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STUDIES ON SOME PROPERTIES OF STARCH ISOLATED FROM *SONNERATIA* SPECIES

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SUMMARY

***Sonneratia* is one of the important mangrove genus, having edible properties. The three species of *Sonneratia, i.e. S.alba, S.apetala* and *S.caseolaris* grows along the coast of Maharashtra. The fruits of these species are starchy and edible. Therefore, the starch from these fruits was isolated and properties of the starch were studied. The starch –iodine-potassium iodide test is negative, i.e. instead of blue colour with iodine-potassium-iodide the fruit starch showed reddish colour. Granule size of isolated fruit starch is variable depending on species. The gelatinization temperature range was slightly varied than other starch sources. X-ray diffraction analysis showed some resemblance between standard and plant starch samples.**

***Key words*: Gelatinization, granule morphology, isolation, mangrove, starch.**

INTRODUCTION

Mangroves are a group of salt tolerant plant species, growing along the intertidal estuarine regions. They form very special type of vegetation protecting our sea coast against erosion and work as buffer, reducing fary of violent storms and typhon (Mall 1985). Due to unfavorable climatic and edaphic conditions species growing along coastline in India constitute one of the best coastal vegetation of the world (Armugum *et al.* 1997). With the protective role, mangroves are used as important and potentially sustainable source of energy in the form of wood and charcoal to meet the increasing needs of developing countries for domestic fuel (Untawale 1998). Some species of mangroves are used as source of food, fodder and medicine (Sathe 1991, Bhosale 1998). *Sonneratia* is one of the mangrove genus used as source of food. There are some reports indicating its edible properties (Tanaka 1976, Kurlapkar 1993). *Sonneratia* is represented by three species, viz. *S.alba*, *S.apetala* and *S. caseolaris*, from which *S.apetala* and *S. caseolaris* are eaten either cooked or raw (Naskar and Guhabakshi 1987). Untawale (1998) also reported that the leaves of *Sonneratia* are used as salads.

As the species has edible properties, it was thought to study its biochemical components. Starch being an energy reserve in most of the plants, it has great economic importance. It is used in various forms as a food and in preparation of gels, pastes, adhesives, dried coating films and other high molecular weight polymers for wide range of industrial purpose (Manner 1973). Despite the great variety and rapid distribution of starch supply only a limited number of products are used for manufacture of starch on an industrial scale (Wood 1959). To know the potential of *Sonneratia*, the starch from the fruits was isolated and some of the important parameters like iodine-potassium iodide staining test, granule size and gelatinization temperature has been studied for the

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comparison and characterization. Furthermore, the isolated starch was analyzed by X-ray diffraction and further studied by comparing it with standard laboratory starch (s. d. fine Chem.), isolated potato starch (as source of edible starch) and available literature data. The particular advantage of diffraction analysis is it discloses the presence of substance as the substance exists in that sample (Cullity 1978). This technique has wide application in many diverse areas as a tool to determine the local atomic environment in many classes of material for structure defined analysis by standard technique of diffraction (Gauns 1996).

MATERIALS AND METHODS

For the analysis, the fruits of *Sonneratia* species were collected from different sites. Fruits of *S.alba* and *S.apetala* were collected from Achara and *S. caseolaris* was collected from Nerurpar estuary, in the month of October, November and May respectively to collect the mature fruits as per the phenophases. The mature fruits of all the three species were collected and immediately brought to the laboratory and processed further for analysis. The starch was isolated from the mature fruits, following the method as described by Badenhuizen (1964). The isolated starch powder was dried in oven at 50^o C (up to dryness) and stored in freeze at 4^o C in closely packed glass bottle. Starch- iodine-potassium iodide test is carried out by routine staining method, but this method fails to give blue colour with iodine-potassium iodide, so the method of Daniel (1991) was followed. The granule size of the starch powder was studied microscopically. The micrometry technique was employed for further measurement. Gelatinization temperature was determined by the method of Daniel (1991). The X-ray diffraction analysis was carried out on Phillips/panalytical X-ray diffractometer -3710. Standardization of this method was achieved by running laboratory starch (s.d.fine chem.) and isolated potato starch (isolated following the method of Badenhuizen 1964). 2 θ and 'd' values were studied with samples and compared with the literature (Jude ® 5.0 database, material data, Inc. Livermore, CA, USA and Manzacco *et al.* 2003). Further the monomer units of starch powder were identified comparing with standard obtained from computer software (JCPDC- internal centre for diffraction data, USA (1977) on X-ray diffraction.

