Preparation and Characterisation of Acetylated Corn Starches

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***Abstract*—In this study, acetylated corn starches with various degree of substitution (DS) were achieved following multiple treatments with acetic anhydride under alkaline conditions. Experimental analyses were performed to investigate the effect of acetylation on a range of starch properties, including granule morphology, solubility, swelling power, paste clarity, syneresis and freeze-thaw stability, and compare them with those of the native corn starch.**

It was found that no significant changes in shape, size or external appearance of the starch granules occurred following any of the treatments. The acetylated starches showed higher solubility and swelling power, and better paste clarity when compared with the corresponding native starch. Acetylation caused reductions in retrogradation tendencies. It was observed that the changes in these properties were proportional to the DS achieved by acetylation.

***Index Terms*—Acetylation, corn starch, physicochemical properties, retrogradation, degree of substitution.**

I. Introduction

In the food industry, starch is utilised to produce various functionalities such as thickening, stabilizing, texturing, gelling, encapsulation, and shelf-life extension. It plays an important role in determining the quality and texture of many foods; controlling the acceptability and palatability of most food products. Despite their advantageous properties, some starches in their native form may pose problems. For example, the tendency of its viscosity to increase rapidly and be thickened during heat treatment may cause difficulties in industrial food unit operations such as in pumps and heat exchangers. Some unmodified starches also have drawbacks such as low shear-stress and thermal resistance, thermal decomposition and high degree of retrogradation which limit their use in industrial food application [1]. Such undesirable characteristics may be overcome by modifying the starch [2]. Modification does not alter the appearance of the starch but can improve the desired properties of the starch.

The most common method of starch modification is acetylation, i.e. esterification usually with acetic anhydride. Acetylation replaces hydroxyl groups in the native starch with acetyl groups. According to Rutenberg and Solarek [3], introduction of these acetyl groups reduces the bond strength between starch molecules and thereby alters the properties. Normally, the resultant acetylated starch will possess good stability at low temperatures and exhibit improved resistance to retrogradation. Acetylation is therefore common for baked, dry, canned, and frozen food products such as gravies, fruit pies, salad dressings, and filled cakes.

Acetylated starches are commercially produced by acetylation with acetic acid, acetic anhydride, ketene, vinyl acetate, or a combination of these reagents [4]. The reagents and solvents used during the acetylation process must yield acceptable products and satisfy the requirement of government regulations especially when the resultant starch acetates are intended for food purposes. Reactant concentration, reaction time, temperature, pH, and the presence of catalyst may influence the final product [5].

The extent of acetylation on the key properties may vary depending on the methods and the conditions involved in acetylation process. The method used in adding acetic anhydride may affect the reaction leading to the variation in the acetyl contents and degrees of substitution (DS) and the resultant acetylated starch properties. Essentially, DS provides information concerning the number of hydroxyl functions substituted per glucose unit. No published study on starch acetylation has been performed using multiple treatments with acetic anhydride. A study on acetylation that approaches the actual scale of industrial production would enable better understanding of potential scale up effects.

The objectives of this study were to prepare and characterise some physicochemical properties of acetylated corn starches. Acetylation was performed at a larger scale using 1 kg of native corn starch. Acetylated starches with various DS were achieved following multiple treatments with acetic anhydride rather than using the conventional strategy of incrementing acetic anhydride concentration. Some physicochemical properties of the starch relevant to food manufacturing were studied simultaneously to investigate the effect of acetylation on starch properties. These included starch granule morphology, swelling power and solubility, paste clarity, and syneresis and freeze-thaw stability.

II. Experimental Procedure

A. Materials

A commercial corn starch was sourced (White Wings Foods, Australia). The moisture and amylose content of the corn starch (NCS) were 10.6 and 20.6%, respectively. Amylose content was quantified using an amylose/ amylopectin assay kit (Megazyme, Warriewood, Australia). All chemicals used were reagent-grade.

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B. Preparation of Acetylated Starch

Acetylated starch samples were prepared following the method of Van Hung and Morita [6] but under slightly different conditions. The samples were prepared in a 5 L acrylic tank equipped with baffles. Mechanical agitation was provided by a stirrer and an electric motor. A three-blade marine propeller was used to ensure homogenous mixing during the process. pH and temperature probes connected to a LabChem CP Conductivity/pH meter (TPS, Brisbane, Australia) were inserted into the starch solution to record pH and temperature reading during the acetylation.

