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Preparation of High Degree Substituted Acetylated Acid Modified Rice Starches, Intermediate Degree Substituted Derivatives of Rice Starch and Zein Proteins and Preparation of Biodegradable Films (Part II)

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Abstract: Starches from Indica, Japonica and Japonica waxy rices were acid modified by treatment at 50°C for 1 h. These were acetylated using acetic anhydride and 11% sodium hydroxide added as 50% aqueous solution for 1 h. These starches were then combined with zein and chemically modified by acetylation for a period of 30 min as before. All acid modified and acetylated starches showed almost the same content of acetyl and same degree of substitution (DS), with acetyl percentage being around 39.5 and DS around 2.4. Starch-zein acetylated one showed almost the same range of acetyl percentage and DS, the values being around 27% and 1.35, respectively. All these modified products dissolved easily in dimethyl formamide. Acid modified and acetylated starches were combined with zein protein after dissolution in the above solvent separately and after mixing, their films were prepared. In a similar way, films were also prepared from starch-zein acetylated products. Among the native starches, japonica waxy showed the highest glass transition temperature (T_g) (~ 232°C) followed by japonica non-waxy (224°C) and the lowest by indica (213°C). Difference in specific heat capacity before and after T_g increased in general from native to acid modified one and acid modified one to acid modified starches cum acetylated one. However the melting points were in the reverse order compared to T_g . Among the acid modified and acetylated ones, the melting point of Indica starch was the highest (~320°C). Glass transition temperature increased to a greater extent acid in modified starches and acetylated japonica waxy starches. Crude corn zein showed three characteristic peaks in DSC thermogram. It had the highest T_g (~258°C) and highest melting point (~306°C) but lowest C_p . After acetylation of starch and zein, all DSC parameters increased along with their respective starch zein mixture values. Decomposition temperature was the highest in japonica-zein acetylated one. Zein showed only endothermic peak. Acetylated japonica-waxy and zein showed the highest heat holding capacity among the three zein starch acetylated ones.

Key words: Bio-degradable films, acid modified and acetylated, indica, japonica, japonica waxy zein acetylated, zein proteins

INTRODUCTION

Preparation of bio-degradable films is recognized as a thrust area of research in the field of carbohydrates, protein chemistry and technology in the 21st century. Generally, native starches are acetylated to higher degree for the preparation of films (Whistler and Hilbert, 1944). But degree of

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substitution (DS) of 3 has not been found much useful as it produces a brittle film (Jarowenko, 1986). Hence, attempts have been made to prepare products of DS less than 3 by many researchers. At the same time, processes for the preparation of films with an intermediate value of DS have been patented (Wurzburg, 1986; Billmers and Tessler, 1993). Singh (1996) isolated starches from foodgrains, modified them by acid treatment and studied their properties at granular and molecular levels. Singh and Ali (2000) studied the acid degradation of starch, the effect of acid and starch type. They observed that after acid modification the Fr.II increased and the effect was least with phosphoric acid. They further suggested that degraded portion had a molecular size similar to that of Fr. II of native starch. Generally, acid modified starches are used for the preparation of candy in food industry, because it gives a gel of higher strength. Now-a-days, corn starch is used commercially either in native or in modified form but not other starches. Only limited information is available about the use of rice starches for the preparation of high degree substituted acetylated starches. Singh *et al.* (2006) recently prepared high degree substituted acetylated rice starches and informed that on acetylation of three kinds of starches under simultaneous conditions showed that waxy acetylated one had highest melting point and they also studied the thermal and physicochemical properties of rice grain, flour and starch (2000). Jane *et al.* (1994), attempted to prepare bio-degradable films by making use of cereal flours or starches and proteins with different types of modifications. A detailed review on edible films and coatings from wheat gluten and corn zein have been made by Gennadios and Weller (1990). Cuq *et al.* (1998) reviewed the studies on the usage of proteins alone from wheat, corn, soya, milk, cottonseed, peanut and also different animal sources for the preparation of bio-degradable films. In the present study, attempts were made to prepare bio-degradable films from a combination of acid modified rice starches, followed by acetylation and physical combination of these with zein proteins. Attempts were also made to combine different types of various rice starches with corn protein and modify them by acetylation for the preparation of films. To characterize these products, their thermal behaviour and other properties were studied.

