for testing. A Shimadzu IRAffinity-1S FTIR equipped with a QATR 10 was used to scan the samples.

Disposal and safety procedures. Wearing of laboratory personal protective equipment (PPE) was observed during the handling of glassware, equipment, reagents, and samples. Used gloves, unused eggshells, and disposable containers were properly disposed into designated disposal bins inside the chemistry laboratory of PSHS-WVC.

Results and Discussion. - The aim of the study is to determine the optimal ratio of eggshell reinforcement to *Manihot esculenta* (cassava) starch. This section includes the results and key findings from the following tests: tensile strength, water absorption, and FTIR analysis.

Tensile Strength. A total of nine (9) trials of bioplastic samples were created and subjected to a feasibility test for the tensile strength test. The feasibility test has three criteria which need to be met. The samples must: (1) have the prescribed minimum operating length of 100 mm as required by the Ametek Brookfield Texture Analyzer; (2) have an even surface area [8]; and (3) be dry after the conduct of the following drying methods: (i) air-drying - the sample is dry at room temperature (30°C) after 48 hours. (ii) oven-drying - the sample is dry after 30 minutes at a temperature of 60°C inside a biobase forced air oven. In the evaluation of the tensile strength of the produced samples, none of the samples were subjected to the procedure due to the failure to produce a viable material for testing.

The key finding which affected the viability of samples is the occurrence of clumping. The clumping of polyvinyl alcohol granules was observed in the circumference and center of the petri-dish containing samples sourced from trials 2, 3, and 4 of all the prepared reinforced samples. Clumping resulted in the strong adhesion of the samples to the casting mold and the uneven distribution of the polymer throughout the mixture. This defect can be further attributed to the undissolved PVA present in the casting slurry. Studies point out that for the PVA to be dissolved it must be within a temperature range of 60°C to 70°C to avoid hydrolysis and must be at a stirring speed of 200 RPM to minimize bubbling at the surface [9,10]. The clumping of the polymer matrix in the samples can be explained by the uneven distribution of components in the mixing and casting process of the bioplastic [11]. Therefore, factors such as the RPM rate of the magnetic stirrer and the temperature range of polyvinyl substances must be monitored to ensure that it can be incorporated into the mixture into a dissolved form. Failure to take note of these components had led to uneven dissolution of granules. Without samples meeting the aforementioned criteria, the conduct of the tensile strength test was no longer feasible.

Water Absorption. The water absorption test involves the use of ASTM-D570 in order to determine the percent of water absorption. The procedure is used to assess the hydrophobic properties brought about by the eggshell reinforcement [4]. The data analysis process consists of the Shapiro-Wilk normality test followed by the Analysis of Variance with 5% confidence level or the Kruskal-Wallis test depending on the normality of the distribution.

The replicates of the reinforced samples were subjected to the water absorption feasibility test. As adapted from previous studies, the following criteria must be met by the samples prior to the test. The samples must: (1) have a size of 1 cm x 3 cm [4]; and (2) not contain signs of fungal growth as it would affect the water absorption of the samples [12].

A key finding observed during the post-casting phase is the occurrence of glycerol leaching which prevented the drying of the samples within the prescribed time period. The humidity of the storage area of the sample undergoing air-drying is also a factor that contributed to the inability of the samples to dry within the prescribed time. Previous studies such as that of Jones et al. [13] have expressed that glycerol leaching would render samples unsuitable for usage and testing [13]. This phenomena negatively affects the viscoelastic properties of the bioplastic and weakens the hydrogen bonds within the product. While the water absorption test does not depend on the weight of the sample to be utilized but rather the percent increase of the sample under water immersion, the failure to extract suitable samples is a hindrance to perform the test on the prescribed amounts of samples. The conduct of the procedure was limited by the presence of moisture which caused the wetness of the sample, which consequently could not be subjected to the test. Additionally, fungal growth in the sample is another factor that prevented the conduct of the procedure.

Statistical Analysis. With the failure to conduct the tensile strength test and water absorption tests, statistical analysis was not conducted. The two aforementioned tests failed to provide any numerical data because of the insufficient number of samples that could be extracted while meeting the prescribed criteria for each test. As previously mentioned, samples that could meet the required size for the conduct of the tensile strength test were not possible. Additionally, a significant number of samples (i.e., samples 1, 2, 5, 6, 7, 8, and 9) presented mold growth and presence of surface moisture which hindered the conduct of the water absorption test. Crops producing tubers or roots have specific requirements for storage because of their high moisture content (60 to 80%) [14]. Flour with high moisture content will be more vulnerable to mold growth [15]. In line with this, proper storage and drying of the cassava starch must be done in order to prevent mold growth during the formulation and drying of samples. Additionally, ensuring that the samples are fully dry is essential for the tests to be conducted.



FTIR Results. FT-IR spectroscopy was only conducted on the samples containing 10% and 15% eggshell reinforcements and the control group. The results of the various treatments were compared with each other in regard to the identification of functional groups present. The analysis of the spectra was limited to the wavelength of the functional group region. Moreover, FTIR analysis through the identification of the functional groups presence confirmed that the dried product is defined as a bioplastic [11]. The presence of C-H bonds and O-H bonds is brought about by the polymerization of starch and water. The two components undergo polymerization with the addition of heat. This reaction results in the presence of C-H, O-H (carboxylic acid) and O-H (alcohol) functional groups, thus forming polymer chains. The common functional groups reflected by the extracted samples with respective starch-to-reinforcement ratios of 0% (control group), 10%, and 15% reinforcement content indicate the presence of the following bonds: C-H, O-H (carboxylic acid) and O-H (alcohol). The analysis of the spectra was restricted to the region to the left of 1400 cm^{-1} which is defined as the “functional group region”. Meanwhile, the range between 600 and 1400 cm^{-1} is defined as the “fingerprint region”. The interpretation of the results was focused on the “functional group region” as it is where most stretching frequencies occur and the “fingerprint region” was not integrated into the findings since the overlapping of bonds is prone to unreliable interpretation. All functional groups that are relevant to the creation of bioplastic are present within the spectra and peaks have also been observed within the prescribed range.

Recommendations. In order to obtain bioplastic that has better characteristics, the researchers highly recommend to:

- Use a desiccator for sample storage to prevent excess moisture and prevent possible mold growth.
- Explore the effects of other starch to reinforcement ratios in the creation of bioplastic. Further modification of the proportion of reagents such as acetic acid to the volume of the mixture should also be taken into consideration to provide a broader range of options in the optimization of the mechanical properties of the product.
- Refine the air-drying method through storing the samples in an area which prevents the build-up of moisture while simultaneously protecting the contents of the casting mold from contamination.
- Consider factors such as temperature and relative humidity of the working and drying area of the samples inside the laboratory.
- Perform FTIR analysis for samples from various trials to discover trends and the consistency of the data procured; it is also

advised to explore other data sets of wavelengths that could be made to determine the presence of other functional groups.

- Compare FTIR graphs of glycerol and the dried casted mixture to confirm that glycerol leaching has occurred in selected samples that feature branch-like patterns on the surface.

Limitations. The bioplastic samples had high moisture content attributed to poor drying conditions and a lack of appropriate facilities. With this, the samples were not extracted and were not viable to be subjected to the tensile strength testing. In addition, the presence of molds contributed to the reason why the samples were not viable to be subjected to the water absorption test. The data analysis through ANOVA or Kruskal-Wallis test was not conducted due to the absence of quantitative data that had to be collected from the tests. With regard to the FTIR analysis, the data set of the wavelength was limited to the functional group region. From a chemical standpoint, it is limited to the enumeration of functional groups within the said region found during analysis. This is defined as the region to the left of 1400 cm^{-1} in the spectra and is where most stretching frequencies occur. As such, peaks in the fingerprint region were not noted in the presentation of the findings. The absence of a confirmatory test for bioplastics hindered the classification of the produced material to a specific type of bioplastic. This limited the researchers to the evaluation of present functional groups based on the necessary functional groups prescribed for the polymerization and formation of bioplastic [15]. The basis for the functional groups evaluation was the library database of the LabSolutions IR software.

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References

- [1] Ramadhan MO, Handayani MN. 2020. The potential of food waste as bioplastic material to promote environmental sustainability: A Review. IOP Conference Series: Materials Science and Engineering 980:012082.
- [2] Xie F, Pollet E, Halley PJ, Avérous L. 2013. Starch-based nano-biocomposites. Progress in Polymer Science. 38(10-11):1590–1628. doi: 10.1016/j.progpolymsci.2013.05.002.
- [3] Maulida, Siagian M, Tarigan P. 2016. Production of Starch Based Bioplastic from Cassava Peel Reinforced with Microcrystalline Cellulose Avicel PH101 Using Sorbitol as Plasticizer. Journal of Physics: Conference Series. 710:012012. doi:10.1088/1742-6596/710/1/012012.



