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Advance Sustainable Science, Engineering and Technology (ASSET) is a peer-reviewed open-access international scientific journal dedicated to the latest advancements in sciences, applied sciences and engineering, as well as relating sustainable technology. This journal aims to provide a platform for scientists and academicians all over the world to promote, share, and discuss various new issues and developments in different areas of sciences, engineering, and technology.

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Editorial Preface

**Advance Sustainable Science, Engineering and Technology (ASSET)
Volume 4 Number 1 April 2022**

It is our great pleasure to present the Volume 4 Number 1 Advance Sustainable Science, Environmental Engineering and Technology (ASSET). This issue includes seven manuscripts. Aryaseta's work on the experimental Investigation to Find the Strength of Ballast. Rakasiwi designed Cnc Machine (Computer Numeric Control) Mini Plotter for Arduino Based Souvenir Craft. Dwiningsih analyzed the Genetics, Biochemistry and Biophysical Analysis of Anthocyanin in Rice (*Oryza sativa L.*). Kusumo investigated Attendance Management system using RFID Technology. Trikusuma developed The Web-based Application of Small and Medium Enterprises (SMEs) Product Distribution Management with Content Management System Shopify Integration in Netasia Singapore. Purwaningtyas investigated Physicochemical Characteristics of White Tea Product of PT. Perkebunan Nusantara IX (Kaligua Gardens) Pandansari Village, Paguyangan District, Brebes Regency. At last but not least, Rahayu studied the Physicochemical Characteristics of Catfish (*Clarias sp*) Sausage with Addition of Moringa Leaf Flour (*Moringa oleifera*).

We thank all of the 17 authors affiliated from University of Arkansas, United States of America; King Saud University, Saudi Arabi; University of Pembangunan Nasional Veteran, Universitas PGRI Semarang, and Universitas 17 Agustus 1945, Indonesia who have contributed to this issue. We believe that all the papers published in this issue will have great influence on the science, environmental engineering and technology.

April 2022
Asst. Prof. Mega Novita
Asst. Prof. Rizky Muliani Dwi Ujianti



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Experimental Investigation to Find the Strength of Ballast

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Abstract. In general, superstructure properties are easier to define. So many researchers and practitioners pay more attention to this structure. In contrast, substructure properties vary widely and are more difficult to study. This particular reason makes the substructure part get less attention and less studied. Although ballast is only part of the substructure, it plays an important role in keeping the railway track in position. Therefore, research about ballast behaviours still needs to be developed. Objective of this research is to use Los Angeles Abrasion (LAA) and Aggregate Impact Value (AIV) test to analyse the strength of nature rock (NR), slag (S) and their different combination. Four different types of samples have been prepared for testing. LAA and AIV test has been performed to assess the strength of the ballast. Finally, the result shows that the combination of slag and nature rock showed good results, even not really significantly. The result for 25%NR+75%S (LAA test) and 1NR+2S (AIV test) is 7,41% and 6,02%, respectively.

Keywords: ballast, LAA, AIV, slag, nature rock

(Received 2022-03-31, Accepted 2022-04-27, Available Online by 2022-05-18)

1. Introduction

The substructure properties are more varies and difficult to define than those of the superstructure. This particular reason makes the substructure part get less attention and less studied. Although ballast is only part of the substructure, it plays an important role in keeping the railway track in position. Therefore, research about ballast behaviours still needs to be developed.

The physical and mechanical characteristics of individual particles significantly influence the behaviours of ballast under both static and cyclic loading. Typically, the size of ballast grains varies in the range of 10-60 mm. Overall characteristics of the granular mass that govern ballast behaviours include particle size distribution, void ratio (or density) and the degree of saturation [1]. Some ballast materials that are often used include granite, basalt, limestone, slag and gravel [2].

Los Angeles Abrasion (LAA) test was used to artificially obtain deteriorated ballast particles [3]. A material's tendency to fracture or abrade during an abrasion test is a function of material type, particle shape, and gradation. In the LAA test, gradation is fixed; therefore, the results will reflect material type and particle shape [4], [5], [6]. Aggregate Impact Value (AIV) is another index parameter that can used





to measure the hardness of the rocks or crushed stones. AIV test provides a relative measure of the resistance of an aggregate to sudden shock or impact [7].

Materials considered to be good ballasts should have properties such as hard, dense, and angular shapes. The angular property with sharp corners will provide interlocking qualities which will grip the sleeper firmly to prevent movement. Furthermore, the stronger the aggregate will certainly make the rail track more stable and also reduce the replacement time of the ballast. In general, slag has better hardness and stability compared to nature rock. The only drawback is that slag has more weight than nature rock. However, a comprehensive lab result is still needed to verify the strength between slag and natural rock.

Researchers have previously done research on mixing materials for asphalt and concrete mixes, but for railway ballasts no one has done this. It is assumed that mixing on the railway ballast material will increase the strength of the ballast itself. Slag has a shape that tends to be rounded, thus reducing the interlocking style. Therefore, in this study, an attempt has been made to mix the slag and nature rock that has a sharp shape corner to support the interlocking style on the railway ballast. If this mixing results in a good result, then ballast durability can be increased so the costs can be reduced and routine maintenance cycles can be extended. Besides that, slag has a pore that allows water to enter, it has possibility to expand due to magnesia and loose lime contained in it. So, this study want to check whether it was decreasing its strength or not. Objective of this research is to use LAA and AIV test to analyse the strength of nature rock, slag and their different combination.

2. Methods

2.1. Ballast Sample Preparation

For LAA test, four different types of samples were used. The first and second sample are 100% nature rock and 100% slag. The third sample is the mixed between nature rock and slag by volume: i) 75% nature rock, 25% slag; ii) 50% nature rock, 50% slag; iii) 25% nature rock, 75% slag. Then the last fourth sample is submerged slag which has been submerged in water at 60° for 3 days and dried in the oven for 4 hours at 110°. The procedure base on ASTM C535.

For AIV test, four different of samples were also used, 100% nature rock, 100% slag and then for making combination between nature rock and slag, three layers has been used:

- i) For 75% nature rock, 25% slag: from bottom 2 layers of nature and 1 layer of slag;
- ii) For 50% nature rock, 50% slag: from bottom first layer of nature rock, second layer is half slag half nature rock then third layer is of slag;
- iii) For 25% nature rock, 75% slag: from bottom first layer is of nature rock and the others two slag.

2.2. Testing Methods

Standard method of LAA and AIV is used for testing. To find the abrasion value,

$$loss = \frac{W_a - W_b}{W_a} \times 100 \quad (1)$$

Where,

W_a = Original weight of the sample (5 kg)

W_b = Weight of the sample retained on 1.70 mm sieve.

To find out impact value,

$$Aggregate\ Impact\ Value = \frac{W_a}{W_b} \times 100 \quad (2)$$

Where,

W_a = Weight of the sample passing through 2.36 mm sieve

W_b = Total dry weight of the sample

3. Results and Discussion

3.1. LAA Test Result

The standard IS 2386: Part IV 1963 set a range of limits for Aggregate Abrasion value is 30% maximum. Figure 1 represents the result of an LAA test in the value of Abrasion Value in percent. In accordance with the predicted earlier, slag has the smallest abrasion value which means better than the nature rock. Surprisingly, the abrasion value of the 25% nature rock and 75% slag has good results also, even not really significantly differ. Submerged slag has almost the same value compare to usual slag. From those four results which have almost the same value, it can be known very clearly that slag has great influence for affecting better result of LAA test.

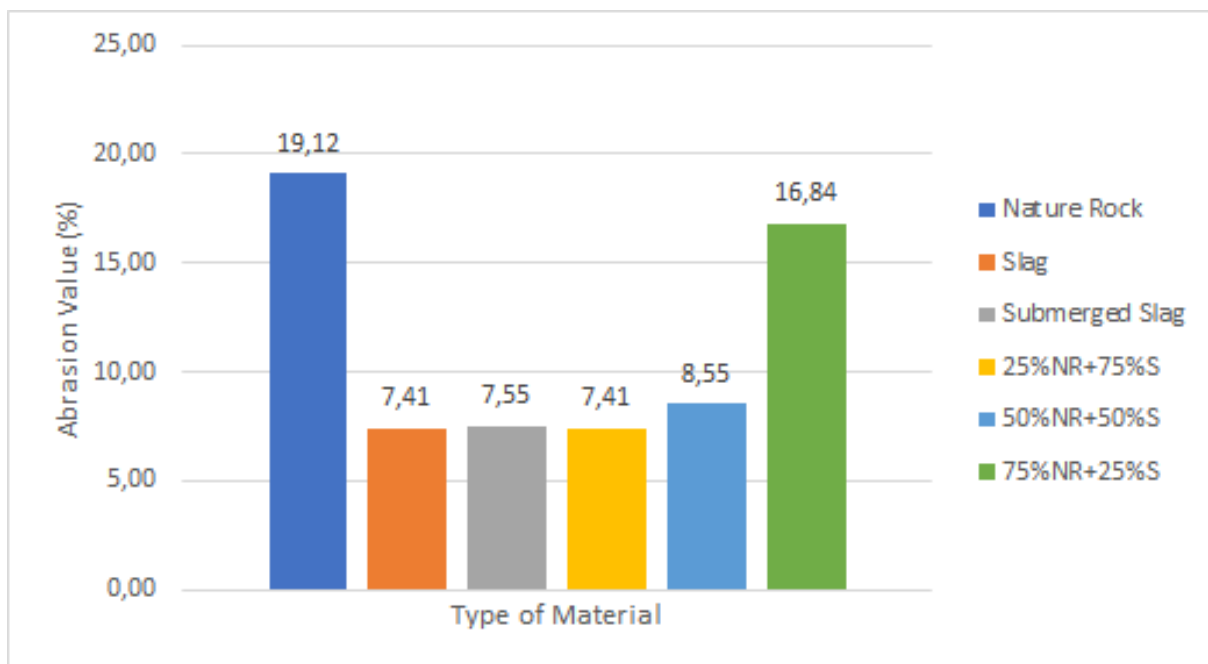


Figure 1. LAA test result of all sample

3.2. AIV Test Result

Figure 2 shows the Impact Value in percent. The smaller the value of impact value the stronger the material. This indicates that not much material has been destroyed due to applied load. It is clear that slag has the smallest value among all materials. Based on AIV classification, slag is considered to be exceptionally strong while nature rock has higher value comparing with other samples. But it is still considered to be strong according to the standard. The impact value of 1NR+2S is 6,02%. However, this result is still in the range of exceptionally strong. It may prove our assumption is correct. This behaviour indicates possibility the interlocking effect of each material. The standard IS 2386: Part IV 1963 set a range of limits for Aggregate Impact value is 20% maximum.

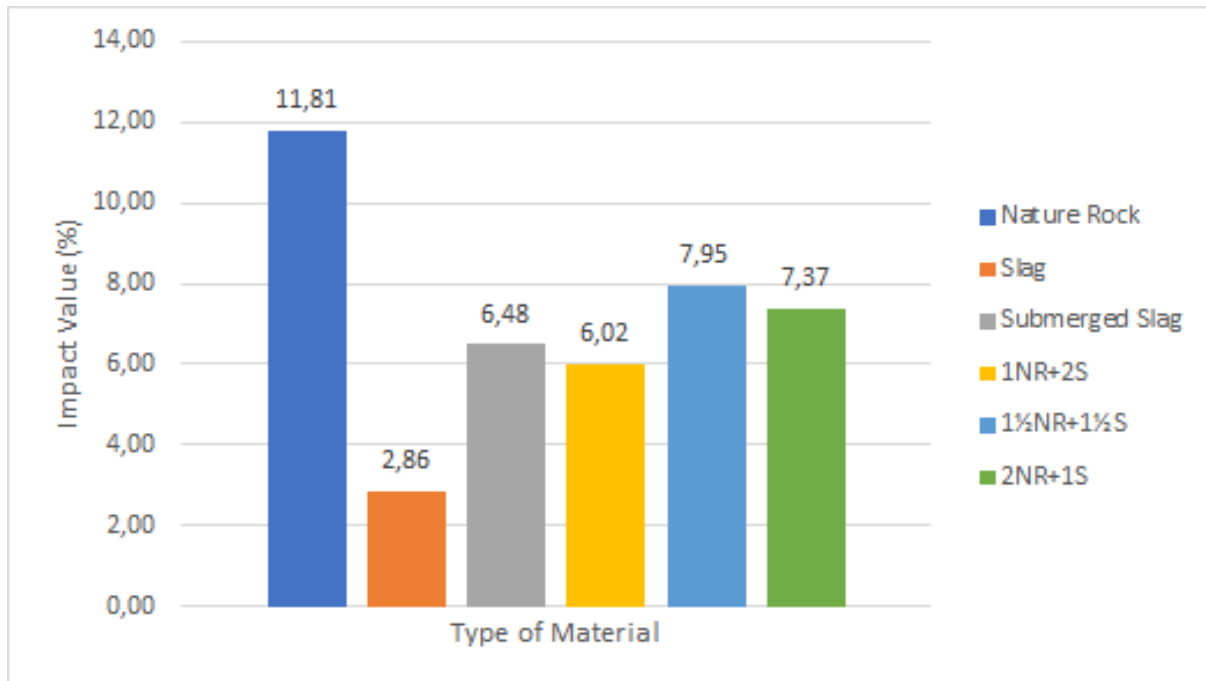


Figure 2. AIV test result of all sample

As it seen from AIV test result, the slag and submerged slag has much difference in value. This difference is not significant in LAA test. In fact, the submerged slag contained water in the void. LAA test is only for surface degradation. So, it can be assumed that the water did not affect the result that much. But, AIV test pound and try to crush it. Then, it is assumed that the water which take some place in voids and not fully removed will affect the result of AIV test.

4. Conclusion

The LAA and AIV test result of slag is 7.41% and 2.86%, respectively. It proves that slag is better materials among the others. Submerged slag also showed good result for both test, again this shows that the quality of slag is good. The test result of slag and natural rock combination surprisingly has good result also, especially for 25%NR+75% (LAA test) and 1NR+2S (AIV test). The result is 7,41% and 6,02%, respectively. However, future research about the combination of slag and nature rock, for ballast railway still need to be developed.

From the test, it is also noticed that that size of particle also affects the strength. Medium size particle between 30mm to 12mm size give better result. It is possible that different results will be obtained if large particles are tested in the LAA test. Whether that result still be reliable or not. So, for further research, it is recommended to examine large-sized particles as well.

From our result it can be seen that besides slag, the combination of nature rock and slag also give good result, but it is suggested doing more experiment on this and also find which one is more economical to use as railway ballast.



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References

- [1] B. Indraratna, M. Shahin, and W. Salim, "Use of Geosynthetics for Stabilizing Recycled Ballast in Railway Track Substructures," *Proc. NAGS2005/GRI 19 Coop. Conf.*, pp. 1–15, 2005, [Online]. Available: <http://ro.uow.edu.au/engpapers/726/>.
- [2] A. Y. Alemu, "Survey of Railway Ballast Selection and Aspects of Modelling Techniques," *Div. Highw. Railw. Eng. Div. Highw. Railw. Eng. Dep.*, pp. 1–61, 2011, [Online]. Available: <http://urn.kb.se/resolve?urn=urn:nbn:se:kth:diva-87466>.
- [3] Y. L. Guo and G. Q. Jing, "Ballast degradation analysis by los angeles abrasion test and image analysis method," *Bear. Capacit. Roads, Railw. Airfields - Proc. 10th Int. Conf. Bear. Capacit. Roads, Railw. Airfields, BCRRRA 2017*, no. June 2018, pp. 1811–1815, 2017, doi: 10.1201/9781315100333-258.
- [4] D. Li, J. Hyslip, T. Sussmann, and S. Chrismer, *Railway geotechnics*. 2002.
- [5] S. Adomako, C. J. Engelsen, R. T. Thorstensen, and D. M. Barbieri, "Review of the relationship between aggregates geology and Los Angeles and micro-Deval tests," *Bull. Eng. Geol. Environ.*, vol. 80, no. 3, pp. 1963–1980, 2021, doi: 10.1007/s10064-020-02097-y.
- [6] M. L. Chen, G. J. Wu, B. R. Gan, W. H. Jiang, and J. W. Zhou, "Physical and compaction properties of granular materials with artificial grading behind the particle size distributions," *Adv. Mater. Sci. Eng.*, vol. 2018, 2018, doi: 10.1155/2018/8093571.
- [7] B. Hr, P. For, S. From, and I. To, "World Journal of Engineering WJERT," vol. 4, no. 2, pp. 302–313, 2018.



Cnc Machine Design (Computer Numeric Control) Mini Plotter For Arduino Based Souvenir Craft

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Abstract. The plotter is a graphic printer that draws using ink pens, the plotter is also the first output device capable of printing large-sized images of architectural and engineering drawings. A plotter is a type of printer that is specifically designed to produce computer output in the form of images or graphics. There are many types of plotters, one of which is the pen plotter, which has one or more colored pens for drawing on transparent paper or plastic and producing a line output. CNC (Computer Numerical Controller) is one of the machine control tools in large industrial factories. With the CNC can control most of the tools one example is as a controller of a 2-dimensional plotter. This research designs a 2-dimensional plotter using Arduino UNO-based CNC. This 2-dimensional plotter has an accuracy of 97.947% and a precision of 99.985%. This 2-dimensional plotter is capable of operating up to a distance of 4cm with a resolution of 0.01cm.

Keywords: CNC, Arduino UNO, Servo Motor, Stepper Motor

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1. Introduction

Technological advances are countless, more and more technology is used and utilized to develop a system that can help work and even daily human activities. So the use of electronic and computer technology is used as much as possible, for example in the field of crafts to get products that have good quality supporting tools are needed including a CNC (Computer Numerically Controlled) machine which is a machine controlled by a computer and uses Arduino and uses numeric language (data





command), with numbers, letters and symbols). For CNC example is CNC Electric Discharge, CNC Plasma Cutter, Router CNC, Milling CNC. The work system of CNC machines is more synchronous between computers and mechanics when compared to similar machine tools, so CNC machine tools are more accurate, precise, and flexible [1].

In the research conducted by the author, the author found one of the merchandising business places where at that place still uses transfer paper to print both images and writing on wood media, where the human resources owned by the company are very limited so that when many merchandise orders experience a little difficult because it meets the many requests from consumers. One of the difficulties experienced was that the company was only able to complete production in a day ranging from 50 pcs to 65 pcs, processing 1 key chain merchandise with writing took 10 minutes, the second difficulty was that the workers had difficulty when consumers asked for the products he ordered to be detailed. and neat because of limited resources, not a few are also less than optimal products [2].

Mini CNC machines are machines that are cheaper in terms of purchase price, maintenance and operational costs, but the complexity of the program is reliable, where the program used is the same as a large CNC router machine. Its use is also very easy because the CNC operation uses a program that is controlled directly by a computer and arduino. The prototyping method is a step aimed at transforming the abstract properties of an idea into a more tangible one. This stage is not only in the form of an idea visualization process but also an idea development process. In general, Prototype has two categories: low-fidelity and high-fidelity. The prototyping process used in Design Thinking is low-fidelity or Rapid Prototyping. This process emphasizes on making a fast, easy, cheap and basic manufacturing process [3].

Based on the problems above in this study the author will make a prototype tool from cnc, using the Arduino Uno microcontroller as the controller of the cnc machine. In this study, the author uses Arduino IDE as a tool to upload programs to control several peripheral sensors from the sensors used. The software interface on the Arduino UNO-based Mini Plotter CNC tool is an image program that has been converted into machine language or G-Code, then this machine language instructs the tool/machine to perform an action, in its use it will use the gctrl.pde processing program, this program sends G-Code image to CNC plotter in other words G-Code is a file with X,Y and Z coordinates. Before installing, first connect the computer with usb to serial communication via a USB cable to the Arduino UNO microcontroller circuit [4].

2. Methods

2.1. Design

Design is a means that has a purpose to complete a system, when the system analysis term is running, the design is the next term. The following are opinions about design based on experts, including:

"Design is the process of planning everything in advance. Design is a visual form resulting from creative forms that have been planned. The initial step in designing the design starts from things that are not regular in the form of ideas or ideas then through the process of cultivation and management will produce things that are orderly, so that things that are already in order can fulfill their functions and uses properly. Design is a drawing, planning, sketching of several separate elements into a unified whole and functioning [5].

"Design is the preparatory stage for the design and implementation of a system that describes how a system is formed which can be in the form of drawing, designing and sketching or arrangement of several separate elements into a unified whole"[6].

Based on the above design theory, it can be understood that the design is a process leading to the development of newer processes, methods, details based on the recommendations of the results of system analysis and is a preparation in the design of a system[7].

2.2. CNC PLOTTER

CNC is a "Computer Numerical Control" with an automatic machine tool system that is executed by commands from an abstract program and will be stored in storage media, making CNC machines different from the way machine tools work in general which are controlled using hand rotation or simple automation. using CAM. To produce certain movements and positions according to the image to be printed, the CNC machine will be controlled electronically.

CNC technology is the key to machine tool technology, which is the basis of industrial computerized units. CNC machines are operated by controllers, each of which has a software module known as an interpreter to retrieve data from the CAM system generated code and convert it to controller motion commands. However, with the development of numerical control technology, existing CNC systems are limited with translators to overcome this problem. A new software system conceptual module is presented [8].

In CNC the assembly will consist of mechanical parts that have three degrees, namely, a driving motor in the form of 2 stepper motors, an electronic circuit that functions as a stepper motor drive, a parallel port interface, and software for G-code translators of CNC machine language. In today's industrial competition, it is very necessary to update tools that can combine electronics, mechanics and software at the same time in order to create tools that can meet the needs in one tool, such as a CNC mini plotter machine controlled by software, mechanisms in designing CNC machines using motors. stepper and pulley and belt, namely a pair of machine elements used to transmit power from one axle to another[9].



Figure 1. CNC Mini Plotter

The way a CNC machine works is that it starts with software that will make the initial design of the image which will then be forwarded via USB (Universal Serial Bus) to the Arduino Uno microcontroller which has loaded the program command code to run the CNC machine, via a stepper motor that gets commands from Arduino in the form of pulses. towards the stepper motor, namely the L293D motor shield. As a result, the stepper controller will control the stepper motor to the X axis and Y axis, the servo motor to the Z system. In this final project, the author has described the integration in electronics and mechanics in the design of a mini plotter CNC machine. X-Y Plotter Prototype Design with Arduino Based G-Code Interpreter was made using 3 servo motors for each axis. The movement of each axis is controlled by instructions G00 and G01 via a computer connected to USB serial communication [10]. The programming methods used in CNC machines are:

1. Incremental Method



A programming method where the reference point is always changing, that is, the last point to be addressed becomes a new reference point for the next measure.

2. Absolute Method

A programming method where the reference point is always fixed, that is, one point/place is used as a reference for all subsequent measures

Models in terms of type, CNC machine tools can be divided into three types, including:

1. The CNC 2A machine is a 2-axis CNC machine, because the tool movement is only in the direction of the two coordinate axes (axis), namely the X-coordinate and the Z-coordinate, otherwise known as a CNC lathe.
2. 3A CNC machines, namely 3 axis CNC machines or machines that have the main axis movement towards the X, Y, and Z coordinate axes, otherwise known as CNC milling machines.
3. Combined CNC machines, namely CNC machines that are capable of doing both lathe and milling work at the same time, can also be equipped with measuring equipment so that they can control the quality of turning/milling on the resulting workpiece. In general, the most common CNC machines are CNC 2A (lathe) and CNC 3A (milling) machines [11].

2.3. G-Code

A programming language is a command format that exists in a block that uses and uses alphabetic codes, numbers, and symbols. Inside the CNC machine tool there is a computer device called the Machine Control Unit (MCU). This MCU serves to translate the coded language into the form of tool movements according to the shape of the workpiece. The language codes on CNC machine tools are also known as G and M codes, where these codes have been standardized by ISO or other international bodies. In the application of alphabetic codes, numbers, and symbols in CNC machine tools, they vary depending on the control system and the type of machine used, but the principle is the same. So that for operation in CNC machine tools using different types, there is no significant difference.

