



Trends in
**Applied Sciences
Research**

ISSN 1819-3579



Academic
Journals Inc.

www.academicjournals.com

Preparation and Properties of High Degree Substituted Acetylated Rice Starches and Preparation of Their Films Part 1

¹Vasudeva Singh, ²Seiichiro Isobe, ²Hidechika Toyoshima,
²Hiroshi Okadome and ²Ken'ichi Ohtsubo

¹Department of Grain Science and Technology,
Central Food Technological Research Institute, Mysore-570013, Karnataka, India
²National Food Research Institute, Kamondai, 2-1-2, Tsukuba, Ibaraki 305-8642, Japan

Abstract: Starches from three varieties of rice of high amylose (indica), intermediate amylose (japonica) and almost negligible amylose (japonica waxy) were isolated. They were acetylated by using four times their weight of acetic anhydride (11% sodium hydroxide as 50% aqueous solution as catalyst) for 15, 30 and 60 min. The acetyl content varied from 30 to 41% and degree of substitution (DS) from 1.63 to 2.55. Under similar conditions of acetylation, waxy rice starch showed highest acetyl content and highest DS. Dimethyl formamide was the solvent used for dispersing these modified starches. Films of indica modified starch of 30% acetyl content and 1.63 DS showed highest strength compared to other varieties of modified starch. Glass transition temperature (T_g) was highest in waxy rice starch (~ 232°C) followed by japonica non-waxy and indica rice starch. After acetylation, the T_g values increased in waxy modified ones, remained almost same in indica modified ones, decreased to some extent in japonica non-waxy modified ones compared to their respective native starches. Difference in specific heat capacity between starting and ending of glass transition point of indica and its modified ones remained almost same, increased in japonica and its derivatized ones, decreased in waxy modified one compared to their respective native starches. Among the native starches, melting point was highest in waxy starch. Modification increased this property in all the starches under all periods of acetylation. Increment was highest in japonica waxy acetylated ones. Degradation or decomposition of these starches occurred at 3-10°C higher than their respective melting points. Heat evolution was very high in japonica native (~ 203 mJ mg⁻¹) and heat absorption was highest in indica 15 min modified one (~ 265 mJ mg⁻¹). Difficulties encountered while determining the dynamic viscosity using the equipment of the synthetic polymer have been explained.

Key words: Degree of substitution, % Acetyl content, glass transition temperature, tensipresser, flow tester, dynamic viscosity

INTRODUCTION

Generally all over the globe research is in progress with regard to preparation of biodegradable plastics by making use of various kinds of starches, popularly corn starch, followed by high amylose corn starch and to some extent different types of starches like tuber (potato), root (cassava/tapioca) depending on the availability of the commodities in the respective places. Lawton (1996) prepared films by cooking native starches and other ingredients in a jet cooker, poured them on a heated glass

Corresponding Author: Vasudeva Singh, Department of Grain Science and Technology,
Central Food Technological Research Institute, Mysore-570013, Karnataka, India
Tel: 91-821-2510843 Fax : 91-821-2517233

plate and exposed them to different relative humidities and he concluded that high amylose corn starch is the best. Generally amylopectin and whole starches are acetylated to form triester of degree of substitution (DS) 3 and was found to form brittle film (Jarowenko, 1986). Mullen and Pacsu (1942) prepared different esters from root, tuber and cereal starches. They studied even rice starch. But the details of these have not been touched by them. Triester of corn starches were prepared by Wolf *et al.* (1951). They used various fatty acids and informed that acetic anhydride was the best one for acetylation of starches. They prepared acetates using pyridine as a catalyst which presently is not economical. Mark and Mehlretter (1970) prepared acetylated high amylose corn starch by acetic anhydride and pyridine method. Mark and Mehlretter (1972) had prepared the acetylated starches by making use of alkali as a catalyst, but duration of the experiment was about 4 h to get higher degree substituted starches. Shogren (1996) prepared high amylose corn starch acetate of higher DS (around 2.5) by using alkali as a catalyst. Singh (1996) isolated starches from various cereals, pulses and acid modified them and studied their properties at granular and molecular level. Singh and Ali (2000) have also studied the starch types and their degradation upon acid modification. Singh *et al.* (2000) have studied the thermal and physico-chemical properties of rice grain, flour and starch. Singh and Ali (2006) have also studied the comparative changes between acid and enzyme modified starches and reported that enzyme modification is always fast. Singh *et al.* (2006) have also studied the crystalline changes of native and different acid modified starches from various food grains. As corresponding studies on various kinds of rice starches for the preparation of high degree substituted acetylated starches are not available, this experiment was planned to isolate starches from different varieties of rice, acetylate them to higher degree using an economically feasible catalyst and study their properties for the preparation of biodegradable plastics/films.

MATERIALS AND METHODS

Materials

Commercial Thailand rice (indica) was procured from market. Brown rices of Japonica viz., Nipponbare and Japonica waxy viz., Himenomochi were procured from the near by Agricultural Research Station which was stated to be stored around 5 to 6°C, harvested in 1997. All chemicals were of analytical grade and for all the studies deionised water was used, unless otherwise stated.

