

## Chrononutrition study of pancakes made with Sago starch

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In recent years, the concept that irregular dietary habits, including missed meals, disrupt the body clock and cause lifestyle-related diseases and metabolic syndrome has become widespread. In the report of the study group for the formulation of the Dietary Reference Intakes for Japanese (2015 version), the viewpoint of chrononutrition was mentioned for the first time, and the effects of disease prevention and improvement based on chrononutrition are expected to increase in the future. In the case of carbohydrates, for example, rice, wheat, and corn starch are good for breakfast, while potato, cassava, and sweet potato starch are good for dinner. In this study, we focused on the issue of when to eat sago starch and used a chrononutrition approach to find the advantages of Sago Palm. We believe that the conscious needs of consumers.

### Materials and Methods

#### 1. Fluctuation of blood glucose level and body clock by transient administration of sago starch

Sago starch (0.2 g/kg) is transiently administered orally to Per2:luciferase knock-in mice, which have been maintained on a two-meal diet (breakfast and dinner) for about one month, and blood glucose fluctuations are monitored over time for 120 minutes. We will also check the phase-shifting effect of the internal clock of the liver using the Lumicycle (ActiaMetrics inc.).

#### 2. Preparation and sensory evaluation of pancakes using sago starch

Pancakes were prepared by substituting 20, 40, 60, 80, and 100% of wheat flour, the main ingredients of pancakes, with sago starch. Sensory evaluation is conducted using the prepared pancakes. The pancakes were evaluated by using the score method to determine the characteristics and preference. In addition, a questionnaire survey will be conducted to determine whether the pancakes can be eaten every day and whether they can be purchased. The panel will be composed of Aikoku Gakuen Junior College faculty and staff, and the amount of food that can be consumed daily as a meal or snack, as well as the taste and texture of the food will be determined.

### Results and Discussion

In the experiment using mice, the dominant inhibitory effect of sago starch on elevated blood glucose levels was confirmed. However, the phase was found to be less advanced than rice and wheat, but slightly regressed compared to potato and sweet potato. This may be due to the size of the starch particles. It was also found that the higher the substitution rate, the less puffy and soggy the pancakes became, and the less palatable they became. Solutions such as increasing the amount of milk were effective, but the pancakes separated, and as a result, only negative data was obtained with this protocol. We believe that further improvements are necessary for commercialization.

## The Recent Sago Business Movements in Sago Palm Producing Countries

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Sago palm has been recognized as a potential plant resource for many occasions for several decades. However, until now there are not so many magnificent movements or products around the sago producing countries. On 7 Nov. 2021, the Agro Inovasi Fair 2021 (Agricultural Innovation Fair) was held in Indonesia. More than 10 tenants exhibited their products and the exhibits by three of them include sago products. One of the three tenants has its clear vision and high aspirations for sago utilization. This report presents the results of the inspection of the fair and the follow-up interview. The results of the latest sago starch and foods awareness survey of people in sago palm producing countries and Japan are also included.

### **Inspection of Agro Inovasi Fair 2021**

Agro Inovasi Fair (AIF) 2021 was held with the Minister of Agriculture at Agricultural Technology Transfer Management Center (BPATP) in Bogor, Indonesia on 7 November, 2021. AIF is an effort to accelerate the dissemination of the findings of the Agricultural Research and Development Agency (Balitbangtan) to the public. AIF is a routine activity of the Agency to downstream the existing research results. It is expected for the national market as well as for export purposes. The minister stated that people cannot rely on rice alone and various other commodities that can replace or substitute them, such as sago. More than 10 tenants that cooperate with Balitbangtan under the Ministry of Agriculture exhibited their products. The exhibits by three of the tenants include sago products such as sago noodle. One of the three tenants producing sago products has their clear vision and high aspirations for national use of sago utilization and export. In the follow-up interview with the tenant, Sagolicious, it was cleared that one of their important achievements was pasta making using sago starch. They got two MURI awards for sago noodles and sago chips (24 November 2021).