MATERIALS AND METHODS

Materials

Indica rice namely Thailand rice was procured from the local super market. Japonica non-waxy (Nipponbare) and Japonica waxy rice (Himenomochi) in the form of brown rice were procured from the nearby National Agricultural Research Station, Tsukuba. It was mentioned that these grains were harvested in the year 1997 and after drying and shelling, brown rice was stored around 5-6°C in cold room. Commercial crude zein was procured from M/s Wako Pure chemicals, Tsukuba. All chemicals were of analytical grade. Unless otherwise stated, deionised water was used for all the studies.

Methods

Brown rice was polished and starch was isolated from these milled rices as per the procedure of Singh *et al.* (2000, 2006).

Acid Modification of Starches

Starches from different rice varieties were acid modified as per the procedure of Singh and Ali (1987). Residue obtained was broken and kept for drying at 40°C for about 18 h. After drying, it was ground by using a Retsch ultra mill by using a 1 mm screen. The quantity of native starch was decided depending on the requirement. Similar procedures were followed for all types of starches.

Acetylation of Acid Modified Starches

Acid modified starches so prepared were used individually as the starting material for this experiment. A 50 g sample of the modified starch was mixed with 184 mL of acetic anhydride slowly

in a round bottomed flask. It was placed on the heating mantle. The flask was fitted with three way necked joints, fixed with the condenser, stirrer. Then 11% sodium hydroxide was added drop by drop as 50% aqueous solution. The mantle regulator was kept in 2.5 position and the instrument was switched on for the temperature to reach about 124°C which took about 20 min. When reactions were going on, various colours were seen. Initially, reactants formed a cottony type, followed by light brownish, ash colour and finally became whitish. Then the products were transferred exactly after 1 h to a blender or mixer containing ice cold water (Mark and Mehlretter, 1972), blended for one minute and filtered through a Buchner funnel. The residue was taken out, broken, mixed with water and kept for continuous stirring. The pH was adjusted to 7 by addition of sodium hydroxide. After further filtration, the residue was washed with water, minimum two times and dried at 40°C for 10 to 12 h. The dried residue was ground as before.

Acetylation of Starch and Zein

In this experiment, the proportion of native starch was 45% and zein was 55%. All rice starches had moisture contents of 9 to 10%. Starch (22.5 g) and commercial crude zein (27.5 g) were taken for acetylation. Initially, acetic anhydride was taken in a round bottomed flask and starch was added with continuous stirring (by using a bead on a magnetic stirrer). Then, protein powder as such, was added and stirred. It was acetylated as before by addition of 11% sodium hydroxide as 50% aqueous solution as a catalyst. (the quantity of alkali was decided by using required amount of alkali for starch portion and 27.5 g zein was converted to half, i.e., 13.75 g and total weight became 36.25 g, for this quantity, the alkali used was 5.3 mL). The total duration of reaction was 30 min. While carrying out the reaction, in indica rice starch, there was no reaction till the temperature reached to 120°C. Afterwards, it went up to 128°C. After switching off the current, the temperature came down to 123°C in 4 min. After about 10 min the mantle was put on. In the reaction that followed a viscous mass with dark, orange colour appeared at 125°C. In japonica starch, a brownish viscous mass, turning to dark orange mass appeared at 120°C. In all the cases the experiment time was counted as soon the temperature reached to around 123 to 125°C. The reaction was stopped exactly after 30 min. After this, the contents were carefully transferred to a blender containing ice cold water, blended for a minute and filtered through a Buchner funnel. The residue was then mixed with water, pH was adjusted to 7, filtered, dried and powdered as before.

Determination of Acetyl Group and Degree of Substitution

The procedure of Mullen and Pacsu (1942) was followed for the determination acetyl group. Percent acetyl and degree of substitution were calculated as before by using the procedure of Wurzburg (1964).

Differential Scanning Calorimetry

About 5 mg of sample was taken in a silver crucible and sealing was carried out as before (2006). Heating from 100 to 400°C at the rate of 10°C min⁻¹. was carried out. An empty crucible was used as a reference and other procedures are as mentioned in Part I (2006). Cooling was carried out without any cooling agent. The instrument DSC 22°C instrument from Seiko Instruments Incorporation, Japan was used and it was calibrated using the melting temperature and enthalpy of indium.

For studying the scanning thermogram of protein, crude zein was powdered in a Retsch Ultra Mill and passed through 1 mm screen.

Solubility

Solvents used for solubility test were acetone, chloroform, dimethyl formamide, dimethyl sulphoxide, ethyl alcohol and acetonitrile. A pinch of powder was taken in a test tube with the required solvent (around 10 mL) and mixed thoroughly on a cyclo mixer to know the solubility.

