Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 339 (2004) 2673–2682
Carbohydrate RESEARCH

Succinyl and acetyl starch derivatives of a hybrid maize: physicochemical characteristics and retrogradation properties monitored by differential scanning calorimetry

Olayide S. Lawal*

Department of Chemical Sciences, Olabisi Onabanjo University, P.M.B 2002, Ago-Iwoye, Ogun State, Nigeria

Received 22 April 2004; accepted 19 August 2004
Available online 12 October 2004

Abstract—Starch isolated from a hybrid maize (8535-23) was chemically modified by succinylation and acetylation. No pronounced difference was observed between the X-ray pattern of native starch and modified starch samples, and the samples gave the characteristic A pattern of cereal starches. Onset temperature (T_o), peak temperature (T_p), concluding temperature (T_c) and enthalpy of gelatinisation (ΔH), reduced after succinylation and acetylation, but gelatinisation temperature range increased following starch modifications. Modifications reduced starch retrogradation.

Keywords: Maize starch; Succinylation; Acetylation; Retrogradation

1. Introduction

The significance of various modifications on starches of different origins has been expounded in the literature.[1–6] These modifications are carried out to improve the functional and physicochemical parameters of the starches in various industries, particularly where native starch itself cannot give optimal performance. Previously, many types of chemical modifications have been applied to starches of various plant sources. These include acid hydrolysis,[7] oxidation,[8] etherification, esterification and cross-linking.[9,10] Specific chemical modifications are applied to starches to meet the requirements of various industrial applications.

Derivatisation of starch with an ionic substituent group such as succinate at low degree of substitution (DS) converts it into a polyelectrolyte, which makes it acquire typical properties of a polyelectrolyte like increased hydrophilic character and increased solution viscosity.[11] This modification is also known to weaken the internal bonding that holds the granules together.[6,12] In addition starch succinate offers very desirable properties such as low-temperature stability, high-thickening power, low-gelatinisation temperature, clarity of cooked food, good film-forming properties and reduced tendency to retrograde.

Organic bases such as pyridine, α-picoline, pyrrole, triethylamine and piperidine have been used as catalysts for the esterification of starch.[13,14] In the literature, low-DS starch succinates have been obtained by refluxing starch in pyridine at 115 °C in the presence of succinic anhydride for varying reaction times without prior gelatinisation.[15,16]

The base-catalysed reaction of pyridine with starch is a nucleophilic substitution (S_N2) reaction, and it passes through the formation of an intermediate complex. The substitution takes place mainly by an addition–elimination mechanism.[17] The purpose of pyridine is to increase the initial reactivity of the granule and to work as a catalyst for the reaction through the formation of the succinyl–pyridium intermediate.

Acetylation of starches by placing acetyl groups along its polymeric backbone decreases its gelatinisation temperature, increases its translucency, viscosity, freeze–thaw stability and reduced retrogradation.[18] Acetylation

* Tel.: +234 802 829 3036; e-mail: laidelawal2@yahoo.com

0008-6215/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2004.08.015

depends upon factors such as reactant concentration, reaction time, pH and presence of catalyst,[19] which determines the number of acetyl groups incorporated into the molecule.

When starches gelatinise, they become thermodynamically unstable structures, and as cooling progresses, a tendency to reassociate sets in among the starch molecules. A collective term for this process is retrogradation. Amylose retrogradation is known to be a rapid process taking only few hours, because of its linear structure, which facilitates faster reassociation, while amylopectin retrogradation, on the other hand, develops over a period of several days.[1,3,20]

Differential scanning calorimetry (DSC) has proven to be a vital tool in the thermal analysis of starches and retrograded starches.[21] In this method, the difference in energy input into a particular substance and a reference material is measured as a function of temperature while both materials are subjected to programmed heating or cooling. DSC has been used to study several factors that influence retrogradation of starches.[22,23] It has been reported that starch retrogradation enthalpies are usually 60–80% smaller compared with gelatinisation enthalpies, while retrogradation temperature range is usually broader than the gelatinisation temperature range for a given sample.[24]

The aim of the work reported herein was to investigate the effect of succinylation and acetylation on physicochemical properties of hybrid maize. The research also hopes to study the effect of such modifications on the retrogradation behaviour of starch using differential scanning calorimetry.

2. Materials and methods

2.1. Materials

Hybrid maize seeds (8535-23) were gifts from the maize breeding unit of the International Institute of Tropical Agriculture (CGIAR/IITA) Ibadan, Nigeria. All other reagents used in this work were of analytical grade.

2.2. Isolation of starch

Maize seeds (1 kg, winnowed) were steeped in 10 L of 0.2% aq SO_2 for 28 h at 30 °C, following which treatment, the steeping solution was discarded, and the swollen grains were washed with water. The sample was blended for 30 min using a blender (Braun Multimix de luxe MX40, type 2291). The slurry obtained following blending was resuspended in distilled water (5 L), screened using a 75-μm sieve and centrifuged for 30 min at 10,000*g* (Type GLC-1 Ivan sorvall, Inc, USA). Starch obtained after centrifugation was reslurried in distilled water (2 L), and protein was separated from the starch by toluene emulsification. Toluene (20 mL) was added to the starch suspension, and it was thoroughly mixed for 30 min and allowed to stand for another 2 h. An emulsion layer of denatured protein formed at the toluene–water interface and was discarded. The process was repeated for the starch slurry until the emulsion layer became negligible. The starch slurry was then washed with acetone and air dried for 24 h at 30 °C. The yield of native starch was 58.7% on the basis of the total weight of dry seeds.

