Physico-Chemical Characteristics of Starches from Sal *(Shorea robusta)* and Dhupa *(Vateria indica)* Seeds

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Pure, white starches were isolated in ~30% yields from defatted sal and dhupa meals. Both starches consisted of granules of varying size and shape characteristics, and contained considerable amounts of protein and lipid constituents. C 18:1, C 16:0 and C 18:0 were the major fatty acids present in both free and bound lipid fractions; whereas the latter in addition contained C 18:2 (~20%). Both starches exhibited two-stage swelling in water; for sal starch the solubility was markedly lower but its swelling power was considerably higher. In DMSO the sal starch was readily soluble but not dhupa starch (only ~20% solubility). The hot paste viscosity as well as set back viscosity of dhupa starch was much higher in comparison to those by sal starch. Very highly purified sal starch virtually exhibited no hot paste viscosity, and behaved like amylopectin-rich material. The latter had only 1.5% amylose as against of ~24% in original sal starch. X-Ray powder patterns revealed sal starch to be of A-type and dhupa starch to be of B-type. Both the starch granules were susceptible for *in vitro* attack by human salivary α-amylase.

Physikalisch-chemische Eigenschaften der Stärken aus Sal- (Shorea robusta)- und Dhupa- (Vateria indica)-Samen. Reine weiße Stärken wurden in 30%iger Ausbeute aus Sal- und Dhupa-Mehlen isoliert. Beide Stärken bestanden aus Körnern verschiedener Größen- und Gestaltscharakteristika und enthielten beträchtliche Mengen an Protein- und Lipidbestandteilen. C 18:1, C 16:0 und C 18:0 waren die hauptsächlichen, sowohl in freien als auch in gebundenen Fettfraktionen, wobei die letztere zusätzlich C 18:2 enthielt (ca. 20%). Beide Stärken zeigten zweistufige Quellung in Wasser. Die Löslichkeit von Salstärke war bedeutend niedriger, jedoch war ihr Quellvermögen erheblich höher. In DMSO war Salstärke gut löslich, die Dhupastärke jedoch nicht (nur ca. 20% Löslichkeit). Die Heißpastenviskosität von Dhupastärke sowie die „set back"-Viskosität von Dhupastärke war wesentlich höher im Vergleich zu denen der Salstärke. Sehr hoch gereinigte Salstärke zeigte keine wesentliche Heißpastenviskosität und verhielt sich wie amylopektinreiches Material. Letzteres hatte nur 1,5% Amylose gegenüber ca. 24% in der ursprünglichen Salstärke. Das Röntgen-Pulvermuster zeigte, daß Salstärke zum A-Typ und Dhupastärke zum B-Typ gehört. Beide Stärkekörnerarten waren *in vitro* durch menschliche Speichel-α-Amylase angreifbar.

1 Introduction

Starch is the principal dietary carbohydrate of a majority of legume and cereal-based foods. In addition to its dietary significance, the primary role of starch, from the technological point of view, is its ability to dictate or modify the texture and consistency of finished food products [1]. The modern food processing industries are increasingly dependent on the use of both native and modified starches (and gums as well) for the manufacture of various fabricated foods. As a result there is a growing demand to look for alternative-new sources of starch from abundantly available but so far under-utilized raw materials, for use in both food and non-food industries. The present communication is concerned with the isolation and physico-chemical characterization of two new sources of starch derived from sal *(Shorea robusta)* and dhupa *(Vateria indica)* seeds. Sal and dhupa are forest trees and their seeds contain oil to a considerable extent, which find commercial applications, for example, as substitute for cocoa butter in chocolate manufacture and in confectionaries [2, 3]. The defatted seed meal is a waste by-product, sometimes posing disposal problems. In a recent communication some of the physico-chemical properties of sal starch are reported [4].

2 Materials and Methods

2.1 Materials

Sal *(Shorea robusta)* and dhupa *(Vateria indica)* seeds were obtained from Orissa Oil Industries Ltd., Sambalpur, Orissa; and National Education Society, Village Industries Section, Shimoga, Karnataka, respectively.

