

Full Length Research Paper

Effect of acid hydrolysis on the physicochemical properties of cola starch

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Cola starch from *Cola nitida (rubra)* was isolated using 1% (w/v) sodium metabisulphite solution and was treated with 0.1 and 0.2 M HCl solution differently at 80 and 100°C, pH (6 to 7.9) and reaction time (30 min to 3 h). The physicochemical and functional properties of the hydrolyzed starch were studied. The hydrolysis reaction presented important changes in the pasting, thermal transition and morphology of the native starch. Reaction time, temperature and concentration of the acid were observed to influence the reactions. The acid modified starch has the following properties; swelling 6.13 to 7.21% solubility 14.83 to 16.65% and amylose content 17.28 to 21.69%, while the corresponding values for the native cola starch were swelling 8.85%, solubility 7.48% and amylose 24.76%. The rapid visco analysis (RVA) of the acid modified starch demonstrated low peak viscosity ranging from 52.71 to 197.22 as against 314.42 reported for the native starch. Breakdown viscosity and the setback values also exhibited the same decreasing trend; 30.24 to 73.17 and 10.18 to 34.91 respectively, as against that of native cola starch that has a breakdown and setback viscosity of 179.25 and 74.42 respectively. The observed trends are consistent with other modified starches that have found useful applications in pharmaceutical, food and confectionary industries.

Key words: Cola, native starch, acid modified (thinned) starch, hydrolysis, composition.

INTRODUCTION

Native (unmodified) starches have different functional properties depending on the crop source and are considered a primary resource that can be processed into a range of starch products. The limited application of native starches is due to low shear resistance, thermal resistance, thermal decomposition and high tendency towards retro gradation, high syneresis, extreme processing conditions such as pH, temperature etc., (Cousidine, 1982). The limitations experienced from native starch may be overcome by various modifications, Jacobs and Delcour (1998). The basis of starch modification lies in the improvement of its functional properties by changing the physical and chemical properties of such native starch (Ortoefer, 1984).

Starch modification which involves the alteration of the physical and chemical characteristics of the native starch can be used to improve its functional characteristic thereby tailoring it to specific applications (Hermansson

and Svegmarm, 1996). It is generally achieved through derivatization such as etherification, esterification, cross linking and grafting of starch, acid or enzymatic hydrolysis, oxidation or physical treatment of starch using heat or moisture. The modified starches generally show better paste clarity, better stability, increased resistance to retro gradation and increased freeze- thaw stability (Zheng et al., 1999).

Recently, cola starch was isolated from *Cola nitida (rubra spp)* and characterized in our laboratory, (Omojola et al., 2010). Its physicochemical characterization showed high industrial potentials in the pharmaceutical, food and confectionary industries. The present study is to modify the starch through acid hydrolysis, evaluate its physicochemical properties, compared with that of the native starch and other acid thinned starches and suggest possible industrial applications.

MATERIALS AND METHODS

C. nitida (rubra) were procured directly from the farm source in

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Ondo, Ondo State, Nigeria. The nuts were removed from the pods, wrapped in green leaves and kept in a basket at room temperature until required. This is necessary to ensure the retention of the quality of the nuts. Sodium metabisulphite (BDH, UK) was purchased from Zion Chemicals, Abuja. Corn starch (BP) and analytical grade chemicals and reagents were obtained from the Pharmaceutical Technology Department, National Institute for Pharmaceutical Research and Development, Idu, Abuja and Sheda Science and Technology Complex Sheda, Abuja, Nigeria.

Extraction of starch from *Cola nitida*

The extraction procedure as described by Omojola et al. (2010) was adopted. 5 kg of the *C. nitida (rubra)* was crushed and soaked for one and half hour in 4 L of 1% w/v sodium metabisulphite solution to ensure proper hydration. The soaked nuts were removed and wet milled into homogenous fine paste using a big grinding machine. The fine paste was dispersed in 2 L of 1% w/v sodium metabisulphite solution to form a large solution of starch paste. The solution was filtered using muslin cloth, the suspension was then allowed to settle for 1 h and the supernatant decanted. The starch layer was washed three times each with 100 ml of 1% sodium metabisulphite acid and at each stage of washing, the suspension was allowed to stand for 2 h for proper sedimentation, and the supernatant was decanted. The mucilage on the starch layer was scrapped to obtain pure white starch. The starch was air dried at room temperature and pulverized to fine powder with a blender.