Preparation of Film

The modified starch (5 to 6%) was dispersed in the selected solvent for about 60 min on a magnetic stirrer with a magnetic bead. It was then placed in a water bath at 75°C bath for 7 min. Glycerol was added to a level of 20 to 25% on the basis of concentration of modified starch. Heating continued for another 8 min with occasional stirring with a glass rod or a steel spatula. The sample was removed and kept aside for 2 to 3 min to avoid the air bubbles. It was then poured on a glass plate (6×6" or 3×3"), which was placed on a horizontal levelled table. Immediately afterwards it was spread depending on the consistency of the dispersion and placed in an oven at 50°C and kept for 2 to 3 h. After removal from the oven the product was kept for about 2 to 3 h at room temperature. Film formed was removed by spray of water drops on the corners and with the help of sharp knife.

Different proportions of acetylated acid modified rice starches were dispersed in the selected solvent and also proper proportions of zein were also dispersed in the same solvent, mixed together and stirred for about 30 min in a water bath, as mentioned above, glycerol (25%) was added and the stirring was continued as before.

Five percent of the rice zein acetylated compound was taken with required quantity of the solvent and stirred for about 1 h on a magnetic stirrer. Afterwards, this was placed in a water bath at 76°C, for 15 min, with addition of glycerol as mentioned above. The film formed were placed in 51% RH for about 8 to 10 days.

Tensipresser

A tensipresser was used for the measurement of Young's modulus. The procedure was as mentioned in the Part I (2006). Films of 20×40 mm after storing at 51% RH for 10-12 days, were held in the adopter of Tensipresser Model TTP-50 BXII from Taketomo Elec. Co. Japan. Various parameters like Young's Modulus, maximum point etc. were recorded at a temperature of about 23-25°C.

RESULTS AND DISCUSSION

Selection of Acid Modified Starches

Generally, it is the common practice to use the starches of higher amylose content for the preparation of films. It is well known that if amylose content is higher the gel forming tendency will also be higher. Also, if waxy starch is acid modified, the branch points are broken and possibly, smaller chains of alpha 1→4 linkages may increase. It has been reported that in a starch granule, amylopectin molecule is degraded at a considerably faster rate than amylose (Cowie and Greenwood, 1957). Ruggeberg (1950) observed that rice starch was more resistant to acid hydrolysis. Singh and Ali (1987) also observed that on acid hydrolysis of rice starch, the molecular weight at the end of 45 or 180 min, will not change drastically. Hence in this experiment the starches were hydrolysed for 60 min.

Role of Zein

Though films prepared from acetylated acid modified starches look good initially, however cracks appear while drying. To overcome this problem it was thought that some protein could be added to give strength to the films. In this context, crude zein was tried after purification by using 70% alcohol, but this did not give the desired result. In the next trial, crude zein as such was dissolved in the solvent and mixed with the dispersed acetylated acid modified starch. The result was that film formation was clear. But when this dispersion was heated at 50°C due to crystallization of proteins in the films and the films were very good (Fig. 1). Therefore attempts were made to use a acetylated starch and zein together for the preparation of films.

