Effect of Carboxymethylation on Porosity and Flow Property of Mango Starch

Agwamba C.E., Maxwell T.K., Nwasuka S.

Abstract-Native Mango starch (NMS) was carboxymethylated in an organic slurry at constant temperature ($30^{o}C$), and concentration of 2M NaOH, and SMCA but at varying time from 0.00 to 4 hrs. Seven (7) of carboxymethyl mango starch derivatives was obtained with varying degree of substitution. The result showed that DS is 3hours gave highest DS of 0.308 while CMS-1 was the lowest with DS 0.089. Carr's Index (CI) and Hausner's Ratio (HR) are indicators for flow property and Powder porosity of the derived carboxymethyl mango was further evaluated by measuring the tapped, bulk, and true density, and compared with NMS and commercial Carboxymethyl starch (CCMS). CMS-2 showed high powder porosity while. CMS-1 has the lowest value. Carr's index and Hausner's ratio value showed that CMS-5, CMS-4, and CMS-2 show good flowability. The result shows that modified Mango starch can be conveniently utilize as excipient in tableting.

Index Terms – Carboxymethylation, Carr's index, Degree of substitution (DS), Flow property, Hausner's ratio, Mango starch , Powder Porosity.

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1 INTRODUCTION

Starch is a polysaccharide of glucose, also called *Amylum* which is the reserved form of energy biosynthesized by all green plants, stored in various organs like fruit, seeds, rhizomes and tubers. It is produced in high enormous amount in some plants like Potatoes, Barley, Peas, rye, sorghum, maize, tapioca, yam (AGROSYNERGIE 2010).

Starch is a low cost polymer that is abundantly available with large number of glucose unit, a semi-crystaline biopolymer, composed of two types of glucose polymers; a straight chain amylose and a branched chain amylopectin. Amylose are formed when D-glucopyronosyl units linked by a-1, 4 bond. Amylopectin are made of short chain glucose unit link to one other via reducing end side by a-1,6 bond (Hai-pu, et al., 2011 and *Wang et al., 1949 and* Manchun *et al,* 2012). The ratios of amylose and amylopectin composition in isolated crude starch depend on the plant source (Manchun *et al.,* 2012); this means that native starches from different botanical sources have their own incomparable properties.

As stated in AGROSYNERGIE (2010), "Starch molecules produced by each plant poses a unique structures and compositions, both in the length of glucose chains or proportion of the amylase/amylopectin content, and the protein composition and aggregation of the storage organs may differ significantly. Therefore the applications of starch industrially depend on the botanical raw material from which it was isolated".

Unmodified Starch, is insoluble in aqueous medium and resistant to most chemical and enzymatic attacks (Manchun *et al.,* 2012), but products made from starch have some advantages like renewable, biodegradable, and environmentally friendly (Eyler *et al.,* 1947). Notwithstanding, native starch in its raw form poses vital properties that limits its application, such as lack of free-flow, compressibility, compactability, poor rheology after gelatinization and sensitivity of cooked starch , and insolubility in cold water(Spychaj, *et al.,* 2013, and Jubril *et al.,* 2012).

However, due to the presence of hydroxyl groups in repeated unit of the biopolymer chain – the functional properties that are draw-backs to native starch could be improved on by chemical, physical or enzyme modification (Bos, *et al.,* 1992 and Okafor *et al.*, 2000). Modification alters the properties of the starch. (Jubril *et al.,* 2012)

Therefore, starch modification is done to improve the shortcomings of native starches and increase the value of starch for domestic and industrial utilization (Manchun, *et al.,* 2012). A chemical modification result to significant alteration in chemical and the physical nature of granular starch and molecular structure, which in turn result to a change in starch properties.

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According to Heinze (2005) "the chemical modification of polysaccharides is the most vital avenue to alter the characteristics of native biopolymers and to use this renewable resource in the context of sustainable development". Chemical modification of starch properties gives an outcome to a change that is irreversible.