G-Code is a language that will be used in the Numerical Control programming language which contains information on the position of a tool to do a job. The G-Code is a preparatory code, contained in their CNC program starting with the letter G and driving the machine. Actions that are generally directed by the G-Code include: turning a pallet, rapid movement, a series of controlled feed movements, producing a piece of goods, drilled holes or decorative shapes, controlling feed movement, in arcs or straight lines, and setting tool information [12].

3. Results and Discussion

3.1. Research Results

CNC Mini Plotter is a combination of computer software and mechanical electronic structure. The stage in the design of the tool is divided into two, namely designing a software program as the operation of the tool and also as an initial design and converting it into GCode which is understood by the tool so that it can be continued to print as the desired output.

The second stage is to design the hardware components that will be used, with the aim of producing the desired output and also shortening the time required for work.

In this chapter is a discussion related to the design of the tool from the block diagram that the author is doing and researching. Based on the previous chapter, the tool block diagram that the author will describe in the following sub-chapter is the result of how the tool runs according to the steps in Figure 3.1.

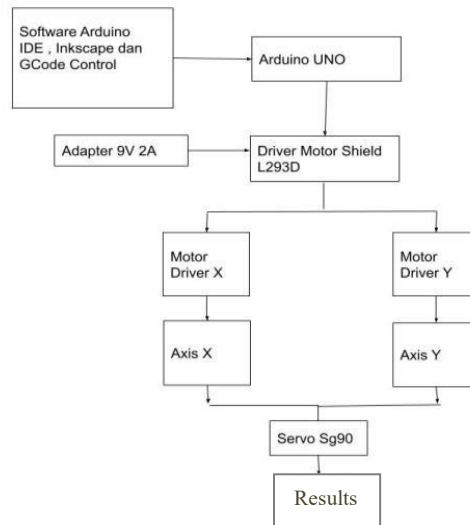


Figure 2. Diagram Blok CNC Mini Plotter

The first step that must be done in running the program is to open the Arduino IDE, Inkscape and GCode Controller software and initialize the Arduino UNO and GCode Controller ports. Then open the Inkscape software and enter the images, sentences, and both that you want to print, the images and text will be converted into G-Code format by re-saving with the .gcode format in the Inkscape application, which has previously installed the additional MarketBot Unicorn G-Code library. so that images and text can be read by the GCode Controller application.

The next step is to design an Arduino program to operate the tool and control the movement of the servo and motor shield, which aims to calibrate the axis which will then calculate the movement of the y-axis and x-axis in a matter of millimetres. Then a compilation test of the Arduino program that has been made will be carried out, this is intended to test whether there are errors in the program created. After doing the compile test, the program will be uploaded to the Arduino. If there is an error in the program, the program will be reviewed and then tested, compile again and then forwarded to upload to the Arduino Uno, upload here is to enter the program as the main Arduino Uno command.

Furthermore, Arduino Uno will forward the command to the Motor Shield Driver, which is first given a 9v 2A adapter voltage to be able to drive and control the main axis, the motor driver available on the DVD Rom, each axis that is connected to the Motor Shield will move according to the Gcode.

The servo motor will then operate according to the command, which must go up or down to adjust the pen that is drawing the output so as not to produce streaks of the y-axis movement later.

3.2. Tool Design

Based on the results of conducting research and the diagrams that have been made in the previous chapter, it results in an implementation in the form of a prototype. The design of the tool that the author has done is based on the actual situation so as to get an overview of the tool to be designed later. This design can be seen in the following figure:

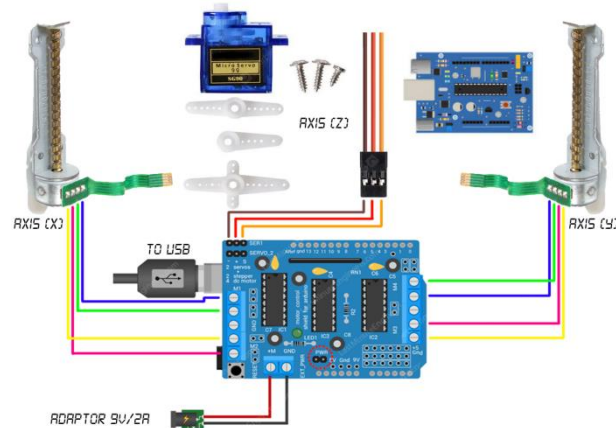


Figure 3. Tools design

4. Conclusion

The conclusion that can be drawn from this research is that the accuracy of the appropriate tool is 97.947% and the precision of the appropriate tool is 99.985% and the proper width (range) is 4cm for the X-axis and Y-axis and the resolution of the appropriate tool is 0.1mm.

References

- [1] I. Alzusnan, J. Teknik, E. Politeknik, and N. Lhokseumawe, "RANCANG BANGUN SISTEM MONITORING LEVEL FLUIDA BERBASIS," vol. 5, no. 1, pp. 15–20, 2021.
- [2] I. V. Choirony, M. S. Hariyanto, M. Ulum, A. Ubaidillah, H. Haryanto, and R. Alfita, "Rancang Bangun Acrylic Engraver and Cutting Machine Menggunakan CNC Milling 3 Axis Berbasis Mikrokontroler," *Elektrika*, vol. 13, no. 1, p. 13, 2021, doi: 10.26623/elektrika.v13i1.3071.
- [3] S. Raad, H. Mohammad, and M. Falah, "Accurate and Cost-Effective Mini CNC Plotter," *Int. J. Comput. Appl.*, vol. 178, no. 48, 2019, doi: 10.5120/ijca2019919370.
- [4] I. D. Febryanto and S. D. Kartikasari, "PERANCANGAN MESIN CNC ROUTER 3 AXIS BERBASIS METODE QUALITY FUNCTION DEPLOYMENT (QFD)," vol. 17, no. 1, pp. 109–120, 2022.
- [5] H. Kusumo and S. Rakasiwi, "Information System Supply Chain Management with FIFO Pertetual Method," *Adv. Sustain. Sci. Eng. Technol.*, vol. 3, no. 2, 2021, doi: 10.26877/asset.v3i2.9722.
- [6] M. M. Hasan, M. R. Khan, A. T. Noman, H. Rashid, N. Ahrned, and S. T. Reza, "Design and Implementation of a Microcontroller Based Low Cost Computer Numerical Control (CNC) Plotter using Motor Driver Controller," 2019, doi: 10.1109/ECACE.2019.8679123.
- [7] J. E. Bresenham, "Algorithm for computer control of a digital plotter," *IBM Syst. J.*, vol. 4, no. 1, 2010, doi: 10.1147/sj.41.0025.



- [8] V. F. Goszal, H. H. Nuha, and M. Abdurohman, “Manufacturing a Plotter Printer with Computer Numerical Control Based Pen Ink Using CoreXY Mechanisms,” *J. MEDIA Inform. BUDIDARMA*, vol. 5, no. 2, 2021, doi: 10.30865/mib.v5i2.2783.
- [9] E. Poustinchi, “Subtractive digital fabrication with actual robot and virtual material using a MARI platform,” *Int. J. Archit. Comput.*, vol. 16, no. 4, 2018, doi: 10.1177/1478077118801594.
- [10] A. Örtorp and T. Jemt, “Clinical experiences of computer numeric control-milled titanium frameworks supported by implants in the edentulous jaw: A 5-year prospective study,” *Clin. Implant Dent. Relat. Res.*, vol. 6, no. 4, 2004, doi: 10.1111/j.1708-8208.2004.tb00036.x.
- [11] D. Mejia, A. Moreno, A. Arbelaiz, J. Posada, O. Ruiz-Salguero, and R. Chopitea, “Accelerated Thermal Simulation for Three-Dimensional Interactive Optimization of Computer Numeric Control Sheet Metal Laser Cutting,” *J. Manuf. Sci. Eng. Trans. ASME*, vol. 140, no. 3, 2018, doi: 10.1115/1.4038207.
- [12] L. Luo, Z. X. Yang, L. Tang, and K. Zhang, “An ELM-Embedded Deep Learning Based Intelligent Recognition System for Computer Numeric Control Machine Tools,” *IEEE Access*, vol. 8, 2020, doi: 10.1109/ACCESS.2020.2965284.



Genetics, Biochemistry and Biophysical Analysis of Anthocyanin in Rice (*Oryza sativa* L.)

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Abstract. Rice (*Oryza sativa* L.) is the primary staple food for half of the world population. It is generally classified based on the grain color into black, red, purple, brown, green, and white. These colored rice are determined by the composition and concentration of anthocyanin pigments in different layers of aleurone, pericarp, and seed coat. Anthocyanins are also accumulated in various tissues of the rice plants, mostly in the grain, but are also presents in leaves, leaf sheath, floral organ, and hull. The type and concentration of the anthocyanins in rice tissues are influenced by the cultivars and developmental stages. Anthocyanin-enriched rice is related to the health effects, including antioxidant, antibacterial, and anti-inflammation activities that potentially use as functional food ingredients, dietary supplements, and natural colorants. Structural and regulatory genes are involved in anthocyanin biosynthesis of rice. Various molecular biology techniques have been applied to improve productivity, nutritional contents, and market value of pigmented rice. This review focused on the genetics, biochemistry and biophysical analysis of anthocyanin in rice that will facilitate rice breeding program to develop new high-yield pigmented rice varieties.

Keywords: rice, anthocyanins, genetics, biochemistry, biophysics

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1. Introduction

Rice (*Oryza sativa* L.) has become the major staple food for almost half of the global population due to the nutrients composition, including carbohydrate, protein, oil components, and other micronutrients [1-5]. There are various kinds of rice are consumed that can be classified based on the grain color into black, red, purple, brown, green, and white [6-9]. White rice is generally consumed, while pigmented rice, such as black, red, purple, and brown contain natural pigment anthocyanins that accumulate in the particular layers of the seed coat, pericarp, and aleurone [10-18]. Total anthocyanins in each pigmented





rice varieties are diverse in the range of 0-493 mg/100 g [19]. Black rice is the most common pigmented rice in the market due to their sensory characteristics and organoleptic properties, such as good taste, fragrant aroma, fluffiness texture, high nutrition values, and positive health effects [20-27]. China is the biggest black rice producer followed by Sri Lanka, Indonesia, India, Philippines, Bangladesh, Malaysia, Thailand, and Myanmar [17]. Pigmented rice genotypes have been cultivated in Asia for a long time, such as Chinese black rice, Indonesian black rice, and Thai black rice [20]. Anthocyanins of the pigmented rice has the potential to be applied as a dietary supplements, functional food ingredient, and natural colorant for food, beverages, and pharmaceutical products. Anthocyanins show positive effects for the human health, including antioxidant, antibacterial, anti-inflammatory, anticancer, anti-diabetic, antitumor, anti-allergic agents, anticarcinogenic, anti-atherosclerosis, and others [23], [28-37], [38-40]. Consequently, anthocyanins play important roles in preventing human diseases, such as atherosclerosis [12], [28], [41], diabetes [42-44], and cancer [45-49]. In the rice plants, anthocyanins involve in pollination to attract the insects, UV-B protection, hormone responses regulation, photo-perception in autumn leaves, stabilize photosynthetic activity, biotic and abiotic stress defense system [50-59].

Anthocyanins are water-soluble natural pigments classified to the phenolic compounds of flavonoids group which responsible for attractive colors, such as purple, red or brown in different tissues of the rice plants with various concentration and composition of anthocyanins [60-64]. There are six anthocyanin types, including malvidin, peonidin, cyanidin, delphinidin, petunidin, and pelargonidin [65]. Different combinations of anthocyanins lead to different colors, the higher anthocyanins concentration and combination, the blacker the color. The concentration and composition of anthocyanins in the rice plants are vary depend on the rice variety and developmental stages [66-68]. The common anthocyanin types in the pigmented rice are cyanidin-3-glucoside, while the minor is peonidin-3-glucoside [69-70]. Anthocyanins are important secondary metabolites in the rice plants and accumulate in various tissues and organs that related to photosynthesis, reproduction, and defense, such as pericarp, aleurone, awn, leaf blade, leaf sheath, palea, lemma, internode, stigma, apiculus, and root [71-74]. Different level of purple color are identified in these tissues and organs of the rice plants. For example, purple and red rice contain 20 times higher concentrations of the anthocyanin in their aleurone layer compared with brown rice [75]. Black rice contain higher total antioxidant capacity compared to red rice; black rice have 0.5-2.5% while red rice only 0.03-0.1% [76]. The purple pigmentation is regulated by the allelic variation of genes, the co-segregation of the alleles do not always happen [72]. Anthocyanin biosynthesis is controlled by the structural and regulation genes, and the stability of the anthocyanins also influenced by the environmental conditions, including temperature, pH value, lights, enzymes, oxygen, and metallic ions [51], [77-84]. Anthocyanin biosynthesis can be enhanced by environmental modification, such as maintaining temperature ranging from 22 to 27oC and light intensity between 301-600 lx. Maintaining the stability of anthocyanins is a crucial factor in food and pharmaceutical industry [85]. Accumulation of anthocyanins in the rice plants are depend on the rice developmental stages, including seedling, vegetative, reproductive, and mature stages. During maturity stage, black rice contains higher anthocyanins in the aleurone layer compared with grain filling stage [75].

Anthocyanins have been used in human diet for centuries as herbal medicines to cure several health problems, such as cold, diarrhea, and hypertension. Recently, anthocyanins are being applied as natural colorant of food and beverages, and also dietary supplements due to their attractive colors and health benefits. In the United States, estimated anthocyanins consumption is around 12.5 mg/day [86]. Black rice as the common pigmented rice in the market that contain various combinations of anthocyanins, such as cyanidin-3-glucoside, peonidin-3-glucoside, and petunidin-3-glocoside in the aleurone layer of the rice grain [13], [17], [70], [76], [87-88]. Cyanidin-3-glucoside is the most anthocyanins composition in black rice around 631 mg/100 g, while peonidin-3-glucoside is around 363 mg/100 g [13], [70], [88-89]. Southeastern Asian countries are the primary producer of black rice. Recently, California also produces black rice due to high market demand [90]. European countries that cultivate black rice are Italy and Greece [23]. In Asian countries such as Thailand, China, Indonesia,



India, Korea, and Japan, black rice is usually combine with white rice to increase the flavor [12], [91-94]. The color of cooked black rice become regal purple [95]. Black rice is usually use as food ingredients in fried rice, paella, risotto, porridge, bread, pasta, and rice cake [96-97]. Black rice also become an important material to produce alcoholic beverages with red color. There are many varieties of black rice, including short and long grains, glutinous and non-glutinous rice, early and late maturity period [98-100]. Red rice is generally used as a natural colorant in ice cream, bread, and liquor [11], [101]. Pigmented rice also contains higher concentration of micronutrients, such as iron, manganese, and zinc in the grain compared to white rice [102-107]. These pigmented rice has a potential to decrease malnutrition around the world [66]. Many studies have been reported the nutritional values of black and red rice [23], [41], [108-111]. Powdered anthocyanins extracts from black rice was produced with spray-drying and freeze-drying processes of Italian black rice that contain rich anthocyanins and antioxidant activity [112]. These powdered anthocyanins are more stable from environmental conditions of storage and food processing, including temperature, pH value, and lights that give high economic value to use for functional foods and pharmaceuticals products [6], [79], [113-114].

In China, red rice has been approved by the Chinese Ministry of Health as a food colorant in soybean products, meat, and fish [115]. Meanwhile, in United States, Canada, European Union, Japan, Australia, New Zealand, and South Africa restrict anthocyanins as a food colorant [116]. Red rice is commonly use as food colorants and dietary supplements in China due to attractive and high stability color from high light exposure, pH changes, and heat conditions; good taste and flavor; cheap; high availability; and high yield rice variety [117]. The anthocyanin concentration in red rice is lower than black rice around 1.5 to 9.4 mg/100 g [70], [118]. Fermented rice as a dietary supplement to decrease cholesterol accumulation of the blood circulation has been marketed in China. Red rice as a herbal medicine to treat cardiovascular disease and abdominal pain [11].

Analysis of biosynthesis, storage, and transportation mechanisms in anthocyanins have been achieved a significant progress due to the development of molecular biology. These progress give a positive impact on the food industry, pharmaceuticals, flavors, rice breeding program [119-121]. Identification of physicochemical properties, quantification, and extraction of anthocyanins in the rice plants was based on Ultraviolet-Visible (UV-Vis) absorption spectrophotometer, High Performance Liquid Chromatography (HPLC), mass spectrometry, liquid chromatography, and paper chromatography [101], [104], [122-124]. Based on the UV-Vis absorption spectrophotometer data, anthocyanins show maximum absorption range in the region 500-535 nm of the blue spectrum; malvidin at 530 nm, peonidin at 517-520 nm, cyanidin at 512-520 nm, delphinidin at 525 nm, petunidin at 526-529 nm, and pelargonidin at 502-506 nm [70]. By identification of phytochemical properties of anthocyanins in pigmented rice gives insights to the application of pigmented rice as health promotion agents [122], [125-126]. Based on the genetic analysis of pigmentation in pigmented rice, there are three genes that regulates the pigmentation, including Ra, Rc, and Rd genes. The intensity of the pigmented rice coloration is influenced by the presence of genes and the genes status (dominant or recessive). Ra genes regulated purple pericarp, which purple color is dominant and white color is recessive. Brown pericarp is produced when Rc gene presence and Rd gene absence. Both of the genes, Rc and Rd genes are presence produce red pericarp. Meanwhile, if only Rd gene presence, it will not produce any color [99], [127-128]. The alleles segregation of coloration in pigmented rice also presence, for example F2 population of the crossing between black rice and white rice varieties showed three phenotypes; black, brown, and white color with the segregation ratio 9:3:4.

Pigmented rice cultivation since ancient time and in 1970s become more popular due to the development of genetic engineering [129]. The famous pigmented rice varieties in Korea are dark red Heugjinjubyeo, dark purple Heugnabyeo, dark blue Jakwangdo, red brown Sanghaehyeolla, black purple Hongmi, and dark red-purple Kilimheugmi [130]. The quality of pigmented rice varieties have been improve by employing recent technologies, including genome sequencing, gene expression analysis, gene editing, and omics technologies [102], [131-134]. High-yield pigmented rice varieties



have been developed by applying the characteristics of grain pigmentation inheritance, tagging the key genes that controlled the rice quality traits, and identifying markers of these rice quality traits [135-136]. The advanced pigmented rice varieties can be developed by understanding the molecular basis of anthocyanin biosynthesis in several organs of the rice plants [50]. In Japan, improved pigmented rice variety was developed by crossing black rice variety 'Okunomurasaki' with high quality-white rice variety 'Koshihikari' [137]. In Kazakhstan, adapted pigmented rice variety was developed by crossing pigmented and non-pigmented rice varieties [138]. In Thailand, a new deep purple rice variety 'Riceberry' was developed by crossing between non-glutinous purple rice and an aromatic white jasmine rice variety [139-140]. Brazil has been released two advanced pigmented rice varieties; the red rice 'Rubi' and the black rice 'Onix' [141]. In China, biofortified purple endosperm rice called 'Zijingmi' with high anthocyanins concentration was developed by editing anthocyanin biosynthesis [142]. New foods and beverages from pigmented rice also have been developed by using improved processing technologies. This review provides an update information on the genetics, biochemistry and biophysical analysis of anthocyanin in pigmented rice that will facilitate rice breeding program to develop improved pigmented rice varieties.

Anthocyanins in Rice Grains

Article title, author's name (without academic title), affiliation and author's affiliate address are placed on the first page under the article title. The distance between the lines between the title and the author's name is 2 spaces, while the distance between the author's affiliate address and the abstract title is 1 space. Keywords must be written below the abstract text for each language, arranged in alphabetical order and separated by commas with 3-5 words.

The author of the responsible or corresponding author must be marked with an asterisk *. At the bottom of the left column of the first page, you must write the Corresponding Author and also write the email address (using an email containing the address of the institution).

If there are more than one author, all authors' names are separated by commas (.). If the author has more than one affiliate, the affiliation is written in sequence. Superscript marks in the form of numbers, for example 1, are given behind the author's name. If all authors are from one affiliation, this sign does not need to be given.

Anthocyanins in Rice Grains

Anthocyanins is mainly accumulated in the pericarp of the rice grains (Figure 1). Purple bran color showed the highest total anthocyanin (2874 cyanidin 3-O-glucoside equivalent (CGE)/100g) followed by black (1884 CGE/100 g), red (8.78 CGE/100g), and brown (3.09 CGE/100g). These rice bran color has been reported to be correlated to the seed dormancy, red bran color rice have a longer dormant compare to the white rice [143]. Based on the quantitative trait loci (QTL) analysis, one QTL qSD7/qPC7 that regulated rice bran color and seed dormancy was identified on chromosome 7 [144]. Anthocyanin contents in the rice pericarp were significantly influenced by environmental conditions and rice developmental stages (Figure 2). Rice developmental stages influence the anthocyanin concentration in caryopsis that shows gradual color changes at each developmental stage. The anthocyanin level increases as the increasing developmental stages and gradual grain filling. At 8-14 days after flowering (DAF), anthocyanins start accumulate in the caryopsis. At the milk stage, caryopsis becomes black and at the maturity stage (35-45 DAF) is the highest anthocyanins concentration accumulate in the caryopsis. The gradual changes of the anthocyanin concentration correlated with the gene expression that control anthocyanin biosynthesis [145]. During maturity stage, the gene expression level of OsDFR, OsF3H, OsAns, and OsCHS are increasing [146]. The anthocyanin accumulation in black rice also influenced by photosynthetic activity.

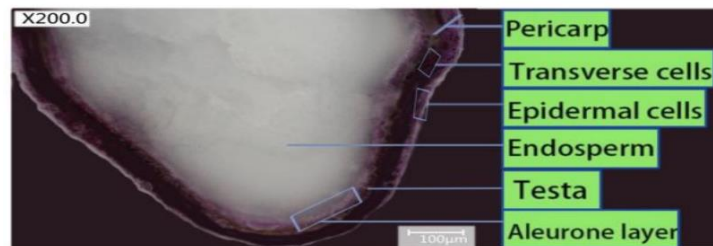


Figure 1. Cross-section of black rice grain showing anthocyanin accumulation in pericarp [66]

Anthocyanin is synthesized on the endoplasmic reticulum, transported through the Golgi apparatus, and accumulated in the vacuole cells of vegetative and generative organs. Anthocyanin biosynthesis is influenced by environmental conditions, such as salinity, drought, abscisic acid (ABA), and rice diseases [147-149]. Black rice pigmentation is regulated by key activator loci for anthocyanin (KALA), such as Kala1, Kala3 or MYB3, and Kala4 [137]. Based on the QTL analysis, Kala1 was identified on chromosome 1 between SSR markers RM7405 and RM7419, Kala3 on chromosome 3 between RM15191 and RM3400, and Kala4 on chromosome 4 between RM1354 and RM7210. LOC_Os04g0557500 within Kala4 region controls the purple color in the rice pericarp. Anthocyanin concentration in the rice pericarp can be enhanced by overexpress LOC_Os04g0557500 [20]. Red rice pericarp is controlled by a QTL rg7.1 on chromosome 7 and LOC_Os07g11020 was identified within the QTL region [150]. LOC_Os07g11020 encodes a bHLH TF. Two genes PURPLE PERICARP A (Pp, Prpa and Prp1) on chromosome 1 and PURPLE PERICARP B (Pb, Prpb and Prp2) on chromosome 4 are regulates the purple color of rice pericarp [151-155].