RESULTS AND DISCUSSION

Starch- iodine-potassium iodide test: Colour is an important indicator of starch, when stained with iodine. The starch when stained with iodine-potassium iodide, it gives an intense blue colour. The colour of starch with iodine-potassium iodide is dependent on amylose-amylopectin concentration. In the present study, the method described by Daniel (1991) was followed, as the starch isolated from fruits of *Sonneratia* fails to give blue colour with iodine-potassium iodide. Daniel (1991) method showed that the colour of starch is dependent on the concentration of amylose in the starch. If the amylose concentration is more it gives dark blue colour. But if this concentration is lowered (ca. 8 %) in starch, then colour of starch appear red. By following this method, the starch obtained from the fruits of *Sonneratia* was analyzed, which showed different colour indicating variation in the concentration of amylopectin in the starch. It is observed that in *S.alba* the colour of starch was dark reddish which became light in *S. apetala* and *S. caseolaris*.

Pomeranz and Meloan (1971) suggested that amylopectin gives red or violet red colour with iodine while amylose gives intense blue colour. Whistler and Corbett (1957) shows amylopectin solutions are blue violet or purple coloured, while Akazawa (1965) stated that the branched amylopectin chains stains purple to red. As there is variation in colour, the amylose- amylopectin concentration was further confirmed by estimating the amylose percentage of starch (Table 1). The lowest percentage is in *S. alba* as compared to other species. It is more in *S. caseolaris*. These results are supported by the work of Mulik and Bhosale (1983). They suggested that, iodine test for starch always does not give blue colouration but the staining may be faint or even

Table 1. Percentage of amylose in isolated starch from fruits of *Sonneratia* species

Species	Powder	Fruit
Sonneratia alba	0.26	29.36
Sonneratia apetala	0.33	8.25
Sonneratia caseolaris	0.49	35.06

reddish brown indicating the presence of other polysaccharides.

Granule Morphology: Starch isolated from fruits of *Sonneratia* further studied for its granule size. It was observed that, the three species showed remarkable variation regarding the size of the granule. According to Akazawa (1965) the different species have different starch granule, varying in characteristics such as size, shape and structure of hilum. Manner (1973) also stated that, the starch granules are organized structures, which vary in both size and shape. In the present investigation, it was observed that, the granules of *S.alba* fruit starch are comparatively larger in size. It ranges between 7.4 to 8.0 ìm, while the granules of *S.apetala* are smaller with 1.85 to 2.0 ìm, and that of *S.caseolaris* was 3.7 to 4.0 ìm (Table 2). The granule size is dependent on species and is species specific. Danial (1991) studied granules from other starch sources and showed variation in size and shape and observed that the size of potato granules was about 45-70 ìm, while the rice granules are minute having 6 ìm diameter. Manner (1973) also reported the granules of potato, wrinkled peas and certain seeds with various sizes.

