

## Cultivation History of Sago Palm (*Metroxylon sagu*) in Leyte, Philippines

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It takes more than 10 years to extract enough amount starch from stem of sago palm (*Metroxylon sagu*). Therefore, intercropping of sago palm and taro (*Colocasia esculenta*) has begun in 2005 in Pangasugan, western part of Leyte, Philippines. Suckers of sago palm were introduced from Dulag, where is one of place sago palm that has distributed along creeks, valleys, and streams of fresh water and marshlands (Quevedo et al., 2005). Sago palm cultivation has continued for a long period in eastern Leyte, based on the results of phytoliths in soils (Baba et al., 2021) and of pollen grains in soils (Okazaki et al., 2021). However, the evidence of sago palm cultivation by phytoliths and pollen grains in Pangasugan was found in the surface soil layer only. While in Julita and Dulag spheroid echinate phytolith and pollen derived from sago palm were observed in subsurface layers, which showed the long cultivation of sago palm.

**Keywords:** cultivation history, intercropping of sago palm and taro, phytolith, pollen

Sago palms (NDP) were transplanted from Dulag to a Pangasugan experimental field, Leyte, Philippines in 2005. The mean height of NDP was  $844 \pm 202$  cm in 2011, which were comparable to  $837 \pm 105$  cm of Indonesian tissue-cultured (ITC) sago palms, which transplanted to Pangasugan in 2007. Consequently, the growth rate of NDP sago palms was less than that of ITC sago palms. Sago palms in Pangasugan were damaged due to typhoon Yolanda on November 8th, 2013. Then, NDP and ITC sago palms recovered to  $1050 \pm 280$  cm and  $781 \pm 90$  cm in 2016, respectively. That is, recovery rate of NDP sago palms after wind damage was faster than that of ITC sago palms.

Starch yield was 8.05 kg D.W. in 9 years after transplanting and increased to 85.7 kg D.W. in 12 years. These results were less than those in Malaysia (Yamamoto, 1998). Even taking into account the impact of Typhoon Yolanda, the accumulation of sago starch in Pangasugan was slower than in Malaysia.

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## Phytolith Assemblages in Spine and Spineless Sago Palm (*Metroxylon sagu* Rottb.) Leaflets and Their Transport into Soil

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The particle size, conical projection spine (CPS) number, CPS vertex angle, length of the bottom of CPS and height of CPS distribution of spheroid echinate phytoliths in the spine and spineless sago palm (*Metroxylon sagu* Rottb.) leaflets from Pangasugan, Leyte, Philippines, were determined under a microscope. These sago palms were transplanted from Dulag area, eastern part of Leyte, in 2005 and their leaflet samples were taken in 2019. The spine sago palm provided the smaller mean particle size, larger number of CPS, larger CPS vertex angle, larger length of the bottom of CPS and smaller height of CPS than those of the spineless sago palm. It is concluded that the spheroid echinate phytoliths in the surface soil were transported from both of the spine and spineless sago palm in the sago palm field of Pangasugan, based on the characteristics of CPS.

The spine and spineless sago palms, which were originally taken and transplanted from Dulag in 2005, were grown in Pangasugan, Leyte, Philippines. The leaflet samples for phytolith analysis were collected from the third leaf from the basal one in 2019. The Surface soil sample (0-16.5 cm in depth) in Pangasugan sago palm field was taken from the intermediate point between spine and spineless sago palm growing site in 2019 and air-dried in Leyte, Philippines. The air-dried soil sample was sieved with a 2 mm mesh sieve and set to Japan to analyze phytoliths and primary minerals. In 2019 the leaflet samples were cut by a knife and scissors, air-dried, dried at 70°C for 24 hours in an oven and ignited at 500°C for 4 hours in an electric furnace. The ashed sample was washed with distilled water and 0.001 mol/L HCl and collected via decantation using distilled water. The residue

was sieved with a 0.045 and 0.250 mm sieve and washed with distilled water. The air-dried surface soil samples (< 2 mm) were treated with 30% hydrogen peroxide solution to remove organic substances. Iron oxides that cover the phytolith surface were removed with a mixture solution (an 8 : 1 volume ratio of sodium citrate and sodium bicarbonate) and sodium dithionite. The soil samples which washed with 0.001 mol/L HCl and distilled water were sieved with a 0.250 and 0.045 mm sieve and stored in plastic bottles (less than 0.045 mm and 0.045mm to 0.250 mm fraction) separately after air-drying. The particles with less than 0.045 mm in diameter and the particles between 0.045 mm and 0.250 mm were mounted on a slide glass using a Matsunami MGK-S embedding agent (polystyrene). The phytoliths (ca. 100 grains) were counted under a polarized transmitted-light microscope (Mt-5000, Meiji Techno) and microscope images were taken by a camera (EOS Kiss X5, Cannon). The particle size of CPS, CPS number, CPS vertex angle, length of the bottom of CPS and height of CPS of spheroid echinate phytoliths in the spine and spineless sago palm leaflets and the surface soil samples in Pangasugan were described.

## Results

Spheroid echinate phytoliths in the spine sago palm are lining up in a row along parallel veins. The spine sago palm produced smaller spheroid echinate phytoliths in diameter ranging from less than 5  $\mu\text{m}$  to 15-20  $\mu\text{m}$  and CPS vertex angles were not sharper than those of spineless sago palm phytoliths, although the number of CPS was similar. The spineless sago palm indicates larger spheroid echinate in diameter, sharper CPS vertex angle, smaller length of the bottom of CPS and larger height of CPS than those of the spine sago palm. Two kinds of spheroid echinate phytoliths from the surface soil layer; large CPS angle (A) and small CPS angle (B) spheroid echinate phytolith with similar diameter.