Carboxymethyl starch (CMS) is a green polymer with enormous relevance in food, pharmacy, medicine, agriculture, cosmetics, oil and gas industry, environmental protection and many more utilization (Spychaj, *et al.,* 2013).
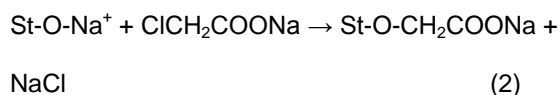
Novel route for the Chemical modification of starches have been studied in recent times, this includes carboxymethylation of native starch. The introduction of bulky hydrophilic groups to polysaccharide chains resulted in reduced starch tendency to retrogradation (recrystallization), and improves its cold water solubility giving a transparent, viscous, and colourless solution (Spychaj, *et al.,* 2013).

As shown in *Wang et al.,*(1949)*,* and Heinze (2005) when a combination of native starch and alkali is treated with chloroacetic acid, or sodium chloracetate, a water-soluble starch ether known as starch glycolate is produced. Using a more simplified formula [-O-Gl(OH)$_3$-O-] to show the anhydroglucose repeating unit found in starch, then [-O-G1(OH)$_2$(OCH$_2$-COOH)–O-] would represent a mono-substituted carboxymethyl ether of this repeating unit.
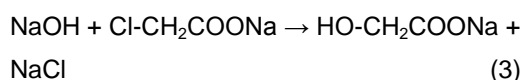
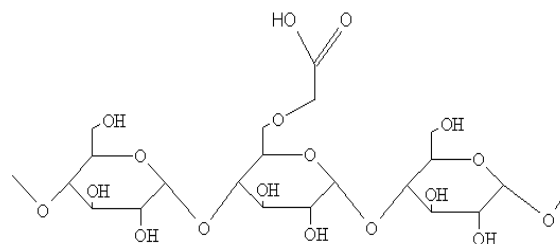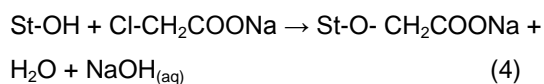This is a two-step reaction, first step being the alkalization of starch as shown by Finch, (1983):

$$St\text{-}OH + NaOH \rightarrow St\text{-}O\text{-}Na^+ + H_2O \qquad (1)$$
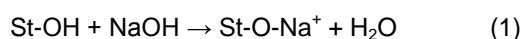
In the second step etherification occurs:

$$St\text{-}O\text{-}Na^+ + ClCH_2COONa \rightarrow St\text{-}O\text{-}CH_2COONa + NaCl \qquad (2)$$

An undesired side reaction of SMCA with NaOH can also occur.

$$NaOH + Cl\text{-}CH_2COONa \rightarrow HO\text{-}CH_2COONa + NaCl \qquad (3)$$

Summary of the reaction

$$St\text{-}OH + Cl\text{-}CH_2COONa \rightarrow St\text{-}O\text{-} CH_2COONa + H_2O + NaOH_{(aq)} \qquad (4)$$

Figure 1: Chemical Structure of Carboxymethylated Starch

Carboxymethylation reaction kinetics is substitution-nucleophilic in nature. The reaction rate is directly proportional to the reactivity of the hydroxyl groups.

Heinze, (2005) states that mega production of CMS is exclusively carried out by slurry processes, i.e., by the conversion of the alkali starch, respectively, swollen in aqueous NaOH and an organic liquid with monochloroacetic acid or its sodium salt.

The synthesis, the distribution of the carboxymethyl groups within the AGU, and properties are well recorded.

CMS are classified based on the total degree of substitution (DS), that is the mean value of the functional groups introduced into the polymer, which is a measure of the polysaccharide derivative properties including carboxymethylated products i.e. both starch and cellulose. To understand the structure-property relationship, the accurate DS and functional pattern must be ascertained as a vital requirement (Heinze, 2005).

According to Mumtaz, (2013), ''Bulk density is defined as the geometric space occupied within the confines of a solid material including any interior cracks, pores, voids of space. This is called geometric enveloped in the material being determined. True density equates bulk density only when there are no internal openings in the material being measured.''

The true density of a powder is seldom same with that of the bulk materials due to the process of pulverisation or milling will alter the crystal structure of ever particle present, which in turn changes the density of the particle in powder.

