# CHARACTERIZATION OF CARBOXYMETHYL *Plectranthus esculentus* STARCH.A POTENTIAL BIOMATERIAL FOR PHARMACEUTICAL APPLICATION

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## ABSTRACT

P. *esculentus* starch was chemically modified via carboxymethylation. The product was characterized using 1D- Nuclear Magnetic Resonance (NMR) spectroscopy. Physicochemical characteristics of the native and carboxymethyl starch such as Viscosity, solubility, Swelling power and viscosity-average molecular weight were also determined. The result showed that the starch from *P. esculentus* starch is composed of α −glucose linked together at 1→4.The bulky carboxymethyl groups introduced into the native starch was detected by $^{13}$C-NMR around 178.5ppm. The carboxymethyl starch with degree of substitution 0.40±0.02 had higher physicochemical properties than the native starch. The result showed that chemical modification by carboxymethylation may improve the physicochemical properties of polysaccharides resulting in higher efficacy for effective utilization in starch-based industries.

**Keywords:** Carboxymethylation, *Plectranthus. esculentus*, starch, NMR.

## 1 INTRODUCTION

Modified starches have played a major role in the food and pharmaceutical industries over the past few decades. They posses unique properties not found in natural starches, which are suitable for the development of new products [2]. The chemical modification of polysaccharide is the most important route to modify the properties of the naturally occurring biopolymers and to use this renewable resources in the context of sustainable development [5-6].Starch is a natural, cheap, available, renewable and biodegradable polymer produced by many plants as a source of stored energy [1]. It has found wide use in the food, textiles, cosmetics, plastics, adhesives, paper and most importantly pharmaceutical industries [4]. In the year 2000, the world starch market was estimated to be 48.5 million tons including native and modified starches. The value of the output is worth €15 *billion* per year [3]. Worldwide, the main sources of starch are Maize (82%), wheat (8%), potatoes (5%) and cassava (5%). [3].

Carboxymethyl starch is a popular chemically modified starch. It is prepared by the reaction of starch (st-OH) and monochloroacetic acid in the presence of sodium hydroxide [7-9]. This is a two-step reaction, according to the Williamson ether synthesis yielding the carboxymethyl polysaccharide derivative [9].

$$St - OH + NaOH \rightleftarrows St - O^{-}Na^{+} + H_2O - - - -(1)$$

$$St - O^{-}Na^{+} + ClCH_2COO^{-}Na^{+}\ St - O - CH_2COO^{-}Na^{+} + NaCl - - - (2)$$

An undesired reaction of sodium monochloroacetic acid with NaOH can also occur

$$NaOH + ClCH_2COO^{-}Na^{+} \rightarrow H - O - CH_2COO^{-}Na^{+} + NaCl - (3)$$

The amount of substituted carboxymethyl group is indicated by the degree of substitution (DS). The DS is defined as the average number of substituents per glucose unit. Each glucose unit contains three hydroxyl

groups ($C_2$, $C_3$ and $C_6$); therefore, the DS lies between zero and three. From the studies of Volkert *et al.*,[9] and Heinze*et al.*,[10], substitution in the order $C_2>C_6>C_3$ was indicated. The plant *Plectranthus* belong to the mint family *Lamiaceae* to which many aromatic plant belong. It is a perennial herb, which is sparsely branched and grow up to 2m in height [11]. Despite this fact, this species is a kind of potatoes [12]. The tubers are cultivated for food in Africa, particularly in the Northern parts of Nigeria around Adamawa, Bauchi, Niger, Kaduna and most importantly Jos, Plateau State [13]. The tubers are rich in Vitamin A and Iron. The leaves are used in the treatment of various ailments such as respiratory and digestive problems [14]. Their utilization as excipient in drug formulation have been reported[36]. Nutrient content of *P. esculentus* starch and tuber had also been reported [36]. Therefore, this study was designed to modify the starch from *P. esculentus* by carboxymethylation, evaluate the effect of carboxymethylation on some physicochemical properties of the native starch and characterize the product by NMR spectroscopy to confirm the modification. The result of this research may likely highlights the effect of carboxymethylation on the physicochemical properties of *P. esculentus* starch for possible application as excipient in drug formulation.

