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The Identification of Local Upland Rice from Central Sulawesi Using PCR Technique and Chemical Properties

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Abstract: **Restu Dwi Nugroho, Rostiati Dg Rahmatu, Nur Edy (Identification of Local Upland Rice from Central Sulawesi Using PCR Technique and Physicochemical Properties. (Supervised by Rostiati, Nur Edy) Rice is a staple food in Indonesia and one of the crops that provides 20% of the total food calories globally. National rice production has so far been focused on irrigated rice fields, especially on the island of Java, while the contribution of upland or rainfed rice, which is scattered across various islands in Indonesia, remains very limited. This could pose a problem in the future due to the rapid population growth, increased food demand, especially for rice, and the reduction of rice fields, which have been the primary land for rice production. Although national production of upland rice is relatively small compared to irrigated rice, upland rice has great potential to be developed, particularly in drylands. Efforts to promote the development of upland rice in Central Sulawesi include optimizing high-quality planting materials or seeds. One approach to identifying the diversity of upland rice in Central Sulawesi is through the identification of its physicochemical and genetic properties. Physicochemical analysis was carried out to assess antioxidants, moisture content, fat content, amylose, amylopectin, carbohydrate content, protein content, and fiber content. Genetic identification was performed to determine genetic diversity. Genetic diversity identification was conducted using PCR sequencing. The results of the study showed that the phytochemical analysis through various tests demonstrated superior chemical properties. In the DNA analysis, DNA amplification was conducted to observe DNA bands using the rbcLa-F and Rbcl-724R primers, which produced distinct bands in the five local upland rice cultivars from Central Sulawesi, yielding amplicons ranging in size from 100 to 1000bp.**

Keywords: **Genetic Diversity, Physicochemical, PCR, Local Upland Rice, Sequencing.**

I. INTRODUCTION

Rice is a plant belonging to the Poaceae (Gramineae) family and serves as a staple food in Indonesia, contributing 20% of the total food calories worldwide. Based on the type of land, rice is categorized into paddy rice, upland rice (gogo), and swamp rice (Simarmata & Rustikawati, 2015). So far, national rice production has focused on irrigated paddy fields, primarily on the island of Java, while the contribution from upland rice remains limited. This could become an issue in the future due to increasing food demand and decreasing paddy fields. Although upland rice production is small compared to paddy rice, upland rice has great potential for development in drylands (Simarmata & Rustikawati, 2015).

Upland rice has long been cultivated by farmers in Central Sulawesi, yet it has received little attention in agricultural development programs, resulting in low production. In 2022, the area of upland rice in Central Sulawesi was approximately 13,420 hectares with a production target of 39,328 tons and a productivity rate of 29.31 quintals per hectare. To support upland rice development, the use of high-quality seeds is essential. Currently, upland rice cultivation technology is lagging, as the seeds used are generally traditional cultivars that farmers have continuously regenerated (Simarmata & Rustikawati, 2015).

An approach to identifying the diversity of upland rice in Central Sulawesi involves identifying genetic diversity and physicochemical properties using molecular markers.

According to Andarini and Nugroho (2023), one widely used molecular marker for studying genetic diversity in rice plants is the microsatellite marker or Inter Simple Sequence Repeats. This method is advantageous due to its codominant nature, high abundance in plant genomes, high reproducibility, high polymorphism, and ease of scoring, and it is PCR-based (Salgotra et al., 2015; Vieira et al., 2016).

The identification of physicochemical characteristics is conducted through analyses of antioxidants, moisture content, fat content, ash content, carbohydrate content, protein content, and crude fiber content. Accurate identification of both genetic and physicochemical properties is expected to reveal the best diversity of upland rice and support optimal cultivation in Central Sulawesi. With the growing use of upland rice in the food industry, there is an opportunity for five types of upland rice from Central Sulawesi to become high-value food commodities. To support the use of Central Sulawesi's upland rice, it is essential to characterize the chemical properties of these five local varieties.a

II. METHODOLOGY

This research took place in Tanambulava District, Central Sulawesi and at the Seed Science and Technology Laboratory, Faculty of Agriculture, Tadulako University, Palu. This research was carried out from June 2023 to August 2023. The tools that will be used in this research are meters, label paper, ruler, digital camera, GPS type Montana 650, Android (auto distance), stationery, machetes, sacks, and the materials that will be used are Moringa seeds, sand, and Aquades. The research was carried out in two stages, namely, three stages, namely the first stage of morphological identification, the second stage of viability testing in the nursery, and the viability test in the nursery.