Table 2. Granule size and gelatinization temperature range of starch isolated from fruits of *Sonneratia* species

Species	Granule size (µm)	Gelatinization temperature (^{O}C)	
		Lower limit	Upper limit
Sonneratia alba	7.4 to 8.0	57	61
Sonneratia apetala	1.85 to 2.0	61	63
Sonneratia caseolaris	3.7 to 4.0	58	62

Gelatinization: Atwell *et al.* (1988) defined the term gelatinization as the collapse of molecular orders within the starch manifested in irreversible changes in properties such as granule swelling, native crystalline melting, loss of birefringence and starch solubilization. Heating starch granule in water initiates series of irreversible changes in which the native starch granule first swells. Then at critical temperature it undergoes a rapid gelatinization with many folds increase in volume and total loss of native granule organization (French 1975). The gelatinized starch is used as a thickener, as an adhesive and for sizing of textile and paper (French 1975). Since, each starch is having a definite temperature range when gelatinization occurs, the gelatinization temperature is used in the identification of starches from different sources (Daniel 1991).

The starch isolated from the fruits of *Sonneratia* was used for determining the gelatinization temperature. It was observed that the gelatinization temperature range for each species varies considerably. The range for *S. alba* is 57-61 ^{o}C, in *S. apatala* it is 61-63 ^{o}C, while *S. caseolaris*, starch showed its gelatinization at 58-62 ^{o}C temperature. The range for *S.alba* and *S.caseolaris* is slightly broader and up to 4^{o}C while in *S.apetala* fruit starch it is narrow (2^{o}C). The range of temperature for gelatinization of *Sonneratia* fruit starch is comparatively lower as compared with other starch sources. It was reported that the range of temperature for gelatinization as 8-10 ^{o} C. The starch from the wheat get gelatinized at temperature of 64-65.5 ^{o} C, maize gelatinized at 62-71^{o} C temperature, while the lower and upper limit of gelatinization of potato is 66^{o} C.

X-ray diffraction analysis: The starch isolated from the fruits of *Sonneratia* was used for X-ray diffraction studies. The X-ray diffraction pattern showed variation in the three species. From the diffraction peaks the intensive peaks were identified and used for standardization following the procedure described by Cullity (1978). Cullity (1978) gives some suggestions regarding the comparison of experimental data of unknown substance/ molecules. As per his suggestions, in comparing experimental and tabulated (known samples) ‘d’ values, the set of ‘d’ will be allowed for comparison with an error of ± 0.01A^{o} (error between ‘d’ values of known and unknown samples). Though one substance has more than one same or nearby‘d’ values. The‘d’ value can be acceptable at two similar or nearby values. Hanawalt *et al.* (1978) is of the opinion that, in characterization of any substance, three strongest/ nearer values are sufficient for to study the pattern of unknown material. Further according to Cullity (1978), if there is any doubt about the validity of particular identification, own standard pattern of known samples can be obtained by maintaining the same experimental condition and the data can be compared. This comparison of two patterns will furnish positive proof of identity. By using this method, the laboratory starch and isolated potato starch run simultaneously as a standard samples for comparison. The comparison of X-ray diffraction pattern of the laboratory starch, isolated potato starch and *Sonneratia* starch was made on the basis of 2 θ and 'd' values. Table 3, shows the comparison of 2 θ angles. It is observed that, the laboratory starch showed maximum 12 values for 2 θ angle. This angle is compared with the samples. Potato starch showed one nearby value, while *Sonneratia* fruit starch, from which *S.alba*, *S. apetala* and *S. caseolaris* showed 9, 7 and 9 values closer to that of laboratory starch. This showed that the isolated fruit starch from *Sonneratia* species has comparative angle 2è with laboratory starch. Out of the three isolated fruit starch sample, *S.caseolaris* fruit starch showed more values which are almost nearer with laboratory starch is significant. The'd' values of all sample were compared as per suggestion of Hanawalt *et al.* (1978). According to him, the d' value with three strongest line i.e. d_1, d_2 and d_3 are sufficient to characterize the pattern of unknown sample. It was observed that the'd' values of all the *Sonneratia* fruit sample showed more than three strongest line comparable with laboratory starch and this was used for confirmation of fruit starch. Table 4, shows that the laboratory starch has 12'd' values, and all the three studied species shows 8 values comparable with laboratory starch indicating its positive resemblance. According to Cullity (1978) the'd' values with an error of ±0.01A^o is allowed for comparison.