Methods

Brown rice was polished to about 10% degree of polish or 90% milling yield using a Yamamoto rice polisher viz., Rice Pal 31, by keeping a flow rate of 4 and resistance at 3. Thailand rice was slightly polished, as it was available as polished rice. Starch was isolated from these rice varieties by following the procedure of Singh and Ali (1987). The wet residue prepared was dried at 40°C for about 18-24 h with stirring the mass at regular intervals in order to dry uniformly. It was ground using a Retsch centrifugal ultra mill using 1 mm screen. Protein content of these starches were estimated by micro Kjeldhal method, which varied from 0.2-0.9% (w.b). Careful steps were taken to isolate starch from japonica rice. Amylose equivalent or amylose content in these starches were estimated as per the procedure of ISO 6647 (1987).

Acetylation

To start with 50 g (w.b) starch of around 11% moisture content was taken. Depending on the moisture content, its weight was converted to dry basis. Acetic anhydride, four times of this weight, was taken. Around 178 g acetic anhydride (~165 mL) was taken in a round bottom flask. The contents were stirred with a bead and starch was added slowly. This round bottom flask was placed in a heating mantle (100 volts, 400 watts, maximum temperature 400°C). It was fixed with a 3 necked

stopper, for fixing the mechanical stirrer, reflux condenser and a thermometer with the latter two, on both the sides of mechanical stirrer. Sodium hydroxide (11%, w/w starch) catalyst was added as 50% aqueous solution, drop by drop with constant stirring. Heater was put on, the regulator of the mantle was adjusted between 2.5 and 3.0. When the temperature reached 122 to 123°C, the time of reaction being considered from the point when refluxing began. The reaction was carried out for different timings 15, 30 and 60 min. All care were taken for proper combination of the reactants. Carefully the product was transferred to a mixer containing ice cold water. It was ground for a minute as suggested by Mark and Mehlretter (1972). The starch acetate was precipitated as a coarse wet mass. It was filtered in a Buchner funnel. The residue was re-suspended in water, pH adjusted to neutral, washed several times with water and dried in an oven at 40°C for about 10 h after breaking the lumps. Dried lumps were powdered in a Retsch ultra centrifugal mill using a 1 mm screen.

Assay of Acetyl Content

Acetyl contents were determined as per the procedure of Mullen and Pacsu, (1942). Acetyl content and degree of substitution (DS) were estimated as per the procedure of Wurzburg (1964):

$$\% \text{ Acetyl} = \frac{(\text{mL blank} - \text{mL sample}) \times \text{Normality of acid} \times 0.043 \times 100}{\text{Sample weight in grams (d.b)}}$$

$$\text{Degree of substitution (DS)} = \frac{162 \times \% \text{ Acetyl}}{4300 - (42 \times \% \text{ Acetyl})}$$

Solubility

The acetates so prepared were tested for their solubility in the following solvents. Ethyl acetate, dimethyl sulphoxide, dimethyl formamide, 1-propanol, 2-propanol, isobutanol, acetone, chloroform, ethanol, acetonitrile, benzene, ethyl ether, toluene and acetic acid. A pinch of sample was taken and with above solvents, where about 5 to 10 mL of the solvent was used and solubility was confirmed.

Film Preparation

A small concentration of modified starch (5-6%) (Whistler and Hilbert, 1944) was dispersed in the selected solvent for 45 to 60 min by using a magnetic bead and stirred on a magnetic stirrer. This dispersed material was kept in a water bath at 76°C and heated for 15 min. Initially, after heating for 7 min, required amount of plasticizer, about 20%, (here glycerol) was added and heating was continued for another 8 min. The dispersion was stirred with a glass rod at regular intervals. After this the sample was removed outside and kept aside for 2 to 3 min, to avoid air bubbles. Later it was poured on a glass plate of sizes 6×6 or 3×3 inches and spread with a glass rod and placed in a good ventilated oven at 50°C for 2 to 2.5 h. Next it was removed from the oven and kept at room temperature for 1 to 4 h. The edges of the film was wetted with few drops of water and removed carefully with the help of a sharp edged knife and kept in a desiccator containing saturated solution of calcium nitrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$) which gives an RH of 51% as suggested by Lawton (1996).

Strength of Film

The films so prepared after storing in desiccator at 51% relative humidity for about 10 days were removed and cut into standard rectangular sizes of 20×40 mm. Minimum three pieces and maximum 5 pieces were prepared. Each film was held in the holder or adopter of Tensipresser Model TTP-50 BX II from Taketomo Electric Company, Japan. Strength of film was measured. Various parameters like Young's modulus, maximum point etc were noted down. This testing was carried out at around 23-25°C temperature, for each test around 5 min was required, (starting from fixing the film, till the end of testing). The clamp distance was around 18 inches. The thickness of the film varied from 0.05 to 0.07 mm.

Flow Tester

To study the dynamic properties of these native and modified starches, a device namely Shimadzu Flow Tester CFT-500, a machine of capillary extrusion type was used. About 1.5 g of native starch (or about 1 g of modified starch) was weighed and poured into a hollow cylinder. Slowly the sample was compressed with a plunger by hand and later in a compressor using the fly wheel supplied by the manufacturer (to get a compact sample). This device was removed and kept on a hollow cylinder and again the plunger was compressed by the fly wheel at a stretch, by which the compacted sample fell down. This compact sample was used for the experiment. The instrument was programmed with minimum and maximum position of the plunger, temperature was programmed which was equal to the melting point of material which was determined from differential scanning calorimeter. Required pressure was added as load and the value was programmed. Preheat time of 50 sec was also programmed. In the Flow meter, a hollow cylinder, above which a die of 0.5 (length)×1.00 mm (diameter) was placed. This whole system was fixed in the heating compartment aperture of Flow tester. The die aperture was closed at the bottom with the screw supplied by the manufacturers.