The spine sago palm gave higher percentages of smaller spheroid echinate phytoliths, 5 to 10  $\mu\text{m}$  (41%) and 10 to 15  $\mu\text{m}$  (37%) in diameter. The spineless sago palm supplied 5 to 10  $\mu\text{m}$  (33%) to 10 to 15  $\mu\text{m}$  (43%) in diameter, suggesting that the phytoliths in spine sago palm leaflets was slightly larger than those in spineless sago palm leaflets. The size frequency percentages of spheroid echinate phytoliths in the surface soil layer (0-16.5 cm) were found to be higher percentages of 5 to 10  $\mu\text{m}$  (44%) and 15 to 20  $\mu\text{m}$  (10%) in diameter. The CPS angle, length of the bottom of CPS and height of CPS in the spine and spineless sago palm and the surface soil layer were clearly shown. The mean vertex angle of CPS was  $131.8 \pm 12.5^\circ$  for the spine sago palm,  $85.1 \pm 10.9^\circ$  for the spineless sago palm and  $107.3 \pm 10.9^\circ$  for surface soil layer. The mean length of the bottom of CPS was  $2.0 \pm 0.3 \mu\text{m}$  for the spine sago palm,  $2.5 \pm 0.6 \mu\text{m}$  for the spineless sago palm and  $3.3 \pm 0.6 \mu\text{m}$  for surface soil layer, respectively. The mean height of CPS was  $0.4 \pm 0.1 \mu\text{m}$  for spine sago palm,  $1.4 \pm 0.4 \mu\text{m}$  for spineless sago palm and  $1.2 \pm 0.3 \mu\text{m}$ . Two contributors of sago palm phytoliths from the spine and spineless sago palms to the phytoliths in the surface soil layer determined the characteristics of phytolith distribution in the surface soil layer. The transportation of sago palm phytoliths through the pores and channels in the surface soil layer to the subsurface soil layers might provide higher frequency percentages in <5  $\mu\text{m}$  and 5 to 10  $\mu\text{m}$  fraction than those of the spine and spineless sago palm and lower frequency percentages in 10 to 15  $\mu\text{m}$  and more than 20  $\mu\text{m}$  fraction except for 15 to 20  $\mu\text{m}$  fraction.

## Spore Density of Arbuscular Mycorrhizal Fungi in Rhizosphere Soil of Sago Palms in Trang and Nakhon Si Thammarat Province in Thailand

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### Introduction

Arbuscular mycorrhizal fungi (AMF; phylum Glomeromycota) can have mutualistic associations with the roots of most terrestrial plants and enhance the plant's nutrient uptake by extending the root absorbing area. They formulate large spores, and their number of spores is used to indicate AMF abundance in the soil. In the case of sago palm (*Metroxylon sago*), Asano et al. (2021) reported that colonization of several types of AMF in the roots was detected. This study investigated the density of AMF spores in sago palms in Trang and Nakhon Si Thammarat Province, Thailand.

### Materials and Methods

The soils (0-15 cm below the ground surface) were collected from the 1 m distance from the three mother palms with three replications in every five different sites, two upland sites beside the water canal in Trang Province (T1 and T2: 7°30.528'N, 99°43.032'E and 7°31.805'N, 99°41.875'E), two waterlogging sites near seashore (N1 and N2: 8°32.337'N, 99°55.888'E and 8°29.362'N, 99°55.362'E), and one upland site (N3: 8°32.465'N, 99°54.933'E). Additionally, we collected soil samples in the oil palm plantation (N4: 8°26.753'N, 99°56.517'E) as a reference. Soil physicochemical properties were analyzed by conventional methods. Rhizosphere soil (1-5 g) was used for the AMF spore extraction using the wet sieving method with 5 min sonication, and AMF spore numbers were counted under a stereo microscope (×40 - 80) on a 90 mm glass plate. Spore density of AMF were calculated on the basis of unit soil volume. Statistical analysis was performed for the spore number by Tukey's honestly significant difference test among the sites.

### Results and Discussion

Soil pH (H<sub>2</sub>O) in sampling sites ranged from 4.6 to 5.6. N1 and N2 showed remarkably higher moisture content (0.76 to 2.19), lower bulk density (0.30 to 0.80 g cm<sup>-3</sup>) with higher organic matter content (5 to 28%), higher EC 1:5 value (0.67 to 2.16) with higher exchangeable K (73 to 362 g m<sup>-3</sup>) and Ca (396 to 1148 g m<sup>-3</sup>) content. N4 showed a higher available P content (Bray II), about 23 to 39 P<sub>2</sub>O<sub>5</sub> g m<sup>-3</sup>, than sago palm fields (1.7 to 23.4 P<sub>2</sub>O<sub>5</sub> g m<sup>-3</sup>). Various structures, colors, and sizes of AMF spores were observed. The spore densities (cm<sup>-3</sup>) were significantly highest in T2 (111), followed by N2, N4, and T1 (23 to 39), and the lowest in N3 (10). The relationships between soil physicochemical properties and spore density were not apparent. However, it was found that the sago palm fields maintained a higher density of AMF spores even in saline, waterlogged conditions in N1 and N2. From these results, it is considered that AMF spores existing in N1 and N2 may tolerate waterlogged and saline conditions.