- [4] Abidin NDZ, Azhar NS, Sarip MN, Hamid HA, Nasir NAHA. 2021. Production of bioplastic from cassava peel with different concentrations of glycerol and CaCO₃ as filler. 4TH International Sciences, Technology and Engineering Conference (ISTEC) 2020: Exploring Materials for the Future. doi:10.1063/5.0043482.
- [5] Dasumiati, Saridewi N, Malik M. 2019. Food packaging development of bioplastic from basic waste of cassava peel (*Manihot utilisima*) and shrimp shell. IOP Conference Series: Materials Science and Engineering. 602:012053. doi:10.1088/1757-899x/602/1/012053.
- [6] Kasmuri N, Zait SMA. 2018. Enhancement of Bio-plastic using Eggshells and Chitosan on Potato Starch Based. International Journal of Engineering & Technology. 7(3.32):110–115. doi:10.14419/ijet.v7i3.32.18408. [accessed 2020 Oct 5]. <https://www.sciencepubco.com/index.php/ijet/article/view/18408/8333>.
- [7] Mohamad SFS, Ramli NIT, Shohaimi NAM, Zainuddin NS, Lothfy FA. 2018. Production of Biodegradable Plastic from Eggshell.
- [8] Nandiyanto A, Triawan F, Suryani I, Budiman B, Suryani I. 2020. The Influence of Turmeric Microparticles Amount on the Mechanical and Biodegradation Properties of Cornstarch-based Bioplastic Material: From Bioplastic Literature Review to Experiments. Materials Physics and Mechanics. 46(1): 99–114. doi:10.18720/MPM.4612020_10
- [9] Syamani FA, Kusumaningrum WB, Akbar F, Ismadi, Widyaningrum BA, Pramasari DA. 2020. Characteristics of bioplastic made from modified cassava starch with addition of polyvinyl alcohol. IOP Conference Series: Earth and Environmental Science 591:012016.
- [10] Putra EP, Thamrin ES, Saputra H. 2019. Effect of dragon fruit skin extract (*hylocereus costaricensis*) on bio-plastic physical and mechanical properties of cassava starch and polyvinyl alcohol. IOP Conference Series: Earth and Environmental Science 258:012047.
- [11] Asrofi M, Sapuan SM, Ilyas RA, Ramesh M. 2021. Characteristics of composite bioplastics from tapioca starch and sugarcane bagasse fiber: Effect of time duration of ultrasonication (bath-type). Materials Today: Proceedings 46:1626–1630.
- [12] Heimerdinger G. The Science of Mold. Restoration Remediation Magazine RSS. 2020 Sep 3 [accessed 2023 Mar 21]. <https://www.randrmagonline.com/articles/88929-the-science-of-mold>
- [13] Jones A, Zeller MA, Sharma S. 2013. Thermal, mechanical, and moisture absorption properties of egg white protein bioplastics with natural rubber and glycerol. Progress in Biomaterials. 2(1). doi:10.1186/2194-0517-2-12
- [14] Hayma J. 2003. AD31E The storage of tropical agricultural products. Agromisa Foundation.
- [15] Wullandari P, Sedayu BB, Novianto TD, Prasetyo AW. 2021. Characteristic of semi refined and refined carrageenan flours used in the making of biofilm (bioplastic). IOP Conference Series: Earth and Environmental Science 733:012112.
- [16] Amin MdR, Chowdhury MA, Kowser MdA. 2019. Characterization and performance analysis of composite bioplastics synthesized using titanium dioxide nanoparticles with corn starch. Heliyon 5.
- [17] Yusoff ZB. 2016. Production of Biodegradable Plastic from Banana Peel. Journal of Petrochemical Engineering Department. 1(1):1-7.
- [18] Syuhada M, Sofa SA, Sedyadi E. 2020. The Effect of Cassava Peel Starch Addition to Bioplastic Biodegradation Based On Chitosan On Soil and River Water Media. Biology, Medicine, & Natural Product Chemistry. 9(1):7–13. doi:10.14421/biomedich.2020.91.7-13.
- [19] Jiang B, Li S, Wu Y, Song J, Chen S, Li X & Sun H. 2018. Preparation and characterization of natural corn starch-based composite films reinforced by eggshell powder, CyTA - Journal of Food. 16(1): 1045-1054. doi:10.1080/19476337.2018.1527783.



Abundance of microplastics in a river-estuary along a rural-urban gradient pathway

J IAH G. SARGADO, HANSEN MAEVE C. QUINDAO, and MARIA DEANNA JOLITO

Philippine Science High School Western Visayas Campus - Department of Science and Technology (DOST-PSHS WVC), Brgy. Bito-on, Jaro, Iloilo City 5000, Philippines

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Abstract

Microplastics are rapidly accumulating in quantity. Exposure to microplastics can be a threat to ecosystems as mammals ingest 0.1 to 5 grams of microplastics per week. This study sought to determine the abundance of microplastics found along a rural-urban gradient in Iloilo River. Samples were gathered from five sites of differing parameters, treated to remove non-microplastic substances, and identified through a compound microscope. Results showed that the abundance along the river ranged between 1 to 4 particles per liter. The unmonitored rural sites had the highest abundance and the monitored urban sites had the lowest abundance. Additionally, most of the microplastics gathered were in the size range of 150 to 500 μm , transparent in color, and are film morphological type. Investigation of additional parameters like area usage may be needed to understand microplastic abundance patterns as there was no strong correlation between urbanization and microplastic abundance.

Introduction. - Plastic is one of the most widely accessible and mainstream production resources. It is reported that the Philippines uses roughly 59.7 billion sachets and 20.6 billion shopping bags annually [1]. The staggering amount of single-use plastic consumed yearly is one of the greatest obstacles in current efforts in waste management [1].

Microplastics are a type of plastic debris that is less than five millimeters (5 mm) in length, and they originate from the degradation of macro plastics and manufacturing runoffs [2, 3]. Its existence has been seen in the air, waterways, sediments, and in the digestive system of animals [4, 5]. The threat of microplastics has prompted research on the material and its effects on ecosystems and human life [6]. Humans have been heavily exposed to microplastics through ingestion, inhalation, and dermal contact [7, 8]. Ingestion is thought to be the main pathway, due to humans' high trophic level in the food chain [2]. Ingestion allows humans to consume 0.1 to 5 grams of microplastics per week, or 1,000 to 3,000 microplastic particles per year into the body via food products [7]. This may lead to chromosome alteration, cancer, and increased vulnerability to neuronal disorders [8].

Abundance is the measure used to determine how much microplastics are present in the water, usually measured per m^3 or liter [9]. Understanding the microplastic abundance along a river's course is a critical factor in identifying the key sources of microplastic and in developing management interventions. Moreover, the morphological type and the physical characteristics of the microplastics are also important in identifying the possible sources of the microplastics. They are one of the many ways to tell the origins of the plastic as certain

morphological types retain their characteristics despite degradation. A rural-urban gradient pathway is a river with one continuous pathway that crosses through a mixture of rural and urban areas. Rivers in urban areas can contribute to the microplastic concentrations in the ocean, specifically the microplastics found in the surface water since they can easily be transported by tides.

Iloilo City (10.7202° N, 122.5621° E), the provincial capital of Iloilo, Philippines, has a population density of 5,842 people per km^2 [10]. Due to its high population density, the presence of microplastics can pose a hazard to humans and animals alike [11]. The Iloilo River is a river-estuary landmark that spans over 10 km in length [12]. It starts in the municipality of Oton where the surrounding barangays have less than 5,000 inhabitants and thus are classified as rural [13]. The river's surroundings transition from rural to urban as it enters the populated city, thus making it a rural-urban gradient pathway. There is a need for microplastic studies in freshwater bodies, specifically in South East Asian countries, where there is a trend of rapid urban development but poor waste management [14].

This study aims to provide information regarding the abundance of microplastics in the surface water of a rural-urban gradient pathway. The microplastics were identified through sample collection, filtration of microplastics, microscopy, and classification. Additional data such as microplastic physical characteristics and morphological type were also collected. It specifically aims to:



- (i) To quantify the number of microplastics found per area via visual counting;
- (ii) To calculate the abundance (in particles per liter) of microplastics found per site;
- (iii) To determine the most prevalent physical characteristics (size and color) among the microplastics found;
- (iv) To determine the most prevalent morphological type among the microplastics found; and
- (v) To determine the trend line in the abundance of microplastics in different sections of the Iloilo River.