Preparation of acid thinned cola starch

The method of Ahmed et al. (2003) for the preparation of acid thinned starch was adopted. 10 g of cola starch was put in a boiling tube, 12.5 ml of distilled water and 12.5 ml of 0.1 M HCl were added. The slurry was heated in a thermo controlled water bath at 80°C with constant stirring. The heating was done at 30 min, 1, 2 and 3 h respectively. The reaction was repeated in 0.2 M HCl at 100°C. After each reaction, the samples were rapidly cooled under running tap water, few drops of distilled water were added, and neutralized by adding drops of 0.5 M NaOH solution. The acid thinned cola starch residues were allowed to stand for 30 min and the modified starch recovered by decantation and filtration through a fine pore sized small sieve. The recovered residues were washed several times with distilled water until all traces of salt were removed by testing for the absence of chloride in the liquor.

The recovered samples were dried in an oven at 28°C for 48 h and milled into powder. The milled samples were weighed and the differences in the weight of starting materials were recorded. The (%) hydrolysis and (%) starch recovery were calculated, and recorded. The pH during the reaction period was also recorded. The experiments were carried out in triplicate. The mean and standard deviation were calculated and recorded.

Determination of chemical composition and physicochemical properties

The swelling and solubility profiles were determined over a temperature range between 50 and 100°C as follows: The acid thinned starch, 1 g and native cola starch were poured separately into calibrated boiling tubes of known weight and the initial volume was recorded. 10 ml of distilled water was added and it was subjected to a vortex mixer (Vortex-2 Genie, set at shake 8) for 5 min and allowed to settle. The tubes were immersed in thermo stated water bath, at the different temperatures and heated for 30 min after which the tubes were centrifuged at 3000 rpm for 10 min. The supernatant was decanted into Petri dishes of known weight

and dried in an oven. The difference in weight was calculated as the solubility index. Also, the weight of the sediment was determined and used to calculate the percent (%) swelling capacity. This was carried out for every 5°C rise in temperature at 30 min interval till 95°C (Omojola et al., 2010).

Amylose content determination was carried out using a colorimetric iodine affinity procedure (Williams et al., 1958). The starch sample (0.1 g) and standard were weighed into separated test tubes. To each was added 1 ml of 95% ethanol and 9 ml mol dm^{-3} NaOH. The tubes were covered with foil, thoroughly mixed and heated for 10 min in boiling water bath to gelatinize the starch, thereafter, it was cooled very well, and the suspensions were diluted 10 times. An aliquot of 0.5 ml of the extract was used for analysis where 0.1 ml of acetic acid solution was added, followed by the addition of 0.2 ml of iodine solution. This was made up to 10 ml mark with distilled water. The solution was left for 20 min for colour development and absorbance was read at 630 nm.

The pasting temperature and viscosity of 12% w/v of the acid thinned starch was assessed using the Rapid ViscoAnalyser model RVA-3D made compatible with a computer. The samples were assessed for peak viscosity, trough, breakdown, and final viscosity, set back, peak time and pasting temperature. Gelatinization properties of the samples were characterized using a Netzsch DSC 204 F1 Phoenix (Netzsch, Germany), Nitrogen, at the rate of 20 ml/min was used as purge gas, 2.7 mg of powdered material was sealed in an aluminum pan and heated from 30 up to 400°C at the rate of 10°C per min, followed by a cooling cycle back to 30°C at the same rate. The onset gelatinization temperature (T_o), peak temperature (T_p) and gelatinization temperature at end (T_e) were recorded. A light microscope with a micrometer (Nikon AFX-35DX microscope, Japan) connected to a computer was used to determine the particle sizes at 40X magnification and the photomicrograph recorded.

RESULTS

The photomicrograph of native and acid thinned starches at various concentrations of HCl, reaction time and heating temperature are shown in Plates 1 to 6. The results of the acid hydrolysis of the native cola starch using 0.1 and 0.2 M HCl solutions at 80 and 100°C at different heating times from 30 min to 3 h are as shown in Tables 1 to 8, respectively. The swelling and solubility profiles of the acid thinned starch are shown in Table 5, while Table 6 shows the percent amylose/amylopectin contents at different acid concentrations, reaction time and temperature. The pasting properties and DSC thermo grams are given in Tables 7 and 8, respectively.