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## Photosynthesis and Morphological Performance of Sago Palm (*Metroxylon sagu* Rottb.) Seedlings Under Saline Conditions

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A former study on sago palm seedlings exposed to NaCl reported that leaf gas exchange of sago palm decreased by 40% under NaCl stress due to a reduction of stomatal conductance for maintaining water status in the leaves. This study aimed to evaluate the efficiency of light energy utilization for photochemical quenching under salt stress which correlated to the growth performance of sago palm.

## Materials and Methods

This experiment was conducted in a greenhouse at Inagro Inc. Ltd, Bogor West Java, Indonesia, from October to December 2021. Germinated sago seeds obtained from West Papua were grown in 500 ml plastic cups filled with vermiculite. After four-month-old, the spiny sago seedlings were transplanted into a polybag (30 cm x 30 cm in size) filled with zeolite. All the seedlings were placed in trays filled with Kimura-B culture solution. Two months after transplanting, the sago seedlings were exposed to 224 mM NaCl for six weeks. The level of culture solution in the tray was maintained every day by adding Kimura-B culture solution with 224 mM NaCl until it reached the decided level. The culture solution in the trays was replaced once a week. Sago palm morphological traits such as plant height and leaflet area were recorded every two weeks. Leaf greenness (SPAD) and OJIP fluorescence transient were observed after the seedlings were exposed to NaCl for five weeks using a portable fluorometer (Pocket PEA, Hansatech Instruments Ltd., Norfolk, England). After six weeks of NaCl treatment, all sago palm seedlings were harvested to measure the lignin deposition in the roots and dry matter weight.

## Results and Discussion

Six weeks of NaCl treatment did not significantly reduce the plant height. However, the leaflet area and leaf dry matter weight were reduced under salt stress. Salt stress caused faster leaf senescence to the older leaves as the

color turned brown. Salt stress also reduced root growth of sago palm as total root dry weight was low under salt treatment. The leaf greenness (SPAD) of the sago palm was not significantly affected by salt stress. There was also no rise in OJIP fluorescence transient under salt stress. Low values of specific energy flux per reaction center derived from OJIP curves such as ABS/RC, TRo/RC, ETo/RC, and DIo/RC, and higher performance index on absorption basis ( $PI_{abs}$ ) under salt stress indicating leaf photosynthetic efficiency of sago palm was not affected by salt stress. Under salt stress, lignin deposition was found in the root tissue, especially in exocarp and stele.

## Soil Physico-Chemical Properties of Lower Agusan River Riparian Zone Towards Sago-Based Ecobelt Establishment for CCA and DRR

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Riverbank zones and its riparian plants plays an important role in reducing the impact of soil erosion process along river systems. However, many of its vegetation are cleared off as these areas are slowly inhabited by human settlements resulting in the occurrence of riverbank mass failures. This condition can be observed in some areas along the Agusan River of Butuan City. With the advent of climate change and the associated risk it can bring, the City Government of Butuan in cooperation with Caraga State University has proposed the establishment of a narrow man-made strip of Sago palm (*Metroxylon sagu* Rottb.) vegetation known as “ecobelt” that would serve as soil stabilizer of riverbank along the lower Agusan river. In support to this plan, the soil physico-chemical properties of proposed ecobelt sites were evaluated in the laboratory. Three barangays were piloted for this study namely: Pagatpatan, Banza and Mahay. Moreover, assessment of the plant composition and diversity of species within the proposed ecobelts were also made for baseline information. Soil chemical data obtained in this study revealed that not all areas in Brgy. Pagatpatan are suitable for the planting of Sago palm. While the other two barangays (Banza and Mahay) are suitable for the planting of Sago palm.

## Variation in the Consumer Prices of Sago Starch and Other Starch in Jakarta Indonesia

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Sago palm has been recognized as a potential plant resource can be utilized in many occasions for several decades. The starch, from such a potential resource can be purchased at various places nowadays, for example at the local traditional markets, the modern supermarkets or retailers and online shops. Since 2020 the Covid-19 pandemic, the life styles of consumers had been changed all over the world, and here in Jakarta also. People more tend to purchase by online shopping so the goods would be delivered directly from the store to their houses. On the other hand, after May 2022, Covid-19 infection has been started to settle down and that consumer's purchasing style are becoming back to the era of before Covid-19. This report shows the result of the prices of sago starch and similar kinds of starch at various places including retail stores and online stores.

### **The economic growth of EC (Online shopping) in Indonesia**

According to the research of JETRO, the number of Indonesian internet users increase a lot. In 2018 was just 171 million people, but on the middle of 2020, it was 196 million people and the Internet penetration rate increased around 8.9 points from the previous year which was 73.7%. Indonesia's total e-commerce transaction price in 2020 was US\$ 32 billion, which is up to 54% compare to the previous year. As those data shows, Indonesian people's purchase life styles have been changing during Covid-19 pandemic. Not only that, the total EC retail price in 2020 was about US\$ 16.9 billion, with the growth rate of 27.0% compare to the previous year, and the analysts said it would be grow to US\$ 28.4 billion by the time of 2024. It is easy to guess the online purchasing will be more common. On the other hand, the percentage revenue of the EC purchasing is still for only 6.1% of total retail sales, so even this movement shows quiet big amount of money, the impact to the total scale is still small.

### **In comparison with other tropical starch and flours**

The price researching of sago this time is simple, checking the price of sago and resemble starch at the picked up several retail stores from every level (which means the target of consumer's income classes) and the official online stores. Since sago starch is not that common yet, unfortunately sago starch could not be seen in all picked up retail stores, meanwhile Flour was seen at all stores, and the retail store that did not provide tapioca starch (cassava starch) was just 1 retail store.