Methods. - The data gathering procedure was divided into four groups: sample collection, sample treatment, sample identification, and data analysis.

Sampling Site. The research study took place in the Iloilo River in Iloilo, Philippines, which is approximately 14.5 km in length. The river's starting point is located in the municipality of Oton. It stretches across the Iloilo city districts and ends at the Iloilo Port located between the Iloilo City Proper and the Lapuz district.

There are three categories for a barangay to be considered urban [13]. The first category of an urban barangay should have at least 5000 inhabitants. The second category requires at least one establishment with a minimum of 100 employees in the barangay. The third category states that there should be five or more establishments with 10 to 99 employees within the two kilometer radius of the barangay hall [13]. Meanwhile, a barangay is considered rural if it does not fit any of the urban criteria.

The Iloilo River is considered a rural-urban gradient pathway. A rural-urban gradient pathway is a body of water that is surrounded by both rural and urban areas. The river's origin point, Oton, is a rural zarea because it has less than 5000 inhabitants. Meanwhile, the Iloilo Port area fits all of the criteria,

and is thus labeled as urban. On the other hand, there is a mixed gradient of urban and rural barangays surrounding the area from the start to the end of the river.

Table 1. Sampling Sites Details

Site	Code	Distance	Coordinates	State of Area
1	U _M R1	1.46 km	10.69479°N, 122.49888°E	Unmonitored Rural Area
2	U _M R2	4.54 km	10.69632°N, 122.52166°E	Unmonitored Rural Area
3	U _M U3	7.76 km	10.70032°N, 122.54170°E	Unmonitored Urban Area
4	MU4	9.67 km	10.70167°N, 122.55886°E	Monitored Urban Area
5	MU5	12.08 km	10.69618°N, 122.57207°E	Monitored Urban Area

Sample Collection. Systematic sampling was used to determine the five general sites, with equal distance between each site as shown in Table 1. Ten liters of water samples were collected from each of the five sites from Iloilo River, Philippines as shown in Figure 1.

The sampling area proper is in the center of the bodies of water: each site was at least 2.4 km away from the other, at least 5 meters away from the riverside, and 0 to 12 centimeters below the surface for each site [15]. Moreover, the weather, time, and pin location of the collection was documented for each site.

The water samples were wet-sieved using stacked mesh sieves (5 mm, 2 mm, 1 mm, 0.3 mm, and 0.15 mm) in order to obtain the particle samples [15, 16]. These were then transported to the lab.

Sample Treatment. Microplastics are removed from the sieve using a vacuum pump with a 0.47 µm pore size filter paper [16]. Next, samples underwent Wet Peroxide Oxidation (WPO). The samples were

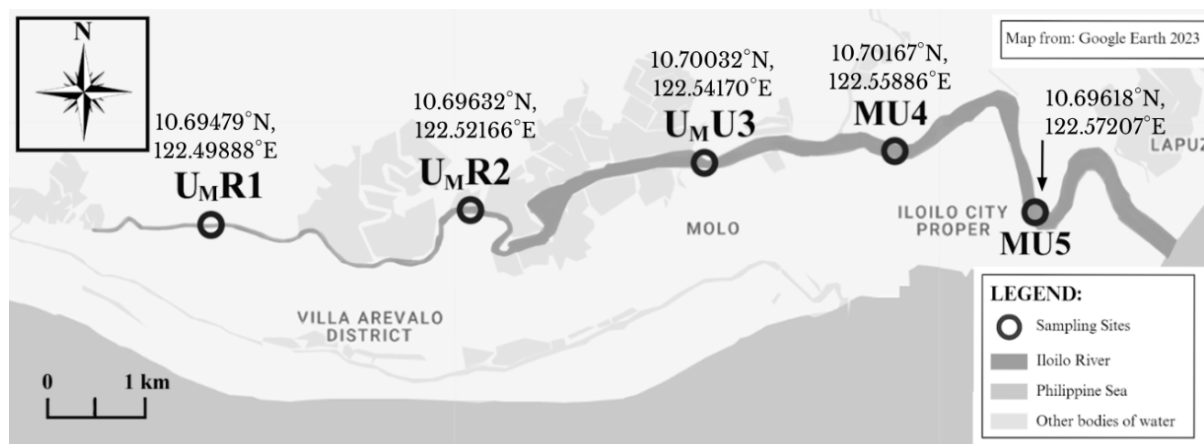


Figure 1. Map of the Iloilo River, Philippines and the five encircled sites (from Google Earth)



mixed with 5 mL of aqueous 0.05 M iron (II) solution and 5 mL of 30% hydrogen peroxide (H_2O_2), and then heated to 75 °C and stirred for 45 minutes at 200 rpm to remove organic material [5, 15].

The remaining particles underwent density separation to remove the inorganic particles with a higher density than plastics. This was done using a 10 mL sodium iodide (NaI) (1.55 to 1.6 g per mL) brine solution and left overnight to settle [17]. Next, a peristaltic pump with a 0.47µm pore size filter paper was used to drain the water out of the mixture and left to air dry overnight [5,15].

Sample Identification. The treated samples were individually isolated with tweezers and placed on glass slides for identification. For the smaller samples, the filter paper was placed under the microscope at full brightness in order to observe the particles. A compound microscope at 40x magnification [4] was used to identify its physical characteristics as shown in Figure 2. Free et al.'s [18] table, Lusher et al.'s [19] description, and Dehghani et al.'s [4] study served as libraries for confirmation. This process was done for each microplastic.

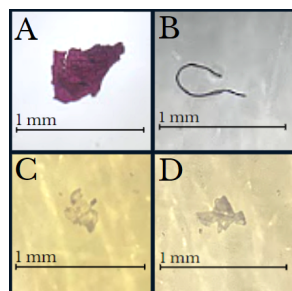


Figure 2. Photographs of different microplastics; (A) Violet fragment, (B) Black fiber, (C&D) Transparent film

The software *ImageFocus4 Version 3.1.2* [20] was used to photograph the particles and used to measure the size of each particle sample. This was done using a 1 mm / 100 div calibration slide for calibration. Samples below 150 µm were disregarded due to difficulty in identifying their characteristics [21]. The background color of the photos was caused

by the reagent (NaI) during density separation. These shades of yellow are from the differing I2 amounts in the solution due to possible amounts of chlorine in the freshwater.

Data Analysis. The data gathered was analyzed by calculating the abundance per area and determining the trend line. The characteristics and morphological types of the microplastics were placed in bar graphs, while the abundance was shown in a table. A scatter plot was used to show the trend line of the data. No specific statistical analysis test was performed due to the descriptive nature of the study.

The abundance of the microplastics was calculated using the following equation:

$$\text{Abundance} = \frac{\# \text{ of particles}}{10 \text{ L}}$$

Safety Procedure. Face masks, lab gowns, and nitrile gloves were worn for all laboratory procedures with adult supervision.

Results and Discussions - The results show that the number of microplastics found in the river ranged from 9.7 to 38 samples per 10L. Meanwhile, the microplastic abundance has a range of 0.97 to 3.80 par/L. The microplastic abundance average of the rural areas was 3.4 par/L and the average of the urban areas was 1.85 par/L.

Table 2. Microplastic Results Per Sampling Site

Site	U _M R1	U _M R2	U _M U3	MU4	MU5
Number	38	30	30	9.7	13.3
Abundance	3.80	3	3	0.97	1.33
Most Abundant Size(in µm)	150 to 500	150 to 500	150 to 500	150 to 500	150 to 500
Most Abundant Color	Black	Black	Trans-parent	Black	Black
Most Abundant Morphological Type	Film	Film	Film	Film	Film

Legend: U_M for Unmonitored, M for Monitored. R for Rural, U for Urban. The number indicates the site number.