### **Sago starch and foods awareness of people**

The questionnaire on sago starch and foods awareness of people in sago palm producing countries in Southeast Asia and Japan was conducted by using social media. The survey was held from 24 - 30 Nov, within 1 week there were 107 respondents, among 20 questions. There were 5 categories, attribute, interest, experience of knowing sago, knowledge about sago and experience of eating sago. Interestingly, even more than 90% answered that they have ever heard about sago and around 80% of them answered already experienced eating sago, 67% of them don't know the starch is extracted from the trunk, 47% of them don't know sago starch is gluten free, 55% of them thought from one sago tree can get less than 100 kg of sago starch, 56% of them did not know that sago starch has been used in Japan as well. This result shows that how much sago information could be distributed to the society. From the result of questionnaire, the awareness of sago starch seems to be not deeply spread. After Covid-19 ruined the all over the world, the awareness of health has been increasing in sago producing countries as well. In Indonesia, the media have broadcasted the importance of healthy lifestyle like gargling and hand washing, eating nutritious foods, regular exercise. Those movements might have various possibilities to boost sago industries for only its producing countries but also for partner countries such as Japan.

## Production and breaking resistance of sago (*Metroxylon sagu*) cookies containing taro (*Colocasia esculenta*) starch

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The intercropping of sago palm (*Metroxylon sagu*) and taro (*Colocasia esculenta*) was started in Pangasugan, Leyte, Philippines in 2005. Taro (*Iniito*) corms were harvested to get taro starch in 2006. Sago starch was extracted in 2017 from the same field as taro. Sago cookies containing 0 to 10% of taro starch were produced and the bearing resistance (BR) of cookies were measured with the penetration resistance meter in 2021. Sago starch was temple bell shape, ranging from 35 to 40  $\mu\text{m}$  in diameter and taro starch was irregular spherical, 1 to 2  $\mu\text{m}$ . The mean value of BR for sago cookies varied from 10.0 to 26.7 kPa, indicating that the lowest mean value of BR was found in large size of 100% sago cookie (roll dough, cut out shape) and that the highest mean value of BR was detected in small size of 100% sago cookie (flat dough, cut by a knife). Sago cookies containing taro starch is possible to release after some ingenuity.

### Materials and Methods

#### 1. Sample collection and starch preparation

In 2017 sago trunks were collected from the sago and taro intercropping experimental field developed in 2005, Pangasugan, Baybay, Leyte, Philippines. Sago grated materials from the trunk pith was collected and squeezed by a netting cloth with tap water. Sago starch was air-dried after washing with tap water several times in Leyte and stored in a vinyl bag. Meanwhile, taro cultivar (*Iniito*, *Ito ini*) corms from the intercropping field in Pangasugan were washed to remove soil and hand-peeled and trimmed to remove the skin and defective parts in 2006. Taro slurry was treated by the wet cloth sieving after grazing by a grater and dispersed in 0.2 mol/L sodium chloride solution at the pH of 8.6 using sodium hydroxide solution. Finally, taro starch was collected from the middle portion of the suspension by centrifuge. The taro starch was steeped in 0.05 % sodium hydroxide for 2 hours and shaking for 2 minutes. Taro starch was separated from the residue. The shaking procedure was repeated three times. The crude starch was collected and centrifuged (800 $\times$ g, 30 minutes). Granule size, color and shape of starch granules were evaluated by a light-transmitting microscope (Meiji Techno MT5000). The X-ray diffraction pattern of sago and taro starches was obtained by an X-ray diffractometer (Rigaku SmartLab).