## 2 MATERIALS AND METHODS

### 2.1 Extraction of *P. esculentus* Starch

*P. esculentus* tubers were obtained from Angwa-rukuba market, washed, peeled, and trimmed to remove defective parts. The tubers were then sliced, diced, and blended with distilled water in a food blender. The mixture was sieved through an 80-mesh screen, and the retained solid was exhaustively rinsed on the sieve with distilled water. The filtrate was allowed to stand overnight at $15^0$C, the precipitate was collected, and the supernatant was discarded. Resuspension and sedimentation operations were repeated until white starch was obtained. The starch was dried at $50^0$C for 6hrs. finally, the dried potato flir was ground and passed through a 100-mesh sieve. starch was kept in a tight light-resistant container.

### *2.2 Preparation of Carboxymethyl P. esculentus Starch*

In the standard preparation, the native potato starch (10.0g) was suspended in 2-propanol (100mL). An aqueous sodium hydroxide solution was added (3% W/v (10ml)). The mixture was stirred at controlled temperature ($30^0$C) for 10min. 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. The carboxymethyl starch was filtered and washed with aqueous ethanol. The modified starch was dried at $50^0$C for 6hrs, the dried carboxymethyl starch was passed through a 100-mesh sieve.

### *2.3 Determination of the Degree of Substitution (DS)*

The DS of carboxymethyl starch (CMS) was determined in accordance with the method reported by Stojanovic*et al.*, [15]. The carboxymethyl groups in the CMS were first converted to an acid form with hydrochloric acid (HCl). The acidified starch was then recovered by precipitation with methanol, Filtration, washing with methanol and drying. Then, 0.2 M NaOH (10ml) was added to a suspension of accurately 1g weighed CMS in 10 mL of purified water. The mixture was transferred to a 100-mL volumetric flask and adjusted to the mark with purified water. The solution (25 mL) was transferred to an Erlenmeyer flask and titrated with 0.04M HCl using phenolphthalein as the indicator. The titration was

repeated three times, and the average value of HCl volume was used for the calculations. A blank was also titrated. The DS was calculated using followed equations:

$$DS = \frac{162 \times nCOOH}{mds - 58 \times nCOOH} - - - - - - - - - (4)$$

$$mds = \frac{(1 - W_{water})}{100} \times ms - - - - - - (5)$$

$$nCOOH = (V - V_n) \times C_{HCl} \times 4 - - - (6)$$

Where 162 is the molar mass of anhydrous glucose unit (in g/mol); *nCOOH* (in mol) is the amount of COOH; mds (in g) is the mass of dry sample; ms (in g) is sample mass; $W_{water}$ (%) is water content; $V_0$ (in mL) is the volume of HCl used for the titration of the blank; $V_n$(in mL) is the volume of HCl used for the titration of the sample; $C_{HCl}$(in mol/L) is the HCl concentration; and 4 is the ratio of the total solution volume (100mL) and the volume taken for titration (25 mL).

## 2.4 DETERMINATION OF PHYSICOCHEMICAL PROPERTIES OF STARCH

### *2.4.1 Viscosity*

The viscosity of a 2% *w/v* starch suspension was determined using a viscometer (Brookfield, RVDV-II$^+$PRO, USA) with a spindle no. RV-02 and a speed of 200 rpm of 200 at 25$^0$C. the readings of viscosity were taken after 30s of rotation. All measurements were performed in triplicate.

### *2.4.2 Swelling Power*

The swelling power (by weight) of starch was measured using a method modified from the one reported by Tester and Marison [16]. Potatoe starch (0.2g) was dispersed in water (20 mL). The suspension was heated to 85$^0$C in a water bath for 30min with vigorous shaking every 5min. the starch gel was then centrifuged at 2,200 rpm for 15 min. the weight of sediment was used to calculate the swelling power, and expressed in percent. The determination was done in triplicate. The swelling power was calculated as follow:

$$Swelling\ power = \frac{Weight\ of\ sediment}{(Weight\ of\ dry\ starch - Weight\ of\ dissolve\ starch)} - - - - (7)$$

### *2.4.3 Solubility*

The solubility of starch was determined. Starch sample 10g was suspended in 40ml of distilled water. It was heated to the desired temperature 60$^0$C, 70$^0$C or 80$^0$C for 30 minutes with continous shaking. The mixtures were centrifuged at 100rpm for 15 minutes. An aliquot of supernatants (5ml) were evaporated at 130$^0$C and weighed. The solubility's of the starch were the percentage ratio in mass(g) of the dried supernatant to the initial mass (g) of the dry starch.

