International Journal of Agricultural Technology 2013 Vol. 9(1): 193-200
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Correlation of the browning of starch extracted from sago palm (*Metroxylon sagu* Robb) to the phenolic content and ecosystem conditions of growth

Hiroyuki Konuma[1*], Rosa Rolle[2] and Somsak Boromthanarat[3]

[1]Assistant Director General and Regional Representative, FAO Regional Office for Asia and the Pacific, Bangkok, Thailand, [2]Senior Agro-Industry and Post-harvest Officer, FAO-Regional Offoce for Asia and the Pacific, Bangkok, Thailand, [3]Director, Asian Coastal Resources Institute Foundation, Asian Institute of Technology, Bangkok, Thailand

Hiroyuki Konuma, Rosa Rolle and Somsak Boromthanarat (2013) Correlation of the browning of starch extracted from sago palm (*Metroxylon sagu* Robb) to the phenolic content and ecosystem conditions of growth. International Journal of Agricultural Technology 9(1):193-200.

Abstract Studies were undertaken to correlate sago starch browning to the phenolic content and the conditions of the ecosystem in which the sago palms (*Metroxylon sagu* Robb) were grown. Starch extracted from sago palms grown in low pH soils, under high sulphur conditions (peat swamp), was observed to have significantly higher concentrations of phenolic compounds (0.115 mg of catechin equiv/g dry weight) when compared to that extracted from palms grown in neutral clay-loamy soils (0.089 mg of catechin equiv/g dry weight). Starch extracted from sago palms grown on peat swamp exhibited a rapid rate of browning during the extraction process yielding a final brown product, suggesting the presence of highly active polyphenoloxidases in the pith from which the starch was extracted. Starch extracted from neutral soils of low sulphur content on the other hand were white on extraction, but underwent browning after an extended incubation period (four hours) at room temperature, suggesting the predominance of latent polyphenoloxidases in the pith from which the starch was extracted.

Key words: sago palms, Starch, peat swamp, *Metroxylon sagu* Robb

Introduction

Polyphenoloxidase (PPO) - catalyzed browning reactions are of significant importance in plant foods. These reactions occur when plant cells are ruptured by wounding, cutting or crushing, wherein indigenous phenolic compounds are oxidized in the presence of molecular oxygen (Mayer, 1986; Mayer and Harel, 1981) to produce brown pigments.

[*] **Corresponding author**: Hiroyuki Konuma; **e-mail**: Hiroyuki.Konuma@fao.org, Rosa.Rolle@fao.org, somsak.b@corin-asia.org

Browning is an important contributor to the quality deterioration of sago starch and has been associated with low marketability of that product (Yatsugi, 1986; Ahmad, 1991; Okamoto *et al*., 1988 and Onsa *et al*., 2000). The occurrence of browning during extraction of starch from the sago pith, tremendously reduces the economic value of the starch.

Our previous findings (Konuma *et al*., 2012) identified significant correlation between the color of sago starch and the characteristics of the ecosystems from which the sago palm (*Metroxylon sagu* Robb) was harvested. This paper report findings on the phenolic content of sago starch extracted from two of the ecosystems previously studied, as well as a qualitative assessment of browning reactions in the starch following extraction.

Materials and methods

Samples of the sago trunks for starch extraction were obtained from 7-10 year old trees growing in two distinctly different ecosystems in Nakhom Si Thamarat Province in Southern Thailand, according to the method described by Konuma *et al*., (2012). The first of these two ecosystems, (Ronpiboon) consisted of mineral soils, while the second ecosystem (Kreng) consisted of swampy peat soils.

Sampling of the sago-trunk

Sago trees were felled, following which the sago trunk was separated into lower, middle and upper portions according to the method of Srioroth *et al.* 1999, as described by Konuma *et al.* (2012). A stainless steel axe was used to separate the bark from the sago pith for each portion of the log.