The result of analyzing sago starch price shows interesting results. In comparison with the major starches that are similar with sago such as flour, tapioca, the sticky rice starch, the price of sago starch places quite high position when the price calculated per 100g. Of course, there were rarer starches such as "the black potato starch" "the banana roots starch" "the red rice starch" "the sweet potato starch" and those placed more expensive price. Nowadays, it is expected that as broadcasted, the Russian-Ukraine war will make some impacts to those starch prices. From these results, we could know that the price of starches has been slightly moving day by day, this kind of market information should be looked with keen interest during those global movements.

## Suggestions for the Use of Indonesian-Made Sago Starch Products in Japan

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Sago starch, which accumulates in the trunks of sago palms, is prepared for use in foods in various unique ways in the countries in which it is produced and is often used as a staple food (Takahashi and Hirao, 1992). Pearl-like products and noodles made from sago starch are common secondary products (Hirao, 2016). In Japan, processed sago starch is used as a dusting flour for noodles. However, raw sago starch is difficult to obtain and its low whiteness is considered a food-production issue in Japan, meaning its usage has been limited until now (Takahashi et al, 1995). However, in an effort to expand the use of sago starch in Japan, we have been clarifying its physicochemical properties and specific cooking and processing characteristics. In this context, at last year's 30th Conference of the Society of Sago Palm Studies (December 4, 2021), Ehara (2022) introduced an Indonesian company's recent sago starch-based secondary products. This company has developed many products that consumers in Indonesia find appealing, and the company's web site introduces the advantages of sago starch, such as its resistant starch content, low GI, antioxidant capacity from polyphenols, and gluten-free properties. Through these activities, the company is expanding the use of sago starch in Indonesia. The sago starch products containing secondary ingredients in this study are characterized by their bright colors, due to sago starch being mixed with many vegetables, potatoes, and fruits. The products also have functional properties and can be promoted to the health conscious. We believe that it is necessary not only to consider and spread the use of sago starch, but also to focus on the spread of secondary products. Using results from property and sensory evaluations, this report discusses and proposes methods for sago starch products developed in Indonesia to be used in Japan.

### **Samples and Methods**

Sago products from Sagolicious (Jakarta, Indonesia), including eight kinds of mie noodles, five kinds of pasta, five kinds of fettuccine, five kinds of lasagna were used in this study. Each product was tested in its plain (sago starch was the main ingredient) state and also with secondary ingredients—vegetables and algae (mustard green (A), moringa (B), spirulina (C)), potatoes (purple yam (D), turmeric (E)), fruits (Papua red fruit (F), dragon fruit (G)), and others. Somen noodles, Chinese noodles, harusame noodles, pasta made from durum semolina, fettuccine, lasagna, and macaroni sold in Japan were used for comparing with the sago products.

Sago noodles were subjected to physical and physicochemical measurements of thickness, changes in water absorption (before immersion in water, after immersion in water, and after boiling), and texture. For the sensory evaluation, a two-point discrimination test and a two-point preference test were conducted on sago, pasta, fettuccine, and lasagna noodles, and respondents were asked to give their opinion on the products freely. The two-point discrimination test included smell, taste, hardness, elasticity (firmness), and chewiness, while the two-



point preference test had an overall evaluation parameter added to the above. Open written responses were requested in the comparison of plain sago products with those having added secondary ingredients. The products were then rated as to their compatibility with soups and sauces on a scale of ◎ (very compatible), ○ (compatible), △ (neutral), and × (not compatible). The panel consisted of 11 to 28 expert panel from Aikoku Gakuen Junior College or faculty members and students from Kyoritsu Women's University.

## Results

(1) Noodles: The thickness of sago noodles with added secondary ingredients was less than that of plain sago noodles, but by boiling after soaking in water, Sample A had a greater rate of change in thickness and had about the same thickness as the plain noodle. Water absorption was higher for B, C, and G than for the plain noodle. Somen, Chinese noodles, and potato harusame noodles have a thickness similar to that of plain sago noodles. They were boiled under the same conditions and compared. Rupture measurements showed that potato harusame noodles had similar values in terms of rupture stress to that of plain sago starch, and although there were many descriptions in the free comments stating that it would be difficult to substitute sago starch flour products for wheat flour products, it was deemed possible to substitute sago starch products with potato harusame noodles. The results also clearly showed that some types of sago noodles kneaded with secondary ingredients were compatible with Japanese noodle soup and Chinese soup in terms of taste and smell.

(2) Pasta: Plain sago starch pasta was rated significantly higher than wheat flour pastas in the sensory evaluation parameters of elasticity (firmness) and chewiness. Sago pasta with added secondary ingredients E and F was evaluated as being a suitable match for meat sauce and carbonara. It was thought that one of the reasons for this was its pasta-like yellowish color.

(3) Fettucine: When comparing plain sago and wheat flour fettucine in a two-point discrimination test, sago fettucine was deemed hard and was not preferred in any item in the two-point preference test. As the core of the fettucine was deemed "powdery" in the free comments, despite the fact that it was soaked in water for 30 minutes before heating, it suggested that the soaking time in water or boiling time was too short. Plain sago was rated as the best for meat sauce, while fettucine with secondary ingredient A was rated as the best for carbonara.

(4) Lasagna: Plain sago lasagna was evaluated as having more smell, taste, and chewiness in the two-point discrimination test as compared to flour lasagna, and no significant difference was found in the two-point preference test except in the parameter of elasticity. In sago lasagna, those with secondary ingredients were preferred as compared to the original, especially secondary ingredients C and E. The plain sago noodle, E, and F were rated as the best matches for meat sauce.