When the required temperature was reached in the device, the sample was inserted at the top aperture, immediately another plunger was slowly fixed and compressed from the top with the load. Now the instrument was activated by pressing the start button. As soon the preheating time was reached in the heating compartment, the plug was released. The melted sample extruded through the die of cross section as mentioned above, the equipment measured the samples dynamic viscosity between the two setting positions. Immediately position was noted on the display arrangement of the instrument and other dynamic properties like shearing force in dynes cm^{-2} , shearing velocity in per second and flowing quantity in mL/sec were noted down.

Differential Scanning Calorimetry (DSC)

Thermal analysis was performed with a DSC 220C instrument from Seiko Instruments Incorporation (Japan). Silver crucible, P/N 560-004 AG15 capsule was used for the experiment. Initial probing studies gave an hint that the melting point of high DS starches were higher than 200°C. Hence temperature range was fixed from 100°C onwards. Also to reach 100°C from room temperature, the system took about 60 to 90 sec. About 5 mg of sample was used in each experiment. The sample was transferred into the silver cup (it was placed only at the center, without spreading on the sides of the crucible). It was covered with the silver cover and sealed very carefully with the help of a sealer supplied by the manufacturers. Now the crucible with sample was weighed. Difference between the initial empty cup weight and this gave the weight of the sample. Another empty cup was used as reference with sealing as before and heating was carried out from 100 to 400°C at the rate of 10°C per min. Initiation of glass transition, glass transition (T_g), end point of glass transition, melting point and dextrinization or degradation or decomposition point were noted down. For all calculations, the T_g was calculated in the peak just before the melting peak as suggested by Maurice and co-workers (1985). For the purpose of comparison the same procedure was followed in all the calculations.

RESULTS AND DISCUSSION

Degree of Substitution

Table 1 shows the amylose equivalent of native starches, the acetyl percentage and degree of substitution of three varieties of rice starches at three different periods of acetylation. Some interesting features of the data were, acetylation was carried out under identical conditions for different periods, the values indicated that the Thailand starch i.e., indica had shown lowest acetyl content and lowest

Table 1: Amylose equivalent (db) of native starches, percent acetyl contents and degree of substitution with different periods of acetylation

Property	Indica	Japonica (non-waxy)	Japonica (waxy)
Amylose eqvt. (%)	34.72	22.99	<1.00
Starch	Period (min)	Acetyl (%)	Degree of substitution (DS)
Indica rice	15	30.44	1.63
	30	35.66	2.06
	60	37.96	2.27
Japonica (non-waxy)	15	34.74	1.98
	30	37.66	2.27
	60	39.81	2.24
Japonica waxy	15	38.82	2.33
	30	38.00	2.30
	60	40.76	2.55

Table 2: Solubility of starch acetates

Solvents	Appearance and inference
Ethyl acetate	Cloudy appearance but goes into solution very slowly
Dimethyl Sulphoxide	Completely soluble
Dimethyl formamide	Completely soluble
1-Propanol	Insoluble
2-Propanol	Insoluble
Isobutanol	Insoluble
Acetone	Cloudy appearance but goes into solution very slowly
Chloroform	Completely soluble
Ethanol	Insoluble
Acetonitrile	Cloudy appearance but goes into solution very slowly
Benzene	Soluble but some particles are seen
Ether	Insoluble
Toluene	Insoluble
Acetic acid	Cloudy appearance but goes into solution very slowly

degree of substitution under all periods of modification and highest exhibited by waxy rice starch and in between was by japonica starch and its modified ones. In other words, higher the acetyl content, higher will be the degree of substitution. The amylose equivalent content in indica starch was around 35%, japonica rice starch was around 23% and in japonica waxy starch was negligible (less than 1%). Now in the present work it was observed that higher the amylose equivalent content lower was the acetyl content and lower degree of substitution. Under the conditions employed even at 15 min period acetylation, the acetyl content was 30% in indica starch whereas ~ 39% in waxy starch, indicating the differences in the granular, molecular make up of each type of rice starch granule (in each variety of rice starches studied, the molecule of amylose and amylopectin, or the extent of amorphous and crystalline content will vary in each granule of the starches studied). Even at the end of 30 min acetylation the acetyl content and DS remained almost the same in the waxy rice starch, but its increase was not significant even at the end of 60 min acetylation, where as in indica there was a gradual increase from 15 to 60 min and the increase was to a lesser extent in japonica starch. So it can be seen from this data, that with low amylose equivalent content, increase in acetyl content and DS was not effective after 15 min of acetylation.

Solubility

Table 2 shows the solubility pattern of starch acetates in general. Solvents in which the starch acetate was completely soluble were considered here for discussions, the behaviour of other solvents are clearly seen from the data in Table 2. It is observed that some solvents were suitable, but handling them was a problem. (Dimethyl sulphoxide is generally used to disperse native starches with little

proportion of water 5 to 15% (French, 1984), but the same solvent dissolves the starch acetates without any addition of water, but handling this solvent is not easy, as the preparation of film using this solvent needs very high temperature. While dispersing the material and drying the films, longer duration is required. It is also a carcinogenic solvent hence it was not considered to be a suitable. Chloroform was a good solvent, but because of its anaesthetic effect, this was also not considered. Benzene being inflammable and dangerous to handle, this too was not considered. Finally, dimethyl formamide (DMF) was found to be a suitable solvent as it was easy to disperse, temperature of heating in water bath, spreading on the glass plate and drying in the ventilated oven were within limits, hence this solvent was selected for further studies on film preparation.

