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Investigation into Native Mango Starch Carboxymethylation

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Abstract

The optimum reaction conditions for the preparation of carboxymethylated starch from native mango (Mangifera indica) starch were investigated. This was in a bid to utilizing the abundant mango waste seeds in the environment as alternative unconventional resource for both domestic and industrial applications. Organic slurry method was employed in the carboxymethylation process. While the effects of NaOH and SMCA concentrations, reaction time and temperature on Reaction Efficiency (RE) and Degree of Substitution (DS) of the products were studied. The DS obtained for the 18 carboxymethylated starches are CMS-1 (0.184), CMS-2 (0.156), CMS-3 (0.139), CMS-4 (0.258), CMS-5 (0.201), CMS-6 (0.197), CMS-7 (0.299), CMS-8 (0.258), CMS-9 (0.308), CMS-10 (0.164), CMS-11 (0.099), CMS-12 (0.143), CMS-13 (0.214), CMS-14 (0.058), CMS-15 (0.081), CMS-16 (0.258), CMS-17 (0.039), and CMS-18 (0.172). As expected, the results showed that the DS and RE were affected by all parameters investigated with CMS-9 and CMS-17 having the highest and lowest DS and RE respectively. It was concluded that the optimum reaction conditions for carboxymethylation of native mango starch are concentration of nNaOH/nAGU and nSMCA/nAGU (2M), and reaction time (2 h).

Keywords*: Mango; Investigation; Carboxymethylation; Industrial; Applications*

1.0 INTRODUCTION

Functionalization of native starches has greatly extended their applications far beyond their original use as sources of biological energy from plant materials (Gunorubon *et al*, 2012). Virtually every industry in existence today, uses starch or its derivatives in one form or another. In foods and pharmaceuticals, starch is used to influence or control characteristics such as texture, moisture, consistency and shelf stability (Kozich *et al*, 2012).

Carboxymethyl starch (CMS) is a popular chemically modified/functionalized starch, which is prepared by the reaction of starch (St-OH) and sodium monochloroacetate (SMCA) ($ClCH_2COO{-}Na^+$) in the presence of sodium hydroxide (NaOH). Carboxymeththyl substitution of starch hydroxyl group gives rise to derivatives that are cold water-soluble and are suitable for applications in the pharmaceutical industry as a disintegrant and as sizing and printing agent in the textile industry (Spychaj e*t al*, 2013). This starch derivative was first produced in 1924 by the reaction of starch in an alcoholic solution with sodium monochloroacetate (Chowdhury, 1924). From that time various production methods of CMS have been carried out to optimize reaction conditions and improve applied properties of the product for various applications. The most important methods include aqueous method, dry method, extrusion technique and organic solvent slurry.

Carboxymethyl starch, under the name sodium starch glycolate as mentioned earlier, is used in the pharmaceutical industry as a disintegrant and as sizing and printing agent in the textile industry (Spychaj *et al*, 2013). Also, solubility of CMS in cold water, water absorption, adhesiveness and film forming characteristics increases as the degree of substitution (DS) increases (Zhou *et al*, 2007). Similarly, paste and film clarity as well as paste and gel storage stability are significantly improved (Tatongjai *et al*, 2010). Equally, carboxymethylated starch derivatives exhibit lower gelatinization temperature, specific changes in rheological properties and pH stability (Bhattacharyya *et al*, 1995; Lawal e*t al*, 2007).

The work of our group has partly focused on the development of functionalized starches from waste materials such as mango seeds for applications in various industries. We had previous reported the isolation and characterization of starch from *Mangifera indica* seeds (Uba *et al.*, 2011). Herein, we report some results on the carboxymethylation studies of extracted native mango starch.