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## Preliminary Study on Micro-Structure of Sago Based Noodle

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Microscopy provides a clue to understanding the relationship between the product's microstructure and functionality of a food material. Scanning Electron Microscopy (SEM) was applied to investigate stereoscopic images of sago based noodle.

### Materials and Methods

Simple extrusion equipment was applied to produce noodle. Three different type of sago noodle were investigated for its micro structure. Sago noodle of two different variant was prepared from native sago starch with or without addition of salt. Noodle specimens of surface and cross section were observed under scanning electron microscope (SEM). Cooking time and proximate composition of noodle was also measured,

### Result and Discussion

Surface and cross section images showed the structural formation of pores with a fine connective network. Salt was added in order to reduce noodle cooking time, Salt addition significantly reduce cooking time by approximately 40%

### Conclusion

Scanning Electronic Microscopy (SEM) analysis confirmed the modifications in noodles microstructure as salt affected sago starch granule structure. The result can support innovation in the further development of sago based noodle as a special food.

## Sago of South Sulawesi Indonesia, from Staple Food to Entertainment and Healthy Food

Dorothea Agnes Rampisela

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Sago in South Sulawesi is almost entirely only left in the Tana Luwu area which is historically under the rule of Luwu Kingdom which is currently covers 4 regencies/city namely Palopo City, Luwu Regency, North Luwu

Regency and East Luwu Regency, each of which borders to the Gulf of Boni. The land area is only 3400 ha left, but this semi-cultured managed sago has high productivity, an average of 500 kg per tree or 25 tons / ha.

Sago is traditionally the main local food and until now in the countryside, almost every meal there must be sago plates such as dange or kapurung. The decreasing land area causes imbalance of supply and demand which trigger the increasing of unit price. The decreasing of land area means limitation of source of seedlings because until now sago breeding still relies on the removal of suckers that grow in the clumps of the parent tree.

The effort to develop sago has experienced many obstacles, so that various strategies have been carried out whose main initiatives are still from sago researchers. Lately, efforts to the awareness of the important function of sago have succeeded motivate the local government, especially North Luwu regency, and have also succeeded in inviting collaboration of central level as well as the private sector. These various efforts and collaboration with Universities will be discussed so that they can become a model for the development of small holders.sago

## Utilization of Sago Bark for Paper Products

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### Introduction

Sago palm is economically valuable crop for producing carbohydrate starch. Processing of sago stems produces approximately 25% of sago flour and about 75% waste (McClatchey et al. 2006). Sago pith fiber (hampas) and sago bark are the solid sago waste, and the bark accounts 17.0% of processed log (Aziin & Rahman 2005). Most of the sago bark are left behind the sago factory and destroyed through open or controlled burning, since leaving the sago bark to naturally degrade is consume time and space. Siruru et al. (2019) has identified the characteristics of sago bark waste; which consisted of 66.8% of holocellulose, 44.0% of cellulose, 41.9% of  $\alpha$ -cellulose, and 22.8% of hemicellulose. Those components are potential to be utilized as a source for paper raw materials. But some undesired components such as lignin and starch should be removed from the biomass to produce sago fiber paper. This research explored the utilization of sago bark as a raw material for paper product, and identified its characteristics.

### Materials and Methods

1. Preparation of sago bark flours
2. Delignification by alkaline hydrothermal

3. Bleaching and pulping by using alkaline hydrogen peroxide
4. Paper making from wet pulp
5. Characterization of paper products

### Results and Discussion

Paper is a material made of pulp, which consists mainly from cellulose. The cellulose can be obtained from fibrous plants, namely from various type of woody plants and non-wood plants. The sago bark as lignocellulose biomass mainly contained crude fiber (37%) and starch (22%). In order to remove the lignin component, alkaline hydrothermal also removed the hot soluble materials such as starch and hemicellulose. Delignification also caused to the conversion of fiber to pure cellulose as wet pulp. The cellulose sago fiber is categorized as long fiber, has a typical dimension with average number 0.656 mm for fiber length and 14.01  $\mu\text{m}$  for fiber diameter.

The paper produced from sago bark fiber have non glossy or rough surface, and can be categorized as art paper with grammage of 85-123  $\text{g/m}^2$ , 0.70-0.77 mm of paper thickness, 0.200-0.279  $\text{kgf/mm}^2$  of the paper tensile strength, 0.14-0.35  $\text{Nm}^2/\text{kg}$  of tear strength. The strength of paper is determined by several factors such as the average length of the fibre, the interfibre bonding ability of the fibre, which is enhanced by delignification and bleaching treatment, and the structure and formation of the sheet. Because paper from sago bark fiber is composed of a randomly felted layer of fibre, the structure has a varying degree of porosity, and caused high ranges of physical properties. The paper has unique natural color, and can be used for decorative paper or artistic paper bag.

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## Short Communication: New Milestone Sago of Indonesia

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Sago is a plant source of carbohydrates. Its existence cannot be excluded from the history of Indonesia as a nation. An image of the sago tree can be found in the reliefs of Borobudur Temple, built in the first century<sup>1</sup>.

Sago is distributed throughout Indonesia's region, and it is especially prevalent in big islands, such as Papua, Sumatra, Kalimantan, Sulawesi, and Java<sup>2</sup>. The sum total of sago in the world is estimated to be around 2,942,278 ha, from which as many as 1,843,278 ha (63%) is from Indonesia<sup>3</sup>. Thus, Indonesia is one of the largest exporters of sago commodities in the global market, exporting about 400,000 tons of sago each year and a total estimated volume ranging from 10,000 to 13,000 tons each year<sup>4</sup>.