### *2.4.4 Weight-average Molecular Weight (Mw)*

Native and modified starch samples with a range of molecular distributions were analysed by dilution solution viscosity. Based on the $M_w$ (weight average molecular weight) and intrinsic viscosity $[\eta]$ values of a standard starch solutions, a plot of log $[\eta]$ against log $M_w$ yielded a straight line. From the plot, K and a values were determined (slope and intercept). The molecular weight of the starch was determined by

Mark-Houwink equation, $[\eta]=KM_w^a$, where $M_w$ is the viscosity-average molecular weight and the parameters K and a are related to local stiffness of the polymer and depend on the nature of the polymer, solvent and temperature. The molecular weight ($M_w$) of the polysaccharide was measured using viscometer and the molecular weight obtained by this technique called viscosity-average molecular weight.

### *2.4.6 Nuclear Magnetic Resonance (NMR)*

NMR of *P. esculentus* starch and carboxymethylated starch were recorded in an NMR (600 MHz) spectrometer (Agilent technologies, America).

## 3. Results and Discussion

**Fig 1***: P. esculentus tubers*

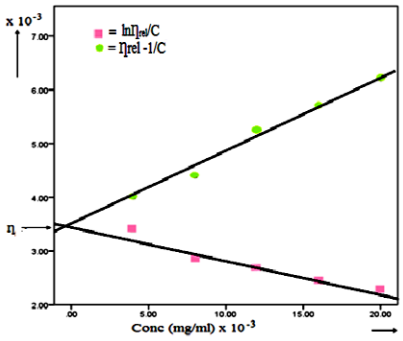


**Fig 2:** Intrinsic viscosity of native *P.esculentus* starch

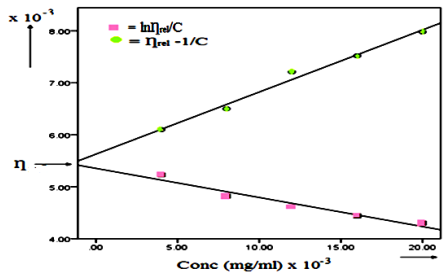


**Fig 3:** Intrinsic viscosity of *CarboxymethylP.esculentus*starch

**Table 1:** Physicochemical characteristics of the native and carboxymethyl *P. Esculentus* starch

| Type | | Solubility (%) | Swelling power (%) | Viscosity (M.Pa.S) | Molecular weight (g/mol) |
|---|---|---|---|---|---|
| Native starch | $60^0$C | 12.0±0.01 | 7.54±0.13 | 24.64±0.90 | $6.02 \times 10^5$ |
| | $70^0$C | 20.50±0.2 | | | |
| | $80^0$C | 45.40±0.1 | | | |
| Carboxymethyl starch | $60^0$C | 38.4±1.5 | 19.90±0.25 | 40.90±0.20 | $10.4 \times 10^5$ |
| | $70^0$C | 54.5±2.7 | | | |
| | $80^0$C | 89.7±1.6 | | | |