## 2. Blending and production of cookies

Sago starch was substituted with different levels of taro starch: 0% (control), 5% and 10%. Five g of microwave-heated butter to 50 g of sago and taro starch for the control cookie mixture was stirred in a blender for 1.5 min and added 10 g of sugar. Mixed manually them for 1.5 min. Then, added 1/3 of egg to the mixture A. The mixed powder of sago and taro starch passed through a sieve and the mixture powder (mixture B and C) were put to make dough. The dough samples were in vinyl bags and stretched to roll or flat and stock in a refrigerator for 60 min. Thereafter, drew it to cut with a knife to 12 mm in height and 24 mm in diameter or with a round molding cutter to 12 mm in height and 35 mm in diameter. The samples were baked in an electric toaster (Mitsubishi RG-GS1, Japan) at 180 °C for 17 min. The baked cookies were cooled down in the air and stocked in the desiccators to keep the moisture condition in vinyl bags for 2 days. Roll and flat dough were cut with a knife or cut out shape.

## 3. Texture analysis of cookies

The texture analyzer (Push-Cone, DIK-5561, Daiki, Japan) was used to measure the maximum stress (bearing resistance) of cookies using the spring with 39.2 N / 40 mm. Cookies were laid in the palate container and the Push-Cone was pressed onto the samples with the slow measuring speed. Crystalline components in cookie dough come to non-crystalline components, accompanied to hydration and swelling by heating. The non-crystalline components and melted sucrose provide crispy texture of cookies (Kawai, 2016).

## Results

### 1. Properties of sago and taro starch

Sago starch indicated light yellow due to weak browning reaction and its granules were round or oval shapes with diameter, ranging from 30 to 40  $\mu\text{m}$ . The color of sago starch was pale yellow (2.5Y 8/4). Taro starch granules were small, irregular and polygonal shapes (Setiarto et al. 2020). Particle size of taro starch granules varied from 1 to 3  $\mu\text{m}$  under a light-transmitting microscope. The diffraction peaks at  $2\theta$  are clearly exhibited at 5.6 (shoulder peak), 17, 18 and 23 degree for sago starch and 17, 18 and 23 degree for taro starch, respectively. The results of sago and taro starch exhibited a C type and A type X-ray diffraction pattern.

### 2. Physical morphology of cookies

The size of cookie ranged from 22 to 26 mm in diameter and from 11 to 14 mm in height for cookie A to C (cutting roll with a knife) and varied from 34 to 38 mm in diameter and 11 to 13 mm in height for cookie D. Moisture content of all cookie samples, related to the maximum strength (Wada and Higo, 2007), ranged from 5.5 to 5.8 % with the mean value of 5.6 % and the standard deviation of 0.1.

### 3. Texture analysis of cookies

Bearing resistance (BR) values of cookies in Table 4 ranged from 22.7 to 26.7 kPa with large standard deviation, varying from 3.4 to 6.8 kPa. These values of BR were remarkably lower than the breaking stress values (271 to 405 kPa) of cookies (Wada, 1988) and the maximum hardening values (1120 to 1150 kPa) of cookies with low gelatinization characteristics reported by Wada and Higo (2007). The addition of different percentages of taro starch provided slightly different BR of cookies. Small amount (up to 10%) of taro starch brought low BR. However, there was not clear effect of taro starch addition to sago starch cookie dough on the BR of cookies.

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## Sensory evaluation of sago (*Metroxylon sagu*) cookies containing taro (*Colocasia esculenta*) starch

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Sago (*Metroxylon sagu* Rottb.) starch is utilized as sago pearl in Philippine. We performed a sensory evaluation of sago cookies made from sago and taro (*Colocasia esculenta*) starch.

Keywords: cookie, sago and taro starch, sensory evaluation

## Materials and Methods

Cookies were made from sago starch and taro starch with different composition: 0% (control), 5% and 10% of taro starch was used instead of sago starch, which were named cookie A, B, and C, respectively. Dough samples of each cookies were in vinyl bags and stretched to roll or flat and stock in a refrigerator for 60 min. Thereafter, drew it to cut with a knife to 12 mm in height and 24 mm in diameter. Concerning dough of 100% of sago starch, dough was cut to 12 mm in height and 35 mm in diameter (cookie D) and 14mm in height and 24 mm in diameter (cookie E). The samples were baked in an electric toaster (Mitsubishi RG-GS1, Japan) at 180 °C for 17 min. The baked cookies were cooled down in the air and stocked in the desiccators to keep the moisture condition in vinyl bags for 2 days