2.3. Starch succinylation

Starch succinylation was carried out using the method described by Trubiano.[25] Solutions of succinic anhydride (2%, 3% and 4% w/v) were prepared separately with distilled water to obtain starch succinate of three different degrees of substitution. Native starch (100 g) was added to each solution in a 500-mL round-bottom flask, and constant heating and stirring were supplied with a hot plate–magnetic stirrer (Galenkamp). The liquid catalyst pyridine was added to the mixture at a ratio of 1:1 starch–pyridine. The mixture was then refluxed for 2 h at 115 °C, after which time it was cooled to room temperature (30 °C). The starch succinate obtained was then isolated from the pyridine mixture by precipitating in absolute EtOH, and the precipitate was washed with EtOH to remove traces of pyridine from the mixture. Traces of pyridine that remained in the starch were removed by adding the calculated amount of dil HCl (determined by titration) to the mixture.

2.4. Starch acetylation

The method of Sathe and Salunkhe[26] was used. Starch (100 g) was dispersed in 500 mL of distilled water and stirred magnetically for 20 min. The pH of the slurry obtained was adjusted to pH 8.0 using 1 M NaOH. Ac_2O (5, 10 and 15 g) was added to three separate mixtures over a period of 1h while maintaining a pH range of 8.0–8.5. The reaction proceeded for 5 min after the addition of Ac_2O. The pH of the slurry was adjusted to pH 4.5 using 0.5 M HCl. The mixture was filtered, and the solid material was washed four times with distilled water and air dried at 302 °C for 48 h.

2.5. Determination of degree of modification

The method of alkali saponification, followed by titration of the excess alkali as described by Genung and Mallatt[27] was employed for the determination of succinyl content. A sample (1 g) was weighed into a conical flask, 50 mL of 75% EtOH was added, and the flask was covered with aluminium foil. The mixture was refluxed for 30 min while maintaining a temperature of 50 °C. After cooling to room temperature, 40 mL of

0.5 M NaOH was added. The flask was covered again, and it was allowed to stand at room temperature for 72 h with occasional shaking. Saponification occurred with addition of NaOH, and the excess alkali was determined by titrating with 0.5 M HCl using phenolphthalein indicator. Additional alkali that probably leached from the sample was titrated after the mixture was permitted to stand for an additional 2 h. Native starch was treated in the same manner to obtain a value for the blank. The percentages of succinyl and the degree of substitution of the sample were calculated as indicated in Eq. 1:

$$\%\ \text{succinyl} = \frac{(\text{Blank titre} - \text{Sample titre}) \times 0.1 \times \text{Molarity of acid} \times 100}{\text{Weight of the sample (g)}}$$
$$\text{Degree of substitution (DS)} = \frac{162 \times \%\ \text{succinyl}}{1000 - (99 \times \%\ \text{succinyl})} \quad (1)$$

The content of acetyl groups (expressed as percentage in dry basis) and the degree of substitution of acetylation were determined according to Smith.[28] Acetylated starch (5 g) was placed in a 250-mL flask, and distilled water (50 mL) was added upon mixing. A few drops of phenolphthalein indicator were added, and the suspension was titrated with 0.1 M sodium hydroxide to a permanent pink end point. After addition of 0.45 M sodium hydroxide (25 mL), the flask was sealed tightly with a rubber stopper and shaken vigorously for 30 min. After shaking, the stopper was carefully removed and washed down, together with the walls of the flask, with distilled water. The saponified mixture containing excess alkali was then titrated with standard 0.2 M HCl solution until disappearance of the phenolphthalein colour. The native starch was treated in the same manner to obtain a blank value.

$$\%\ \text{acetyl (dry basis)} = \frac{(\text{Blank titre} - \text{Sample titre})\ \text{mL} \times \text{Acid Molarity} \times 0.043 \times 100}{\text{Sample weight in gram (Dry basis)}}$$
$$\text{Degree of substitution (DS)} = \frac{162A}{4300 - 42A} \quad (2)$$
in which A = percent acetyl (dry basis).

2.6. Proximate analysis

Standard Association of Official Analytical Chemistry methods,[29] were adopted for estimating moisture, ash, crude fibre, amylose, protein and fat content.

2.7. Physicochemical properties

2.7.1. Wide-angle X-ray diffraction of starch granules. X-ray diffraction measurements of the powder samples were performed on the crystallography beamline of the Elletra storage ring at Trieste, Italy. The powder sample was ground manually and loaded in glass capillaries of 0.3 mm diameter. The capillaries were sealed with wax and immersed in a sonic bath to obtain a more compact sample. The X-ray beam emitted by the wiggler source on the 2-GeV electron storage ring was monochromatised by a Si(1 1 1) double-crystal monochromator and focused on the sample. Data were collected by rotating the sample to achieve better homogeneity. A photon wavelength of 1.0 Å was used, and, a Mar 345 Imaging plate (MarResearch GmbH, Norderstedt, Germany) served as the detector. Sample to detector-to-detector distance and wavelength were calibrated using lanthanum hexaboride. The scanning region of the diffraction angle (2θ) was from 5° to 45°.

2.7.2. Swelling power and solubility. Swelling power and solubility determinations were carried out in the temperature range of 55–95 °C, using the method of Leach et al.[30]

2.7.3. Effect of pH on swelling power and solubility. A 1% w/v slurry was prepared with distilled water, and the pH was adjusted to desired value (pH 2–12) with 0.1 M HCl or 0.1 M NaOH. The slurries were allowed to stand for 1 h at 30 °C, and centrifuged at 5000*g*, for 15 min. An aliquot (5 mL) of the supernatant was dried to constant weight at 110 °C to determine percentage solubility of the starch.