Extraction of sago starch

Sago starch was extracted from the top, middle and bottom parts of the trunk by splitting the trunk of the sago palm lengthwise and removing the pith. The pith was subsequently grated and kneaded to release the starch. The starch thus obtained was washed and filtered in order to separate out any fibrous residues. The raw starch suspension thus obtained was allowed to settle out in a container by precipitation. The precipitated sago starch was subsequently washed twice with water and then oven-dried. Three hundred grams of the dried starch was transferred to a Ziploc plastic bag for laboratory testing to determine total phenolic content.

Qualitative assessment of sago starch browning

Extracted samples of sago starch were transferred in triplicate to beakers containing methanol. The samples were periodically photographed at intervals over a 4 hour period.

Extraction of polyphenols from sago starch

Phenolic compounds were extracted from sago starch following the methodology of Aguilera, *et al*., 2011. Samples (2 g each) of the starch were incubated with 10 mL of a solution of methanol-HCl (1%): water solution (80:20 v/v) at 37°C for 2 hr. The samples were subsequently filtered using Whatman No.1 filter paper. A 10 ml aliquot of the methanolic filtrate thus obtained was mixed with 10 mL of methanol for measurement of the total phenolic content.

Determination of total polyphenol content of extracted starch

Total phenolic content was measured using the modified Folin-Ciocalteu method (Singleton *et al*., 1999). An aliquot (0.1 ml) of the methanolic extract of the starch was added to 2 ml of Folin-Ciocalteu Reagent (1:10 v/v with water). The mixture was allowed to equilibrate for 5 min, mixed with 1.5 ml of sodium carbonate solution (60 g/l) and incubated at room temperature for 90 min. The absorbance of the mixture was read at 725 nm with distilled water as a blank. Total phenolic content was reported as milligrams of catechin equivalents per gram dry weight of starch.

Results

Browning of debarked sago pith

Debarked pith of sago palms was observed to undergo pink discoloration, followed by the development of brown to black pigments, when the pith was wounded by several small incisions (Figure 1). The intensity of this pink discoloration was apparently influenced by the extent of wounding of the trunk (Figure 1). The larger and deeper the wounds, the more intense and widespread was the pink discoloration of the pith. Different trees from the same ecosystem also appeared to show different rates of discoloration (Figure 1).

Fig. 1. Discoloration of 7-10 year old debarked sago palms harvested from the Ronpiboon ecosystem. The severely wounded trunk (left) shows more intense of pink discoloration, and some browning.

Browning of sago starch

We recently reported that sago palms grown in fresh water swamp under acidic conditions and high soil sulphur concentrations (samples obtained from Kreng), yielded starch that was brown in color and of a high ash content. Sago palms grown in neutral soils of a low sulphur and ash content, yielded starch that was white in color. A mixture of sago palm varieties was observed to grow under netural brackish conditions in the Ronpiboon ecosystem (Konuma *et al*. 2012) yielding starch that was white to pale pink in color. In an effort to further study the correlation between sago starch browning and the ecosystems in which the palms were grown, the total phenolic content of sago starch extracted from the pith of sago palms obtained from Ronpiboon and Kreng ecosystems, was quantitated using the modified Folin-Ciocalteu method (Singleton *et al.*, 1999). The rate of browning of sago starch slurries in methanol was qualitatively evaluated by photographing samples of the slurries maintained at room temperature, over four hours for comparison.

Starch extracted from the pith of palms grown in Ronpiboon, an ecosystem having clay-loam soils of neutral pH (6.5) and comparably low sulphur content (11.61 mg kg-1), was white in color immediately following extraction, but progressively underwent browning over time, during incubation at room temperature (Figure 2) over four hours. The formation of brown polymers was particularly noticeable on the surface of the slurries after four hours of incubation, owing largely to direct exposure of the surface of the slurry to oxygen in the environment.