2.0 MATERIALS AND METHODS

2.1 Sample collection and preparation

Ripe Mango was procured from local market (Kasuwar Daji) in Sokoto metropolis, Sokoto State, Nigeria and was identified at the Botany department of Biological Sciences, Usmanu Danfodiyo University, Sokoto. The mango was eating with the help of local people and the waste mango seeds were collected, washed thoroughly with distilled water, dried, decorticated to remove skin and seed kernel was grounded to powder before extraction.

2.2 Extraction of starch

Starch extraction was carried out using hot distilled water method as described by Uba *et al.* (2011).

2.3 Preparation of sodium carboxymethyl starch

Organic slurry method of modification was employed as described by Lawal e*t al.* (2007). The native mango starch (10.0 g) was suspended in 2-propanol (200 ml). $20cm^3$ of various concentrations (1.0M, 1.5M or 2.0M) of aqueous sodium hydroxide solution was added. The mixture was stirred at controlled temperature (30°C) for 10 min. $80cm^3$ of various

concentrations (1.0M, 1.5M or 2.0M) of Sodium monochloroacetate was added and stirring was continued up to the designated time. The pH of the mixture was adjusted to about 5.0 by addition of 50% glacial acetic acid and the carboxymethyl starch was filtered, washed with 80% aqueous ethanol until the pH of the liquid was neutral (7.0) and dried at 50°C for 6 h. The dried carboxymethyl starch was passed through a 100-mesh sieve. This procedure was repeated 18 times with variation in the concentration of SMCA, NaOH, and reaction time and the products of the reactions were labelled CMS-1 to CMS-18.

2.4 IR Spectroscopy

Infrared (IR) spectra of the native mango starch and CMS were recorded using KBr disks on a Shimadzu-8400SFTIR Fourier transform infrared (FT-IR) spectrophotometer. Substitution was confirmed by the presence of carbonyl groups in the IR spectrum.

2.5 Determination of the Degree Substitution of sodium carboxymethyl mango starch

The degree of substitution (DS) was determined with flame atomic absorption spectrometry based on the sodium content of the CMS as describe by Lawal e*t al.* (2009). Each sample (50 mg) was dissolved in concentrated nitric acid (4 cm^3) in a glass vessel and heated with a hot plate. The digested sample was made up to 100 cm^3 with distilled deionized water before analysis with the spectrometer (flame photometer, Corning 400). The flame composition was air–acetylene while the wavelength of sodium was 589.0 nm. The degree of substitution was determined as follows:

$$DS = \frac{162\%Na}{(2300 - 80\%Na)} \quad \text{.....(1)}$$

%Na of the unmodified starch was predetermined by flame atomic absorption spectrometry and it was corrected in the CMS derivative.

R.E = DS/DSt x100 ----------(2)

DS of 3 is the maximum any starch carboxymethylation can reach, therefore Reaction Efficiency (R.E) is a percentage comparison between the Degree of Substitution (DS) obtainable from the reaction, and the theoretical degree of substitution (DSt = 3) this show the extent to which the carboxymethyl group substitutes hydroxyl group on the starch molecule.

3.0 RESULTS AND DISCUSSION

FT-IR Studies

The spectra in Figs. 1 and 2 showed the characteristic bands for native and carboxymethyl mango starch, in the region of 970 and 1200 cm^{-1}. These bands were preserved after carboxymethylation and the appearance of new bands at 1646, 1422 and 1360 $cm^{-1}$for carboxylate group (-COO-) were observed in the CMS samples. This result is in agreement with absorption bands reported by Jiang *et al*. (2011) and this confirms that the carboxymethylation of the native starch was successful. From the spectra, protonated carboxylic groups (–COOH) also produced a C—O band at 1735 cm^{-1} (Wang *et al.*, 2009). The broad band between 3600 and 3000 cm^{-1} can be attributed to O–H stretching (due to hydrogen bonding involving hydroxyl groups on the starch molecules) and that at 2929 cm^{-1} to CH_2 symmetrical stretching vibrations. As observed, the intensities of both bands were expected to decrease by carboxymethylation (Lawal, *et al.*, 2008; Li *et al.*, 2010 , Jiang *et al.*, 2011).

