DEVELOPMENT OF CARBOXYMETHYLCELLULOSE FROM NATA DE COCO

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ABSTRACT

Nata de coco was used as raw material for the preparation of food-grade carboxymethylcellulose referred to as carboxymethyl-nata (CMN). The effect of various conditions in the carboxymethylation of nata cellulose was determined. The concentration of NaOH, mercerisation time, shredding time during mercerisation and etherification, and the concentration of monochloroacetic acid significantly affected the following physico-chemical properties of CMN, namely, degree of substitution (DS), viscosity, degree of polymerisation (DP), and solubility. The best processing conditions were: the use of 40% NaOH, 60 mins mercerisation time, 60 mins shredding time during mercerisation and etherification, and 1.35g/mL ClCH_2COOH . CMN with desirable properties was produced using these conditions.

1. INTRODUCTION

Nata de coco is a bacterial cellulose that forms on the surface of an acidic sugary medium by the action of *Acetobacter aceti* subspecies *xylinum*. Traditionally, nata de coco is consumed as a dessert. New recipes have recently been developed that include nata in various dishes and food products. As a bacterial cellulose, nata has great potential not only in food but also in non-food products. Applications of nata as thin films for electronic devices, wound dressings, high-quality additive to paper, audio acoustic diaphragms, and artificial skin and leather^{1,2,3} and as a solid matrix in chromatography⁴ have been reported.

Cellulose of bacterial origin is distinguished by its degree of crystallinity, mechanical strength, absorptive capacity and its overall chemical purity. No further processing is necessary^{1,2}. Nata de coco can be chemically modified into cellulose derivatives like carboxymethylcellulose (CMC). CMC is a multipurpose, "generally regarded as safe (GRAS) substance"⁵ which finds wide use in various processed foods because of its ability to thicken water, suspend solids in aqueous media, stabilise emulsions, absorb moisture from the atmosphere, and form films⁶.

The preparation of CMC from nata de coco is one way of addressing other possible uses of nata de coco. The cellulose derivative from nata de coco is referred to as carboxymethyl-nata (CMN). It is a cellulose ether which contains a carboxymethyl group and it functions

as a hydrocolloid. Since the product is similar to CMC, it could be used in food applications requiring CMC. The development of food-grade CMN is discussed in this paper.

2. MATERIALS AND METHODS

2.1 Preparation of carboxymethyl-nata

Nata pulp was used as the starting raw material for carboxymethyl-nata preparation. Fresh nata cubes (Class B and C) were thoroughly washed to remove adhering dirt. The cubes were then placed in a blender to reduce particle size. A series of pressing and washing were done to remove the acidic odour and taste and to further purify the nata pulp.

The preparation of CMN was based on the general procedure of Nikitin⁷ for CMC and is as described by Manguiat and Sabularse⁸.

2.2 Factors affecting carboxymethylation

Using the above mentioned procedure, the effects of some key factors on CMN properties were determined. These were: (a) concentration of NaOH, (b) mercerisation/steeping time, (c) shredding/mixing time during mercerisation and etherification, and (d) concentration of ClCH_2COOH .

2.2.1 Effect of NaOH concentration

The effect of six levels (% w/w) of NaOH, namely, 25%, 30%, 35%, 40%, 45% and 50% were determined. For this batch, 0.90g/mL ClCH_2COOH , 60 mins shredding during 6 hrs of mercerisation, and 60 mins shredding time during 6 hrs of etherification, including ageing were employed.

2.2.2 Effect of mercerisation time

Mercerisation was conducted 1, 3 and 6 hrs at different levels (% w/w) of NaOH. Four concentrations were tested: 25%, 30%, 35% and 40%. Shredding time was maintained at 60 mins, while shredding time during the 4-hr etherification period was kept at 60 mins. In all treatment combinations, 0.90g/mL of ClCH_2COOH was added.

2.2.3 Effect of shredding/mixing time

Shredding times of 30 and 60 mins were tested for both mercerisation and etherification processes at various levels (% w/w) of NaOH (25%, 30%, 35% and 40%). Mercerisation duration was maintained at 6 hrs, while etherification period including ageing was kept at 4 hrs. For all samples, 0.90g/mL of ClCH_2COOH was used.

