CHAPTER IV

RESULTS AND DISCUSSION

4.1 Studies on the optimum conditions for nata cellulosic gel formation by *Acetobacter xylinum*

4.1.1 Effect of strains of *A. xylinum* on their cellulosic gel formation abilities

Three strains of *A. xylinum* ; AGR60 ,DK and ST were used. After 14 days of static fermentation in 4L coconut water medium (1 inch of medium depth), the gel were lifted from the surface of mash to measure the thickness and weight of gel (Table 4.1)

Table 4.1 Thickness and weight of gel formed by three strains of *A. xylinum* after incubating at 32° C for 14 days in 4L coconut water medium in tray (1 inch of medium depth).

Strains	Gel thick* (cm.)	Cellulosic gel weight* (g)		Yield of cellulosic gel (g wet wt./Vol. of mash)
		wet weight	dry weight	
AGR60	0.90b	1124.0b	45.19b	0.281b
DK	1.95a	2287.5a	171.74a	0.572a
ST	2.10a	2479.0a	182.45a	0.619a

* Means in the same column followed by the same letters are not significantly different at 5% by DMRT.

The gel from DK and ST strains were not significantly different in their thickness and weight (dry and wet) of gel, but significantly thicker ($P \leq 0.05$) than gel from AGR60. The cellulose content (g cellulose/ g dry weight), as presented

in Table 4.2, of gel from these three strains were not significantly different. Cellulose yield (g dry wt basis) was directly related to yield of gel forming ability. It could be concluded that cellulosic synthesis efficiency of DK and ST strains were better than AGR60 strain.

Table 4.2 Moisture Content and Cellulose Yield and Cellulose Content in gel formed by three strains of A. *xylinum* after incubating at 32 °C for 14 days in 4L coconut water medium in tray.

Strains	Moisture content (%)	Cellulose Yield* (g dry wt basis)	Cellulose content (g cellulose/g dry wt.)
AGR60	95.58	30.35b	0.64b
DK	92.49	116.64a	0.70a
ST	92.64	128.90a	0.71a

* Means in the same column followed by the same letters were not significantly different at 5% by DMRT.

However, it might be possible that AGR60 strain would produce higher cellulosic gel yield when it grow in the other medium. But until now, it is still no study about which medium be suitable for each strain. Therefore, coconut water medium was used in this experiment because it was used in most of industrial production.

When the thickness of cellulosic gel produced by these three strains ; AGR60, DK and ST, were compared with other strains as reported by Alaban (1962). Within the same fermentation conditions, incubation at 32 ° C for 14 days at the static condition, but difference in concentration of inoculum used in each experiment ; 10% for AGR60 , DK , ST strains and 20% for N-103,N-106 , N-108, N-109, N-110 and N-111 strains. It was found that thickness of nata cellulosic gel produced by the latter group of strains were in range 2.65-3.50 cm which were higher than thickness of cellulosic gel produced by the former group of strains. That belonged to the difference of strains and inoculum concentration. In addition, Leepipattanawit(1988) reported thickness of cellulosic gel produced from *A. xylinum* TISTR 86 was 2.50 cm after incubation at room

temperature (27-32 °C) for 14 days. Therefore, this result was quite closely to the thickness of gel from DK and ST strains which provided the 1.95 and 2.10 cm (Table 4.1), respectively.

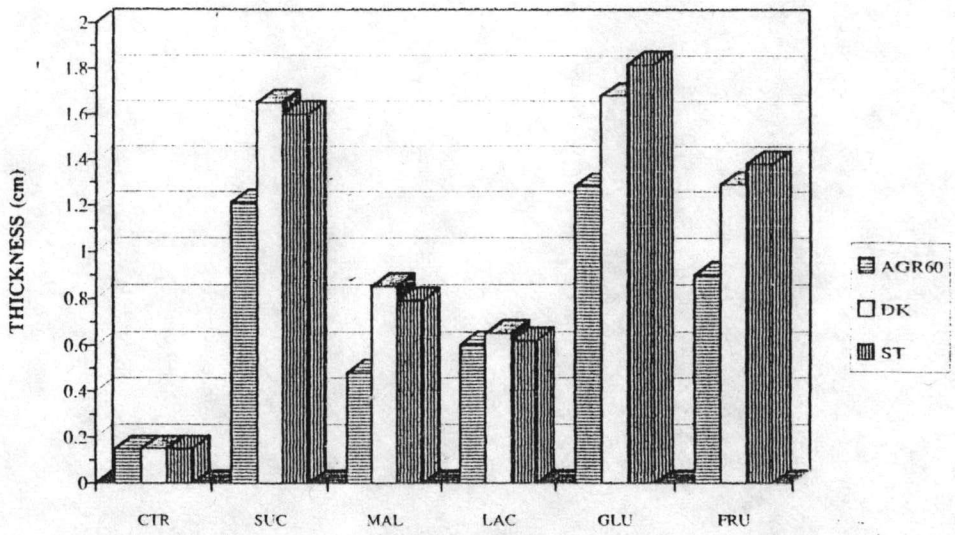
4.1.2 Effect of sugars on gel formation of *A. xylinum* strains