Differential Scanning Analysis

Differential scanning calorimetry thermograms of these native rice starch and different period modified starches have been shown in Fig. 1. Table 3 shows some of the parameters measured by the DSC instrument. Initially, each rice starch and their respective modified ones will be analysed, afterwards a comparison among these will be made.

In indica starch, difference between starting point of glass transition and ending point of glass transition (for convenience this will be abbreviated as A) in native starch and 15 min acetylated starch were almost same i.e., $\sim 12^\circ\text{C}$. Degradation or deformation or breakdown of native starch occurred just 1.6°C higher than the melting point, but in 15 min acetylated starch it was 7.4°C higher than its melting point. In 30 and 60 min acetylated ones, though the thermogram appeared with several peaks (clearly seen by expanding the x-axis scale), only the first peak for T_g calculations and second peak for melting point determination were taken into consideration. The A value was 7.5°C for 30 min and around

Table 3: Some parameters noted down from the differential scanning thermograms of various native and modified starches

Starch	Mel.pt. ($^\circ\text{C}$)	Deg.Pt ($^\circ\text{C}$)	T_1 ($^\circ\text{C}$)	T_g ($^\circ\text{C}$)	C_p mJ mg^{-1}	T_2 ($^\circ\text{C}$)	T_2-T_1 ($^\circ\text{C}$)
Indica native	268.3	269.9	204.8	212.8	0.815	217.4	12.6
15 min modified	270.1	277.5	200.9	207.2	0.773	212.9	12.0
30 min modified	281.8	286.6	206.9	210.6	0.873	214.4	7.5
60 min modified	283.0	288.0	208.9	212.6	0.702	217.6	8.7
Japonica native (non-waxy)	268.2	271.8	219.0	223.9	0.276	228.2	9.2
15 min modified	278.0	288.1	210.5	216.0	1.512	221.9	11.4
30 min modified	300.6	303.4	198.7	206.6	0.826	214.3	15.6
60 min modified	302.7	308.4	203.6	210.8	1.234	217.7	14.1
Japonica native (waxy)	272.6	275.0	227.5	231.7	0.669	237.3	9.8
15 min modified	307.8	311.2	229.4	232.8	0.363	237.2	7.8
30 min modified	308.9	310.5	270.7	271.5	0.221	272.4	1.7
60 min modified	315.2	320.0	274.9	276.0	0.087	278.5	3.6

Note: Mel pt.-melting point, Deg.pt.: Degradation/decomposition/dextrinisation point. T_1 : Starting point of glass transition, T_g : Glass transition point, T_2 : Endpoint of glass transition point. T_2-T_1 : Difference between T_2 and T_1 , named in the text as A

Table 3a: Enthalpy or heat content changes in native and various period modified starches of various rices

Name of the native and respective modified	ΔH Exothermic ($-\text{H}$) mJ mg^{-1}	ΔH Endothermic (H) mJ mg^{-1}
Indica native	-59.0 \pm 0.1	119.4 \pm 2.4
Indica 15 min modified	-110.4 \pm 2.2	265.2 \pm 5.3
Indica 30 min modified	-17.6 \pm 0.4	2.3 \pm 0.1
Indica 60 min modified	-7.6 \pm 0.2	25.5 \pm 0.5
Japonica native	-202.8 \pm 4.1	125.9 \pm 2.5
Japonica 15 min modified	-185.5 \pm 3.7	99.6 \pm 2.0
Japonica 30 min modified	-77.5 \pm 1.6	19.2 \pm 0.4
Japonica 45 min modified	-91.0 \pm 1.8	6.5 \pm 0.1
Japonica waxy native	-34.1 \pm 0.0	109.4 \pm 2.2
Japonica waxy 15 min	-154.4 \pm 3.1	31.4 \pm 0.6
Japonica waxy 30 min	-88.6 \pm 1.8	45.7 \pm 0.9
Japonica waxy 60 min	-91.0 \pm 1.8	2.8 \pm 0.1

