THE EFFECTS OF HYPOCHLORITE-OXIDATION ON THE PHYSICO-CHEMICAL PROPERTIES OF STARCH OBTAINED FROM *TACCA INVOLUCRATA*

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Abstract

Tacca involucrata tubers are a staple source of starch in the Middle belt region of Nigeria. In this study, hypochlorite oxidation is utilized as a means of improving the physicochemical properties of the starch, to obviate problems posed by high moisture absorbing capacity (risk of microbial contamination) and poor flow properties, etc. Samples of the starch were treated with calcium hypochlorite to provide an oxidative concentration of 10 g per litre of active chlorine, for times ranging from 15 minutes to 2 hours at 28 ^{o}C and pH 9. Suitable properties of the bleached starches: particle size, relative viscosities, true and loose densities, gelatinization temperature and equilibrium moisture content, were assessed in relation to the native unoxidized starch. The results indicate that oxidation produces starch of whiter colour and optically clearer mucilage. It causes depolymerisation of the starch, hereby yielding lower viscosity grades with lower molecular weights. The process produces starch with smaller grain particles due to structural breakdown along fissure lines. The gelatinization temperature became lowered on oxidation. The moisture absorbing capacity and hence risk of microbial attack was also reduced, but starch of slightly poorer flow was produced.

Keywords: *Tacca involucrata;* starch; modification; hypochlorite; oxidation physiochemical

INTRODUCTION

Starch can be found in numerous organs of many higher plants. In addition to higher plants, starch is also found in mosses and ferns, and in some protozoa, algae and bacteria (Shannon and Garwood, 1994). Starch is a granular mixture of two polymers, namely amylose and amylopectin. Different starches have different compositions of amylose and amylopectin, and these are responsible for their different properties, such as gelatinization temperature (Trease and Evans, 1983).

Sometimes, chemical modification of starch is necessary. This is because of such problems as insolubility, instability, large molecular size, moisture content and susceptibility to microbial oxidation. Chemical modification may also be undertaken to satisfy a given pharmaceutical end (Abdel-Hafiz, 1997). Modification may be achieved by oxidation, acid treatment (Rafai *et al.,* 1992, Hebeish

et al., 1985), etherification (Radley, 1976), grafting (Metratro and Barnby, 1977) and poly (viny1) starch derivatisation.

Starches oxidized by hypochlorite are termed "chlorinated starches", though no chlorine is introduced into the starch by this treatment (Rutenberg and Solarek, 1994). Oxidation gives starch of lower viscosity, high solid dispersions and resistance to viscosity changes on storage. Hypochlorite oxidation is useful in the paper industry (Rutenberg and Solarek, 1994), the viscous stability of oxidized starch as well as the range of viscosities possible make them suitable for paper and paper board surface sizing. Hypochlorite-oxidised starch has been used as tablet disintegrants where its lower viscosity imparts improved disintegrant effect (Carter, 1975). Oxidized starches are also useful as lubricant for gloves during surgery due to reduced particle size and lower risk of microbial contamination.

The objective of this study was to investigate the suitability of hypochlorite oxidation as a suitable chemical modification method for *Tacca involucrata* starch, in an attempt to solve such problems as its lacklustre colour, high moisture absorbing capacity, poor flow, etc. *Tacca involucrata* plant grows wild in the middle belt region of Nigeria, particularly Benue and Kogi States, where the tubers are consumed as a delicacy.

MATERIALS AND METHODS

Materials
Tacca involucrata tubers, calcium hypochlorite powder (BDH, England) sulphuric acid, sodium metabisulphite, sodium hydroxide (Merck, Germany) ethanol (98%), n-hexane (M&B, England) and chloroform (BDH,

England) were used as procured from the respective manufacturers

Methods

Extraction of starch
The tubers were washed, peeled and washed again with distilled water. They were then milled several times to give a fine pulp. The pulp was passed through muslin cloth to remove fibers. The resulting slurry was left undisturbed for 24 hours during which the starch settled at the bottom, and was collected by decanting the upper layer. The starch paste was soaked in 0.1 N sodium metabisulphite for 24 hours, then washed with distilled water. It was soaked in 0.1 N sodium hydroxide for 24 hours and washed again with distilled water till it became neutral. Finally, the slurry was treated with 0.1 N sulphuric acid for 12 hours, then washed severally with distilled water till it became neutral again. It was then allowed to settle and the starch collected and dried at 40 $^{\circ}$C.