4.1.2.1 Coconut water medium

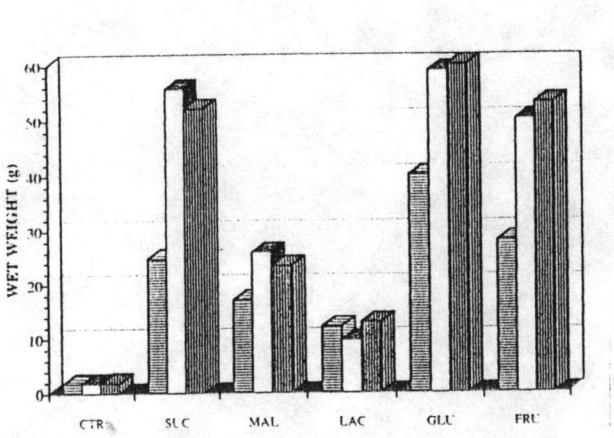
After incubation at 32°C for 14 days in coconut water medium consisting of 6% sucrose, 0.02% acetic acid (1 inch of medium depth), the gels were determined thickness and weight (Figure 4.1).

The effect of medium as C-source on production of cellulosic gel were studied. Five different sugars were chosen for studying the cellulosic gel forming abilities of these three strains in both media ; coconut water and synthetic media, were considered. As shown in Figure 4.1a, b and c, these three strains could produce high yield of celluloic gel when they were in sucrose, glucose and fructose. The gel formed by ST strain in coconut water medium added with glucose gave the thickest gel but not significantly different from gel produced by DK strain in coconut water medium added with sucrose,as shown in Figure 4.1a. In addition, gel formation of DK and ST strains were not significantly different but significantly different from AGR60 strain.

However, a thin pellicle forming though no sugar addition (as control) was investigated because *A. xylinum* can use the remaining sugars and growth factors in coconut water for its growth and cellulosic gel formation. Comparison among the various types of sugar that converted to the thickest gel by these three strains ; AGR60, DK strains were glucose and sucrose, while glucose for ST strain. The results showed that wet weight (Figure 4.1b) and dry weight (Figure 4.1c) were most related to the thickness (Figure 4.1a) of gel. This relation was the same as reported by Krusong and Tantratian (1994). Among five kinds of sugar used for *A. xylinum*, three kinds of them consisting of sucrose, glucose and fructose were suitable for gel formation.



(a)



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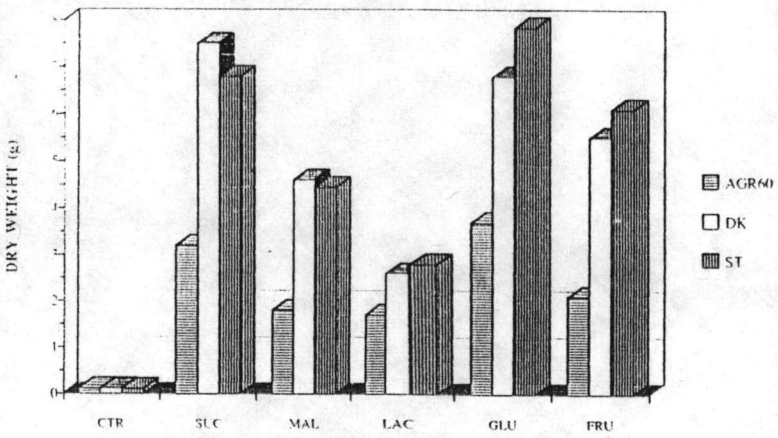

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Figure 4.1 The cellulosic gel formed by three strains of *A. xylinum* ; AGR60, DK and ST; in coconut water medium (1 inch of medium depth) with various types of sugars : SUC,sucrose ;MAL, maltose ; LAC,lactose; GLU,glucose; FRU, fructose and CTR, control (no sugar addition) after incubation at 32 °C for 14 days,under static condition.
(a)Thickness, (b) wet weight and (c) dry weight