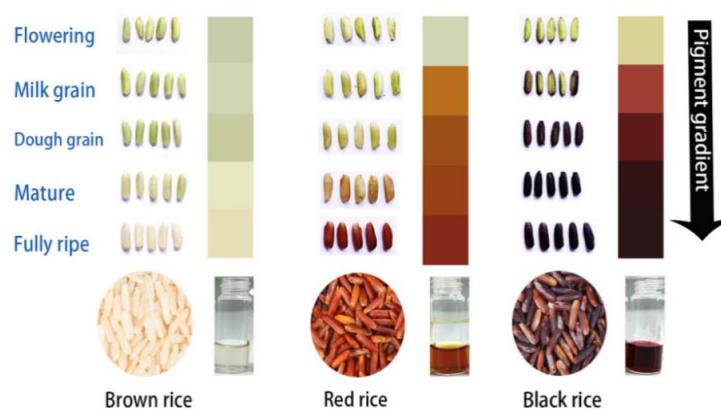


Figure 2. Pigment gradient in brown rice, red rice, and black rice during developmental stages [66]

Transgenic pigmented rice varieties were developed by using transgene stacking system that have higher nutritional and medical values for food and pharmaceutical industries [137]. Anthocyanin concentration in the rice pericarp can be improved by using this genetic engineering technique that can be enhanced their antioxidant activity and seed dormancy period [162]. By enhancing the anthocyanin concentration in the rice pericarp may enhance the abiotic and biotic resistance [50]. Important genes regulating the anthocyanin biosynthesis, such as CHS (chalcone synthase), F3H (flavanone 3]3-hydroxylase), DFR (dihydroflavanol), and ANS1 (anthocyanin synthase) were identified by using whole genome sequencing and transcriptomic sequencing in the pigmented rice plants [156]. Pigmented rice produce lower yield and lower grain weight than white rice varieties [157-158]. Lower grain yield of pigmented rice due to the anthocyanin deposition that reduce chlorophyll content in spikelet, decrease photosynthetic rate and also grain filling rate [159]. The accumulation of anthocyanin in pericarp of the

pigmented rice cause lower grain weight [145]. The lower grain yield and decreased grain size in black rice near isogenic lines (NILs) population was identified in Japan [137].

Anthocyanins in Rice Floral Organs

Floral organs of the rice plants including stigma and apiculus showing red, purple, or brown color because of anthocyanins accumulation (Figure 3). These obvious color is important in pollination to attract insects and other animals but does not apply in the rice plants due to self-pollination. Anthocyanins content in the floral organs also important as protective agents from ultraviolet radiation and strong light intensity, and also become defense system from abiotic and biotic stresses including salinity, drought, cold, heat, and diseases [160-162]. The specific color of stigma and apiculus is important for rice taxonomy [73], [163-164]. Investigation about purple stigma and apiculus started in 1957 [165]. OsB2 is an important gene regulating anthocyanin biosynthesis in floral organs of the rice plants. The variation color of apiculus is regulated by locus C, OsC1 [50].

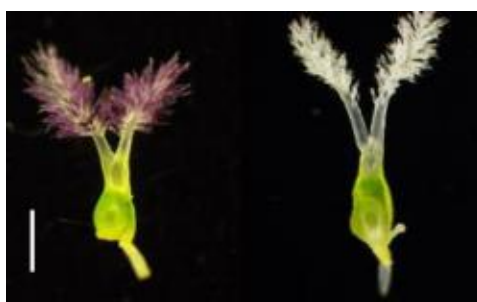


Figure 3. Purple and white stigma of the rice plants [73]

The variation color of apiculus and stigma are regulated by several QTL regions. Diverse color of apiculus is controlled by C-gene located between SSR markers RM19552-RM19565 [166-167]. Purple apiculus is regulated by Pa-6 and red apiculus is coordinated by OSC [163], [166]. Purple stigma is controlled by Ps-4(t) located in RM253, RM111, and RM6917 [168]. The first purple stigma gene OsC1 was identified on chromosome 6. Based on the map-based cloning strategy, two genes OsC1 and OsDFR that responsible for the purple color of stigma and apiculi were identified in pigmented indica rice cultivar Xieqingzao. A R2R3-MYB transcription factor is encoded by OsC1 on chromosome 6, while a dihydroflavonol 4-reductase is encoded by OsDFR. OsPa gene responsible for apiculi color and OsPs gene regulating the stigma color were identified by transcriptional expression analysis and CRISPR/Cas9. Variety color of stigma and apiculus can be produced by gene interaction of OsC1, OsDFR, OsPa, and OsPs. The purple color of stigma and apiculi is the result of genes interaction OsC1, OsPa or OsPs, and OsDFR. Brown apiculi can be produced by gene interaction OsC1 and OsPa. Knock-out of OsDFR resulting straw-white color stigma [169].

Anthocyanins in Rice Leaves

Leaves as the primary organ in photosynthesis promote the biosynthesis of anthocyanin and starch. Anthocyanin accumulation in the rice leaves reduces the efficiency of photosynthetic activity and consequently decrease the rice yield [170]. On the other hand, reducing the anthocyanin concentration in the leaves will increase the photosynthetic activity and subsequently improve rice yield. Consequently, in the rice variety selection process, purple leaf trait become a negative marker [50]. Based on the genomic sequence analysis, accumulation of anthocyanin in the rice leaves is regulated by OsC1 and OsDFR [170]. OsC1 controls cyanidin 3-O-glucoside concentration in the rice leaves [171]. Rb gene on chromosome 1 involves in anthocyanins biosynthesis in the rice leaves was identified by GWAS analysis. LOC_Os04g0577800 and LOC_Os04g0616400 on chromosome 4 also involve in

anthocyanin biosynthesis in the purple rice leaves that identified by using bulk segregant and transcriptome analysis [172].

Anthocyanins in Rice Leaf Sheath

Purple leaf sheath in rice due to the accumulation of anthocyanins. Leaf sheath color trait also become a marker in rice variety selection. The level of anthocyanin accumulation in the leaf sheath also influenced by the developmental growth stages. At V4 stage with 4-leaf, the anthocyanin concentration in the leaf sheath ranging from 0.01 $\mu\text{mol/g}$ until 0.06 $\mu\text{mol/g}$. The highest concentration of anthocyanins starting at active tillering stage until maturity stage around 1.16 $\mu\text{mol/g}$ [73]. Accumulation of the anthocyanins in the leaf sheath also correlated with defense system to the abiotic stresses, including soil acidity, ultraviolet radiation, and temperature [173-174]. Diverse leaf sheath color is regulated by OsC1 gene that has co-segregation with apiculus color [167]. A mutant rice plant Z418 showing purple leaf sheath which was developed from C418 rice variety with green leaf sheet color by modifying OsC1 gene [175]. OsC1 gene also identified in the F2 population of crossing between purple leaf sheath rice (Tainung 72 / TNG72) and green leaf sheath rice (Taichung Sen 17 / TCS17) [176]. There a segregation in that F2 population with ratio 3:1, 3 for purple leaf sheath and 1 for green leaf sheath, indicating that OsC1 is the dominant gene. Based on the RT-PCR analysis, the gene expression of OsC1 in the leaf sheath tissue started at 5-leaf stages [177]. Another gene that regulating the rice purple leaf sheath is PSH1(t) on chromosome 1 that identified in a recombinant inbred line (RIL) population resulting from crossing rice variety IRBB60 and 9407 [152]. Variation of the leaf sheath color also controlled by two QTLs on chromosome 1 and 6 [73], [178-179]. LOC_Os06g10350 on chromosome 6 as a gene controlling leaf sheath color was also identified by using F2 rice population and 117 markers. LOC_Os06g10350 belong to the MYB family transcription factor.

Variation of leaf sheath color ranging from light to dark purple showing tyran rose, pansy purple, red purple, and blackish purple. Anthocyanin accumulation in the leaf sheath ranged from 1.04 to 42.77 $\mu\text{mol/g}$, tyran rose color has the least anthocyanin concentration and blackish purple color has the most anthocyanin content (Figure 4). The diverse leaf sheath color also associated with the rice varieties [73].

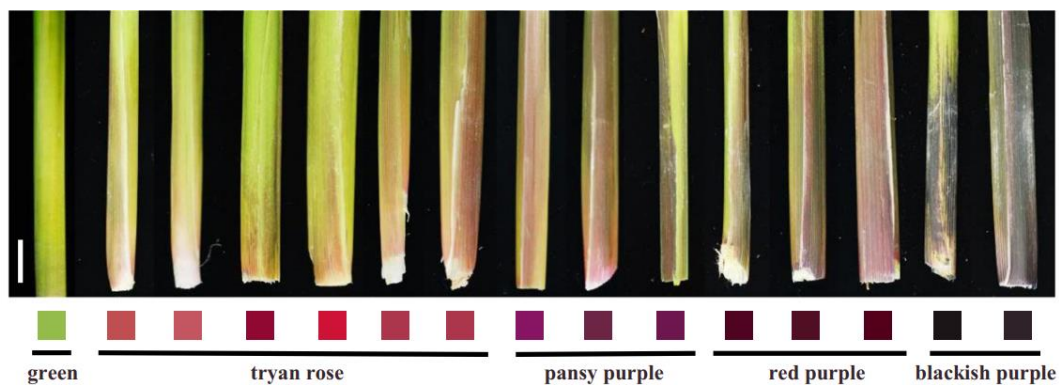


Figure 4. Diverse rice leaf sheath color [73]

Anthocyanins in Rice Hulls

Colored rice hulls which are black and red due to the accumulation of anthocyanins [36]. About 15% of the rice varieties are colored hull and the most rice varieties (85%) have white-hulled. Colored rice hull responsible to protect rice grain from oxidative stress [180]. China and Japan have been cultivated rice variety with colored hulls since ancient time due to the health positive effects. Recently, rice variety with colored hulls are cultivated in South Asia countries, United States, Italy, and Greece

[181]. The type of anthocyanin in the purple rice hull is cyanidin 3-O-glucoside with concentration 2.8 mg/g [182]. Lemma and palea of the rice hull are associated with the rice floral organs and seed characteristics including grain length, grain width, and grain weight [183-188]. The highest anthocyanins accumulation is in purple hulls. Rice hull has been treated as one of residue material from the rice plants, but right now colored rice hull is become antioxidant and anti-cancer sources. Straw-white hull correlated with non-shattered rice grains and became an important marker during rice domestication [189].









Pheno-type	Hull color	Straw-white				Brown		Purple	
						Apiculus	Hull	Apiculus	Hull
									
Geno-type	C1								
	S1								
	A1								

Figure 5. C-S-A gene system of rice hull pigmentation [182]

Methanol extracts of colored rice hulls from rice variety Heuginju with black hull, WD-3 with purple hull, Jeoginju with red hull, and Ilpum with light-brown showed significantly high anti-cancer and antioxidant activities. Black hull of Heuginju showed the highest anti-cancer and antioxidant activities compared to the others colored hulls [190]. Acetone extract of rice hull contain procyanidins [36]. Based on the molecular, genetic, metabolic, and phylogenetic analysis; colored rice hulls were regulated by C-S-A gene system, which C1 encoding MYB transcription factor and become gene that produce color, S1 encoding bHLH protein and acting as a tissue specific regulator, A1 encoding dihydroflavonol reductase and only express when C1 co-ordinate with S1 (Figure 5). Brown hull color is formed when A1 is not expressed. One QTL responsible for black hull trait is on chromosome 4 [191]. Gene Phr1 on chromosome 4 encoding polyphenol oxidase is found to be responsible for black hull color of rice [192-193]. Black hull is also regulated by Bh4 gene on chromosome 4. Another genes that responsible for black hull are Bh-a, Bh-b, and Bh-c as complementary genes [194]. Two QTLs qHC4 and qHC7 also responsible for black hull coloration, these QTLs were identified by using an F2 population of crossing between SS18-2 and EM93-1 [194].

Conclusions

Pigmented rice has been popular among rice consumers and increasing demand of pigmented rice have become motivation for rice breeder to develop high yield pigmented rice. Anthocyanin is a source of functional food ingredients, natural colorants, pharmaceuticals, and other industrial biochemical products with the high health benefits. Anthocyanin accumulation in the rice plants can be enhanced by genetic molecular techniques and environmental regulation. Advanced pigmented rice varieties with enhanced anthocyanins content have been developed. To fulfill the consumer increasing demand, it is important to explore deeply the genetic bases of pigmented rice in order to enhance pigmented rice quality, sensory properties, and nutritional content. In this review provides important information for rice breeder to develop high quality pigmented rice based on consumer demand.

References

- [1] Gregorio, G.B., Senadhira, D., Htut, T., & Graham, R.D. (1999). Improving iron and zinc value of rice for human nutrients. *Agr. Develop.* 23, 68-87.



- [2] Tsuda, T., Watanabe, M., Ohshima, K., Norinobu, S., Choi, S.W., Kawakishi, S., & Osawa, T. (1994). Antioxidative activity of the anthocyanin pigments cyanidin-3-O- β -D-glucoside and cyanidin. *J. Agr. Food Chem.* 42, 2407-2410.
- [3] Chung, Y.M., Lee, J.C., Kim, K.S., & Eun, J.B. (2001). Chemical compositions of 26 varieties of Korean rice straw. *Food Sci. Biotechnol.*, 10, 267-271.
- [4] Lee, Y.R., Kim, J.Y., Woo, K.S., Hwang, I.G., Kim, K.H., Kim, K.J., Kim, J.H., & Jeong, H.S. (2007). Changes in the chemical and functional components of Korean rough rice before and after germination. *Food Sci. Biotechnol.*, 16, 1006-1010.
- [5] Dwiningsih, Y. (2020). Molecular genetic analysis of drought resistance and productivity traits of rice genotypes. University of Arkansas, Fayetteville, USA.
- [6] Ito, V.C., Zielinski, A.A.F., Demiate, I.M., Spoto, M.H.F., Nogueira, A., & Lacerda, L.G. (2019). Effects of gamma radiation on the stability and degradation kinetics of phenolic compounds and antioxidant activity during storage of (*Oryza sativa* L.) black rice flour. *Brazilian Archives of Biology and Technology*, 62, 01–14.
- [7] Chen, X., Itani, T., Wu, X., Chikawa, Y., & Irfune, K. (2013). Physiological factors affecting transcription of genes involved in the flavonoid biosynthetic pathway in different rice varieties, *Plant Signaling & Behavior*, 8:12, e27555. Doi: 10.4161/psb.27555
- [8] Chung, H.S. & Woo, W.S. (2001). A quinolone alkaloid with antioxidant activity from the aleurone layer of anthocyanin-pigmented rice. *J. Nat. Prod.*, 64, 1579-1580.
- [9] Dwiningsih, Y., Kumar, A., Thomas, J., Ruiz, C., Alkahtani, J., Baisakh, N., & Pereira, A. (2021). Quantitative trait loci and candidate gene identification for chlorophyll content in RIL rice population under drought conditions. *Indonesian Journal of Natural Pigments*, 3, 2, 54-64. Doi: 10.33479/ijnp.2021.03.2.54
- [10] Lee, H.J., Oh, S.K., Choi, H.C., & Kim, S.U. (1988). Identification of anthocyanins from pigmented rice seed. *Agric. Chem. Biotechnol.*, 41, 257-265.
- [11] Yoshinaga, K. (1986). Liquor with pigments of red rice. *Journal of Brewing Society of Japan*, 81, 337-342.
- [12] Ling, W.H., Wang, L.L., & Ma, J. (2002). Supplementation of the black rice outer fraction to rabbits decrease atherosclerotic plaque formation and increase antioxidant status. *J. Nutr.*, 132, 20-26.
- [13] Hu, C., Zawistowski, J., Ling, W., & Kitts, D.D. (2003). Black rice (*Oryza sativa* L. indica) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. *J. Agr. Food Chem.*, 51, 5271-5277.
- [14] Middleton, Jr.E., Kandaswami, C., & Theoharides, T.C. (2000). The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer., *Pharmacol. Rev.* 52: 673-751.
- [15] Jang, M., Cai, L., Udeani, G.O., Slowing, K.V., Thomas, C.F., Beecher, C.W., Fong, H.H., Farnsworth, N.R., Kinghorn, A.D., Mehta, R.C., Moon, R.C., & Pezzuto. (1997). Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science*, 275: 218-220.
- [16] Shim, S.I., Chung, J.W., Lee, J.M., Hwang, K.T., Sone, J., Hong, B.S., Cho, H.Y., & Jun, W.J. (2006). Hepatoprotective effects of black rice on superoxide anion radicals in HepG2 Cells. *Food Sci. Biotechnol.*, 15, 993-996.
- [17] Chaudhary, R.C. (2003). Speciality rices of the world: Effect of WTO and IPR on its production trend and marketing. *Journal of Food, Agriculture and Environment*, 1, 2, 34–41.
- [18] Dwiningsih, Y., Rahmaningsih, M., & Alkahtani J. (2020). Development of single nucleotide polymorphism (SNP) markers in tropical crops. *Adv. Sustain. Sci. Eng. Technol.*, 2, 2, 14065.
- [19] Ryu, S.N., Park, S.Z., & Ho, C.T. (1998). High Performance Liquid Chromatographic Determination of Anthocyanin Pigments in Some Varieties of Black Rice. *Journal of Food and Drug Analysis*, 6, 4. Doi: [10.38212/2224-6614.2893](https://doi.org/10.38212/2224-6614.2893)



- [20] Oikawa, T., Maeda, H., Oguchi, T., Yamaguchi, T., Tanabe, N., Ebana, K., Yano, M., Ebitani, T., & Izawa, T. (2015). The Birth of a Black Rice Gene and Its Local Spread by Introgression. *Plant Cell*, 27, 2401-2414.
- [21] Kristantini, P.H. (2009). Potensi pengembangan beras merah sebagai plasma nutfah Yogyakarta. *Jurnal Litbang Pertanian*, 28, 3, 88-95.
- [22] Pratiwi, R. & Purwestri, Y.A. (2017). Black rice as a functional food in Indonesia. *Functional Foods in Health and Disease*, 7, 3, 182-194.
- [23] Ling, W.H., Cheng, Q.X., Ma, J. & Wang, T. (2001). Red and black rice decrease atherosclerotic plaque formation and increase antioxidant status in rabbits. *J. Nutr.*, 131, 1421-1426.
- [24] Xia, M., Ling, W.H., Ma, J., Kitts, D.D., & Zawistowski, J. (2003). Supplementation of diets with the black rice pigment fraction attenuates atherosclerotic plaque formation in apolipoprotein E deficient mice. *The Journal of nutrition*, 133, 3, 744-751.
- [25] Xu, Z., Hua, N., & Godber, J.S. (2001). Antioxidant activity of tocopherols, tocotrienols, and γ -Oryzanol components from rice bran against cholesterol oxidation accelerated by 2,2'-Azobis (2-methylpropionamide) dihydrochloride. *Journal of Agricultural and Food Chemistry*, 49, 4, 2077-2081.
- [26] Sitarasi, R., Nallal, U.M., Razia, M., Chung, W.J., Shim, J., Chandrasekaran, M., Dwiningsih, Y., Rasheed, R.A., Alkahtani, J., Elshikh, M.S., Debnath, O., & Ravindran. (2022). Inhibition of multi-drug resistant microbial pathogens using an ecofriendly root extract of *Furcraea foetida* silver nanoparticles. *Journal of King Saud University-Science*, 34, 2, 101794. Doi: 10.1016/j.jksus.2021.101794
- [27] Dwiningsih, Y. (2014). Nutritional characterization of bread contained natural pigment from insect. *Indonesian Scientific Research Olympic*.
- [28] Xia, M., Ling, W.H., Ma, J., & Zawistowski, J. (2002). Supplementation of diets with the black rice pigment fraction attenuates atherosclerotic plaque formation in apolipoprotein E deficient mice. *J. Nutr.*, 133, 744-751.
- [29] Chiang, A.N., Wu, H.L., Yeh, H.I., Chu, C.S., Lin, H.C., & Lee, W.C. (2006). Antioxidant effects of black rice extract through the induction of superoxide dismutase and catalase activities. *Lipids*, 41, 797-803.
- [30] Choi, Y.M., Jeong, H.S., & Lee, J.S. (2007). Antioxidant activity of methanolic extracts from some grains consumed in Korea. *Food Chem.*, 103, 130-138.
- [31] Samyor, D., Das, A.B., & Deka, S.C. (2017). Pigmented rice a potential source of bioactive compounds: a review. *International Journal of Food Science and Technology*, 52, 1073-1081. Doi:10.1111/ijfs.13378
- [32] Kang, M., Rico, C.W., & Lee, S. (2011). Varietal Difference in Physicochemical Properties of Pigmented Rice Varieties. *J. Crop Sci. Biotech*. Doi: 10.1007/s12892-010-0041-8
- [33] Yawadio, R., Tanimori, S., & Morita, N. (2007). Identification of phenolic compounds isolated from pigmented rices and their aldose reductase inhibitory activities. *Food Chem.*, 101, 1644-1653.
- [34] Nam, S.H., Choi, S.P., Kang, M.Y., Koh, H.J., Kozukue, N., & Friedman, M. (2006). Antioxidative activities of bran extracts from twenty one pigmented rice cultivars. *Food Chem.*, 94, 613-620.
- [35] Toyokuni, S., Itani, T., Morimitsu, Y., Okada, K., Ozeki, M., & Kondo, S. (2002). Protective effect of colored rice over white rice on Fenton reaction-based renal lipid peroxidation in rats. *Free Radic. Res.* 36: 583-592
- [36] Oki, T., Matsuda, M., Kobayashi, M., Nishiba, Y., Furuta, S., & Suda, I. (2002). Polymeric procyanidins as radical-scavenging components in red-hulled rice. *J. Agric. Food Chem.*, 50, 7524-7529.
- [37] Adom, K.K. & Liu, R.H. (2002). Antioxidant activity of grains. *J. Agric. Food Chem.*, 50, 6170-6182.



- [38] Lazze, M.C., Pizzala, R., savio, M., Stivala, L.A., Prosperi, E., & Bianchi, L. (2003). Anthocyanins protect against DNA damage induced by tert-butyl-hydroperoxide in rat smooth muscle and hepatoma cells. *Genetic Toxicology and Environmental Mutagenesis*, 535, 103-115.
- [39] Dwiningsih, Y. (2012). Margarine with high antioxidant content. *Natural Pigment Conference*.
- [40] Dwiningsih, Y. & Notoesoedarmo, S. (2012). Antioxidant sources from insects. *Natural Pigment Conference*.
- [41] Hou, Z., Qin, P., & Ren, G. (2010). Effect of Anthocyanin-Rich Extract from Black Rice (*Oryza sativa* L. Japonica) on Chronically Alcohol-Induced Liver Damage in Rats. *J. Agric. Food Chem.*, 58, 3191-3196. Doi: 10.1021/jf904407x
- [42] Hemamalini, S., Umamaheswari, D.S., Lavanya, D.R., & Umamaheswara, R.D.C. (2018). Exploring the therapeutic potential and nutritional properties of 'KaruppuKavuni' variety rice of Tamil Nadu. *Int. J. Pharma Bio Sci.*, 9, 88-96.
- [43] Konczak, I. & Zhang, W. (2004). Anthocyanins—more than nature's colours. *J. Biomed. Biotechnol.*, 239-240.
- [44] Yazhen, S., Wenju, W., Panpan, Z., Yuanyuan, Y., Panpan, D., Wusen, Z. & Yanling, W. (2019). Anthocyanins: Novel Antioxidants in Diseases Prevention and Human Health. In *Flavonoids-A Coloring Model for Cheering Up Life*; IntechOpen: London, UK.
- [45] Kocic, B., Filipovic, S., Nikolic, M. & Petrovic, B. (2011). Effects of anthocyanins and anthocyanin-rich extracts on the risk for cancers of the gastrointestinal tract. *Off. J. Balk. Union Oncol.*, 16, 602-608.
- [46] Woo, D.S., Jun, Y.K., Mi, J.P., Sang-Ik, H., Hang, W.K., Ji-Eun, R., Sea, K.O., Jin, H.L., Ki, C.J., & You, C.S. (2011). Relationship of Radical Scavenging Activities and Anthocyanin Contents in the 12 Colored Rice Varieties in Korea. *J Korean Soc Appl Bi*, 54, 693-699.
- [47] Chen, P.N., Chu, S.C., Chiou, H.L., Chiang, C.L., Yang, S.F. & Hsieh, Y. (2005). Cancer, Cyanidin 3-glucoside and peonidin 3-glucoside inhibit tumor cell growth and induce apoptosis in vitro and suppress tumor growth in vivo. *J. Nutr.*, 53, 232-243.
- [48] Luo, L.P., Han, B., Yu, X.P., Chen, X.Y., Zhou, J., Chen, W., Zhu, Y.F., Peng, X.L., Zou, Q., & Li, S.Y. (2014). Anti-metastasis activity of black rice anthocyanins against breast cancer: Analyses using an ErbB2 positive breast cancer cell line and tumoral xenograft model. *Asian Pac. J. Cancer Prev.*, 15, 6219-6225.
- [49] Umar Lule, S. & Xia, W.J.F.R.I. (2005). Food phenolics, pros and cons: A review. *J. Food Rev. Int.*, 21, 367-388.
- [50] Xia, D., Zhou, H., Li, P., Wu, B., Zhang, Q., & He, Y. (2020). How rice organ painted: the genetic basis of anthocyanin biosynthesis in rice. *Research Square*. Doi: 10.21203/rs.3.rs-101298/v1
- [51] Dooner, H.K., Robbins, T.P. & Jorgensen, R.A. (1991). Genetics and development control of anthocyanin biosynthesis. *Annu Rev Genet.*, 25, 173-199.
- [52] Kootstra, A. (1994). Protection from UV-B-induced DNA damage by flavonoids. *Plant Mol. Biol.* 26, 771-774.
- [53] Reddy, V.S., Goud, K.V., Sharma, R., & Reddy, A.R. (1994). Ultraviolet-B-responsive anthocyanin production in a rice cultivar is associated with a specific phase of phenylalanine ammonia lyase biosynthesis. *Plant Physiol.*, 105, 1059-1066.
- [54] Holton, T.A., & Cornish, E.C. (1995). Genetics and Biochemistry of Anthocyanin Biosynthesis. *The Plant Cell*, 7, 1071-1083.
- [55] Dixon, R.A. & Paiva, N. (1995). Stress-induced phenylpropanoid metabolism. *Plant Cell* 7, 1085-1097.
- [56] Shirley, B.W. (1996). Flavonoid biosynthesis: new functions for an old pathway. *Trends Plant Sci.* 1, 377-382.
- [57] Chalker-Scott, L. (1999). Environmental significance of anthocyanins in plant stress responses. *Photochem Photobiol.*, 70, 1-9.



