The Effect of Hypochlorite Oxidation and Acetylation on Some of the Physicochemical Properties of Icacina Trichantha Starch

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Abstract

The study evaluated the effect of hypochlorite oxidation and acetylation on some physicochemical properties of Icacina trichantha starch. The native and modified (oxidized and acetylated) starches were studied with respect to Infrared spectroscopy(IR), microscopy, gelatinization, swelling power, solubility index, amylose content, paste clarity and proximate compositions. Oxidized starch had the highest paste clarity, followed by the acetylated starch. The paste clarity of all the starches were found to be pH dependent. The modified starch also had higher solubility index compared to the native starch but lower gelatinization temperature. The modified starches had lower swelling power but higher amylose content. The properties of the starches were found to be dependent on the amylose content.

Keywords: Icacina trichantha starch, Oxidation, Acetylation, Physicochemical properties

Introduction

Starch is the only qualitatively important digestible polysaccharide and has been regarded as nutritionally superior to low molecular weight carbohydrate or sugars (ASP, 1996). The modern food processing industries are increasingly dependent on the use of both native and modified starches for manufacture of various fabricated food products. It is an important ingredient for the food industries. Starches with specific properties are necessary to impart functionality desirable attributes (Tharanahan *et al.,* 1990) and to impart viscosity to foods. The clarity of starch paste is one of its important attributes. Starch used to thicken fruit pie filling is preferably transparent but starch used in spoonable salad dressing should be opaque. Clarity varies considerably with the sources of starch and can be altered by chemical modification of the starch, (Stuart *et al.,* 1989). Mild oxidation of starches with hypochlorite yields a system with greater stability and clarity and less tendency towards gelatinization and retrogradation (Ihekoronye and Ngoddy 1985).

Though starch is mainly used as food, it can also be readily converted chemically and biologically into many useful and diverse products such as paper, textiles, adhesive beverages, confectionaries, pharmaceuticals and plastics. *Icacina trichantha* (family: Icacinacea) is a shrub up to 2m with scandent growth above. It produces very large tuber of forest and jungle vegetation in south-east Nigeria. The plant is reported to be found as weed of rice paddies in Bendel State (Gill and Ene, 1978)

Although icacina trichantha is not included on the sources of starch as reported above but report has shown that it contains starch. The tubers are up to 50kg and when food is insufficient, some people of the upper Shari region used the seeds as meal or more rarely, the tuber (Mabberley 1997).
While various attempts have been made to characterize starches derived from cassava, taro, sweet potato, red cocoyam, rice etc no such studies have been reported on Icacina trichantha. During the present study, starch was extracted from tubers of the icacina trichantha and modified chemically in order to evaluate the proximate compositions and other functional properties.

Materials and Methods

Extraction of Icacina trichantha starch: The method of Attama *et al.* (2003) was used for the isolation of starch from the Icacina trichantha tubers. In this method, the tubers were washed thoroughly, peeled, rewashed, cut into small pieces and milled into a pulp. The pulp was then soaked in water and sieved using muslin cloth. The filtrate was allowed to stand for 24hours and the starch separated by decantation. The separated starch was washed with distilled water four times and then soaked for further 24h in 0.1N sodium metabisulphite solution and thereafter washed thoroughly (three times) to free it of this reagent. The slurry was then soaked in 0.1N sodium hydroxide solution for 24h and subsequently washed repeatedly with distilled water until neutral to litmus. It was soaked in 0.1N sulphuric acid for 12h and washed thoroughly until neutral to litmus. The resulting starch slurry was decanted after 24h standing and dried at 40oC in an oven.

Preparation of hypochlorite (oxidized) starch: The method of Ogungbenle (2007) with little modification was used. A 30g quantity of the starch was dispersed in 150cm^3 distilled water. The pH of the slurry was adjusted to 9.0 using 3% NaOH. A 3g NaOCl was added and the dispersion stirred for 15min using a magnetic stirrer. The pH of the mixture was then adjusted to 7.0 with 0.5M HCl and the slurry later filtered through Whatman No1 filter paper. The residue obtained was washed four times with distilled water to remove completely some acids that may be present in the product and finally

dried at 40°C. The above procedure was repeated with four other samples while stirring at 30mins, 60min, 90min and 120min respectively.