2.2 Isolation of Starch

2.2.1 Defatting Process

The seeds were powdered in a plate grinder to pass through 60 mesh sieve and repeatedly (4×) extracted with a mixture of hexane-$CHCl_3$-CH_3OH (1:2:1, v/v/v) at reflux temperature.

2.2.2 Decolourization Process

The defatted materials were successively treated with chlorine water (1%), sulphurous acid (0.5%), hydrogen peroxide (0.5%) and finally with acidic organic solvents and then air dried.

2.2.2.1 Crude starch recovery

The defatted and decolourized materials were steeped in water containing $HgCl_2$ (100 ppm) for 16 h at room temperature and then mascirated in a Waring Blender. Crude starch granules were separated by filtration through 200–240 mesh sieves, and centrifuged (5000 r. p. m., 10 min).

2.2.2.2 Starch purification

The crude starch granules were successively treated, with dil. NaOH (0.1 M, 5 min at room temperature) and NaCl (0.1 M)-toluene (10:1, v/v). The treatments were repeated four times, and after each treatment the granules were sedimented by centrifugation and the sediments were thoroughly washed with water. The final sediment was washed twice with methanol and dried by solvent exchange.

2.2.3 Viscosity determination

2.2.3.1 The relative viscosity (η_r) of starch solution (0.5% in 1 N KOH) was determined in an *Ostwald* capillary viscometer. The intrinsic

viscosity η_i (dl/g) was computed by $\ln \cdot \eta_r/0.5$.

2.2.3.2 The hot paste viscosity as well as set back viscosity of the starch slurry (5 or 10%) was measured (in B.U) in a Brabender amylograph model E with the following temperature programme:

$$50°C \xrightarrow[1.5°C/min]{} 95°C \xleftrightarrow[20\ min]{} 95°C \xrightarrow[1.5°C/min]{} 50°C.$$

Samples were withdrawn at regular intervals and viewed under microscope for determining the (initial, middle and final) gelatinization temperature range, based on the loss of birefringence of the granules.

2.2.4 X-Ray studies

The X-ray powder patterns of pure starch granules were taken with Carl-Zeiss *Debye-Scherrer* powder camera of 114.6 mm diameter. The granules were moistend with distilled water and then exposed for 5 h at 10 Å/30 kW to Co-K_α filtered radiation. Diffraction angle measurements were done using a Carl-Zeiss glass scale with an accuracy of 0.1 mm.

2.2.5 Lipid analysis

The free lipids were extracted with water saturated 1-butanol and the covalently bound lipids in the defatted granules were extracted with hexane-$CHCl_3$ (1:1) after prior acid hydrolysis (2 N HCl, 100°C, 2 h). The constituent fatty acids in the respective lipid fractions were simultaneously liberated and esterified by refluxing with methanolic hydrogen chloride (4 M) for 2 h. The resulting fatty acid methyl esters were analysed by Packard model 427 GLC fitted with flame ionization detector and 10% DEGS (on Chromosorb W) column (stainless steel, 8 ft × 1/8″ o. d.) at 180°C (isothermal).

2.2.6 *In vitro* digestibility of native and gelatinized starch

Starch (100 mg) was gelatinized by boiling with water (10 ml) followed by the addition of acetate buffer (0.1 M, pH 4.6, 10 ml). To the resulting suspension incubated at 55°C was added amyloglucosidase (380 units/mg starch, Sigma Chemical Co., USA) and left for 4 h. Later it was found to be starch negative (I_2-KI reagent). An aliquot (0.1 ml) of this solution was then estimated for glucose content by the glucose-oxidase method. The true estimate of starch was obtained by 0.9 × glucose content.