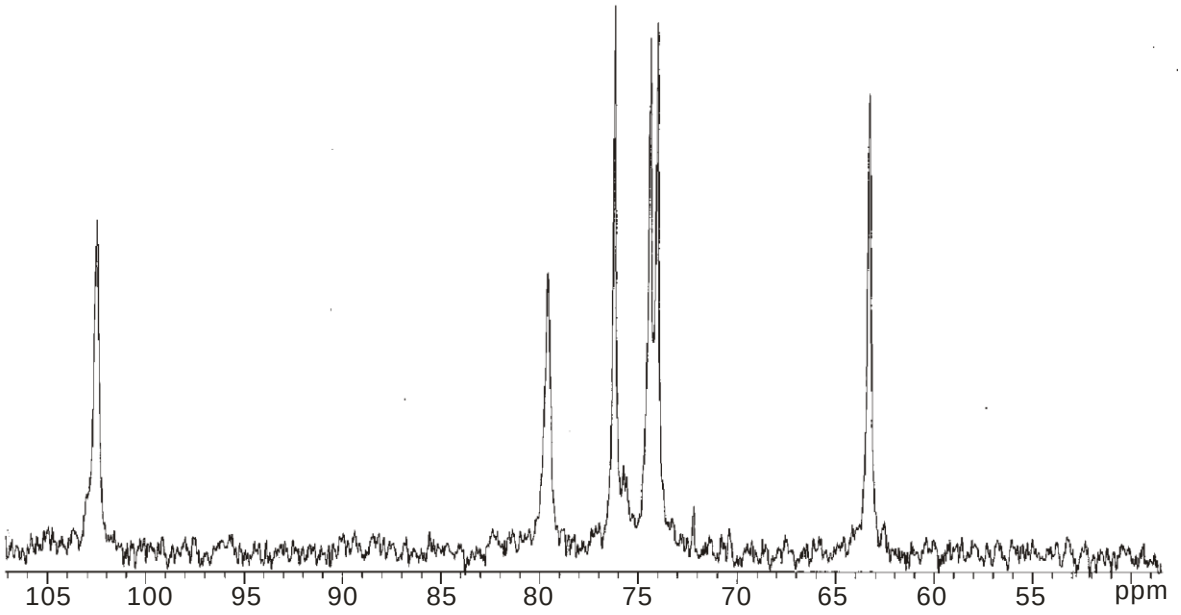


**Fig 4:** $^{13}$C. NMR Spectrum of *P.esculentus* starch

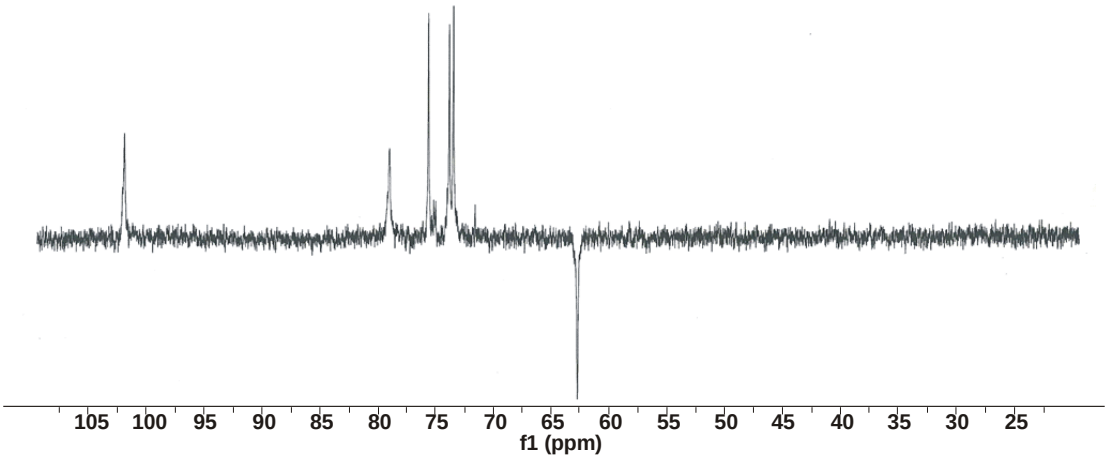


**Fig 5:** $^{13}$C.- DEPT NMR Spectrum of *P.esculentus* starch

## 4 DISCUSSION OF RESULTS

The viscosity, swelling power and solubility compared favourably with other commercial starches previously studied by other authors [17, 18]. Swelling is a primary mechanism in diffusion controlled release dosage form [19]. The viscosity, swelling power, solubility and molecular weight for carboxymethyl starch 40.90±0.20 MPa.S, 19.90±0.25%, 89.7±1.6% (at $80^0$C) and 10.4 X $10^5$ g/mol were higher than the native starch 24.64±0.90 MPa.S, 7.54±0.13%, 15.40±0.1% (at $80^0$C) and 6.02 X $10^5$ g/mol. (Table 1).