Preparation of acetylated starch: The method of Ogungbele (2007) was used. Starch (30g) was dispersed in 150cm^3 of distilled water and constantly stirred for 30min. The slurry was adjusted to pH 8.0 with NaOH, 3.6g of acetic anhydride was then added to the slurry and the reaction was allowed to proceed for another five minutes. The pH of the starch was adjusted to 4.5 with 0.5M HCl and filtered through Whatman No 1 filter paper. The residue obtained was washed severally with distilled water to remove completely residual acids that may be present in the product and finally oven dried at 40°C.

Proximate analysis: Proximate composition of samples were determined according to the method of AOAC (1980) for moisture, total ash, total crude fibre, crude fat and total crude protein respectively. Carbohydrate was obtained by difference. All results were the average of triplicate analyses.

Determination of amylose: Mc-Gready and Hassid colourimetric (1943) method was used to determine amylose content in starch samples. A 0.1g dried defatted sample was dispersed in 2ml ethanol, then 10ml distilled water and 2ml of 10% NaOH were added. The mixture was heated on a hot plate until a clear solution was obtained. The clear solution was made up to mark in 100ml volumetric flask. A 5ml of the solution was pipetted into 100ml flask, 3 drops of 6N HCl and 5ml iodine solution (0.2% iodine in 2% potassium iodide) were added and the volume made up to mark with distilled water. It was allowed to stand for 20min for maximum colour development, and absorbance recorded at 640nm. The concentration of the amylose was determined from a standard curve prepared from pure amylase.

Determination of swelling power: This was determined in accordance with the method described by Daramola *et al.* (2006). To a 0.1g of sample in a weighed test tube was added 10ml of distilled water and heated in a water bath at temperature of 85oC for 30min with continuous shaking. In the end, the test tube was centrifuged at 1000xg for 15min in order to facilitate the removal of the supernatant which was carefully decanted and the weight of the starch paste taken. Swelling power was calculated as follows: Swelling power = weight of starch paste / weight of dry starch sampled

Determination of solubility index: Solubility index was evaluated by adding 1g of starch to 20ml of distilled water in a test tube. This was subjected to heating in water bath at a temperature of 85°C for 30m in. It was subjected to centrifugation at 1200xg for 20min. A 10ml of the supernatant was decanted and dried to constant weight. The solubility was expressed as the percent by weight of dissolved starch from a heated solution (Daramola *et al.,* 2006).

Size of starch granules: The method of Attama et al., (2003) was used for determination of the size of starch granules. Dry starch samples were dispersed in water and viewed under a microscope at a magnification of 400 x. The micrographs were used to compare the morphology of the starch granules.

Determination of the gelatinization temperature: The method of Attama *et al. (*2003) was used for determination of gelatinization temperature. A 1g quantity of each starch sample (native, acetylated and oxidized) was put in 20ml beaker and 10ml of distilled water was added. The dispersion was heated on a hot plate. The gelatinization temperature was read with a thermometer suspended in the starch slurry.

Determination of pH of starch: The method reported by Benesi *et al.* (2004) was used for pH determination. Approximately, 5g of starch sample was added to 20ml of distilled water in a beaker. The contents were stirred for 5min and allowed to settle. The pH of the water phase was taken using a calibrated pH meter.
Determination of starch paste clarity

Paste clarity: The method of Ashveen et al. (2008) was used for paste clarity determination. Starch samples in screw cap tubes were suspended in distilled water to yield 1% (w/v) slurries. The pH of the slurries were adjusted to 2, 4, 6, 8, 10 and 12 by the addition of 0.1M HCl or NaOH as required. The tubes were then heated in a boiling water bath (with occasional shaking) for 30min. After cooling to ambient temperature, the percent transmittance % at 650nm was determined against water as a blank using spectrophotometer.

