

Effect of Sulphiting on the Physical and Functional Properties of Acetylated Cassava (*Manihot esculenta*) Starch

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ABSTRACT

Native cassava starches from a mixed cultivar and clone TMS 30572 were sulphited using a graded amount of sodium sulphite (Na_2SO_3) to obtain 0-5000 mg SO_2/kg starch. The differently sulphited starches were acetylated with 41 mL of acetic anhydride using 3% NaOH as catalyst, washed, centrifuged and dried at 30°C. The degree of acetylation of the starches was determined. The physical (bulk density, sedimentation, whiteness, and water and oil absorption capacities) and functional properties (swelling power, solubility, viscosity, paste clarity and freeze-thaw stability) of the starches were also determined. Sulphiting inhibited acetylation. There was a negative correlation between the level of sulphiting and degree of acetylation for TMS30572 and a mixed cultivar. The yield of the sulphited starches ranged between 94-97% and 89-96% for TMS 30572 and the mixed cassava cultivar, respectively. Optimum yield was 152 and 1250 mg SO_2/kg starch for TMS 30572 and the mixed cultivar, respectively. Sulphiting improved the whiteness of the starches but reduced some of the important functional properties. The whiteness ranged between 93.6-96.6% and 89.1-93.5% for TMS 30572 and mixed cultivar starches, respectively. At low concentrations of less than 75 mg SO_2 , cassava starches showed improved paste clarity while sulphiting at concentrations higher than 75 mg SO_2/kg starch reduced paste clarity. Sulphited cassava starches did not freeze until the fourth freeze-thaw cycle and exuded high water content at the fifth freeze-thaw cycle. Sulphiting of acetylated cassava starch is not encouraged in food ingredients when swelling and freeze-thaw stability is required.

Keywords: acetylation, cassava starch, functional, physical, properties, sulphiting

INTRODUCTION

Cassava (*Manihot esculenta*) originated from South America and was transported by sailors to Africa in the 16th century (Leotard *et al.* 2009). Cassava is one of the most important staple food crops in the world feeding more than 600 million people worldwide and more than 200 million people in sub-Saharan Africa alone (Sautter *et al.* 2006). It plays a major role in efforts to alleviate the African food crisis as it can tolerate extreme stress and produce food energy all-year round. Cassava is essentially a starch-producing and starch-storing plant, with 20-40% of its fresh root weight being starch. Cassava is used for many products locally in Nigeria which do not add much value to it (Hahn 1989; FAO 1994).

Starch is not used in its native form. Modified starches (acetates) are used for industrial purposes where they have been tailor-made to meet the requirement of end users with much added value. They find uses in fast food, sweets and sausages and play a prominent role in our everyday life. Cassava starch may be used in the manufacture of sweeteners, thickeners and stabilizers in the food system (International Starch Institute 2005). Sulphur dioxide (SO_2) and its derivatives have long been used in foods as preservatives. They are added to food to inhibit non-enzymic and enzymic browning, inhibit and control microorganisms and act as an antioxidant (Nogueira *et al.* 2007; Ukpabi 2010). SO_2 and its derivatives are metabolizable to the sulphate and excreted in the urine without obvious pathological results (Fazio and Warner 1990; Lindsay 1996). Additions of SO_2 to starch during production have been reported to prevent fermentation oxidation and formation of coloured agents (Kordylas 1988). The effects of sulphiting on the rheological and functional properties of native starch have been reported (Paterson *et al.* 1994), but none on acetylated cassava starch. Golachowski (2003) reported a reduced number of

linkages to acetyl groups in potato starch by increasing SO_2 . Information on the effects of acetylation and sulphiting on the physico-chemical properties of cassava starch is important in order to obtain shelf-stable acetylated starch with the desired quality. Clone TMS 30572 is a new high-yielding variety being distributed to farmers by the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

While extensive work has been done on the chemical modification of starch (Adebowale and Lawal 2003), to the best of our knowledge there is no report on the effect of sulphiting on acetylated cassava starch. The present work investigates the effects of using graded levels of SO_2 and acetylation on the physical and functional properties of cassava starch.

MATERIALS AND METHODS

Raw materials

Cassava tubers were obtained from the Federal College of Agriculture (FCA) farm in Akure, Ondo State Nigeria. The clone TMS 30572 was harvested 18 months after planting. The mixed cultivar was obtained from Matna Food Company Limited, Akure, Ondo State, Nigeria; a factory that processes fresh cassava tubers into starch using rasping process.