DISCUSSION

Morphology

The morphology of the native cola starch as depicted in Plate 1 had earlier been reported (Omojola et al., 2010). The photomicrographs of the acid thinned starches show that some of the samples still possess similar morphology with the native starch especially at lower heating time, but as the heating time increases, the granules showed traces of irregular shapes and some aggregation. At 3 h heating time, the starch granules had undergone

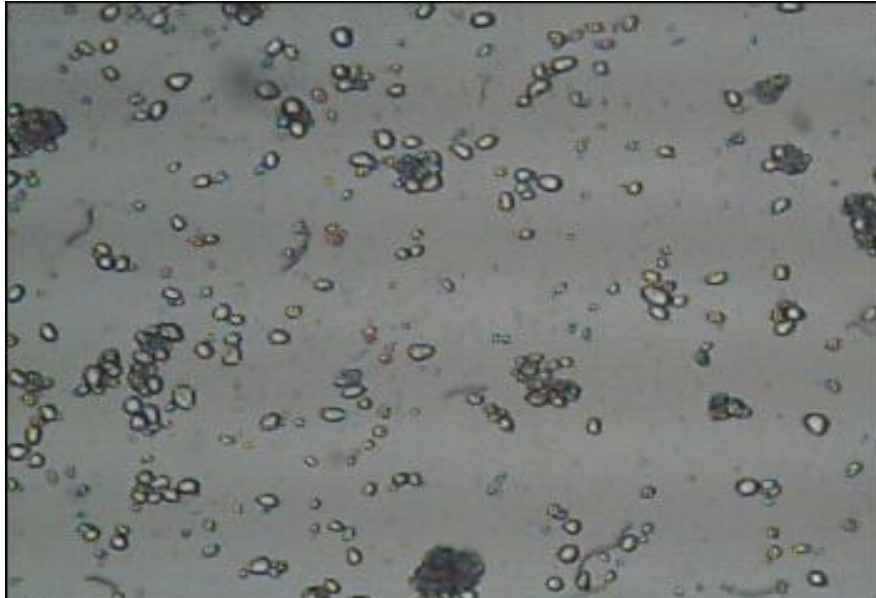


Plate 1. Photomicrograph of native cola starch.

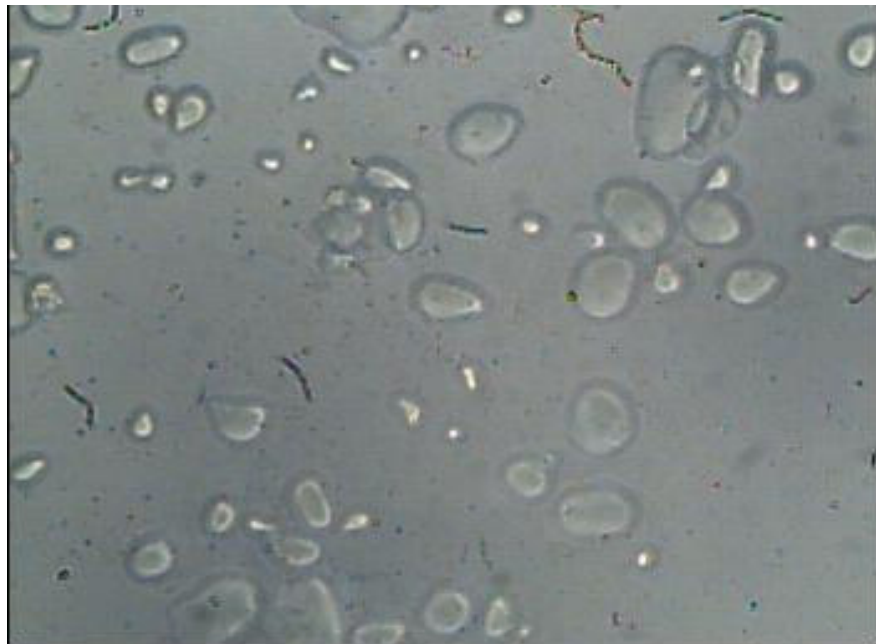


Plate 2. Photomicrograph of acid thinned cola starch (0.1 M HCl, 100°C 1 h).

morphological alterations. This is similar to the reported thermal behavior of corn starch modified by acid treatment (Bennica et al., 2008).