The processing of the sago starch as a source of carbohydrates in Indonesia is not varying. The sago starch is commonly used as the main raw ingredient for snacks such as shrimp crisps (kerupuk) and other types of traditional snacks. This is due to the fact that the processing of other sources of carbohydrates, like rice and wheat, is favored more. The people tend to consume rice and derived products of wheat flour (noodles, bread, and cereal). This shifting of the trends in consumption is because the protein, fat, and other nutrition contained in sago is lower than other foodstuffs, hence affecting the flavor of sago. The development of sago starch processing is expected to answer the problems for the increasing demands of food due to the shifting of the people's diet pattern, as a consequence of industrialization, urbanization, globalization, and socioeconomic developments. The shifting of diet may activate dormant degenerative diseases such as diabetes, obesity, cancer, and cardiovascular disease<sup>5</sup>.

Establishing a new perspective regarding sago will have to be done to increase the people's reception toward a food product processed from sago. The development of a sago-based product to increase the economic value and people reception can be done in collaboration between policymakers and industrialists. The National Research and Innovation Agency of Indonesia, the government's research organization, has done several studies on the benefits of sago as a food source. Sago starch is known to have low glycemic index and the potential to be a resistant starch. Several food products from sago starch include analog rice, pasta, noodles, and cookies.

The research and development done on sago starch culminated in processing the ingredient into a food product that is already popular in society, i.e., instant noodles. Instant noodles are a well-received product in both traditional markets and modern markets. The National Research and Innovation Agency of Indonesia worked together with Langit Bumi Lestari, LLC to conduct production and commercialization of sago-based instant noodles. Langit Bumi Lestari, LLC (ASINDO Group) produced the brand "Sagomee", sago-based instant noodles with the benefits of being gluten free and is expected to compete in the instant noodle market in Indonesia.

The sago starch used as the raw ingredient for "Sagomee" is a production of Bangka Asindo Agri, LLC (ASINDO Group), an industry of sago starch and modern tapioca which incorporates zero waste technology. Bangka Asindo Agri, LLC is located in Bangka Island, Indonesia, and processes sago with expert European technology, without food bleaching agents or preservatives. It also boasts a high-technology process, food-grade equipment, hygiene, and a closed system. The interest of Indonesian citizens toward the launching of these sago-based instant noodles. This claim can be supported by the production results of "Sagomee" that increases exponentially each year (LBL data).

A continual innovation on sago-based fusion food is prompted from this example, creating a new variety of snacks such as cookies, brownies, bolu cakes, Surabaya spekkoecks, black oreo swiss rolls, and other dry cakes that can be beneficial as the people's food sources.



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## Evaluation of Critical Control Point in Small-Scale Sago Processing Unit (Case Study in Bogor, West Java)

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### Introduction

Sago has great potential as a source of carbohydrates and as raw material for both food and non-food industries. Sago is processed by both large- and small-scale industries. Small-scale sago starch processing generally uses simple equipment, has no production planning, and is less efficient and hygienic. It causes the resulting quality is quite low. There is no further processing for the wastes produced, which can cause pollution to the environment (Budiman 2016).

One of the small-scale sago starch processing units is located in Tanah Baru, Bogor, West Java. It has been operating for 30 years with 70 tons/year production capacity. One-time production input is 8 tons of sago trunks with an output of 1.5 tons of sago starch. The target consumer of this small-scale sago processing unit is the food industry, such as meatball producers.

The government pays attention to the safety of food industry products by implementing a food safety system rule called Hazard Analysis Critical Control Point (HACCP). In this study, the determination of critical control points in small sago processing industries aims to prevent or eliminate and minimize the impact of physical, chemical, and biological hazards to an acceptable level to produce safe and quality sago starch as a raw material for the food industry.

### Materials and Methods

This research was conducted in the Tanah Baru area on a small-scale sago processing unit in North Bogor, West Java. The activities are carried out in several stages, including:

1. Identification of problems in the small-scale sago starch processing industry
2. Determination of critical control points for sago starch processing based on the Hazard Analysis Critical Control Point (HACCP) principle.
3. Selection of alternative solutions for corrective actions.

### Results and Discussion

#### *Identification of the problems*

Barks of sago stems were cut and stripped using simple equipments, which left a lot of dirt and foreign matter attached to the sago to be shredded. The filtering process produces waste in the form of sago dregs. The wastes were not further processed and accumulated at the waste disposal site. The settling process was carried out for 36 hours in an unhygienic open place. The washing process was carried out once by workers who did not use personal protective equipment, such as footwear, in the washing container. The drying process used the energy

of sunlight in an open space to allow contamination due to dust, dirt, and vehicle fumes, as well as the high-moisture content of the sago produced. The moisture content of processed sago is 18% (maximum 13% based on SNI 01-3729-2008). A sieve with a small mesh size caused the resulting starch in the form of lumps and non-uniform size. Mold analysis showed  $4.0 \times 10^4$  colonies/g material (maximum  $10^3$  colonies/g material based on SNI 01-3729-2008). The processing did not pay attention to the cleanliness of the environment, tools, and workers, causing mold growth in sago.

#### *Determination of the Critical Control Point for Small-Scale Sago Processing*

The critical control point is one of the HACCP principles to determine important parts that must be controlled in the production process to prevent hazards or eliminate and minimize the impact of hazards to an acceptable level (Bakri *et al.* 2016). Critical control points in the processing of sago starch include the processes of settling, washing, drying, and sifting. The length of the settling time process will affect the level of acidity and microbial activity due to spontaneous fermentation. The washing process affects the quality of the sago produced, especially the cleanliness and the presence of foreign matter or dirt. Drying affects the moisture content of sago. The high moisture content will cause sago to clump, bacteria and molds are easy to grow and shorten the shelf life. This sifting can affect the quality of sago starch, especially the presence of dirt and foreign matter, as well as the uniformity of sago size.