2.2.7 Assay of salivary α-amylase

Corn starch granules (600 mg) were gelatinized in boiling water (15 ml) for ~30 min followed by the addition of 0.04 M phosphate buffer (15 ml, pH 6.9 containing 0.013 M sodium chloride). Freshly drawn human saliva (1 ml), equally diluted with phosphate buffer, was added to this and incubated at 37°C for 10 min. Ethanol (3 vol) was then added and centrifuged. One unit of amylase activity was defined as the amount of enzyme required to release 1 μmole of maltose per min under the above conditions.
Raw starch granules were digested by suspending them (100 mg) in 0.02 M phosphate buffer having 6.7 mM NaCl (pH 6.9, 100 ml) and incubating at 37°C with freshly drawn human salivary α-amylase (0.5 ml enzyme in 0.5 ml phosphate buffer), ~35 units per mg starch. At regular intervals aliquots (5 ml) were withdrawn and added to ethanol (10 ml), centrifuged and the supernatant was assayed for reducing sugar content.

2.2.8 Miscellaneous methods

Swelling power and solubility behaviour in water and DMSO, ionic character, and estimation of amylose content were all done by the methods reported earlier [5].

3 Results and Discussion

Sal and dhupa seeds contain ~20% oil, which finds use in both food and non-food industries [6]. The defatted seed meals were found to contain considerable amount of starch (~40–50%). Since this material was too highly coloured a method was devised for its decolourization and isolation of pure white starch. The method involved successive treatments with organic solvents and a variety of oxidising-bleaching agents over a period of time. Although the method was a bit laborious it finally yielded pure white starch in good yields (~ 25–30% of the defatted material), which was further purified by the usual methods.
Both the starches contain small spherical to big oval shaped granules of varying size (see Table 1 and Fig. 1). There exists a range of granule populations of different sizes. The hilum and striations were clearly observable in most of the granules, and all granules exhibited characteristic birefringence under polarized light (see Fig. 1). Except a few majority of the granules appeared smooth. Comparatively the dhupa starch granules were bigger in size than those of sal starch granules. The gelatinization temperature range of sal starch was higher, indicating probably, a tight macromolecular association of the granule constituents. This was in agreement with the earlier data [4].

Table 1.
Characteristics of Sal and Dhupa Starches.

	Sal	Dhupa
Yield (%)	28	31
Granule characteristics		
Shape	Round-oval	Oval
Size (μm)	7.5–10	20–25
G. T. Range (°C)	74–86	69.5–80
Starch content (%)		
By enzymatic method	93.0	87.8
By Phenol-H_2SO_4 method	90.0	92.0
Protein (%)*	3.9	0.5
Lipids, free lipid	1.1	1.2
bound lipid (%)	2.9	2.8
Amylose content (%)	24.0	22.0
X-Ray type	A	B
Viscosity		
η_r	1.76	1.62
η_i (dl/g)	1.13	0.97

* By micro-Kjeldahl, N × 6.25.

The protein content in sal starch was more (~3.9%) than that of dhupa starch (~0.5%). It is not known whether the protein is a contaminant or covalently bound to the starch molecule. The glycoprotein nature of starch has recently been reported, which might have many biological-physiological implications [7]. Both the starches were non-ionic like those of legume and cereal starches [8]. The amylose content of the starches ranged between 22–24%. The λ_{max} of the blue-coloured complex derived from sal starch was 600 nm, whereas the λ_{max} of that of dhupa starch was around 585 nm. Whether such subtle variations of absorption maximum are due to fine differences in the overall make up-architecture of starch components remains to be elucidated.
In Figs. 2 and 3 are presented the swelling power and solubility in water and DMSO of sal and dhupa starches, respectively. Both of them exhibited a two stage swelling behaviour like some cereal starches [9]. However, the solubility of sal starch was markedly lower, but its swelling power was considerably higher (over 70% at 90°C). At 90°C only 10% solubles were