Acid hydrolysis

The results of the acid hydrolysis of the native cola starch using 0.1 and 0.2 M HCl at 80, 100°C at different heating

times from 30 min to 3 h are as shown in Tables 1 to 4 respectively. The results show that heating temperature, time and acid concentration affected the extent of hydrolysis or starch recovery. Any increase in the aforementioned parameter increases the extent of starch hydrolysis. This trend is similar to the results obtained in the acid modification of cassava starch (Ahmed et al., 2003) on prolonged treatment. Acid will attack both the amorphous and crystalline regions of the starch granule

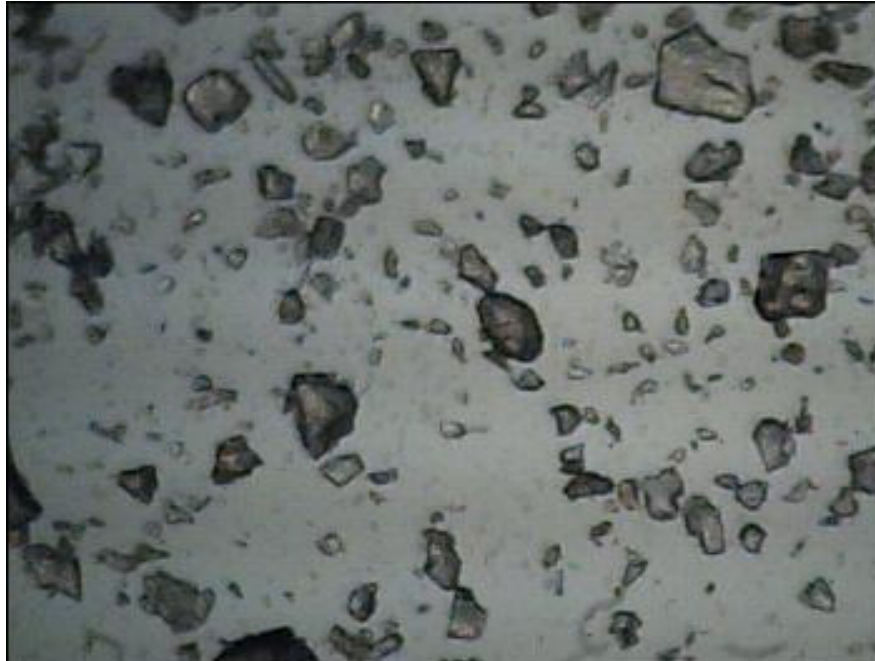


Plate 3. Photomicrograph of acid thinned cola starch (0.2 M HCl, 80°C 1h).