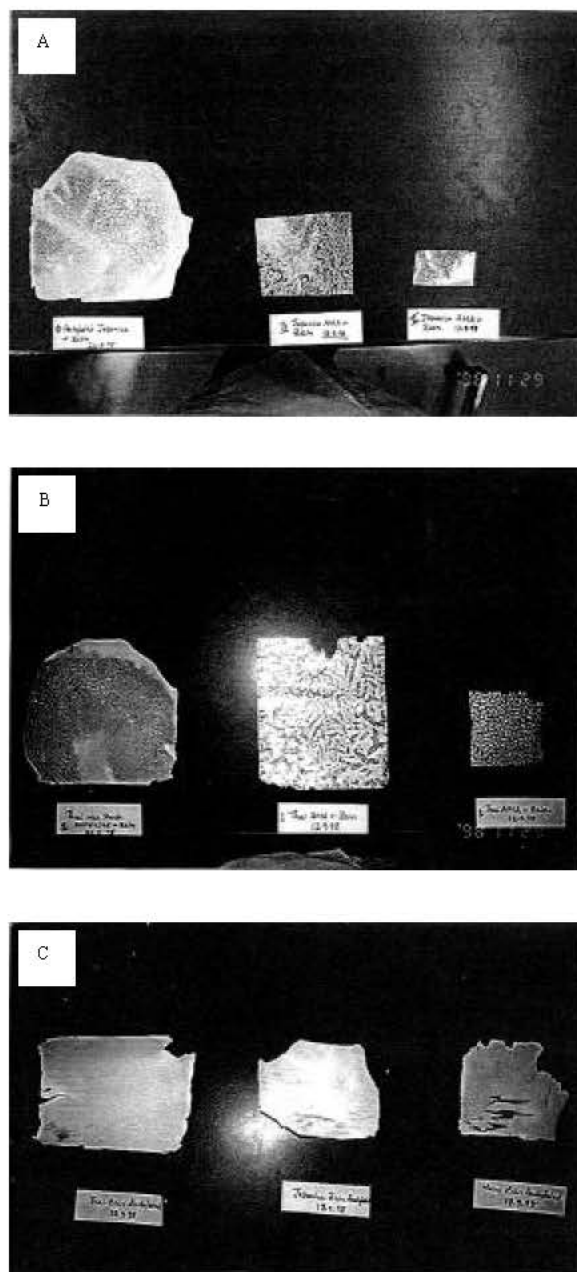


Fig. 1: (A) Films prepared from physical mixing of i. Acetylated japonica and zein ii. Japonica acid modified and acetylated in combination with zein iii. Same as in ii. (from left to right) (B) Films prepared from physical mixing of i. Indica rice starch acetylated and zein ii. Indica acid modified and acetylated and zein iii. Same as ii, but with different proportions (from left to right) and (C) Films prepared by chemical modification of i. Indica rice starch and zein ii. Japonica rice starch and zein iii. Waxy rice starch and zein

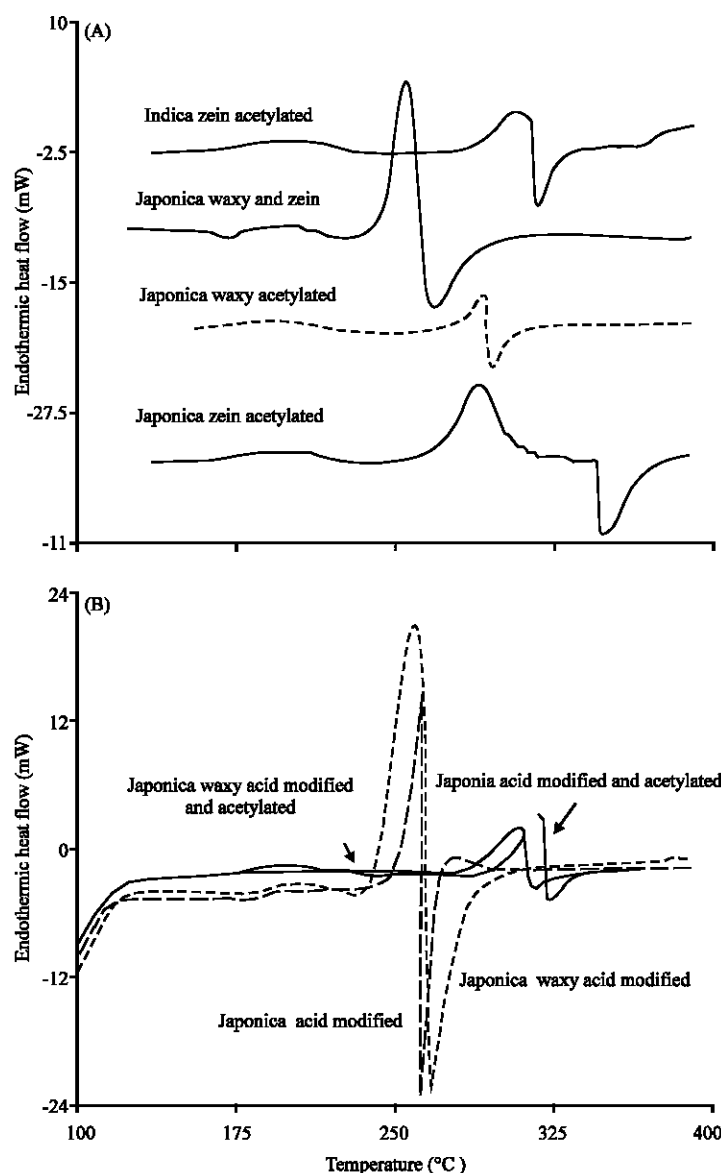


Fig. 2: (A) DSC thermograms of Indica zein acetylated, Japonica waxy and zein, Japonica waxy acetylated and Japonica zein acetylated and (B) DSC thermograms of Japonica acid modified and Japonica acid modified and acetylated. And Japonica waxy acid modified and japonica waxy acid modified and acetylated. Figures are overlapped