It can be concluded that true density can be referred to as particle density of a particulate solid or powder, that is to say the density of the particles that make up the powder, which differ from bulk density that depends on the mean density of a large volume of powder in a precise medium, usually air.

Porosity or void fraction is a measure of the void, or empty space in a material, and is a fraction of the volume of the void over the total volume, between 0-1 or as percentage between 0-100%.

2 Materials and Methods

2.1. Materials

Mango Fruit was obtained from Shehu Shagari Central Market Sokoto, Nigeria. The fleshy edible mesocarp was removed, kernel was opened with a hammer to reveal the seed, which was grounded and dried , and all chemicals used was provided by Faculty of Pharmaceutical Science, of Usmanu Danfodiyo University, Sokoto.

2.2. Methods

2.2.1. Isolation of Starch from Mango Starch

starch was isolated from mango seed using the hot water method described by Chavan *et al.*, (2010) and Kevate *et al.*,(2010) with few modification.

Soak about 100.00 g of powder mango seed with 200 cm³ of distilled water in 1000 cm³ beaker in a thermostatic water bath at a constant temperature of 40⁰C for about 24 hours. One part of the soaked powder mango seed kernel and three parts of distilled water would be blended for 3 min at medium and high speed. Pass the resultant slurry through a double layer of muslin cloth and then centrifuged at 5000 rpm for 20 min. The supernatant decanted and discarded while the sediment re-suspended in excess 0.02% $NaOH_{(aq)}$ to remove any residual proteins and phenolic compounds. After standing for 4 hours the decant supernatant and discarded. Repeated procedure 6-8 times until the supernatant became colourless. Suspend final sediment in distilled water and then subjected to filtration through 0.045 mm sieve, neutralized to pH 7.0 by washing with distilled water, filtered on Buchner funnel and thoroughly washed with distilled water. Dry filtered cake overnight at room temperature, ground to powder, weighed and stored in an air-tight glass bottle before further analysis.

2.2.2. Preparation of Carboxymethyl Mango Starch

Organic slurry method of modification was employed as described by Lawal *et al.,* (2007). The native mango starch (10.0 g) was suspended in 2-propanol (200 cm³). 20cm3 of 2.0M of aqueous sodium hydroxide solution was added. The mixture was stirred at controlled temperature (30°C) for 10 min. 80 cm³ of concentrations 2.0M of Sodium monochloroacetate was added and stirring was continued up to the designated time (1 1.3, 2, 2.3, 3, 3.3, or 4 hours). The pH (Jenway 3510 pH meter) 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 is neutral (7.0) and dried in an oven (Nuve Oven/FN-055) at 50°C for 6 hours. The dried carboxymethyl starch was passed through a 100-mesh sieve. This procedure was repeated (1 1.3, 2, 2.3, 3, 3.3, or 4 hours) for 7 times with same concentration of SMCA, NaOH, and carboxymethyl mango starch derivatives obtained was labelled CMS1 to CMS-7. While NMS is native mango starch and CCMS is commercial carboxymethyl starch as shown in the result table 1.0 below.

2.2.3. Determination of Degree of Substitution (DS)

Method described by Spychaj, *et al.,* (2013), was used to determine the DS of modified mango starch.

About 0.5 g of the CMS derivative sample was dissolved in 20 cm³ of 0.2 M NaOH and 50 cm³ of distilled water was added. The solution was transferred to a 100 cm³ volumetric flask, which was then filled up to the mark with distilled water. 25 cm³ of the solution was transferred to an Erlenmeyer flask and diluted by addition of 50–100 cm³ of distilled water. The excess of NaOH was back-titrated with standard 0.05 M HCl using phenolphthalein as the indicator. The titration was repeated three times and the average value of the HCl volume was used for the calculations. A blank was also titrated. The DS was calculated using Equation (6),

The amount of COOH being equal to:

$$n_{COOH} = (V_b - V) \times C_{HCl} \times 4 \qquad (5)$$

$$DS = \frac{162 \times n_{COOH}}{mds - (58 \, x \, n_{COOH})} \qquad (6)$$

Where 162 g/mol is the molar mass of an anhydroglucoseunit (AGU); nCOOH (in mol) is the amount of COOH calculated from the obtained value of the equivalent volume.
58 g/mol is the net increase in the mass of an AGU for each carboxymethyl group substituted, and $m$ds (in g) is the mass of dry sample calculated from known sample mass (in g) and the water content, $w_{water\%}$.