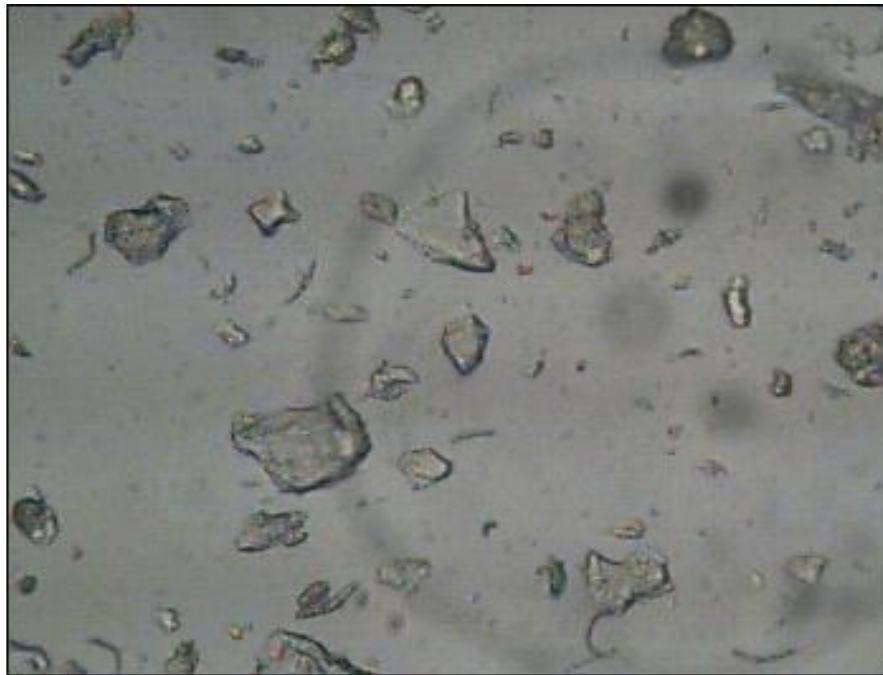


Plate 4. Photomicrograph of acid thinned cola starch (0.2 M HCl, 100°C 1 h).

to obtain water soluble molecules.

Swelling and solubility

The percent swelling and solubility profiles of the native

cola and acid thinned starches are shown in Table 5. It can be seen that the swelling profile of acid thinned starch is lower than that of the native starch. This may be related to the changes in the surface characteristics of starch granules. At higher temperature, starch appears to lose its granular structure faster resulting in a low

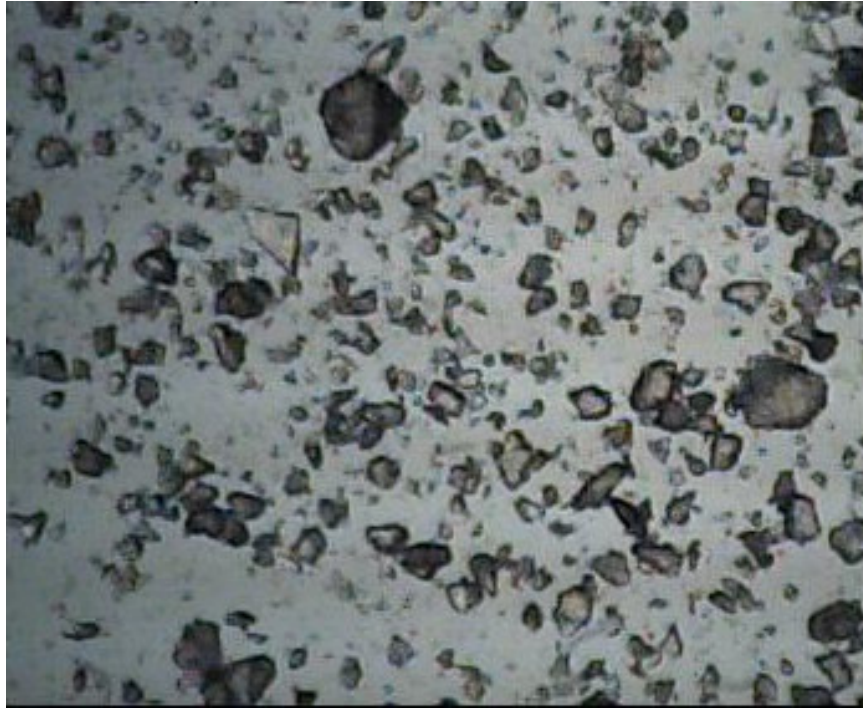


Plate 5. Photomicrograph of acid thinned cola starch (0.1 M HCl, 80°C 3 h).