2.7.4. Oil- and water-absorption capacity. The method of Beuchat[31] was used to determine oil- and water-absorption capacity of the starch. Distilled water (10 mL) or oil (Executive Chef Oil, Lever Brothers (Nigeria) Plc, Lagos, Nigeria, density 0.9 g/mL) was added to 1 g of sample. The mixture was mixed thoroughly with a Variwhirl mixer (Model A901, Salver Chem, Chicago,

IL, USA) for 30 s and allowed to stand for 30 min, after which time the volume of the supernatant was recorded. The mass of oil or water absorbed was expressed as percentage of the starch on a dry-weight basis.

2.7.5. Gelation studies. Samples of starch (2–18% w/v) were prepared in test tubes with distilled water (5 mL). The starch suspensions were mixed with a Vari-whirl mixer for 5 min. The test tubes were heated for 30 min at 80 °C in a water bath, followed by rapid cooling under running cold tap water. The test tubes were further cooled at 4 °C for 2 h. Least gelation concentration was determined as that concentration when the sample from the inverted test tube did not fall down or slip.

2.7.6. Brabender viscography. The Brabender viscographic pattern of 8% starch paste (36 g of starch on a dry-weight basis in 450 mL of water) was obtained on a Brabender viscograph (Type 8012003 W-G) equipped with a 700 cm g sensitivity cartridge. The starch suspension was heated from 50 to 95 °C. It was kept at this temperature for 30 min, then cooled to 50 °C and held at this temperature for 30 min. The speed of the rotor was fixed at 75 rpm, and the heating as well as cooling rate was 15 °C/min throughout the range of gelatinisation holding and cooling steps.

2.7.7. Light transmittance. Paste clarity was studied using the method of Bhandari and Singhal,[6] with modifications. Native and modified starches (50 mg, dry wt) were suspended in distilled water (5 mL) using 10 mL of cotton-plugged test tubes. The test tubes were then heated in a boiling water bath (with occasional shaking) for 30 min. After cooling to ambient temperature, the percentage transmittance (%T) was determined at 650 nm against water blank using a spectrophotometer (Hewlett-Packard). Also to monitor the tendency for retrogradation, samples were stored for 24 h at 4 °C to effect nucleation, after which time they were stored at 30 °C for 1–9 days before determining the absorbance.

2.7.8. Thermal properties and retrogradation studies. Gelation and retrogradation of starches were measured using a Perkin-Elmer DSC6 (Norwalk, CT) differential scanning calorimeter equipped with thermal analysis software, Pyris windows (Perkin-Elmer). Distilled water (6.0 μL) was added to starch (2.0 mg) in the DSC pans (BO14-3017). The pans were sealed, reweighed and kept at 30 °C for 24 h to ensure equilibration of the starch samples and water. The samples were scanned from 30 to 130 °C at 10 °C/min using an empty pan as reference. The heated pans were then immediately cooled and kept at 4 °C inside a refrigerator for 24 h, following which time they were kept for one or six days at 30 °C in order to make complete storage days of two and seven, respectively. Following these periods of storage, the samples were scanned under the same conditions as for the first scanning. Indium and zinc were used for calibration, while the empty pan was used as the reference. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy (ΔH, J/g) for gelatinisation and retrogradation were determined. Experiments were replicated three times.

2.8. Statistical analysis

Analyses were done in triplicate. Analysis of variance was performed to calculate significant differences in treatment means, and LSD ($p < 0.05$) was used to separate means.[32]

3. Results and discussion

3.1. Degree of modification

Preparation of starch succinate in 2% and 3% solution succinic anhydride results in starch succinate of 0.04 and 0.11 degrees of substitution (DS) with 3% and 6.15% succinyl, respectively. The starch samples were coded sMS1 and sMS2, respectively. In a similar development, concentrations of acetic anhydride at 10 g and 15 g produced starch acetate of 0.03 and 0.04 DS, which corresponded to 0.79% and 1.05% acetyl, respectively. They were also labelled aSM1 and aSM2 in order of increasing % acetyl group. In previous works, variations in degrees of substitution have been achieved by varying other parameters like time and ratio of catalyst to starch. The % succinyl content increased from 0.99 to 6.13 with an increase in reaction time from 1 to 5 h for cornstarch. Also, % succinyl of amaranth starch increased from 1% to 5% following an increase in reaction time from 1 to 5 h.[11]

The increase in % succinyl and acetyl following increase in concentration of succinic anhydride and acetic anhydride could be attributed to an increased rate of collision with the starch, which facilitated formation of starch derivatives with higher degree of substitution.

3.2. Proximate analysis

The results of the proximate analysis of derivatised and native hybrid maize starch presented in Table 1 indicates increase in moisture content following succinylation and reduction in moisture content after acetylation. No significant ($p < 0.05$) reduction was observed between the ash content of the derivatised samples and native starch. Protein content reduced significantly ($p < 0.05$) after acetylation and succinylation. A similar trend was observed in fat content and fibre content of the starches, except that the reductions were not significant at sMS1 in both cases. Both amylose content and pH values were

Table 1. Proximate composition of native, succinylated and acetylated starch derivatives of hybrid maize[a]

Sample	Parameter (%)						
	Moisture	Ash	Protein[b]	Fat	Fibre	Amylose	pH
Native	10.34 ± 0.6a	0.33 ± 0.01a	0.62 ± 0.02a	0.85 ± 0.04a	0.66 ± 0.64a	20.42 ± 0.56a	6.97 ± 0.01a
sMS1	13.56 ± 0.4b	0.33 ± 0.04a	0.57 ± 0.07b	0.81 ± 0.02a	0.62 ± 0.75a	20.22 ± 0.81a	6.23 ± 0.01a
sMS2	13.66 ± 0.5b	0.32 ± 0.01a	0.52 ± 0.02b	0.73 ± 0.03b	0.51 ± 0.11b	20.21 ± 0.94a	6.01 ± 0.01a
aMS1	9.22 ± 0.6ab	0.31 ± 0.05a	0.41 ± 0.04b	0.64 ± 0.02c	0.35 ± 0.34c	19.34 ± 0.77a	5.54 ± 0.02a
aMS2	8.54 ± 0.7a	0.30 ± 0.01a	0.32 ± 0.03c	0.64 ± 0.01c	0.33 ± 0.44c	19.11 ± 0.66ab	5.52 ± 0.01a