V_b (in cm^3) is the volume of HCl used for the titration of the blank, V (in cm^3) is volume of HCl used for titration of the sample, C_{HCl} (in mol/dm^3) is the HCl concentration, and 4 is the ratio of the total solution volume (100 cm^3) and the volume taken for titration (25 cm^3).

2.3. Characterisation *methods*
2.4. Powder flow properties

Powder flow characteristics were evaluated by various methods. These methods include measurement of bulk density, tapped density, Carr's index and Hausner's ratio .

a. Determination of True Density

The true density (D$_T$) of the starch was determined by the liquid displacement method using 5_+xylene as the immersion fluid as described by Ohwoaurhua *et al,* (2004) and computed according to the following equation.

$$D_T = \frac{w}{(a+w)-b} \times SG \qquad (7)$$

Where
w: weight of the powder,
SG: specific gravity of liquid,
a: weight of bottle + liquid and
b: weight of bottle + solvent + powder.

b. Determination of Bulk Density

Bulk densities (D$_b$)of the samples were measured by the weight and volume procedure. Exactly 10.00g quantity of the powdered sample was placed in 50 cm^3 clean, dry measuring cylinder and the volume V_o occupied by the sample without tapping determined.

$$D_b = \frac{w}{V_O} \qquad (8)$$

Where
w: weight of powdered starch
V_o: volume occupied by powered starch without tapping

c. Determination of Tap Density

Exactly 10.00g quantity of the powdered sample was placed in 50 cm^3 clean, dry measuring cylinder and the volume V_{500} occupied by the powdered sample after tapping with a spatula 500 times was determined.

$$D_t = \frac{w}{V_{500}} \qquad (9)$$

Where
w: weight of powdered starch

V_{500}: volume of by powdered starch after tapped 500 times

d. Carr's Compressibility Index and Hausner ratio

Carr's Compressibility index and Hausner ratio are indicators for flow properties and compressibility of the powders, and were derived from Bulk and tapped densities using equation (11) and (12) below.

$$CI = \left(\frac{D_t - D_b}{D_t}\right) \times 100 \qquad (10)$$

$$HR = \frac{D_t}{D_b} \qquad (11)$$

e. Determination of Powder Porosity

This was determined from the value of true and bulk densities when fitted into the equation according to the method of Ohwoauvohrua *et al.,* (2004).

$$E = (1 - \frac{D_b}{D_T}) \times 100 \qquad (12)$$

Where
E: Powder Porosity
D$_b$: Bulk density
D$_T$: True density

3.0 Statistical analyses

The data reported in the tables were average of triplicate observations. Data obtained were analyzed by one –way analyses of variance (ANOVA) using Minitab 17 for windows version 8.1. Confidence interval of sample means was reported at the 95% confidence probability. Comparisons of means were made at 5% significance level (*P<0.05*).

4.0. Result and Discussion

CMS derivatives obtained was a fine, white powder with good-flowing properties.
The highest Degree of substitution (DS) was 0.308 while lowest is 0.089. Physicochemical properties of carboxymethyl derivatives CMS-1 to CMS-7 in comparison to NMS and CCMS are presented in table 1.

Table 1: shows result of physical and flow properties, porosity of powder (PP)