Type of research, the research is an experimental study with a quantitative descriptive approach. Quantitative descriptive analysis is conducted through direct experiments on the research objects, namely five upland rice cultivars from Central Sulawesi, to determine their chemical properties.

Chemical Analysis: Antioxidant The antioxidant activity test was conducted using the DPPH method (Mu'awwanah & Ulfah, 2015). Crude extracts of upland rice were dissolved in analytical-grade methanol to obtain concentrations of 0, 100, 200, 400, 600, and 800 ppm. Synthetic antioxidant BHT was used as the control (0, 2, 4, 6, and 8 ppm). The DPPH solution was prepared by dissolving DPPH crystals in analytical-grade methanol at a concentration of 1 mM. The preparation of the 1 mM DPPH solution was conducted at low temperatures and protected from sunlight. A volume of 4.50 ml of each extract solution and the BHT antioxidant solution was reacted with 500 µl of the 1 mM DPPH solution in separate test tubes. The reaction was carried out at 37°C for 30 minutes, after which absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 517 nm. The absorbance of the blank solution was measured to calculate the percent inhibition. The blank solution was prepared by reacting 4.50 ml of methanol solvent with 500 µl of the 1 mM DPPH solution in a test tube. Antioxidant activity is expressed as percent inhibition, calculated using the following formula:

$$\%\ \text{inhibisi} = \frac{\text{absorbansi blanko} - \text{absorbansi sampel}}{\text{Absorbansi blanko}}\ X\ 100\%$$

The sample concentration and its percent inhibition were plotted on the x and y axes, respectively, in a linear regression equation. This equation was used to determine the IC50 (50% inhibitor concentration) value for each sample, with a y-value of 50, and the resulting x-value being recorded as the IC50. The IC50 value indicates the concentration of the sample solution (either extract or BHT) required to reduce DPPH free radicals by 50%.

The sample was weighed in a dish at 4 grams, recorded as the weight of the material in the dish, and placed in an oven at 100°C for 6 hours. The sample was then placed in a desiccator for 15 minutes, and the dish with the sample was weighed again to obtain the final weight of the sample. The moisture content was calculated using the following formula:

$$Moisture\ Content\ (\%)\ \frac{Initial\ Mass - Final\ Mass}{Sample\ Mass} \text{x } 100$$

Fat Content Analysis (AOAC, 2005)

The fat flask to be used was dried in an oven at 100°C for 24 hours, then placed in a desiccator for 15 minutes. The weight of the dried fat flask was recorded. A sample weighing 1 gram was placed in filter paper, weighed, and tied with fat-free wool thread. The fat solvent was added to the fat flask, and the sample assembly was placed in a Soxhlet extraction apparatus.

The sample was heated for 5 hours until the solvent returning to the fat flask appeared clear. The solvent was then distilled, and the fat flask was removed and dried in an oven at 100°C. The fat flask containing the sample was cooled in a desiccator for 15 minutes, and the final weight of the fat flask was recorded. The weight of the oil was determined as the difference between the initial and final weights of the flask.

$$\text{Fat Content } (\%) = \frac{\text{Fat Weigh}}{Sample\ Weight}\ x\ 100\%$$

Amylose Content Analysis (Juliano, 1971)

The amylose content of the material was measured using the IRRI method. A 100 mg sample of flour was placed in a test tube, then 1 ml of 95% ethanol and 9 ml of 1 N NaOH were added. The mixture was heated in boiling water for approximately 10 minutes to form a gel, which was then transferred entirely into a 100 mL volumetric flask, shaken, and filled to the mark with water. A 5 mL aliquot of this solution was pipetted into a separate 100 mL volumetric flask. Then, 1 ml of 1 N acetic acid and 2 ml of iodine solution were added, and the solution was filled to the mark with water, shaken, and allowed to stand for 20 minutes. The color intensity was then measured with a spectrophotometer at a wavelength of 625 nm. The amylose content in the sample was calculated using an amylose standard curve.