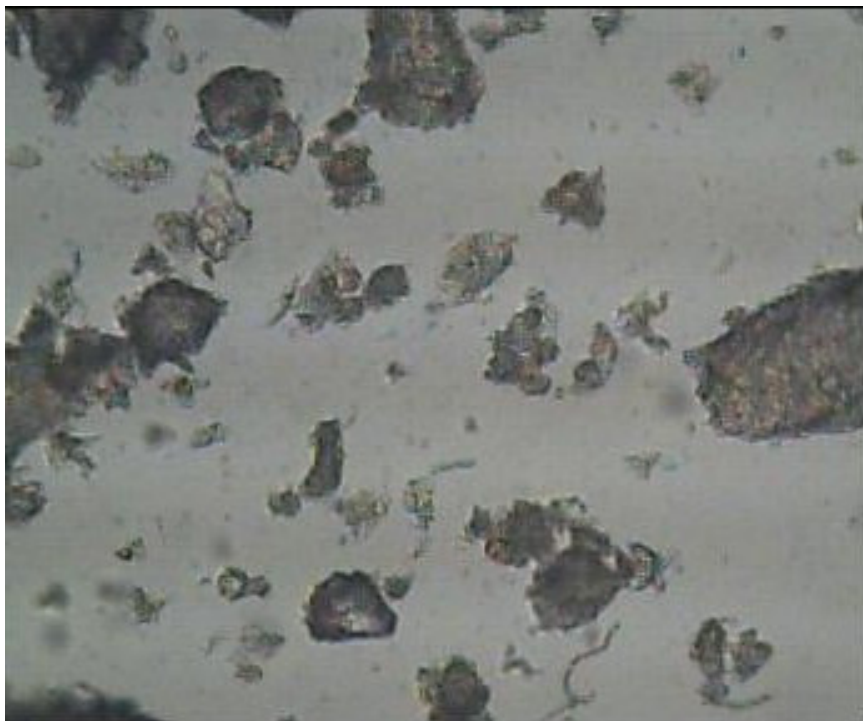


Plate 6. Photomicrograph of acid thinned cola starch (0.2 M HCl, 100°C 3 h).

swelling capacity (Chang et al., 1995). Acid thinned starches are more soluble than native starches. The increase in solubility values may be due to shortening of

the chain lengths of the starch, corresponding to the weakening of the hydrogen bonds (Osunsami et al., 1989), or due to the increasing hydroxyl groups (Aiyeye

Table 1. Acid hydrolysis of the native cola starch using 0.1 M HCl at 80 °C.

Parameter	Heating period			
	30 min	1 h	2 h	3 h
Extent of hydrolysis (%)	15.00± 0.05	21 ± 0.00	24.99± 0.05	29.97±0.03
Starch recovery (%)	85.00 ± 0.05	79.00± 0.00	75.01± 0.05	70.03±0.03
Final weight of starch (g)	8.5	7.9	7.5	7.0
Moisture content (%)	9.14± 0.01	9.25± 0.00	9.45± 0.01	10.32±0.00
pH	7.2	6.6	6.2	6.0
Final colour of starch	Off- white	Off- white	Off- white	Off- white

Table 2. Acid hydrolysis of the native cola starch using 0.1 M HCl at 100 °C.

Parameter	Heating period			
	30 min	1 h	2 h	3 h
Extent of hydrolysis (%)	16.00± 0.00	20.00±0.05	25.58±0.15	29.98±0.04
Starch recovery (%)	84.00± 0.00	80.00±0.05	74.42±0.15	70.02±0.04
Final weight of starch (g)	8.4	8.0	7.42	7.0
Moisture content	9.25± 0.01	9.34± 0.01	9.66± 0.00	9.86± 0.02
pH	7.5	7.5	6.9	6.2
Final colour of starch	Off- white	Off- white	Off- white	Off- white

Table 3. Acid hydrolysis of the native cola starch using 0.2 M HCl at 80 °C.

Parameter	Heating period			
	30 min	1 h	2 h	3 h
Extent of hydrolysis (%)	20.00± 0.01	25.98± 0.04	30.00± 0.00	32.95± 0.15
Starch recovery (%)	80.00± 0.01	74.04± 0.04	70.00± 0.00	67.05± 0.15
Final weight of starch (g)	8.0	7.9	7.5	7.0
Moisture content	9.18± 0.01	9.23± 0.00	9.44± 0.02	9.62± 0.02
pH	7.3	7.3	6.9	6.4
Final colour of starch	Off- white	Off- white	Off- white	Off- white

Table 4. Acid hydrolysis of the native cola starch using 0.2 M HCl at 100 °C.

Parameter	Heating period			
	30 min	1 h	2 h	3 h
Extent of hydrolysis (%)	21.02± 0.05	25.00± 0.01	30.00± 0.00	35.00± 0.00
Starch recovery (%)	78.98± 0.05	75.00± 0.01	70.00± 0.00	65.00± 0.00
Final weight of starch (g)	7.9	7.5	7.0	6.5
Moisture content	9.19± 0.02	9.23± 0.01	9.34± 0.02	9.45± 0.00
pH	7.4	7.3	6.8	6.0
Final colour of starch	Off- white	Off- white	Off- white	Off- white

et al., 1983). It has also been reported that the high solubility of acid modified starch with increasing temperature may be due to the loss of granular structure and release of amylose fraction of the starch, as the amylose molecules are preferentially solubilized and leached from swollen granules (Stone et al., 1984).