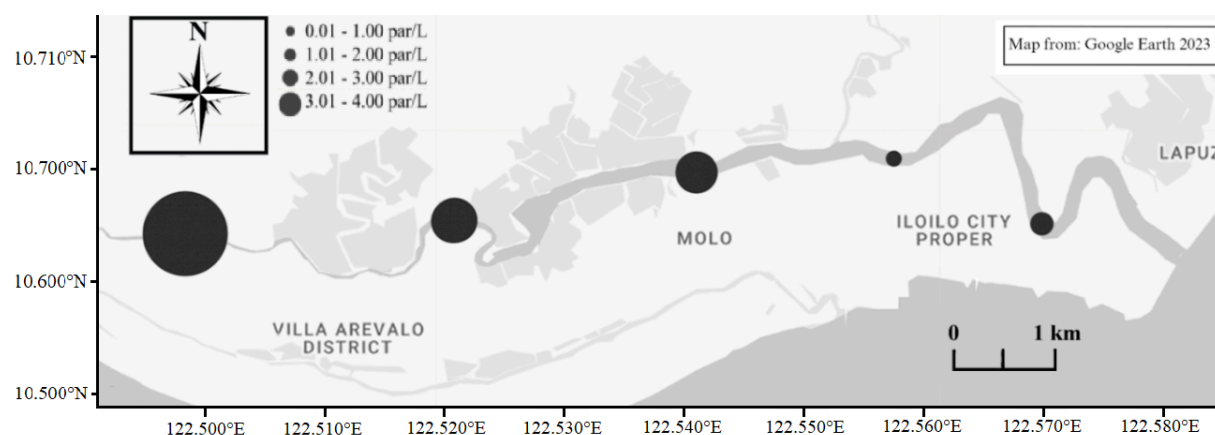


Figure 3. Iloilo River Map with Microplastic Spatial Distribution.



Observations suggest that the site's monitoring status affects the microplastic abundance in the area. The abundance of the Iloilo River has similar values to that of Cañas River (1.58 par/L), Pasig River (3.41 par/L), and Parañaque River (5.02 par/L) [15]. These similarities may be due to their shared history and surroundings. In the study conducted by Osorio et al. (2021), the Cañas river with the least microplastics was far from sources of plastic emissions, while the rivers with higher abundance values were heavily polluted and surrounded by plastic manufacturing industries [15]. On the other hand, the Pasig River, despite being in a polluted area, had a lower abundance due to a rehabilitation program, similar to Iloilo River [15]. However, there are other studies that have lower microplastic ranges than this study; which could have been affected by the difference in water sample amount [5, 15]. The counting of microplastics was limited to microplastics within the size range of 150 μm to 5 mm because the available equipment is not able to handle microplastics less than 150 μm .

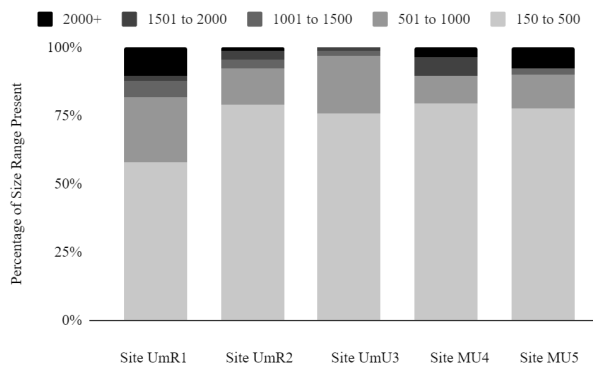


Figure 4. Bar Graph of Microplastic Sizes per site

As seen in Figure 4, the majority of microplastics were characterized within the size range of 150 to 500 μm . It is to be noted that this software provides low quality images, and can lead to inaccurate particle measurements. The results were triangulated to eliminate such limitations. It is hypothesized that the abundance of 150 to 500 μm sized microplastics originated from severe degradation and fragmentation of waste in the surrounding areas. These results align with microplastic findings and observations in the Parañaque River, where their most abundant size range is 250 to 500 μm [15]. It contains a public seafood market - this meant that its main source possibly had more degradable plastic products. The results also align with microplastic findings in the Chi River Basin, which has their most abundant size range at 51 to 500 μm [22]. One of the Chi River Basin sampling sites was surrounded with light industry plants.

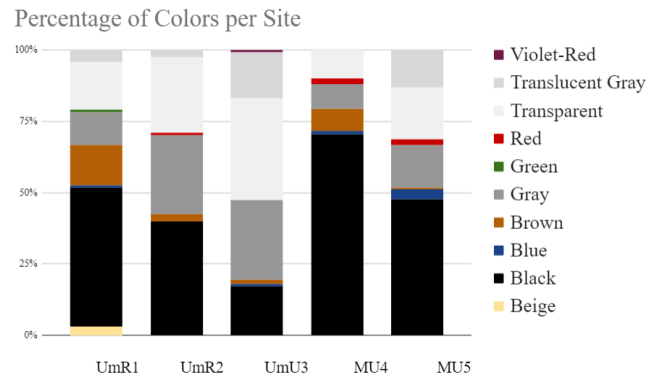


Figure 5. Bar Graph of Microplastic Colors per site

Figure 5 shows that the most prevalent colors among the microplastics found were black, translucent, and gray. This suggests that the majority of the microplastics found can be traced back to plastics of those colors. Although the transparent opacity was also prevalent in other studies, the majority of the surface water microplastic studies had blue and white as their most abundant colors [23, 24].

For studies with vibrant colors common in their findings, it was found that these studies were conducted in rivers lined with industrial and commercial areas where colored plastic is commonly used. This description does not fit Iloilo River's surroundings, and asserts the small percentage of vibrant plastics in the study. Only three out of the five river sites were near industrial and commercial areas (U_{MR1} , U_{MU3} , and $MU5$). U_{MR1} had the most diversity in color. U_{MR2} , a site surrounded by rice fields and the least industrialized, had the least color diversity. Additionally, the abundance of transparent and non-vibrant colored microplastics is due to the microplastic degradation that causes the color to fade overtime [3, 15]. However, the degradation rate of the microplastics found is not constant, as the age of the particles and their duration in the river are uncontrollable variables. Additionally, because the samples are in the surface water, there is a lack of ultraviolet B-rays to initiate the extreme degradation process which can cause the microplastics to compile in the river [3].

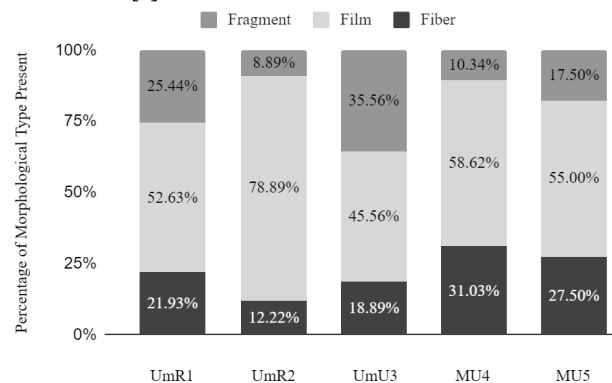


Figure 6. Bar Graph of Microplastic Morphological Types per Site



Only three morphological types were identified across the five sites (film, fragment, and fiber), with film being the most abundant. This aligns with a study in a Mongolian lake; however, their results showed that fiber and fragments were more prevalent [11, 15, 18]. Their findings were caused by waste dumping and plastic manufacturing industries within the area. The Iloilo River, especially in urban protected areas, is mostly free from plastic waste and has little fishery activity. The only macro plastic waste encountered were sachets and plastic bags, which are film types [17], suggesting that the waste surrounding the area influences the prevalence of morphological types found. It also suggests that wrappers, plastic bags, and other film plastics are the main cause of pollution along the Iloilo River. The microplastics found in the river differ from previous microplastic studies in abundance and morphological type due to undetermined factors such as the waste management in the sampling site areas.

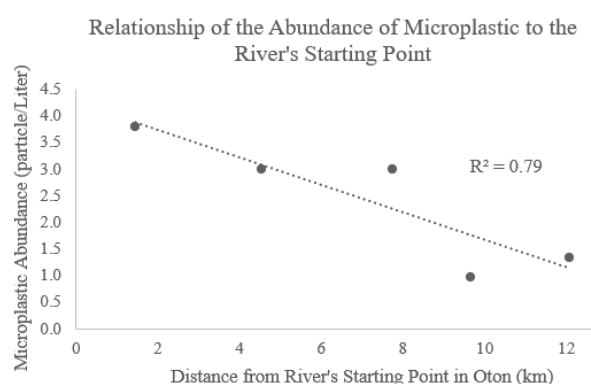


Figure 7. Relationship of the Abundance of Microplastic to the River's Starting Point

Given by the negative trendline, there is an inverse correlation between microplastic abundance and distance from the origin point, indicating that rural areas have less microplastic abundance. This corresponds to a study indicating a positive correlation between high microplastic abundance and lack of solid waste management despite population density [11, 18]. However, the same studies also stated that there is a high positive correlation between microplastic abundance and population density [22, 23]. This has opposite results to our study, as the urbanized and densely populated areas of the Iloilo River are rehabilitated [12]. However, this rehabilitation did not apply to the rural areas, especially in the UMR1 site, where the area was a mix of populated and heavily vegetated, suggesting that the state of urbanization itself may not be a factor in the increase of microplastic abundance in the Iloilo River, but rather the human activities in the affected areas. The unmonitored areas had higher values than the monitored areas. It is to be noted that the number of sites used might have affected the trendline of such phenomena as this study was limited to having only five sites. Additionally, only the population density and visual observation of surroundings were taken into account. Other factors such as waste management practices and regulations of the barangays were outside the scope of the study.