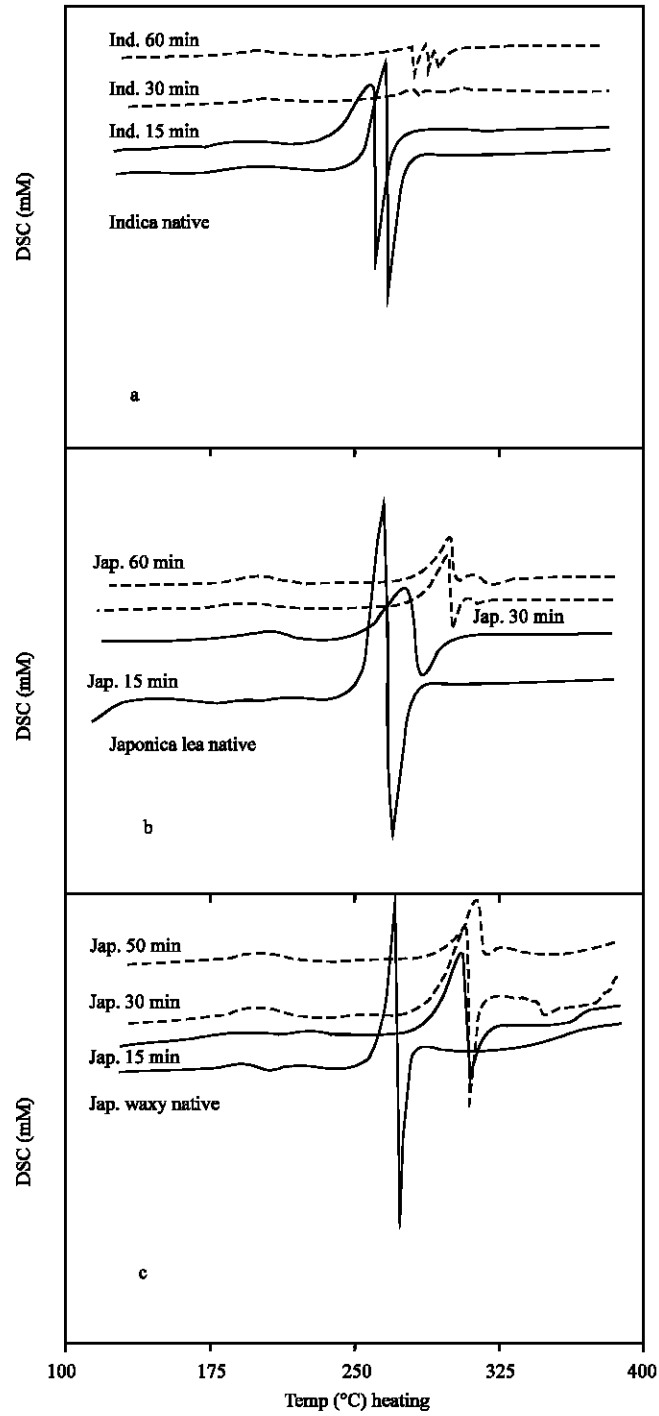


Fig. 1: Differential scanning calorimetry thermograms of the high degree substituted acetylated rice starches, their periods of acetylation are shown on each Figure. a) Indica starch and its modified ones, b) Japonica starch and its modified ones, c) Japonica waxy starch and its modified ones

9°C for 60 min acetylated ones, indicating the transition completed in a gap of $p \sim 8$ to 9°C in 30 and 60 min modified ones. The degradation occurred about 5°C higher than the melting point in 30 and 60 min modified ones. Glass transition point appeared to be almost same in native and modified ones with an exception of 15 min modified one where it came down by around 5.5°C. Difference in heat capacity at T_g appeared to be almost same starting from native to all the modified starches, being slightly low in 15 and in 60 min modified one and slightly greater in 30 min modified ones. Melting point increased from native to 60 min modified ones. Much increase was not there between native and 15 min one, but there was an increase in melting point in others i.e., 14 to 15°C, indicating with higher content of acetyl group from 15 to 60 min, the hardness in the derivatives have increased and hence melting point and degradation point have increased in indica starch which had highest amylose equivalent content among the three varieties of starches used for the experiment.

The behaviour was quite different in japonica (non-waxy) compared to that of indica. In native starch the A value was 9.2°C, after modification this value increased in all periods of acetylation, highest being in 30 min acetylated one, indicating a gap of $\sim 6.4^\circ\text{C}$ between native and this. Glass transition was highest in native, after modification this value decreased in all periods of acetylation indicating acetyl group makes the molecule of amylose and amylopectin bulky. [These bulky groups were of flexible nature, hence the T_g value decreased (Armeniades and Baer, 1977)]. Melting point increased with all periods of modification, the increase was around 10°C in 15 min modified ones but ~ 32 to 35°C in 30 and 60 min modified ones. These results suggested that esterification made the japonica starch molecules harder and harder, hence a higher amount of thermal energy was required to melt the modified ones. Degradation started within 3 to 10°C higher than the corresponding melting point in native as well as acetylated ones. This degradation can be considered as a decomposition of starch acetate molecules just after melting but at almost same temperature as in native and different period acetylated ones. In 15 min modified one it occurred around 10°C higher than the melting point, but in 30 and 60 min about 3 to 5°C higher than the melting point. Even degradation point increased from native to different period modified ones by 17 to 36°C as in the case of melting point. There was increase in heat capacity (C_p) after modification compared to native, which means the heat holding capacity or enthalpy of japonica acetylated ones were higher than the native, the order of increasing heat capacity follows 30, 60 and 15 min, indicating the significance of 15 min modified one. Even lower acetyl content in 15 min modified one ($\sim 35\%$, Table 1) has shown a higher C_p value whereas higher acetyl content shown in 30 and 60 min acetylated ones ($\sim 38\%$ and 40%, Table 1) have exhibited lower C_p values. These values are quite interesting and need in depth studies in future.

Waxy starch, almost completely a branched molecule in its starch granule had thermograms which were different in nature. The value of A was highest in native and it decreased continuously with different period of acetylation except the degree of decrease is reverse in 30 and 60 min. Among T_g values, there is continuous increase from native to different periods in acetylated ones. Much difference was not observed between native and 15 min modified one. But the value rose tremendously in higher periods of acetylation, indicating the special nature of acetylated amylopectin molecule in the waxy starch. Also according to Armeniades and Baer (1977) these acetyl groups which replaces -OH groups in several chains of amylopectin molecules of starch will be bulky or cross links occurs between the different chains of amylopectin molecule (as this starch contains less than 1-2 % linear portion), which decreases the mobility of the amylopectin acetate molecule and hence increases the T_g . Specific heat capacity decreased with modification continuously and was least in 60 min modified one, indicating japonica waxy starch after acetylation loses its heat holding capacity. In a branched molecule like japonica waxy starch acetate, why, how and where this phenomenon occurs is to be thought over with further research. The melting points increased continuously with modification, as in the case of japonica rice starch. Waxy starch which has a huge number of branches in its molecules (amylopectin), the acetyl content even at 15 min modification, was very high, about 39%, indicating amylopectin in

particular undergoes acetylation to a greater extent compared to other varieties of starches studied. The degradation or decomposition of modified starches occurred just 5°C above the melting point of each waxy acetylated starch.