One kilogram of commercial cornflour (dry basis) was combined with 2.5 L of distilled water to provide a starch-water ratio of 0.40. The starch slurry was mixed for 1 h at room temperature to fully suspend the starch granules. After adjusting the pH to 8.0 with 1 M aqueous sodium hydroxide, acetic anhydride (8%, w/w, on a starch dry basis) was added drop wise. Sodium hydroxide (1 M) was added simultaneously at a rate sufficient of 0.02 L/min using a pump controller (Dynavac, Brisbane, Australia) equipped with pump motor (Emerson, St Louis MO, USA) to maintain the pH of the suspension between 7.8 to 8.4 during the reaction. The reaction was allowed to proceed for 60 min (i.e. reaction time) following completion of acetic anhydride addition. The slurry was then adjusted to pH 5.5 with 1 M hydrochloric acid to quench the reaction. The slurry was vacuum-filtered and the resulting cake was mixed with 2 L of distilled water and refiltered. It was washed three times with distilled water to remove residual acid and then oven dried overnight at 40^{o}C.

Repeated treatments (maximum of 3) with acetic anhydride were performed. Repetition of the treatment implies the starch was treated again with another portion of acetic anhydride following careful washing with distilled water. The same amount of sodium hydroxide was consumed for each treatment with acetic anhydride.

C. Determination of Acetyl Content and DS

Measuring the acetyl content and degree of substitution of the acetylated starch is a prime method in recognizing the result of acetylation. In this study, the percent acetylation (% acetyl) and degree of substitution (DS) were determined titrimetrically following the method of Wurzburg [7] with minor modification.

One gram of an acetylated corn starch sample was suspended in 50 mL of a 75% ethanol solution. The slurry was kept in a water bath at 50^{o}C for 30 min with constant stirring. The slurry was then cooled at room temperature and 40 mL of 0.5 M potassium hydroxide was added. The slurry was allowed to stand for 72 h at room temperature with occasional swirling. The excess alkali was titrated with 0.5 M hydrochloric acid using phenolphthalein as an indicator. The solution was allowed to stand for another 2 h. Any additional alkali that might leach from the sample was titrated. Blanks with native starch were analysed concurrently. The sample volume, the hydrochloric acid normality and the volume of hydrochloric acid required to titrate the blank and sample were recorded. Duplicate measurements were performed for each starch sample.

D. Characterization of Starches

Morphological properties of the native and acetylated starch granules, especially the shape and diameter size, were observed using a scanning electron microscope (SEM) Philips XL20 (FEI Company, Eindhoven, The Netherlands) at an accelerating potential of 10.0 kV. All samples were sputter-coated with carbon prior examination. The images obtained were then analysed using the image analysis software AnalySIS® (Soft Imaging System, Adelaide, Australia) to deduce the particle-size frequency distribution.

Starch swelling power and solubility were determined by following the method proposed by Subramanian *et al*. [8] with minor modification. An Eppendorf centrifuge type 5415D (Eppendorf South Pacific, New South Wales, Australia) was used throughout the analysis.

Clarity of starch pastes (expressed as % light transmittance) was determined as described by Perera and Hoover [9]. A graph of light transmittance (%) versus storage time (h) was plotted presenting the effect of storage on starch paste clarity for different starches.

The method described by Liu *et al*. [10] was followed to characterized syneresis and freeze-thaw stability of starches. For each freeze-thaw cycle, the tubes were placed in a freezer for 22 h then thawed at 30^{o}C for 2 h in a water bath. After each cycle, the sample was centrifuged at 3,100 rpm for 20 min in a Sigma 3-18K centrifuge (Sigma, Germany).

III. Results and Discussion

A. Acetyl Content (%) and Degree of Substitution (DS)

The extent of acetylation for various starches is primarily influenced by the introduction of acetyl groups into the starch structure, and is simply expressed as DS. Singh *et al.* [11] has reviewed the DS for acetylated starches prepared from diverse origins and reported significant variation. The individual characteristics of the starch and difference in acetylation conditions may account for this variation.