Means within columns with different letter are significantly different ($p < 0.05$).
[a] All values are means of triplicate determinations ± standard deviation.
[b] N × 6.25.

reduced following chemical modifications. Introduction of succinyl groups enhanced hydrophilic capacities of the derivatised starches, and this probably facilitated increases observed in the % moisture level. The reduction in moisture content after acetylation has been reported in previous publications.[1,2,4,33] Wootton and Bamunuarachchi[34] suggested that the decrease in moisture content after acetylation is due to the substitution of some of the available hydroxyl groups of the glucose units by acetyl groups. Reduction in ash, protein, fat, fibre and amylose content are due to structural disintegration and losses during chemical modification processes.

3.3. Wide-angle X-ray diffraction of starch granules

Figure 1 depicts the X-ray diffraction pattern of native, acetylated and succinylated starch samples. All the samples gave the characteristic A pattern of maize starch[35] with strong peaks at 15.9°, 17.2°, 18.8° and 25.0° 2θ. No pronounced difference was observed between the X-ray pattern of the native and modified starches apart from reduced peak intensities in the succinylated and acetylated starches. In previous publications, similar X-ray patterns have been reported for native cornstarch and its chemically modified derivatives.[36,37]

3.4. Swelling power and solubility

Effect of temperature on swelling power and solubility is presented in Figures 2 and 3, respectively. Both swelling power and solubility were temperature dependent, and values increased with an increase in temperature. At the temperature range studied, acetylation and

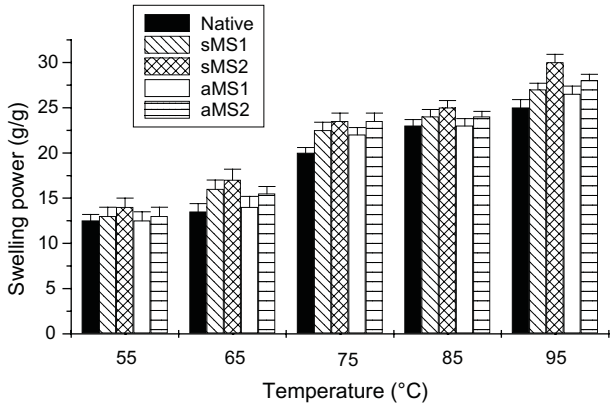

Figure 2. Effect of temperature on swelling power of native, succinylated and acetylated starches of hybrid maize. Error bars: standard deviations; results are means of triplicate determinations.

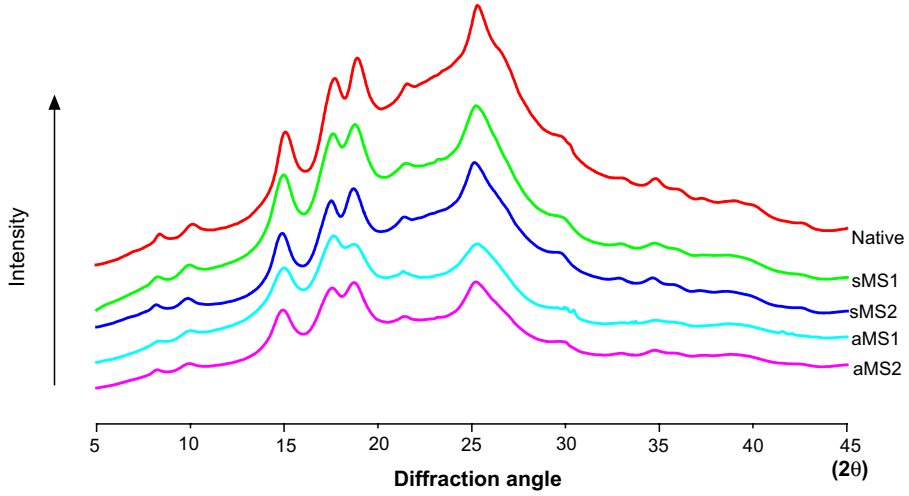

Figure 1. Wide-angle X-ray diffraction pattern of native, succinylated and acetylated starches of a hybrid maize.

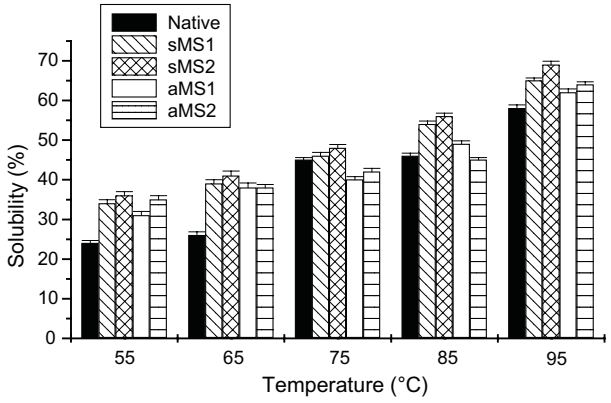

Figure 3. Effect of temperature on solubility of native, succinylated and acetylated starches of hybrid maize. Error bars: standard deviations; results are means of triplicate determinations.