Amylose/ amylopectin contents of acid thinned starch

The percent amylose/ amylopectin contents of acid thinned starch as presented in Table 6 shows the effect of reaction time on the amylose content of acid thinned

Table 5. Percent swelling power and solubility of acid thinned cola starch.

Starch properties	% Swelling	% Solubility
Native cola starch	8.85	7.48±0.02
0.1 M HCl, 80 °C, 1 h	7.21±0.02	14.83±0.02
0.1 M HCl, 80 °C, 3 h	6.47±0.00	15.98±0.00
0.1M HCl, 100 °C, 1 h	7.05±0.02	15.00±0.01
0.1M HCl, 100 °C, 3 h	6.13±0.01	16.65±0.02

Table 6. Percent amylose/amylopectin contents of acid thinned cola starch at different acid concentrations, reaction time and temperatures.

Acid concentration	Temperature of heating (°C)	Heating period	% Amylose	% Amylopectin
0.1 M HCl	80	30 min	21.69±0.03	78.31±0.03
0.1 M HCl	80	1 h	20.18±0.04	79.82±0.04
0.1 M HCl	80	2 h	20.09±0.00	79.91±0.00
0.1 M HCl	80	3 h	19.66±0.02	80.34±0.02
0.1 M HCl	100	30 min	21.56± 0.03	78.44 ±0.03
0.1 M HCl	100	1 h	20.15±0.00	79.85±0.00
0.1 M HCl	100	2 h	20.10±0.00	79.90±0.00
0.1 M HCl	100	3 h	19.35±0.01	80.65±0.01
0.2 M HCl	80	30 min	21.46±0.01	78.54±0.01
0.2 M HCl	80	1 h	20.04±0.00	79.96±0.00
0.2 M HCl	80	2 h	20.03±0.04	79.97±0.04
0.2 M HCl	80	3 h	17.82±0.00	82.18±0.00
0.2 M HCl	100	30 min	20.88±0.02	79.12±0.02
0.2 M HCl	100	1 h	19.99±0.00	80.01±0.00
0.2 M HCl	100	2 h	18.14± 0.02	81.86±0.02
0.2 M HCl	100	3 h	17.28±0.03	82.72± 0.03

N/B: The percent amylose/ amylopectin of native cola starch = 24.76/ 75.24.

starch. The amylose content of acid modified starch is lower than the unmodified one. This is in line with earlier reported work on tapioca and corn starches (Napporn et al., 2001; Ya-June et al., 2003). The decreasing trend in the amylose content of the acid thinned starch as the reaction time increases corresponds to different concentrations of the HCl used and for the different heating temperatures.

Pasting properties

Table 7 shows the comparative RVA of the acid thinned and native cola starches. The peak viscosity of the acid thinned ranged from 52.71 to 197.32 and is lower than that of native cola starch of 314.42. The table also shows that the peak viscosity decreases as the reaction time increases. Similar trends have earlier been reported for tapioca starch (Napporn et al., 2001). The low peak viscosity suggests that it can be used in forming gels in gums and jellies. The breakdown viscosity ranged from

30.24 to 73.17 as compared to 179.25 for the native one. The value decreases as the reaction time increases. The final viscosity showed a reduction from 209.58 for the native starch to 21.18 to 157.93 for the acid thinned starch. It also shows a decreasing trend as the reaction time increases. The setback viscosity which is lower than that of native starch suggests that such starch may find application in the food industry.

Gelatinization properties

The data obtained for the DSC thermo gram of the acid thinned starch is as shown in Table 8. The results showed variations in the onset, peak and gelatinization temperatures, of both the acid thinned and native cola starches.

Native starch has onset temperature (T_o) as 85.5°C and a peak temperature (T_p) of 318.1°C, while the 0.1 M HCl, 80°C, 3 h hydrolyzed cola starch, recorded (T_o) of 37.7°C, with a (T_p) of 252.9°C and 0.2 M HCl, 100°C, 1 h

Table 7. Pasting properties (RVA) of acid thinned cola starch at different acid concentrations, heating periods and temperatures.