Oxidation of starch with hypochlorite
A 25 ml volume of 3 % solution of sodium hydroxide was prepared in five 50 ml flat bottom flasks. A 10 g quantity of the dry starch was added into each of these flasks to obtain 40% w/v slurry. A 2.5 g quantity of calcium hypochlorite [Ca $(OCl)_2$] was wrapped in a filter cloth and suspended in the flask. The contents of the flask were agitated for 15 minutes using a magnetic stirrer to yield approximately 10 % calcium hypochlorite solutions. The temperature of the system was maintained at 21-38 $^{\circ}$C. The process above was performed for five samples of treatment times of 15 minutes, 30 minutes, 1 hour, 1.5 hours and 2 hours. At the end of these times, the oxidative action was stopped by diluting the slurries with copious amounts of distilled water repeatedly and then decanting. The deposited starch was dried in each case at 40 $^{\circ}$C. The sixth

sample (control) was unoxidised starch.

Organoleptic examination
After oxidation, the colour, odour, taste and feel of the oxidized and unoxidized starch samples were assessed. A 10 % gelatinized dispersion of each sample was formed in water, and the different samples were compared.

Particle size
A little quantity of each oxidized starch sample under study was placed on a glass slide, covered with a cover slip and then distilled water introduced into the space such that the samples were suspended in the water without the formation of bubbles. The preparation was then viewed through a microscope (x 200) and photographed as magnified.

Effects of oxidation time on molecular weight
A 20 ml volume of 1 % w/v slurry was prepared by gentle heating. From the gels, dilutions were done to obtain 25 ml volumes of 0.05 %, 0.10 %, 0.15 %, 0.2 % and 0.25 % w/v starch gels. Twenty milliliters of each concentration was introduced into an Ostwald U-tube viscometer and the elution times taken, as well as that of distilled water at 25 ^{o}C. This determination was also carried out for different grades (low viscosity, medium viscosity and high viscosity) of gelled sodium carboxy methylcellulose using the same concentrations. The relative viscosities were calculated. A plot of reduced viscosities (specific viscosity divided by concentration in g per deciliter, where specific viscosity is one unit less relative viscosity) against the concentration yielded the intrinsic viscosity of each oxidized sample (intercept on the ordinate). From the plots, it was possible to compare

(without determining accurately) the molecular weights of the different starch samples via the Mark – Houwink equation (Eqn 1), using constants derived for the sodium carboxy methylcellulose dispersions.

$$Log\ [\eta] = \log k + \alpha \log M$$

Eqn 1

Where [η] refers to the intrinsic viscosity (intercept on Y-axis of the curve of reduced viscosity against concentration, in grams per deciliter), M is the average molecular mass of the sample while α and k are constants characteristic of polymer-water dispersions. These constants were obtained from the SCMC dispersions and only served to compare the molecular weights of the starch samples.

Moisture absorption capacity
Samples from the six batches were dried to a constant weight at 40 ^{o}C, then exposed to normal atmospheric humidity and temperature for one week and weighed again. The moisture difference per gram of dry sample was calculated**.**

True density determination
 A 50 ml pycnometer of known weight was filled with alcohol, stoppered and weighed. After emptying the container, 1 g of the native starch was added, and the volume occupied by it obtained by determining the amount of alcohol needed to fill the container again, with the stopper on. The true density of the powder was obtained thus:

$$True\ density\ (\rho)\ =\ \frac{\rho_l}{W}$$

Eqn 2

where ρ_l is the density of the ethanol employed in the test and ΔW is the weight of the ethanol displaced by 1 g of the starch sample.

Loose densities determination
The bulk volumes (and hence bulk densities) were obtained by pouring 5 g of each starch sample into a 10 ml

graduated cylinder and recording the volumes occupied.

The tapped volumes (hence tapped densities) were obtained by carefully tapping at the base of the cylinder till the volume became constant.