The phenolic content of starch samples extracted from this ecosystem ranged from 0.063 to 0.119 mg of catechin equivalents /gram of dry weight with an average phenolic concentration of 0.089 catechin equivalents /gram of dry weight (Table 1). Okamoto *et al*., 1985, identified DL-epicatechin, D-

catechin and procyanidin in sago palm pith and noted that DL-epicatechin and D-catechin produced colored substances by oxidation with enzymes prepared from the sago palm. Shirlene (2002) also reported the identification (+)-catechin and (-)-epicatechin in sago pith.

Table 1. Total phenolic content of starch extracted in triplicate from the pith of five trees, from each of the two ecosystems studied

Ecosystems	Replications	Mean TPC (mg of catechin equiv./g dry weight)
Ronpiboon	Tree1	0.063
	Tree2	0.077
	Tree3	0.119
	Tree4	0.105
	Tree5	0.078
	Average	0.089
Kreng	Tree1	0.183
	Tree2	0.131
	Tree3	0.061
	Tree4	0.117
	Tree5	0.083
	Average	0.115

TPC = Total phenolic content

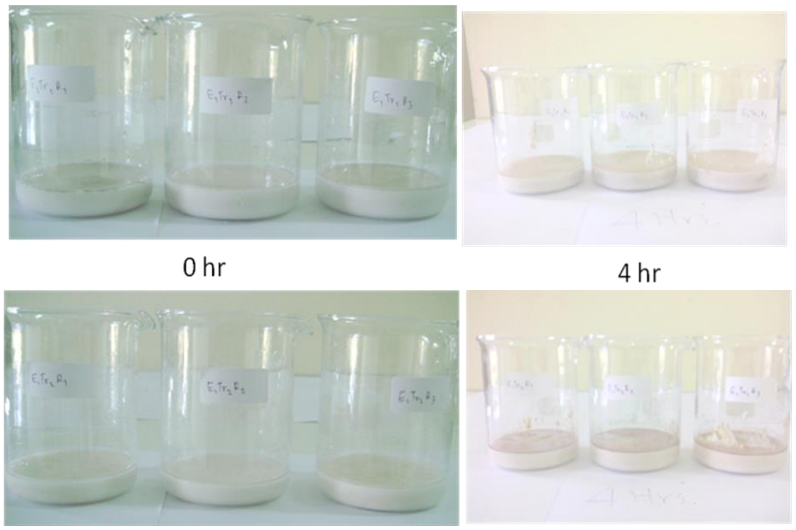

Fig. 2. Samples of methanolic starch slurry extracted from the Ronpiboon ecosystem, incubated at room temperature under ambient conditions over a four hour period. Samples at 0 hr (L); Samples at 4 hr (R).

Binn Sari (2004) studied the effect of holding time, pH and temperature of sago pith slurry on browning and determined that the holding time of sago

pith slurry was an important factor in determining the amount of soluble phenolic compounds that were oxidized. Onsa *et al.* (2007) reported the occurrence of latent and soluble polyphenoloxidases in the sago palm. The slow onset of browning observed in starch samples extracted from the Ronpiboon ecosystem seems to suggest the presence of a latent polyphenoloxidase in the sago palms extracted from that ecosystem.

Starch samples extracted from palms grown in Kreng, an ecosystem with acidic soil conditions (pH 4.91) and a significantly higher sulphur content 67 mg kg-1 (Konuma *et al.*, 2012) than that of the Ronpiboon ecosystem were uniformly pink in color after extraction (Figure 3), suggesting the presence of high polyphenoloxidase activity during the extraction of these starch samples from the pith. The phenolic content of these starch samples was also comparably higher than that of starch extracted from the Ronpiboon ecosystem and ranged from 0.06 mg of catechin equivalents/gram of dry weight to 0.18 mg of catechin equivalents /gram of dry weight, with an average of 0.115 mg of catechin equivalents /gram of dry weight for that ecosystem (Table 1). These starch samples underwent a uniform color change from pale pink to pale brown when incubated at room temperature over four hours (Figure 3). This color change appears to have been largely due to the polymerization of pink quinones present in the starch, to melanins during room temperature incubation. In contrast to the samples extracted from Ronpiboon, there was no evidence of a dependency on the requirement for oxygen as a substrate in this reaction, suggesting that the color change which took place on incubation was not catalysed by polyphenoloxidases despite the comparably high phenolic content of the starch.