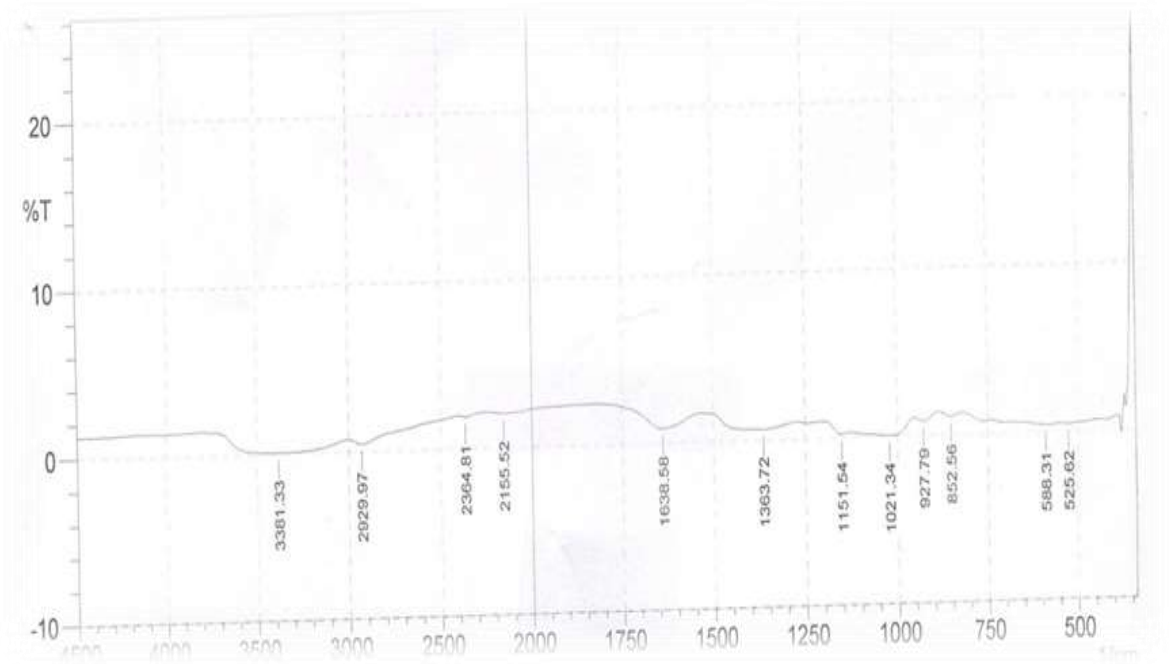


Fig. 1: FT-IR spectrum for native mango starch (M0)