The results showed that these three strains could produce high yield of celluloic gel when they were in sucrose, glucose and fructose. That might be due to glucose and fructose are monosaccharide therefore, these three strains of *A. xylinum* could converted glucose and fructose to cellulosic gel easily. However, in sucrose which is disacchrides,it also provided high yield. That might be due to sucrose composed of glucose and fructose. And the important reason that glucose and fructose could be coverted to cellulose effectively because they were in the cellulose biosythesis pathway (Richmon, 1991). About others sugar such as maltose although, it was composed of 2 molecule of glucose ,but it provided lower cellulose yield. That might be due to *A. xylinum* could produce in a little amount of α-glucosidase enzyme or could not produce.

From table 4.3 , the cellulose content (g cellulose/ g dry wt) in gel from these three strains in all three kind of sugars were not significantly different. The cellulose yield of ST strain gel in glucose was the highest, but not significantly different by DK strain gel in sucrose. Comparing among three strains, the cellulose formation of AGR60 was less than the others. And comparing among three kind of sugars, it was found that glucose and sucrose gave the maximum quantity of cellulose. But in the selection of sugar to be used as carbon source, it may be disadvantageous and nonprofitable to use glucose in commercial scale since it is costly. So, sucrose was suitable for adding in coconut water medium in cellulosic gel production

Within the same culture conditions, fermentation in coconut water medium with 1inch of medium depth and incubation at 32°C for 14 days at static condition were used in both studies. The volume of medium were different ; 4 L and 150 ml of section 4.1.1 and 4.1.2 , respectively. The results showed that when the fermenting volume increased, the high production of cellulose was obtained. Because the thick of gel from section 4.1.1 was higher. In case of cellulose content (Table 4.2 and Table 4.3 in section 4.1.1 and 4.1.2 ,respectively), it also could be said that at the volume of fermentation medium could affect cellulose content of nata cellulosic gel.

Table 4.3 Cellulose content and cellulose yield in gel formed by three strains of *A. xylinum* in three kinds of sugars added in 150 ml coconut water medium (1 inch of medium depth).

Strains	Cellulose Yield** (g dry weight basis)			Cellulose Content (g cellulose/ g dry weight)		
	SUC*	GLU*	FRU*	SUC*	GLU*	FRU*
AGR60	1.67b	2.03a	1.27c	0.54	0.52	0.54
DK	3.61b	3.81b	2.82c	0.53	0.54	0.52
ST	4.24a	4.26a	3.44a	0.57	0.54	0.56

*SUC,sucrose : GLU , glucose : FRU , fructose

** Means in the same row followed by the same letters were not significantly different at 5% by DMRT

4.2.1.2 Synthetic medium

After fermentation at 32°C for 14 days , the gel formation were measured as their thickness and wet and dry weight (Figure 4.2a). The thickest gel obtained from DK and ST strains in glucose and sucrose medium. The gel forming ability of DK and ST strains were not significantly different in all kind of sugars but significantly different by AGR60 strain except in control (0% sugar) and LAC, lactose. The highest gel produced by AGR60 strain was harvested from glucose treatment.

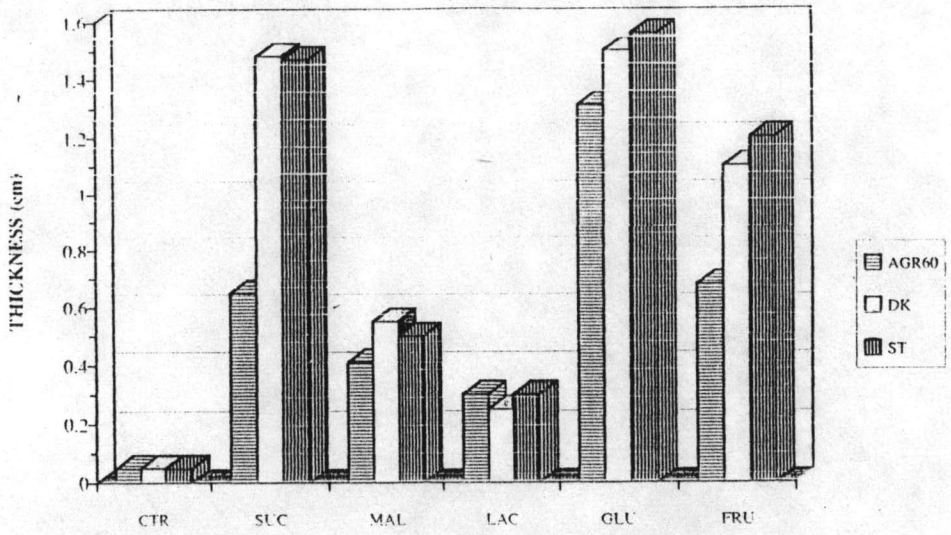
From the data, it should be observed that different kind of sugars was suitable for different strain. However, trends of sugar utilization of these three strains in synthetic medium still provided the same results as shown in section 4.2.1.1 (coconut water medium). That was three kinds of sugar ; sucrose, glucose and fructose, could be converted to thicker cellulosic gel than other sugars, because of the similar reason presented in previous section. That is, glucose and fructose are monosaccharide therefore, these three strains of *A. xylinum* could converted glucose and fructose to cellulosic gel easily. However, in sucrose which is disacchrides,it also provided high yield. That might be due to sucrose composed of glucose and fructose.