Results and Discussion

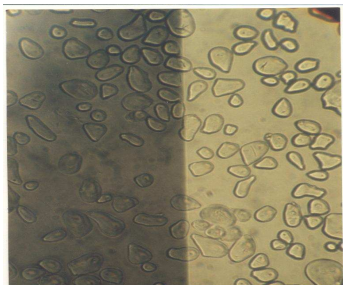
Infra red spectra: The IR spectra of the native and modified starches showed the following vibrational sequences: For the native starch they include 1156 and 1083cm-1, 1023 and 982cm-1 respectively. The peaks at 1083 and 1023cm-1 are characteristic of the anhydroglucose ring (O-C stretch). A characteristic peak occurred at 1642cm-1 which is presumably a feature of tightly bound water present in the starch. An extremely broad band due to hydrogen- bonded hydroxyl group (O-H) appeared at 3400cm-1 which was attributed to the complex vibrational stretches associated with free, inter and intra-molecular bound hydroxyl groups which make up the gross structure of starch. In the oxidized starches, peaks at 1752cm-1 to 1680cm-1 were observed (carboxyl group). The acetylated starch showed peaks at 1749cm-1(carboxyl group).

Amylose content: The apparent amylose content of the native (26.5%) oxidized (30%) and acetylated (32%) starches respectively varied significantly. The acetylated starch had the highest value while the native starch had the lowest. Previous report (Riley *et al.,* 2006) has shown that the amylose content plays a key role in the digestion of starch. Starches with low amylose contents were found to be more digestible than starches with high amylose content.

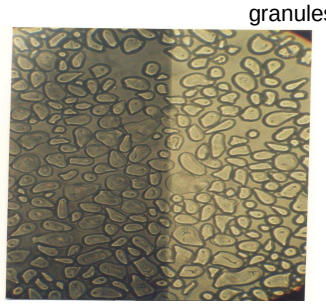
Table1: Properties of native and modified Icacina trichantha starches

Properties	Native starch	Oxidized starch (15min)	Oxidized starch (30min)	Oxidized starch (60min)	Oxidized starch (90min)	Oxidized starch (120min)	Acetylated starch
Gelatinization temperature (^oC)	73	67	64	60	58	50	70
Swelling power	13.42	12.62	ND	ND	ND	13.32	8.80
Solubility (%)	10.40	11.60	ND	ND	ND	15.20	13.40
Amylose content (%)	26.50	ND	ND	ND	ND	30.0	32.0

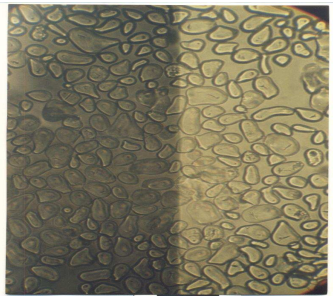
key: *ND = not determined*



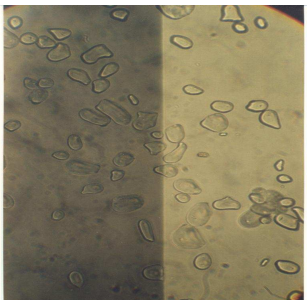
(a) *Icacina trichantha* starch oxidized (120 min)



(b) *Icacina trichanta* starch oxidized (15min



(c) Acetylated *Icacina trichantha* starch



(d) Native *Icacina trichantha* starch

Fig. 1: Photomicrograph of *Icacina trichantha* starch for (a) oxidized (120min), (b) oxidized (15min), (c) acetylated and (d) native

In comparism, the amylose content of native and modified *Icacina trichanta* starches were within the range of (17 to 35%) earlier reported by Mbofung *et al.* (2006), for six varieties of taro starches. The high amylose content of this sample suggests that the starch content should be high also, as previously suggested by Ikegwu *et al.* (2009).

Proximate compositions of the native *Icacina trichantha* starch were, fat 0.47%, protein 0.88%- asi,!0.50%, moisture 6.50% and carbohydrate, 91.63%. The oxidized starches were seen to be progressively finer to touch with increasing duration of oxidation. The oxidized and acetylated starches produced aqueous dispersion of greater clarity, less gelhing tendency than those of the native starch. The longer the time of hypochlorite treatment, the lesser the gelatinization and the greater the clarity obtained.

The photomicrographs of the oXidized, acetylated and native starches are presented in figure 1. There were distortions of the starch granules of the oxidized and acetylated starches particularly at the central points called the hilum.