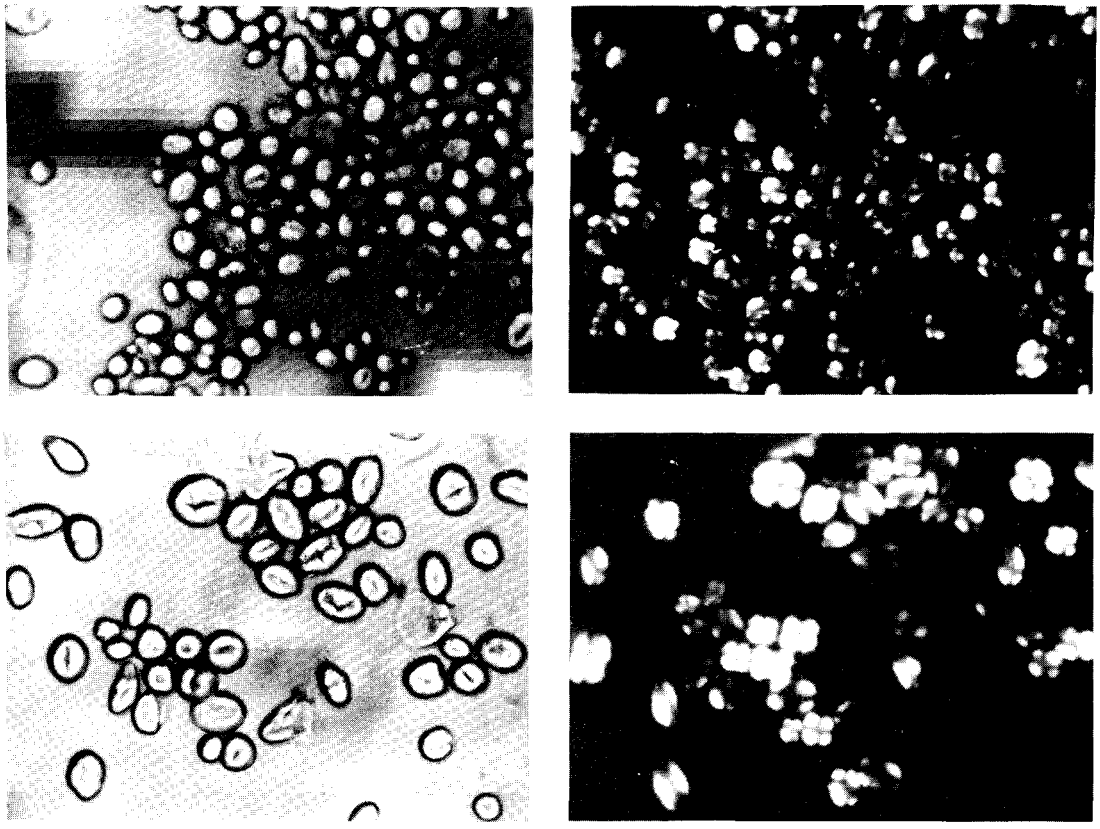

Fig. 1 Light and polarized photomicrographs (× 100) of sal (a) and dhupa (b) starch granules.

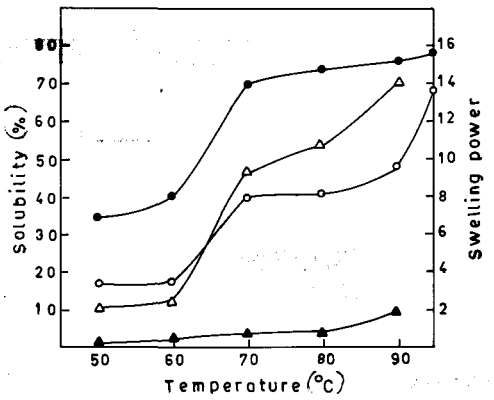

Fig. 2. Swelling power (−△−/−○−) and solubility behaviour (−▲−/−●−) of sal and dhupa starches, respectively, in water.