Cookies characteristics were evaluated by appearance, sweetness, hardness, crispness and comprehensive evaluation by the panelists recruited from the 10 university students of 20s. The appearance including color of

baked cookies is one of the important characteristics when the consumers picked up cookies. The appearance, sweetness, hardness and crispness were scored by the 10-point hedonic scale. Comprehensive evaluation was scored by the 9-point evaluation after Kawasome et al. (1971): “the most favorite” was given 9 point and “not like eating” was given 1 point.

The difference between cookies for each score was tested by Fisher’s least significant difference method using Excel Statistics 2012 for Windows (SSRI Co., Ltd., Tokyo, Japan). Factors affecting comprehensive evaluation were examined by stepwise multiple regression analysis using Excel Statistics 2012 for Windows. We chose appearance, sweetness, hardness, and crispness as explanatory variables. We used the forward selection method for variable selection and chose 2 as the threshold for the F-value.

### Results and Discussion

The score of appearance, sweetness, and comprehensive evaluation of cookie D was the highest, 7.4, 7.8, and 6.0, respectively. Although no significant difference was found in appearance, sweetness and comprehensive evaluation of cookie D was significantly higher than other cookies. The score of hardness, crispness of cookie C was the highest, 6.6 and 4.8. Hardness and crispness of cookie D were significantly less than those of cookie C.

Standardized partial regression coefficient of sweetness was the highest except cookie D, which score of sweetness varied from 7 to 10. Crispness was also chosen as a factor that influenced comprehensive evaluation including cookie D. Concerning cookie D, score of comprehensive evaluation was significantly correlated with score of crispness ( $p < 0.05$ ).

Although sugar content was same for all cookies, score of sweetness was different for each cookies and was the most effective for comprehensive evaluation of sensory evaluation.

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## Growth Characteristics and Starch Productivity of Oil Palm (*Elaeis guineensis* Jacq.) and Palmyra Palm (*Borassus flabellifer* L.)

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### Introduction

Palms belonging to 8-14 genera of palm family are known as palms that accumulate starch in the trunk, but

there are very few reports on their starch productivities except for sago palm (*Meteroxylon sagu* Rottb.). From such a background, the authors have been conducting researches on starch-accumulating palms other than sago palm such as *Agenga*, *Corypha*, *Caryta* etc. In this presentation, we report on the starch productivities of oil palm, which is not recognized as starch-accumulating palm, but can be expected to have considerable starch accumulation (Haska 2001), and palmyra palm.

### Materials and Methods

The surveys on oil palm and palmyra palm were conducted in August 2009 at the state-owned oil palm plantation (PT. Perkebunan Nusantara VIII) in Kertajaya Village, Banten Province, Java, Island, and at a farmer's garden in Naibonat Village on the outskirts of Kupang City, East Nusa Tenggara Province, Indonesia, respectively. For the former, three 28 year-old palms to be replanted were cut down, and for the latter, one female and one male plant with an estimated age of 13 years were cut down to investigate the growth characteristics of the above-ground part and the trunk. The starch and total sugar contents in the pith were measured for 6 positions at equal intervals on the trunk, and the starch content per trunk was calculated.

### Results and Discussion

The plant and trunk lengths of oil palm and palmyra palm were 17-19 m; 9-10m and about 12 m; about 8 m, respectively, and their diameters were approximately 40-45 cm and 35 cm. The trunk weight was 1100-1500 kg for oil palm and about 800 kg for palmyra palm. The number of leaves was 20-34 for oil palm (pinnate compound leaf) and 19-37 for palmyra palm (palmate compound leaf), and a large difference between the plants was observed. The differences in leaf characteristics depending on the leaf position were small in both species. The leaf lengths of oil palm and palmyra palm were 5-7 m and 3-4 m, respectively, and the number of leaflets was 270-380 and 80-90, respectively. The longest leaflet length and its maximum leaf width were approximately 1 m; 5-6 cm for oil palm and 1 m; 8 cm for palmyra palm, and the leaflet thickness was 0.2-0.3 mm and 0.4-0.5 mm, respectively. The SPAD value of the longest leaflets was 60-80 for oil palm and 40-50 for palmyra palm.

