



Physicochemical and release characteristics of acetylated Indian palmyrah retrograded shoot starch



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ABSTRACT

The aim of the present study is to determine the influence of serial modifications, including retrogradation followed by acetylation on morphological, physicochemical and drug release properties of retrograded Indian palmyrah (*Borassus flabellifer* L.) shoot starch. The acetylated retrograded starches prepared by using different concentrations of acetic anhydride were shown a degree of substitution (DS) in the range of 0.16–0.55. Acetylation of retrograded starch produced significant morphological changes from rough to smooth surface. The amylose content, water holding capacity, swelling and solubility power tend to increase with increase in DS. A strong peak at 1751 and 1032 cm^{-1} confirms the formation of acetylated retrograded starch. The TGA data reveal that with increase in DS there is an increased thermal stability and decreased bound water of starch. The elemental analysis also confirms the addition of acetyl groups because of increased carbon and hydrogen content. The matrix tablets of acetylated retrograded starch with high DS showed a delayed release in gastric pH and sustained release in simulated intestinal fluid. Overall, this result suggested that acetylated retrograded starch with high DS are thermally stable and can be used for formulating protein and peptide drugs for colon targeting.

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1. Introduction

Palmyrah (*Borassus flabellifer* L.) also known as toddy palm belonging to the family Arecaceae and commonly cultivated in tropical Asian countries such as India, Thailand, Bangladesh, Myanmar, Sri Lanka, Malaysia, etc. [1]. The palm produces a several beneficial products like sweet fruit pulp, the inflorescence sap, the seed-shoots and the kernel of young and mature nuts [2]. During the seed germination, the excess of carbohydrate is stored in the form of starch, which leads to the formation of the palmyrah seed shoot (Fig. 1). As reported earlier, the higher starch content of palmyrah shoots can be used in the formulation of sustained release tablets [1].

Starch a natural, biodegradable polymer which is extensively used as pharmaceutical excipient because of its high abundance and cheap in nature to that of synthetic polymer. It is widely used as excipient in binding and to control the release of drug from tablets. However the native starches have limited usage due to their poor

thermal resistance, high moisture sensitive and tendency towards retrogradation [3]. Therefore an alternative way to improve the usage of starches is by developing modified starches which increase the positive attributes. The modification of native starch can be done by physical or chemical method. This enables the changes in structural and physicochemical characteristics of native starches.

Retrogradation is one of the physical means of producing a resistant starch, which on further chemical modifying can reduce the susceptibility towards amyolysis [4]. During retrogradation, the starch recrystallization and re-association occurs by means of forces and inter or intramolecular hydrogen bonding [5]. The retrograded starch can further be chemically modified by means of acetylation.

Acetylation of starch is one of the chemical modifications, where the starch is esterified with CH_3CO in varying degree of substitution. It was found that starch acetates are less susceptible towards the amylase activity than the unmodified starch [6]. Nowadays the starch acetates have been widely used because of their low gelatinization temperature, storage stability, good swelling and solubility power [7].

The objective of this study was to prepare starch acetates from retrograded starch with different degree of substitution and

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Fig. 1. Palmyrah seed shoot.

to investigate the physicochemical and release characteristics of acetylated retrograded starches.

2. Materials and methods

2.1. Materials

The palmyrah seed shoots were obtained from the local market of Visakhapatnam, Andhra Pradesh and authenticated at Andhra University with voucher specimen number 22086. Drug diclofenac sodium was kindly received as gift sample from the Metrix Healthcare Private Limited, Ahmedabad, India. All chemicals used were of analytical grade and were procured from Sigma–Aldrich (India).

2.2. Starch isolation

The shoots were washed with distilled water to remove the traces of contaminants. Then the shoots are chopped and wet ground to get thick slurry. The slurry obtained was allowed to steep in 0.05% NaOH solution for 24 h [8]. The resultant slurry was subjected for repeated washings with distilled water until the supernatant liquid becomes clear. Then the slurry was allowed to pass through a sieve to remove thick fibrous matter and a filtrate of white starch suspension was allowed for settling. The supernatant liquid was decanted and the sedimented starch was air dried for 48 h. Finally the crude starch obtained was tightly packed in polyethylene bags.