The derived parameters were calculated from the densities as follows:

Compressibility
index (C. I) = TD - BD
$$\text{--------- X100} \qquad \text{Eqn 3}$$
TD

Hausner's quotient (H.Q) =TD
BD

Eqn 4

TD refers to the tapped density of each sample, and BD, its bulk density. By relating the true densities to the bulk densities, it was possible to calculate the porosities of the samples.

$$\text{Porosity} = \frac{\Delta\rho}{\rho} \times 100$$

Eqn 5

Where $\Delta\rho$ refers to the difference between the true and bulk densities, and ρ is the true density of each sample, as determined previously.

Angle of repose determination
A 10 g weight of each of the six powders was used. A glass funnel was suspended with the orifice at about 10 cm distance above the table surface. The powder sample was poured into the funnel with the orifice closed, then the orifice was sharply opened, to allow the powder to flow uniformly onto a white sheet of paper on the table. The height of the powder heap was measured using a metre rule, and the radius of the powder base determined by tracing. The angle of repose (Θ) was calculated as

$$\Theta = \tan^{-1}\left[\frac{\text{height of heap}}{\text{Base radius}}\right]$$

Eqn 6

Solubility tests
The solubility of each starch sample was determined in various solvents, namely distilled water, ethanol 98% w/v, chloroform and n-hexane, relative to that of the unoxidized starch. In this study, a 1 g quantity of each powder sample was placed into a 20 ml flat bottom flask, and then 10 ml of distilled water was added. The flask and contents were covered tightly, then left undisturbed for 24 hours at room temperature. At the end of the 24 hours, a filter paper of known weight was used to filter the content of each flask. The filter and residue were then dried and the amount of residue determined by difference. The same procedure was repeated for the other solvents.

Gelatinization temperature determination
A 1 g quantity of the unoxidized starch sample was weighed and placed in a 20 ml flat bottom flask. Slurry was made by adding 5 ml of distilled water, and heat was applied with careful agitation on a magnetic stirrer-hot plate assembly. The temperature was monitored on a thermometer. This temperature (gel point) was determined in triplicate. The same procedures were repeated for each oxidized starch sample.

RESULTS AND DISCUSSION

Organoleptic microscopic examination
Hypochlorite treatment caused a bleaching effect (whiter colour) which increased proportionally with the duration of oxidation. The starch samples became whiter and finer in texture, though taste and smell differences were undetectable. Gels of the longer oxidized samples had

greater clarity than the less oxidized and unoxidized samples. The sample oxidized for 2 hours gave an optically clear dispersion. Oxidation caused the development of a fissure at the helium, extending to the distal poles. It increased in width with the duration of hypochlorite treatment. This confirms that oxidation takes place in the interior of the granule as well as on its surface, as previously suggested (Rutenberg and Solarek, 1994). The fissures created on the grains are evident in the photomicrographs presented in Fig. 1.

Rheological tests

The relative viscosity was lower for the oxidized samples than the unoxidised sample. The starch oxidized for 2 hours exhibited the least viscosity for all concentrations. This is attributable to the structural degradation of the molecules, producing starch of smaller molecular weights (depolymerization). The fall in molecular weight may be attributed to scission of the glucosidic linkages. The effect on molecular weight is presented in Table 1.

Equilibrium moisture content tests

Hypochlorite treatment markedly impaired the water absorbing capacity of the oxidized starches. The change was proportional with the duration of oxidation. The rapid fall in moisture gain is evident in Fig. 2.

The higher moisture absorbing capacity of the unoxidised starch exposes it to microbial contamination and degradation, and explains the high bacterial level of native potato starch (Carter, 1975) used as a disintegrant in tablets. High moisture absorbing capacity may also affect the structural integrity of tablets formulated with the untreated starch.

Density/Flow parameters

The bulk and tapped densities decreased with the duration of oxidation. The oxidized samples therefore exhibited lesser tendencies to consolidate as oxidation progressed. Rapid consolidation is essential to uniform filling of tablet machine die cavity. A decrease in bulk density is attributed to a reduction in particle size, producing the characteristic poor flow behaviour of fine particles. Fines are also known to exhibit static charges, which impede flow. The flow properties parameters are shown in Table 2.