The important reason that glucose and fructose could be converted to cellulose effectively because they were in the cellulose biosythesis pathway (Richmon, 1991). About others sugar such as maltose although, it was composed of 2 molecule of glucose, but it provided lower cellulose yield. That might be due to *A. xylinum* could produce in a little amount of α-glucosidase enzyme or could not produce.

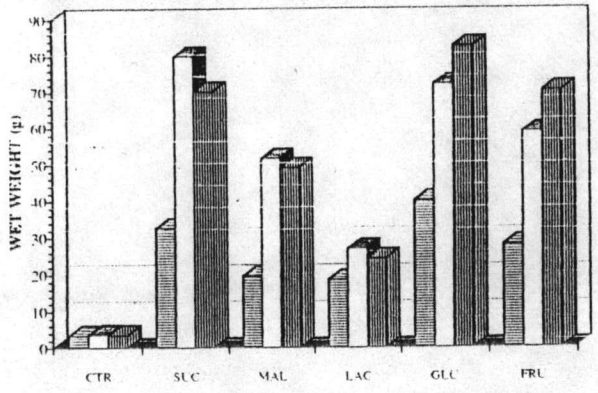
In a treatment of no sugar added medium (as control) a thin pellicle forming was investigated because *A. xylinum* can use the remaining sugars and growth factors in coconut water for its growth and cellulosic gel formation.

DK and ST strains, which could efficiency utilize sucrose and glucose, were suitable for gel formation. However, it was shown that sucrose was more advantage since its low costs.

Determination of cellulose (cellulose content and cellulose yield) in thick gel obtained from sucrose, glucose and fructose (Table 4.4). The cellulose content (g cellulose/ g dry wt) in gel produced these three strains in three kind of sugars were not significantly different because cellulose composition in gel by dry wt was nearly constant value (0.5 g cellulose/ g dry wt). On the other hand, the highest yield of cellulose in gel were obtained from ST and DK strains in glucose, followed closely by sucrose. For decreasing cost of gel production, it should be selected sucrose as carbon source for DK and ST strains.



(a)



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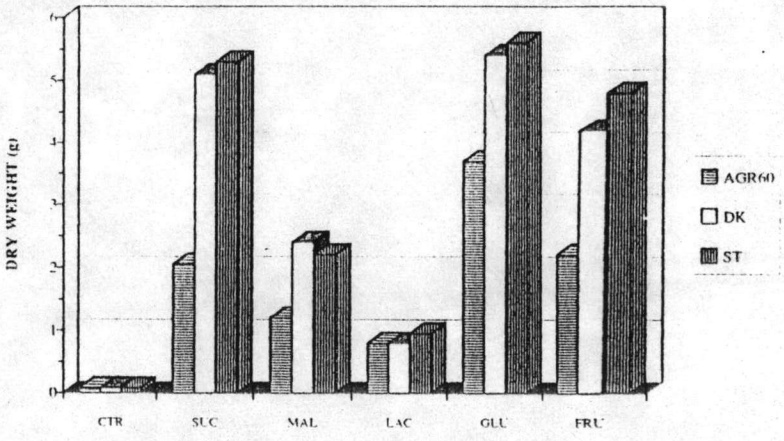

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Figure 4.2 The cellulosic gel formed by three strains of *A. xylinum* ; AGR60, DK and ST; in synthetic medium (1 inch of medium depth) with various type of sugars : SUC,sucrose ; MAL, maltose ; LAC,lactose; GLU,glucose ; FRU, fructose and CTR, control (no sugar addition) after incubation at 32 °C for 14 days, under static condition.
(a) Thickness, (b) wet weight and (c) dry weight

Table 4.4 Cellulose Content and Cellulose Yield in gel formed by three strains of *A. xylinum* in three kinds of sugars added in 150 ml synthetic medium (1 inch of medium depth).