Starch extraction

Starches were extracted from cassava tubers (TMS 30572) according to the procedure described by Kordylas (1990). Cassava tubers were peeled, washed and grated in a mechanical driven cassava grater. The grated pulp was mixed with sufficient amount of water to form slurry and sieved using a muslin cloth. The starch was allowed to settle and the supernatant decanted. Repeated washing was done three times. The starch obtained was spread in thin layer on a tray and left to dry in an air oven (Labcon air oven)

Table 1 Quantity of Na₂SO₃ used for sulphiting at graded level.

Na ₂ SO ₃ (mg)	0	72.84	147.66	295.32	590.64	1181.28	2460.94	4921.88	9843.75
SO ₂ required (mg)	0	37	75	150	300	600	1250	2500	5000

at 55 ± 2°C for 48 h. The dried starch was pulverized using marlex electroline food mixers model 4250 England and sieved using 254 µm sieve.

Sulphiting of cassava starches

Sulphiting of the cassava starches was carried out according to the procedure of Golachowski (2003) on potato starch with slight modification. 200 g dry basis of each starch sample was weighed into conical flask and 200 mL of distilled water was added. A graded amount of sodium sulphite solution that gives 0-5000 mg SO₂/Kg starch (Table 1) was added to the starch preparations.

Acetylation of sulphited cassava starches

Each of the sulphited starch milk from above (TMS 30572 and the mixed cultivar starches) was acetylated by adding 41 mL of acetic acid anhydride using 3% NaOH as catalyst following the procedure of Golachowski (2003). The acetic acid anhydride was added at a constant rate of 1 mL per min while maintaining a pH range of 8-9 using pH meter (Jenway pH meter model 3015). After all the acetic acid anhydride was added, the pH was finally adjusted to 5.2-5.6 with 10% HCl. The modified starch slurries were centrifuged at 1000 rpm for 10 min. The residue obtained were washed three times with distilled water and dried in the air oven at 30°C for 24 h and weighed to determine the yield. The dried, sulphited and acetylated starches were ground using the marlex electroline food mixers and sieved to pass through 254 µm sieve and packaged in polyethylene bag for further analysis.

Determination of the degree of acetylation of starches

The degree of acetylation of the sulphited starches was determined using the modified procedure of Golachowski (2003). 10 g starch db was mixed with 65 mL distilled water in a conical flask and neutralized by adding few drops of 0.1M NaOH to obtain a faint pink colour with phenolphthalein indicator. 25 mL of 0.5 M NaOH was added to the mixture and mixed thoroughly for 35 min using magnetic stirrer at 1000 rpm. The resultant mixture was titrated against 0.5 M HCl until the pink colour disappeared.

$$\text{Acetylation \%} = \frac{(25 - X) \times 0.043 \times 0.5 \times 100}{\alpha}$$

where X = amount of 0.5M HCl used for titration of a sample; α = weight of starch on dry basis (db).

Water and oil absorption capacity (WAC, OAC)

The water absorption capacity of each starch sample was determined using the method of Sathe *et al.* (1982). A suspension of 1g of starch db in 10 mL of distilled water or [10 mL of oil (executive chef oil with density of 0.92 g/mL)]. The suspensions were stirred for 5 min using magnetic stirrer (Stuart scientific Co Ltd model 7664) at 1000 rpm. The mixture was then centrifuged (MSE minor 35 England) for 30 min at 3500 rpm. The free water or oil obtained was removed carefully and the volume of the water or oil was determined. The water or oil absorbed by the starches was calculated as the difference between the initial water or oil used and the volume of the supernatant obtained after centrifuging. The result was expressed as a percentage g/g of water or oil absorbed by the starch.

Whiteness

The whiteness of the starches was determined using the Kett electric laboratory C-103-3 whiteness determination machine which is calibrated into percentage whiteness Kett scale.

Bulk density (BD)

The procedure of Narayana and Narasinga Rao (1984) was used with slight modification. A specified amount of the starches were put in a pre-weighed (W₁) 5 mL measuring cylinder, it was gently tapped and the volume was noted. The new mass of the sample and measuring cylinder was recorded as (W₂). The bulk density (g/mL) was computed as:

$$BD = \frac{W_2 - W_1}{\text{Volume of sample}}$$

Swelling power and starch solubility

The swelling power and solubility of the starches were determined by using the method of Leach *et al.* (1959). One gram of starch (dry weight basis) was weighed into centrifuge tube and 50 mL distilled water added. These tubes were immersed in water bath at temperature range from 50 to 90°C at 10°C intervals for 30 min and thoroughly and constantly stirred with glass rod during the heating period. The tubes were removed, cooled to room temperature and centrifuged at 5000 rpm for 15 min. The supernatant was carefully transferred into a conical flask and 5 mL out of it was pipetted into a pre-weighed glass Petri dish, evaporated over a steam bath and dried in the air oven at 110°C for 4 h. The weight of the pastes were determined and used to calculate the swelling power as gram of sediment paste per gram starch. The difference in weight of the Petri dish after drying the supernatant gave the weight of the soluble starch. Percentage solubility was calculated as gram of soluble starch per gram starch.