S/n	Sample Code	DS	Time (Hr)	PP (%)	Bulk Density	Tapped Density	True Density	CI	HR
1	NMS	0	0	81.12	0.459	0.607	2.429	24.378	1.322
2	CMS-1	0.089	1	68.83	0.499	0.584	1.602	14.430	1.169
3	CMS-2	0.164	1.5	86.71	0.423	0.484	3.179	12.596	1.144
4	CMS-3	0.214	2	81.03	0.602	0.696	3.172	13.578	1.157
5	CMS-4	0.258	2.5	77.91	0.556	0.628	2.519	11.331	1.128
6	CMS-5	0.308	3	71.44	0.802	0.910	2.808	11.842	1.134
7	CMS-6	0.299	3.5	81.97	0.537	0.642	2.979	16.316	1.195
8	CMS-7	0.257	4	78.34	0.544	0.674	2.512	19.210	1.238
9	CCMS	N.A	N.A	77.79	0.529	0.585	2.383	9.444	1.104
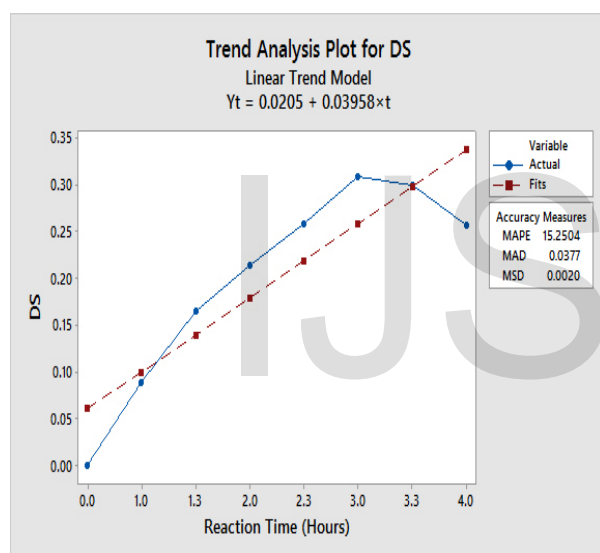
**Fig. 1: Effect of reaction time on DS**

Influence of reaction time

Fig. 1 illustrates the effect of reaction time on DS for a concentration of NaOH equal to 2M. The reaction is carried out at a temperature of $30^{o}C$. Optimal DS of 0.308 was obtained over a period of 3 hr for CMS5 and has no significant difference from CMS-6. Overall observation show that The DS increases with the reaction time increase.

This increase is as a result of a better contact time of the etherification reagents with native starch macromolecules.

It can also be implied that increase in reaction time improves the uniformity within reaction mixture, helps the starch granules to swell, and making it more accessible to the reagents (Khalil *et al.,* 1990), and this in line with observation by Lawal *et al.,* (2007), and Stojanovic *et al.*, (2000)

show that no remarkable further increases were observed in both DS and RE after 3 hours of reaction, which is in agreement with higher DS and RE obtained for 3 hr and decrease of DS obtained for 3.3 hr and 4 hr under same conditions. This decrease in DS as time increases could be due to supersaturation in the reaction medium, Which favours the side reaction, causing a reaction of between nNaOH/nAGU and nSMCA/nAGU, this side reaction in turn does not favour Carboxymethyl starch formation.

Influence of DS on Powder Porosity
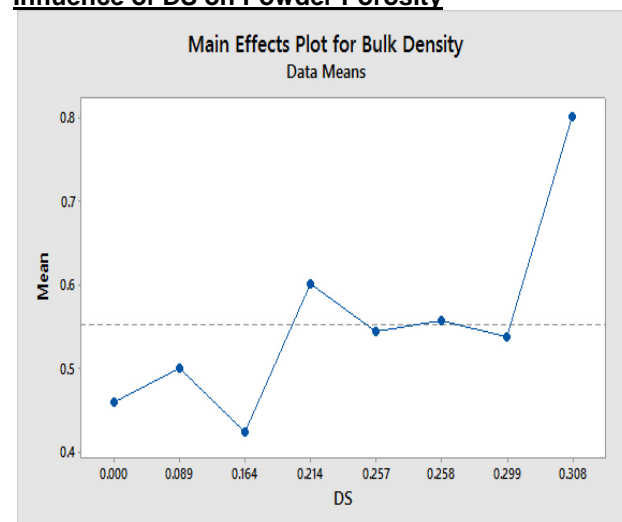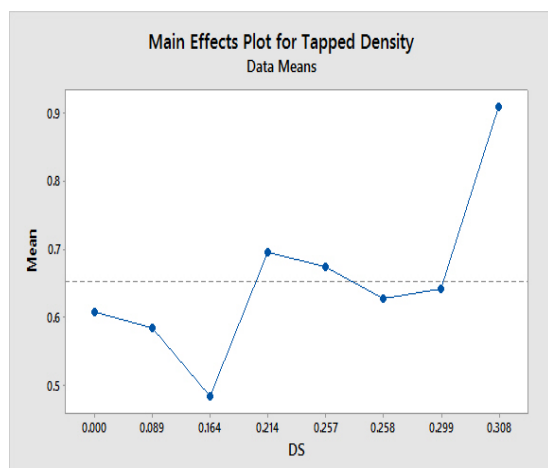