Strains	Cellulose yield** (g dry weight basis)			Cellulose content (g cellulose/ g dry weight)		
	SUC*	GLU*	FRU*	SUC*	GLU*	FRU*
AGR60	1.10b	1.94a	1.29b	0.52	0.53	0.56
DK	2.98a	3.03a	2.45b	0.57	0.55	0.58
ST	2.78b	3.17a	2.51b	0.53	0.57	0.54

*SUC,sucrose : GLU , glucose : FRU , fructose

** Means in the same row followed by the same letters were not significantly different at 5% by DMRT

Comparison the effect of media on thickness of cellulosic gel, coconut water medium provided the thicker nata cellulosic gel than that in synthetic medium. This might be due to in coconut water medium consisiting of higher amount and quality of growths factors as vitamin and other trace elements, than containing in synthetic medium.

4.1.3 Effect of surface area of fermentation medium on nata cellulosic gel formation

From previous data, the ST strain of *A. xylinum* performed the highest effective ability of cellulosic gel production ,so it was used as inoculum in this study. After 14 days of fermentation time at 32 °C in different size of trays containing coconut water medium, but maintained the 1 inch of medium depth in all tray sizes. The cellulosic gel were harvested from the mash to determined the thick and weight of gel as presented in Table 4.5.

The various size of fermentation trays were used for containing coconut water medium. The cellulosic gels formed by *A. xylinum* ST strain were determined yield of the cellulose products as described in the term of thick and weight of gels as shown in

Table 4.5. It was shown that the highest yield of cellulose was obtained from the 168.75 cm^2 of surface area of tray. Because it gave highest value of g gel wet weight to volume of mash ratio. Although, the cellulosic gel from 1350 cm^2 of surface area of tray gave the thickest gel, it did not mean the highest cellulosic gel yield obtained because it displayed lower ratio of g gel wet wight to volume of fermentation medium.

Table 4.5 Cellulosic gel yield in cellulosic gel production by *A. xylinum* ST strain in various size of fermentation trays. After 14 days of fermentation time at 32°C in coconut water medium.

Surface area of tray (cm^2)	Volume of medium (cm^3)	Thick (cm)	wet weight (g)	dry weight (g)	Yield of cellulosic gel* (g wet wt/vol. of medium)
84.74	200	1.60	159.60	9.93	0.798c
168.75	400	2.05	402.15	21.47	1.005a
337.50	800	2.00	793.28	46.96	0.992a
675.00	1600	1.95	1517.10	90.42	0.948b
1350.00	3200	2.10	2939.70	201.37	0.919b

* Means in the same column followed by the same letters were not significantly different at 5% by DMRT

The maximum cellulose content was obtained from the cellulosic gel from 168.75 and 337.5 cm^2 of surface area of tray. The cellulosic gel forming ability of *A. xylinum* ST strain in these tray sizes were not significantly difference. The cellulosic gel from the smallest tray provided the lowest cellulose content. On the other hand, the biggest tray also gave the low cellulose content . Therefore, it could be assumed that cellulose content and yield of cellulose were influenced by surface area of tray.

In addition, influence of the surface /volume (S/V) ratio of the culture recipient was studied by Joris, Billiet, de Wulfand and Vandamme (1993).They concluded that cellulose production were influenced by S/V ratios. At high S/V ratios decreased cellulosic gel formation of *A. xylinum*. While at low S/V ratios limited the

growth of *A. xylinum*. However, though in this experiment used the constant ratio of S/V. It still provided the similar trend of cellulose production by *A. xylinum*. That is, at high dissolved oxygen (DO) levels (similarly to at high S/V ratios in the former experiments) in the biggest tray decreased the need for cellulose formation of *A. xylinum*, while low DO levels (similarly to at low S/V ratios) in the smallest one limit the growth of this microorganism.

Table 4.6 Moisture content, cellulose yield and cellulose content in cellulosic gel produced by ST strain of *A. xylinum* in various size of fermentation tray after 14 days of fermentation time at 32° C in coconut water medium.