Gelatinization temperature: Table 1 shows the effect of oxidation and acetylation on the gelatinization temperature of the starch. The table shows that gelatinization temperature decreased with increase in oxidation duration. The gelatinization temperature for the acetylated was higher than that for the oxidized starches. The decrease in the gelatinization temperature with duration of oxidation is suggestive of proportional variation of degree of oxidation with the tendency of starch towards thermal gelatinization. Oxidation weakens the inter-particle bond of starch making it easier for heat to effect gelatinization at lower temperature. The higher value of gelatinization temperature of native starch compared to acetylated arises from the fact that the introduction of acetyl groups stabilizes starch by interrupting the linearity of amylose and segments of amylopectin branches, thereby sterically interfering with intermolecular alignment. In addition, there is higher degree of orderliness in the native starch, Ashveen (2008).

The solubility profiles of the oxidized and acetylated starches resembled that of native Icacina trichantha starch. They were insoluble in the organic solvents such as ethanol, acetone and toluene. They were insoluble in cold water, hence their solubility in water were determined at 85^{o}C. The modified starches (oxidized and acetylated) had higher solubility in water. Their results are presented in table 1. The unmodified *Icacina trichantha* starch had the highest swelling power (13.4%w/w) when compared to oxidized (13.3%w/w) and acetylated starch (8.7%w/w) as shown in table 1. This could be attributed to the higher amylose content of the modified starches. The swelling power of starches is of great significance in tablet and capsule formulations, as it is believed that disintegrants work through a swelling and wicking action (Adebayo and Itiola, 1998).

Table 2: Paste clarity of native and modified starches

pH	Percent Transmittance			
	Native starch	Acetylated starch	Oxidized Starch (15 min)	Oxidized Starch (120 min)
2	69	83	68	96
4	48	44	40	27
6	29	48	28	31
8	36	46	29	51
10	61	55	33	58
12	79	92	78	84

The results of paste clarity are shown in Table 1. The paste clarity of all starches were moderately high at pH 2 which decreased sharply up to pH 4. In very acidic solution negatively charged phosphate groups are neutralized, and the ionization of hydroxyl groups is suppressed. Therefore lysophospholipid complexed amylose chains contain only electropositive nitrogen. Coulombic repulsion between these positive nitrogens on adjacent amylose chains decreases the compactness of the amorphous region, thus increasing the transmission (Hoover and Vasanthan, 1997). A significant increase was observed after pH 10. This can be explained in terms of granular swelling, resulting from repulsion between adjacent negative charges centred on the hydroxyl groups of complexed lysophospholipid molecules (Ashveen et al., 2008). The oxidized and acetylated starch showed better paste clarity than native icacina trichantha starch. The native starch showed 69% at pH 2 while icacina trichantha starch oxidized for 2h had 96% transmittance which is in agreement with the value obtained for potato starch at 650nm reported by(Stuart et al., (1989) and compared to that obtained by (Ogurgbenle, 2007) for gourd seed which showed 95% transmittance. Stuart *et al.* (1989) observed that more opaque paste gave a lower % transmittance. The high clarity observed for oxidized starch pastes could signify that the starch granules of these samples are fragile during pasting and remnants of granules are absent from the paste.

Conclusion: This work has highlighted some important effects of oxidation and acetylation on the physicochemical properties of Icacina trichantha starch. The swelling power of both native and modified fall on the group of highly restricted-swelling starches. As a result, they may be used for the manufacture of value-added products such as noodles and composite blends with cereals. The physicochemical, pasting and functional properties obtained indicate that Icacina trichanta starch has useful technological properties for many applications such as in food processing, paper and textile industries. The low amylose content makes it a good choice food for diabetics and other health conscious individuals. Research is in progress to utilize these starches for the preparation of biodegradable materials.

References

Adebayo A.S. and Itiola O.A (1998). Evaluation of breadfruit and cocoyam starches as exodisintegrants in a paracetamol tablet formulation. In: Riley, C.K., wheathey, A.O. and Asemota,H.N. (2006). Isolation and characterization of starches from eight Dioscorea alata cultivars grown in Jamaica. Afri. J.Biotechnol. 5: 1528-1536.