- [58] Shirley, W.B. (2002). Biosynthesis of flavonoids and effects of stress. *Curr. Opin. Plant Biol.*, 5, 218–223.
- [59] Dwiningsih, Y. & Notoedarmo, S. (2008). Chlorophyll and Carotenoid in C4, C3, and CAM plants. Indonesian Scientific Research Olympic.
- [60] Reddy, C.K., Kimi, L., Haripriya, S., & Kang, N. (2017). Effects of polishing on proximate composition, physico-chemical characteristics, mineral composition and antioxidant properties of pigmented rice. *Rice Sci.*, 24, 241–252.
- [61] Zhang, H., Shao, Y., Bao, J., & Beta, T. (2015). Phenolic compounds and antioxidant properties of breeding lines between the white and black rice. *Food Chem.*, 172, 630–639.
- [62] Maqsood, A., Khan, Z.I., Ahmad, K., Akhtar, S., Ashfaq, A., Malik, I.S., Sultana, R., Nadeem, M., Alkahtani, J., Dwiningsih, Y., & Elshikh, M. (2022). Quantitative evaluation of zinc metal in meadows and ruminants for health assessment: implications for humans. *Environmental Science and Pollution Research*, 29, 21634–21641. Doi: 10.1007/s11356-021-17264-1
- [63] Ge, X., Khan, Z.I., Chen, F., Akhtar, M., Ahmad, K., Ejaz, A., Ashraf, M.A., Nadeem, M., Akhtar, S., Alkahtani, J., Dwiningsih, Y., & Elshikh, M.S. (2021). A study on the contamination assessment, health risk and mobility of two heavy metals in the soil-plants-ruminants system of a typical agricultural region in the semi arid environment. *Environmental Science and Pollution Research*, 29, 14584–14594. Doi: 10.1007/s11356-021-16756-4
- [64] Dwiningsih, Y., Kumar, A., Thomas, J., Ruiz, C., Alkahtani, J., Al-Hashimi, A., & Pereira, A. (2021). Identification of Genomic Regions Controlling Chalkiness and Grain Characteristics in a Recombinant Inbred Line Rice Population Based on High-Throughput SNP Markers. *Genes*, 12, 1690. Doi: 10.3390/genes12111690
- [65] Clifford, M. N. (2000). Anthocyanins: Nature, occurrence and dietary burden. *J. Sci. Food Agric.*, 80, 1063-1072.
- [66] Mackon, E., Jeazet Dongho Epse Mackon, G.C., Ma, Y., Haneef Kashif, M., Ali, N., Usman, B., & Liu, P. (2021). Recent Insights into Anthocyanin Pigmentation, Synthesis, Trafficking, and Regulatory Mechanisms in Rice (*Oryza sativa* L.) Caryopsis. *Biomolecules*, 11, 394. Doi: 10.3390/biom11030394
- [67] Xu, T., Sun, J., Chang, H., Zheng, H., Wang, J., Liu, H., Yang, L., Zhao, H., & Zou, D.T. (2017). QTL mapping for anthocyanin and proanthocyanidin content in red rice. *Euphytica*, 213, 243.
- [68] Dwiningsih, Y. & Notoedarmo, S. (2012). Rice contained carotenoid. Indonesian Scientific Research Olympic.
- [69] Escribano-Bailón, M.T., Santos-Buelga, C., & Rivas-Gonzalo, J.C. (2004). Anthocyanins in cereals. *Journal of Chromatography A* 054, 129e141.
- [70] Abdel-Aal, E.S.M., Young, J.C., & Rabalski, I. (2006). Anthocyanin composition in black, blue, pink, purple, and red cereal grains. *Journal of Agricultural and Food Chemistry*, 54, 4696–4704.
- [71] Reddy, V.S., Dash, S., & Reddy, A.R. (1995). Anthocyanin pathway in rice (*Oryza sativa* L.): identification of a mutant showing dominant inhibition of anthocyanins in leaf and accumulation of proanthocyanidins in pericarp. *Theor Appl Genet.*, 91, 301–312.
- [72] Sakamoto, W., Ohmori, T., Kageyama, K., Miyazaki, C., Saito, A., Murata, M., Noda, K., & Maekawa, M. (2001). The purple leaf (Pl) locus of rice: the Plw allele has a complex organization and includes two genes encoding basic helix-loop-helix proteins involved in anthocyanin biosynthesis. *Plant Cell Physiol.*, 42, 982–991.
- [73] Chin, H., Wu, Y., Hour, A., Hong, C., & Lin, Y. (2016). Genetic and evolutionary analysis of purple leaf sheath in rice. *Rice*, 9, 1, 8. Doi: 10.1186/s12284-016-0080-y
- [74] Dwiningsih, Y., Kumar, A., Thomas, J., Gupta, C., Ruiz, C., Alkahtani, J., Baisakh, N., & Pereira, A. (2021). Identification and expression of abscisic acid-regulated genes in US RIL rice population under drought conditions. 82nd Meeting of Southern Section of the American Society of Plant Biologists.



- [75] Goffman, F.D. & Bergman, C.J. (2004). Rice kernel phenolic content and its relationship with antiradical efficiency. *J Sci Food Agric*, 84, 1235-1240. Doi: 10.1002/jsfa.1780
- [76] Chen, X.Q., Nagao, N., Itani, T., & Irifune. (2012). Anti-oxidative analysis, and identification and quantification of anthocyanin pigments in different coloured rice. *Food Chemistry*. 135, 2783-2788. Doi: 10.1016/j.foodchem.2012.06.098
- [77] Koes, R., Verweij, W., & Quattrocchio, F. (2005). Flavonoids: a colorful model for the regulation and evolution of biochemical pathways. *Trends Plant Sci.*, 10, 1360–1385.
- [78] Brenda, W.S. (2001). Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. *Plant Physiol.*, 126, 485–493.
- [79] Loypimai, P., Moongngarm, A., & Chottanom, P. (2016). Thermal and pH degradation kinetics of anthocyanins in natural food colorant prepared from black rice bran. *Journal of Food Science and Technology*, 53, 1, 461–470.
- [80] Zhang, Y., Hu, X. S., Chen, F., Wu, J. H., Liao, X. J., & Wang, Z. F. (2008). Stability and color characteristics of PET-treated cyanidin-3-glucoside during storage. *Food Chemistry*, 106, 2, 669–676.
- [81] Tiwari, B.K., O' Donnell, C.P., Muthukumarappan, K., & Cullen, P.J. (2009). Anthocyanin and color degradation in ozone treated blackberry juice. *Innovative Food Science and Emerging Technologies*, 10, 1, 70–75.
- [82] Gradinaru, G., Biliaderis, C. G., Kallithraka, S., Kefalas, P., & Garcia-Viguera, C. (2003). Thermal stability of *Hibiscus sabdariffa* L. anthocyanins in solution and in solid state: Effects of copigmentation and glass transition. *Food Chemistry*, 83, 3, 423–436.
- [83] Dwiningsih, Y., Kumar, A., Thomas, J., Yingling, S., & Pereira, A. (2020). Identification of QTLs associated with drought resistance traits at reproductive stage in K/Z RILs rice population. 5th Annual Meeting of the Arkansas Bioinformatics Consortium AR-BIC 2020, Bioinformatics in Food and Agriculture.
- [84] Dwiningsih, Y. (2013). Thermostability of natural pigment from secang bark (*Caesalpinia sappan* Linn.). *Indonesian Scientific Research Olympiad*.
- [85] Hou, Z., Qing, P., & Ren, G., (2010). Effect of anthocyanin-rich extract from black rice (*Oryza sativa* L. Japonica) on chronically alcohol-induced liver damage in rats. *Journal of Agricultural and Food Chemistry* 58, 3191e3196.
- [86] Shipp, J. & Abdel-Aal. (2010). Food Applications and Physiological Effects of Anthocyanins as Functional Food Ingredients. *The Open Food Science Journal*, 4, 7-22.
- [87] Tian, Q. G., Giusti, M. M., Stoner, G. D., & Schwartz, S. J. (2005). Screening for anthocyanins using high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry with precursor-ion analysis, product-ion analysis, common-neutral-loss analysis, and selected reaction monitoring. *Journal of Chromatography A*, 1091, 72–82.
- [88] Yao, Y., Sang, W., Zhou, M. J., & Ren, G. X. (2010). Antioxidant and a-glucosidase activity of colored grains in China. *Journal of Agricultural and Food Chemistry*, 58, 770–774.
- [89] Lee, J.H. (2010). Identifications and quantification of anthocyanins from the grains of black rice (*Oryza sativa* L.) varieties. *Food Science and Biotechnology*, 19, 391e397.
- [90] Hiemori, M., Koh, E., & Mitchell, A.E. (2009). Influence of cooking on anthocyanins in black rice (*Oryza sativa* L. japonica var. SBR). *J. Agric. Food Chem*, 57, 1908-1914. Doi: 10.1021/jf803153z
- [91] Yoshida, H., Tomiyama, Y., & Mizushima, Y. (2010). Lipid components, fatty acids and triacylglycerol molecular species of black and red rices. *Food Chemistry*, 123, 210e215.
- [92] Kong, L., Wang, Y., & Cao, Y. (2008). Determination of Myo-inositol and D-chiroinositol in black rice bran by capillary electrophoresis with electrochemical detection. *Journal of food composition and analysis*, 21, 6, 501-504.
- [93] Han, S.J., Ryu, S.N., & Kang, S.S. (2004). A New 2-Arylbenzofuran with Antioxidant Activity from the Black Colored Rice (*Oryza sativa* L.) Bran. *Chem. Pharm. Bull*, 52, 11, 1365-1366.



- [94] Dwiningsih, Y. & Notoedarmo, S. (2012). Rice contained carotenoid. Indonesian Scientific Research Olympic.
- [95] Kushwaha, U. K. S. (2016). Black Rice: Research History and Development. Springer International Publishing, 21–31.
- [96] Surh, J., & Koh, E. (2014). Effects of four different cooking methods on anthocyanins, total phenolics and antioxidant activity of black rice. *Journal of the Science of Food and Agriculture*, 94, 15, 3296–3304.
- [97] Dwiningsih, Y. & Notoedarmo, S. (2012). Potential of green microalgae (*Haematococcus pluvialis*) as food supplement. Indonesian Scientific Research Olympic.
- [98] Duyi, S., Baran, A., & Chandra, D. S. (2017). Pigmented rice a potential source of bioactive compounds: A review. *International Journal of Food Science & Technology*, 52, 5, 1073–1081.
- [99] Jeng, T. L., Lai, C. C., Ho, P. T., Shih, Y. J., & Sung, J. M. (2012). Agronomic, molecular and antioxidative characterization of red- and purple-pericarp rice (*Oryza sativa* L.) mutants in Taiwan. *Journal of Cereal Science*, 56, 2, 425–431.
- [100] Dwiningsih, Y., Thomas, J., Kumar, A., Gupta, C., Ruiz, C., Yingling, S., Crowley, E., & Pereira, A. (2020). Molecular genetic analysis of drought resistance and productivity mechanisms in rice. Plant and Animal Genome XXVIII Conference, January 11-15, 2020.
- [101] Kim, M., Kim, H., Koh, K., Kim, H., Lee, Y.S., & Kim, Y.H. (2008). Identification and quantification of anthocyanin pigments in colored rice. *Nutrition Research and Practice*, 2, 1, 46-49.
- [102] Mbanjo, E.G.N., Kretschmar, T., Jones, H., Ereful, N., Blanchard, C., Boyd, L.A., & Sreenivasulu, N. (2020). The Genetic Basis and Nutritional Benefits of Pigmented Rice Grain. *Front. Genet.*, 11, 229. Doi: 10.3389/fgene.2020.00229
- [103] Hurtada, W.A., Barrion, A.S.A., & Nguyen-Orca, M.F.R. (2018). Mineral content of dehulled and well-milled pigmented and non-pigmented rice varieties in the Philippines. *Int. Food Res. J.*, 25, 2063–2067.
- [104] Shao, Y., Hu, Z., Yu, Y., Mou, R., Zhu, Z., & Beta, T. (2018). Phenolic acids, anthocyanins, proanthocyanidins, antioxidant activity, minerals and their correlations in non-pigmented, red, and black rice. *Food Chemistry*, 239, 733–741.
- [105] Raghuvanshi, R.S., Dutta, A., Tewari, G., & Suri, S. (2017). Qualitative characteristics of red rice and white rice procured from local market of Uttarakhand?: a comparative study. *J. Rice Res.*, 10, 49–53.
- [106] Hashmi, M.I. & Tianlin, J.S. (2016). Minerals contents of some indigenous rice varieties of Sabah Malaysia. *Int. J. Agric. For. Plant.*, 2, 31–34.
- [107] Dwiningsih, Y., Kumar, A., Thomas, J., Yingling, S., & Pereira A. (2019). Molecular genetic analysis of drought resistance and productivity in K/Z RIL rice population. Arkansas Bioinformatics Consortium 2019.
- [108] Frank, T., Reichardt, B., Shu, Q., & Engel, K. (2012). Metabolite profiling of colored rice (*Oryza sativa* L.) grains. *Journal of Cereal Sciences* 55, 112-119. Doi:10.1016/j.jcs.2011.09.009
- [109] Morimitsu, Y., Kubota, K., Tashiro, T., Hashizume, E., Kamiya, T., & Osawa, T. (2002). Inhibitory effect of anthocyanins and colored rice on diabetic cataract formation in the rat lenses. *International Congress Series* 1245, 503-508.
- [110] Frei, M. & Becker, K. (2005). Fatty acids and all-trans- β -carotene are correlated in differently colored rice landraces. *J Sci Food Agric*, 85, 2380-2384. Doi: 10.1002/jsfa.2263
- [111] Kim, J.Y., Do, M.H., & Lee, S.S. (2006). The effects of a mixture of brown and black rice on lipid profiles and antioxidant status in rats. *Annals of Nutrition and Metabolism*, 50, 347e353.
- [112] Dwiningsih, Y. (2014). Production of secang bark (*Caesalpinia sappan* Linn.) as natural pigment for food with spray drying method. Indonesian Scientific Research Olympic.



- [113] Hou, Z., Qin, P., Zhang, Y., Cui, S., & Ren, G. (2013). Identification of anthocyanins isolated from black rice (*Oryza sativa* L.) and their degradation kinetics. *Food Research International*, 50, 2, 691–697.
- [114] Dwiningsih, Y., Thomas, J., Kumar, A., Gupta, C., Crowley, E., Ruiz, C., & Pereira, A. (2019). Drought stress response in US recombinant inbred line of rice population. *National Science Foundation (NSF) 26th National Conference 2019*, 26, 76, 127.
- [115] Ma, J., Li, Y., & Li, J. (2000). Constituents of red yeast rice, a traditional Chinese food and medicine. *J Agric Food Chem.*, 48, 5220-5225.
- [116] Wrolstad, R.E. (2004). Anthocyanin pigments – bioactivity and coloring properties. *J Food Sci.*, 69, C419-C425.
- [117] Stintzing, F.C. & Carle, R. (2004). Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends Food Sci Technol.*, 15, 19-38.
- [118] Ali, M.H., Khan, M.I., Bashir, S., Azam, M., Naveed, M., Qadri, R., Bashir, S., Mehmood, F., Shoukat, M.A., & Li, Y. (2021). Biochar and *Bacillus* sp. MN54 Assisted Phytoremediation of Diesel and Plant Growth Promotion of Maize in Hydrocarbons Contaminated Soil. *Agronomy*, 11, 1795. Doi: 10.3390/agronomy11091795
- [119] Khoo, H.E., Azlan, A., Tang, S.T., Lim, S.M. (2017). Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food Nutr. Res.*, 61, 1361779.
- [120] Turturică, M., Oancea, A.M., Râpeanu, G., & Bahrim, G. (2015). Anthocyanins: Naturally occurring fruit pigments with functional properties. *Ann. Univ. Dunarea Jos Galati Fascicle VI-Food Technol.*, 39, 9–24.
- [121] Holton, T.A. & Cornish, E.C. (1995). Genetics and Biochemistry of Anthocyanin Biosynthesis. *The Plant Cell*, 7, 1071-1083.
- [122] Zhang, M.W., Zhang, R.F., Zhang, F.X., & Liu, R.H. (2010). Phenolic Profiles and Antioxidant Activity of Black Rice Bran of Different Commercially Available Varieties. *J. Agric. Food Chem.*, 58, 7580-7587. Doi: 10.1021/jf1007665
- [123] Pang, Y., Ahmed, S., Xu, Y., Beta, T., Zhu, Z., Shao, Y., & Bao, J. (2018). Bound phenolic compounds and antioxidant properties of whole grain and bran of white, red and black rice. *Food Chemistry*, 240, 212–221.
- [124] Dwiningsih, Y. (2012). Spectroscopy and natural pigment structural determination. *Natural Pigment Conference*.
- [125] Dwiningsih, Y., Rondonuwu, F.S., & Martosupono, M. (2009). The role of curcumin in bacteriochlorophyll a activity. *Satya Wacana Christian University, Salatiga, Jawa Tengah, Indonesia*.
- [126] Rafael, A., Dwiningsih, Y., Tuririday, H., & Karwur, F.F. (2009). Natural colorant and biopsychology. *Biopsychology: Improving the Quality of Life, First National Conference on Biopsychology, Gadjah Mada University, Yogyakarta, Indonesia*.
- [127] Finocchiaro, F., Ferrari, B., & Gianinetti, A. (2010). A study of biodiversity of flavonoid content in the rice caryopsis evidencing simultaneous accumulation of anthocyanins and proanthocyanidins in a black-grained genotype. *Journal of Cereal Science*, 51, 28e34.
- [128] Furukawa, T., Maekawa, M., Oki, T., Suda, I., Lida, S., Shimada, H., Takamura, I., & Kadowaki, K. (2006). The Rc and Rd genes are involved in proanthocyanidin synthesis in rice pericarp. *Plant Journal* 49, 91e102.
- [129] Chung, H.S. & Woo, W.S. (2001). A quinolone alkaloid with antioxidant activity from the aleurone layer of anthocyanin-pigmented rice. *J. Nat. Prod.*, 64, 1579-1580.
- [130] Hyun, J.W. & Chung, H.S. (2004). Cyanidin and Malvidin from *Oryza sativa* cv. Heugjinjubyeo Mediate Cytotoxicity against Human Monocytic Leukemia Cells by Arrest of G2/M Phase and Induction of Apoptosis. *J. Agric. Food Chem.*, 52, 2213-2217. Doi: 10.1021/jf030370h



- [131] Li, Y., Teng, F., Shi, F., Wang, L., & Chen, Z. (2017). Effects of high-temperature air fluidization (HTAF) on eating quality, digestibility, and antioxidant activity of black rice (*Oryza sativa* L.). *Starch – Stärke*, 69, 7–8, 1600274-n/a.
- [132] Laokuldilok, T. & Kanha, N. (2015). Effects of processing conditions on powder properties of black glutinous rice (*Oryza sativa* L.) bran anthocyanins produced by spray drying and freeze drying. *LWT – Food Science and Technology*, 64, 1, 405–411.
- [133] Meng, L., Zhang, W., Wu, Z., Hui, A., Gao, H., Chen, P., & He, Y. (2018). Effect of pressure-soaking treatments on texture and retrogradation properties of black rice. *LWT – Food Science and Technology*, 93, 485–490.
- [134] Dwiningsih, Y., Kumar, A., Thomas, J., Yingling, S., & Pereira, A. (2019). Molecular genetic analysis of drought resistance and productivity in US rice cultivars. *Plant and Animal Genome XXVII Conference* (January 12-16, 2019).
- [135] Arbelaez, J.D., Moreno, L.T., Singh, N., Tung, C.W., Maron, L.G., Ospina, Y., et al. (2015). Development and GBS-genotyping of introgression lines (ILs) using two wild species of rice. *O. meridionalis* and *O. rufipogon*, in a common recurrent parent, *O. sativa* cv. Curinga. *Mol. Breed.*, 35, 81. Doi: 10.1007/s11032-015-0276-7
- [136] Bhuiyan, M.A.R., Narimah, M.K., Rahim, H.A., Abdullah, M.Z. & Wickneswari, R. (2011). Transgressive variants for red pericarp grain with high yield potential derived from *Oryza rufipogon* × *Oryza sativa*: field evaluation, screening for blast disease, QTL validation and background marker analysis for agronomic traits. *Field Crops Res.*, 121, 232–239. Doi: 10.1016/j.fcr.2010.12.012
- [137] Maeda, H., Yamaguchi, T., Omoteno, M., Takarada, T., Fujita, K., Murata, K., et al. (2014). Genetic dissection of black grain rice by the development of a near isogenic line. *Breed. Sci.* 64, 134–141. Doi: 10.1270/jsbbs.64.134
- [138] Rysbekova, A.B., Kazkeyev, D.T., Usenbekov, B.N., Mukhina, Z.M., Zhanbyrbaev, E.A., Sartbaeva, I. A., et al. (2017). Prebreeding selection of rice with colored pericarp based on genotyping *Rc* and *Pb* genes. *Russ. J. Genet.*, 53, 49–58. Doi: 10.1134/s1022795416110119
- [139] Waiyawuththanapoom, P., Waiyawuththanapoom, W., & Tirastittam, P. (2015). Social media as a channel for Thailand's Rice Berry Product. *Int. J. Econ. Manag. Eng.* 9, 904–907.
- [140] Gene Discovery Rice and Rice Science Center. (2017). Riceberry. Available online at: <http://dna.kps.ku.ac.th/index.php/news-articles-rice-rsc-rgdu-knowledge/rice-breeding-lab/riceberry-variety> (accessed March 15, 2022).
- [141] Wickert, E., Schiocchet, M.A., Noldin, J.A., Raimondi, J.V., De Andrade, A., Scheuermann, K.K., et al. (2014). Exploring variability?: New Brazilian varieties SCS119 Rubi and SCS120 Onix for the specialty rices market. *Open J. Genet.* 4, 157–165. Doi: 10.4236/ojgen.2014.42016
- [142] Zhu, Q., Yu, S., Zeng, D., Liu, H., Wang, H., Yang, Z., Xie, X., Shen, R., Tan, J., Li, H., Zhao, X., Zhang, Q., Chen, Y., Guo, J., Chen, L., & Liu, Y.G. (2017). Development of "Purple Endosperm Rice" by Engineering Anthocyanin Biosynthesis in the Endosperm with a High-Efficiency Transgene Stacking System. *Mol Plant*, 10, 918-929.
- [143] Gu, X.Y., Kianian, S.F., Hareland, G.A., Hoffer, B.L., & Foley, M.E. (2005). Genetic analysis of adaptive syndromes interrelated with seed dormancy in weedy rice (*Oryza sativa*). *Thero Appl Genet.*, 110, 1108-1118.
- [144] Gu, X.Y., Foley, M.E., Horvath, D.P., Anderson, J.V., Feng, J., Zhang, L., Mowry, C.R., Ye, H., Suttle, J.C., Kadowaki, K., & Chen, Z. (2011). Association between seed dormancy and pericarp color is controlled by a pleiotropic gene that regulates abscisic acid and flavonoid synthesis in weedy red rice. *Genetics*, 189, 1515-1524.
- [145] Rahman, M.M., Lee, K.E., & Kang, S.G. (2016). Allelic Gene Interaction and Anthocyanin Biosynthesis of Purple Pericarp Trait for Yield Improvement in Black Rice. *Journal of Life Science*, 26, 6, 727-736. Doi: 10.5352/JLS.2016.26.6.727