Surface area of tray (cm^2)	Volume of medium (cm^3)	Moisture content (%)	Yield of cellulose (g dry wt basis)	Cellulose content* (g cellulose/g dry wt)
84.74	200	93.78	6.75	0.68c
168.75	400	94.66	15.89	0.74a
337.50	800	94.08	35.69	0.76a
675.00	1600	94.04	64.20	0.71b
1350.00	3200	93.15	140.95	0.70b

* Means in the same column followed by the same letters were not significantly different at 5% by DMRT

4.2 Factors affecting on carboxymethylcellulose production

4.2.1 Effect of cellulosic materials on carboxymethylcellulose production

Three forms of cellulosic materials ; blended cellulosic gel , dried cellulosic gel (nata cellulose powder) and wood cellulose powder, were used in the amount of 180g and 8.1g and 8.9 g, respectively. Each form contained the same amount of cellulose with moisture content of 95.5, 1.4 and 1.4%, respectively. All cellulosic materials were treated by modified method of Dhariyal and Chilpalkatti (1958) by mixing with distilled water, sodiumhydroxide and sodiumchloroacetate at amount of 20.25 ml , 12.8 g and 5.94 g, respectively.

Table 4.7 Weight, moisture content , L-value and pH of carboxymethylcellulose (CMC) produced by different cellulosic materials compared.

cellulosic materials	blended cellulosic gel	nata cellulose powder	wood cellulose powder
weight of CMC (g)	2.35	7.97	8.56
moisture content (%)	13.68	8.11	7.95
L-value	76.84	78.37	72.11
pH (1% solution)	10.46	8.05	7.93

The quantity and moisture content of CMC from nata cellulose powder were closed to CMC from wood cellulose powder which used as control. The CMC from blended cellulosic gel gave the product with the lowest yield and highest moisture content. The L-value; as described the whiteness, of CMC powder from blended cellulosic gel was 76.84 which was similar to L-value of CMC from nata cellulose powder (78.37). The lowest L-value of CMC obtained from CMC of wood cellulose powder. The solution of CMC from nata cellulose powder and wood cellulose powder presented at 1%CMC solution were 8.05 and 7.93 respectively. The solution of CMC from blended cellulosic gel had the pH of 10.46 which was higher than the acceptable pH range of food grade CMC (pH 6.5-8.5).

Table 4.8 Viscosity of 1% CMC solution , degree of substitution (DS) and impurities of CMC produced by different cellulosic materials

cellulosic material	blended cellulosic gel	nata cellulose powder	wood cellulose powder
viscosity*(cP)	24.0	104.0	168.0
DS	0.040	0.246	0.281
NaCl** (%)	7.52	4.41	3.37
Na-glycolate**(%)	10.89	5.15	4.68

* 1% CMC solution

** impurities from side reaction of the etherification reaction of CMC production

The viscosity and DS of CMC from nata cellulose powder were higher than CMC from blended cellulosic gel but lower than CMC from wood cellulose powder which used as control. The impurities matters, in the form of sodiumglycolate and sodiumchloride, in CMC from nata cellulose powder were 5.15% and 4.41% which were lower than CMC from blended cellulosic gel. The CMC from wood cellulose powder provided the lowest amount of impurities.

The properties of CMC produced from several cellulosic materials were considered. The L-value of CMC from nata cellulose powder was higher than CMC from wood cellulose because of effect of raw material colour. The wood cellulose powder was brown and the nata cellulose powder was pale yellow in color. The CMC produced from nata cellulose powder provided better qualities and higher quantities than from blended cellulosic gel. The high water content of blended cellulosic gel caused the higher impurities as sodiumchloride and sodiumglycolate. The water content in reaction mixture could activate the sodiumhydroxide to react with sodiumchloroacetate to form the impurities.(McLaughlin and Herbst, 1950). The CMC production using nata cellulose powder as raw material had low water content. The lower impurities obtained. The DS of CMC from nata cellulose powder was higher than CMC from blended cellulosic gel. Because of the higher concentration of sodiumhydroxide and sodiumchloroacetate in reaction mass of nata cellulose powder.

It was found that the CMC from nata cellulose powder were higher DS and viscosity than CMC from blended cellulosic gel and were closed to CMC from wood powder. The DS and viscosity of CMC from nata cellulose powder were lower than CMC from wood cellulose powder because the condition used was the optimum condition for wood (Dhariyal and Chipalkatti ,1958). The nata cellulose powder was more appropiate to produce CMC. It was chosen as substrate for the following experiment.