Amylopectin Content Analysis (Forsyth et al., 2002)

Amylose and amylopectin were separated by mixing 1 M KOH with the flour at 2°C until dissolved. A 2 mL aliquot of the solution was passed through a Sepharose CL-2B column and eluted with water. Fractions were collected every 40 minutes (starch presence was confirmed with Lugol's solution and observed with a spectrophotometer at a wavelength of 500 nm). The fraction from the first peak contained amylopectin, which was then dried and weighed.

Carbohydrate Content Analysis (Nurmalasari, 2023)

The carbohydrate content analysis was conducted as follows: pipette 2 ml of the sample and place it in a hydrolysis beaker. Add 200 ml of water and 3–4 drops of antifoam, then hydrolyze overnight for 3 hours (timed after boiling). The sample was cooled and neutralized with a 30% NaOH solution. Add 3 drops of 3% CH_3COOH, transfer the solution to a 500 ml volumetric flask up to the mark, and homogenize it. Pipette 10 ml of the filtrate into a 250 ml Erlenmeyer flask, add 15 ml of distilled water and 25 ml of Luff Schoorl’s solution, then heat and allow to stand for 10 minutes before cooling. Then, add 25 ml of 6 N H_2SO_4, followed by 25 ml of KI until the solution turns a yellow-brown color. Next, titrate with sodium thiosulfate, adding 5 ml of 1% starch midway through titration. Continue titration until the color changes from purple to milky white. The carbohydrate content was calculated based on this method (SNI 01-2891-1992).

The data analysis technique used in this research is quantitative descriptive analysis. The sample contents that have been determined will be read and presented in comparative diagrams.

III. DISCUSSION

1. Moisture Content

Based on the moisture content test results for the five upland rice cultivars, as shown in Figure 1, the Paebohe cultivar has the highest moisture content at 9.88%, followed by the Menso cultivar at 9.50%, Puyutas at 9.42%, Pulu Konta at 9.22%, and the lowest moisture content is found in the Kalendeng cultivar at 9.14%.

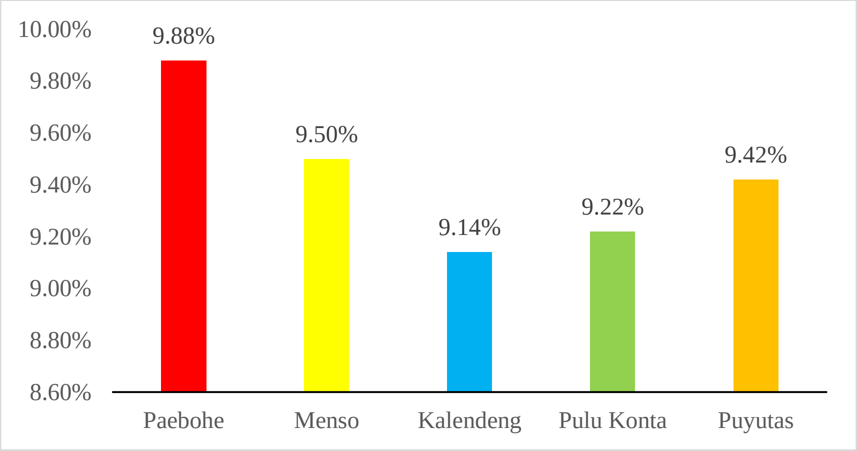

Figure 1. Average Moisture Content in Various Upland Rice Cultivars

Based on the results from the chemical analysis of moisture content, the highest moisture content was observed in the Paebohe cultivar at 9.88%, while the lowest was in Kalendeng at 9.14%. This is because the Paebohe upland rice cultivar has several advantages, making it an attractive choice for farmers, especially in dry land areas. Some key advantages of this cultivar include:

Drought Tolerance: Paebohe rice is known for its ability to grow in upland (dryland) areas that do not require intensive irrigation. According to Sharp (2002), studies indicate that drought resistance is associated with increased proline content, which plays a crucial role in maintaining root growth under low water osmotic potential.

Osmotic Adjustment: Similarly, Hamim et al. (1996) and Naiola (1996) reported that lowering osmotic potential is an effective plant response to drought stress. By accumulating dissolved compounds for osmotic adjustment, plant cells are able to maintain their turgor pressure.

Environmental Sustainability: According to Winarno (2004), Paebohe upland rice supports sustainable agriculture because it requires minimal water and chemical inputs, making it environmentally friendly.These benefits make Paebohe an effective choice to enhance food security in areas with challenging land and climate conditions.