Limitations. The flow rate of the water's current during the collection and its area could not be measured. The water volume (L) was used as a replacement for the collection area measurement. Only ten liters of water were collected per site, and only microplastics within the size range of 0.15 mm to 5 mm were investigated. Due to limited chemical supplies, the reagents used in the Wet Peroxide Oxidation method used in the sample treatment were scaled down; thus, no blank samples were used during the study.

Conclusion. - The abundance of the microplastics throughout the river is 0.97 to 3.8 par/L, with an inverse correlation between microplastic abundance and the distance from the starting point. Microplastics commonly are in gray colors, a size range of 150 to 500 μ m, and a film morphological type. There is a weak correlation between rural-urban and microplastic abundance, but a higher correlation with monitoring status, and thus its lack of waste management.

Recommendations. - A higher quality microscope camera lens is recommended to accurately count a larger microplastic size range. For abundance, future studies should have a higher water volume quantity per site for a more detailed data set. For the color, size, and morphological type, high quality software is recommended for more accurate measurements and identification of the characteristics. It is also noted that the opacity of the microplastic may also be investigated as it could be related to the other characteristics. More sampling sites are needed to provide a more accurate and detailed trendline. Further investigation is needed regarding possible correlations between the abundance in the river and other influencing factors, such as the surroundings' waste management.

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References

- [1] Global Alliance for Incinerator Alternatives. 2019. PLASTICS EXPOSED: How Waste Assessments and Brand Audits are Helping Philippine Cities Fight Plastic Pollution. <http://tinyurl.com/y873x3zd>.
- [2] Koehler A, Anderson A, Andrady A, Arthur C, Bouwman H, Gall S, Hidalgo-Ruz V, Law K, Leslie H, Kershaw P, et al. 2015. Sources, Fate And Effects Of Microplastics In The Marine Environment: A Global Assessment Science for Sustainable Oceans. doi: 10.13140/RG.2.1.3803.7925
- [3] Andrady AL. 2011. Microplastics in the marine environment. Marine Pollution Bulletin. 62(8): 1596-1605. doi: 10.1016/j.marpolbul.2011.05.030
- [4] Dehghani S, Moore F, Akhbarizadeh R. 2017. Microplastic pollution in deposited urban dust,



- Tehran metropolis, Iran. Environmental Science and Pollution Research. 24(25): 20360–20371. doi.org/10.1007/s11356-017-9674-1.
- [5] Espiritu EQ, Dayrit SASN, Coronel AS, Paz NS, Enriquez EP. 2019. Assessment of Quantity and Quality of Microplastics in the Sediments, Waters, Oysters, and Selected Fish Species in Key Sites Along the Bombong Estuary and the Coastal Waters of Ticalan in San Juan, Batangas. Philippine Journal of Science. 148 (4): 789-801.
- [6] Colacion DAB, San Diego EMM, Secondes JR, Oberio ZL. 2020. Microplastic occurrence in the coastal sediments of Anilao, Iloilo. Publisience. 3(1):122-126.
- [7] Ragusa A, Notarstefano V, Svelato A, Belloni A, Gioacchini G, Blondeel C, Zucchelli E, De Luca C, D'Avino S, Gulotta A, et al. 2022. Raman Microspectroscopy Detection and Characterisation of Microplastics in Human Breastmilk. Polymers. 14(13):2700. Doi: 10.3390/polym14132700.
- [8] Prüst M, Meijer J, Westerink RHS. 2020. The plastic brain: neurotoxicity of micro- and nanoplastics. Particle and Fibre Toxicology, 17(1). doi:10.1186/s12989-020-00358-y.
- [9] Michida Y, Chavanich S, Chiba S, Cordova MR, Cabanas C, Glagani F, Hagmann P, Hinata H, Isobe A, Kershaw P, et al. 2020. Guidelines for Harmonizing Ocean Surface Microplastic Monitoring Methods. Version 1.1. Ocean Best Practices. hdl.handle.net/11329/1361.
- [10] Philippine Statistics Authority Region VI - Western Visayas. 2018. Iloilo Provincial Statistical Office. Psa.gov.ph. http://rso06.psa.gov.ph/psailoilo/statistics.
- [11] Arcadio CGLA, Navarro CKP, Similatan KM, Inocente SAT, Ancla SMB, Banda MHT, Capangpangan RY, Torres AG, Bacosa HP. 2022. Microplastics in surface water of Laguna de Bay: First documented evidence on the largest lake in the Philippines. doi:10.21203/rs.3.rs-1891626/v1.
- [12] Department of Environment and Natural Resources - Environmental Management Bureau - Region VI. 2020. ILOILO RIVER: Annual Assessment Report, CY 2020. http://tinyurl.com/yp24n2ch.
- [13] Philippine Statistics Authority. 2013. Urban Barangays in the Philippines. PSA. http://tinyurl.com/2pak8eez.
- [14] Chen HL, Gibbins CN, Selvam SB, Ting KN. 2021. Spatio-temporal variation of microplastic along a rural to urban transition in a tropical river. Environmental Pollution. 289:117895-117895. doi: 10.1016/j.envpol.2021.117895
- [15] Osorio ED, Tanchuling MAN, Diola MBLD. 2021. Microplastics Occurrence in Surface Waters and Sediments in Five River Mouths of Manila Bay. Frontiers in Environmental Science. 9. doi: 10.3389/fenvs.2021.719274.
- [16] Prume JA, Gorka F, Löder MGJ. 2021. From sieve to microscope: An efficient technique for sample transfer in the process of microplastics' quantification. MethodsX. 8:101341. doi:10.1016/j.mex.2021.101341.
- [17] Mattsson K, Ekstrand E, Granberg M, Hasselöv M, Magnusson K. (2022). Comparison of pre-treatment methods and heavy density liquids to optimize microplastic extraction from natural marine sediments. Sci Rep 12, 15459. oi: 10.1038/s41598-022-19623-5.
- [18] Free CM, Jensen OP, Mason SA, Eriksen M, Williamson NJ, Boldgvid B. 2014. High-levels of microplastic pollution in a large, remote, mountain lake. Marine Pollution Bulletin. 85(1):156–163. doi:10.1016/j.marpolbul.2014.06.001.
- [19] Lusher AL, Bråte ILN, Munno K, Hurley RR, Welden NA. 2020. Is It or Isn't It: The Importance of Visual Classification in Microplastic Characterization. Applied Spectroscopy. 74(9):1139–1153. doi:10.1177/0003702820930733.
- [20] Braña ASS, Celestial MLP, Moline RJM, Mediodia CJA. 2021. Microplastics in farmed oysters (*Crassostrea iridalei*) from Capiz, Philippines. Publisience. 4(1):8-13.
- [21] Castro JH. 2022. Physical Characterization and Spatial Variability of Microplastics in Coastal Surface Waters in Roxas, Palawan, Philippines. Journal of Science, Engineering and Technology (JSET). 9:1-11. http://tinyurl.com/mrarpebk.
- [22] Department of Environment and Natural Resources. 2020. Pasig River Rehabilitation Program. http://tinyurl.com/2s438mn6.
- [23] Thamsenanupap P, Tanee T, Kaewsuk J. 2022. Evidence of microplastics in the Chi River Basin, Thailand: Anthropogenic influence and potential threats to edible arthropods. Limnologica - Ecology and Management of Inland Waters. https://doi.org/10.1016/j.limno.2022.126030.
- [24] Kunz A, Schneider F, Anthony N, Lin H. 2023. Microplastics in rivers along an urban-rural gradient in an urban agglomeration: Correlation with land use, potential sources and pathways. Environmental Pollution. 321:121096–121096. https://doi.org/10.1016/j.envpol.2023.121096.