Profile of the starch	Peak visc.	Trough visc.	Breakdown visc.	Final visc.	Set back	Peak time	Pasting temp.
Native cola starch	314.42	135.17	179.25	209.58	74.42	4.80	74.50
0.1 M HCl, 80°C 1 h	115.51±0.00	69.08±0.00	46.42±0.00	96.40±0.02	25.48±0.04	4.27	85.60
0.1 M HCl, 80°C 2 h	114.16±0.01	69.58±0.03	44.58±0.04	91.65±0.04	23.93±0.03	4.27	85.75
0.1 M HCl, 80°C 3 h	60.50±0.00	30.09±0.01	30.42±0.01	43.51±0.00	13.44±0.00	4.20	85.80
0.1 M HCl, 100°C 1 h	82.23±0.00	33.91±0.02	48.33±0.02	48.18±0.03	13.66±0.01	4.20	84.65
0.1 M HCl, 100°C 2 h	62.84±0.03	16.69±0.02	46.15±0.04	46.91±0.02	10.67±0.01	4.13	84.70
0.1 M HCl, 100°C 3 h	61.91±0.02	31.67±0.00	30.24±0.02	41.33±0.00	10.24±0.03	4.13	84.70
0.2 M HCl, 80°C 30 min	197.32±0.03	124.16±0.00	73.17±0.02	157.93±0.02	33.74±0.02	4.27	84.88
0.2 M HCl, 80°C 1 h	82.31±0.03	19.72±0.00	62.60±0.02	49.93±0.02	10.18±0.02	4.13	85.85
0.2 M HCl, 80°C 2 h	79.84±0.02	33.67±0.00	46.17±0.01	46.78±0.02	12.93±0.02	4.22	85.90
0.2 M HCl, 80°C 3 h	56.82±0.03	21.40±0.00	35.41±0.01	34.34±0.02	12.71±0.02	4.27	85.90
0.2 M HCl, 100°C 30 min	182.83±0.00	116.34±0.00	65.67±0.00	151.27±0.03	34.91±0.01	4.27	84.05
0.2 M HCl, 100°C 1 h	85.63±0.03	19.97±0.00	65.67±0.00	30.32±0.03	10.33±0.01	4.20	84.80
0.2 M HCl, 100°C 2 h	74.81±0.03	30.11±0.01	44.70±0.01	23.91±0.00	10.03±0.03	4.13	85.80
0.2 M HCl, 100°C 3 h	52.71±0.03	21±0.00	31.53±0.04	21.18±0.04	9.71±0.02	4.13	85.80

Table 8. Gelatinization properties of acid thinned cola starch.

Starch properties	Glass transition temp. (°C)	Peak temp. Tp (°C)	Endset temp. (°C)	ΔH (Tc-To)	Pasting temp. (°C)
Native starch	301	321.1	340	39	74
0.1 M HCl, 3 h	37.7	252.9	66.5	28.8	65.80
0.2 M HCl, 1 h	42.4	226.9	66.7	24.3	64.70

hydrolyzed cola starch has (T_0) of 42.4°C and (T_p) of 226.9°C. The gelatinization temperature of the native cola starch was recorded as 74.00°C, while that of the acid treated starch ranged between 64.70 to 65.80°C. The lower gelatinization properties observed for the acid thinned starches may be due to the weakening of the hydrogen bond during acid hydrolysis. The formation of new chemical group in starch granule and de-polymerization of starch granules result in the lowering of transition temperature.

Conclusion

Acid hydrolysis of cola starch affected its physicochemical, thermal and morphological properties. The extent of modification depends on the acid concentration, reaction time and temperature. Acid hydrolysis increased starch solubility and lowers its swelling capacity. It also resulted in lower peak, set back and breakdown viscosities than was reported for the native starch. The lower peak viscosity which may be related to increasing crystallinity suggests that such starch could be employed as tablet filler in the pharmaceutical industry. Acid thinned starches may also be utilized in the candy industry in the manufacture of

syrops, jellies and gum products. They can also be employed as stabilizers in sausages and dressings.

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