The result shows that the carboxymethyl starch when used as a binder/disintegrant could absorb sufficient moisture to swell and cause tablets to disintegrate and burst and free up sufficient energy to release drug content. [20]. Carboxymethylation generally increases the viscosity and water holding capacity [10]. During carboxymethylation process, the interaction in the granules are weakened by the introduction of carboxymethyl groups, this makes the starch to be more attracted towards water molecules [5-8].

Henry [23[ reported that introduction of carboxyl group reduces the bond strength between starch molecules (amylase/amylopectin) and thereby increases the swelling power and solubility of the starch granules. This facilitates access of water to amorphous areas, enhancing the viscosity [20]. The superior solubility and swelling power of the carboxymethyl starch compared with the native starch may be due to the presence of hydrophillic substituting groups ($CH_2C=O$) which allow the retention of water molecules because of their ability to form hydrogen bonds [20]. The increase in the viscosity of the carboxymethyl starch may also be due to the steric hinderance exhibited by the bulky carboxymethyl groups which obstruct the proper alignment of starch chain for maximum retrogation [20].

Adding bulky functional groups like carboxyethyl and carboxymethyl groups reduces the tendency of the starch to recrystallize and make the starch less prone to damage by heat and bacteria [20]. NaOH increases the reactivity of starch towards chemical reaction as compared to the untreated starch. Thus, it means that etherifying or esterifiying reagents are able to penetrate the swelled starch structure more easily and thus substitution of the hydroxyl group of the anhydrous glucose unit becomes easier [8-10].

Molecular weight and concentration are two most important characteristic of polymers synthesized through chemical or microbial processes [21]. However, current methods for characterizing polymer molecular weight such as Multi-Angle Laser Light Scattering (MALLS) or Gel-Angle Laser Light Scattering (GPC) require long processing and analysis, expensive equipment, high sample concentration and high sample volume [21]. Therefore in this study, viscometry method was used to determine the molecular weight of the polysaccharides. This is because of its low cost and is able to evaluate the differences in polymer viscosity for varying molecular weight and solvent conditions.

The experimentally determined relative viscosity ($\eta_{rel}$) were used to plot $\eta_{rel}$-1/c vs concentration and to determine the intrinsic viscosity by extrapolating to concentration C=0, if $\eta = \eta_o$, it can be shown that ($\eta/\eta_0$)-1. [$\eta$] may be determined from the limiting behavior of (1/C) and 1$\eta$ ($\eta/\eta_0$) as C→0. Plotting both functions enables the intrinsic viscosity to be obtained with greater precision. K and a which are constants were derived by charting out a series of [$\eta$] values Vs the known molecular weight in a log-log plot. This relationship is derived from Mark-Houwink equation [$\eta$]=KM$^a$ and log[$\eta$]=log(k) + a (log M).

The viscosity-average molecular weight of starch and its derivative is shown in Table 1 and figure 2 and 3. There is a significant increase in the molecular weight of the native starch after carboxymethylation.

The Mark-Houwink relationship [$\eta$]=KM$^a$ was evaluated as K=2.0X10$^4$ and a=0.75 for a standard starch in 0.1M aqueous Nacl at 25$^0$C, the low value of the exponent a is characteristic of a branched polymer [22]. The viscosity average-molecular weight of native and carboxymethylated starch (Table 1) were found to be 6.02 x 10$^5$g/mol and 10.40 x 10$^5$g/mol respectively. The increase in the molecular weight could be due to the presence of bulkier carboxymethyl groups in the native starch [22].