- [146] Kim, B.G., Kim, J.H., Min, S.Y., Shin, K., Kim, J.H., Kim, H.Y., Ryu, S.N., & Ahn, J. (2007). Anthocyanin content in rice is related to expression levels of anthocyanin biosynthetic genes. *J Plant Biol.*, 50, 156-160.
- [147] Ithal, N. & Reddy, A.R. (2004). Rice flavonoid pathway genes, OsDfr and OsAns, are induced by dehydration, high salt and ABA, and contain stress responsive promoter elements that interact with the transcription activator, OsC1-MYB. *Plant Sci.*, 166, 1505-1513.
- [148] Gandikota, M., Kochko, D.A., Chen, L.L., Ithal, N., Fauquet, C., & Reddy, A.R. (2001). Development of transgenic rice plants expressing maize anthocyanin genes and increased blast resistance. *Mol Breeding*, 7, 73-83.
- [149] Dwiningsih, Y., Thomas, J., Kumar, A., Gupta, C., Yingling, S., Basu, S., & Pereira, A. (2018). Circadian expression patterns of the HYR gene. *Arkansas Bioinformatics Consortium 2018*, 7, 11, 34.
- [150] Septiningsih, E.M., Trijatmiko, K.R., Moeljopawiro, S., & McCouch, S.R. (2003). Identification of quantitative trait loci for grain quality in an advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogon*. *Thero Appl Genet.*, 107, 1433-1441.
- [151] Shao, Y., Jin, L., Zhang, G., Lu, Y., Shen, Y., & Bao, J. (2011). Association mapping of grain color, phenolic content, flavonoid content and antioxidant capacity in dehulled rice. *Thero Appl Genet.*, 122, 1005-1016.
- [152] Wang, W.Y., Ding, H.F., Li, G.X., Jiang, M.S., Li, R.F., Liu, X., Zhang, Y., & Yao, F.Y. (2009). Delimitation of the PSH1(t) gene for rice purple leaf sheath to a 23.5 kb DNA fragment. *Genome*, 52, 268–274.
- [153] Wang, C.X. & Shu, Q.Y. (2007). Fine mapping and candidate gene analysis of purple pericarp gene Pb in rice (*Oryza sativa* L.). *Chin Sci Bull.*, 52, 3097-3104.
- [154] Hu, J.P., Anderson, B., & Wessler, S.R. (1996). Isolation and Characterization Of Rice R Genes Evidence for Distinct Evolutionary Paths in Rice and Maize. *Genetics*, 142, 1021-1031.
- [155] Mathilde, A.C., Theresa, M.F., Yong, G.C., Sang, N.A., Julapark, C., WU, K.S., Xiao, J.H., Yu, Z.H., Pamela, C.R., Sandra, E.H., Gerard, S., Susan, R.M., & Steven, D.T. (1994). Saturated Molecular Map of the Rice Genome Based on an Interspecific Backcross Population. *Genetics*, 138, 1251-1274.
- [156] Oh, J.H., Lee, Y.J., Byeon, E.J., Kang, B.C., Kyeoung, D.S., & Kim, C.K. (2018). Whole-genome resequencing and transcriptomic analysis of genes regulating anthocyanin biosynthesis in black rice plants. *3 Biotech.*, 8, 115.
- [157] Peng, Z.M., Zhang, M.W., & Tu, J.M. (2004). Breeding and nutrient evaluation on three-lines and their combination of indica black glutinous rice hybrid. *Acta Agronomica Sinica*, 30, 342-347.
- [158] Yoon, H.H., Paik, Y.S., Kim, J.B., & Hahn, T.R. (1995). Identification of anthocyanidins from Korean pigmented rice. *Agric. Chem. Biotechnol.* 38, 581-583.
- [159] Rahman, M.M., Lee, K.E., & Kang, S.G. (2015). Studies on the effects of pericarp pigmentation on grain development and yield of black rice. *Indian J. Genet.* 75, 426-433.
- [160] Petroni, K. & Tonelli, C. (2011). Recent advances on the regulation of anthocyanin synthesis in reproductive organs. *Plant Sci.*, 181, 3, 219–229. [Doi: 10.1016/j.plantsci.2011.05.009](https://doi.org/10.1016/j.plantsci.2011.05.009)
- [161] Lin-Wang, K., Bolitho, K., Grafton, K., Kortstee, A., Karunairetnam, S., McGhie, T.K., Espley, R.V., Hellens, R.P., & Allan, A.C. (2010). An R2R3 MYB transcription factor associated with regulation of the anthocyanin biosynthetic pathway in Rosaceae. *BMC Plant Biol.*, 10, 1, 50. [Doi: 10.1186/1471-2229-10-50](https://doi.org/10.1186/1471-2229-10-50)
- [162] Dwiningsih, Y. (2010). Lichenes: pigmented pollution bioindicator. *BioS Popular Biology Magazine*. Satya Wacana Christian University.
- [163] Zhao, S., Wang, C., Ma, J., Wang, S., Tian, P., Wang, J., Cheng, Z., Zhang, X., Guo, X., & Lei, C. (2016). Map-based cloning and functional analysis of the chromogen gene C in rice (*Oryza sativa* L.). *J Plant Biol.*, 59, 5, 496–505. [Doi: 10.1007/s12374-016-0227-9](https://doi.org/10.1007/s12374-016-0227-9)



- [164] Dwiningsih, Y. & Notosoedarmo, S. (2012). Carotenoid biosynthesis in the anther. Natural Pigment Conference.
- [165] Takahashi, M. (1957). Analysis on apiculus color genes essential to anthocyanin coloration in rice. *J Fac Agr Hokkaido Univ.*, 50, 266–362.
- [166] Liu, X., Sun, X., Wang, W., Ding, H., Liu, W., Li, G., Jiang, M., Zhu, C., & Yao, F. (2012). Fine mapping of Pa-6 gene for purple apiculus in rice. *J. Plant. Biol.*, 55, 218–225.
- [167] Fan, F.J., Fan, Y.Y., Du, J.H., & Zhuang, J.Y. (2008). Fine Mapping of C (Chromogen for Anthocyanin) Gene in Rice. *Rice Science*, 15, 1-6.
- [168] Chen, Z., Deng, W., Li, F., Zhou, J., Li, J., Xu, P., Deng, X., Hu, F., Wang, L., Chen, S., et al. (2010). A genetic study on the purple stigma genes and their locations in *Oryza longistaminata*. *J. Yunnan Univ.-Nat. Sci. Ed.*, 32, 103–107.
- [169] Meng, L., Qi, C., Wang, C., Wang, S., Zhou, C., Ren, Y., Cheng, Z., Zhang, X., Guo, X., Zhao, Z., Wang, J., Lin, Q., Zhu, S., Wang, H., Wang, Z., Lei, C., & Wan, J. (2021). Determinant Factors and Regulatory Systems for Anthocyanin Biosynthesis in Rice Apiculi and Stigmas. *Rice*, 14, 37. Doi: 10.1186/s12284-021-00480-1
- [170] Zheng, J., Wu, H., Zhu, H., Huang, C., Liu, C., Chang, Y., Kong, Z., Zhou, Z., Wang, G., Lin, Y., & Chen, H. (2019). Determining factors, regulation system, and domestication of anthocyanin biosynthesis in rice leaves. *New Phytol.*, 223, 2, 705–721. Doi: 10.1111/nph.15807
- [171] Chen, C., Wu, W., Sun, X., Li, B., Hu, G., Zhang, Q., Li, J., Zhang, H., & Li, Z. (2014). Fine-mapping and candidate gene analysis of BLACK HULL1 in rice (*Oryza sativa* L.). *J. Plant Omics.*, 7, 12.
- [172] Gao, J., Dai, G., Zhou, W., Liang, H., Huang, J., Qing, D., Chen, W., Wu, H., Yang, X., Li, D.J., et al. (2019). Mapping and identifying a candidate gene Plr4, a recessive gene regulating purple leaf in rice, by using bulked segregant and transcriptome analysis with next-generation sequencing. *Int. J. Mol. Sci.*, 20, 4335.
- [173] Reddy, K.R., Kakani, V.G., Zhao, D., Koti, S., & Gao, W. (2004). Interactive effects of ultraviolet-B radiation and temperature on cotton physiology, growth, development and hyperspectral reflectance. *Photochem Photobiol.*, 79, 416–427.
- [174] Dwiningsih, Y., Mangimbulude, J. & Krave, A.S. (2007). Leachate denitrification activity from Semarang Jatibarang landfill. Satya Wacana Christian University, Salatiga, Jawa Tengah, Indonesia.
- [175] Gao, D., He, B., Zhou, Y., & Sun, L. (2011). Genetic and molecular analysis of a purple sheath somaclonal mutant in japonica rice. *Plant Cell Reports*, 30, 901-911.
- [176] Chin, H., Wu, Y., Hour, A., Hong, C., & Lin, Y. (2016). Genetic and evolutionary analysis of purple leaf sheath in rice. *Rice*, 9, 1, 8. Doi: 10.1186/s12284-016- 0080-y
- [177] Procissi, A., Dolfini, S., Ronchi, A., & Tonelli, C. (1997). Light-dependent spatial and temporal expression of regulatory genes in developing maize seeds. *Plant Cell*, 9, 1547–1557.
- [178] Yue, B., Cui, K.H., Yu, S.B., Xue, W.Y., Luo, L.J., & Xing, Y.Z. (2006). Molecular marker-assisted dissection of quantitative trait loci for seven morphological traits in rice (*Oryza sativa* L.). *Euphytica*, 150, 131–139.
- [179] Hadagal, B.N., Manjunath, A., & Goud, J.V. (1980). Linkage of genes for anthocyanin pigmentation in rice (*Oryza sativa* L.). *Euphytica*, 30, 747–754.
- [180] Lee, S., Kim, J., Jeong, S., Kim, D., Ha, J., Nam, K., & Ahn, D. (2003). Effect of Far-Infrared Radiation on the Antioxidant Activity of Rice Hulls. *J. Agric. Food Chem*, 51, 4400-4403. Doi: 10.1021/jf0300285
- [181] Simmons, D. & Williams, R. (1997). Dietary practices among Europeans and different South Asian groups in Coventry. *Br. J. Nutr.*, 78, 5-14.
- [182] Sun, X., Zhang, Z., Chen, C., Wu, W., Ren, N., Jang, C., Yu, J., Zhao, Y., Zheng, X., Yang, Q., Zhang, H., Li, J., & Li, Z. (2018). The C–S–A gene system regulates hull pigmentation and reveals



- evolution of anthocyanin biosynthesis pathway in rice. *Journal of Experimental Botany*, 69, 7, 1485-1498. Doi: 10.1093/jxb/ery001
- [183] Li, X., Sun, L., Tan, L., Liu, F., Zhu, Z., Fu, Y., Sun, X., Sun, X., Xie, D., & Sun, C. (2012). TH1, a DUF640 domain-like gene controls lemma and palea development in rice. *Plant Mol Biol*, 78, 351-359. Doi: 10.1007/s11103-011-9868-8
- [184] Weng, J.F., Gu, S.H., Wan, X.Y. et al. (2008). Isolation and initial characterization of GW5, a major QTL associated with rice grain width and weight. *Cell Res.*, 18, 1199–1209.
- [185] Shomura, A., Izawa, T., Ebana, K., Ebitani, T., Kanegae, H., Konishi, S., & Yano, M. (2008). Deletion in a gene associated with grain size increased yields during rice domestication. *Nat Genet.*, 40, 1023–1028.
- [186] Song, X.J., Huang, W., Shi, M., Zhu, M.Z., & Lin, H.X. (2007). A QTL for rice grain width and weight encodes a previously unknown RINGtype E3 ubiquitin ligase. *Nat Genet.*, 39, 623–630.
- [187] Fan, C.C., Xing, Y.Z., Mao, H.L., Lu, T.T., Han, B., Xu, C.G., Li, X.H., & Zhang, Q.F. (2006). GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. *Theor Appl Genet.*, 112, 1164–1171.
- [188] Dwiningsih, Y., Kumar, A., Thomas, J., & Pereira, A. (2017). Identification drought-tolerance rice variety for reducing climatic impacts on rice production. Fulbright Enrichment Seminar Climate Change, Estes Park, Colorado, USA.
- [189] Zhu, B., Si, L., Wang, Z., Zhou, Y., Zhu, J., Shangguan, Y., Lu, D., Fan, D., Li, C., Lin, H., Qian, Q., Sang, T., Zhou, B., Minobe, Y., & Han, B. (2011). Genetic Control of a Transition from Black to Straw-White Seed Hull in Rice Domestication. *Plant Physiology*, 155, 1301-1311. Doi: 10.1104/pp.110.168500
- [190] Baek., J.A., Chung, N.J., Choi, K.C., Hwang, J.M., & Lee, J.C. (2015). Hull extracts from pigmented rice exert antioxidant effects associated with total flavonoid contents and induce apoptosis in human cancer cells. *Food Sci Biotechnol.*, 24, 241-247.
- [191] Chen, W., Gao, Y.Q., Xie, W.B., Gong, L., Lu, K., Wang, W.S., Li, Y., Liu, X.Q., Zhang, H.Y., Dong, H.X., Zhang, W., Zhang, L.J., Yu, S., Wang, G.W., Lian, X.M., & Luo, J. (2014). Genome-wide association analyses provide genetic and biochemical insights into natural variation in rice metabolism. *Nat Genet.*, 46, 714-721.
- [192] Yu, Y., Tang, T., Qian, Q., Wang, Y., Yan, M., Zeng, D., Han, B., Wu, C.I., Shi, S., & Li, J. (2008). Independent losses of function in a polyphenol oxidase in rice: differentiation in grain discoloration between subspecies and the role of positive selection under domestication. *Plant Cell*, 20, 2946–2959.
- [193] Kuriyama, H. & Kudo, M. (1967). Complementary genes Ph and Bh controlling ripening black coloration of rice hulls and their geographical distribution. *Jpn J Breed.*, 17, 13–19.
- [194] Maekawa, M. (1984). Geographical distribution of the genes for black hull coloration. *Rice Genet Newsl*, 1, 104–105



Attendance Management system using RFID Technology

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Abstract: Attendance management is a problem commonly faced by companies, management using a manual system still causes problems. The problem that is often faced is the difference in employee attendance data reports because they have not been well integrated. The purpose of this study was to design an employee attendance information system at PT. Kartika Utama Semarang. From the above problems, a system is needed to integrate these needs automatically and is managed entirely by a computer using an RFID system in it so that the leadership can monitor attendance and payroll in real time. The application of the author's research and development (R&D) research method in this study. This research resulted in an information system application to facilitate the management of attendance and payroll of PT. Kartika Utama Semarang.

Keywords: Information System, Attendance, Payroll, RFID

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1. Introduction

Technological developments are step forward from in the era of globalization where is science and technology. Science and Technology is highly relied upon by the world community in any field today. This is due to the growing development of human thinking about technology so that humans are able to create technological innovations like today which are useful for helping humans to make it easier to deal with problems related to science and technology [1].

The rapid development of computer technology as a means of processing data and information is used in almost all fields, make information and data processing as part of an organization that is fast, accurate





and has data storage features that are supported by guaranteed data security with an efficient computer system so as to produce a fast and reliable information data [2].

Over time, Radio Frequency Identification (RFID) was developed as one of the many new technologies to make it easier for humans to identify various things. This technology consists of a special chip-shaped tag with a unique information code and a reader that reads the tag code. This system was originally designed to replace product barcode technology, but as it develops, it can be applied in other areas and adopted as a method that will be used in large numbers in the future [3].

Every institution engaged in the industrial sector, the increasing use of information in all fields is strongly influenced by the rapid development of information technology today, one of which is at PT. Kartika Utama Semarang. PT. Kartika Utama Semarang is a fishery company that produces canned crab products for the United States, European Union and Japan target markets. PT. Kartika Utama Semarang is a fairly large company with a large number of employees. The company is run by a factory manager who oversees seven departments, seven of which are run by managers assisted by staff.

The condition faced by this company is that all activities related to attendance, performance appraisal and payroll have not been computerized properly. The process of recording employee attendance and payroll at PT. Kartika Utama Semarang still uses manual attendance, employees arrive before 08.00 am and finish after 04.00pm beyond that hour, the employee is calculated overtime with overtime wages of Rp.8.000/hour. The employee performs the process by taking the timesheet on the timesheet rack next to the attendance machine, then inserting the card into the attendance machine slot for processing which will produce information on when the employee enters and leaves work. If the card is not filled in on time, the card will be printed in red ink as a sign of delay.

The use of the attendance machine which is still manual using this machine is also sometimes damaged, and while the attendance machine is under repair, each employee takes attendance by writing his name and signature on a piece of paper. As a result, the calculation of working hours and overtime hours is not accurate. Periodically the administration section takes attendance card sheets and combines the data into a spreadsheet on the computer. Each of these processes is repeated without making many changes to the attendance and payroll processes. A fully automated reporting process like this is actually very suitable for management with computers that are integrated by an RFID system in it so that managers can monitor attendance and payroll reports in real time per company branch [4].

Method

2.1. Information System

In general, an information system is defined as a system consisting of a series of information subsystems from data processing tests to produce useful information for the decision-making process [5].

Information Systems is a system inside organization which is a combination of person, facilities, technology, media, procedure, and control which aim for look after communication line main, processing type certain routine transactions, signaling managerial and soon as a result of a past event Sending important external factors to external events for the purpose internal and other and provide base information for decisions that appropriate [6].

Information system terms refers to use of computer technology in a organization for provide information to user. Information Systems "Computer Based" is a collection of hardware and software designed to transform data into useful information useful. Information Systems is gathering subsystem physical and non-physical mutually related, functioning in harmony, which achieves one purpose processing data into information useful. Consists of building block components consisting of: :block input, block model, block outputs, block technology, block database, and block control. Six blocks must interact to achieve destination in one units [6] .

The figure below is an information system block that interacts with each other:

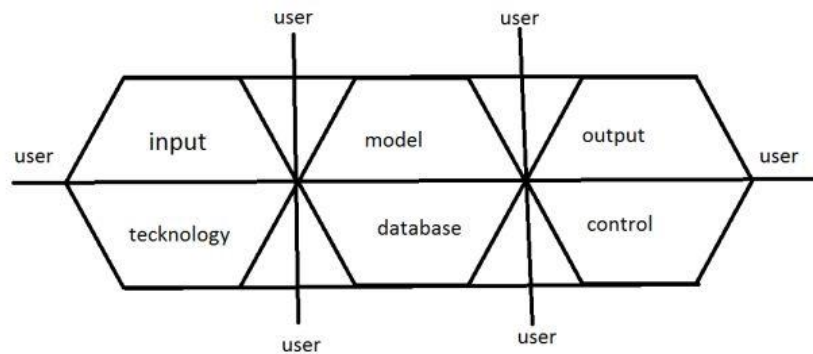


Figure 1. Building Block

2.2. Attendance

Attendance is data collection that is used as evidence of employee attendance at work. According to the Big Indonesian Dictionary, absenteeism is absence or absence. As proof of employee attendance at work, it is mandatory for employees to register attendance times independently on the form available when arriving and returning from work [6].

Absence is also a routine pattern of absence from duties or obligations. Absenteeism has long been a violation of an implicit contract between employer and employee and as an indicator of lazy individual performance. Absence can be considered as an administrative problem from an economic point of view [7].

2.3. Radio Frequency Identification (RFID)

Radio Frequency Identification (RFID) is a wireless identification system that can process non-contact data such as barcodes and magnetic cards such as ATMs. RFID technology is suitable for automated processes because it is easy to use. RFID combines advantages not found in other identification technologies [8].

Close object identification relation with data collection. One of the most advantageous identification methods is Auto-ID or automatic identification. That is, the collection procedure data with identify objects automatically without human involvement [9].

Auto-ID automatically works to increase efficiency and minimize data entry errors. Human resources can be focused on other areas because Auto-ID can work automatically. Technologies that utilize the Auto-ID method include: Barcodes, smart cards, voice recognition, biometric

identification such as retinal scans, Optical Character Recognition (OCR) and RadioFrequency Identification (RFID) [10].

Radio Frequency Identification(RFID) is a method of identification with radio waves. The identification process includes an RFID reader and an RFID transporter (RFID tag). An RFID tag is embedded in the object to be identified. Each RFID tag has a unique identification number (ID number). It is certain that there are no RFID tags with the same ID number. How it works RFID reader reads the ID number contained in the RFID tag to identify object items [11].

Generally, the RFID system consists of 3 parts, namely:

1. RFID Tags

Tags RFID available in the form of decal, paper, or plastic and is available in various sizes. Every tag containing chips and antenna which could keep number ID and some information certain.

2. Antenna

Used for send radio frequency signal between reader RFID and RFID tags. because of the tag RFID and readers RFID is a transceiver, tags RFID and reader RFID each have an antenna internal it self.

3. RFID reader

Radio Frequency Identification (RFID) read number ID and information other saved by RFID tags. Reader RFID must be compatible with tags RFID to read it. The author uses RFID special to detect tags Low frequency passive RFID. This is reader RFID 125 KHz.



Figure 2. RFID JT308 Card Reader USB 125KHz.

2.4. Development style

In developing employee attendance information systems requires careful preparation and planning. The development of this information system refers to the development model Research and Development (R & D) as the basis for product development. Design Development R & D has a goal for product development and validation. According to steps to implement the R&D strategy are carried out to test the effectiveness of the product [12]. The research and development

steps are as follows: (1) Potential and Problems, (2) Data collection, (3) Product Design, (4) Design Validation, (5) Design Revision, (6) Product Test. The chart of research steps used in product development is shown in the following figure.

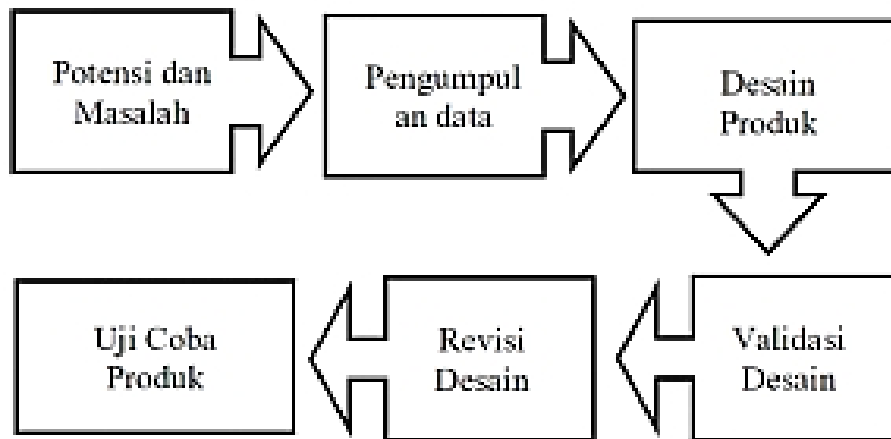


Figure 3. Product Development Procedure

From the scheme above, the author makes a work plan taken from Borg and Gall methods, including:

1. Analyzing the potential and problems contained in PT. Kartika Utama Semarang.
2. Carry out data collection using several approaches.
3. Designing the initial design of the system development to be developed.
4. Submission of the system design that the author made which can then be validated by experts/experts.
5. Revision of the system design that has been designed based on input and suggestions from experts/experts.
6. The system trial is in the form of an application that has been made by the author by the user, the intended user is an authorized device at PT. Kartika Utama Semarang.