Carr's compressibility data indicated poor flow (limits 23% to 28%) or moderate flow (limits 18-%21%) respectively (Well and Aulton, 1988). Hausner's quotient values also indicated poor to moderate flow

Table 1: Mark-Houwink equation values derived from plots of reduced viscosity against concentration for different test samples

Sample*	Plot intercept $[\eta]$	Log $[\eta]$	Molecular weight¶ (Daltons)
15 min	23.08	1.36	4325.05
30 min	22.86	1.36	4078.10
1 hr	22.48	1.35	3694.32
1.5 hr	21.89	1.34	3157.53
2 hr	21.42	1.33	2774.17
Native starch	23.65	1.37	4986.44
SCMC (LVG)	38.58	1.59	90988.02
SCMC (MVG)	45.79	1.66	251450.14
SCMC (HVG)	54.32	1.73	692471.31

The test specimens included T. involucrata starch samples subjected to varying calcium hypochlorite oxidation times, native unoxidized starch used as control and also standard SCMC grades used to determine the relevant constants. ¶Conversion was done by substituting 0.75 (log k) and 0.17 (α) in the equation. These values were obtained using the standard SCMC grades

Table 2: Effects of varying hypochlorite oxidation times on certain properties *T. involucrata* starch.

| Oxidation time (Min)	Bulk density (g/ml)	Tapped density (g/ml)	True density (g/ml)	Carr's Index (%)	Hausner's quotient	Angle of Repose (°)	Porosity (%)
Control	0.69	0.85	0.8970	18.82	1.23	28.30	23.08
15	0.67	0.80	0.9250	16.25	1.19	27.92	27.57
30	0.64	0.78	0.9526	17.95	1.22	28.52	32.82
60	0.63	0.75	0.9702	16.00	1.19	28.20	35.06
90	0.60	0.72	0.9754	16.67	1.20	30.38	38.49
120	0.51	0.70	0.9800	27.14	1.37	33.56	47.96
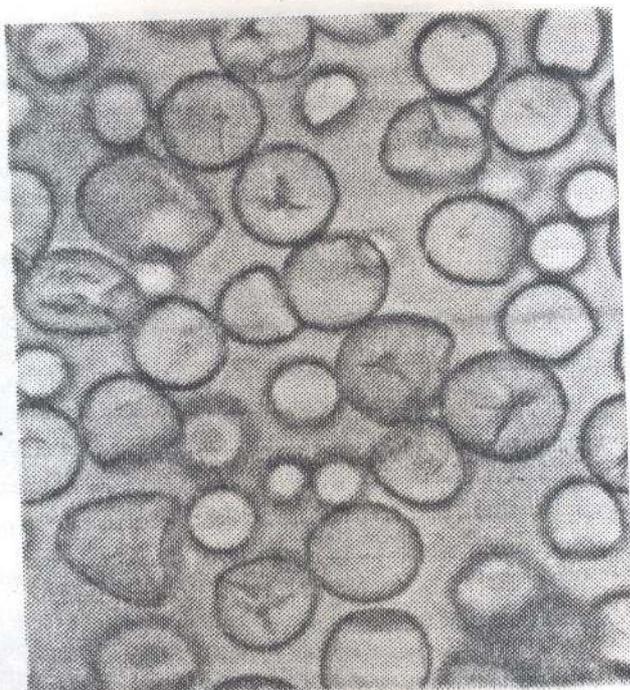
Fig 1: Photomicrograph of native Tacca starch granules.
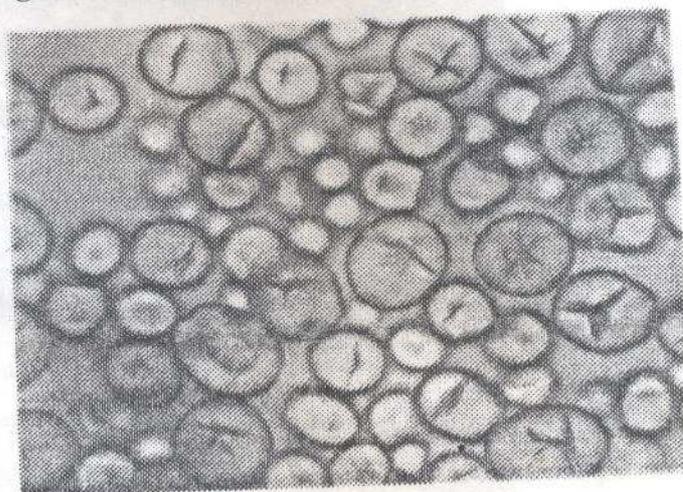
Fig 2: Photomicrograph of Tacca stsrch granules after oxidation for 15 mins