The values of acetyl content (%) and DS achieved by acetylation of the NCS starch are summarised in Table I. The term of ACS 160, ACS 260, and ACS 360 used in Table I and also throughout this paper refers to the acetylated corn starches produced by 1x, 2x, and 3x treatment(s) with acetic anhydride for 60 min, respectively.

TABLE I: The Acetyl Content and DS of Acetylated Corn Starches Obtained from Different Treatments

Starches	Acetyl Content (%)	DS
ACS 160	2.16	0.08
ACS 260	3.88	0.15
ACS 360	5.29	0.21

As shown in Table 1, the range of acetyl content and DS achieved in this study were around 2.16 – 5.29% and 0.08 – 0.21, respectively. Acetyl content and DS increased with number of repeated treatments with acetic anhydride. Wurzburg [7] has previously suggested that repeated treatment with acetic anhydride may be used if a higher degree of acetylation is desired. These results provide experimental validation of this assertion.

B. Starch Granule Morphology

Fig. 1 shows the images of native (NCS) and acetylated corn starch granules observed under SEM. The image illustrates significant variations in granule morphology and size.

Corn starch granules appear to vary from 4 to 18 µm in size (Fig. 1). This is similar to values of 5 to 20 µm for Indian corn starch observed by Singh *et al.* [1]. The shape of the starch granules also vary from angular to polyhedral, which is consistent with observations of corn starch morphology reported by others [1], [12]. Furthermore, there appears to be no significant observable changes in shape, size, or external appearance of the corn starch granules following acetylation (Fig 1b-d).

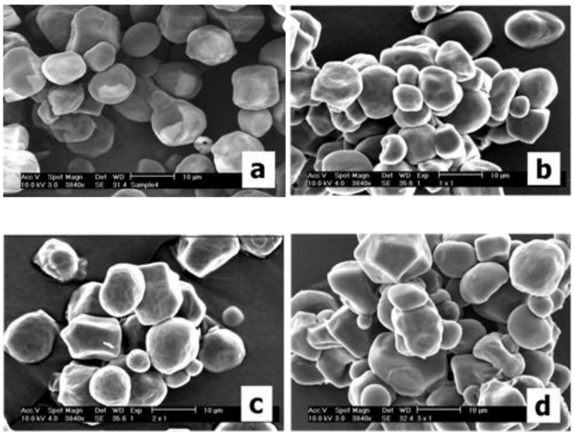

Fig. 1. Scanning electron micrographs of native and acetylated corn starches: (a) NCS, (b) ACS 160, (c) ACS 260, and (d) ACS 360 (Bars = 10 µm).

C. Swelling Power and Solubility

Swelling power and solubility at 90°C of acetylated corn starches with different degrees of substitution (0.08 – 0.21) were in the range of 17.37 to 27.85 g/g and 9.50 to 17.38%, respectively – see Table II. These values are higher than for the NCS (15.15 g/g and 8.50%, respectively). This increase in swelling power and solubility for an acetylated starch is consistent with observations made by other researchers [1], [5], [6], [10], [13]–[18].

TABLE II: SWELLING POWER AND SOLUBILITY OF NATIVE AND ACETYLATED CORN STARCHES OBTAINED FROM DIFFERENT TREATMENTS

Starches	Swelling Power (g/g)	Solubility (%)
NCS	15.15	8.50
ACS 160	17.37	9.50
ACS 260	25.99	12.78
ACS 360	27.85	17.38

Lawal [19] postulated that the introduction of (bulky) acetyl groups into starch molecules following acetylation leads to structural reorganization as a consequence of steric hindrance. This results in repulsion between starch molecules, thus facilitating an increase in water percolation within the amorphous regions of granules and a consequent increase in swelling capacity. The structural reorganization may also weaken the starch granules following acetylation. This enhances the leaching of amylose from the granule and thus increases starch solubility.

As well, the increases in swelling power and solubility were proportional to the degree of substitution (DS) (Table I). ACS 130 starch, which possessed the lowest DS value of 0.0297, exhibited the lowest values of swelling power and solubility (16.66 g/g and 9.07%, respectively). The highest values of swelling power and solubility were observed in ACS 330 starch which possessed the highest value of DS of 0.19. This tendency is in reasonably good agreement findings in previous studies with acetylated potato [1], [17].

D. Paste Clarity

In general, starch paste became clearer and more translucent after being heated at 90°C for 1 h. Results from the studies conducted on starch paste clarity are summarised in Fig. 2. Acetylation improved the paste clarity of the corn starches as shown by an increase in % light transmittance.