4.2.2 Effect of ratio of nata cellulose powder to distilled water on CMC production

Four different ratio of nata cellulose powder to distilled water were used as followed; 1.0 : 1.0, 1.0 : 1.5, 1.0 : 2.0 and 1.0 : 2.5. Other ingredients

and methods followed as described in previous experiment.

The quantity and moisture content of CMC produced by using of nata cellulose powder and water at the ratio of 1.0 : 1.5, 1.0 : 2.0 and 1.0 : 2.5 were closely. The CMC from the ratio of 1.0 : 1.0 gave 8.01 g which was the lowest amount. The CMC from the ratio of 1.0 : 2.0 provided the highest amount (8.94g) which was closed to the amount of CMC from the ratio of 1.0 : 1.5 and 1.0 : 2.5 (8.87 and 8.92g, respectively). The L-value of CMC powder from all ratio were closely. The pH of 1% solution of CMC from all ratio of nata cellulose powder to distilled water were in the range of acceptable value for food grade CMC(6.5-8.5).

The maximum viscosity was 232 cP which obtained from the ratio of 1.0 : 2.0. The DS of CMC from the ratio of 1.0 : 1.5 and 1.0 : 2.0 were 0.252 and 0.250 which were higher than DS of CMC from the other ratio. The lowest impurities were obtained from the ratio of 1.0 : 1.0. The higher amount of water, the more sodiumchloride and sodiumglycolate were detected.

The highest viscosity was obtained from ratio of 1.0 : 2.0. It could be assumed that the ratio of nata cellulose powder to distilled water of 1.0 : 1.0 and 1.0 : 1.5 did not have enough water for the reaction to cause the cellulose to swell. The sodiumhydroxide and sodiumchloroacetate in the mixture of the ratio of 1.0 : 2.5 were too low diluted. They reacted with each other as side reaction instead of with cellulose cause of impurities in the production.

Table 4.9 Weight, moisture content, L-value and pH of CMC produced by different ratio of nata cellulose powder to distilled water (w/v)

nata cellulose powder : H_2O	1.0 : 1.0	1.0 : 1.5	1.0 : 2.0	1.0 : 2.5
weight of CMC (g)	8.01	8.87	8.94	8.92
moisture content (%)	7.11	8.12	8.09	8.20
L-value	74.65	74.23	76.64	77.12
pH (1%CMC Solution)	8.01	7.84	7.76	7.76

Table 4.10 Viscosity, DS and impurities of CMC produced by different ratio of nata cellulose powder to distilled water (w/v)

nata cellulose powder : H_2O	1.0 : 1.0	1.0 : 1.5	1.0 : 2.0	1.0 : 2.5
viscosity *(cP)	120	196	232	112
DS	0.141	0.252	0.250	0.218
NaCl (%)	2.34	2.40	2.43	2.42
Na-glycolate (%)	2.81	3.01	3.66	4.13

* 1%CMC solution

The result of this experiment was similar to the report of McLaughlin and Herbst (1950). They mentioned that the efficiency of etherification reaction could be increased and the side reaction could be decreased at high sodiumhydroxide concentrations, by keeping the water content of system low. However, enough water must be present to insure adequate swelling of the cellulose so that etherification occurs uniformly. To confirm the above result unreacted cellulose was detected. It was found the unreacted cellulose in the mixture of the ratio of 1.0 : 2.5 . The mixture of the ratio of 1.0 : 1.0, 1.0 : 1.5 and 1.0 : 2.0 were not found unreacted cellulose. It could be assumed that the cellulose in these mixture were completely reacted with sodiumhydroxide to form alkali cellulose.

The optimum ratio of nata cellulose powder of distilled water of 1.0: 2.0 gave the highest viscosity of 232 cP and the DS value of 0.250. It was differed from the report of Waldeck and Smith (1952) which mentioned that the optimum ratio of wood cellulose to distilled water of 1.0 : 0.71 gave the highest viscosity of 600 cP and DS of 0.86. The results were differed that might be due to the effect of methods and effect of cellulose characteristics. Waldeck and Smith(1952) used steeping press and passing shredder to produce CMC. In this experiment used all ingredients mixing which was the simplest and economical method. Not only the CMC production method affected to the optimum ratio of cellulose to distilled water, but type of cellulose also affected. McLaughlin and Herbst (1950) reported that the ratio of cellulose to distilled water

depend upon the type of cellulose. The ratio of nata cellulose powder to distilled water of 1.0 : 2.0 was selected to use in further experiment.