Post-harvest quality assessment of *Carica papaya* L. 'Red Lady' papaya coated with rice bran wax (RBW)

ANSLEY JOYCE H. SENDICO, JULIA ALLYZA G. TABANDA, BLEZANIE JOY N. NOBLEZA, and ANGELO P. OLVIDO

Philippine Science High School Western Visayas Campus - Department of Science and Technology (DOST-PSHS WVC), Brgy. Bito-on, Jaro, Iloilo City 5000, Philippines

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Abstract

Carica papaya L. fruits are delicate and perishable due to their high water content and would benefit from preservation methods. Waxing is a preservation method that serves as a barrier equivalent to a laminate film on the peel. Rice bran wax (RBW) as a coating has been noted to have delayed the produce ripening process. This study aimed to determine the quality of papaya coated in 15%(T1), 20%(T2), and 25%(T3) RBW emulsions against a control over a time period by assessing %weight loss, pH, fruit color, and decay incidence. Results showed that T1 had the highest weight loss percentage, pH level, and lowest decay incidence out of all treatment groups. T2 had the highest decay incidence, whereas the T3 showed the lowest %weight loss. Results had statistically insignificant differences between all treatments for all parameters. Thus, RBW is not recommended for *Carica papaya* L. fruit preservation.

Introduction. - *Carica papaya* L. fruit is produced in about 60 countries, especially in developing countries such as the Philippines which mainly grows in home gardens or as an intercrop [1,2]. It is delicate and perishable due to its high water content and therefore lags far behind bananas and pineapples in world trade [3]. Papaya shelf-life is crucial as the papayas are stored for 1 to 2 days before being distributed to wholesaler retailers where they must last at least 3 days at the supermarket or retail shop [4]. Current preservation methods that are used in the country are the usage of modified atmosphere packaging (MAP) [5,6] and the use of 1-methylcyclopropene (1-MCP) [7]. Waxing is another method of prolonging a fruit's shelf life by coating it in an artificial waxing material. One such material used for waxing is rice bran wax (RBW) [8,9,10]. This method does not harm the environment and is a cheaper alternative to other preservation practices [11].

RBW has been noted to inhibit ripening while being utilized as a wax coating for produce with it being found to be an effective way to extend tomato shelf-life of up to 27 days [8,9]. Similar results can also be seen with limes, sweet peppers, and cherry tomatoes [8,9]. The common material for waxing is Carnauba wax which must be imported as its source plant *Copernicia prunifera* can only be found in northeastern Brazil [10]. RBW, on the other hand, can be made and procured locally in the Philippines as a by-product of the biowaste of rice milling.

The physical and chemical properties of a fruit can be analyzed to determine or approximate its maturity, namely %weight loss, pH, color, and decay incidence.

Weight loss percentage is an indicator of the fruit dehydration process due to transpiration. Papayas comprise 80 to 85% water making them highly perishable as a high water content makes them highly susceptible to microbial growth [13]. They are also climacteric fruits that continue to ripen and transpire even after harvesting wherein there would be a rapid rise in the rate of oxygen and carbon dioxide exchange [14]. Papayas couldn't replace water that has been lost as water vapor once they are taken off from the main plant thus contributing to the weight loss products will experience. When moisture loss is too high, produce can experience commodity deterioration which includes symptoms like shriveling [15]. Wax coatings like RBW act as a second layer or barrier to retain moisture that could potentially lead to weight loss due to moisture loss [13].

Ripening and subsequently pH control in fruits are very important to prevent color and texture loss [17]. An increase in total acidity observed during ripening is believed to be associated with an increase in free galacturonic acids [18]. Fruits with a pH range of 4.5 or below do not allow the growth of most of the bacteria with exempted examples being molds, yeasts, and aciduric bacteria [19].

Color is one of the first quality attributes a customer can detect in food products [16]. Color change in papayas can be seen as their smooth thin green skin turns yellow or red as it ripens, and their succulent flesh ranges from yellow to orange to red [20]. Therefore, waxing can slow down the ripening process which can be reflected by the color change of the fruits.



Decay incidence is the occurrence of decay symptoms. Such symptoms can be decay and mold, sunken areas on the skin, discoloration, overripeness, softness, scarring of the skin, bruising of flesh, brown spots on the skin, and shriveled appearances [21]. The high moisture content and soft texture of fruit make it susceptible to mechanical injury throughout its production and retail life [14]. Aside from being a protective barrier, wax coatings can also create a non-water-compatible surface that is not conducive to pathogen growth while sealing small cracks and dents in the exocarp to establish a barrier against pathogen entry [23].

There are limited investigations conducted utilizing RBW as the material for waxing on papaya and significantly bigger fruit sizes than sizes found in current RBW studies.

This study's findings will contribute to the enhancement of papaya preservation techniques for producers, particularly in exploring alternative wax materials like RBW, thereby expanding the existing body of research in this field. The additional information on RBW provided by this study may encourage other researchers to further investigate RBW or present different methods for using it as a wax coating.

This study aimed to determine the quality of *Carica papaya* L. 'Red lady' papaya coated with RBW. The study specifically aimed to:

- (i) measure the papaya fruits' percentage weight loss, pH level, fruit color, decay incidence, and mean values of the treatment groups [(T1) RBW: 15% (w/v), (T2) RBW 20% (w/v), (T3) RBW 25% (w/v)] and the control group;
- (ii) compare the papaya fruits' percentage weight loss, pH level, fruit color, decay incidence, and mean values of the treatment groups and the control group; and
- (iii) determine if there is a statistical difference in the mean percentage weight loss, mean pH level, mode of fruit color, and mode decay incidence of papaya between the treatment and control groups.

Methods. - This study used an experimental approach to examine the quality of Red Lady papaya. The study lasted four weeks, with one and a half weeks allotted for material procurement and authentication and setup preparation, another one and a half weeks for data collection, and the final week used for value computation and data analysis. Each papaya was given a code and was randomly assigned to a group. There were three experimental groups and one control group, with each group having three replicates as per the standard control of the Randomized Complete Block Design (RCBD) with a total of nine papayas per group and a total of 36 papayas overall. The experimental phase was conducted for 9 days, and an assessment of the fruit color was done daily during the whole duration of the data-gathering period. The weight was measured

on day 0 to get the initial weight and on day nine to get the final weight. The pH and decay were analyzed on the last day of the data-gathering period.

Sample Collection. Samples of the papayas were collected from Spring Bloom Farms, Pavia, Iloilo. They were noted to be unripe based on the color maturity index of Basulto et al. [20]. Samples were picked based on the following criteria: (i) must be Red Lady variety with certification from the Department of Agriculture Western Visayas (DA - WV); (ii) must have no decay sign; (iii) must have no pests; and (iv) must not be shriveled.

Wax Preparation. RBW coating emulsion was prepared using a method adapted from Abrihami et al. [10]. The RBW was melted using the double boiler method. This method utilizes the heat of trapped steam between two pots to melt solid wax. A small pot containing the RBW was placed inside a larger pot that was partially filled with water. Using a burner, the water was boiled into a simmer. As adapted from Abhirami et al. [10], the amounts used for the treatments are the following: 15% (T1), 20% (T2), and 25% (T3) were 600 g, 800 g, and 1,000 g of RBW respectively. The hot wax was added to a bucket with 300 mL of emulsifier Polysorbate 80 (Tween 80) and 4 L of hot distilled water, homogenized using a stand mixer for 10 minutes at 18,000 rpm. The treatments were quantified using weight per volume (w/v), which was determined by the ratio of the wax weight to the water volume.

Waxing. The papayas were divided into three blocks with 9 papayas per block. The dip coating method was used to apply the wax on the papayas wherein the whole fruit was dipped in the emulsion for 3 minutes. After waxing, the papayas were left to dry for 5 hours and transferred to their assigned tables. A mosquito net was suspended on each table to prevent insects from contaminating the samples.

%Weight loss. For the weight loss percentage determination, the samples were weighed before (day 0) and after (day 9) storage. The equation for calculating the percentage of weight loss given below was based on the study of Zhang et al. [8]; where m and m_0 are the final and initial weights of each papaya fruit, respectively.

$$\%weight\ loss = (m_0 - m) (m_0^{-1})$$

Fruit color. The fruit color of the papayas was observed and documented every 5 pm from day 1 to day 9. The color change of the papaya was based on the maturity index of Maradol papaya in the study of Basulto et al. [20] (See Figure 1).





Figure 1. Maturity index of Maradol papaya [20]

pH determination. A sample of their mesocarp or the fleshly middle layer of the fruit's pericarp was fed into a juicer. The pH value of the obtained solution was measured with a pH meter.

Decay Incidence. In this study, papaya is considered to be experiencing decay when the visual signs of quality loss can be seen on the exocarp or the outer layer skin of the fruit [19]. One of the symptoms of plant disease is fruit spots, definite and localized areas of softening discoloration, and disintegration of tissue [21]. These spots can merge to form rot. Any fruit with visible microorganism growth is also considered decayed.