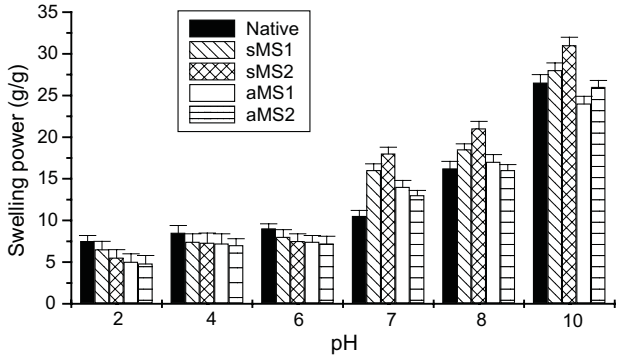

Figure 4. Effect of pH on swelling power of native, succinylated and acetylated starches of hybrid maize. Error bars: standard deviations; results are means of triplicate determinations.

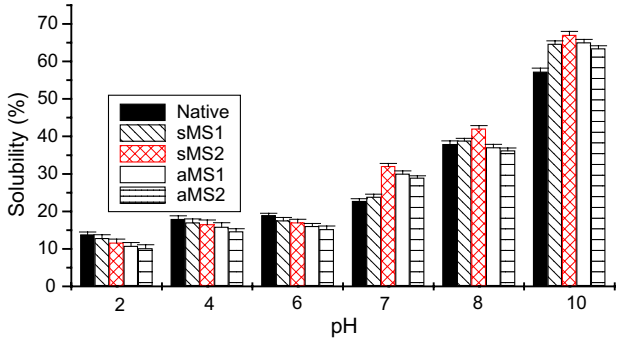

Figure 5. Effect of pH on solubility of native, succinylated and acetylated starches of hybrid maize. Error bars: standard deviations; results are means of triplicate determinations.

succinylation improved the swelling power and solubility of the native starch. Comparing among the derivatised samples, at all temperatures studied, it was observed that succinylated derivates have better swelling power and solubility compared to the starch acetate, and in both cases increases in swelling power and solubility were observed as the level of modification increased. In previous publications, we have expounded on the influence of substituent groups on swelling power and solubility of starches.[1,2,4] It is reasonable that as the temperature of the medium increases, starch molecules become more thermodynamically activated, and the resulting increase in granular mobility enhances penetration of water, which facilitates improved swelling capacities. It is also reasonable that following introduction of acetyl and succinyl bulky groups on starch molecules, structural reorganisation occurs as a result of steric hindrance, and this results in repulsion, thus facilitating an increase in water percolation within the granules with subsequent increase in swelling capacity. Structural disintegration probably weakens the starch granules after modifications, and this enhanced leachates from the starch, a development that improved starch solubility. Similar observations have been reported earlier for starches of rice,[33,38] wheat[34] and the Great Northern bean.[39]

Swelling power and solubility of native and derivatised starches were found to be pH dependent (Figs. 4 and 5). Swelling capacity and solubility of native and derivatised starch samples increased with increase in pH. Maximal swelling powers and solubility were observed at pH 10. In the acidic range, pH 2–6, native starch had better swelling power and solubility than acetylated and succinylated starches. At pHs 7–10, derivatised starches had improved swelling power and solubility over the native starch. And at this range, succinylated starch derivatives had better swelling capacity and solubility than acetylated derivatives. In addition, corresponding increases in swelling capacity and solubility were observed as the level of succinylation and acetylation increased. This observation agrees with the report of Deshpande et al.[40] Under alkaline conditions, starches may undergo partial gelatinisation, thus resulting in higher swelling and solubility. This accounts for higher swelling and solubility of the starches at the extreme of the alkaline range.

3.5. Water- and oil-absorption capacity

Water- and oil-absorption capacities of derivatised starches and native starch are depicted in Figure 6. Water-absorption capacity increased after succinylation and acetylation. In contrast, oil-absorption capacity was reduced following succinylation but increased after acetylation. Increase in water-absorption capacities following esterification reactions have been reported in the literature.[33] The introduction of bulky functional groups and their electrostatic repulsion facilitated percolation and absorption of water within the starch matrices. But following succinylation, long-chain hydrophilic succinyl substituents probably impaired oil absorption of succinylated derivates.

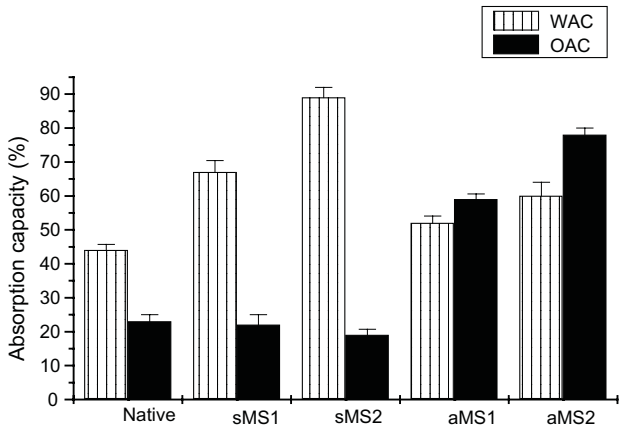

Figure 6. Water- and oil-absorption capacity of native, succinylated and acetylated starches of a hybrid maize. Error bars: standard deviations; results are means of triplicate determinations; WAC: water-absorption capacity; OAC: oil-absorption capacity.

3.6. Gelation properties

In Table 2, gelation properties of native and modified starches are presented. The least gelation concentration (LGC) serves as the index of gelation. The results indicate a reduction in gelation properties of the native starch after succinylation and acetylation. The lowest LGC value, 6, was observed in the native starch, while the highest value, 14, was observed in sMS2. Gel formation in starches involves swelling and hydration of starch granules, which occurs predominantly in the amorphous region of starches. And gel strength depends on strength of intragranular binding forces within swollen starch granules. The intragranular binding forces maintain structural rigidity of the starch gels, and stronger gels are formed when the functional groups facilitating intragranular binding forces, predominantly hydrogen bonding are unaltered. Succinyl and acetyl group substitution on starch molecules hamper these intragranular force interactions by replacing the OH groups on the glucose units, thus limiting formation of strong gels as compared with those of native starch. It is also reasonable that intra- and intermolecular electrostatic repulsion after introduction of acetyl and succinyl groups reduced gel cohesion, thus resulting in weaker gels.