2.2.4 Effect of monochloroacetic acid concentration

Three levels (g/mL) of ClCH_2COOH were tested: 0.9, 1.35 and 1.80 at different levels (% w/w) of NaOH (25, 30, 35 and 40). Mercerisation time was kept at 6 hrs with 60 mins of shredding, while etherification was maintained at 4 hrs with 60 mins of shredding.

2.3 Physico-chemical analysis of CMN

The CMN samples produced were analysed for the following physico-chemical properties: degree of substitution, viscosity, solubility in water and degree of polymerisation.

2.3.1 Degree of substitution

Degree of substitution (DS) of the CMNs produced was conducted according to the American Society for Testing Materials (ASTM) Methods D1439-83A "Testing NaCMC"⁹.

2.3.2 Viscosity

The viscosity of CMN solutions (1%) was determined at room temperature (28 – 30°C) using a Brookfield-type LVT model viscometer following the ASTM method⁹ with some modifications.

2.3.3 Solubility

The solubility of CMN in water was determined according to the method of Schoch¹⁰ with some modifications. A known amount of CMN was dissolved in a known volume of distilled water and the undissolved portion was separated by centrifugation. It was then dried in an oven and weighed. The dissolved portion was then calculated.

2.3.4 Degree of polymerisation and molecular weight determination

The molecular weight (MW) of CMN was determined from viscosity measurements following the procedure of Svenson¹¹. Viscosity was determined using a modified Ubbelohde viscometer. The viscosity data were used to calculate the intrinsic viscosity from which DP and MW were derived using the formula, $[\eta] = KM^a$.

2.3.5 Experimental design and statistical analysis

The Randomised Complete Block Design (RCBD) with two replications were used with a repetition of a whole set of experiments as the replication. Duncan's Multiple Range Test (DMRT) was used to determine differences among means. Simple linear correlation tests were made to ascertain relationships among the CMN properties evaluated. The data were analysed using the Statistical Analysis System (SAS) program for personal computers.

3. RESULTS AND DISCUSSION

3.1 Factors affecting carboxymethylation

3.1.1 NaOH concentration

The physico-chemical properties of the CMN produced at various levels of NaOH are given in Table 1. An increasing degree of substitution (DS) was observed with increasing alkali concentration up to 40%. The highest DS was achieved at this concentration. Above 40% NaOH, no significant effect on DS was recorded.

A similar trend was noted by Khalil and co-workers¹² in their study of carboxymethylation of maize starch. They noted that DS increased significantly by increasing NaOH concentration up to 8 N (32%, w/w). However, further increase in NaOH concentration was accompanied by a sharp decrement in DS. The results were explained by considering the two competitive and simultaneous processes involved during carboxymethylation.

Table 1: Physico-chemical properties of carboxymethyl-nata as affected by varying concentrations of NaOH.

NaOH (%, w/w)	Degree of substitution	Viscosity (1%, R.T.) cps	Degree of polymerization	Solubles (%)
25	0.272 c	2.50 c	59.37 d	0.83 c
30	0.332 b	6.28 b	70.34 d	32.90 b
35	0.383 ab	13.05 a	633.98 b	54.68 ab
40	0.415 a	8.5 b	392.56 c	55.32 ab
45	0.407 a	14.42 a	752.92 b	54.33 ab
50	0.376 ab	16.98 a	1066.18 a	70.85 a
C.V. (%)	1.29	7.15	6.00	13.70

Condition: 0.9 g/mL ClCH₂COOH; 60 mins shredding - 6 hrs mercerisation; 60 mins shredding - 4 hrs etherification. Means followed by the same letter are not statistically different at 0.05 level of significance (DMRT).