2. Ash Content

Based on the ash content test results, the Pulu Konta cultivar has the highest ash content at 1.07%, followed by Menso at 0.95%. Paebohe and Kalendeng each have an ash content of 0.88%, while Puyutas has the lowest ash content at 0.74%, as shown in Figure 2.

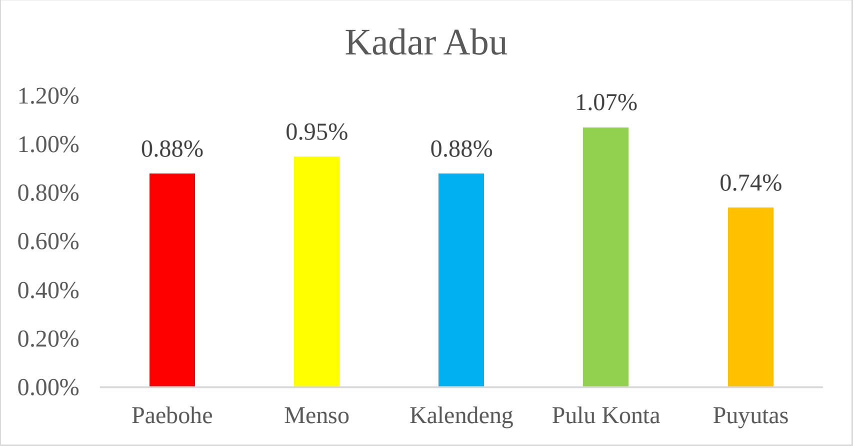

Figure 2. Average Ash Content in Various Upland Rice Cultivars

The chemical analysis results show that the Pulu Konta cultivar has the highest ash content. This higher ash content in upland rice varieties is attributed to genetic factors, soil conditions, and cultivation techniques, all of which contribute to the differences in ash content among upland rice varieties. Research indicates that ash content in rice is influenced by the soil's mineral composition, fertilizer application, and the rice variety's adaptation to environmental conditions. In upland rice, which grows in dryland with limited water access, plants often absorb more minerals from the soil to adapt to harsh conditions.

Studies on upland rice varieties show that environmental factors like drought can also increase ash content, as plants actively accumulate minerals such as potassium (K), calcium (Ca), and phosphorus (P) as a form of adaptation. High nitrogen and potassium fertilization, common in cultivation, may also raise ash content (inorganic mineral residue) in plants. The yield of superior rice varieties is determined by genetics, environment, and plant management. The production capacity of current superior rice varieties is not yet optimized due to genetic influence under environmental conditions (Widyastuti et al., 2013).

Rice yield depends on varietal characteristics, environmental conditions, and management practices (Zou, 2011). Superior rice varieties are cultivated in paddy fields and some in dryland. Character information and relationships between characteristics are essential to increase grain yield (Selvaraj C. et al., 2011). Generally, rice is grown in paddy fields, where environmental and management factors greatly impact yields, resulting in varying outcomes (Rohayana & Asnawi, 2012). Crop management contributes up to 70% of production output (Peng, 2008).

3. Carbohydrate Content

Based on the carbohydrate test results, as shown in Figure 3, the Puyutas cultivar has the highest carbohydrate content at 82.53%, followed by Paebohe at 80.65%, Pulu Konta at 79.66%, Kalendeng at 78.78%, and the lowest in the Menso cultivar at 78.65%.