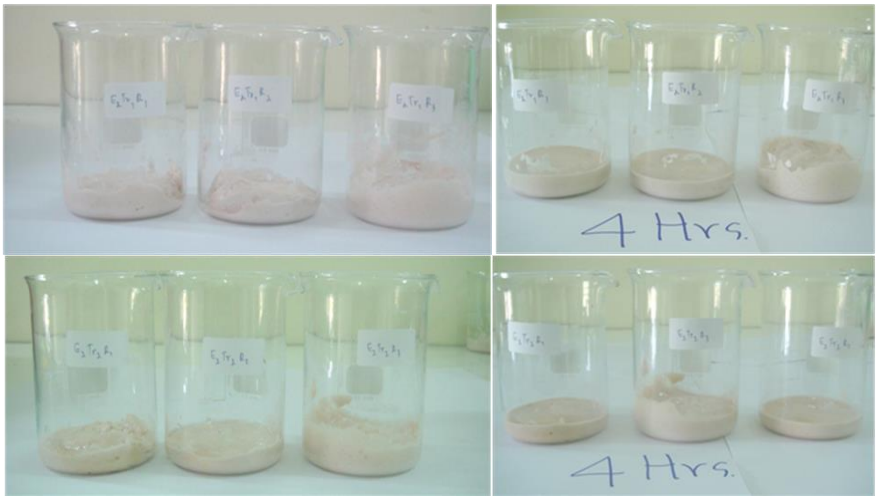

Fig. 3. Samples of methanolic starch slurry extracted from the Kreng ecosystem, incubated at room temperature under ambient conditions over a four hour periods. Samples at 0 hr (L); Samples at 4 hr (R).

It is quite possible that the melanins produced in the starch, had an inhibitory effect on polyphenolxidase enzymes present, thereby precluding further changes in the color of the starch. Shirlene (2007) studied the effect of holding time on the browning of sago pith slurry and determined that colour development was significantly more intense with increased holding time but was not significant ($P<0.05$) after six hours.

An ANOVA analysis comparing the total phenolic content of the starch samples extracted from the two habitats, indicated a significant difference in total phenolic content between starch extracted from the two ecosystems and among starch samples extracted from different trees ($p < 0.05$) within the same ecosystem (Table 2).

Table 2. Analysis of Variance *(*ANOVA) of triplicate measurements of the total phenolic content of starch extracted from five trees obtained from each of the two ecosystems studied

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.038[a]	9	.004	17.584	.000
Intercept	.310	1	.310	1.298E3	.000
Ecosystems	.005	1	.005	21.856	.000
Trees	.007	4	.002	6.996	.001
Ecosystems * Trees	.026	4	.006	27.105	.000
Error	.005	20	.000		
Total	.353	30			
Corrected Total	.043	29			

a. R Squared = .888 (Adjusted R Squared = .837)

Conclusion

Our findings reported here are consistent with our earlier report on the subject and seem to suggest that sago palms grown in low pH soils, under high sulphur conditions (peat swamp), contain comparably high levels of phenolic compounds and active soluble polyphenoloxidase enzymes. Palms grown in neutral soils at comparably lower sulphur concentrations, had lower levels of phenolic compounds and underwent oxidation after a lag period, suggesting the existence of latent polyphenoloxidases in starches extracted from that ecosystem. These findings highlight the potential for controlling the discoloration of sago pith post-harvest by promptly treating debarked sago pith with polyphenoloxidase inhibitors, prior to extraction of the starch.