Decay incidence was based on the visual observation of decay spots recorded using the method of Cao et al. [24]. Their four-grade scale is as described: 0 = no decay; 1 = slight decay, covering up to 25% of the fruit surface; 2 = moderate decay, covering >25% but <50% of the fruit surface; 3 = severe decay, covering >50% of the fruit surface. Results will be expressed as a decay index after using the following formula as used by Cao et al. [24]:

$$DI = \frac{(1 \times N_1 + 2 \times N_2 + 3 \times N_3) \times 100}{(3 \times N)}$$

where DI is the decay index, N is the total number of fruits, and N_1 , N_2 , and N_3 is the number of fruits showing slight decay, moderate decay, and severe decay, respectively.

Data Analysis. Data were plotted using Google Docs and Google Sheets before being processed with the Statistical Package for the Social Sciences (SPSS) ver.28 program. To determine whether the data are statistically significant, one-way ANOVA was utilized at a 95% confidence interval for the parametric data of weight and pH. At the same time, the Kruskal-Wallis H test was used for the nonparametric data of color and decay incidence.

Safety Procedure. Polysorbate 80 poses physical hazards and potential health effects to researchers. It may cause skin and eye irritation. The heated emulsion of RBW, Polysorbate 80, and distilled water may cause burns during the waxing process. To avoid these, proper and cautious handling of the substance was always observed.

Results and Discussion. - The study aimed to determine the quality of *Carica papaya L.* coated with different concentrations of RBW by assessing their weight loss, pH level, fruit color, and decay incidence.

Weight loss analysis. All samples had weight loss. Among the treatment and control groups, treatment 3 yielded the least mean percentage weight loss at 18.54%, followed by control (18.68%), T2 (21.11%), and T1 (21.58%). It could be inferred that T3 allowed for the lowest transpiration rate and water content loss. This may be due to the concentration of the RBW or material component of the film which affects the water vapor permeability properties of the wax [25]. Similarly, the study of Jutamongkon et al. [9] reported that higher RBW concentrations resulted in less weight loss in tomatoes. Zhang et al. [8] also found that RBW-coated cherry tomatoes had a significantly lower weight loss compared to the control group. Percentage weight loss is an indicator of the fruit dehydration process due to transpiration. It can be used as a representation of a way to determine coating efficiency in the preservation of fruit quality [10].

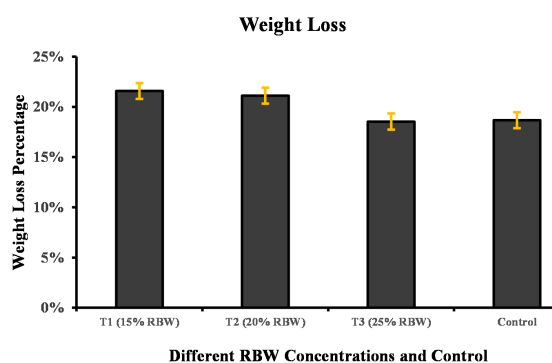


Figure 2. Mean percentage weight loss of the groups

However, one-way ANOVA results revealed that there was no statistically significant difference between the treatment and control groups ($F(3,32)=0.757$, $p=0.527$). The weight loss of the coated fruits was not significantly different from the control group. Furthermore, after the means of Day 1 and Day 9 of each treatment and the control group underwent a paired t-test, it was found that RBW neither slowed down nor sped up the weight loss rate. This indicates that there is not enough evidence to prove that a relationship between RBW coatings at different concentrations and weight loss of fruits exists, which may be attributed to the small sampling size. It is to note as well that there are only two data points observed on day 0 and day 9 which could not be represented entirely and thus inconclusive.

pH analysis. It is observed that all groups have yielded a pH value that is between 5 and 6. The mean pH values of the T1 papayas fall under the pulp's standard pH, which is between 5.5 and 5.9 [26]. The rest of the treatments and the control group fall a little lower than the range stated.



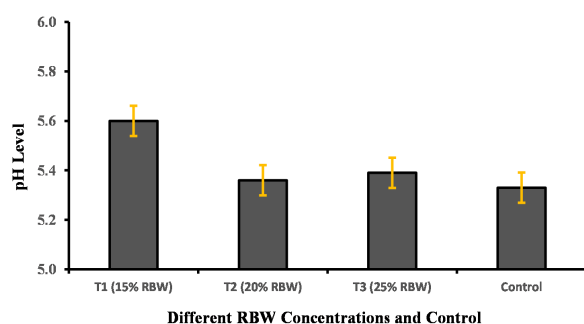


Figure 3. Mean pH levels of the treatments and the control group

One-way ANOVA results revealed that there was no statistically significant difference between the treatment and control groups ($F(3,32)=0.987$, $p=0.411$). This suggests that with or without wax, the pH level of the papayas relatively stayed within the normal range. The results of this analysis denote that RBW did not significantly change the pH of the papaya.

Batista-Silva et al. [27] claimed that the organic acid content of fruits decreases with maturation due to the breathing process or sugar conversion. This results in a rise in pH as the acidity of the fruit decreases. In a study conducted by Gomez et al. [28], it was noted that papayas in the mature green stage were more acidic. However, papayas at the intermediate and ripe stages had less acidity and were found to be sweeter than green papayas. The acidity is low because the papayas in the different treatment groups and the control group have a pH level below but near neutral.

Fruit color analysis. A higher ranking in the color scale means there is a higher number of papayas observed that had higher valued scores on the papaya maturity index. This corresponds to how far along the papaya is in its ripening based on the visual evidence of its pericarp color.

T1 (15% RBW), T2 (20% RBW), and the control group showed similar progression in color wherein they consistently had a color score mode of 2 for the first five days and it increased to 3 on the sixth up until the seventh day. The fruit color on days 8 and 9 was not recorded anymore due to the presence of decay obscuring the color of the papayas. There was no statistically significant difference between the change in fruit color of the treatment groups and the control group as determined by the Kruskal-Wallis H test in the SPSS software. However, the fruit color of treatment 3 was not recorded due to it being obscured by the wax. The results show that the different concentrations of wax did not greatly affect the change in color of the treatments in comparison to the control group. It can be inferred that the RBW did not alter the respiration rate to the extent of significance.

Decay incidence analysis. T1 showed the least decay percentage while the higher RBW concentration tied for the highest decay percentage. This ranking is also reflected in the modes of the decay scores of each treatment (Figure 4).

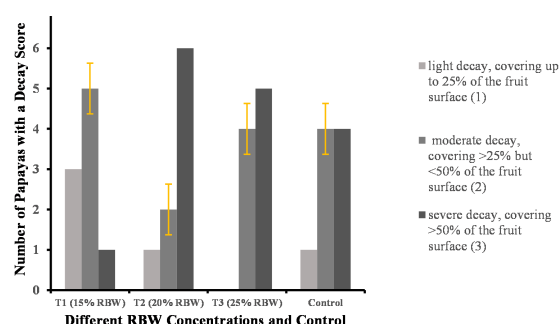


Figure 4. Decay index score of the treatments and control group.

Table 1. Decay index percentages and description of the treatments and the control group

Treatment	Decay Index Percentage	Description
Control	56.70%	severe decay
(T1) RBW: 15% (w/v)	43.20%	moderate decay
(T2) RBW 20% (w/v)	62.10%	severe decay
(T3) RBW 25% (w/v)	62.10%	severe decay

The Kruskal-Wallis H test resulted in no significant differences ($h = 7.179$, $p = .066$, $df = 3$) between the 3 treatments and control for decay incidence. This indicates that coating *Carica papaya* L. 'Red Lady' Papaya with RBW has no significant effect on inhibiting microbial growth or decay symptoms. Waxing creates a hydrophobic film barrier on the surface of the fruit [29], which blocks out pores in the cuticle of the exocarp for less water vapor loss [30]. This could mean that RBW is also sealed in possible moisture which is conducive to microbial growth as water is essential for the normal body functioning of organisms [23]. Although not part of the study's parameters, it has been observed that treated papayas' mesocarp can be considered unripe inside for having firm flesh that would have been softening due to ripening [31]. The flesh is also lacking in the bright orange color that ripened papayas have [19].

Papayas may also be more susceptible to fungal invasion during ripening as their normal range of pH, which the papayas of the study have, is lower than that of the pH range of 4.5 or below which helps in inhibiting the growth of most of the bacteria in fruits [32]. It is also suggested that as the pH of the tissue increases, skin layers soften, soluble carbohydrates build-up, and defense barriers weaken which could explain the eventual microbial growth, discoloration, and sunken areas observed on the papayas [31].



Limitations. It is to note that temperature was excluded as a variable in the experiment as of the time the study was conducted, there was not any available equipment for it and was deemed an uncontrolled variable. It could be that heat from the wax, despite being let cool, influenced the progression of the papaya quality.