Sedimentation volume

Sedimentation volume was determined according to the procedure of Raja *et al.* (1987), 10 g (dry weight basis) of starch was weighed into a graduated 100 mL measuring cylinder followed by addition of 100 mL distilled water. The content was mixed thoroughly. The sediment volume was recorded after 3 h when the level became constant.

Viscosity measurement

The method of Amani *et al.* (2004) was used with slight modification. Starch suspension of 5% was heated to 90°C for 30 min in a temperature controlled water bath (Labon model WBN 007) with continuous stirring. The paste was transferred to a rotatory viscometer (viscotester VT – 04E Rion Co, Ltd Tokyo, Japan) using the rotor no 1. Paste viscosity was measured at 90 to 30°C cooling phase and expressed in centi Pascal second.

Paste clarity determination

The procedure of Craig *et al.* (1989) was used. 1% (dry weight basis) aqueous dispersion of starch was boiled at 100°C for 30 min under constant stirring. Percentage transmittance was measured after cooling to 30°C at 640 nm using Genesys 10UV scanning spectrophotometer (model Genesys 10-5 Thermo Electron Corp., Madison, USA).

Freeze-thaw stability determination

The freeze-thaw stability was investigated using the method of Singh and Kaur (2004). An aqueous suspension 5% w/v (dry weight basis) was prepared using distilled water. The suspension was heated to 95°C for 30 min in water bath and then cooled with continuous stirring to prevent skin formation. The paste was subjected to alternate freezing and thawing (18 h and 3 h, respectively) for 5 cycles. This was centrifuged at 5000 rpm for 10 min and the percentage of exudates was determined and plotted against the number of freeze-thaw cycle.