3. Result & Discussion

The Design of this system aim of developing an old system that is costumized to the needs company related to problems that faced by the company. In designing the new system, several main steps need to be taken, namely conducting data analysis and evaluating the system that has been used by the company.

3.1. ERD (Entity Relationship Diagram)

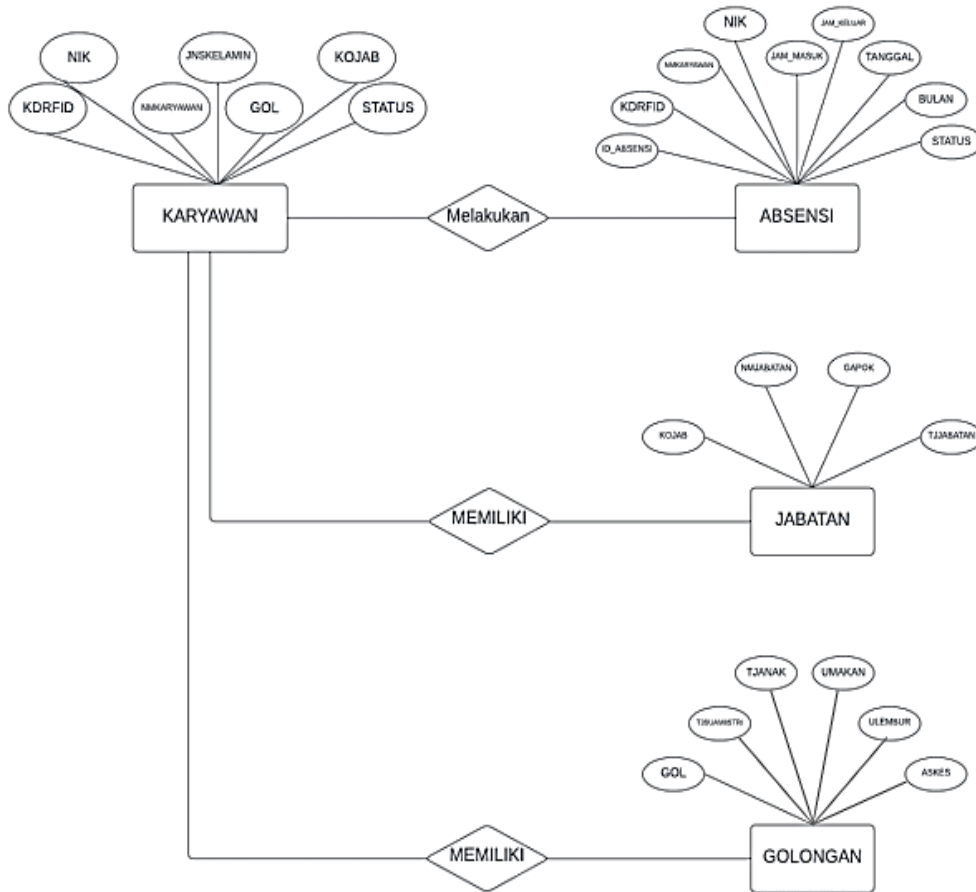


Figure 4. Entity Relationship Diagram

ERD (Entity Relationship Diagram) above is a data modeling design or system in databases. The function of ERD is to model structures and relationships among very complex data on the system created by PT.Kartika Utama Semarang. The existence of an ERD system is very important for companies to manage attendance employee data.

3.2. System Implementation

From the results of the system design that has been carried out, the implementation of the Project Evaluation and Review Technique can be implemented as follows.



Figure 5. Login Page

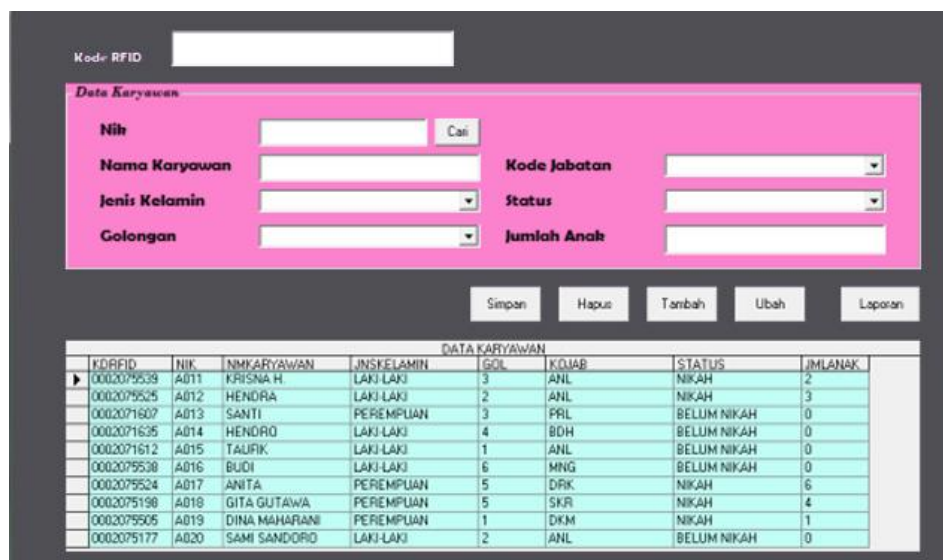
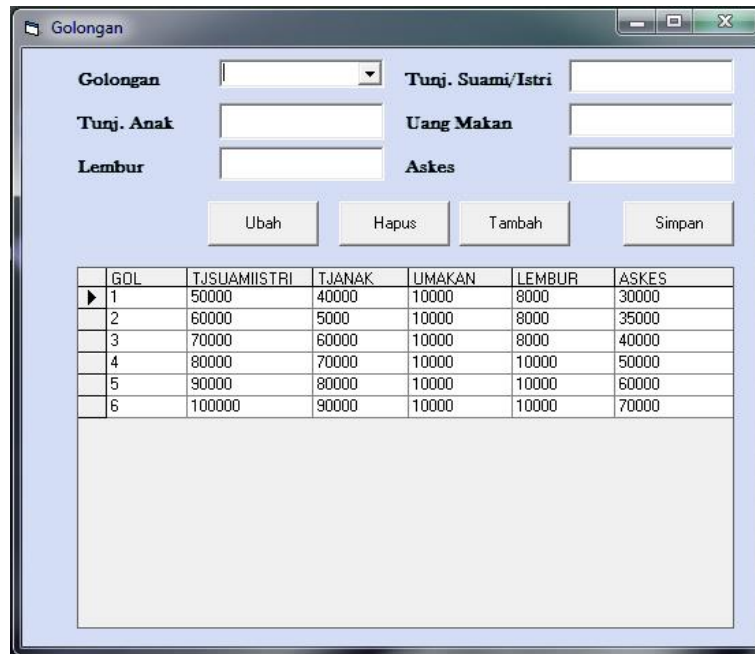
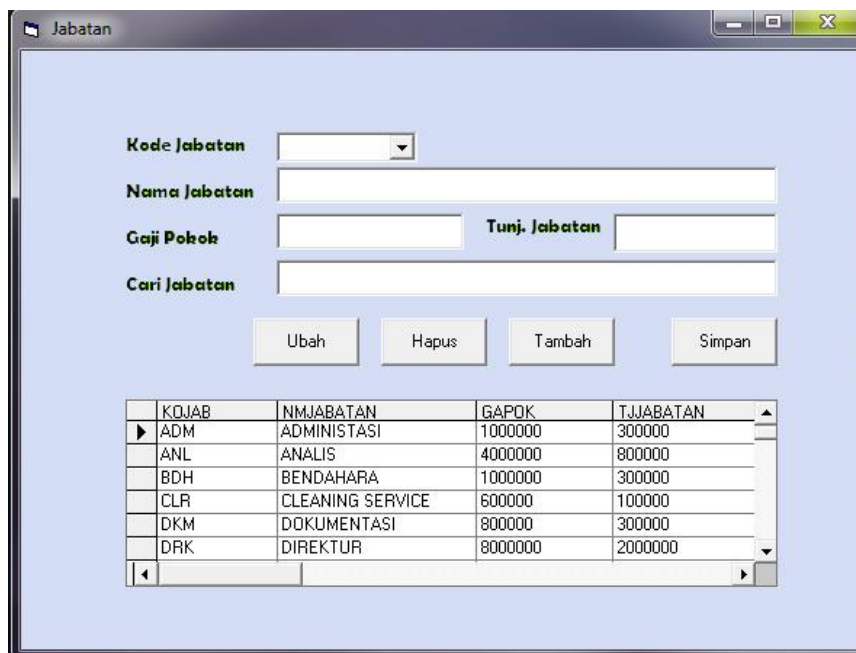


Figure 6. Employee Data Input Page



GOL	TJSUAMIISTRI	TJANAK	UMAKAN	LEMBUR	ASKES
1	50000	40000	10000	8000	30000
2	60000	5000	10000	8000	35000
3	70000	60000	10000	8000	40000
4	80000	70000	10000	10000	50000
5	90000	80000	10000	10000	60000
6	100000	90000	10000	10000	70000

Figure 7. Employee Class Page



KQJAB	NMJABATAN	GAPOK	TJJABATAN
ADM	ADMINISTRASI	1000000	300000
ANL	ANALIS	4000000	800000
BDH	BENDAHARA	1000000	300000
CLR	CLEANING SERVICE	600000	100000
DKM	DOKUMENTASI	800000	300000
DRK	DIREKTUR	8000000	2000000

Figure 8. Employee Job Page



ID/RFID	NIK	NIM/AFYAWAN	Data Absensi Masuk	JAM MASUK	JAM KELUAR	TANGGAL	BULAN	STATUS
0002071607	AD13	SANTI				30/08/2016	8	ALFA
0002071635	AD14	HENDRIO				30/08/2016	8	ALFA
0002075530	AD16	BLUDI				30/08/2016	8	ALFA
0002075524	AD17	ANITA				30/08/2016	8	ALFA
0002075190	AD18	GITA GUTAWA				30/08/2016	8	ALFA
0002075505	AD19	DINA MAHAFANI				30/08/2016	8	ALFA
0002075177	AD20	SAMI SANDIRO	05:00:17	16:00:37		30/08/2016	8	HADEH
0002075539	AD11	KRISNA H.				30/08/2016	8	ALFA
0002071612	AD15	TALRIK				30/08/2016	8	ALFA
0002075525	AD12	HENDRA				31/08/2016	8	ALFA
0002071607	AD13	SANTI				31/08/2016	8	ALFA
0002071635	AD14	HENDRIO				31/08/2016	8	ALFA
0002075530	AD16	BLUDI				31/08/2016	8	ALFA
0002075524	AD17	ANITA				31/08/2016	8	ALFA
0002075190	AD18	GITA GUTAWA				31/08/2016	8	ALFA
0002075505	AD19	DINA MAHAFANI				31/08/2016	8	ALFA
0002075177	AD20	SAMI SANDIRO				31/08/2016	8	ALFA
0002075539	AD11	KRISNA H.				31/08/2016	8	ALFA
0002071612	AD15	TALRIK				31/08/2016	8	ALFA
0002075525	AD12	HENDRA				01/09/2016	9	ALFA
0002071607	AD13	SANTI				01/09/2016	9	ALFA
0002071635	AD14	HENDRIO				01/09/2016	9	ALFA
0002075530	AD16	BLUDI				01/09/2016	9	ALFA

Figure 9. Attendance Form Page

3.3. System Testing

Test run for verify that systems are designed and built to meet the specifications pre-set. This test uses the method by running the system directly from a multi-user PC/laptop and using Visual Basic6.0 to access the information system.

In system attendance, the stages used are input, process and outputs. Administrator or employee are required to login first to be able to access the system. After logging in, administrators or employees can access several menus including employee data input, incoming attendance, outgoing attendance.

4. Conclusion

Employee attendance information system developed with RFID can facilitate attendance employee in PT.Kartika Utama Semarang. This multiuser attendance information system makes it easier for leaders to monitor attendance in real time, so that the employee attendance system can run effectively and efficiently.

References

- [1] H. D. Rjeib, N. S. Ali, A. Al Farawn, B. Al-Sadawi, and H. Alsharqi, "Attendance and information system using RFID and web-based application for academic sector," *Int. J. Adv. Comput. Sci. Appl.*, 2018, doi: 10.14569/IJACSA.2018.090137.
- [2] Q. Miao, F. Xiao, H. Huang, L. Sun, and R. Wang, "Smart attendance system based on frequency distribution algorithm with passive RFID tags," *Tsinghua Sci. Technol.*, 2020, doi: 10.26599/TST.2018.9010141.
- [3] F. Akter, A. Bushra Akhi, N. Jahan Farin, M. M. Khondoker, and M. G. Saklayen, "IoTSAMS: A Novel Framework for Internet of Things (IoT) Based Smart Attendance Management System," *Intell. Control Autom.*, 2018, doi: 10.4236/ica.2018.93006.
- [4] R. Qureshi, "The Proposed Implementation of RFID based Attendance System," *Int. J. Softw. Eng. Appl.*, 2020, doi: 10.5121/ijsea.2020.11304.



- [5] Meiryani, P. Siagian, R. A. A. W. Puspokusumo, and Lusianah, "Decision making and management information systems," *Journal of Critical Reviews*, vol. 7, no. 7. 2020, doi: 10.31838/jcr.07.07.52.
- [6] D. Berdik, S. Otoum, N. Schmidt, D. Porter, and Y. Jararweh, "A Survey on Blockchain for Information Systems Management and Security," *Inf. Process. Manag.*, vol. 58, no. 1, 2021, doi: 10.1016/j.ipm.2020.102397.
- [7] M. S. P. Hasibuan, "Manajemen Sumber Daya Manusia," *Ed. Revisi Jakarta Bumi Aksara*, 2011.
- [8] S. Rakasiwi and H. Kusumo, "Utilization of E-money for School Payments Using Web-Based RFID Sensors," *Adv. Sustain. Sci. Eng. Technol.*, vol. 3, no. 2, 2021, doi: 10.26877/asset.v3i2.9721.
- [9] A. Motroni, A. Buffi, and P. Nepa, "A Survey on Indoor Vehicle Localization through RFID Technology," *IEEE Access*, vol. 9, 2021, doi: 10.1109/ACCESS.2021.3052316.
- [10] Y. B. Kushermanto and A. Mulyanto, "Penerapan Teknologi RFID Modul RC522 Berbasis Raspberry Pi B + Pada Sistem Absensi Siswa di SMK At-Taqwa Cabangbungin Kabupaten Bekasi," *J. Inform. SIMANTIK*, 2017.
- [11] P. Parkhi, S. Thakur, and S. Chauhan, "RFID-Based Parking Management System," *Int. J. Adv. Res. Comput. Commun. Eng.*, 2014.
- [12] P. D. Sugiyono, *metode penelitian kuantitatif, kualitatif, dan R&D*. 2016.



The Web-Based Application of Small and Medium Enterprises (SMEs) Product Distribution Management with Content Management System Shopify Integration in Netasia Singapore

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Abstract. Netasia is a brand activation of the company named Golden Style Management Lte. Ptd. in Singapore which acts as a local partner and distributor for Small and Medium Enterprises (SMEs) from Indonesia. Netasia utilizes Content Management System (CMS) named Shopify to reach end-users. But currently, they do not have a platform for managing product distribution from preorders to vendors until distribution to stores. This research was conducted to develop a web-based application of SMEs product distribution management with CMS Shopify integration in Netasia Singapore. This research was conducted using Research and Development (RnD) method for research approaches and prototyping method for software development. The prototyping method often involves communication between developers and the company as application users, so that the application undergoes several changes. As the result of this research, the application has been successfully integrated with the data from the company's CMS Shopify through the API using cURL. The application was tested with several testing methods such as 1) black-box testing with 100% succeed percentage so the application is proper to use, 2) white-box testing with cyclomatic complexity of 4 and 100% succeed percentage for value test so the application passed the software engineering criteria, 3) User Acceptance Testing (UAT) with 94% average percentage so the application meets user expectations, and 4) browser application compatibility testing with the results that the application is compatible with all of the browser applications specified in this research for the menus function aspect, and less compatible with the Internet Explorer browser for the user interface aspect.

Keywords: web-based application, content management system, Shopify, API, cURL

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1. Introduction

Netasia also known as Netasia Singapore is a brand activation of the company named Golden Style Management Lte. Ptd. in Singapore. Netasia acts as a local partner and distributor for Small and Medium Enterprises (SMEs) from Indonesia in Singapore. The products distributed also adapt to the needs and



market trends in Singapore. Netasia takes care of products from arrival to stores, monitoring sales, and penetrating to new places or new target markets. Netasia acts to preorder SMEs products from some vendor partners in Indonesia, then distributes them both to end-users and to several store partners in Singapore.

To optimize the distribution of SMEs products, Netasia started to reach end-users by utilizing a Content Management System (CMS) named Shopify. Content Management System (CMS) is a system that helps users to manage web content more easily without doing a programming [1]. Users just upload the content to the CMS and configure it through the features provided by the CMS. Meanwhile Shopify is an e-commerce CMS that is used as a marketplace for selling product by online. Netasia uses the Shopify to sell several products from SMEs vendors to anyone as an end-user.

Besides, Netasia also needs to manage the distribution of SMEs products from preorders to vendors until distribution to stores in Singapore. But currently, Netasia does not have a platform for managing product distribution from preorders to vendors until distribution to stores. They have been managed product distribution manually using communication apps such as WhatsApp and manual recording using Google Sheets or Microsoft Excel. It can stack files that can complicate product distribution management. The process of product distribution management in Netasia is illustrated in Figure 1.

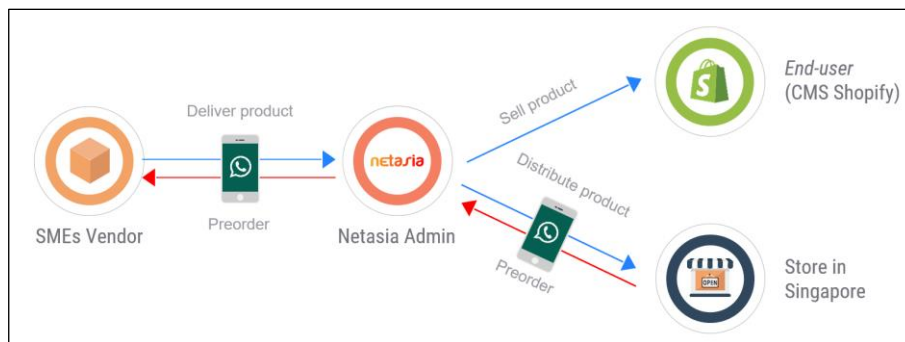


Figure 1. Product Distribution Management Process in Netasia

The way that can be used to manage product distribution more easily is create a web-based application. Web-based application is an application that runs on a web server and can be accessed using a web browser [2]. Web-based application is more interactive and responsive to user actions than website [3]. The web-based application developed is also integrated with the data from Netasia's CMS Shopify through the Application Programming Interface (API) using a client URL (cURL) method. Application Programming Interface (API) is a library that acts as an intermediary for an application to interact with other applications [4]. While the client URL (cURL) is a command line to transfer data through the server using the URL syntax through several protocols [5]. Shopify provides procedures for accessing these APIs.

Based on the conditions and problems that have been described above, Netasia needs a platform that can be used to manage the distribution of SMEs products from preorders to vendors until distribution to stores in Singapore. Therefore, this research was conducted to develop a web-based application of SMEs product distribution management with CMS Shopify integration in Netasia Singapore. This application is expected to help Netasia manage the distribution of SMEs products more effectively and efficiently.

2. Methods

The research was conducted using the prototyping method as one of the methods from the System Development Lifecycle (SDLC). Prototyping is a system development method that uses an approach to create a software quickly and gradually so that it can be immediately evaluated by users [6]. There are several stages in the prototyping method as can be seen in Figure 2 [7].

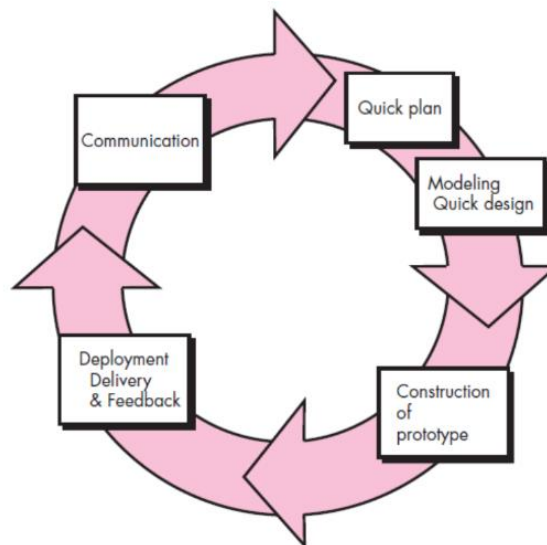


Figure 2. Prototyping Method

2.1. Communication

Collecting data and information with literature study, interviews with the company, and observation of the data in the company's CMS Shopify. The prototyping method that used in this web-based application development often involves communication between developers and the company as application users. In this method, the application is developed as a prototype that can be changed or updated according to the company's needs.

2.2. Quick Plan

Consists of problem analysis and needs analysis which includes data requirements, system functional requirements, and system non-functional requirements for the web-based application development. Data requirements include vendor data and vendor product data obtained from the company's CMS Shopify, and also store data obtained from interviews with the company. System functional requirements include menus and features in the application desired by the company. System non-functional requirements include software requirements and hardware requirements to support the development process.

2.3. Modeling Quick Design

The data obtained from the previous stage is then visualized in a system model using the Unified Modeling Language (UML), database design, and user interface design. Unified Modeling Language (UML) is a method that provides visual modeling that allows developers to create designs or blueprints in a standardized form, easy to understand, and equipped with effective mechanisms for sharing and communicating the designs that have been made [8]. UML diagrams used in this research include use case diagram, sequence diagram, activity diagram, and class diagram.

Use case diagram are used to describe the sequence of actions performed by the system from each entity or actor. There are several use cases for each actor in a use case diagram. Based on the use case diagram as can be seen in Figure 3, there are three actors in the web-based application developed in this research, namely admin, vendor, and store. Each actor has their own use case. This use case is also used for making sequence diagram, activity diagram, and class diagram. Sequence diagram are used to describe the behavior of objects in the use case by describing the life time of objects and messages sent and received by objects. Activity diagram is used to model the workflow of a business process and the sequence of activities in a process, which are arranged based on the use case scenarios that have been made previously. Class diagram are used to describe the state (attributes/properties) of a system, as well

as manipulate the state (methods/functions). These four diagrams are related to each other.