Degree of Substitution

Table 1 gives the percentage of acetyl content and corresponding degree of substitution of acetylated acid modified starches and acetylated starch and zein. In order to get a higher DS product, the reaction was carried on for a period of 1 h. It may be seen from the table, that in all the three types of starches, the acetyl contents varied from 38.4 to 39.4% and corresponding DS varied from 2.3 to 2.4. The values appear to be same as observed in native starches (Singh *et al.*, 2006).

Table 1: Percent acetyl and degree of substitution of various modified starches

Type of modification and period of modification	Acetyl content (%)	Degree of substitution (DS)
Indica starch		
Acid modified and acetylated (1 h)	39.24	2.40
Japonica starch		
Acid modified and acetylated (1 h)	39.39	2.41
Japonica waxy starch		
Acid modified and acetylated (1 h)	38.42	2.32
Starch and zein protein acetylation (0.5 h)		
Indica starch + zein	28.20	1.47
Japonica starch + zein	26.40	1.34
Japonica waxy rice starch + zein	26.70	1.36

Table 2: Solubility of various modified starches

Solvent	Indica AMA	Japonica AMA	Japonica waxy AMA
Acetone	Almost soluble	Almost soluble	Soluble
80% acetone	Soluble	Soluble	Almost soluble
Chloroform	Soluble	Insoluble	Soluble
Dimethyl formamide	Soluble	Soluble	Soluble
Dimethyl sulphoxide	Soluble	Soluble	Soluble
Alcohol	Insoluble	Insoluble	Almost soluble
Acetonitrile	Almost soluble	Almost soluble	Almost soluble
80% acetonitrile	Soluble	Soluble	Soluble

Solubility of acetylated starch and zein

Solvent	Indica and zein acetylated	Japonica and zein acetylated	Japonica waxy and zein acetylated
Dimethyl formamide	Soluble	Soluble	Soluble
Dimethyl sulphoxide	Insoluble	Insoluble	Insoluble
Chloroform	Almost soluble	Almost insoluble	Almost insoluble
Aceto nitrile	Insoluble	Insoluble	Insoluble
70% Ethyl alcohol	Insoluble	Insoluble	Insoluble
Acetone	Insoluble	Insoluble	Insoluble
80% Acetone	Soluble	Soluble	Soluble

AMA: Acid modified and acetylated

When acetylated rice starch and zein were used, the end point identification with phenolphthalein was difficult, but its control sample helped to identify the end point accurately. As it is seen from the results, the acetyl content varied from ~26 to 28% and DS varied from 1.36 to 1.47, highest being by indica rice starch. It is clear when starch and zein were acetylated, much variation in acetyl content and DS could not be observed.

Solubility

Table 2 shows the solubility pattern of acid modified and acetylated starches. Clear solubility was achieved with dimethyl formamide as well as dimethyl sulphoxide, while it was not so, with other solvents. Though chloroform is considered to be one of the best solvents for dissolving starch acetates, it did not dissolve japonica acid modified and acetylated starch. Therefore dimethyl formamide was selected for dispersion.

Solubility pattern of acetylated starch and zein are quite different. As the proportion of zein is high compared to starch, the solubility in dimethyl sulphoxide was not there. Basically dimethyl formamide, a solvent for proteins, this compound dissolved easily with dimethyl formamide. Pure acetone was not a suitable solvent, but when water was added to the extent of 20%, the nature was acquired by the starch-zein acetate. Though 80% acetone was a good solvent for dispersion, after heating and while drying lot of problems were faced. Hence dimethyl formamide (DMF) was selected as a solvent.

Preparation of Films

Generally films are prepared using acetylated rice starches. Acetylated indica rice starch and acetylated japonica starch have also given good films. Different properties of films prepared by using acetylated starches mixed with zein are depicted in Fig. 1. As the mechanical properties of the films appeared to be good, the following formulations containing 40 parts of 10% indica rice starch acetate of ~38% acetyl content and 2.3 DS and 70 parts of 20% zein was used.