The same explanation may be given to the insignificant effect of increasing NaOH concentration above 40% on DS of CMN. The first process involves the reaction of cellulose (*Nata*-OH) with monochloroacetic acid in the presence of NaOH to yield carboxymethyl-nata as suggested by Equation 1:

The second process involves the reaction of NaOH with ClCH_2COOH to yield sodium glycolate as shown in Equation 2:

It is more probable that the first reaction predominated over the second when the concentrations of $\leq 40\%$ were used. Above these concentrations, the second reaction was favoured and apparently may have started to prevail with the formation of larger amounts of sodium glycolate. Also, the side reaction may have led to the consumption of ClCH_2COOH for the side product rather than for the carboxymethylation process¹².

One of the main factors that influenced the formation and composition of the alkali-cellulose was found to be the concentration of alkali¹³. Consequently, the etherification process was dependent on the alkali-cellulose formed and the quality of CMN produced greatly relies on this.

Another possible explanation that might have contributed to the non-increase in DS at NaOH concentrations $> 40\%$ is by considering the very nature of the nata cellulose itself. Nata, being a bacterial cellulose, was reported to be highly crystalline in nature². The difficulty in modifying its configuration during the mercerisation process even at NaOH concentrations $> 40\%$ may be attributed to its well-ordered structure. Possibly, substitution in the nata cellulose took place only in the amorphous (disordered) regions, which occupy only a small portion in the whole cellulose structure. Hence, at 40% NaOH, maximum swelling was probably attained in these region and this solely contributed to the overall substitution in this particular sample.

A pre-treatment may be necessary to break the crystalline regions and expose reactive sites to attain a high DS using nata cellulose. The use of isopropyl alcohol was mentioned by several researchers^{6, 14, 15}.

In terms of viscosity, values were observed to gradually increase with increasing NaOH concentration up to 35% . At 40% NaOH, a decrease in viscosity was noted as shown in Table 1. This unexpected decrease in viscosity can be accounted for by the reduction in chain length of the polymer during mercerisation at 40% NaOH. A shorter polymer chain length gives a less viscous solution⁵. Another possible explanation could be that with this treatment combination (40% NaOH and $0.9 \text{ g/mL ClCH}_2\text{COOH}$), the polymer may have taken a coiled configuration that resulted in a derivative with a characteristic low viscosity. Viscosities of 1% CMN solutions produced using 35% , 45% and 50% NaOH were statistically the same.

The DP of the CMN samples followed the same trend as that of viscosity. The highest DP was attained at 50% NaOH. The DP of CMN produced using 35% and 45% NaOH were statistically the same but lower than that of 50% . At 40% NaOH, a decline in DP was observed. DP is largely responsible for the thickening action. The longer the average chain length of the polymer, the more viscous is the solution. The DP for most commercial types ranges from 500 to 2000⁶.

The amount of solubles were also noted to increase slightly with increasing NaOH concentration. The highest value of 70.8% solubles was realised at 50% NaOH. The amount of solubles of CMN in 35%, 40% and 45% NaOH solutions were not significantly different from each other. All samples were considered partially soluble in water except the 25% NaOH sample which was practically insoluble.

3.1.2 Effect of mercerisation/steeping time

The effect of mercerisation on the properties of CMN are shown in Table 2. Increasing the reaction time between the NaOH and cellulose significantly raised the DS, viscosity and amount of solubles of the CMN within the time range examined. Longer steeping time resulted in higher values for the three parameters. This implied better CMN quality. Highest values were achieved at 6 hrs steeping time, the longest time duration used in the experiment. The improvement of the various properties of CMN by prolonging the duration of alkalisation is a direct consequence of its favorable effect on the swellability of cellulose resulting in efficient diffusion and absorption of reactants and better contact between the etherifying agent and cellulose. This observation conformed with the finding of Khalil *et al*¹².

Table 2: Physico-chemical properties of carboxymethyl-nata as affected by varying concentrations of NaOH and mercerisation time.