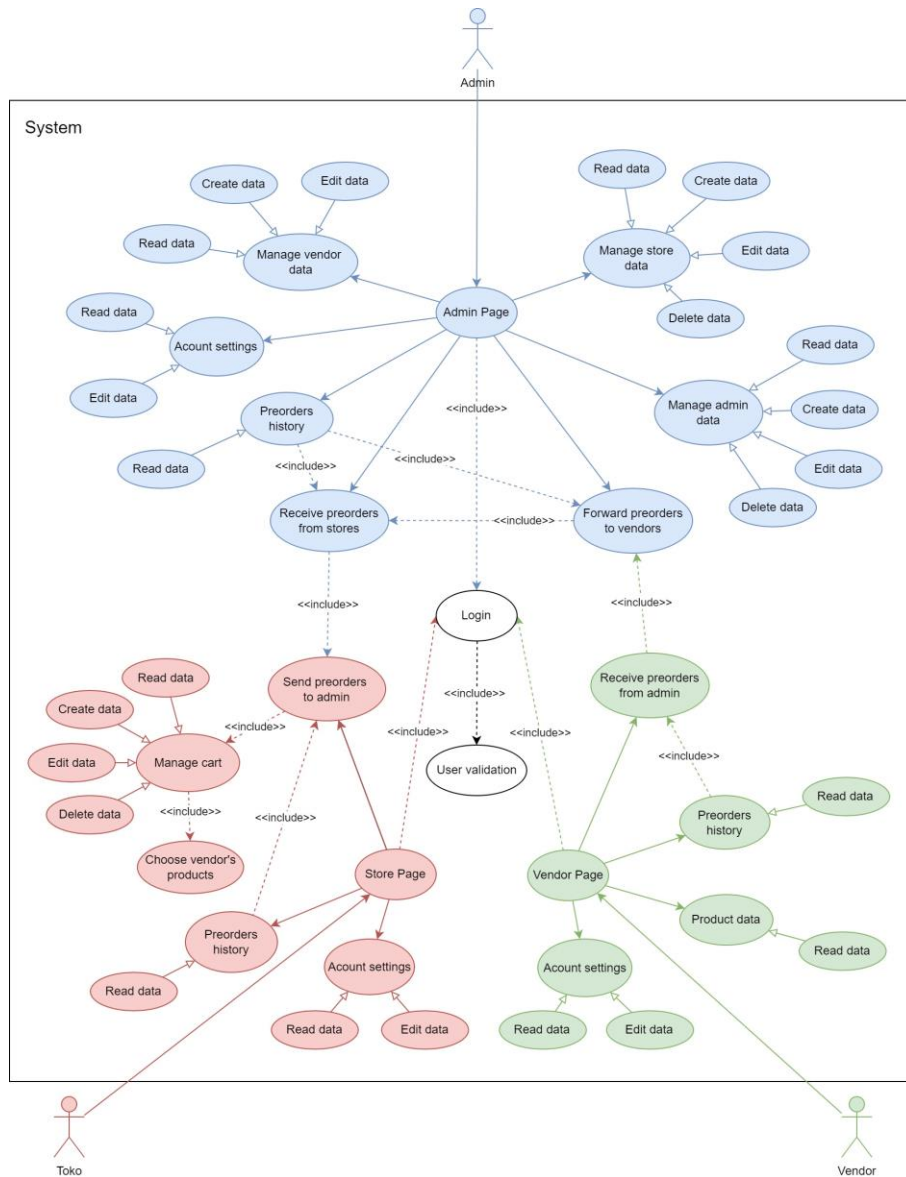


Figure 3. Use Case Diagram

2.4. Construction of Prototype

Develop the software based on the results of some analysis and system design that have been carried out in the previous stage. This stage becomes the main focus in the prototyping method because at this stage the software has begun to be developed. The software developed then will deliver to the company as application users to receive feedbacks. It can be used as an evaluation and improvement of the software developed, so that the application can be proper to use.

2.5. Deployment, Delivery, and Feedback

If the prototype of the software has been completed, then the software is delivered and used by users to get feedback. This stage also includes the software testing stage which consists of black-box testing,

white-box testing, User Acceptance Testing (UAT), and browser application compatibility testing. The instrument used in the software testing is a questionnaire using a form.

3. Results and Discussion

3.1. Software Development Results

The results of the application development were obtained after going through several stages of the prototyping method which lasted for about eight months. The web-based application was developed using the Codeigniter framework. This web-based application is also integrated with the data from the company's CMS Shopify through the API using cURL. The prototyping method that used in this web-based application development often involves communication between developers and the company as application users. It also makes the development of this application undergoes several changes and updates based on the communication with user and user feedback.

Before being implemented, the application developed in this research will go through the construction of prototype stage to make the application prototype. There have been several changes in this application according to feedback from the company as application users at the communication stage and deployment, delivery, and feedback stage. The construction of prototype stage in the development of this application can be seen in Figure 4.

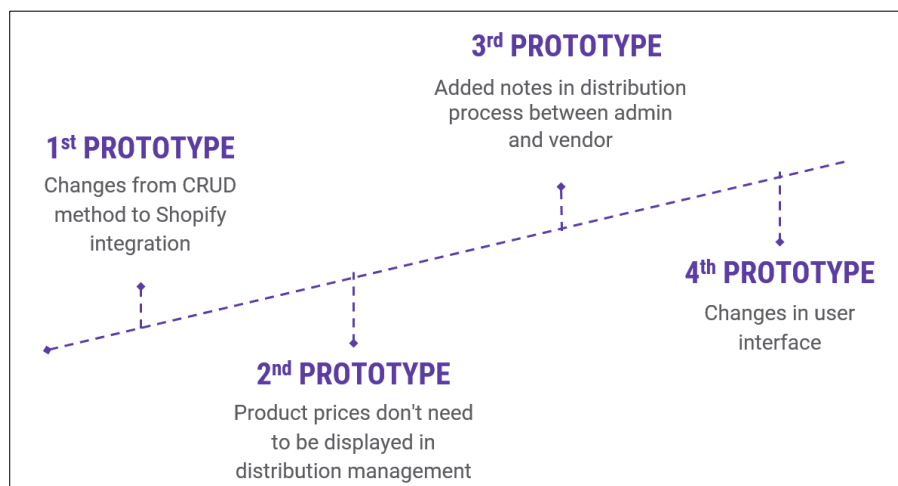


Figure 4. Construction of Prototype Stages

After going through several changes in construction of prototype stage, the application is then integrated with the data from CMS Shopify through the API. The data in the company's CMS Shopify includes vendor data and vendor's product data. This data can be integrated because Shopify as a CMS provides a web service that allows other applications to access some of its features, through the Application Programming Interface (API) using the client URL (cURL) method. The data is presented in JavaScript Object Notation (JSON) format and will be called in the PHP code structure.

There are four stages in using cURL method [9], including 1) initializing the cURL function using `curl_init()` command, 2) setting the value options (set options) such as the URL address, the result format, headers, etc. using `curl_setopt()` command, 3) executes cURL to make an HTTP request according to the options given in the previous stage using `curl_exec()` command, and 4) closes cURL using `curl_close()` command because the process is deprecated. The data retrieved from the API is presented in JSON (JavaScript Object Notation) format. Then the data decoded using `json_decode()` command. This function is used to parse JSON format and make it is easy to processed into PHP variables. The

data integration process from the CMS Shopify through the API can be seen in Figure 5.

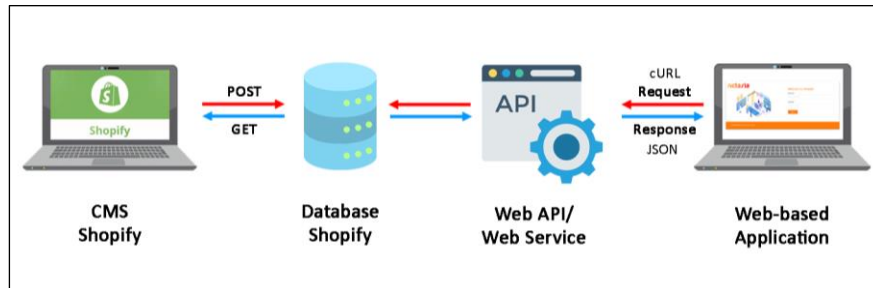


Figure 5. Data Integration Process through API

The application developed in this research consists of four main pages, such as login page, admin page, vendor page, and store page. When the user accesses the application, the first page that is shown by the system is the login page. This page consisting a form to enter a username and password, and a button to login. The user will login to the application and directed to the dashboard page according to their respective levels as admin, vendor, or store by the username and password that have been entered. The login page can be seen in Figure 6.

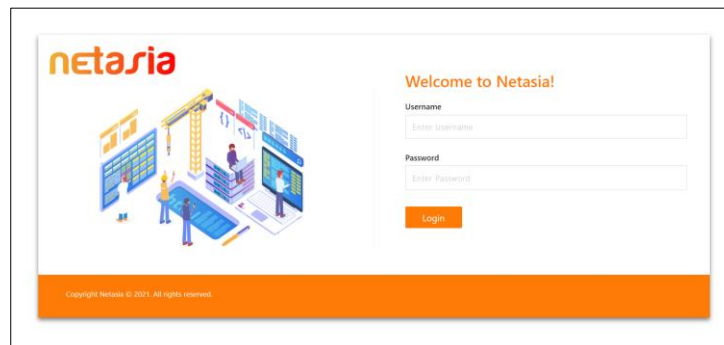


Figure 6. Login Pages

The admin page has six main menus including dashboard, vendor partner, store partner, preorders, preorder history, and admin accounts. The admin page also has other features such as vendor details, store details, processing preorder, sending preorders to vendors, preorder details, and account settings.

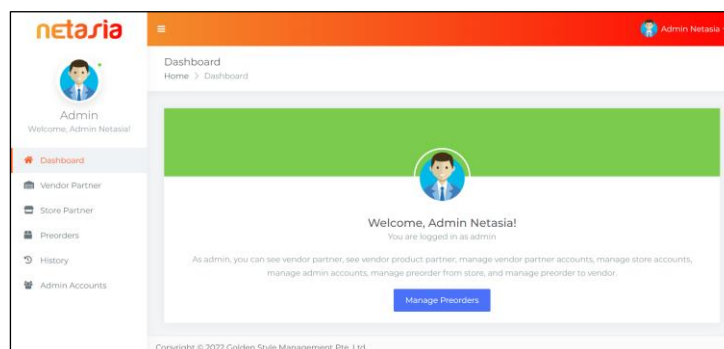


Figure 7. Admin Dashboard Page

The admin dashboard page along with the menus and features can be seen in Figure 7.

The vendor page has four main menus including dashboard, products, preorders from admin, and preorder history. The vendor page also has other features such as processing preorder, preorder details, and account settings. The vendor dashboard page along with the menus and features can be seen in Figure 8.

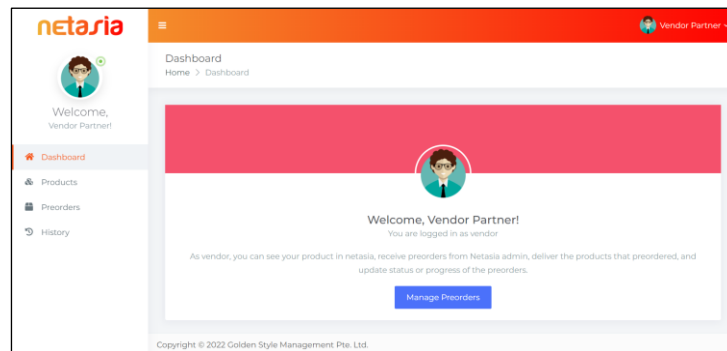


Figure 8. Vendor Dashboard Page

The store page has several main menus including dashboard, preorders, cart, and preorder history. The store page also has other features such as vendor details (containing list of vendor's products), product details, preorder details, and account settings. The store dashboard page along with the menus and features can be seen in Figure 9.

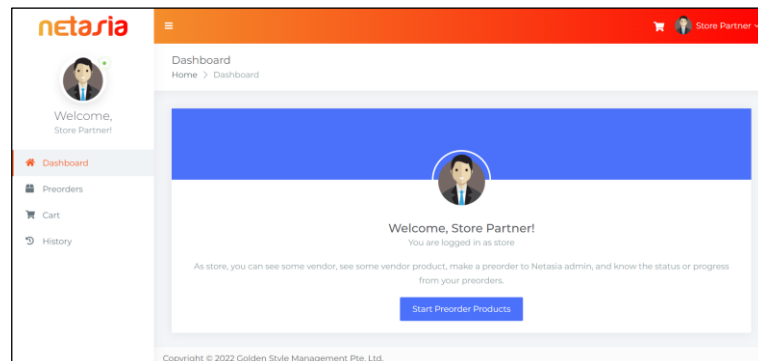


Figure 9. Store Dashboard Page

3.2. Software Testing Result

Software testing is one of the important stages in software development. Software testing is carried out to determine whether the software is in accordance with the criteria and objectives of the software design. Software testing used in this research included black-box testing, white-box testing, User Acceptance Testing (UAT), and browser application compatibility testing. The results of software testing for the web-based application developed in this research are described as follows:

a. Black-box testing

Black-box testing is used to test the functional specifications of a system or software [10]. Black-box testing was carried out by involving three lecturers from the Informatics Study Program of Universitas PGRI Semarang as respondents using a questionnaire. There are 76 test cases in this test with details of 4 test cases for login page, 41 test cases for admin page, 15 test cases for vendor page, and 16 test cases for store page. The results of the black-box testing from three respondents



obtained 100% succeed percentage in overall with the details of calculation as follows:

$$\text{Succeed} = \frac{\text{Number of succeed}}{\text{Number of all aspects}} \times 100\% = \frac{76}{76} \times 100\% = 100\% \quad (1)$$

$$\text{Failed} = \frac{\text{Number of failed}}{\text{Number of all aspects}} \times 100\% = \frac{0}{76} \times 100\% = 0\% \quad (2)$$

The percentage from each respondent's then averaged and the results of the black-box test were obtained as follows:

$$\text{Succeed} = \frac{100\% + 100\% + 100\%}{3} = \frac{300\%}{3} = 100\% \quad (3)$$

$$\text{Failed} = \frac{0\% + 0\% + 0\%}{3} = \frac{0\%}{3} = 0\% \quad (4)$$

b. White-box testing

White-box testing is used to test the detail or internal structure of the software design and uses the control structure of the procedural program design to divide the test into several test cases [10]. White-box testing was carried out by the researchers themselves by testing the code structure of the web-based application developed in this research. The code structure tested is a program flow that has a looping, such as the preorder process from store to admin and the preorder process from admin to vendor. The white-box test results that have been obtained are then processed using several techniques, such as basis path testing, cyclomatic complexity, independent path, and value test. The result obtained the cyclomatic complexity of 4 and 100% succeed percentage for value test.

c. User Acceptance Testing (UAT)

User Acceptance Testing (UAT) is a verification process that the solution made in the system or software is suitable for the user. In contrast to black-box testing or white-box testing that focusing on the functionality and structure of the system or software, the UAT focuses on whether the solution in the system or software is accepted by the user [11]. The UAT test was carried out by involving 3 respondents consisting of 1 representative from company as admin, 1 representative from the SMEs vendor, and 1 representative from the store, using a questionnaire. There are 13 questions which are divided into three aspects such as usability, ease of use, and user interface. The UAT results obtained a percentage of respondent 1 was 93%, respondent 2 was 98%, and respondent 3 was 92%. The percentage from each respondent's then averaged and the results of the UAT test were obtained as follows:

$$\text{UAT Result} = \frac{\text{Total percentage}}{\text{Number of respondents}} = \frac{283\%}{3} = 94\% \quad (5)$$

d. Browser Application Compatibility Testing

Browser application compatibility testing was carried out by the researchers themselves by testing the functionality and user interface of the web-based application developed in this research on several predetermined samples of browser applications. The results of browser application compatibility testing can be seen in Table 1.



Table 1. Browser Application Compatibility Testing

Num.	Aspects	Google Chrome	Internet Explorer	Mozilla Firefox	Opera Browser	Safari Browser
1	Menus function	Normal	Normal	Normal	Normal	Normal
2	User interface	Normal	Changed	Normal	Normal	Normal

4. Conclusion

Based on the research that has been done, it can be concluded that the web-based application of SMEs product distribution management with CMS Shopify integration in Netasia Singapore was successfully developed using the prototyping method which consists of several stages including communication, quick plan, modeling quick design, construction of prototype, and deployment, delivery, and feedback. This application has also been successfully integrated with the data from the company's CMS Shopify through the API from Shopify using cURL method. So, the company does not need to re-upload vendor data and vendor's product data. The application was tested with several testing methods such as 1) black-box testing with 100% succeed percentage so the application is proper to use, 2) white-box testing with cyclomatic complexity of 4 and 100% succeed percentage for value test so the application passed the software engineering criteria, 3) User Acceptance Testing (UAT) with 94% average percentage so the application meets user expectations, and 4) browser application compatibility testing with the results that the application is compatible with all of the browser applications specified in this research for the menus function aspect, and less compatible with the Internet Explorer browser for the user interface aspect.

Acknowledgements

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References

- [1] A. Hendriyanto and D. Edi, "Integrasi Data Tokopedia dengan Content Management Systems Point of Sales," *J. Strateg.*, vol. 3, no. November, pp. 311–319, 2021.
- [2] Sulton and R. Setiawan, "Rancang Bangun Aplikasi Computer Laboratory Resource Inventory System Berbasis Web pada Sekolah Menengah Kejuruan Plus Pontren Yabafa," *J. Algoritma. Sekol. Tinggi Teknol. Garut*, pp. 50–57, 2014, [Online]. Available: <http://sttgarut.ac.id/jurnal/index.php/algoritma/article/view/301/275>.
- [3] Feradhita NKD, "Web App VS Website – Apa Saja Perbedaan Keduanya?," *Logique*, 2019. <https://www.logique.co.id/blog/2019/05/10/web-app-> (accessed Nov. 27, 2021).
- [4] Y. Maulanadireja, "Optimalisasi Distribusi Barang PT. XYZ Menggunakan Hill Climbing Berbasis Android," *Tesis*, 2020, [Online]. Available: https://elibrary.unikom.ac.id/id/eprint/4178/%0Ahttps://elibrary.unikom.ac.id/id/eprint/4178/8/11.UNIKOM_YUSUP_MAULANADIREJA_BAB2.pdf.
- [5] R. Yusuf, H. Kusniyati, and R. E. Dewi, "Aggregator Otomatis Pencari Produk dengan Pemberitahuan Melalui Surel Menggunakan Fungsi cURL," *J. Tek. Inform.*, vol. 8, no. 1, pp. 48–56, 2015, doi: 10.15408/jti.v8i1.1936.
- [6] R. C. Noor Santi, "Perancangan Interaksi Pengguna (User Interaction Design) Menggunakan Metode Prototyping," *J. Tek. Inform.*, vol. 9, no. 2, pp. 108–113, 2018, doi:



- 10.15408/jti.v9i2.5599.
- [7] A. Abdullah and R. Kurniawan, “Perancangan Sistem Informasi Akuntansi Desa Wisata Pentingsari Menggunakan Metode Prototyping,” *Automata*, p. 7, 2021, [Online]. Available: <https://journal.uui.ac.id/AUTOMATA/article/view/17340>.
 - [8] H. F. Siregar and N. Sari, “Rancang Bangun Aplikasi Simpan Pinjam Uang Mahasiswa Fakultas Teknik Universitas Asahan Berbasis Web,” *J. Teknol. Inf.*, vol. 2, no. 1, p. 53, 2018, doi: 10.36294/jurti.v2i1.409.
 - [9] B. E. W. Asrul, “Sistem Temu Kembali untuk Data Harga Murah Barang Toko Online Menggunakan Web Service,” *J. IT STMIK Handayani*, vol. 14, pp. 13–19, 2014, [Online]. Available: https://spectrum.ieee.org/riskfactor/computing/it/estonia-manages-legacy-it?utm_campaign=COVID19&utm_source=Social+Alias&utm_medium=Email.
 - [10] T. Hidayat and M. Muttaqin, “Pengujian Sistem Informasi Pendaftaran dan Pembayaran Wisuda Online menggunakan Black Box Testing dengan Metode Equivalence Partitioning dan Boundary Value Analysis,” *J. Tek. Inform. UNIS JUTIS*, vol. 6, no. 1, pp. 2252–5351, 2018, [Online]. Available: www.ccsenet.org/cis.
 - [11] E. Suprpto, “User Acceptance Testing (UAT) Refreshment PBX Outlet Site BNI Kanwil Padang,” *J. Civronlit Unbari*, vol. 6, no. 2, p. 54, 2021, doi: 10.33087/civronlit.v6i2.85.



Physicochemical Characteristics of White Tea Product of PT. Perkebunan Nusantara IX (Kaligua Gardens) Pandansari Village, Paguyangan District, Brebes Regency

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Abstract. White tea is a type of tea that still sounds foreign, the high price makes white tea sometimes used as a symbol of one's social status. Due to the high price, not many people consume it, so it is less popular. PT. Perkebunan Nusantara IX (PTPN-IX) which is located at the west foot of Mount Slamet, precisely in Pandansari Paguyangan Village, Brebes Regency, Central Java, with an altitude of 1,500-2,050 meters above sea level and a temperature between 8-280 Celsius. One type of tea product PTPN-IX that has not been studied physically, chemically and its characteristics is white tea. This study aims to examine the characteristics of white tea produced by PTPN-IX in Brebes. This research was carried out at the request of the PTPN-IX, the methodology used was to test some of the core components contained in white tea, some tests were carried out 3 times and the average value was taken. The components tested included: ash content, crude fiber, Pb/Cu/Zn/Hg content, total plate count, coliform, water content, carbohydrates, protein, pectin, tannins and caffeine. The results showed that white tea produced by PTPN-IX were: ash content of 5.70%; Crude fiber 13.40% ; Pb 2.35 mg/Kg ; Cu 12.94 mg/Kg ; Zn 22.30 mg/Kg ; Hg (negative) ; Total plate number 1.3 x 10³ cfu/g ; Coliform (negative). For chemical components, the results are: water 5.65%; carbohydrates 3.9% ; 18% protein; pectin 5.85% ; tannins 5.25% ; caffeine 2.4 – 4.5.

Keywords: caffeine, pectin, tannin, white tea

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1. Introduction

Recently, the international community has begun to show an interest in white tea (Fig.1). The unique taste that comes from the umami (fifth taste) of white tea and the abundance of tea polyphenols which are very beneficial for the health and fitness of white tea drinkers are the main attractions of this special tea. Although in limited quantities, Indonesia has been able to produce silver needle quality white tea (the highest grade for white tea). White tea looks white because the tops of the leaves are still covered with white fine hairs. White Tea is the finest and rarest tea in the world, because it is only produced in



Fujian, China. And only picked from *Cammellia sinensis* Shui hsien varieties and dai bai varieties, in certain seasons. White tea contains nutritious substances that are very beneficial for health and fitness, making white tea the most superior health drink compared to health drinks from other types of tea.[1]



Figure 1. White Tea

The picking process is a very important process, so it must be picked carefully. Picking only the two youngest shoots that have not yet opened are still covered with fine silver hairs. Picked when the shoots have not opened, and done before the sun has risen to keep the moisture from the tea shoots. Good quality white tea is usually picked in early spring, harvesting time is usually only 2 days to 2 weeks.

The withering process plays a very important role in determining the final quality. The tea leaves that have been picked are spread evenly on a container made of woven bamboo, then dried in a place where the sun is not too hot or stored in a room that has good air circulation. The container for withering should not be placed directly on the floor.

White tea is naturally dried with the help of the wind and the sun's rays, without going through a fermentation or grinding process so as not to damage the actual shape of white tea. Drying in white tea aims to reduce the moisture content of the shoots to 3-4%.

The sorting process aims to obtain uniform shape and size of white tea so that it meets the standards for being marketed abroad with guaranteed quality. Storage and packaging after sorting is done to maintain the fragrant aroma of white tea, white tea is usually packaged in paper bags or plastic bags.

The components contained in white tea are: polyphenols (antioxidants), catechins (lowers cholesterol), flavonols (stimulates blood vessels), caffeine (stimulates the nervous system), tannins (source of umami taste), essential oils (source of aroma), fluoride (prevents odor, tooth decay), vitamins A-B-C, monocatechin, chlorophyll (prevents bad breath), theophylline (prevents asthma), hydroxy coumarin (prevents blood clots), zinc, calcium (essential minerals) and amino acids (regenerates cells). [2]

From the research that has been done by researchers on green tea and black tea, with the aim of knowing the difference antioxidant activity between green tea and black tea and its correlation with polyphenol content in tea produced by PT. Tambi Wonosobo. The results showed that the phenol content and antioxidant activity of green tea is higher than the phenol content and antioxidant activity black tea. Phenol content affects antioxidant activity the higher the phenol content, the higher antioxidant capacity in tea.

2. Methods

2.1. Materials

Sample preparation was carried out by taking much as 1 kg, fresh white tea leaves, dried. It is declared dry if it has reached a constant weight on two weighings. (The sample used for the laboratory is 200 grams)

2.2. Methods

This research conducted in Basic Chemistry Laboratory Program Bachelor Degree in Chemical Engineering, University of 17 August 1945, Semarang. Research begins by testing according to the parameters in SNI, some parameters are carried out manually and other parameters using instruments such as spectrophotometers UV-Vis, FTIR.