Acknowledgements

This study was undertaken by the Asian Coastal Resources Institute Foundation, with technical and funding support from the FAO Regional Office for Asia and the Pacific, Bangkok, Thailand.

References

Aguilera, Y., M. Dueñas, I. Estrella, T. Hernãndez, V. Benitez, R.M. Esteban and M.A. Martín-Cabrejas (2011). Phenolic profile and antioxidant capacity of chickpeas (*Cicer arietinum* L.) as affected by a dehydration process. Plant Foods Hum Nutr. 66:187-195.

Ahmad, M.N. (1991).Optimizing starch granule extraction using a leaching prototype machine. In N. Thai-Tsiung, T. Yiu-Liong and K. Honh-Siong, (eds). Proceedings of the 4th International Sago Symposium, August 1990. Kuching, Sarawak, Malaysia. pp. 188-193.

Binn Sari, N. (2004) . Browning of sago (*Metroxylon sagu*) pith slurry as influenced by holding time, pH and temperature. Journal Food Biochem 28:91-99.

Konuma, H., Rolle R.S. and Boromthanarat, S. (2012). Color characteristics of sago starch as they relate to the growth environment of the sago palm (*Metroxylon sagu* Robb). Journal of Agricultural Technology 8:273-287.

Mayer, A. M. and Harel, E. (1981). Polyphenol oxidases in fruits. Changes during ripening. In Friend, J. and Rhodes, M. J. C. (eds.), Recent Advances in the Biochemistry of Fruits and Vegetables, Academic Press, New York, USA., pp 161-182.

Mayer, A.M. (1986). Polyphenol oxidases in plants-recent progress. *Phytochemistry* 26:11-20.

Onsa, G.H., Nazamid, S., Jinap, S. and Jamilah, B. (2000). Latent polyphenol oxidases from sago log (*Metroxylon sagu*): Partial purification, activation and some properties. Journal of Agriculture and Food Chemistry 48:5041-5046.

Onsa, G.H., Saarin, N.B., Asizah, O. and Bahri, N. (2003). Membrane bound polyphenoloxidases and peroxidases from *Metroxylon sagu* : changes in activity and isoenzyme profiles during maturation. http://www.aseanfood.info/Articles/11019982.pdf

Onsa, G.H., Bin Sarri, N., Selmat, J., Nakar, J., Mohammed, A.S. and Bahri, S. (2007). Histochemical localization of polyphenoloxidase and peroxidase from *Metroxylon sagu.* Asia Pacific J. Mol. Biol. Biotechnol. 15(2): pp. 2007.

Okamoto, A., Ozakawa, T., Imagawa, H. and Arai, Y. (1985). Polyphenolic compounds related to browning of sago starch. J. Agric. Chem Soc. Japan 59:1257-1261.

Okamoto, A., Imagawa, H., Arai, Y. and Ozawa, T. (1988). Partial purification and some properties of polyphenoloxidases from sago palm. Journal of Biological Chemistry 52:2215-2222.

Shirlene, M.A. (2002). Factors affecting browning of sago (*Metroxylon sagus* Rottb.) pith and their effects on sago starch. http://psasir.upm.edu.my/8479/1/FSMB_2002_16_A.pdf

Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Oxidants and Antioxidants 99:152-178.

Sriroth, K., Piyachomkwan, K., Chotineeranat, S., Chollakup, R., Hicks, A. and Oates, C.G. (1999). Structural and functional properties of Thai sago (*Metroxylon* spp.) starch extracted from different trunk portions.

Yatsugi, T. (1986). Problems of sago starch manufacturing. Proceeding of the Third International Sago symposium "The Equatorial Swamp as a Natural Resource" 5-7 July 1986, Tokyo, Japan, pp. 201-207.

(Received 3 November 2012; accepted 30 December 2012)