2.3. Preparation and acetylation of retrograded starch

Retrogradation of starch was done according to the method described by Zieba et al. [4]. Acetylated retrograded starch (AS) was prepared by following the method of Wang and Wang [9] with slight modification. A 10% retrograded starch suspension was prepared by dissolving 8 g of retrograded starch in distilled water and magnetically stirred for 30 min. The pH of the suspension was maintained throughout the process at 8.0–8.5 pH with 1 M NaOH with constant stirring. To the uniform suspension, acetic anhydride of varying quantities 0.8 g, 1.6 g and 2.4 g was added drop wise while maintaining the pH between 8.0 and 8.5. The stirring continued for 90 min and later on the pH adjusted to 4.5 with 1 M HCl. Finally the suspension was filtered through Whatmann filter paper 4. The obtained residue was washed with distilled water for five times and left for air drying at room temperature. Thereby producing three starch acetates (AS-1, AS-2 and AS-3) varying in acetyl content and finally the percentage yield was calculated.

2.4. Determination of acetyl percentage and degree of substitution

Acetyl percentage (AC%) and degree of substitution (DS) was determined by the method of Wurzburg [10]. An acetylated starch of 1 g was taken in a stoppered volumetric flask containing 50 mL of 75% ethanol. The flask was subjected for agitation, warmed to a temperature of 50 °C for 30 min and cooled. To this 40 mL of 0.5 M KOH was added and the excess KOH was back titrated with 0.5 N HCl using phenolphthalein as an indicator. The above solution was left aside for 2 h in order to leach out alkali which may present in the sample. A blank was performed by using unmodified retrograded palmyrah starch.

$$AC\% = \frac{[B - S] \times N \times 0.043 \times 100}{W} \quad (1)$$

where 'B' and 'S' are the volume of titrant used for blank (mL) and sample (mL) respectively. 'N' is the normality of 0.5 N HCl and 'W' is the weight of the sample (g) taken.

AC% was used to calculate the DS, according to the following equation:

$$DS = \frac{162 \times \% \text{Acetyl group}}{4300 - (42 \times \% \text{Acetyl group})} \quad (2)$$

2.5. Physicochemical characteristics of acetylated starch

2.5.1. Elemental analysis, pH and amylose content determination

The moisture content of each starch sample was determined according to the method described in AOAC [11]. The amylose content of each starch sample was determined by the method described by Juliano [12]. pH of starch samples (1%, w/v) was determined by means of digital pH metre. The elemental analysis was performed for elements like carbon and hydrogen by using an Elemental Analyzer (Make-M/s Elementar, Germany; Model-Vario EL III).

2.5.2. Water holding capacity

Water holding capacity of acetylated starch was determined by the method described by Deepika et al. [13]. A suspension of 1 g starch in 15 mL of distilled water was prepared, agitated for 1 h and centrifuged at 3000 rpm for 10 min. The supernatant liquid thus obtained was decanted and the wet starch was weighed.

$$WHC(\%) = \frac{W_{WS}}{W_S} \times 100 \quad (3)$$

Here, W_{WS} is the weight of wet starch (g) and W_S is the initial weight of starch (g) on dry basis.

2.5.3. Swelling and solubility

The suspensions of each starch (1%, w/v) was prepared by using distilled water and are subjected to a different temperatures of 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C, 90 °C for 30 min in a temperature controlled water bath. The samples were left for cooling at room temperature and are centrifuged at 3000 rpm for 15 min. In a pre-weighed Petridish, the supernatant liquid thus obtained was carefully decanted and the swollen sedimented starch was weighed [14,15]. Then the supernatant liquid was evaporated overnight at 110 °C and residue was obtained. The weight of this residue represents the solubilized portions of starch in water [16,17]. Swelling and solubility power were calculated according to the equation,

$$\text{Swelling power}(\%) = \frac{W_{WS}}{W_S \times (100 - \% \text{Solubility})} \times 100 \quad (4)$$

$$\text{Solubility}(\%) = \frac{\text{weight of soluble starch} \times 100}{W_S} \quad (5)$$

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