#### *Alternative Solution*

Based on the various problems in the small-scale sago processing unit, the following alternative solutions are proposed namely, improving the production schedule with a maximum 8-hour settling process, making a water filter with foam material, making a drying house from polycarbonate material with a capacity of 456 kg, and making a sifter with the driving force of a grinder with a capacity of 600 kg/hour.

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## Microsatellite Marker Analysis of Sago Palm Seedlings from a Spiny Mother Palm and Some Other Spinless and Spiny Adult Palms Growing in the Same Field

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Sago palm is resource plant that adapt well to various problem soils such as swampy or saline and can produce a large amount of starch. It grows hermaphrodite flowers and staminate flowers, but the pollen of the hermaphrodite flowers is considered to be sterile, and its pollination is assumed to be cross-pollination because the hermaphrodite flowers and staminate flowers have different flowering periods. However, the process of seed development is still unclear in genus *Metroxylon*. In this study, we aimed to understand the pollination under natural condition that is thought to regulate seed formation of sago palm. Thus, we applied microsatellite sequencing for some seedlings with their mother palm and also for some other adult palms growing in the same field.

### Materials and Methods

On January 30, 2019, the leaflets were collected from the seedlings of sago palm (*Metroxylon sagu*) and their mother palm with the other adult palms that were considered to have simultaneous flowering period with the seedlings' mother palm grown in the same field in Abelisawa, Konawe, Southeast Sulawesi, Indonesia. The mother palm was folk variety 'Manno' (spiny type), and the other adult palms as candidates for pollen parent were 2 plants of folk variety 'Roe' (spinless type) and 7 plants of 'Rui' (spiny type). *M. vitiense* from Fiji, *M. warbrugii* from Samoa, and *M. salomonense* from the Solomon Islands also were included as related species. The DNA samples extracted from the leaflets using the DNeasy Plant Mini Kit (QIAGEN) were used for PCR with a primer (sv513907) that leads the microsatellite region for a large number of alleles in the previous studies (Kumekawa et al. 2013, Devit et al. 2019). The sequence of the mother palm and pollen parents was analyzed using SeqScanner 2 (Applied Biosystems) and Geneticx Ver.14.

### Results and Discussion

The sequence of CTCTCTC was present following a (CT)14 repeat of the candidates as pollen parent. In the seedlings that have a repeat of (CT)17, there was the sequence of CTCTCT following the repeat of (CT)17. On the other hand, no specific sequence including C was observed following the repeat of (CT)17 in the mother palm. Therefore, it was considered that these seedlings might have been pollinated with the candidates as pollen parent. In several seedlings, a sequence that was not found in the mother palm was shared with the candidates as pollen parent. Consequently, it was clear that sago palm's pollination could be crossed.

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## Sago Palm (*Metroxylon sagu* Rottb.) Enzymes Influencing the Trunking Formation

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Sago palm is a member of the Palmae family, subfamily Calamoideae, tribe Calameae, subtribe Metroxylinae, and genus *Metroxylon* Rottboell, abundantly found in India, Oceania, and Southeast Asia. This palm is an economically significant agricultural species to the people who live in the area where it is planted. The primary product of the sago palm is sago starch, which accumulates in the tree trunk. Sago starch consumption is evident in areas where the palm grows. Therefore, it is critical to identify the enzymes which influence the trunking, hence contributing to the starch production in sago palm. This paper presents the enzymes that are found differentially expressed in the trunking sago palm using the Annealing Control Primer (APC)-based GeneFishing technique, Representational Difference Analysis (RDA) method, and RNA sequencing of the transcriptome. The three approaches revealed enzymes that are differentially expressed in trunking and non-trunking palm. These enzymes are involved in many areas of plant development contributing to the development of the trunk and starch in sago palm.

### Materials and Methods

1. The ACP-based GeneFishing technology was performed through the amplification of cDNA converted from the total RNA extracted from the leaf tissues sampled of mature trunking and non-trunking sago palm using 20 different arbitrary ACPs provided in the GeneFishing DEG Premix Kit (SeeGene, Seoul, Korea). Bands of interest were excised, underwent DNA purification using GF-1 Gel DNA recovery kit (Vivantis, Malaysia) and cloned into a pGEM-T easy cloning vector (Promega, USA). The cloned plasmids were sequenced and BLAST searched for sequence similarity.

2. In the RDA technique using Oligo(dT)18 as a primer, total RNA from trunking and non-trunking sago palm was converted to cDNA. The cDNA samples were digested using the enzyme DpnII (NEB), ligated with the RBgl 12 and RBgl 24 adaptors, and amplification was performed for RDA analysis. The non-trunking cDNA was labelled as a tester, whereas the trunking cDNA was labelled as a driver. The common sequences would be removed, while the sequence of interest would be exponentially expanded. Following amplification, differential cDNA fragments were cloned, and transformed plasmids containing the differential fragments were sequenced and BLAST-analyzed.

3. The transcriptomics analysis method was performed to identify the enzymes involved in trunk formation in sago palm based on the RNA transcriptomes. Three biological replicates each of trunking and non-trunking sago palm leaf tissues were sampled. The total RNA of the samples were purified using an improved CTAB RNA extraction protocol. The RNA samples were sequenced using the BGISEQ-500 platform. RNA transcriptomic sequence is registered under NCBI BioProject PRJNA781491. Gene expression and annotation information are

accessible in the public functional genomics data repository Gene Expression Omnibus (GEO) with accession number GSE189085. Differentially expressed genes (DEGs) analysis was performed using DEseq2.