Comparison Among the Three Native and Respective Modified Starches

The T_g was highest in japonica waxy and lowest in indica and in between for japonica non-waxy. The A value was highest in indica and almost same in other two natives, after acetylation the A values decreased gradually in indica, drastically in japonica waxy but increased to various extents in japonica non-waxy. The melting points of all the native starches remained almost same with an exception in waxy japonica where about 4°C higher value was noticed. After modification melting points increase was noticed in all three varieties but the extent of increase was high in waxy japonica. Highest specific heat capacity was in indica followed by waxy and least in japonica. Acetylation imparted least changes in indica, drastic reduction in japonica waxy and tremendous increase in japonica non-waxy. Glass transition temperature remained almost same in indica and its modified ones, but in japonica the values decreased after modification indicating acetyl groups were flexible in nature, but in waxy acetylated starches there was increase in T_g specially in higher period of modification, reasons are as informed before. The melting point difference between native and 60 min modified ones was highest in waxy japonica (42.6°C) and lowest in indica (~15°C) and in between occupied by japonica (34.5°C). But in waxy modified ones specially in the 60 min one there was a jump about 43°C compared to its native (Further detail research in these aspects are required).

Thus we can conclude each type of starch behaves differently after acetylation. It appears amylose equivalent content in each, plays a special role. Glass transition temperature appears to increase to a greater extent in waxy rice starch after acetylation but the same appears to decrease in non-waxy rice starches. Thus special behaviour of japonica waxy is noticed in all the properties.

Heat Contents and Their Changes after Modification

Table 3a shows the enthalpy changes in the DSC melting as well as endothermic peaks. The top peaks of Fig. 1 were of exothermic and bottom peaks were of endothermic in nature.

In indica native 59 mJ mg^{-1} was the heat evolved during melting. After 15 min acetylation heat lost by the system was more, up to an extent of 50 mJ mg^{-1} extra compared to the native (-110.4-(-59)). But in 30 and 60 min, the values of ΔH increased and heat holding capacity had increased compared to native. In other words there was an absorption of heat during melting i.e., 17.6-(-59) = +32 mJ mg^{-1} . Under endothermic peaks, in 15 min modified one, good amount of heat absorption was seen but after 30 and 60 min acetylation the absorption capacity decreased indicating the special role of substituted acetyl groups in these modified starches. Thus 15 min acetylated indica starch show special behaviour with respect to exothermic and endothermic compared to 30 and 60 min acetylated ones.

In japonica native, heat evolved was ~203 mJ mg^{-1} , which was highest among all the native starches implying the fact that the heat content in native japonica was highest. After modification the evolution of heat decreased gradually from 15 to 60 min indicating the heat holding capacity had increased after acetylation i.e., -185.5-(-202.8) = 17.3 implying that holding of heat in 15 min modified one was high compared to its native. Similarly this heat capacity was high with 30 min as well as 60 min acetylated ones. Quite interesting observation was that endothermic changes were reducing very gradually from native to the 60 min modified one, whole absorption of heat was least in 60 min acetylated one. This indicates the special nature or property of acetyl groups in the starch acetate molecule of japonica starch.

In Japonica waxy native heat evolved was very low, after modification the values increased, showing the special behaviour of acetylated amylopectin molecule of starch. In 15 min acetylated one,

highest evolution of heat was observed but after 30 and 60 min the evolution decreased. Heat absorption capacity was least in 60 min modified one and also absorption decreased gradually except in japonica waxy 30 min modified one.

Comparison

Among the three native starches, highest evolution of heat was seen in japonica starch followed by indica native and least by japonica waxy, which implies that the japonica varieties behaved differently compared to indica starch. After acetylation the evolution of heat came down continuously in indica over a period from 15 to 60 min. In japonica the same phenomenon occurred but the extent of decrease was less compared to indica modified ones; but in the modified japonica waxy, there was more evolution of heat compared to its native. In endothermic peaks, enormous changes were seen only in 15 min indica acetylated one, but less changes in 30 and 60 min acetylated ones. Japonica and Japonica waxy and their modified ones behaved almost in similar way. Endothermic changes are also predominant in japonica native followed by indica and japonica waxy. Indica acetylated for 15 min, showed highest change followed by japonica and japonica waxy. Abnormality was seen in other two periods of acetylation.

To conclude, exothermic changes are predominant in japonica (non-waxy) native, 15 min acetylated ones brought out major changes in all the starches and abnormality was seen in higher periods of acetylated ones only in the case of japonica non-waxy and waxy ones. Under endothermic peaks, gradation was observed in japonica waxy and non-waxy varieties but abnormality was seen in indica acetylated ones.

Film Properties

Figure 2 clearly shows the films prepared from these modified starches. Among the films prepared following modified ones could give good films:

Indica starch of 15, 30 and 60 min acetylated. In Japonica starch only 15 and 30 min modified ones. Waxy acetylated could form film but, after drying, it could not be peeled off from the plate or cracks use to appear while drying. (It is well known from the previous researchers that amylopectin starch is not suitable for preparation of films).