3.7. Pasting properties

Pasting properties of native and derivatised starch samples are presented in Table 3. After succinylation and acetylation, gelatinisation temperature (T_p) of native starch is reduced. A similar trend was observed in peak viscosity (P_v), hot paste viscosity (H_v), cold paste viscosity (C_v).

Among the succinylated starch derivatives, the most marked reduction was observed in sample aMS2, while the least was observed in sMS1. The results also indicate a reduction in setback value of the native starch after modifications. Highest reduction in setback value was observed in sMS2. However, increases were observed in the breakdown value of native starch after succinylation and acetylation. Reduction in gelatinisation temperature following derivatisation is due to weakening of intragranular and intergranular binding forces within

Table 3. Pasting properties of native succinylated and acetylated starch derivatives of hybrid maize[a]

Sample	Parameter						
	T_p (°C)	P_v (BU)	H_v (BU)	H_{v30} (BU)	C_v (BU)	SB (BU)	BD (BU)
Native	86	165	155	150	520	355	15
sMS1	82	145	135	120	230	85	25
sMS2	81	135	120	105	200	70	30
aMS1	78	155	140	125	260	105	30
aMS2	73	135	125	100	235	100	35

T_p: initial pasting temperature; P_v: peak viscosity during heating; H_v: hot paste viscosity (at 95 °C); H_{v30} viscosity after 30 min holding at 95 °C; C_v: cold paste viscosity (at 50 °C); SB: setback value = $C_v - P_v$; BD: breakdown = $P_v - H_{v30}$; BU: Brabender unit.

[a] Values are means of triplicate determinations.

Table 2. Gelation properties of native, succinylated and acetylated starch derivatives of hybrid maize

Concentration (%w/v)	Starch sample				
	Native	sMS1	sMS2	aMS1	aMS2
2	−Liquid	−Liquid	−Liquid	−Liquid	−Liquid
4	−Viscous	−Liquid	−Liquid	−Liquid	−Liquid
6	+Gel	−Liquid	−Liquid	−Liquid	−Liquid
8	+Gel	−Viscous	−Liquid	+Gel	−Liquid
10	+Gel	+Gel	−Viscous	+Gel	−Viscous
12	+Firm gel	+Gel	−Viscous	+Firm gel	+Gel
14	+V.firm gel[a]	+Firm gel	+Gel	+Firm gel	+Firm gel
16	+V.firm gel	+Firm gel	+Firm gel	+V.firm gel	+Firm gel
18	+V.firm gel	+V.firm gel	+Firm gel	+V.firm gel	+V.firm gel
LGC[b]	6	10	14	8	12

[a] Very firm gel.

[b] LGC: least gelation concentration.

starch molecules. When a starch granule is heated in excess water, it leads to further granule swelling, additional leaching of soluble components and total disruption of granules. This process results in formation of a viscous starch paste. And the viscosity of starch, as a food component is a vital factor for consideration in its applicability to food systems. Although in the literature increases in viscosity values after succinylation[6] and acetylation[33,38,41] have been reported, reduction in viscosity values after succinylation and acetylation as observed in this work agrees with data in some previous publications.[6,42,43] It has also been established that increased or reduced viscosity after acetylation depends on the starch source and esterification method.[44] Generally, cold paste viscosities were higher than the peak viscosities, and this is a consequence of the reassociation of starch molecules following gelatinisation.

Reduction in setback value following acetylation and succinylation could be attributed to limitations caused by the succinyl and acetyl groups introduced in the starch molecules. Breakdown value is a measure of fragility of the starch. Following modifications, the modified starches become partially degraded and this partially degraded, network was not resistant to shear and could not maintain the integrity of the starch granule. This accounts for the higher breakdown value observed in modified starches.

3.8. Light transmittance

Results of studies conducted on starch paste clarity is presented in Table 4. The % transmittance (%T) of all the starches was reduced as the length of storage days increased from 1 to 10. However, pronounced reduction in %T was observed in native starch. Among the derivatised starch samples, least reduction in % transmittance was observed in sMS2, and the reductions observed following starch derivatisations increased as the degree of substitution of the starches increased. Similarly, in the report of Bhandari and Singhal,[6] succinylation improved the paste clarity of corn and amaranth starches. Also, in a previous study conducted on new cocoyam starch,[1] %T increased following acetylation. Chemical substitution of the OH groups on the starch molecules by succinyl and acetyl moiety hampered the formation of ordered structure following gelatinisation, and these developments retarded retrogradation, which resulted in a more fluid paste with improved clarity. Improved paste clarity is a useful property in the manufacture of some confectionary products.

3.9. Thermal properties and retrogradation studies

From Table 5, where thermal properties and retrogradation parameters of native and derivatised starch samples

Table 4. Effect of storage time on paste clarity of native succinylated and acetylated starch derivatives of hybrid maize[a]

Starch sample	Percentage transmittance (650 nm)					
	First day	Second day	Fourth day	Sixth day	Eighth day	Tenth day
Native	24.67 ± 0.05a	20.45 ± 0.15a	14.65 ± 0.55a	11.77 ± 0.64a	8.54 ± 0.01a	6.61 ± 0.04a
sMS1	25.55 ± 0.01a	23.34 ± 0.07b	21.45 ± 0.66b	20.55 ± 0.04b	19.78 ± 0.06b	19.55 ± 0.01b
sMS2	28.45 ± 0.05b	27.43 ± 0.05c	27.01 ± 0.55c	26.43 ± 0.01c	25.02 ± 0.01c	20.56 ± 0.94b
aMS1	26.99 ± 0.05a	22.34 ± 0.05b	20.34 ± 0.76b	19.44 ± 0.01b	18.89 ± 0.06b	17.94 ± 0.99c
aMS2	27.64 ± 0.04b	24.64 ± 0.02b	21.34 ± 0.94b	20.34 ± 0.05b	19.33 ± 0.07c	18.55 ± 0.84c

Means within columns with different letter are significantly different ($p < 0.05$).
[a] All values are mean of triplicate determinations ± standard deviation.