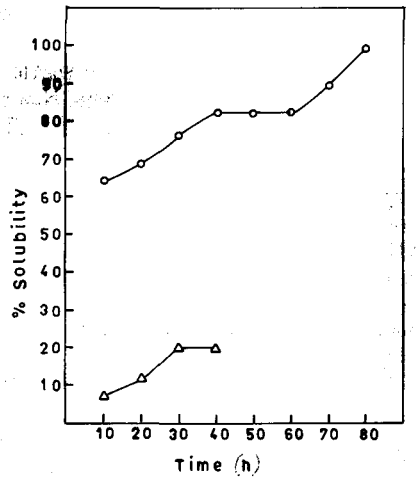

Fig. 3. Solubility (%) of sal (−○−) and dhupa (−△−) starch in DMSO.

recovered from the sal starch indicating a very slow relaxation of the bonding forces within the granule. Nevertheless, the granules imbibed a high degree of swelling in water. On the contrary, dhupa starch showed over 70% solubility at around 90°C and its swelling power was more or less comparable to that of sal starch. It is likely that the inter- and intra-molecular association forces were different in dhupa and sal starch. In consonance with this, the dhupa starch showed only 20% solubility in DMSO even after 72 h and later on became a soft gelly like mass, not at all sedimentable even at 10,000 rpm for 40 min, whereas the sal starch was readily soluble in DMSO (see Fig. 3). Thus, it appears very likely that the polymer-polymer interactions as well as intra-molecular bonding forces within these starch granules are of entirely different magnitude. Such associative and bonding forces are labile in the case of sal starch and are therefore easily permeable to the solvent molecules. This property is reminescent of that of pigweed starch [10], corn starch [10] and varagu starch [11]. Previous report on sal starch has also indicated its low and restricted swelling despite the fact that at any particular degree of swelling the sal starch was more soluble [4]. This was explained as due to low molecular weight amyloses being loosely associated and therefore getting released during the swelling process [4].

The hot paste viscosities of the two starches as determined in Brabender amylograph are shown in Fig. 4. Comparatively, dhupa starch showed considerable peak viscosity as well as set back viscosity than the sal starch. The latter showed very moderate PV and SBV on cooling. However, the starch paste appeared to be stable during continued cooking and holding at 95°C in the case of sal starch than dhupa starch. The latter showed some tendency to breakdown during continued cooking. However, in a separate experiment, where the sal starch was extensively purified (~95%) by repeated bleaching treatments, it was found that the starch virtually showed no hot paste viscosity and it behaved like waxy starch having little (or no) amylose. It is plausible that such high purification steps have resulted in some degree of modification or leached out the

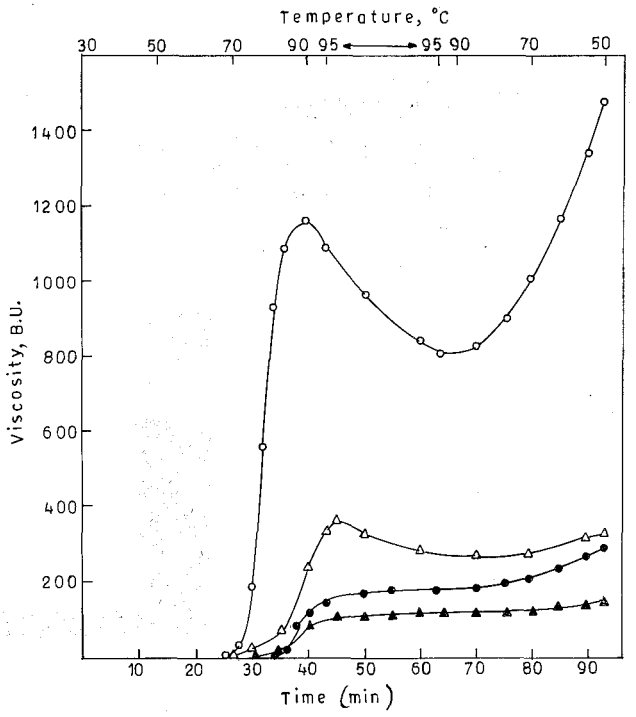

Fig. 4. Brabender amylogram, at 10% concentration, of starches (–●–/–○–) and flours (–▲–/–△–) from sal and dhupa, respectively.

linear (amylose) component or both, and has yielded an amylopectin- rich fraction [12]. Interestingly the amylose content of the highly purified starch was only ~2% and its starch content as estimated enzymatically was ~95%. In Fig. 4 is also depicted the viscosity pattern of sal and dhupa flours.