NaOH (%, w/w)	Mercerisation Time (hrs)			
	1	3	6	Mean
Degree of substitution				
25	0.136	0.112	0.123	0.124 c
30	0.106	0.212	0.174	0.164 c
35	0.198	0.225	0.361	0.261 b
40	0.320	0.359	0.427	0.369 a
Mean	0.190 b	0.227 ab	0.271 a	
C.V.	28.80			
Viscosity, cps (1%, R.T.)				
25	2.38 e	2.45 e	2.48 e	
30	2.38 e	3.00 d	2.45 e	
35	2.45 e	2.70 e	13.25 b	
40	6.50 c	12.00 b	24.45 a	
Mean	3.43	5.04	10.66	
C.V. (%)	13.90			
% Solubles				
25	0.86	3.75	2.90	2.50 b
30	0.00	13.65	3.78	5.81
35	7.34	17.84	33.90	19.69 a
40	30.92	38.90	42.92	37.59 a
Mean	9.78 b	18.53 a	20.88 a	
C.V. (%)	39.80			

Conditions: 0.9g/mL ClCH₂COOH; 60 mins shredding – mercerisation; 60 mins shredding – 4 hrs etherification. Means followed by the same letter are not statistically different at 0.05 level of significance (DMRT).

3.1.3 Effect of shredding/mixing during mercerisation and etherification

Table 3 shows the physico-chemical properties of CMN as affected by varying mixing time during mercerisation and etherification. No remarkable effect on degree of substitution was noted at any mixing time and with any level of NaOH used. On the other hand, the duration of mixing during the etherification process had a notable effect on DS. At 60 mins mixing, higher DS was attained as compared to when 30 mins was employed.

Table 3: Physico-chemical properties of carboxymethyl-nata as affected by varying concentrations of NaOH and shredding time during mercerisation and etherification.

Shredding time during mercerisation (min)					
		30	60		
NaOH	Shredding time during etherification (min)				Mean
(%, w/w)	30	60	30	60	
Degree of substitution					
25	0.123	0.172	0.098	0.272	0.131 d
30	0.174	0.348	0.140	0.332	0.221 c
35	0.362	0.378	0.233	0.383	0.324 b
40	0.427	0.429	0.358	0.414	0.405 a
Mean	0.272	0.332	0.207	0.350	
Mercerization Means	0.302			0.279	
Etherification Means	0.239 b			0.341 a	
C.V. (5%)	18.20				
Viscosity, cps (1%, R.T.)					
25	2.48 d	2.50 d	2.20 d	2.50 d	
30	2.45 d	14.60 ab	2.40 d	6.28 c	
35	13.25 b	23.32 a	2.78 d	13.05 b	
40	24.45 a	19.20 a	13.40 b	8.50 c	
C.V. (5%)	24.80				
% Solubility					
25	2.90 c	0.12 c	3.10 c	0.83 e	
30	3.78 e	18.52 d	1.39 e	32.90 c	
35	33.90 c	29.48 c	0.73 e	56.69 a	
40	42.94 b	31.27 c	39.99	55.32 a	
C.V. (5%)	39.70				

Conditions: 0.9g/mL ClCH₂COOH; 6 hrs mercerisation; 4 hrs etherification. Means followed by the same letter are not statistically significant at 0.05 level of significance.

The influence of the mixing process on viscosity properties of CMN solutions was not very apparent. There were three CMNs from different treatment combinations involving varied concentrations of NaOH, mixing time during mercerisation, and mixing time during etherification, that yielded the highest viscosity readings. These were: 40% NaOH, 30 mins mixing during mercerisation and 30 mins mixing during etherification; and 40% NaOH, 30 mins mixing during mercerisation and 60 mins mixing during etherification;

and 35% NaOH, 30 mins mixing during mercerisation and 60 mins mixing during etherification. Among these combinations, the common factor is the 30 mins mixing during mercerisation, which implies that this mixing time is favorable for the resulting CMN to possess desirable viscous properties.

In relation to solubility, CMN samples that employed 35% and 40% NaOH and 60 mins mixing during mercerisation and etherification attained the highest amount of solubles. The effect of the duration of mixing was not distinct and no pattern was established with regards to the solubility properties of the CMN samples in solution with the duration of mixing during mercerisation and etherification.

3.1.4 Effect of monochloroacetic acid concentration

The properties of different CMN samples as affected by varying levels of ClCH₂COOH are presented in Table 4.