Table 5. Thermal and retrogradation properties of native, succinylated and acetylated maize starch[a]

Sample	Native starch					Second day retrograded starch						Seventh day retrograded starch					
	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	ΔH_{R2} (J/g)	$\%R_2$	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	ΔH_{R7} (J/g)	$\%R_7$
Native	77.6	82.6	85.4	7.8	14.3	42.7	47.1	55.4	12.7	4.2	29.3	52.1	57	63.4	11.3	7.5	52.4
sMS1	73.1	78.6	84.8	11.7	10.6	37.9	43.6	54.8	16.9	2.6	24.5	47.3	53.4	62.6	15.3	4.2	39.6
sMS2	71.4	76.1	84.9	13.5	9.8	36.4	41.6	54.0	17.6	2.3	23.5	46.2	51.4	61.8	15.6	3.5	35.7
aMS1	69.2	74.9	80.1	10.9	9.2	34.2	40.3	49.8	15.6	1.8	19.5	43.9	50.0	57.6	13.7	3.1	33.6
aMS2	65.5	71.4	77.3	11.8	7.4	31.3	36.4	47.3	16.0	1.4	18.9	42.0	46.2	54.3	12.3	2.4	32.4

T_p: peak of gelatinisation temperature.
T_c: conclusion of gelatinisation temperature.
ΔH: enthalpy of first scanning.
ΔH_{R2}: enthalpy of retrogradation after two days of storage.
ΔH_{R7}: enthalpy of retrogradation after seven days of storage.
%R: percentage retrogradation.
[a] T_o: onset of gelatinisation temperature.

are presented, it can be observed that onset temperature (T_o), peak temperature (T_p), concluding temperature (T_c) and enthalpy of gelatinisation (ΔH), which represents the amount of thermal energy involved gelatinisation process, are reduced after succinylation and acetylation. However, the gelatinisation temperature range increased following starch modifications. Reductions in gelatinisation temperatures after derivatisations lend credence to the structural reorganisation and consequent weakening of intragranular and intergranular binding forces of the starch molecules. Also, the introduction of bulky groups into the backbone of the biopolymer enhances structural flexibility, and this also contributes to the reduction of gelatinisation temperature of the modified starches. These observations are consistent with previous reports on corn and amaranth starches,[11] new cocoyam starch[1] and potato starch.[10] Expectedly, enthalpy of gelatinisation is reduced following modification because less energy is required to gelatinise the modified starches compared with the unmodified starch.

Broadening of the gelatinisation band after modifications is attributed to an increase in inhomogeneity in the starch molecules as a result of acetyl and succinyl moieties. When starch pastes were stored for two and seven days to monitor retrogradation, it was observed that for the gelatinisation temperature range, the band increased for all the starches but shifted to lower values. Enthalpy of regelatinisation after the second day (ΔH_{R2}) and seventh day (ΔH_{R7}) were also reduced. It was also observed that percentage retrogradation (%R) was reduced following modifications, and among the derivatised starch samples the highest level of reduction was observed in aMS2 after the second day and the seventh day of storage. %R also decreased as the DS increased among the modified starches. It is also noteworthy that %R and enthalpy of regelatinisation increased with the storage days of the gelatinised starch. Recrystallisation of starch molecules occurred during gel storage, and reheating of aged starch gel in a DSC produced an endothermic transition that was absent in freshly gelatinised samples. The enthalpy of retrogradation values were also observed at lower temperature ranges than for those gelatinisation. Starch molecule recrystallisation occurs in a less ordered manner in stored starch gels than in native starches.[20] In view of this, less heat is needed to regelatinise stored starch gels. This explains the observation of retrogradation endotherms at a temperature range below that for gelatinisation. Since retrogradation is a time-dependent process, longer days of storage enhances structural reordering after gelatinisation. This explains the higher values of ΔH_{R7} compared with ΔH_{R2} Succinylation and acetylation decrease such rearrangement of molecular chains to the ordered structure after gelatinisation, and this explains reductions in %R of modified starches compared with the native starch.

Acknowledgements

The author is grateful to Dr. K. O. Adebowale and Professor Atilio Cesaro of the Department of Biochemistry, Biophysics and Macromolecular Chemistry, University of Trieste, Trieste, Italy for providing facilities for DSC analyses. Dr. Luisa Barba of the ELETTRA beamline, Area Science Park, Basovissa, Trieste, Italy, assisted in the X-ray diffraction studies. The Abdus Salam International Centre for Theoretical Physics, Italy, through their Young Collaborator Programme, enhanced this research work.