The ratio of pith weight to trunk weight was 82-85% for oil palm and about 72% for palmyra palm. The pith dry matter percentage was 30-38% in the former and 22-32% in the latter, showing a large difference between the plants. The pith dry weight was 320-390 kg for oil palm and 130-180 kg for palmyra palm, and the total sugar percentage in the pith was 9-15% and 8-11%, respectively. The starch percentage was 8-16% for oil palm and 1-10% for palmyra palm, and there was a large difference between the plants in both species, and additionally the percentages of female plant in palmyra palm were higher than those in male plant. The total sugar percentages of oil palm were higher above the central part than in the lower part in one plant, while in the other two plants, there was no clear difference depending on the position at about 10%. On the other hand, the starch percentages of each plant were higher above the central part of the trunk than those below the central part, and the maximum value of each plant was 21-25%. In palmyra palm, the difference in total sugar percentages depending on the position of the trunk was small, and they were mostly about 10%. On the other hand, the starch percentages were very low in all positions in the male plant, and the maximum value was only 2.4%. In the female plant, those were higher above the central part of the trunk, similar to oil palm, and the maximum value was about 20%. The starch content was in the range of 30-60 kg in oil palm, and there was a large difference between the plants. In the palmyra palm, the starch content was extremely low, about 1 kg in the male plant and 17 kg in the female plant.

From the above, the starch content in the oil palm at the time of replanting is as low as 30-60 kg per plant due to the low starch percentage in the pith. However, it should be considered as a starch resource due to the annual enormous amount of discarded oil palms. On the other hand, it was estimated that the starch productivity of palmyra palm was very low, although it is necessary a further study considering the number of survey plants and growth stages after trunk formation.

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## Feasibility Study for Copper Application to Sago Palm Leaf

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Nutrient deficiency is one of the major growth-limiting factors for crop cultivation in peatland, including sago palm. Among the nutrients, copper deficiency is one of the most common symptoms (Nitta et al. 2000). Several soil fertilization experiments were conducted, however no appreciable results were reported (Kakuda et al. 2005). This study aims to investigate the feasibility of applying copper to sago palm leaves in order to develop an alternative fertilization method to improve the performance of sago palms grown on peatlands.

### Materials and Methods

Sago leaflets were detached from three-years-old sago seedlings that grown in Phytotron at Nagoya University, and individually stuck into a watery sponge. Copper fertilizer (CuSO<sub>4</sub>), mixing with 0.05% tween20, was applied as 40 droplets (10µl each) on a specific area (40 cm<sup>2</sup>) drawn on the prepared leaflets. The treated leaflets were allowed to stand 24 h (Knoche et al. 1992) in a growth chamber with the following conditions: 28-30°C, 75% relative humidity, and dark (night) or 250-300 µmol m<sup>-2</sup> s<sup>-1</sup> light intensity (daytime). After treatment, the treated leaflets was cut at the specific area and washed with 60% acetone to remove residues. Copper concentration in the leaflet was determined by ICP-AES. Copper absorption were evaluated by conducting two experiments using different leaflet side (adaxial, abaxial) and light condition (light, dark). Twelve leaflets from three sago seedlings were used for each treatment. Stomatal density of both sides and stomatal opening in both light conditions were also measured to investigate the effect of leaf property on copper absorption. To validate



the copper absorption, SEM and X-ray micro-analysis of the treated and untreated leaflets were employed with energy-dispersive X-ray spectroscopy attached to a scanning electron microscope.