Conclusion. - The results obtained from each parameter showed that there was no significant difference in weight loss, pH, fruit color, and decay incidence between the treatment groups and the control group.

Recommendations. - It is recommended to use an advanced tool or software such as ImageJ to measure the total area of decay and the fruit color of the papaya. The use of a homogenizer is also advised to homogenize the papaya flesh in measuring the pH level. Furthermore, it is advised to replace the Brand X used by the study with a self-produced RBW as well as the characterization of said RBW. The addition of parameters to be analyzed such as firmness, ethylene production, titratable acidity, flesh characterization, microbial infestation, and soluble solids content is also recommended to examine further the ripening of the fruit physically and chemically. The cosmetic attributes that wax coatings can provide for papayas can also be investigated for consumer preferences.

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References

- [1] Evans E, Ballen F. 2018. An Overview of Global Papaya Production, Trade, and Consumption. <https://edis.ifas.ufl.edu/publication/FE913>.
- [2] Chua Y. 2018. The Philippines Fresh Papaya. Export Value Chain. Australia: University of Adelaide. <http://bitly.ws/vtxW>.
- [3] Villegas, VN, 1991. Carica papaya L. In: Verheij, E.W.M. and Coronel, R.E. (Editors): Plant Resources of South-East Asia No 2: Edible fruits and nuts. PROSEA Foundation, Bogor, Indonesia. Database record: prota4u.org/prosea.
- [4] Maunahan M, Absulio W, Esguerra E, Sun T, Collins R. 2015. A value chain approach to developing export markets for 'solo' papaya farmers in the Philippines. Leuven, Belgium: International Society for Horticulture Science.. doi: 10.17660/ActaHortic.2015.1088.45
- [5] Floros J, Matsos K. 2005. Introduction to modified atmosphere packaging. Elsevier LTD: In Food Science and Technology, Innovations in Food Packaging, Academic Press. doi: 10.1016/B978-012311632-1/50042-5.
- [6] Castro S. Post-Harvest Technology in the Philippines. <https://un-csam.org/Activities%20Files/A20/10%20Philippines.pdf>.
- [7] Fabi J, Cordenunsi B, De Mattos Barreto G, Mercadante A, Lajolo F, Nascimento J. 2007. Papaya Fruit Ripening: Response to Ethylene and 1-Methylcyclopropene (1-MCP). Journal of Agricultural and Food Chemistry. doi: 10.1021/jf070903c.
- [8] Zhang L, Chen F, Zhang P, Lai S, Hongshun Y. 2016. Influence of rice bran wax coating on the physicochemical properties and pectin nanostructure of cherry tomatoes. New York: Springer Science+Business Media.
- [9] Jutamongkon R, Praditdoug S, Vananuvat N. 2011. Effect of Rice Bran Waxing on Fruit and Vegetable Storage. Thailand: Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University. <http://bitly.ws/vtyV>.
- [10] Abhirami P, Modupalli N, Natarajan V. 2020. Novel post-harvest intervention using rice bran wax edible coating for shelf-life enhancement of Solanum lycopersicum fruit. India: India Institute of Food Processing Technology. 10.1111/JFPP.14989.
- [11] Hagenmaier, R., Shaw P. 1992. Gas Permeability of Fruit Coating Waxes. U.S. Citrus and Subtropical Products Laboratory, Agricultural Research Service, U.S. Department of Agriculture. 117(1):105-109. doi:<https://doi.org/10.21273/jashs.117.1.105>.
- [12] Ohashi T, Pilon L, Spricigo P, Miranda M, Corrêa D, Ferreira M. 2015. Postharvest quality of 'golden' Papayas (Carica papaya L.) coated with carnauba wax nanoemulsions. Revista Iberoamericana de Tecnología Postcosecha, vol. 16, núm. 2, 2015, pp. 199-209. <http://bitly.ws/vtIp>.
- [13] Ayala-Aponte A, Ocoró-Zamora M. 2013 April 26. Influence of thickness on the drying of papaya puree (Carica papaya L.) through refractance window™ technology. Dyna (Medellin, Colombia). <http://bitly.ws/vx8c>.
- [14] Food and Agriculture Organization. 1989. Prevention of Postharvest Food Losses: Fruits Vegetables and Root Crops. Daya Publishing House. <https://www.fao.org/3/T0073E/T0073E00.htm>
- [15] Becker B, Fricke B. 2002. Transpiration and Respiration of Fruits and Vegetables. https://b.web.umkc.edu/beckerb/publications/chapters/trans_resp.pdf.



- [16] Rhim J, Shellhammer T. 2005. Lipid-based edible films and coatings. Elsevier LTD: In Food Science and Technology, Innovations in Food Packaging, Academic Press. doi:10.1016/B978-012311632-1/50053-X.
- [17] Andres-Bello A, Barreto-Palacios V, Garcia-Segovia P, Mir-Bel J, Martinez-Monzo J. 2013 May 9. Effect of pH on color and texture of food products. Springer Science+Business Media New York 2013: Food Eng Rev (2013) 5:158–170]. 10.1007/s12393-013-9067-2.
- [18] Hewajulige IGN, Dhekney SA. 2016. Papayas. Elsevier LTD: Encyclopedia of Food and Health. <http://dx.doi.org/10.1016/B978-0-12-384947-2.00517-1>.
- [19] Bozoglu T, Erkman O. 2016. Spoilage of vegetables and fruits. United States: John Wiley & Sons, Ltd. 10.1002/9781119237860.ch20.
- [20] Basulto F, Duch E, Espadas y Gil F, Plaza R, Saavedra A, Santamaria A. 2009 Aug. Postharvest ripening and maturity indices for Maradol papaya. Interciencia. <http://bitly.ws/vxbo>.
- [21] Teixeira da Silva J, Rashid Z, Nuot D, Sivakumar D, Gera A, Teixeira Souza M, Tennant P. 2007 Nov 1. Papaya (*Carica papaya* L.) Biology and Biotechnology. Global Science Books: Tree and Forestry Science and Biotechnology. <http://bitly.ws/vxbS>.
- [22] Carmen D, Esguerra E, Absulio W, Maunahan M, Masilungan G. 2012 Aug 1. Understanding Consumer's Preference For Fresh Table-Ripe Papaya. Philippine Journal of Crop Science (PJCS): Crop Science Society of the Philippines. <http://bitly.ws/vxew>.
- [23] Kider J, Raja S, Badler N. 2011 April. Fruit senescence and decay simulation. Eurographics. 10.1111/j.1467-8659.2011.01857.x.
- [24] Cao S, Hu Z, Pang B, Wang H, Xie H, Wu F. 2010. Effect of ultrasound treatment on fruit decay and quality maintenance in strawberry after harvest. Food Control. 21(4):529–532. doi:10.1016/j.foodcont.2009.08.002.
- [25] Goslinska M, Heinrich S. 2019. Characterization of waxes as a possible coating material for organic aerogels. Powder Technology. 357:223–231. doi:10.1016/j.powtec.2019.08.096.
- [26] Okonko I, Chukwuka K, Adekunle A. 2010. Microbial Ecology of Organisms Causing Pawpaw (*Carica papaya* L.) Fruit Decay in Oyo State, Nigeria. Journal of Toxicological Sciences. 2(1):43–50. <https://bit.ly/3T6h1YT>.
- [27] Moy 2003. PAPAYAS. ScienceDirect. 4345–4351. <https://www.sciencedirect.com/science/article/pii/B012227055X008816>.
- [28] Gomez M, Lajolo F, Cordenunsi B. 2002. Evolution of Soluble Sugars During Ripening of Papaya Fruit and its Relation to Sweet Taste. Journal of Food Science. 67(1):442–447. doi:10.1111/j.1365-2621.2002.tb11426.x.
- [29] Bourliew-Lacanal C, Guillard V, Vallès-Pàmies B, Gontard N. 2007. Edible moisture barriers: materials, shaping techniques and promises in food product stabilization. Food Materials Science: Principles and Practice, Editions Springer, 616 p., 2007, Food Engineering Series, 978-0387719467. fflal-0145449
- [30] Chan M. 2016 July. The coat on fruits - wax?. Hong Kong: Center for Food Safety. <http://bitly.ws/vtVg>.
- [31] Payasi A, Sanwal G. 2010. Ripening Of Climacteric Fruits And Their Control. Journal of Food Biochemistry. 34(4):679–710. doi:10.1111/j.1745-4514.2009.00307.x.
- [32] Nishimwe G, Okoth E, Rimberia F. 2018 Dec 8. Evaluation of physicochemical, nutritional and sensory quality characteristics of new papaya hybrid fruits developed in JKUAT.