Strength of Film

All the films were tested in the Tensipresser tensile strength unit. Table 4 shows some of the properties measured in these films. Indica modified for 15, 30 and 60 min as well as japonica modified for 15 and 30 min showed Young's modulus. Among the films tested high value for Young's modulus is shown by indica 15 min modified one followed by 60 min and least by indica 30 min. Among japonica modified ones, the one with 15 min could register higher Young's modulus and lesser by japonica 30 min, thus indicating lower period of acetylated starch has higher Young's modulus compared to higher period of acetylation. Braking force again followed same pattern, requiring highest force in the case indica 15 min and least in japonica 30 min modified one. Maximum strain of 1.5×10^{-3} was seen in indica 15 min modified one whereas in japonica 15 and 30 min modified one the same phenomena occurred at 0.25×10^{-3} and at 0.05×10^{-3} , respectively implying that the weaker films are formed by japonica modified ones. Work involved i.e., the area under the curve from starting point to the maximum point followed same pattern as in Young's modulus and braking force. Highest work was involved in indica 15 min modified one and least in japonica 30 min. Presence of higher quantity of linear component (amylose equivalent) plays a key role for the strength of film. Among the studied samples, indica starch with ~ 35% amylose equivalent content, with 1.63 DS is very good, compared to higher degree substituted starches. Hence we can conclude that DS of 2 and above is not good to obtain film of proper strength.

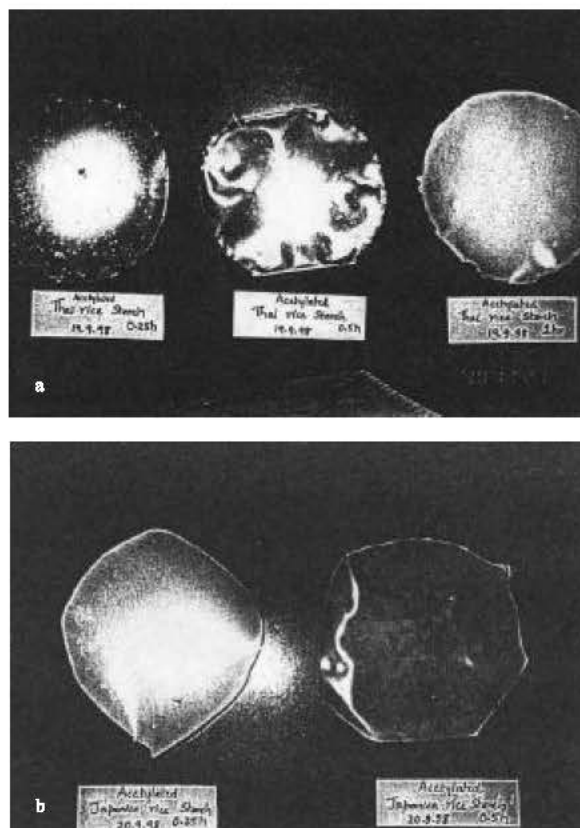


Fig. 2: Films prepared from high degree substituted rice starches. a) Indica rice starch 15, 30 and 60 min, respectively acetylated, b) Japonica rice starch 15 and 30 min acetylated

Table 4: Some properties from the Tensipresser with respect to the strength of the films

Properties/mod.sta.	Indica 15	Indica 30	Indica 60	Jap. 15	Jap. 30
Young's modulus	1.100×10^6	0.464×10^5	1.184×10^5	0.525×10^5	0.16×10^5
Max. point (Breaking force)	0.183×10^6	0.464×10^5	1.162×10^5	0.525×10^5	0.16×10^5
Max. strain	1.500×10^{-3}	0.250×10^{-3}	0.625×10^{-3}	0.250×10^{-3}	0.050×10^{-3}
Work	0.139×10^7	0.853×10^5	0.370×10^6	0.726×10^5	0.426×10^5

Indica 15, 30 and 60 are indica rice starch modified for these periods in min, Jap.15 and 30 are japonica non-waxy rice starch modified for these periods. The unit of Young's modulus is in $N\ m^{-2}$, Maximum point is in dynes and work is in Joules

To conclude, among the films studied for their properties, it has been noted that either in indica or japonica, 15 min acetylated one appears to be stronger compared to higher period modified ones.

Flow Meter or Flow Tester

Initially to get a proper film, proportion of material as well as quantity of material required is standardized while manufacturing films. After knowing these parameters, before making the sheet, another important property namely dynamic flow is also required to be studied. This could be studied by making use of a Flow tester or a flow meter.

From Table 5 it is clear, that for every type of starch, value of maximum and minimum position of the plunger plays a key role. As seen the value for indica starch was different compared to japonica

Table 5: Conditions applied on the instrument and response given by the Flow meter/Flow tester

Sample	Conditions				Response by the instrument			
	Min. posn.	Max. posn.	Temp. (°C)	Preht. (Sec)	Viscosity in poise	Sh.stress dynes/cm ²	Sh.vel. per sec	Flow (mL sec ⁻¹)
Indica starch	5	7	273	50	3.43×10 ⁴	1.47×10 ⁸	42.78	0.42
Waxy (Japonica)	2	4	278	50	1.99×10 ⁸	1.47×10 ⁸	73.84	7.25×10 ⁻³
starch	2	4	278	50	2.75×10 ⁶	1.47×10 ⁸	53.47	5.25×10 ⁻³