Table 4: Physico-chemical properties of carboxymethyl-nata as affected by varying concentrations of NaOH and ClCH₂COOH.

NaOH	Monochloroacetic Acid (g/mL)		
(%,w/w)	0.90	1.35	1.80
Degree of substitution			
25	0.272 b	0.102 d	0.128 d
30	0.332 a	0.113 d	0.114 d
35	0.383 a	0.252 c	0.257 b
40	0.414 a	0.492 a	0.438 a
C.V. (%)	11.28		
Viscosity, cps (1%, R.T.)			
25	2.5 d	2.45 d	2.35 d
30	6.28 c	2.32 d	2.50 d
35	13.05 a	3.00 d	2.50 d
40	8.50 b	12.50 a	6.25 c
C.V. (%)	17.48		
% Solubles			
25	0.83 e	0.00 e	0.00 e
30	32.90 d	0.00 e	0.00 e
35	54.68 b	6.98 e	44.89 c
40	55.32 b	78.00 a	86.03 a
C.V. (%)	35.65		

Conditions: 60 mins shredding - 6 hrs mercerisation; 60 mins shredding - 4 hrs etherification. Means followed by the same letter are not statistically different at 0.05 level of significance (DMRT).

Though the highest DS of 0.492 was achieved with 1.35g/mL of ClCH_2COOH at 40% NaOH, the DS values obtained from the following combinations of NaOH and ClCH_2COOH levels were statistically the same as the former: 35% – 0.90g/mL, 40% – 0.90 g/mL and 40% – 1.8g/mL. It is consistently evident that again, at 40% NaOH, DS values recorded were relatively high at any ClCH_2COOH concentration. It is also noticeable that the three CMN samples treated with 0.90g/mL ClCH_2COOH obtained statistically the same DS. With these observations, no clear pattern was established with regards to the sole effect of ClCH_2COOH on DS. This is a clear indication that there are specific levels of NaOH and ClCH_2COOH that should be combined in order to obtain relatively high DS.

Two CMN samples obtained the highest viscosity reading: one produced from 35% – 0.90g/mL, and the other from 40% – 1.35g/mL NaOH - ClCH_2COOH combinations. Again, no exact pattern was demonstrated with regards to the effect of ClCH_2COOH concentration on viscosity.

CMNs possessing high degree of solubility were obtained using 1.35 and 1.80g/mL of ClCH_2COOH at 40% NaOH. As with viscosity, no pattern was demonstrated with regards to the sole effect of the concentration of the etherifying agent on solubility.

Table 5: Relationship among characters of CMN under increasing levels of NaOH at each level of monochloroacetic acid.

Character	Level of ClCH_2COOH (g/mL)	Correlation Coefficient
DS x Viscosity	0.90	0.80 ns
	1.35	0.93 ns
	1.80	0.91 ns
DS x DP	0.90	0.77 ns
	1.35	-
	1.80	-
DS x Solubility	0.90	0.97*
	1.35	0.95*
	1.80	0.99**
Viscosity x DP	0.90	0.95
	1.35	-
	1.80	-
Viscosity x Solubility	0.90	0.89 ns
	1.35	1.00**
	1.80	0.87 ns
DP x Solubility	0.90	0.81 ns
	1.35	-
	1.80	-

ns - Not significant

* - Significant at the 0.05 level of probability

** - Significant at the 0.01 level of probability

In general, the effect of ClCH_2COOH concentration on the properties of CMN relies on its final reaction with the state of the alkali cellulose. Again, this clearly indicates that specific combinations of the reactants are vital in order to obtain CMN with desirable qualities. In this experiment, the use of 40% NaOH – 1.35g/mL ClCH_2COOH combination resulted in a sample possessing overall satisfactory qualities, namely, highest DS, viscosity and solubility.

3.2 Correlation among properties

The result of simple linear correlation analysis among characters is presented in Table 5. DS and viscosity, DS and DP, and DP and solubility have no relationship within the range of NaOH and ClCH_2COOH concentration studied. On the other hand, only DS and solubility were found positively correlated at any level of ClCH_2COOH tested. As DS increased solubility also increased.