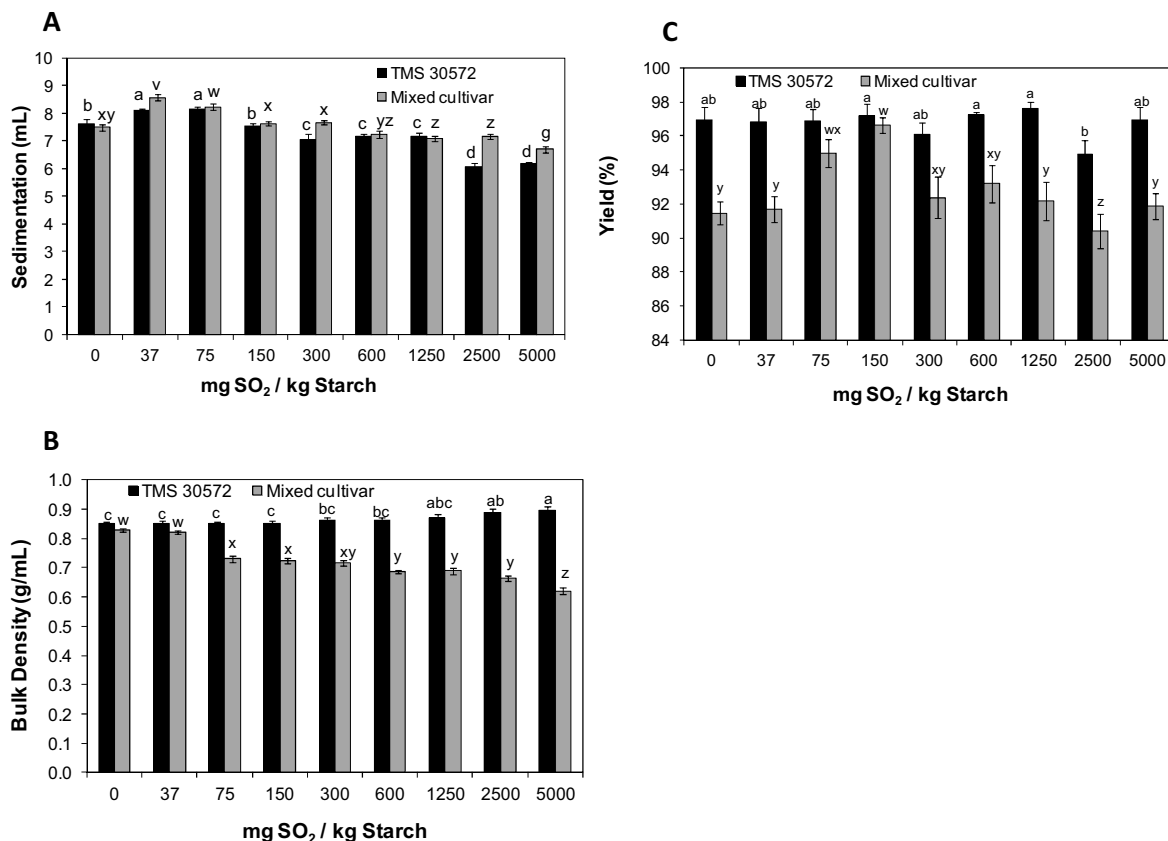


Fig. 1 Effect of sulphiting on (A) Sedimentation, (B) Bulk density and (C) yield of cassava starches. All the data represents mean \pm SE ($n = 3$). Mean value of samples with different letters are significantly different ($P < 0.05$) according to DMRT.

Statistics

Determinations were carried out in triplicate; errors were calculated as standard deviation from the mean. One way ANOVA was used to find statistical difference between the means of the values reported. The means were separated by using Duncan's multiple range test (DMRT). SPSS (version 17.0) was used for the analyses.

RESULTS AND DISCUSSION

Effect of sulphiting on bulk density, sedimentation and yield of acetylated cassava starches

Sulphiting had significant effect on the sedimentation of the starches. In both TMS 30572 and mixed cultivar starches, the sedimentation increased between 37 and 75 mg SO₂/kg starch substitution and thereafter began to decrease significantly as the level of sulphiting increases (Fig. 1A). Sulphiting increased the bulk density of the TMS30572 starch although it was only significant at higher level of sulphiting (Fig. 1B). On the other hand, the bulk density of mixed cultivar starch was decreased significantly as the level of sulphiting increased (Fig. 1B). The difference may be due to the bleaching effect of the sulphiting which removed impurities and made the starch lighter. For TMS30572 starch, it is in line with the result obtained for starches acetylated without sulphiting, which increased the bulk density (Gonzalez and Perez 2002).

Sulphiting did not have significant influence on the yield obtained for TMS30572 starch (Fig. 1C). The yield was 97% when the starch was not sulphited and 92-98% when sulphited. However, there was a slight improvement on the yield obtained for mixed cultivar starch which was highly significant; rising up to 96% when sulphited at 150 mg SO₂/kg starch compared with 90% for starches that were not sulphited. Beyond the 150 mg SO₂/kg starch substitution, the yield significantly decreased (Fig. 1C). The slight improvement in the yield can be linked to the bleaching effect of sulphiting on the impurities present in mixed cultivar starches thereby militating against their removal

during washing. Sulphiting agents have been reported to have bleaching power Kordylas (1990) and BeMiller and Whistler (1996).

Starch solubility

Sulphited cassava starches exhibited decreased solubility as the SO₂ concentration increased (Figs. 2A, 2B). In both TMS 30572 and mixed cultivar starches, sulphiting levels of 37-150 mg SO₂/kg starch have the highest solubility. The solubility is all time lower than those obtained for native cassava starch. As the temperature increases solubility also increased albeit with inverse correlation to SO₂ concentration. This is consistent with the observations of Leach *et al.* (1959), Adebowale *et al.* (2005) and Lawal (2004) that solubility is a function of temperature. Narayana and Narasinga Rao (1984) stated that the solubility of hydrophobic chemicals decrease in salt solution such as NaCl.

Effect of sulphiting on the appearance of cassava starches

Sulphiting improved the appearance of the starches in both TMS 30572 and the mixed cultivar. There was a sharp increase in the whiteness of the mixed cultivar starch when sulphited with 37 mg SO₂ compared with the unsulphited sample (Fig. 3). The whiteness ranged from 93.6-96.1% and 89.1-93.8% for TMS 30572 and the mixed cultivar, respectively (Fig. 3). There was no significant difference in the whiteness of mixed cultivar starch at 37 mg SO₂ substitution level; however, when the level was increased to 75 mg SO₂ it resulted in a significant increase in the whiteness which continued to increase as the level of SO₂ increased. Sulphiting have been reported to improve the appearance of starch during processing by preventing chemical reactions that cause colouration (Golachowski 2003). All the starches meet the colour standard of 90% for cassava starches use in food products (ISI 2005).