Fig. 2: Effect of DS on Bulk Density
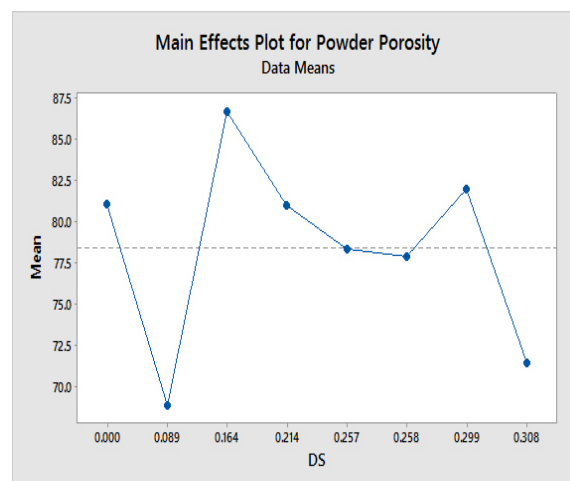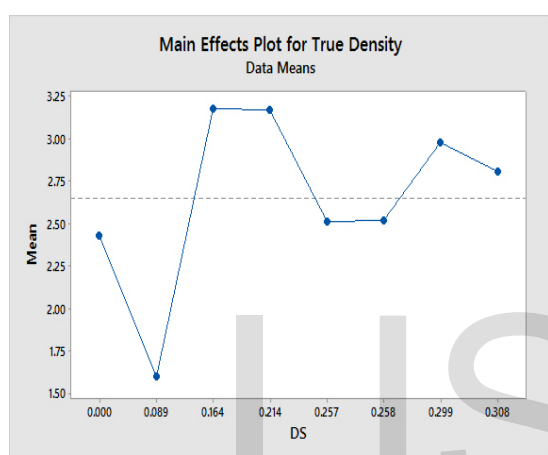
Fig. 3: Effect of DS on Tapped Density

Fig. 4: Effect of DS on True Density

As shown in table 1 and Fig. 2 and 3, generally there is a gradual increase in tapped density and bulk density as DS increases. CMS5 has the highest tapped and bulk density which high significant difference with NMS and other derivatives of carboxymethyl mango starch. From figure 3 The true density, CMS2 -CMS3 has no significant difference and, CMS4- CMS7 has no significant difference but significantly different from the other derivatives, CMS1 has lowest true density, and is significantly different from native starch and other derivatives. While CMS2 had the highest value of true density.

Fig.5: Effect of DS on Powder Porosity

From **Fig. 5** CMS1 has the lowest powder porosity and did not differ significantly from CMS-5 despite has highest Bulk and Tapped density, but both differs significantly from the rest while CMS-2 has the highest value and differs significantly from CMS-1, NMS, and CMS-5. These disparities in porosity could be attributed to starch densities, Particle size, and particle shape.

Spherically shaped particle with small sizes occupy more interparticle space, and decreases the pore or channel in the particle and subsequently result to a reduction in porosity.

Since capillary action as a result of porosity proceeds swelling then rupture in the mechanism of disintegration of tablets (Keith, 1976) it would be expected that the modified mango starch (CMS-2, CMS-6 and CMS3) would act more as a suitable disintegrating agent due to higher value of porosity as compared to the unmodified mango starch(NMS).

Influence of DS on Powder Flowability

The Hausner ratio and Carr's index represents the inter particle friction state. More HR is close to 1, the better is the flowability. In general, the least favourable flowability outcome is observed when HR ratio is greater than 1.25 (US Pharmacopeia XXXI, 2008). As shown in Fig, 6. It can be said that some of modified derivatives possess (CMS-2, CMS-4, and CMS-5,) approximately better flowability due to less interparticle friction within the powder sample when compare to native mango starch (NMS)

As explained by Jubril, *et al.,* 2012 "To get an insight into the degree of densification which could happen during tableting, there is need to understand the Hausner's ratio and Carr's index."