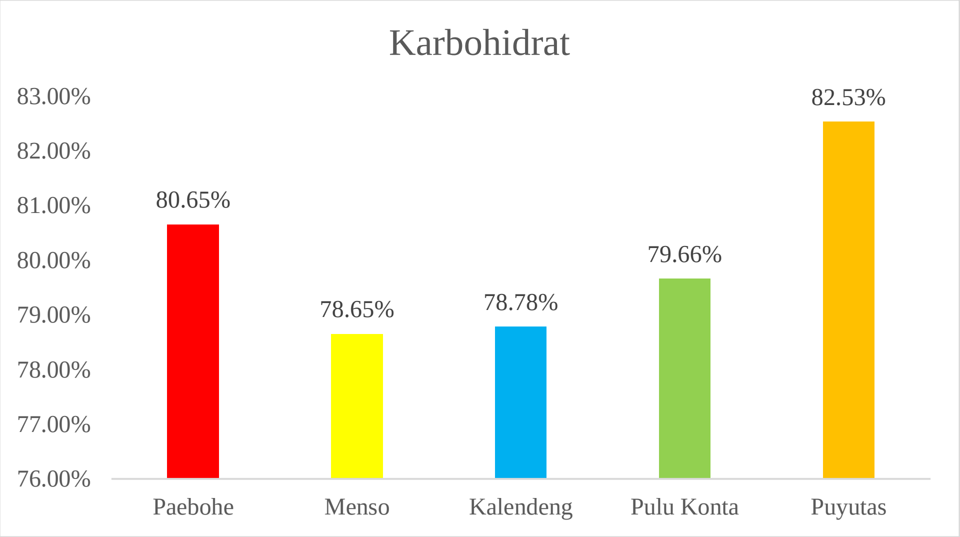

Figure 3. Average Carbohydrate Content in Various Upland Rice Cultivars

The carbohydrate testing results indicate that the Puyutas cultivar has the highest carbohydrate content at 82.53%, while the Menso cultivar has the lowest at 78.65%. This difference in carbohydrate content between the Puyutas and Menso cultivars is likely due to a combination of genetic factors, metabolic capability, and environmental conditions that support carbohydrate accumulation in rice grains.

Metabolic System: The Puyutas cultivar may possess more active metabolic enzymes involved in starch synthesis, such as ADP-glucose pyrophosphorylase, which plays a key role in starch formation in rice endosperm. More efficient metabolism contributes to higher carbohydrate levels.

Upland rice varieties also have complex, multifunctional plant cell walls composed of polysaccharides and proteins. The configuration and abundance of these cell wall components influence cell elongation and plant growth (Desmet, 2024).

4. Fat Content

Based on the fat content test results shown in Figure 4, the Pulu Konta cultivar has the highest fat content at 1.18%, followed by Menso at 1.05%, Kalendeng and Puyutas each at 0.85%, and Paebohe with the lowest fat content at 0.65%.