AOAC (1980). Official methods of analysis 13th edition, Association of official analytical chemist. Washington DC.

Ashveen, V..N.,Randhir, P., David ,R. and Jagjet R.K. (2008).Isolation and properties of starch from some local cultivars of cassava and taro in Fiji. Journal of Natural Science, 26: 45-48.

Asp, N. G. (1996). Dietary carbohydrates: classification by chemistry and physiology. Food chem, 7: 9-14 .

Attama, A.A., Nnamani, P.O., Mbonu, I.K.. and Adikwu, M.U. (2003). Effect of Hypochlorite oxidation on the physicochemical properties of gladiolus starch Jounal of pharm..and allied sci.:1;(1) 28-35,

Benesi, I.R.M.,labuschagne, M.T., Dixon, A.G.O. and Mahungu, N.M. (2004). Stability of native starch quality parameters, starch extraction and root dry matter of cassava genotypes in different environments, Journal of Science Food and Agriculture. 84: 1381-1388.

Callghan, P.J. and lelieve, J.(1985). In: Ogungbenle, H.N., (2007). Effect of chemical modification on starch of some legume flours. Pakistan Journal of Nutrition. 6: (2)167-171.

Daramola, B. and Osanyinlusi, S.A. (2006). Investigation on modification of cassava starch using active components of ginger roots (Zingiber officinale Roscoe), Afri. J. Biotechnol. 5: 917-920.

Gill and Ene 182-183 (1978) In: Burkill, H.M (1985).The useful plants of west tropical Africa 2: 1-2.

Hoover, R. and Vasanthan, T. (1992). Studies on isolation and characterization of starch from oat (Avena nuda) grains. In: Ashveen, V..N., Randhir,P.C., David, R. and Jagjit, R.K.. (2008). Isolation and properties of starch from some local cultivars of cassava and taro in Fiji The Pacific Journal of Natural Science. 10: 45-48

Ihekoronye, A.I and Ngoddy, P.O. (1985). Integrated food science and technology for the topics. Macmillan, New York, P178,190.

Ikegwu, O..J., Nwobasi, V.N., Odo, M.O. and Oledinmma, N.U. (2009). “Evaluation of the pasting and some functional properties of starch isolated from some improved cassava varieties in Nigeria.” EJEAFChe 8 (8): 64

Lineback, D.R and Ke, C.H. (1975). Starch and low molecular weight carbohydrates from chick pea and horse bean flour, Cereal chemistry 52: 334-347.

Mabberley, D. (1997). The plant-Book 2nd edition. Cambridge University Press Gt Britain.

Mbofung, C.M.F., Aboubakar, Y.N., Njintang, A., Abdou, B. and Balaam F. (2006), Plyscio chemical and Functioning Propertus of six varieties of Taro (colocasi esculenta L. Schott) flour. Journal of food Technology (4) 2: 135-142.

McCready, R. M. and Hassid, W. F. (1943). The separation and quantitativeestimation of amylose and Amylopectin in Potato starch., Journal of the American Chemical Society, 65: 1154

Ogungbenle H.N. (1985). Effect of chemical modification on starch of some legume flours. Pakistan journal of nutrition 6:(2) 167-171.

Riley, C.K.., Wheatley, A.O., and Asemota, H.N. (2006). Isolation and characterization of starches from eight Dioscorea alata cultivars grown in Jamaica Afri. J. Biotechnol, 5(17): 1528-1536.

Stuart, A.S.C., Cladualdoc, M., Paul, A.S. and Hosenney, R.C. (1989).“Starch paste clarity’’. cereal chemistry 66: 173-182 .

Tester, R.F, Karkalas J. and Qi, X. (2004). starch structure and digestibility: Ezyme substrate relationship, world’s poultry science Journal 60: 186-195.

Tharanahan, R.V.G.; Changala, R. and Muralikrisha, G.M.(1990) ,In: Ogungbenle H.N (2007). “Effect of chemical modification on starch of some legume flours’’ Pakistan Journal of Nutrition, 6(2): 167-171.