### Results and Discussion

Based on the result, both experiments showed a significant increase of copper concentration in sago palm leaflets after the foliar solution was applied. It was revealed that a certain amount of copper in the foliar solution possibly penetrates the sago palm leaf. Based on SEM and X-ray microanalysis, the specific distribution of copper was not observed in both leaflets. Regarding this result, the feasibility of copper penetration into the sago leaflet remained unclear. As an alternative, another experiment was performed. Sago leaflets were treated and immediately washed with 60% acetone soon after the treatment was applied. The control leaflets were also prepared. Based on the result, no significant difference was observed between the control and the treated leaflet. This experiment proved that only a small residue remained. Thus, sago leaflet feasibly absorb copper after 24 h of treatment. Foliar uptake may occur through a number of pathways. Among these, the stomatal pathways are the most studied. Some studies have found higher penetration in the presence of stomata and in response to stomatal opening (Eichert and Goldbach 2008). In this study, higher copper penetration with application on the abaxial side and in a light condition was expected due to higher stomatal density and stomatal opening. However, no difference in copper concentration was observed among the treatments applied. This tendency showed that, in the sago palm, the role of the stomata on foliar uptake may be limited.

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## Growth Responses of Manno Sago Seed on Organic Fertilizer Application

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Seed of Manno sago (*Metroxylon sago* Rottb.) origin from Sentani, Jaya Pura Regency, Province of Papua, was cultivated in nursery for 9 months and then transplanted in 7 m x 7 m in Halu Oleo University's Farm at July

2012 under collaboration: Ministry of Agriculture, Forest and Fisheries of Japan, Kochi University and Halu Oleo University. This experiment concluded that Manno sago rising from seed was grown perfectly in a new agro-climate. After 9 years old, more than 80% of clumps was produced trunk length up to 5 m clump<sup>-1</sup>, and one of 27 plants was bolting in October 2021.

### Materials and Methods

Seedling of Manno sago was rising in pot culture with 30 kg air dry soil, watering and puddling. In this experiment, we applied four levels of organic fertilizer (MOF): 0, 2.0, 5.0 and 10 %. Each pot was planting with one seedling. This experiment purpose was to study an effect of organic treatment on the growth of Manno seedling. SPAD values and dry matter production were measured from 4 to 20 weeks after planting (WAP). Chemical properties of soil and MOF were analysed.

### Results and Discussion

In this experiment we show that Organic Fertilizer (MOF) application was induced leaf number up to 12 WAT and decreased at 16 and 20 Weeks after Treatment (WAT) (Fig.1.A). MOF application 10 and 5% was significantly induce leaf length from 12 to 20 WAT (Fig. 1.B), respectively. We suggest that the benefit of organic MOF application effect due to the higher content of organic-C, P<sub>2</sub>O<sub>5</sub>, and exchangeable cations as shown in Table 1. SPAD values were decreased from 8 to 20 WAT (Fig.2A). Shoot and root dry weight was increased by 2.0 to 5.0% and decreased by 10% level of MOF application as shown in Fig. 2.B.

### Conclusions

Based on the above results, we concluded that organic fertilizer application on seedling stage of Manno Sago palm was significantly induced palm growth at nursery stage, however, the optimum level of organic fertilizer application was 5%. Higher level of organic fertilizer application was reduced some variables growth of Manno sago seedling.

Table 1. Soil and organic MOF chemical properties

Elements	Unit	Soil	MOF	Elements	Unit	Soil	MOF
pH (H <sub>2</sub> O)		6.76	6.98	Total-K	me 100 g <sup>-1</sup>	10.8	28
Organic-C	%	2.1	10.08	Ca <sup>2+</sup>	ppm	<i>nd</i>	10.2
Total-N	%	0.37	0.54	Mg <sup>2+</sup>	ppm	<i>nd</i>	1.73
Available P <sub>2</sub> O <sub>5</sub>	ppm	17.52	44	<b>Micro Nutrients</b>			
Soil texture		<i>Sandy loam</i>	<i>nd</i>	Cu	ppm	<i>nd</i>	71.49
				Zn	ppm	<i>nd</i>	18.22

*nd.* =not determined