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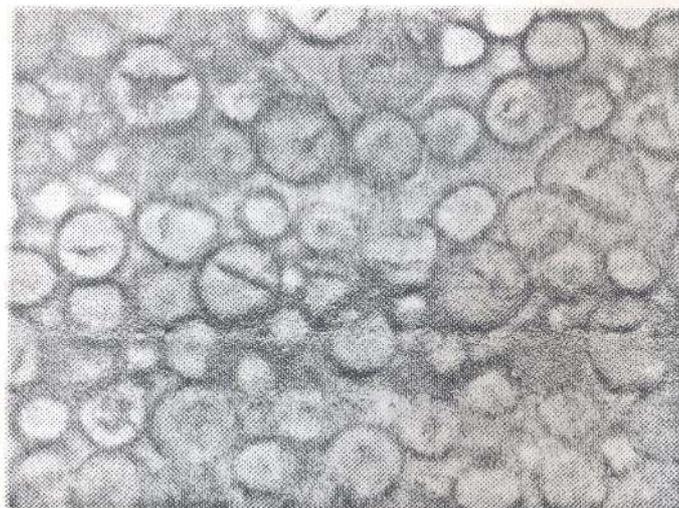
Fig 3: Photomicrograph of Tacca starch granules after oxidation for 30 mins
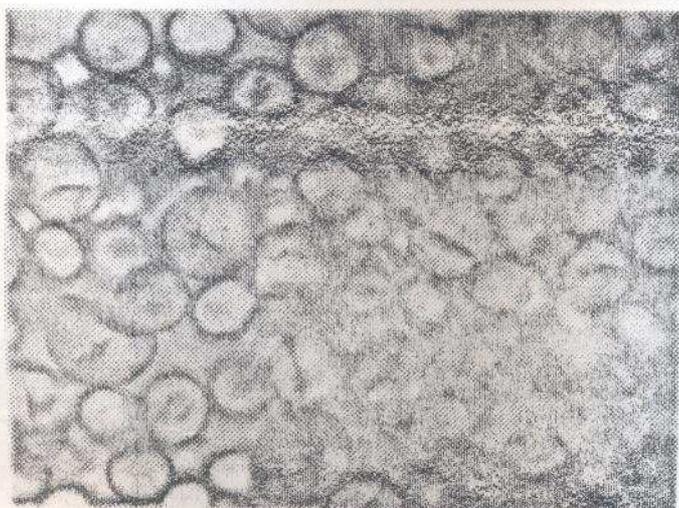
Fig 4: Photomicrograph of Tacca starch granules after oxidation for 1 hour.
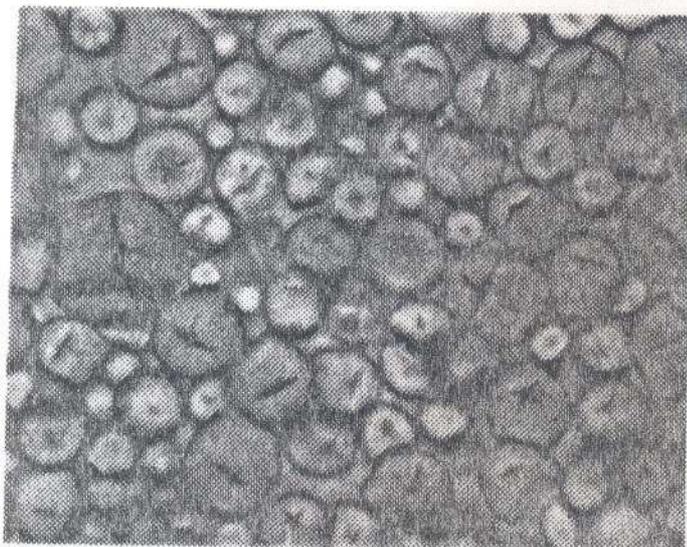
Fig 5: Photomicrograph of Tacca starch granules after oxidation for 1 hour 30 mins.
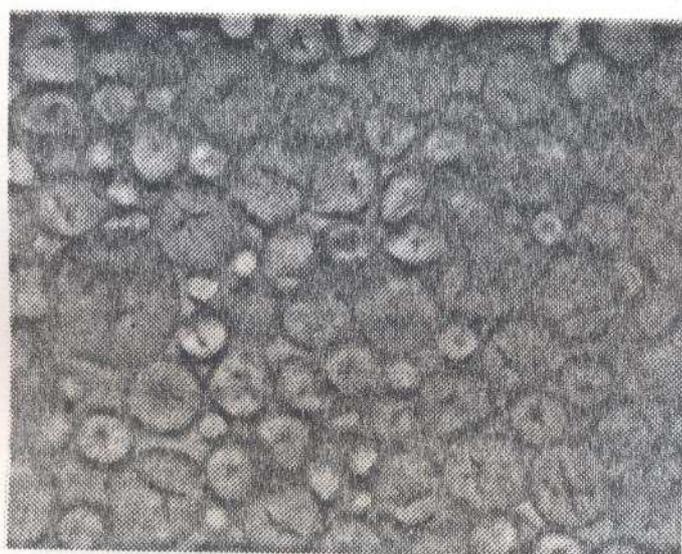