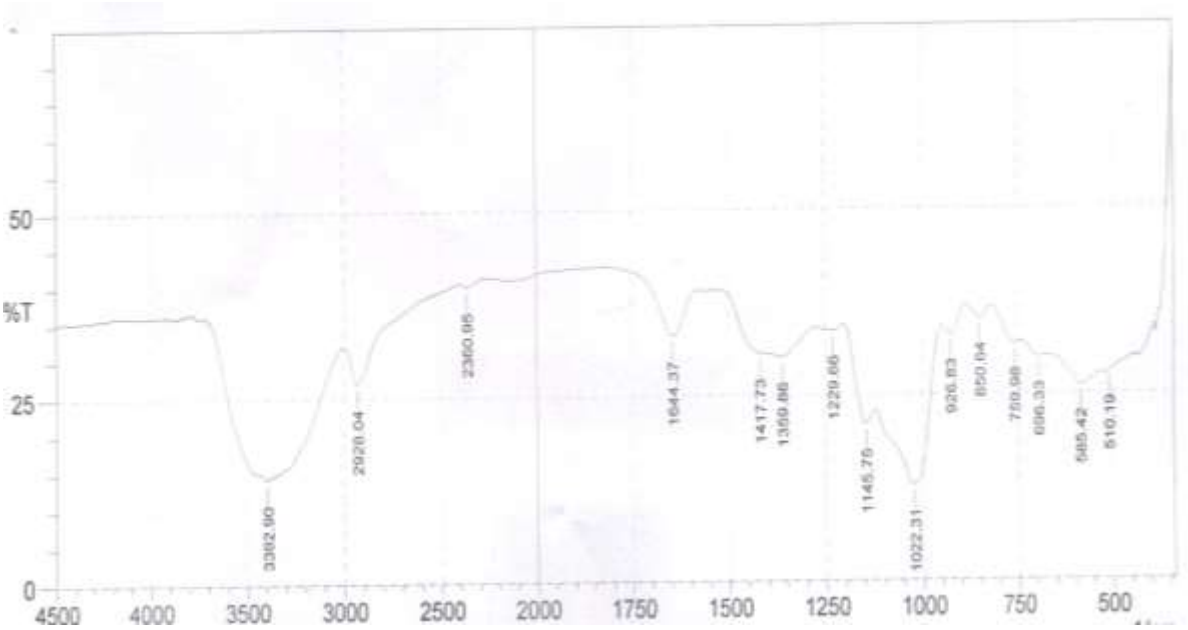


Fig. 2: FT-IR spectrum for carboxymethyl mango starch (CMS^{-1})

3.2 Carboxylmethylation studies

The total degree of substitution (DS), which is an indication of the average number of functional groups introduced into the polymer, and the functionalization pattern greatly influence the properties of polysaccharide derivatives like carboxymethylated starch products. Generally, the results of the carboxymethylation studies showed that the DS and RE of the various starch derivatives were affected by variation in the amount of nNaOH/nAGU, nSMCA/nAGU, and the reaction time (Table 1).

Table 1: Degree of substitution (DS) and reaction efficiency (RE) of carboxymethylation of Native Mango Starch at varied concentrations of NaOH and SMCA and reaction time.

Sample	nNaOH:nAGU (mol/mol)	nSMCA:nAGU (mol/mol)	Time (hour)	D.S	R.E (%)
CMS-1	1.00	1.00	2.00	0.184	6.130
CMS-2	1.00	1.50	2.00	0.156	5.200
CMS-3	1.00	2.00	2.00	0.139	4.642
CMS-4	1.50	1.00	2.00	0.258	8.600
CMS-5	1.50	1.50	2.00	0.201	6.700
CMS-6	1.50	2.00	2.00	0.172	5.730
CMS-7	2.00	1.00	2.00	0.299	9.970
CMS-8	2.00	1.50	2.00	0.258	8.600
CMS-9	2.00	2.00	2.00	0.308	10.281
CMS-10	1.00	1.00	4.00	0.164	5.470
CMS-11	1.00	1.50	4.00	0.099	3.300
CMS-12	1.00	2.00	4.00	0.143	4.770
CMS-13	1.50	1.00	4.00	0.214	7.130
CMS-14	1.50	1.50	4.00	0.058	1.930
CMS-15	1.50	2.00	4.00	0.081	2.700
CMS-16	2.00	1.00	4.00	0.258	8.600
CMS-17	2.00	1.50	4.00	0.039	1.300
CMS-18	2.00	2.00	4.00	0.172	5.730

$p<0.001$, F= lowest and confidence interval (C.I) = 95%

CMS-9 showed the highest DS, which varied significantly ($p<0.001$) from all other modified derivatives (Table 1). While CMS-17 showed the lowest DS, which also varied significantly ($p<0.001$) from others (Table 1). This may affect the swelling capacity, hydration capacity and water solubility index of the modified derivatives.