Though films with acid modified and acetylated starches could be made, there were problems while drying these films due to different proportions of starches used. Here, a 5% concentration of indica rice acid modified and acetylated could be ideal to give film of good quality. Hence these acid modified and acetylated rice starches were dispersed in DMF. While mixing, followed by heating in waterbath and pouring on the plate, clear uniformity was seen, but while drying in the oven, precipitation of proteins was seen which is shown in Fig. 1. Following were the proportions used:

- (i) Six percent indica starch acid modified and acetylated + 6% zein
- (ii) In a total weight of 1.2 g, 30 parts was indica acid modified and acetylated starch of 6% concentration and 70 parts was zein of 6% concentration.
- (iii) Six percent japonica starch acid modified and acetylated + 6% zein
- (iv) In a total weight of 1.2 g, 30 parts was japonica starch acid modified and acetylated and 70 parts was zein as before (ii).

In order to get uniformity of the film, chemical reaction was carried out on starch and zein by acetylation and after usual procedures, the films prepared (5% concentration) were found to be very good and these were kept in an oven. After removal from the oven, the films were placed at room temperature for 1 to 2 h and then in a desiccator, at 51% RH and were removed after 10 days. It was found that there was no change in the films, indicating that the films were of good quality (Fig. 1). It was very interesting to observe that even waxy rice starch which was acetylated with zein could also give good film. Further work is needed to understand the behaviour of japonica waxy rice starch with zein.

The following combination was the best to adopt - 5% concentration of each of indica rice and zein acetylated, japonica and zein acetylated and japonica waxy and zein acetylated.

Tensile Strength

This aspect of study was carried out in all the films, after keeping in a desiccator at 51% RH for minimum 10 days. The thickness of films varied from 0.05 to 0.07 mm. Though all films could register some values for Young's modulus, maximum point, deformation, low break, deformation at low break etc. only 1. indica rice acid modified and acetylated and mixed with zein and 2. indica rice acetylated for 30 min + zein added could only register an Young's modulus value of $1.02 \times 10^6 \text{ N m}^{-2}$ and $9.48 \times 10^6 \text{ N m}^{-2}$ others could register other data except Young's modulus. Indica acetylated to which zein protein added gave higher value for Young's modulus.

It is evident that good films could be prepared with ideal proportion of waxy starch acetylated with zein. However, further work is needed to confirm the above results. Perhaps the quality of films could be improved with further changes of conditions of preparation, RH conditions, as it has been suggested that 51% RH is the condition for the films prepared from starch in native form along with the ingredients like polyvinyl alcohol and polyethylene co-acrylic acid by Lawton (1996).

Differential Scanning Thermogram Analysis

Figure 2 and Table 3 present data on parameters measured from the thermograms of various native and modified starches.

Table 3: Some parameters measured from differential scanning calorimetry thermograms of various rice starches, their acid modified, acid modified and acetylated

Native and modified ones	Starting point of glass transition temperature (SPT _g T) (°C)	Glass transition temperature (°C)	Difference in specific heat capacity before and after T _g (C _p)	End point of glass transition temperature (EPT _g T) (°C)	Melting point (°C)	Decomposition point or degradation or breaking point in °C
Indica native	204.8	212.8	0.815	217.4	268.3	269.9
Indica AM	204.2	209.6	0.734	215.0	267.1	269.5
Indica AMA	209.9	215.6	0.866	221.6	326.3	334.0
Japonica native	219.0	223.9	0.276	228.2	262.2	271.8
Japonica AM	202.2	204.8	0.307	208.3	264.1	264.4
Japonica AMA	208.5	214.5	1.692	237.5	319.7	325.3
Japonica waxy, native	227.5	231.7	0.669	237.3	272.6	275.0
Japonica waxy, AM	213.0	230.7	1.858	233.0	260.5	270.2
Japonica waxy, AMA	240.5	251.5	1.610	257.9	307.0	318.0

Note: The values of native starches have been taken from the part I paper

In the case of indica starch, starting point of glass transition (SPT_gT) temperature decreased marginally in acid modified (AM) one but increased in acid modified and acetylated one (AMA) compared to the native starch. The glass transition temperature (T_g) decreased after acid modification but increased after AMA, though not to a greater extent. The difference in specific heat capacity before and after glass transition (C_p) decreased after acid modification and increased after AMA. Similar changes had occurred in end point glass transition temperature (EPT_gT). Melting point remains almost same after acid modification but increased enormously after AMA i.e., about 58°C. The decomposition temperature remained almost same in native and acid modified one but increased after AMA by 64°C. This observation indicated that acid modified starch was almost similar to native starch and also probably the granular structures of these starches remains almost same. The melting point increase in AMA indicates that acetylation occurred after granular structure breaking up. Acetylation increased the strength of linear and branched polymers in the disrupted granule with acetyl groups replacing the OH- group to create a bridge between the molecules. If these molecules were to be melted higher energy would be required and in turn, there was increase in the melting point.