References

1. Lawal, O. S. *Food Chem.* **2004**, *87*, 205–218.
2. Adebowale, K. O.; Afolabi, T. A.; Lawal, O. S. *Food Chem.* **2002**, *78*, 305–311.
3. Adebowale, K. O.; Lawal, O. S. *Food Hydrocoll.* **2003**, *17*, 265–272.
4. Adebowale, K. O.; Lawal, O. S. *J. Sci. Food Agric.* **2003**, *83*, 1541–1546.
5. Adebowale, K. O.; Lawal, O. S. *Nahrung/Food* **2002**, *46*, 311–316.
6. Bhandari, P. N.; Singhal, R. S. *Carbohydr. Polym.* **2002**, *48*, 233–240.
7. Singh, V.; Ali, S. Z. *Carbohydr. Polym.* **2000**, *41*, 191–195.
8. Parovuori, P.; Hamunen, A.; Forssel, P.; Autio, K.; Poutanen, K. *Starch/Stärke* **1995**, *47*, 19–23.
9. Hoover, R. *Starch/Stärke* **1986**, 149–155.
10. Perera, C.; Hoover, R. *Food Chem.* **1999**, *64*, 361–375.
11. Bhandari, P. N.; Singhal, R. S. *Carbohydr. Polym.* **2002**, *47*, 277–283.
12. Tuschhoff, J. V. Hydroxypropylated Starches. In *Modified Starches: Properties and Uses*; Wurzburg, O. B., Ed., 2nd ed.; CRC: Boca Raton, Florida, 1987; pp 90–95.
13. Robert, H. J. Nondegradative reactions of starch. In *Starch Chemistry and Technology*; Whistler, R. L., Paschall, E. F., Eds.; Academic: New York, 1967; Vol. 1, pp 439–487.
14. Robert, H. J. Starch derivatives. In *Starch Chemistry and Technology*; Whistler, R. L., Paschall, E. F., Eds.; Academic: New York, 1967; Vol. 2, pp 293–334.
15. Rutenberg, M. W.; Solarek, D. Starch derivatives production and uses. In *Starch Chemistry and Technology*, 2nd ed.; Whistler, R. L., BeMiller, J. N., Paschall, E. F., Eds.; Academic: New York, 1984; Vol. 2, pp 312–314.
16. Bilmers, R. L.; Tessler, M. M. *Polym. Mater. Sci. Eng.* **1995**, *72*, 190–191.
17. Bunton, C. A. *Nucleophilic Substitution at the Saturated Carbon Atom, Reaction Mechanisms in Organic Chemistry Series*; Elsevier: New York, 1963, 1963; Vol. 1, p 172.
18. Gonzalez, Z.; Perez, E. *Starch/Stärke* **2002**, *54*, 148–154.
19. Aiyeleye, F. B.; Akingbala, J. O.; Oguntimein, G. B. *Starch/Stärke* **1993**, *45*, 443–445.
20. Morikawa, K.; Nishinari, K. *Food Hydrocoll.* **2000**, *14*, 395–401.

21. Harwalkar, V. R.; Ma, C. Y. *Thermal Analysis of Foods*; Elsevier Applied Science: London, 1990.
22. Jang, J. K.; Pyun, Y. R. *Starch/Stärke* **1997**, *49*, 272–277.
23. Leon, A.; Duran; deBarber, C. B. *Z. Lebensm. Unters. Forsch.* **1997**, *204*, 316–320.
24. Baker, L. A.; Rayas-Duarte, P. *Cereal Chem.* **1998**, *75*, 301–303.
25. Trubiano, P. C. Succinate and Substituted Succinate Derivatives of Starch. In *Modified Starches Properties and Uses*, 2nd ed.; Wurzburg, O. B., Ed.; CRC: Boca Raton, Florida, 1987; pp 131–148.
26. Sathe, S. K.; Salunkhe, D. K. *J. Food Sci.* **1981**, *46*, 617–621.
27. Genung, L. B.; Mallat, R. C. *Ind. Eng. Chem.*, **1941**, *13*, 369–374.
28. Smith, R. J. Characterisation and analysis of starches. In *Starch Chemistry and Technology*; Whistler, R. L., Paschall, E. F., Eds.; Academic: New York, 1967; Vol. 2, pp 569–635.
29. *Official Methods of Analysis of the Association of Official Analytical Chemists*: Association of Official Analytical Chemists: Arlington, VA, 1996.
30. Leach, H. W.; McCowen, L. D.; Scoch, T. J. *Cereal Chem.* **1959**, *36*, 534–544.
31. Beuchat, L. R. *J. Agric. Food Chem.* **1977**, *25*, 258–261.
32. SAS. SAS/STAT users guide, rel. 6.03, SAS Institute, Inc.: Carey, NC, 1988.
33. Gonzalez, Z.; Pérez, E. *Starch/Stärke* **2002**, *54*, 148–154.
34. Wootton, M.; Bamunuarachchi, A. *Starch/Stärke* **1979**, *31*, 201–204.
35. Lim, S. T.; Chang, E. H.; Chung, H. J. *Carbohydr. Polym.* **2001**, *46*, 107–115.
36. Kuakpetoon, D.; Wang, Y. J. *Starch/Stärke* **2001**, *53*, 211–218.
37. Wang, L.; Wang, Y. *Starch/Stärke* **2001**, *53*, 570–576.
38. Liu, H.; Corke, H. *Starch/Stärke* **1999**, *51*, 249–252.
39. Sathe, S. K.; Yyer, V.; Salunkhe, D. K. *J. Food Sci.* **1981**, *46*, 1914–1917.
40. Deshpande, S. S.; Sathe, S. K.; Rangnekar, P. D.; Salunkhe, D. K. *J. Food Sci.* **1982**, *47*, 1528–1533.
41. Liu, H.; Ramsden, L.; Corke, H. *Carbohydr. Polym.* **1997**, *34*, 283–289.
42. Islam, N. M.; Azemi, B. M. N. M. *Starch/Stärke* **1997**, *49*, 136–141.
43. Jaworenko, W. Acetylated and Miscellaneous Organic Esters. In *Modified Starches Properties and Uses*; Wurzburg, O. B., Ed.; CRC: Boca Raton, Florida, 1986; pp 55–57.
44. Agboola, S. O.; Akingbala, J. O.; Oguntimehin, G. B. *Starch/Stärke* **1991**, *43*(2), 62–66.