A close examination of the effect of sodium monochloroacetate (SMCA) concentration on the carboxylmethylation shows that the DS decreases with increase in SMCA concentration from 1M to 2M at constant temperature, time (2) and nNaOH/nAGU (1M NaOH) (Fig. 3a). It was also observed that when the same synthesis was carried out with change in time to 4 hours, lower values of DS were obtained (Fig. 3a).This observation is not consistent with earlier results on carboxylmethylation of cocoyam starch at various nSMCA/nAGU concentrations (Lawal, *et al*, 2007).

An increased concentration of NaOH to 1.5M gave higher values of DS compared with 1M NaOH but a significant decrease in DS as nSMCA/nAGU increases (Fig. 3b). The DS was also observed to lower significantly with increase in time to 4 and decreases with increase in nSMCA/nAGU.

However, when the synthesis was carried out for 2 h with 2M NaOH, higher values of DS were obtained (Fig. 3c). An increase in nSMCA/nAGU showed in insignificant increase in DS, but gave higher values compared to when 1M and 1.5M nNaOH/nAGU were used. A significant decrease in DS was also observed as nSMCA/nAGU increases from 1M to 1.5M but significantly increased when nSMCA/nAGU was increased to 2M for reaction time of 4 hours.

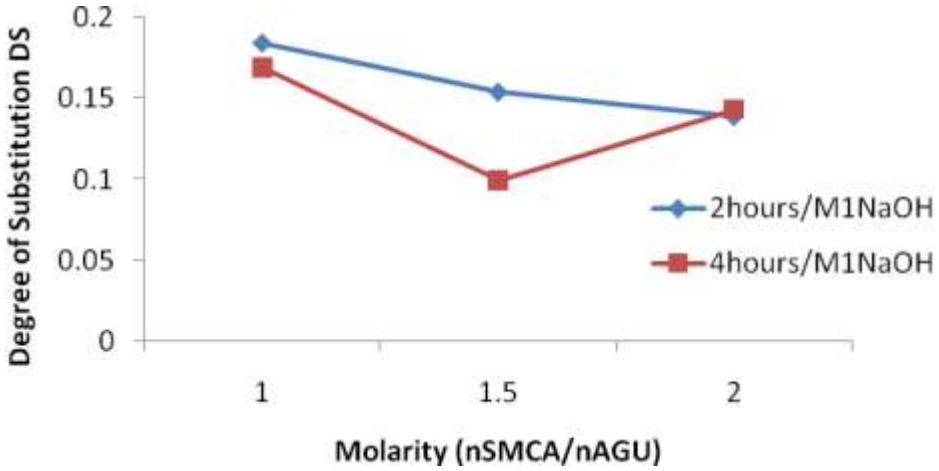


Fig. 3a: Plot of DS against Molarity of SMCA for 2 and 4 h with 1M NaOH.

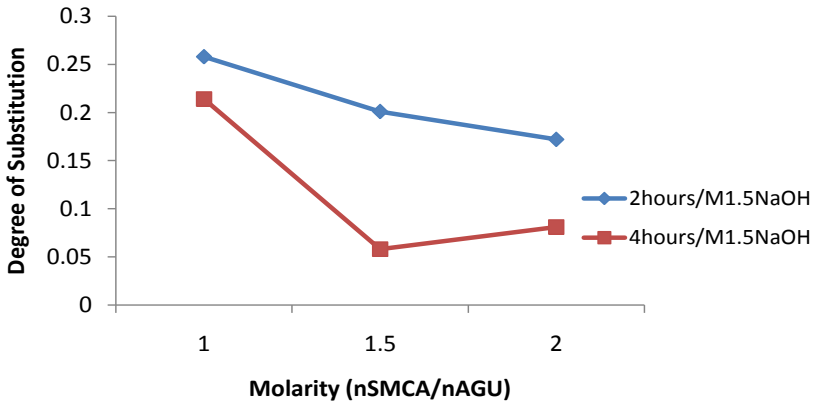


Fig. 3b: Plot of DS against Molarity of SMCA for 2 and 4 h with 1.5M NaOH.