Japonica starch showed an interesting behaviour. Starting point of glass transition temperature decreased in acid as well as in AMA starches, the degree of decrease being greater in acid and less in AMA one. On the otherhand, C_p increased continuously, to a lesser extent in AM and to a greater extent in AMA. End point glass transition temperature occurred at a little higher temperature compared to its native. Much differences are not seen between native and acid modified one but about 60°C increase was seen in AMA one. Therefore, it is clear that linear and branched molecules in japonica starch granule could become stronger and stronger in AMA one, but softer to some extent in AM one which would make the starch acetate molecule to slide one above the other, or mobilize or glass transit at a lower temperature compared to its native.

The phenomenon was quite different in japonica waxy starch. All parameters decreased after AM whereas the value for C_p increased by almost three times compared to its native starch. But in AMA one all parameters increased indicating the special behaviour of branched polymer of japonica rice starch.

Comparison Between Natives and Two Types of Modifications

Among the natives, the highest values were shown by the japonica waxy starch, lowest by the indica starch and in between values were shown by the japonica non-waxy starch for all the parameters. Acid modification induced molecular breakdown and there was decrease in all the parameters except C_p values. But the acetylation of the acid modified starches, increased all the parameters in general. The values appeared to be very high in acid modified and acetylated waxy starches, suggesting the special behaviour of branched alpha 1→4 and alpha 1→6 glucose polymers.

Differential Scanning Thermograms of Rice Starches and Zein Before and After Acetylation

As seen from Table 4 the SPT_gT was almost the same in all starting material before acetylation, i.e., a mixture of rice starch and protein zein except in Japonica-zein where it occurred at a lower temperature by 4°C.

In the DSC thermogram of zein, there were clearly three peaks before melting point. The T_g values were 200.2, 227.6 and 257.7°C, respectively, probably because of the crude nature of the protein which contained lot of impurities. For all comparison purposes, through out these studies, glass transition temperature had been calculated in the peaks just before the melting points. Difference in specific heat capacity before and after glass transition value was 0.294 mJ mg⁻¹ in the case of zein. The melting point (~306°C) and decomposition point (~308°C) appeared almost same with a narrow difference.

When this zein was combined with indica rice starch in the proportion as mentioned in the methods, the parameters completely changed, showing decreased values. This was the general observation in all starches except C_p which increased in starch-zein and further increased in starch-zein acetylated ones. But on acetylation, all values increased compared to starch and zein combinations (Table 4). There was enormous increase in melting point when compared to starch and zein mixture, C_p value increased in indica combination to a greater extent.

Among acetylated ones, indica-zein acetylated ones showed the highest values for all the parameters as compared to indica-zein. Japonica-zein acetylated ones showed higher values as compared to japonica-zein, japonica-waxy acetylated one showed lower values compared to japonica waxy-zein. The enormous change in the decomposition temperature of japonica-zein acetylated one was worth noticing vis-à-vis to its melting point. The melting point increase was 53°C for indica-zein acetylated one compared to its respective starch-zein acetylated one, ~34°C for japonica-zein acetylated, 41°C for waxy-zein acetylated one compared to their respective starch-zein mixture melting points. The difference in decomposition point and melting point temperature at which decomposition in indica-zein acetylated one occurred was 8°C, in japonica-zein acetylated one it was ~60°C and in waxy-zein acetylated one it was 7°C higher than their respective melting points. This observation is interesting, as decomposition of starch-zein acetate followed by denaturation and decomposition, which occurred almost at same temperature in indica and waxy, but at very high temperature in japonica-zein acetylated one.

It has also been observed that all parameters in zein as such were high. Due to the addition of this zein to starch, the values decreased and by acetylation of this starch-zein mixture, the values increased further. Perhaps only one parameter i.e., melting point of indica-zein acetylated one was higher than zein melting point by about 3.5°C.

Therefore, it is evident that addition of zein chemically could bring a lot of changes probably by creating a covalent bond between linear, branched, acetyl and zein, in addition to the hydrogen bonds between the different fractions of the starch granule to make the molecule hard and also the starch zein acetylated one was becoming very strong in all respects.