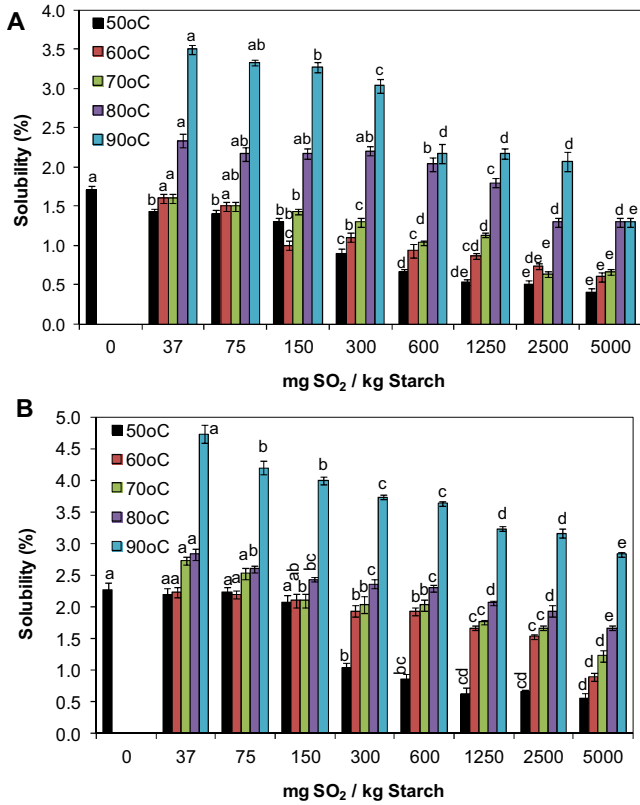


Fig. 2 Effect of sulphiting and temperature on the solubility of (A) TMS 30572 and (B) Mixed cultivar starches. Data represent mean \pm SE ($n = 3$). Mean value of samples with different letters are significantly different ($P < 0.05$) according to DMRT.

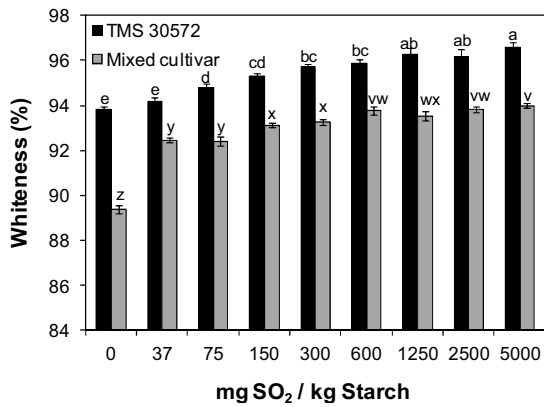


Fig. 3 Effect of sulphiting on the whiteness of acetylated cassava starches. Value are mean \pm SE ($n = 3$). Mean value of samples with different letters are significantly different ($P < 0.05$) according to DMRT.

Effect of sulphiting on the degrees of acetylation

Sulphiting of cassava starch led to a decrease in the degree of acetylation of the starch (Fig. 4). As the concentration of SO₂ increases the degree of acetylation obtain decreased showing an inverse correlation between the levels of sulphiting and degree of acetylation for both TMS 30572 and mixed cultivars starches. The result obtained is similar to those of Golachowski (2003) for SO₂-treated potato starch. The observed differences in the decrease pattern of the mixed cultivar and TMS 30572 may be due to the effect of industrial processing technique used on the mixed cultivar such as rasping which might have ruptured the starch granules, hence, making it more accessible to the effect of SO₂. The reduction in the degree of acetylation due to sulphiting in both starches might be as a result of higher side reactions; the acetyl groups combine more with SO₂ as the concentration increases thereby forming more sodium acetate

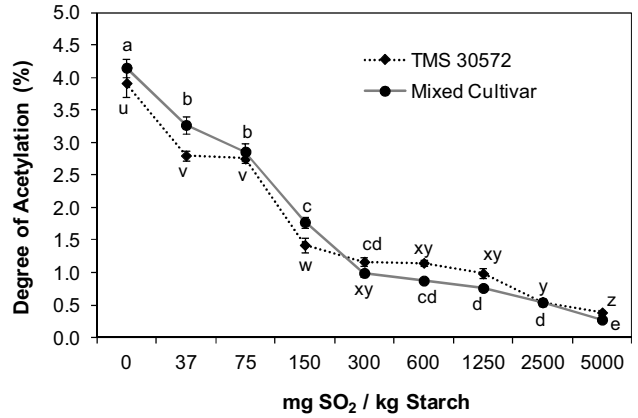


Fig. 4 Effect of sulphiting on acetylation of TMS 30572 and mixed cultivar starches. The degree of acetylation was measured in percentages. Values are mean \pm SE ($n = 3$). Mean value of samples with different letters are significantly different ($P < 0.05$) according to DMRT.