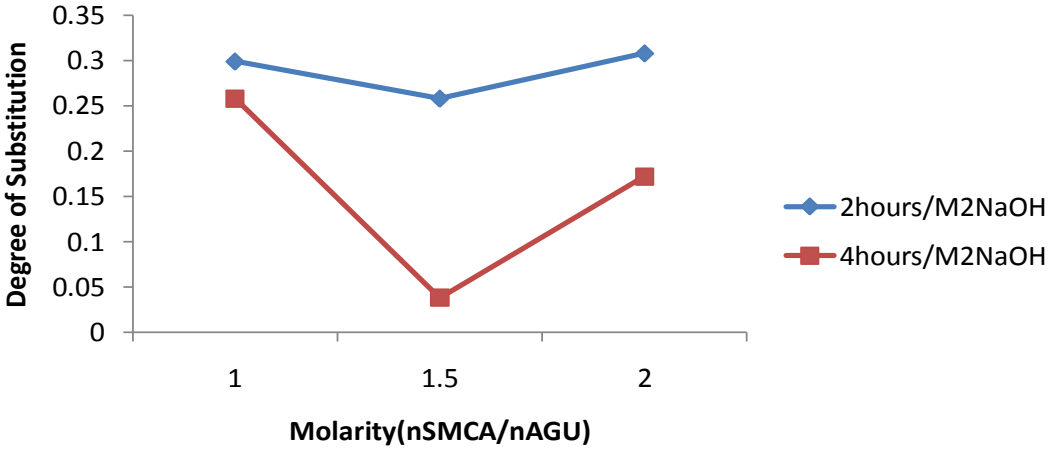


Fig. 3c: Plot of DS against Molarity of SMCA for 2 and 4 h with 2M NaOH.

Investigations into the influence of increasing NaOH concentration on the reaction mixture showed that the initial increase in the amount of NaOH favourably increased DS (Fig. 4a). It was observed that at constant concentration of SMCA (1 M nSMCA/nAGU) and reaction time of 2 h, DS show a significant increase as the concentration of nNaOH/nAGU is increased. The same increase was observed when the reaction time was changed to 4 h even though the DS values were lower than those for 2 h.

When the concentration of SMCA was increased to 1.5M, a significant increase in DS was observed as the concentration of nNaOH/nAGU is increased for 2 h. However, a significant decline in DS was observed for reaction duration of 4 h, which is lower when compared to reaction time of 2 h (Fig. 4b).

Though the variation pattern of DS with concentration of NaOH for 2 h and 4 h reaction times were similar at SMCA concentration of 2M, DS varied greatly for 2 h than 4 h reaction time. Generally, increase in nNaOH/nAGU resulted in a significant rise in DS for 2 h reaction time whereas a lower DS was obtained with increase in nNaOH/nAGU concentration for 4 h reaction time (Fig. 4c).

It is worth noting that during the carboxymethylation process, the NaOH provides the alkaline environment for the reaction as well as serving as the swelling agent to facilitate diffusion and penetration of the etherifying agent to the starch granular structure. The increase in DS observed as the concentration of NaOH increases in the reaction mixture can be explained based on the two competing reactions during the carboxymethylation process. Further increase in NaOH concentration caused an inactivation of sodium monochloroacetate and hence it was consumed in the side reaction.This observation is in line with previous reports on carboxymethyl corn and amaranth starch by Bhattacharyya *et al*. (1995) and carboxylmethylation of cocoyam starch by Lawal *et al* (2007).