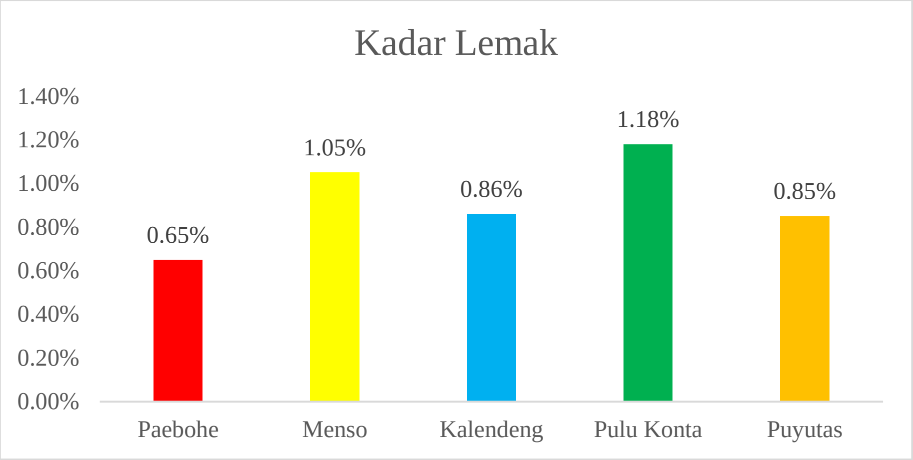

Figure 4. Average Fat Content in Various Upland Rice Cultivars

The chemical analysis results indicate that the Pulu Konta cultivar has the highest fat content, while Paebohe has the lowest. The higher fat content in Pulu Konta rice compared to other cultivars, like Paebohe, may be attributed to several factors: Genetic Factors: Pulu Konta rice may have genetic traits that lead to higher fat accumulation in the rice grains. The fat content in rice is influenced by the genetic composition of the cultivar, which determines how the plant stores energy in the form of lipids (fats). If Pulu Konta has genes that promote or store more fat, this would be reflected in the chemical analysis. Role of Fat in Plants: Fat in rice grains plays a crucial role as an energy source and as a structural component of cell membranes. Cultivars like Pulu Konta with higher fat content may have evolved to store more fat as an energy reserve, potentially aiding in seed development or resistance to specific environmental conditions. Environmental Influence: Environmental factors, such as soil, humidity, water availability, and sunlight exposure, can affect plant metabolism, including how the plant synthesizes and stores fat. If Pulu Konta is grown in conditions that promote higher fat synthesis, this may explain its greater fat content. Cultivation Techniques: In addition to genetic and environmental factors, cultivation practices—such as fertilization, water usage, and harvest methods—can also influence fat content in rice. For instance, if Pulu Konta is cultivated with techniques that promote healthier, more optimal plant growth, this may contribute to its higher fat levels. Impact of Fat Characteristics on Rice Quality: Fat also contributes specific characteristics to the taste, texture, and aroma of rice. Rice with higher fat content, like Pulu Konta, might offer advantages in terms of flavor quality or resistance to oxidation, although these factors need further investigation. For more detailed insights, additional studies could focus on lipid metabolism in Pulu Konta rice or compare its fat content with other cultivars under similar growth conditions (Desmet, 2004).

5. Protein Content

Based on the protein test results for the five cultivars shown in Figure 5, the Kalendeng cultivar has the highest protein content at 10.34%, followed by Menso at 9.85%, Pulu Konta at 8.87%, Paebohe at 7.94%, and the lowest protein content is found in the Puyutas cultivar at 6.45%.

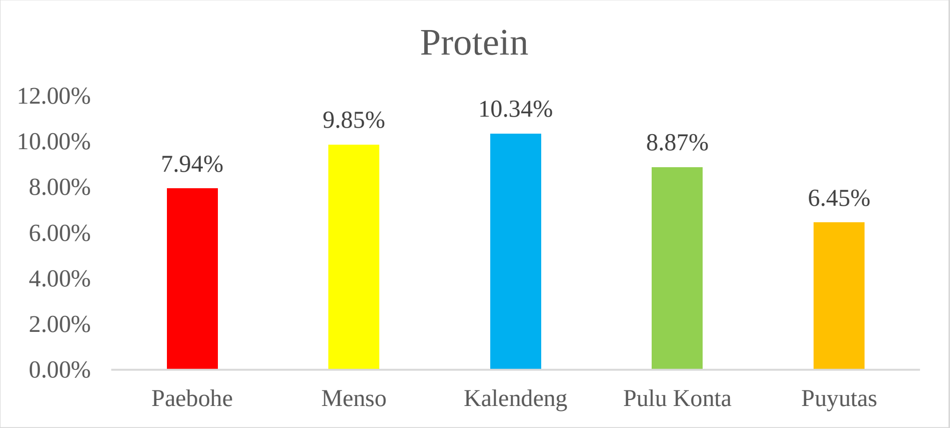

Figure 5. Average Protein Content in Various Upland Rice

Cultivars Research results indicate that the Kalendeng upland rice variety has a higher protein and amylose content. This increase in protein and amylose levels is attributed to genetic factors influencing grain composition and the cultivar's ability to accumulate nutrients. Higher amylose content also suggests that this variety tends to produce firmer rice, suitable for the dry conditions of upland fields. Additionally, Kalendeng may have better metabolic adaptations for nitrogen absorption, which is crucial for protein synthesis. Carbohydrates in rice are primarily composed of starch, which consists of two components: amylose and amylopectin. Rice with a higher amylose content yields a non-sticky, fluffy texture that becomes firm once cooled. In contrast, rice with higher amylopectin content results in a sticky texture that clumps even when cooled (Fitriyah, 2021). Protein is essential for cell formation, repair, and metabolism. Typically, protein, amylose, and lipid content influence rice flavor. Rice with a preferred taste generally has a protein content below 7% and moisture content between 15.5% and 16.5%. Protein content has a negative correlation with rice adhesiveness and a positive correlation with hardness, compactness, and chewiness. High-protein rice requires more water and longer cooking times, resulting in a firmer, less elastic texture when cooked. Correlation analysis among amylose, protein, and fat content indicates that each component affects the taste of cooked rice (Fitriyah, 2021).

VI. CONCLUSION

The highest moisture content is found in the Paebohe cultivar, while the lowest is in Kalendeng. The highest ash and fat content is observed in the Pulu Konta cultivar, and the highest carbohydrate content is in the Puyutas cultivar, which also has high levels of amylose, protein, starch, and antioxidants. The Menso cultivar has the highest amylopectin content, whereas the Kalendeng cultivar has the lowest amylopectin content.

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