3. Results and Discussion

The results of the analysis of white tea from PT. Perkebunan Nusantara IX Brebes based on SNI tea is shown in table 1 and 2 below :

Table 1. Results of the physical analysis of white tea.

No	Parameters	Quality Standard (SNI)	Value
1	Water Content	Max. 8% b/b	5.65
2	Extract content in water	Max.32% b/b	41.08
3	Ash Content	Max. 8%	5.70
4	Water soluble ash alkalinity	1 – 3 %	2.35
5	Coarse Fiber	Max. 16%	13.40
6	Pb	Max.20 mg/kg	2.35
7	Cu	Max. 50 mg/kg	12.94
8	Zn	Max. 40 mg/kg	22.30
9	Hg	Max.0.03mg/kg	Negative
10	Total Plate Number	Max. 3 x 10 ³ efu/g	1.3 x 10 ³
11	Coliform	< 3 APM/g	Negative

Table 2 . Results of the Analysis of the Chemical Content of White Tea (100 grams)

No	Parameters	Percent (%)
1	Polifenol	26
2	Karbohidrat	3.9
3	Pektin	5.8
4	Kafein	40.78
5	Protein	18
6	Tanin	4.26
7	Flavonoid	1.26

4. Green tea contains polyphenols of 588.58 mg/kg – 750 mg/kg which is known as a source of antioxidants and contributes to the sensation of bitter and sour taste.[4] In white tea the polyphenol content of 26% or 26 grams/100gr, this indicates that the polyphenol content of white tea is greater than that of green tea. The initial brewing temperature of 95oC and the brewing time of 15 minutes produced the best extract characteristics with the extract yield of $26.2 \pm 0.50\%$, total flavonoids 252.3 ± 1.71 mg QE/g dry weight of the material, and antioxidant activity of 173.5 ± 1.34 g/ml.[5] The flavonoid content in white tea is 1260 mg/100 gr (1.26%) greater than green and white tea. From the two chemical parameters above, it shows that the content of active substances in white tea is greater than black and green tea. Especially for the white tea produced by PT. Perkebunan Nusantara IX, the Kaligua garden has very good characteristics and meets standards. This can be seen from the physical test of the white tea which has a value below the maximum allowable limit in SNI. The antioxidant content (flavonoids and polyphenols) of white tea is quite high, 26% and 1.26%, respectively. Flavonoids as antioxidants, antimicrobials and also anticancer that can fight free radicals that damage body cells. While these polyphenols substances also act as antioxidants that can fight cancer cells and reduce the risk of inflammation in the body. The antioxidant content in tea is 100 times more than that in fruits and vegetables so that it can increase metabolism and body immunity.



White tea produced by PT. Perkebunan Nusantara IX has good quality and is feasible to produce, in addition to having a high economic value, white tea has health benefits.

5. Conclusion.

The complete characterization physicochemical of white tea from the Kaligua plantation of PT. Perkebunan Nusantara IX is presented in tables 1 and 2, if it refers to the SNI standard, then the white tea from the Kaligua plantation meets the standard.

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References

- [1] NN, 2021, Five White Tea Benefits , <https://www.alodokter.com/manfaat-teh-putih-dan-cara-menyajikannya> , 25 Maret 2022.
- [2] NN, 2021, Tea Leaves As a Source of Antioxidants [https://farmasi.unpak.ac.id/berita/daun-teh-berfungsi-sebagai-sumber-antioksidan#:~:text=Alkaloid%20utama%20dalam%20daun%20teh,cuprum%20\(Andi%2C%202006\)](https://farmasi.unpak.ac.id/berita/daun-teh-berfungsi-sebagai-sumber-antioksidan#:~:text=Alkaloid%20utama%20dalam%20daun%20teh,cuprum%20(Andi%2C%202006),), 25 Maret 2022. [3] C. Choi, J. Kim, J. Kim, D. Kim, Y. Bae, and H. S. Kim, "Development of heavy rain damage prediction model using machine learning based on big data," *Adv. Meteorol.*, vol. 2018, 2018.
- [3] Dini Cahya Chairunnisa, 2021, Isolation of Caffeine from Tea Using Uv-Vis Spectrophotometric and FTIR Methods, Academia Education.
- [4] Nurheni Sri Palupi, Dase Hunaefi, Nugraha Susanto, 2021, Optimization of Green Tea Extraction Based on Polyphenol Content, Antioxidant Activity and Sensor Profile , *Jurnal Industrial Plants and Beferages*, Volume 8 Nomor 2, [dx.doi.org/10.21082/jtidp.v8n2.2021.p87-98](https://doi.org/10.21082/jtidp.v8n2.2021.p87-98) .
- [5] Riza Ibnu Fajar, Luh Putu Wrasati, Lutfi Suhendra, 2018 ,The Content Of The Flavonoid Compound And Antioxidan Activity Of Green Tea Extract In The Treatment Temperature And Time Brewing, *Journal of Agroindustrial Engineering and Management* , Volume 6, No.3, p196-202.



Physicochemical Characteristics of Catfish (*Clarias sp*) Sausage with Addition of Moringa Leaf Flour (*Moringa oleifera*)

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Abstract. Sausage is a processed food product that is generally made of meat, fat, filler, water, binder and salt. Catfish is a source of animal protein that has advantages compared to other animal products, namely catfish which is rich in lysine, and leucine. Moringa leaf flour is Moringa leaves that are given drying, milling treatment to facilitate processing into various food products and as a fortification material with high nutrition. This study aims to determine the effect of differences in concentration of catfish: tapioca flour with the addition of Moringa leaf flour on the physicochemical properties of catfish sausage. The results obtained in the study that the difference in the concentration of catfish: tapioca flour and the addition of Moringa leaf flour affect the analysis of water content 58%; fat content 13%; ash content 1.85%; protein content 18.09%; color parameter L* 47,26-66,48; color parameter a* (-4.43)-5.40; color parameter b* 14.25-26.71.

Keywords: *Catfish, Moringa Leaf Flour, Sausage, Tapioca Flour*

1. Introduction

Indonesia is a country that has various nutritional problems. Lack of Protein Energy is due to the low level of energy and protein consumption in the daily diet, furthermore it is not sufficient for the recommended needs [2]. The prevention of PEM is to increase the consumption of foods that are high in vitamins and protein found in plant and animal food sources [8]. Catfish is a source of animal protein that has advantages compared to other animal products, namely catfish which is rich in lysine and leucine. Sources of animal protein are found in catfish and sources of vegetable protein can be found in fruits and vegetables [10]. Moringa leaves are one type of vegetable as a source of vegetable protein produced from the Moringa plant. Moringa leaves have been widely researched on their usefulness and nutritional content of Moringa leaves. Moringa leaves are known to be rich in nutrients contained in them, namely protein, iron, phosphorus, calcium, potassium, zinc, vitamins A, B, C, D, E, K, biotin, and folic acid [12]. Sausage is one of the processed foods that are widely known and favored by the public. Sausages in Indonesia are readily available, made from processed meats such as chicken and beef. Sausage is a processed product of mashed meat mixed with herbs and spices, then put in a wrapper or casing. Fish sausage is an innovation that is processed from fish meat that is right for consumption



because fish sausage is a food of high nutritional value and a type of ready-to-eat food. This study aims to determine the effect of differences in concentration of catfish: tapioca flour with the addition of Moringa leaf flour on the physicochemical properties of catfish sausage.

2. Materials and Methods

2.1. Material

The ingredients for the manufacturing process are catfish obtained from the Rejomulyo fish market in Semarang with the criteria of 4 months of age for catfish, fine pepper, tapioca flour, Sodium Tripolyphosphate (STPP), granulated sugar, Moringa leaves, ginger, nutmeg, skim milk, garlic, cooking oil, fine salt, ice water. The analytical materials used were concentrated H₂SO₄, NaOH, 0.1 N HCl, aquadest, methyl red, Kjeldhal tablets, and Hexan.

2.2. Method

The design used in this study used a completely randomized design method. This study uses a factorial design method with 2 factors. The factor I was the concentration of Moringa Leaf Flour (0%, 1%, 2%, 3 %). Factor II is the concentration of Catfish Meat: the concentration of Tapioca Flour (70%:30%, 75%:25%, 80%:20%) used.

2.3. Making Moringa Leaf Flour

To make Moringa leaf flour, first, Moringa leaves are sorted to get fresh Moringa leaves that are still green and separated from the twigs. Washing is done using running water to remove dirt on the surface of the leaves that can damage the quality of Moringa leaf flour. Drain the Moringa leaves after washing to speed up the drying process. Drying of Moringa leaves using a cabinet dryer for 4 hours at 50 °C and grinding of dried Moringa leaves using a blender which is then sifted using a 60 mesh sieve to produce fine Moringa leaf powder according to flour standards.

2.4. Sausage Making

Making a catfish sausage with the addition of Moringa leaf flour, the first is the preparation of catfish. Wash the catfish, then wash the spines and skin of the catfish to get the catfish meat. Grinding catfish with ice for 10 minutes and followed by mixing catfish with additional ingredients for 20 minutes and ice water to prevent the dough temperature from being low during grinding so that the stability of the emulsion can be maintained. Printing is done by packaging using a plastic sleeve. Boiling is carried out according to the procedure with a time of 20 minutes.

2.5. Physicochemical Characteristic Test

The observed physio-chemical characteristics of crackers were water content, ash content, protein content based on the method [1]. Color test chromatometer [7].

2.6. Data analysis

Data analysis was carried out using univariate analysis, if a difference was found, it would be continued with the DMRT test with a 95% confidence level. Data analysis was carried out using SPSS version 21 software.

3. Results and Discussion

3.1. Color Analysis (L, a* and b*)*

The results of color analysis showed that there was a significant difference ($p < 0.05$) between the concentration of catfish meat: tapioca flour and moringa leaf flour on the values of L*, a* and b* of catfish meatballs. Color analysis results are presented in Table 1.

Table 1. Catfish Sausage Color Analysis Results

Sample/ sample	Color Value		
	L*	a*	b*
X1Y1	65.20±1.46 ^{abD}	5.16±0.09 ^{bD}	14.25±0.05 ^{abA}
X1Y2	65.73±0.27 ^{bD}	5.4±0.16 ^{aD}	15.59±0.50 ^{bA}
X1Y3	66.48±0.86 ^{aD}	4.87±0.04 ^{cD}	14.46±0.05 ^{aA}
X2Y1	57.41±0.53 ^{abC}	-1.78±0.03 ^{bC}	25.31±0.17 ^{abB}
X2Y2	58.21±0.89 ^{bC}	-1.95±0.44 ^{aC}	24.86±0.81 ^{bB}
X2Y3	57.43±1.52 ^{aC}	-1.72±0.45 ^{cC}	25.03±0.64 ^{abB}
X3Y1	52.17±0.80 ^{abB}	-2.76±0.18 ^{bB}	26.50±0.21 ^{abBC}
X3Y2	55.50±2.16 ^{bB}	-3.1±0.14 ^{aB}	25.76±0.55 ^{bBC}
X3Y3	47.99±1.12 ^{abB}	-1.33±0.10 ^{cB}	24.53±0.40 ^{abc}
X4Y1	47.61±0.58 ^{abA}	-3.30±0.13 ^{bA}	25.38±0.58 ^{abC}
X4Y2	47.41±0.80 ^{bA}	-4.43±0.19 ^{aA}	26.71±0.35 ^{bC}
X4Y3	47.26±0.43 ^{aA}	-2.39±0.75 ^{cA}	25.82±0.28 ^{aC}

Color is a component that has a very important role in determining the acceptability and quality of food ingredients. The degree of color parameter L* represents brightness with a value of 0 indicating black and a value of 100 producing white. The value of L* color in catfish sausage in the control treatment or without the addition of Moringa leaf flour resulted in the highest value compared to catfish sausage which was given the addition of Moringa leaf flour. This is because the process of boiling catfish sausage with the addition of Moringa leaf flour can cause the green color of the chlorophyll of Moringa leaf flour to turn darker than before boiling sausages [6].

The degree of color value a* represents the chromaticity of the mixture of red and green. The color value +a* (positive) has a value scale of 0 to +100 indicating red color and the color value -a* (negative) has a value scale from 0 to (-80) indicating green color. The color parameter a* with the addition of Moringa leaf flour produces a greenish color. The use of Moringa leaf flour as an addition to the catfish sausage dough causes a decrease in the value of the -a* color to greenish. This is due to the damage to chlorophyll in Moringa leaf flour during the boiling process of catfish sausage [11].

The degree of color value b* represents the chromaticity of the blue-yellow color mixture. The color value +b* (positive) has a value scale of 0 to +70 indicating yellow color and the color value -b* (negative) has a value scale of 0 to (-70) indicating blue color. The color parameter b* with the addition of Moringa leaf flour produces yellow and blue colors. The use of Moringa leaf flour as an addition to the catfish sausage dough produces a yellowish color which can be seen from the graph of the +b* color analysis to greenish. The results of the analysis in the control treatment resulted in a lower b* color value compared to sausages that were given the addition of Moringa leaf flour. There is a change in color after adding Moringa leaf flour and heating during boiling which causes damage to chlorophyll [11].

3.2. Water content

The results of the water content analysis showed that there was a significant difference ($p < 0.05$) between the concentrations of catfish meat: tapioca flour and Moringa leaf flour on the water content of catfish sausage. The results of the analysis of water content are presented in Figure 1.

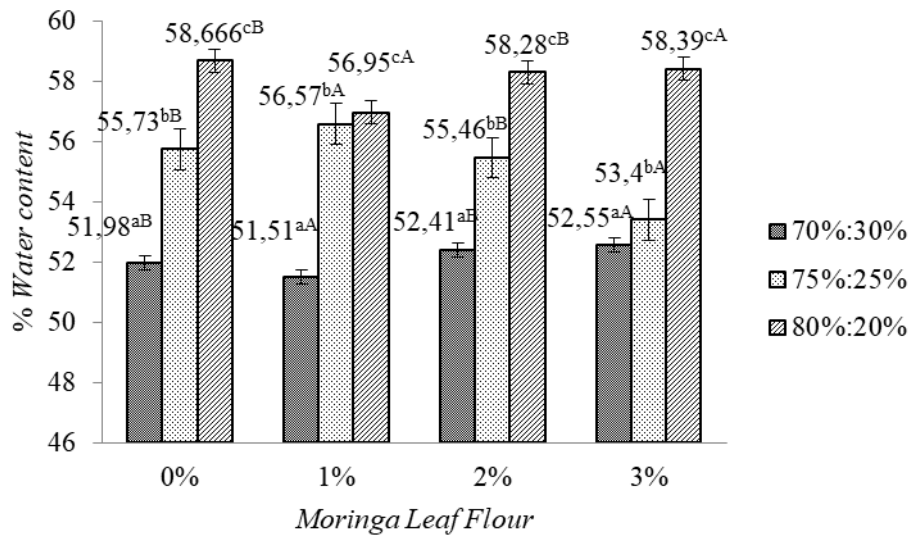


Figure 1. Water Content

Moisture content is a very important parameter in fish sausage products, because it tends to spoil quickly. Based on the quality requirements of SNI (Indonesian National Standard), the water content of fish sausage is a maximum of 68%. The results of the water content of the catfish sausage sample in (Figure 1) obtained the results of the water content of catfish sausage with a value of 51%-58%. Differences in concentration of catfish and tapioca flour affect the water content of sausages. This is because the higher the concentration of catfish used in making sausages, the higher the ability to bind water. The results of the higher water content with the lower concentration of tapioca flour used [3] .

3.3. Ash Content

The results of the ash content analysis showed that there was a significant difference ($p < 0.05$) between the concentrations of catfish meat: tapioca flour and Moringa leaf flour on the water content of catfish sausage. The results of the analysis of water content are presented in Figure 2.

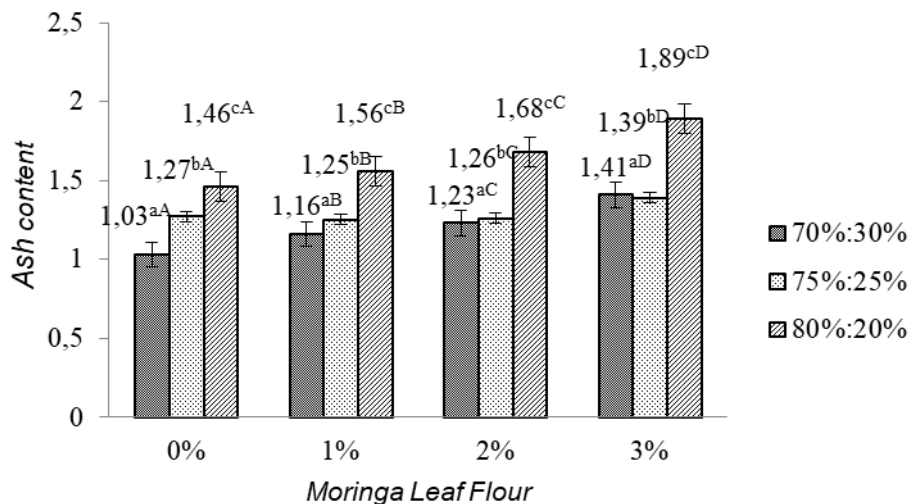


Figure 2. Ash Content

Ash content or inorganic substances known as mineral elements, caused in the combustion process. The combustion process will burn out organic materials and the remaining unburned inorganic materials

are called ash. ash content of catfish sausage with the same letter notation showed results that were not significantly different at 0.05 significance using the Univariate Test with Duncan. Based on SNI quality requirements, the maximum ash content of fish sausage is 2.5 %. The results of the analysis of the ash content of catfish sausage with the addition of Moringa leaf flour (Figure 2) obtained the results of the ash content of the catfish sausage sample with a percentage of 1.03%-1.85%. According to [5] , ash content in food products is an negative substance produced from the residue of the combustion process of organic matter in food products. The higher the concentration of fish used, the higher the ash content. The addition of Moringa leaf flour in sausage dough is higher, the higher the ash content produced in catfish sausage.

3.4. Protein Content

The results of the analysis of protein content showed that there was a significant difference ($p < 0.05$) between the concentrations of catfish meat: tapioca flour and Moringa leaf flour on the water content of catfish sausage. The results of the analysis of water content are presented in Figure 3.

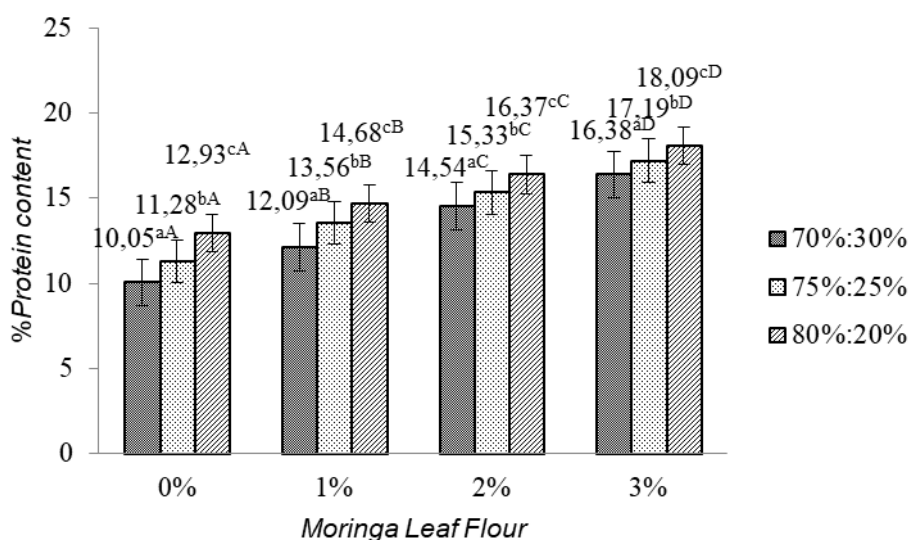


Figure 3. Protein Content

Content of catfish sausage with the same letter notation showed results that were not significantly different at 0.05 significance using the Univariate Test with Duncan. Protein is a substance contained in food that is very important for the body. Protein is a complex compound consisting of amino acids bound by peptide bonds with elements of Hydrogen (H), Oxygen (O), Carbon ©, Nitrogen (N). Proteins function as regulatory substances and building blocks. Based on SNI quality requirements, the protein content of fish sausage is at least 9,0 %. The results of the analysis of the protein content of catfish sausage with the addition of Moringa leaf flour (Figure 3) showed the protein content of the catfish sausage sample with a value of 10.05%-18.09%. The protein content of catfish sausage with the addition of Moringa leaf flour, the higher the concentration of catfish and Moringa leaf flour used in making catfish sausage with the addition of Moringa leaf flour, the higher the value of the protein content of the sausage, it is the same as low and high protein as seen from The high water content in the product is lost from the ingredients. The higher the value of the protein produced if the water loss is high [9] .

4. Conclusions

Differences in concentration of catfish meat: tapioca flour and Moringa leaf flour have an effect on the physical and chemical properties of catfish sausage. The chemical properties of catfish sausage increased the results of the analysis in the treatment of 80% catfish and 20% tapioca flour with the best results being 58.66% water content, 1.85% ash content, 18.09% protein content. The physical properties

of L* color with the best results were 66.48 in the treatment of 80% catfish and 20% tapioca flour. Treatment of 75% catfish and 25% tapioca flour on color a* 5,40 and color b* 26,71. Chemical properties obtained the best results at a concentration of 3% Moringa leaf flour on water content , fat content, carbohydrates and ash content and protein content. Physical properties obtained the best results at 0% concentration of Moringa leaf flour (Color L* 66,48 % , Color a* 5,4%.

References

- [1] AOAC. *Official Methods of Analysis*, 2013
- [2] Adriani, "Introduction to Community Nutrition" , Prenada Media Group, 2012
- [3] D.P. Dewi, "Moringa Leaf Flour Substitution (Moringa Oleifera L.) in Cookies on Physical Properties, Organoleptic Properties, Proximate Content, and Fe Content". *Indonesian Nutrition Sciences*, 2018, 1 (2), p. 104.
- [4] P.S. Eko. Purwiyato, S. (2010). *Food Techno & Agroindustry* .
- [5] Hasniar, Et Al. (2019). Nutritional Content Analysis And Organoleptic Testing On Tempe Meatball With The Addition Of Moringa (Moringa Oleifera) Leaf. *Journal Of Agricultural Technology Education* , 5 , 197.
- [6] Hastuti, S., Suryawati, S., & Maflahah, I. (2016). Sensory Testing Of Moringa Leaf Fortified Chicken Nuggets. *Agrointek* , 9 (1), 71. <https://doi.org/10.21107/Agrointek.V9i1.2126>
- [7] Hutching, Jb (1999). *Food Color And Appearance* . Inc., Maryland.
- [8] Imandira Pan, Fa (2013). The Effect Of Substitution Of Dumbo Catfish Meat (Clarias Gariepinus) And Yellow Sweet Flour (Ipomoea Batatas L.) On Nutritional Content And Acceptance Of High Protein And -Carotene Building Businesses. *Journal Of Nutrition College* , Volume 2 .
- [9] Pratama, R., Rostini, I., & Liviawaty, E. (2014). Characteristics Of Biscuits With Addition Of Jangilus Fish Bone Flour (Istiophorus Sp.). *Indonesian Journal Of Aquatics* , 5 (1), 245040.
- [10] Rustaman. (2015). *Favorite Catfish* .
- [11] Setiaboma, W., Desnilasari, D., Iwansyah, Ac, Putri, Dp, Agustina, W., Sholichah, E., & Herminiati, A. (2021). *Chemical Characterization And Organoleptic Tests Of Manyung Fish (Arius Thalassinus , Ruppell) With The Addition Of Fresh And Steamed Moringa Oleiferea (Moringa Oleiferea Lam) Leaves* . 12 (1), 9–18.
- [12] Syarifah, Ramadhan, Tegar And Yani, M. (2015). The Nutritional Content And Functional Properties Of The Moringa (Moringa Oleifera) Plant. *Urban Agriculture Bulletin* , 5 .

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