Table 4: Some parameters measured from DSC thermograms of rice starch in combination with corn protein zein before and after acetylation

Material	SPT _g T (°C)	T _g (°C)	C _p	EPT _g T (°C)	M. pt. (°C)	D. pt. (°C)	D. pt- M. pt
Zein protein	255.2	257.7	0.294	260.4	306.1	307.8	1.7
Indica zein	208.1	214.5	0.970	219.9	256.9	282.7	25.8
Indica zein acetylated	215.6	222.2	1.414	230.3	309.6	317.9	8.3
Japonica zein	204.2	209.9	0.712	215.6	256.1	278.8	18.7
Japonica zein acetylated	211.0	214.8	0.614	220.3	289.9	349.3	59.4
Japonica waxy-zein	208.7	214.1	1.020	218.8	254.7	268.4	13.7
Japonica waxy zein acetylated	206.4	212.1	0.995	217.5	296.9	304.1	7.2

Note: SPT_gT: Starting point of glass transition temperature, T_g: Glass transition temperature, C_p: Difference in specific heat before and after glass transition, EPT_gT: End point of glass transition temperature, M. pt: Melting point, D. pt: Degradation or decomposition or breaking point

Table 5: Exothermic and endothermic values of native starches, native protein, their combination and their combination along with acetylation

Starch/protein/modified	Delta H (mJ mg ⁻¹) exothermicity	Delta H (mJ mg ⁻¹) endothermicity
Indica starch native	-059.0	119.4
Native zein	No peak	113.5
Indica zein	-155.2	084.0
Indica zein acetylated	-135.6	076.8
Japonica starch native	-202.8	125.9
Native zein	No peak	113.5
Japonica zein	-195.9	085.8
Japonica zein acetylated	+ 231.3	-119.6
Japonica waxy native	-034.1	109.4
Japonica waxy zein	-195.9	085.8
Japonica waxy-zein acetylated	-047.7	052.0

Exothermic and Endothermic Changes in Starch, Zein and their Acetylated Products

Table 5 shows the exothermic and endothermic heat changes in differential scanning thermograms of melting and decomposition peaks of starch, starch-zein and starch-zein acetylated ones. Generally, the top peaks were to be exothermic in nature and bottom peaks were to be endothermic in nature.

With respect to top peaks, all native starches were exothermic. Zein protein had shown endothermic nature with no exothermic peak. The probable reason might be the nature of protein which was very crude.

Indica and zein in combination had shown their exothermic as well as endothermic nature indicating that the combination effected both changes, though zein as an independent one had shown only its endothermic nature. When indica-zein acetylated, the exothermic nature continued, but the amount of heat evolution decreased, as compared to indica-zein (by 19.6 mJ mg⁻¹) indicating that indica-zein acetylated one was having more heat holding capacity.

Japonica-zein mixture had shown both exothermic and endothermic behaviours. On acetylation, i.e., on the chemical combination, the system behaved in a reverse way i.e., endothermicity in exothermic peak and exothermicity in endothermic peak. A detailed plan of work is also to be made in this direction also.

In japonica waxy native, exothermic and endothermic behaviour pattern was shown. On combination of this starch with zein, the behaviour pattern was both exothermic and endothermic. But on acetylation of waxy and zein, the evolution of heat was very less, i.e., around 48 mJ mg⁻¹ indicating that heat holding capacity of japonica waxy zein acetylated one was high.

Notable observations from this study have been that (i) endothermicity reduced in indica and waxy starches starting from the respective rice starch, zein protein and their acetylated combinations, indicating the significance of each compound individually and in combination and (ii) japonica-zein acetylated one had shown exothermic behaviour.

CONCLUSIONS

Three types of rice starches were combined with one of the industrial protein viz. zein (corn protein), individually. They were mixed physically as well as chemically (by acetylation). The rice starches were also acid modified and acetylated. Clear films could be prepared by physical mixing of these modified starches with zein protein in different proportions, but the films had crystal type projections, whereas the chemically prepared films from starch and zein had uniformity, which has been observed for the first time and this concept has been patented at Japan through National Food Research Institute. Starches after acid modification and acetylation, became harder and harder, as seen by their higher melting points and higher glass transition temperatures and higher specific heat capacity. By physical combination of the starches and protein (zein), the mixture became soft, as

indicated by decrease in melting point, whereas on acetylation of these combinations, they became harder and harder as compared to their respective natives. Only endothermic change was noticed in zein protein, but on combination with starch and acetylation, the endothermicity decreased.

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