## Results and Discussion

The ACP-based GeneFishing technology identified differentially expressed transcripts (DETs) trunking sago palm to be matched with ascorbate peroxidase (APX), short-chain dehydrogenase/reductase (SDR), asparagine synthetase C (AsnC), glucosyltransferase (GT) family, O-antigen ligase, beta-glucosidase and glycerol kinase enzymes. The transcripts from trunking showed higher expression of redox-regulating function to reduce the accumulation of reactive oxygen species (ROS) and higher transcript levels of nitrogen utilisation. The enzymes beta-glucosidase and glycerol kinase help in the defence activity of the trunking sago palm (Hussain et al., 2022). Meanwhile, the RDA method found that upregulated genes code for growth development and signalling in neutralising the imbalance of plant stress. The gene product identified in the study; S-adenosylmethionine synthase (AdoMetS), peptidyl-prolyl cis-trans isomerase, and ATPase were found expressed in trunking sago palm. The AdoMetS is an enzyme that catalyses the formation of S-adenosylmethionine (Ado Met/SAM), responsible for normal growth development (Bourjan et al., 1994; Li et al., 2011). The transcriptomic RNA sequencing identified 963 DEGs and out of that 326 were enzymes. After abundance filtering, 181 unique enzymes were identified, of which 80 were upregulated while the other 101 unique enzymes were downregulated in trunking sago palm (Yan et al., 2022). Results of the three different approaches to identify genes and enzymes that are differentially expressed in trunking and non-trunking sago palm showed that these enzymes performed in many different areas of plant development and require plenty of future work in the molecular biology of the sago palm to determine the association of genes for trunking and starch formation.

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## Domestic Rearing of Sago Worm (*Rhynchophorus ferrugineus*)

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The worms of the *Rhynchophorus ferrugineus* weevils have been diet supplements for farmers that own sago plantations or farms as a source of protein. The sago worms are reported to contain 27.97% protein, all 9 essential amino acids, 59.71% fat with three unsaturated fatty acids including the essentially fatty acids: omega-3 and omega-6 (Leatemala et al., 2020). Norazlin Abdullah et al. (2012) produced sago powder using steaming, homogenising and air sprayed method. This process was observed to be able to reduce the original fat content of 50% to less than 30%, increasing carbohydrate content of 15% to 30% while maintaining protein content of 24%. Sago worm regularly are sold in the markets in Sarawak as it has a delicacy by the local community. Due to its long life cycle of 40 days to meet supply, the current price for sago worms is between RM 40 - RM 80 as per kilogram.

The supply of the sago worms is at the expense of the growing *Metroxylon* sago palm being the most common pest in sago cultivation in Sarawak. The sago worms cause damage through burrowing into the trunk and tunnelling through the internal tissues to feed on the soft fibers and terminal buds for about a month. Currently, the sago worms are collected from the farms or plantations through natural breeding. In addition, the rearing is based on traditional knowledge where the source of food is from non-commercial wild sago palms growing sporadically in some villages. The solution is to go into *ex-situ* domestic rearing of the sago worms with modern technology which can offer economic opportunities to not only the rural community who have the resources in their own farms to produce, but to the entrepreneurs. A preliminary study on using combination of sago pith and coconut shred as alternative food source was conducted using plastic containers as rearing containers of 0.6 m long, 0.4 m wide and 0.4 m high. The experimental layout with Randomized Complete Block Design replicated 4 times with four treatments: T1 - 100% sago pith + outdoor; T2 - 100% sago pith + indoor, T3 - 50% sago pith + 50% coconut shreds + outdoor and T4 - 50% sago pith + 50% coconut shreds + indoor was used. Cages for indoor treatment were being kept in the laboratory while cages for outdoor treatment outdoor were placed outside the building in the open space.

The adult palm weevils were collected from the field. A pair of male and female adult palm weevils were conditioned by keeping them in closed, perforated small plastic cups for 48 hours before introducing them into the plastic containers containing the different food formulas based on the treatments. Feeding with newly harvested sago pith and/or coconut shreds were conducted every 2 days. The eggs took approximately an average of 5 days to hatch in all treatments with an average 20 worms seen with naked eye within a week. In T3, there were no surviving worms after 28 days. At Week 6, the survival of the sago worms in T1 was only 10% with 2 worms with a mean of 3.00 cm in length, 1.20 cm in width and mean weight of 4.1 g while in T2, the survival

was 30% with 6 worms with a mean of 4.25 cm in length, 1.50 cm in width and mean weight of 5.29 g. In T4, the survival was 40% with 8 worms with a mean of 3.75 cm in length, 1.30 cm in width and mean weight of 3.86 g each. It was known that the sago weevils do not like sunlight being in a habitat of dense sago palm, thus attributed to the higher survival % and better performance in indoor environment. Zulkifli et al. (2018) reported that although the sago worms consumed more coconut as food source than sago, the protein presence in the digestive system of sago worm was higher with sago diet than coconut.

It can be concluded that indoor environment was more conducive for the sago worm development. However, there was no conclusive observation on the different formulas due to competition based on the number of survival worms, size and weight per worm per cage. Further studies will be conducted on selective uniform size and same number of worms at Week 1, carrying capacity per cage and nutrient content of sago worms growing on different food formula.

**Keywords:** *Rhynchophorus ferrugineus*, sago worm, *Metroxylon* sago palm, domestic culture

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