The degree of substitution (DS) was determined not based on the total monosaccaride unit but on free Unit. The degree of substitution was determined as 0.4010±0.02. modified polysaccharide with low DS (< 0.1) are used in the food industries since they confer consistency, texture and stability while polysaccharide with high DS (> 0.1) are used in the pharmaceuticals [24]. Different values of DS were obtained for other carboxymethyl starches in other studies [7, 25, 26].Although $^{13}$C-NMR has a much weaker signal, it has significant advantages over $^1$H-NMR spectra in the analysis of polysaccharides, because the chemical shift in $^{13}$C-NMR are spread out over a broader range (0-200ppm). This broad distribution of signals helps to overcome the severe overlapping problems associated with the $^1$H-NMR spectra. [37]. In the $^{13}$C spectrum, signals from anomeric carbons appear in the 90 to 105ppm region while the non-anomeric carbons are between 60 and 85ppm for polysaccharide with de-oxygen sugars, the **–CH$_3$** signals appear in a much higher field (15 to 20ppm). The anomeric C-1 carbons are the most diagnostic; thus from C-l alone one can often determine the different types of sequences present and their relative proportions. The resonance of C-2 to C-5 can be found around 65-78ppm. The primary OH (C-6 for pyranoside) resonate at 60-70ppm . (37).Of the two types of sugar residues conformation, signals derived from $\alpha-$anomeric carbons mostly appear in the region of 98 to 100ppm while most of the $\beta-$anomeric carbons will appear between 101 and 105ppm. (37,39).The signal of carbon atoms having primary hydroxyl groups, such as C-6 appear at a higher field of 60 to 64ppm, while the signals of carbon atoms with secondary hydroxyl groups, the non-anomeric carbons for C-5 shifts by 10ppm to a lower field. (38,40). The carbon anomeric region of $^{13}$C NMR of the hydrolyzed starch (fig 4) showed one major signal at the anomeric region which may be attributed to one neutral sugar components of the polysaccharide which was assigned as C-l of $\alpha-$D-glucose at 102.5ppm. The signals due to non-anomeric carbons C-2 to C-5 appear between 60 and 85ppm. The spectrum region of anomeric carbon

(102.5ppm) and the methylene carbons (62.50 and 63.50ppm) are well depicted (fig 5). The resonances of the carbon atoms were well resolved and identified as the resonances of C-2. C-3, C-4 and C-5 of residue A. *The $^{13}C$ NMR spectrum for modified starch derivative (Not shown) shows some differences in relation to unmodified starch.. A new signal at 176.5ppm was observed for the carbonyl carbon of the carboxymethyl groups.* (39). *Results of $^{13}C$-DEPT NMR $135^0$ sub-spectra of the unmodified gum is shown in (fig 5). The $^{13}C$-DEPT NMR experiment was used to identify the methylene groups signals of the carbon atoms bearing two protons which have opposite amplitude to the CH and $CH_3$ carbons. The $^{13}C$-DEPT NMR $135^0$ spectrum for the unmodified gum (fig 5) showed at a high field two inverted signals (62.45 and 63.65ppm) assigned to methylene carbons (C-6) of the sugar residue. Resonance were assigned with the aid of literature data.* (28,29,30).. The resonance of the carbon atoms were resolved (fig 4.5 and 6) and identified as the resonances of C-1 (102.5), C-2 (74.4), C-3 (75.8), C-4 (81.32), C-5 (74.2) and C-6 (62.5) of $\propto -glucose$. The $^{13}C$ NMR for the carboxymethylated starch showed signal at 176.5ppm assigned to the carbonyl carbon of carboxylmethyl groups (Not shown). The carboxymethylated starch $^{13}C$ NMR spectrum showed a decrease in signal intensities at both the C-6 and C-4 peaks indicating that the polysachharide underwent a prefefred degradation of the crystalline region during carboxymethylation [32, 34].

## 5 CONCLUSION

Carboxymethylated *P. esculentus* starch was synthesized and characterized. The new carboxymethyl group was detected by NMR analysis. The carboxymethylation occur preferentially at the primary carbons of glucose units as observed by NMR analysis. A high viscosity, swelling power, solubility and molecular weight were observed for aqueous solutions of carboxymethyl starch in comparism to the unmodified starch. The study confirms that carboxymethylation improves the properties of the native starch. It is more attractive because the starch from *P. esculentus* is a natural, abundant, non-toxic, low cost and regional raw materials. This material may be utilize as binder or disintegrant in solid dosage formulation.

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