The relative viscosity of dhupa starch was less in comparison to sal starch. The η_i was however greater by a few degrees of magnitude for sal starch.

X-Ray diffraction patterns at 5.69, 4.73, 3.57 and 3.26 Å indicated sal starch to be of A-type, whereas strong characteristic lines at 1.82, 2.18, 2.56, 2.78, 3.34 and 3.79 Å revealed dhupa starch to be of B-type [13]. In addition to these lines, more reflections at the higher angle region were diffused and weak indicating probably poor crystallinity in the starch granules.

The fatty acid composition of free and bound lipid fractions of sal and dhupa starches are given in Table 2. Quantitatively bound lipids were more than free lipids. The free lipid content of sal starch reported earlier was only ~0.8%, as this was only a partial estimate of free lipid fraction because the solvents used for its extraction were comparatively milder [4], unlike the water saturated 1-butanol (or 1,4-dioxan), used in the present study, which is known to extract quantitatively most of the surface and firmly bound lipids from starch granules. Analysis of these lipid fractions by GLC as methyl esters revealed the preponderance of C 18:1, especially in dhupa starch free lipid fraction it was present to the extent of ~70%. The next major fatty acid present was C 16:0 (~20%) followed by C 18:0. Interestingly C 18:2 was found only in the bound lipid fractions of both starches (~28%). In addition the sal starch contains traces of C 18:3, a highly unsaturated fatty acid.

C 18:0 followed by C 18:1 were the major fatty acids in the total fat extracted from sal [14] and dhupa [15] seeds. C 16:0 was present to the extent of ~10%. The ratio of unsaturated to saturated fatty acids was 1.59 and 1.05 for sal and dhupa seed fats, respectively. A special feature of sal fat was the presence of epoxy-stearic acid, ~1.2% [16].

Such high lipid contents, particularly the internal lipids, do play a part in amylose complexing (as inclusion compounds) and they modify many of the physico-chemical characteristics, such as gelatinization temperature range, swelling and solubility behaviour, and also their susceptibility for hydrolysis by enzymes [17]. It is suggested that the lipid molecules could act as a template for the construction of the amylose helix [18]. The internal lipids may have a role in starch biosynthesis of non-waxy cereals.

From Table 3 it may be deduced that both starches in their raw, ungelatinized form are quite amenable for amylolysis with salivary α-amylase. The percent hydrolysis value by this enzyme ranged from ~3.5 to 70% for these starches at 37°C over a period of 0–72 h. Cereal but not legume starches are similarly reported to be easily susceptible for amylolysis even in their native state. The ability to quantitatively breakdown raw, ungelatinized starch by amylases *in vitro* is an important development in starch technology, as it saves energy (required for prior starch gelatinization) and thereby helps effective utilization of biomass.

Table 2.
Qualitative and Quantitative Analyses of Fatty Acids Present in the Lipid Fractions of Sal and Dhupa Starches.

Starch	Lipid fra.	Yield* (%)	Fatty acids identified (% wt)							
			12:0	14:0	16:0	18:0	20:0	18:1	18:2	18:3
Sal	Free	1.13	0.4	1.4	18.6	18.6	5.3	55.5	–	0.1
	Bound	2.89	0.9	1.6	19.2	10.6	0.7	39.6	27.5	–
Dhupa	Free	1.19	0.1	0.6	15.9	12.3	1.0	70.1	–	–
	Bound	2.78	0.3	1.3	20.0	9.3	0.5	40.2	28.3	–

* calculated per 100 g dry weight.

Table 3.
In vitro Amylolysis of Sal and Dhupa Starch Granules.

Starch	Incubation with salivary α-amylase										
	0	0.25	0.5	1.0	2	4	8	16	24	48	72 h
Sal	3.4	6.7	8.6	10.3	14.2	19.1	27.9	45.5	57.3	68.7	69.1
Dhupa	3.5	19.1	24.6	29.9	37.7	46.2	54.7	63.5	68.1	73.0	74.6

The values represent percent maltose released.

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