Viscosity and DP were found positively correlated at 0.90 g/mL ClCH_2COOH concentration while viscosity and solubility were found highly correlated at 1.35g/mL ClCH_2COOH .

3.3 Selection of best processing conditions

Based on the results of the study, the processing conditions that gave the best CMN qualities are summarised below:

NaOH	40% (starting concentration)
Mercerisation time	6 hrs
Mixing time during mercerisation	60 mins
Mixing time during etherification	60 mins
ClCH_2COOH concentration	1.35 g/mL (starting concentration)

These conditions were used in the preparation of a larger amount of CMN for characterisation. The physicochemical properties and viscosity characteristics of the developed CMN were found to be comparable to a commercially produced carboxymethyl cellulose product, except for purity which was found to be lower than the USFAD standard⁸. The product in powdered form had a grayish color, a purity of 95.4%, DP of 538.15, MW of 87,180, viscosity of 73 cps for a 2.0% solution at room temperature and a solubility of 78%. The DS of the product was 0.501. A maximum DS of 0.95 per monomeric unit of food grade CMC is allowed by the Code of Federal Regulations (CFR) of the US⁵.

The viscosity characteristics of CMN solutions were found to be affected by concentration, pH and other treatments such as freezing and boiling⁸.

The unique functional properties of hydrocolloids/gums make them important ingredients in food applications. The intended use of the developed CMN is as a food additive specifically to control viscosity flow properties of food systems. The CMC produced imparted stability and viscosity to peanut butter and firmness and body to mayonnaise⁸. It was not able to impart additional thickness to catsup but was able to prevent syneresis or bleeding to a certain extent.

4. SUMMARY AND CONCLUSIONS

Nata de coco pulp was used in the preparation of carboxymethylcellulose, termed carboxymethyl-nata (CMN). Several factors were investigated to determine their effects on the properties of CMN. The effect of NaOH concentration, mercerisation time, shredding/mixing time during mercerisation and etherification, and the concentration of ClCH_2COOH were studied.

An increase in degree of substitution (DS) was noted with increasing NaOH concentration up to 40%. Above this concentration, there was an insignificant change in DS. Likewise, viscosity was also observed to gradually increase with increasing NaOH concentration up to 35%. There was an unexpected decrease in viscosity at 40% NaOH. Viscosities of 1% CMN solutions using 35%, 45% and 50% NaOH were relatively high and statistically the same. Degree of polymerisation (DP) of the CMN samples followed the same trend as that of viscosity. The amount of solubles were observed to increase slightly with increasing NaOH concentration. The highest value of 70.8% solubles was obtained at 50% NaOH.

The effect of mercerisation time on the properties of CMN was found to be significant. Increasing reaction time between NaOH solution and cellulose significantly raised the DS, viscosity and solubility of CMN within the time range examined. Longer steeping/mercerisation time resulted in higher values for the three parameters. The use of 6 hrs mercerisation time, the longest time used was found most favorable.

Time of shredding during mercerisation had no remarkable effect on DS. However, shredding time during etherification had a notable effect on DS. A higher DS was obtained with longer shredding time (60 mins). The influence of the mixing process on viscosity properties of CMN solutions was not very apparent because of interaction effects. Among the treatment combinations used, a 30-min mixing time during mercerisation was found favorable to attain a viscous product. Also the effect of mixing on the solubility characteristic of CMN was not very distinct because of interaction effects. No pattern was established with regard to this character.

No clear pattern was observed for the sole effect of ClCH_2COOH on DS, viscosity and solubility. Significant interactions were noted, implying that there were specific levels of NaOH and ClCH_2COOH that should be combined to obtain CMN with acceptable qualities.

Simple correlation analysis among the parameters indicated that DS and DP; and, DS and solubility had no relationship within the range of variables studied. Both DS and solubility; and, viscosity and DP showed positive correlations.

From the results of the above experiments, optimum processing conditions were selected to prepare a larger volume of CMN for characterisation. These were: 40% NaOH, 6 hrs mercerization time, 60 mins mixing during mercerisation and etherification, and 1.35g/mL of ClCH_2COOH .

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