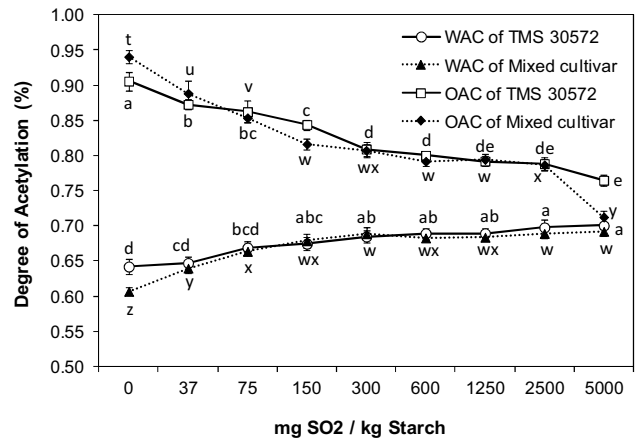


Fig. 5 Effect of sulphiting on water and oil absorption capacities of acetylated TMS 30572 starch and mixed cultivar starch. The degree of acetylation was measured in percentages. Values are mean \pm SE ($n = 3$). Mean value of samples with different letters are significantly different ($P < 0.05$) according to DMRT. WAC: Water Absorption Capacity; OAC: Oil Absorption Capacity.

instead of the starch-acetate. Jarowenko (1986) reported that starch-esters are readily cleaved with alkali salts.

Water absorption capacity (WAC) and Oil absorption capacity (OAC)

The WAC of both TMS 30572 and mixed cultivar starches increased with increasing SO₂ concentration (Fig. 5). This might imply that sodium-acetate formed in the side reactions were more hydrophilic than the starch-acetate which has been confirmed to be hydrophobic, Wurzburg (1987). Also, since more of the acetyl group added combines with the salt, there is reduction in the amount of the hydrophobic acetyl group that combine with the starch molecules. The un-sulphited starches have the highest OAC in both TMS 30572 and mixed cultivar starch, and the OAC decreased with increasing SO₂ concentration (Fig. 5). This might be due to limited substituted acetyl group in the resultant starch due to sulphiting because it is the acetyl group that cause increase in OAC of acetylated starch.

Swelling power of sulphited and acetylated starches

An increase in temperature resulted in an increase in the swelling power of the starches, however, the swelling power of the sulphited cassava starches decreased as the concentration of SO₂ increased (Figs. 6A, 6B). Similar results have been reported for cocoyam and African yambean

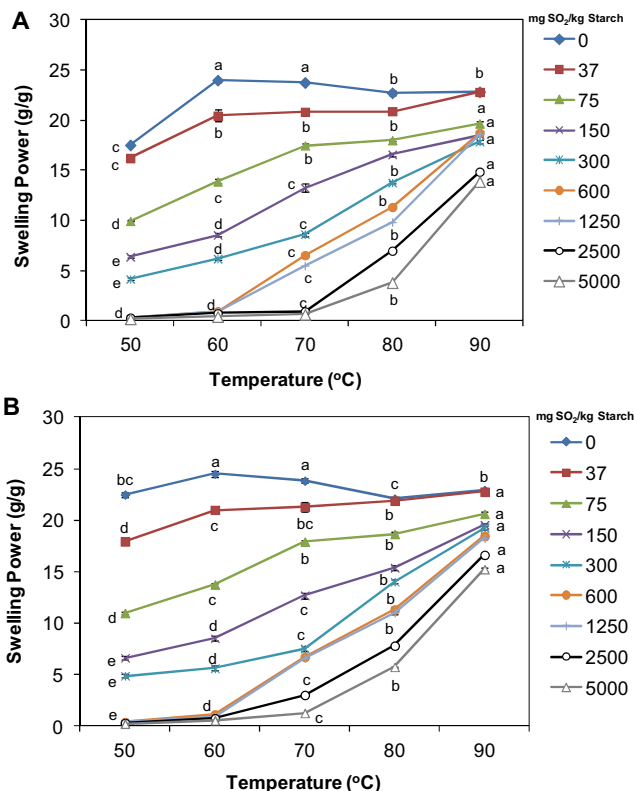


Fig. 6 Effect of sulphiting on the swelling power of acetylated (A) TMS 30572 and (B) mixed cultivar starches. Values are mean \pm SE ($n = 3$). Mean value of samples with different letters are significantly different ($P < 0.05$) according to DMRT.