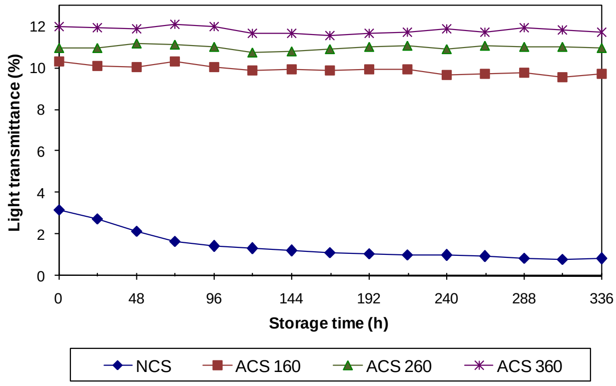

Fig. 2. Effect of storage time on light transmittance (%) of starch pastes.

As shown in Fig. 2, all acetylated corn starches (including MAPS 281) showed a significantly higher % light transmittance when compared to the NCS starch. This indicates that the acetylated starches possessed a better paste clarity and greater resistance to retrogradation. The figure also shows that the % light transmittance of all acetylated corn starches was slightly reduced as the duration of storage increased from 24 to 336 h. By contrast, a pronounced reduction in % light transmittance was observed in NCS over the same period. Improved paste clarity is a useful property in the manufacture of some food products, such as salad dressings and confectionery products. Similar effects of acetylation on paste clarity have been widely reported for various types of starch, as in [1], [16], [19].

E. Syneresis and Freeze-thaw Stability

The retrogradation tendency of gels prepared from native and acetylated corn starches was measured by determining syneresis (percentage of separated water) during storage at 4°C. All starch gels started to retrograde after 24 h, indicated by an increase in % separated water which increased progressively with storage (number of cycles). However, the acetylated starch gels showed a lower increment in syneresis than the NCS gel, as illustrated in Fig. 3. This indicated that acetylation served to reduce syneresis.

The highest resistance to freeze-thawing cycle(s) was observed in ACS 360 starch (lowest % separated water) and the lowest was in native corn starch (highest % separated water). Syneresis in freeze-thawed gels is due to the rearrangement of amylose molecules in starch granules at reduced temperature which acts to exclude water from the gel structure. Retrogradation may take place when a gelatinised starch is cooled. An increase in % separated water indicates a lack of freeze-thaw stability.

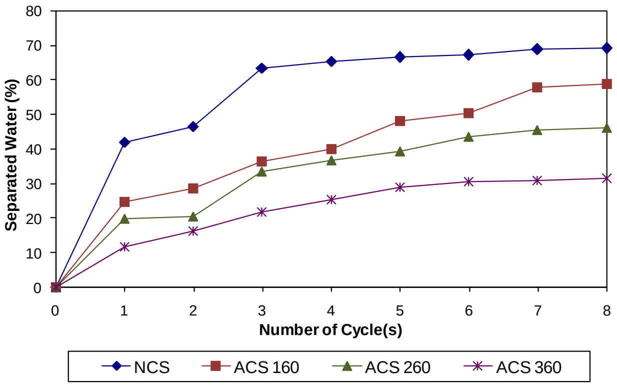

Fig. 3. Syneresis and freeze-thaw stability study of native and acetylated corn starches.

Gonzales and Perez [14] reported that a reduced retrogradation tendency in acetylated rice starch is due to the prevention of alignment and association between macromolecules by the acetyl groups. In addition, Singh, *et al*. [1] postulated that the alteration in the retrogradation properties of acetylated starches may also be due to the increase in water retention capacity of the starch molecules due to the presence of acetyl groups in the refrigerated stored gels. Consequently, acetylation is used to stabilise starch gels in low temperature conditions that may cause retrogradation.

IV. Conclusion

Some important properties of starch relevant to food manufacturing were studied simultaneously following acetylation. These include acetyl content and DS, granule morphology, swelling power and solubility, paste clarity, syneresis and freeze-thaw stability. A comparison between the properties of native starch to those of acetylated starches was also performed. Acetylation has caused an increase in swelling power, solubility, paste clarity, and freeze-thaw stability of corn starch.

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