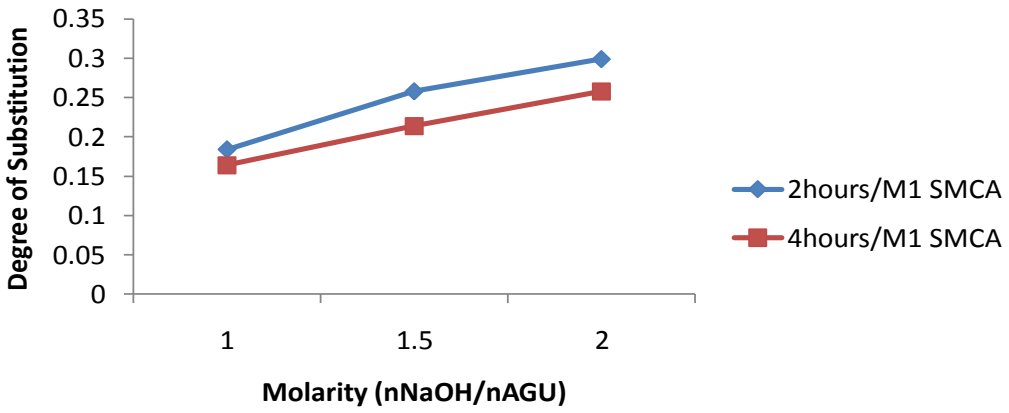


Fig. 4a: Plot of DS against Molarity of NaOH for 2 hour and 4 hours with 1M nSMCA/nAGU.

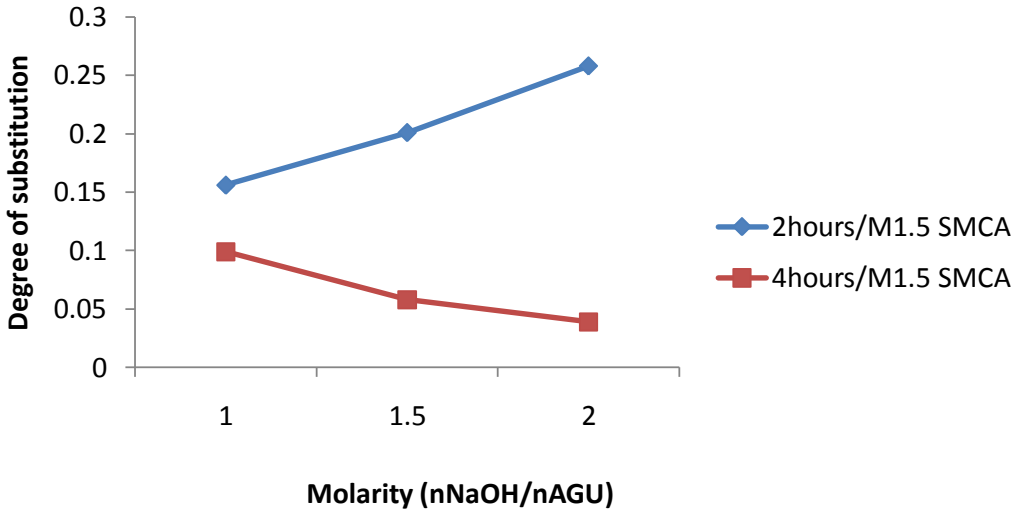


Fig. 4b: Plot of DS against molarity of nNaOH/nAGU for 2 and 4 hour with 1.5M nSMCA/nAGU.