starches (Lawal 2004; Akintayo and Akintayo 2009). The decrease in the swelling power after the addition of more than 75 mg SO_2/kg starch is lower than those obtained for the native cassava starch at temperature 60°C. This might be due to small substituent acetyl group which contribute to the swelling power of cassava starch Wurzburg (1987). Lower gelatinization temperature is also experienced in all sulphited/acetylated samples compare with native starch. Similar result was obtained for potato starch and African yambean (Golachowski 2003; Akintayo and Akintayo 2009). It has been suggested that to prevent swelling of starch under strongly alkaline reaction, sodium chloride or sodium sulphate may be added at concentrations of 10-30%. Thus, when swelling is required to enhance the ability of the starch to act as a thickener in food formulations, sulphited samples cannot be used.

Starch viscosity

Sulphiting decreased the viscosity of acetylated cassava starch (Figs. 7A, 7B). Both the mixed cultivar and TMS 30572 starches followed the same trend. The viscosity is lower than those obtained for the native cassava starch even at lower concentration of 37 mg SO_2/kg starch except at temperature of 40-60°C where the viscosity is higher in non-sulphited TMS 30572 starch. It was observed that no gel was formed at concentrations higher than 75 mg SO_2/kg and no gel was formed at lower temperature when cooled to 30°C. Rogols (1986) obtained similar result when sodium metabisulphite was added to wheat flour dough. Sulphur dioxide effect a reversible cleavage of protein disulphide bonds which reduced the elasticity of the dough and the viscous moduli than dough without metabisulphite. Rogols (1986) asserted that salts can slow pasting especially in alkali systems.

Also, sulphited cassava starches showed resistant to retrogradation in acetylated cassava starches. The hot paste viscosity is lower than those obtained for acetylated non-sulphited cassava starches. At 37 mg SO_2/kg starch, the hot

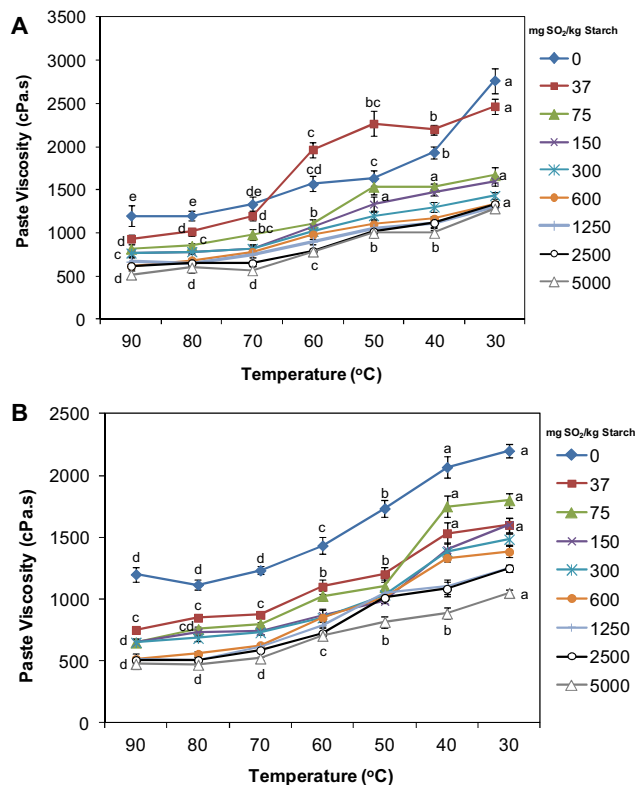


Fig. 7 Effect of sulphiting on paste viscosity of acetylated (A) TMS 30572 and (B) mixed cultivar starches. Values are mean \pm SE ($n = 3$). Mean value of samples with different letters are significantly different ($P < 0.05$) according to DMRT.

paste viscosity was 750 cPa while non-sulphited, but acetylated starch within the same temperature has a hot paste viscosity > 1000 cPa (Figs. 7A, 7B). This was due to gel formation in the acetylated cassava starch while no gel was formed in the sulphited cassava starch. On cooling, the final viscosities at 30°C of sulphited starches were lower than those obtained for native and acetylated starch, hence, sulphiting retard retrogradation tendency. This might be due to small acetyl group in the resultant starch. Sulphiting may retard retrogradation but may not be used in food formulation that require high viscosity and gel formation.