The present results of X-ray diffraction analysis were compared with some other results on X-ray diffraction analysis of starch. Especially the results of potato starch were used for comparison and observed that the results of present investigation were comparable with earlier reports. This comparison is shown in table 5. Least peaks are observed in isolated potato starch. Attempt was also made to identify the monosaccharide units from the studied samples (Table 6) with the help of computer software JCPDS. There are 5 monomer sugars identified i.e. hydrogen tartarate hydrate, D- trehalose dihydrate, D- maltose hydrate, D-fructose and D-galactose. From these 5 sugars, laboratory starch showed 4 sugars, potato starch showed the presence of 3 sugars while the *Sonneratia* fruit starch showed 4, 3 and 3 monomers units in *S.alba*, *S. apetala* and *S.caseolaris* respectively. Out of these identified sugars hydrogen tartarete hydrate and D- trehalose are common in the starch samples.

Table 3. Comparison of angle 2 θ of isolated *Sonneratia* fruit starch with laboratory (s. d. fine chem.) and isolated potato starch

Laboratory starch	Potato starch (isolated)	*S. alba*	*S. apetala*	*S. caseolaris*
21.355	–	21.565	21.380	–
32.125	–	32.275	32.275	32.150
36.780	36.325	–	36.685	–
42.460	–	42.565	42.645	42.475
47.985	–	48.100	–	48.015
49.735	–	48.875	–	48.775
51.635	–	49.925	–	–
61.455	–	61.665	61.690	61.765
67.325	–	67.600	67.380	67.310
70.130	–	–	–	70.115
71.560	–	71.700	71.680	71.500
89.410	–	–	–	89.560

Table 4. Comparison of d value of isolated *Sonneratia* fruit starch with laboratory (s. d. fine chem.) and isolated potato starch

Laboratory starch	Potato starch (isolated)	*S. alba*	*S. apetala*	*S. caseolaris*
6.1790	–	6.1195	6.1719	–
4.1377	–	4.1190	4.1190	4.1346
3.6289	3.6728	–	3.6379	–
3.1616	–	3.1541	3.1485	3.1605
2.8155	–	2.8092	2.8087	2.8139
2.7225	–	2.7673	–	–
2.6288	–	–	–	–
2.2406	–	2.2337	2.2329	2.2305
2.0654	–	2.0580	2.0639	2.0658
1.9928	–	–	–	1.9931
1.9581	–	1.9548	1.9553	1.9595
1.6275	–	–	–	1.6253

Table 5. Standardization and comparison of potato starch with available literature (for d value) for X-ray diffraction.

Sample	Wheat starch	Wheat flour	Corn starch	Potato starch	Potato starch (isolated)
'd' spacing (A^o)	3.85	3.84	3.79	3.71	1.88
	4.41	4.43	4.37	4.00	3.67
	4.86	4.87	4.87	4.55	3.96
	5.10	5.10	5.18	5.15	5.21
	5.81	5.84	5.79	6.15	

Table 6. Monosaccharides identified from different starch powder samples by X-ray diffraction analysis

Sugar identified	Chemical formula	Laboratory Starch	Potato starch	*S. alba*	*S. apetala*	*S. caseolaris*
Hydrogen tartarate hydrate	$C_4H_6O_6.H_2O$	+	+	+	+	+
D-trehalose dihydrate	$C_{12}H_{22}H_{11}.2H_2O$	+	+	+	+	+
D-maltose hydrate	$C_{12}H_{22}O_{11}.H_2O$	+	-	-	-	+
D-fructose	$C_6H_{12}O_6$	-	+	+	-	-
D-galactose	$C_6H_{12}O_6$	+	-	+	+	-

+ indicates presence of sugar

From the above results it is concluded that the starch isolated from fruits of *Sonneratia* has structural similarities with the other known starch samples. Thus it may be used as the source of edible starch.

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