Fig 6: Photomicrograph of Tacca starch granules after oxidation for 2 hours.
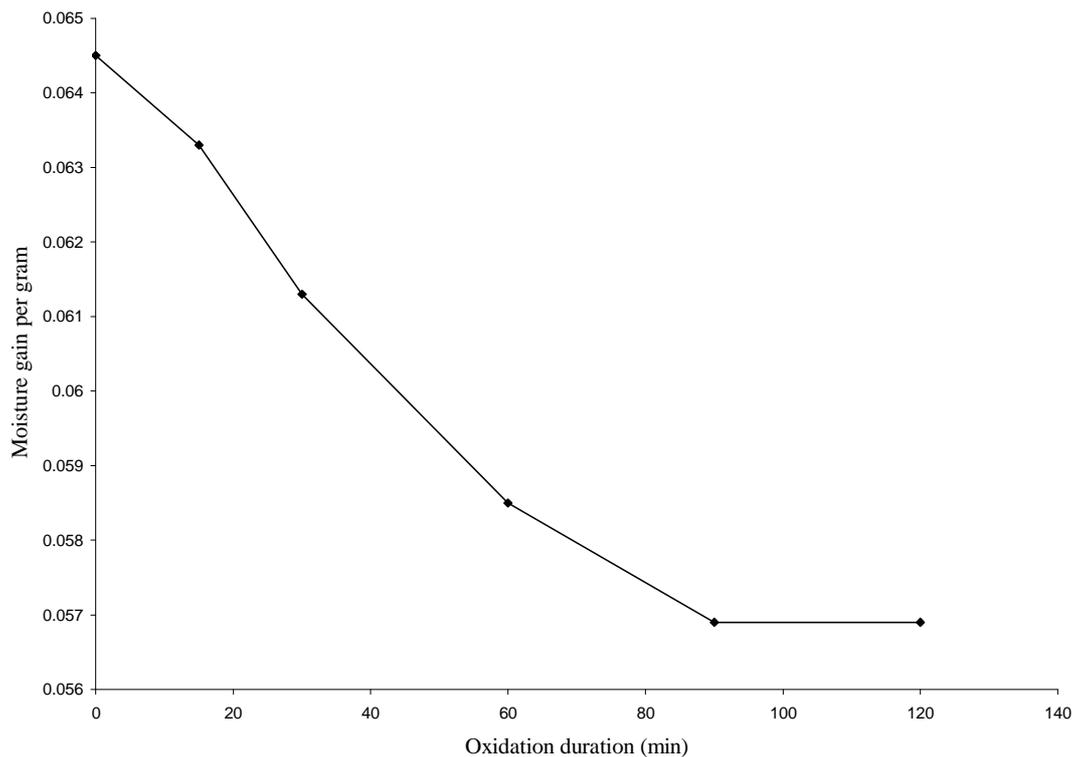
Fig 7:   Effect of varying hypochlorite oxidation times on the moisture absorbing capacity of ***Tacca involucrata*** starch.
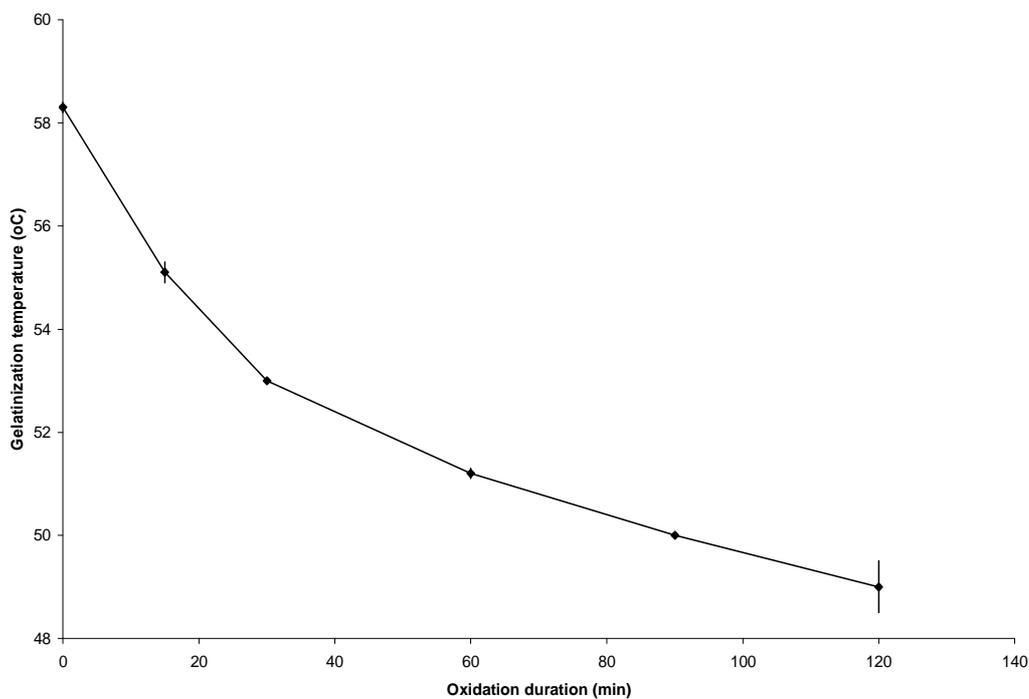