Note: Min. posn.-Minimum position of the plunger, Max.posn.-Maximum position of the plunger, Preht.: Preheat time in seconds, Viscosity of flow: viscosity of the material, in other words dynamic flow of the material which flows through the die, Sh. stress: shearing stress of the material in dynes/cm², Shearing velocity: per second, Flowing quantity: when the sample melts, quantity of flow in mL sec⁻¹

waxy rice starch. The temperature fixed or programmed was always 5°C higher than the melting point of the material which was known from the DSC instrument. The quantity of flow was 0.42 mL sec⁻¹ in the case of indica starch but it was different in the case of waxy starch. Two values of waxy starch have been shown, where we observe changes in viscosity, shearing velocity and flowing quantity though minimum and maximum positions fixed for waxy starch was same. Other details, standardization of experiments are to be carried out in future, in order to understand the dynamic flow behaviour of various starches in native form. There were difficulties in measuring dynamic viscosity of modified ones, which needs further studies and standardization of techniques in this instrument.

CONCLUSIONS

Starches were isolated from three types of rices and they were acetylated for different periods. Native and modified starches found to be easily soluble in dimethyl formamide than other solvents and hence films were prepared from this combination. Under the conditions employed waxy japonica had highest acetyl content as well as highest DS. Japonica waxy and its modified ones found to be toughest by the Differential Scanning Calorimetry studies. Enthalpy changes found to be significant in lower periods of acetylation than in higher periods of acetylation. Film forming ability and strength of the film appeared to be good in lower periods of acetylated indica than in other japonica and its modified starches.

ACKNOWLEDGMENTS

Vasudeva Singh is grateful to the United Nations University, Japan, for the award of the UNU-KIRIN Fellowship (1998-99) and T. Yoshino, at National Food Research Institute, for helping in DSC measurements.

REFERENCES

- Armeniades, C.D. and E. Baer, 1977. Transition and Relaxation in Polymers. Introduction to Polymer Science and Technology: An SPE Text Book: Kaufmen, H.S. (Ed.), Wiley: New York.
- French, D., 1984. Organization of Starch Granules. In: Starch Chemistry and Technology. Whistler, R.L., J.N. BeMiller and E.F. Paschall (Eds.), Academic Press Inc. Orlando, Florida, USA., pp: 241.
- International Standard, 1987. (E). Rice-Determination of amylose content. ISO, pp: 6647.
- Jarowenko, W., 1986. Acetylated Starch and Miscellaneous Organic Esters. In: Modified Starches: Properties and Uses. Wurzbug, O.B. (Ed.), CRC Press, Inc., Boca Raton, FL., pp: 55-78.
- Lawton, J.W., 1996. Effect of starch type on the properties of starch containing films. Carbohydr. Polym., 29: 203-208.

- Mark, A.M. and C.L. Mehlretter, 1970. Acetylation of high amylose corn starch. Influence of pretreatment techniques on reaction rate and triacetate solubility. *Starch*, 22: 108-110.
- Mark, A.M. and C.L. Mehlretter, 1972. Facile preparation of starch triacetates. *Starch*, 24: 73-76.
- Maurice, T.J., L. Slade, R.R. Sirett and C.M. Page, 1985. Polysachharide-Water Interactions. Thermal Behaviour of Rice Starch. Influence of Water on Food Quality and Stability. Simatoa, D., S.L. Multon and M. Nijhoff (Eds.), Publ.: Dordrecht, Netherlands.
- Mullen, J.W. and E. Pacsu, 1942. Starch studies: Preparation and properties of Starch Triesters. *Ind. Eng. Chem.*, 34: 1209-1217.
- Shogren, R.L., 1996. Preparation, thermal properties and extrusion of high-amylose corn starch acetate. *Carbohydr. Polym.*, 29: 57-62.
- Singh, V. and S.Z. Ali, 1987. Comparative acid modification of various starches. *Starch*, 39: 402-405.
- Singh, V., 1996. Studies on modified starches. Ph.D Thesis submitted to and awarded by the University of Mysore, Karnataka, India.
- Singh, V., H. Okadome, H. Toyoshima, S. Isobe and K. Ohtsubo, 2000. Thermal and Physico-chemical Properties of rice grain, flour and starch. *J. Agric. Food Chem.*, 48: 2638-2647.
- Singh, V. and S.Z. Ali, 2000. Acid degradation of starch. The effect of acid and starch type. *Carbohydr. Polym.*, 41: 191-195.
- Singh, V. and S.Z. Ali, 2006. *In vitro* Hydrolysis of Starches by alpha-amylase in comparison to that by acid. *Am. J. Food Technol.*, 1: 43-51.
- Singh, V. and S.Z. Ali, R. Somashekar and P.S. Mukherjee, 2006. Nature of crystallinity in native and acid modified starches. *Intl. J. Food Properties*, (Accepted).
- Whistler, R.L. and G.E. Hilbert, 1944. Mechanical properties of films from amylose, amylopectin and whole starch triacetates. *Ind. Eng. Chem.*, 36: 796-798.
- Wolff, I.A., D.W. Olds and G.E. Hilbert, 1951. Triesters of corn starch, amylose and amylopectin. *Ind. Eng. Chem.*, 43: 911-914.
- Wurzburg, O.B., 1964. Esters-Acetylation. In: *Methods in Carbohydrate Chemistry*, Vol. IV, Whistler, R.L., R.J. Smith and J.N. BeMiller (Eds.), Academic Press, New York, pp: 286.