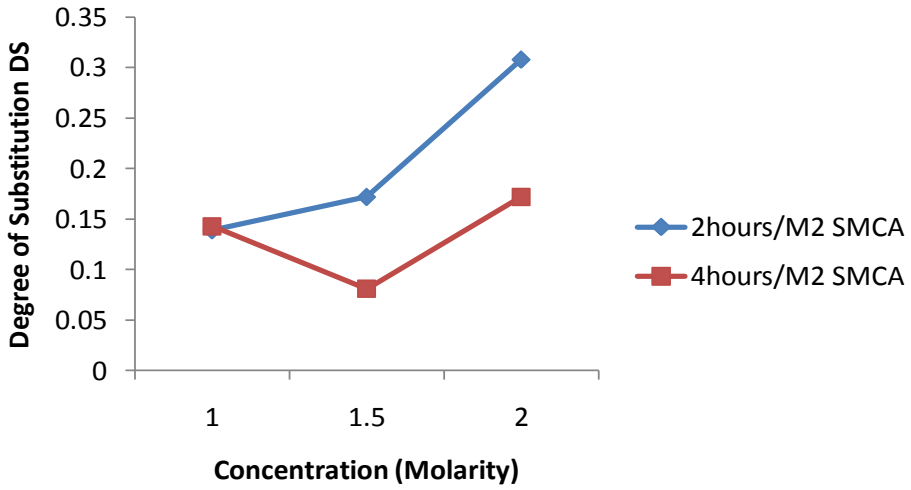


Fig. 4c: Plot of DS against Molarity of NaOH for 2 and 4 h with 2M nSMCA/nAGU.

The results on effect of reaction time on the carboxymethylation showed a surprising decrease in DS and RE with time beyond 2 h (Fig. 5). Though high DS and RE were observed at 2 h, these decrease as the reaction time increased to 4 h and this is attributed to the enhanced period of contact between the etherifying reagent and the starch molecules. This decrease in DS and RE as time increase could be due to shift in equilibrium when the

reaction medium reaches saturation, which favours the backward undesired side reaction of SMCA with NaOH, that inhibits carboxymethyl starch formation.

It is also reasonable that longer reaction time enhanced starch swelling and ultimately improved homogeneity of the reactants, and this had been observed in carboxymethylation studies of cocoyam starch by Lawal *et al.* (2007), where he concluded that no remarkable further increases were observed in both DS and RE after 3 h of reaction, which is in agreement with our results.

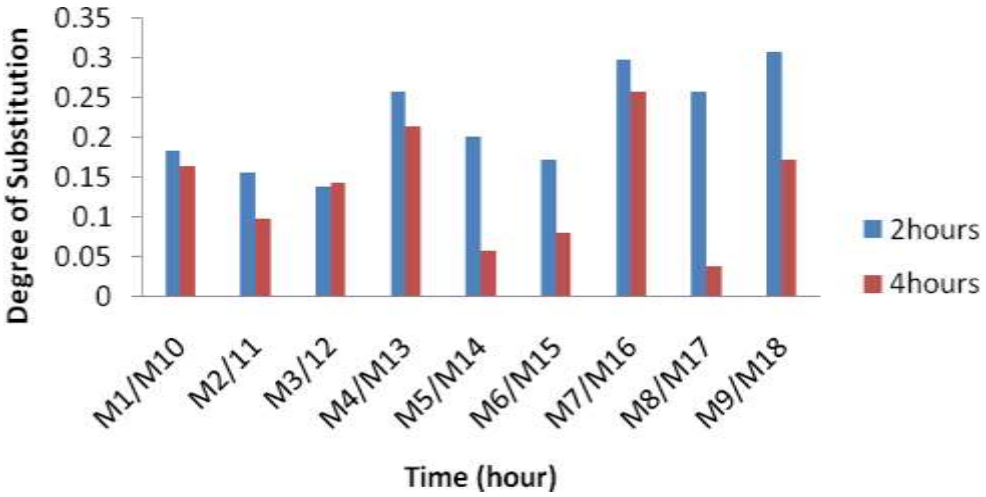


Figure 5: Plot of DS against time.

4.0 CONCLUSION

The study has successfully shown that native mango starch can be converted into functionalized starches like carboxymethyl starch and has demonstrated that nNaOH/nAGU and nSCMA/nAGU concentration, temperature and reaction time are important in the carboxymethylation process. It has established that the optimum reaction conditions for preparation of carboxymethyl mango starch are concentration of nNaOH/nAGU and nSMCA/nAGU (2M), temperature (30 ^{0}C) and reaction time (2 h).

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