Paste clarity

Paste clarity of sulphited cassava starches increased as 37 mg SO_2/kg starch of sulphiting was added, but later decreased as the SO_2 concentration was further increased (Fig. 8). The rate of decrease was similar in both mixed cultivars and TMS 30572 starches however, the paste clarity was higher in TMS 30572 than the mixed cultivar. This might be due to the mechanical effect of rasping which caused rupturing in the starch granules (Sajeev *et al.* 2003). The initial higher paste clarity at concentration level of 37 mg SO_2/kg starch might be due to the bleaching effect of SO_2 on the cassava starch (Kordylas 1990). Paste clarity, however, is a function of various factors such as swelling power, granules size, dispersion and amount of granules remnants in the pastes Craig *et al.* (1989).

The observation and result obtained for swelling power and solubility (Fig. 6) showed that there is a decrease in swelling power as sulphiting concentration increased. Hence, reduction in paste clarity at concentration higher than 37 mg of SO_2/kg starch might be due to decrease in swelling power as observed at these concentrations (Figs. 6A, 6B). Due to limited swelling experienced, the amount of granules ghost is high and the salt will affect refractive index more thereby lowering the paste clarity. From the above observation, we highly recommend that food formulation where paste clarity is important should be sulphited

Table 2 Effect of sulphiting on freeze-thaw stability of TMS 30572 cassava starch.

mg SO ₂ /kg starch	Freeze-thaw cycle (days)/exudate (%)					
	0	1	2	3	4	5
0	-	NF/NE	NF/NE	NF/NE	F/NE	F/6.67
37	-	NF/NE	NF/NE	NF/NE	F/NE	F/9.33
75	-	NF/NE	NF/NE	NF/NE	F/NE	F/20
150	-	NF/NE	NF/NE	NF/NE	F/NE	F/36.66
300	-	NF/NE	NF/NE	NF/NE	F/NE	F/53.33
600	-	NF/NE	NF/NE	NF/NE	F/NE	F/53.33
1250	-	NF/NE	NF/NE	NF/NE	F/NE	F/60
2500	-	NF/NE	NF/NE	NF/NE	NF/NE	F/65
5000	-	NF/NE	NF/NE	NF/NE	NF/NE	F/78

NF/NE: not frozen and no exudates; F/NE: frozen but no exudates

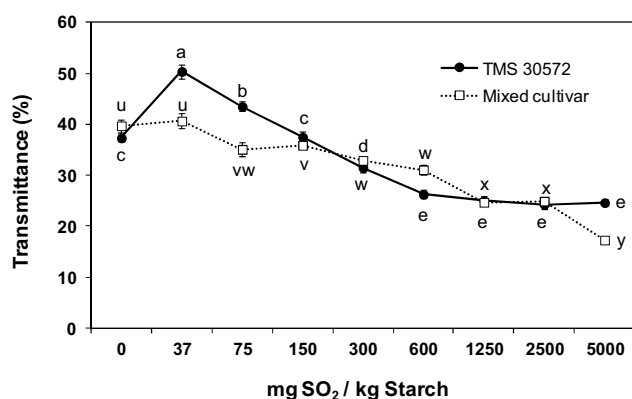


Fig. 8 Effect of sulphiting on paste clarity, measured as percentage transmittance, of acetylated cassava starches. Values are mean \pm SE ($n = 3$). Mean value of samples with different letters are significantly different ($P < 0.05$) according to DMRT.

below 75 mg SO₂/kg starch.

Freeze thaw stability

The sulphited starches did not freeze until the fourth freeze-thaw cycle (**Table 2**). The starches remained in the sol form, and gave no exudates after each freeze-thaw cycle until the fifth cycle. After the fifth freeze-thaw cycle, the percentage exudates released increased as the concentration of SO₂ increased (**Table 2**). The non-frozen of the sulphited starches at the early cycles might be due to decrease in the freezing point of starch paste due to sulphiting. This shows that cassava starch intended for frozen food formulations may not be sulphited.

CONCLUSIONS

Sulphiting was found to improve the appearance and WAC and it also reduces the tendency of acetylated starch to retrograde. Notwithstanding, sulphiting inhibited the effectiveness of some functional and desirable physical properties of acetylated cassava starches. When desired for use in food formulation, the concentration should not be above 75 mg SO₂/kg starch.

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