It is important to note that lower Hausner ratio, implies decrease in the tendency for densification to occur. The flow of the powder descends, as the values of these indices increase, (Staniforth, 1996) and this improves the probability of producing tablets with greater degree of weight variation (Olayemi *et. al,*2008).

It can be observed from fig. X, that CI steadily rises with increase of DS from CMS-4 to CMS-7, this may be due to more water that is absorbed as the starch derivative spend more time in the aqueous reaction medium. Overall CMS-7 show the highest value, followed by CMS-6, which is significantly different from NMS and CCMS.

According to Lefnaoui and Mostefa, (2015) the flowability powder is excellent if (CI ≤ 10), good if (11< CI < 15), fair if (16 <CI <20), passable if (21< CI < 25) and lastly poor if (CI> 25). From the above statement, flowability of CCMS excellent, CMS-4, CMS-5, CMS-2, CMS-3, and CMS-1 in increasing order have good flowability. CMS-6 is fair, while NMS has a passable flowability.
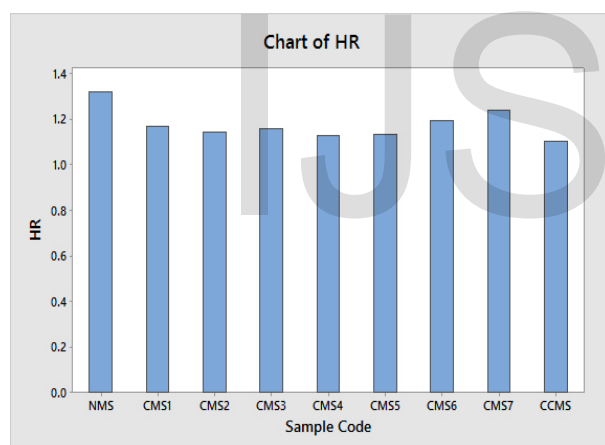

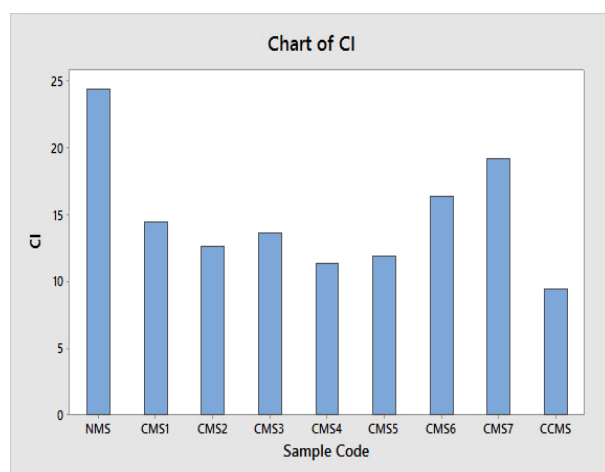**Fig. 6: Effect of DS on Huasner's Ratio**

Fig.7: Effect of DS on Carr's Index

4.0 Conclusion

This research has been able to establish that Mango starch can be chemically modified to give carboxymethyl starch which has improved powder porosity and flow properties, and reduces the limitations associated with native starch in its various ends applications, and confer it with advantage that will allow for it wider, suitable, and adaptable industrial utilization especially in pharmaceuticals where it can either be used as disintegrate, filler or binder in tablet manufacturing.

The result shows that DS is a function of reaction time between 0.0 to 3 hr, the relation between DS and reaction time are directly proportional, any further increase in time above 3 hours result the reaction equilibrium shifting to the right, thereby favouring the opposite direction of the reaction or encourages side reactions.

Carboxymethylation enhances flowability and porosity of native mango starch powder, but most times it is independent of DS but more dependent on time.

Therefore it can be said that Mango seed represents a huge potential to become a major unconventional source of starch because of its affordability, and abundance, also this could serve as means to mitigate the problems associated with agricultural waste pollution.

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