Fig. 8: The effect of hypochorite oxidation duration on the gelatinization temperature of *T. involucrata* starch

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Angle of repose increased as the duration of oxidation increased. This increase may be due to the smaller particles produced which showed poorer flow or due to the geometry of the granules produced on fissure. The angle of repose for all the starch samples was within limits for moderate flow ($<50^{o}$). Angles of less than 25° indicate very good flow (Well and Aulton, 1988).

Solubility and gelatinization temperature tests

The solubility test revealed that hypochlorite oxidation does not elicit any appreciable improvement in the solubility of starch in the solvents tested. Gelatinization test results are depicted in Fig. 3.

The gelatinization temperature showed a gradual fall as duration of oxidation increased. This enhanced sensitivity to heat may be due to introduction of polar groups in the course of oxidation, leading to enhanced intermolecular interaction.

CONCLUSION

Hypochlorite treatment gives *Tacca involucrata* starch with improved physical appearance (whiter colour and greater optical clarity of mucilage). The treatment does not alter the solubility of the starch in common solvents. Oxidation possibly produces depolymerization of starch chains, giving lower viscosity grades with lower molecular weights and slightly poorer flow properties. The enhanced optical clarity and reduced viscosity are advantageous and could increase the utility of the starch in external preparations where high viscosity would be a disadvantage. The finer particles as well as reduced moisture absorbing capacities are also advantageous in its use as a lubricant for surgical gloves and equipment, after sterilization.

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