4.2.3 Effect of ratio of nata cellulose powder to sodiumhydroxide on CMC production

The ratio of nata cellulose powder to distilled water of 1 : 2.0 (w/v) was used in this experiment. The various ratio of cellulose powder to sodiumhydroxide were used as followed; (w/w) 1.0 : 1.5, 1.0 : 3.0, 1.0 : 4.5 and 1.0 : 6.0, respectively. Other compositions and methods followed as described in previous experiment.

As the amount of sodiumhydroxide in the mixture increased, the CMC production yield was greater. The highest viscosity and DS of CMC were obtained from the ratio of nata cellulose powder to sodiumhydroxide of 1.0 : 3.0. The highest L-value was obtained from CMC produced from the ratio of 1.0 : 1.5 of nata cellulose cellulose powder to sodiumhydroxide. The L-value was related to sodiumhydroxide concentration as the higher sodiumhydroxide concentration, the lower L-value obtained. The pH of CMC solution from all ratio were closely. The highest viscosity was 236 cP which obtained from the ratio of 1.0 : 3.0. That was closed to viscosity of CMC from the ratio of 1.0 : 4.5 (228 cP). The lowest viscosity of CMC solution obtained from the ratio of 1.0 : 1.5 (34cP). As the highest concentration of sodiumhydroxide in reaction mixtures, the viscosity decreased.

Table 4.11 Weight ,moisture content ,L-value and pH of CMC produced by different ratio of nata cellulose powder to sodiumhydroxide (w/w).

nata cellulose powder : NaOH	1.0 : 1.5	1.0 : 3.0	1.0 : 4.5	1.0 : 6.0
weight of CMC (g)	8.52	9.14	9.71	9.96
moisture content (%)	8.22	8.27	8.71	8.86
L-value	80.75	79.54	77.02	77.13
pH (1%CMC Solution)	7.87	7.98	7.96	8.01

Table 4.12 Viscosity, DS and impurities of CMC produced by different ratio of nata cellulose powder to sodiumhydroxide (w/w)

nata cellulose powder : NaOH	1.0 : 1.5	1.0 : 3.0	1.0 : 4.5	1.0 : 6.0
viscosity *(cP)	34	236	228	134
DS	0.144	0.264	0.262	0.262
NaCl (%)	1.56	2.38	2.55	2.89
Na-glycolate (%)	2.41	3.10	3.16	3.23

* 1%CMC solution

Sodiumhydroxide concentration could directly affected the CMC production because it caused cellulose to swell. It was important that cellulose must be swell and form alkali cellulose before it was reacted with sodiumchloroacetate to form CMC. The cellulosic fiber could not completely swell at low concentration of sodiumhydroxide (Wise, 1946). The higher concentration of sodiumhydroxide caused the efficiency of carboxymethylation and the product viscosity decreased (McLaughlin and Herbst, 1950). The excess sodiumhydroxide also affected on CMC colour from white to cream or yellow, which the lower L-value was obtained. The creamy or yellow powder of CMC was not good physical property (Toyoshima, 1993).

It was found that the amount of sodiumhydroxide in the reaction mixtures of the ratio of 1.0 : 1.5 provided the lowest DS and viscosity. That might be due to the cellulose could not swell at too diluted sodiumhydroxide concentration. On the other hand, too high concentration of sodiumhydroxide in the mixtures, the ratio of 1.0 : 4.5 and 1.0 : 6.0, the high impurities and low viscosity were detected. It was similar to the report of Kloow (1985) that the excess sodiumhydroxide absorbed water content to CMC powder and that also caused viscosity reducing.

The optimum ratio of nata cellulose powder to sodiumhydroxide of 1.0 : 3.0, provided the highest viscosity of 236 cP and DS of 0.264. This was differed from the previous investigations, Dhariyal and Chilpakatti (1958) who reported that the optimum ratio of wood cellulose to sodiumhydroxide of 1.0: 3.0 gave the highest

viscosity (250 cP) and DS (0.41). Waldeck and Smith (1952) mentioned that the optimum ratio of wood cellulose to sodiumhydroxide of 1: 0.44 presented the highest viscosity of 600 cP and DS of 0.86. Both investigations, using the method of CMC production by steeping press and passing shredder and then, milling. The cellulose could be swell effectively at low sodiumhydroxide concentration under pressure. The different method of CMC production, the different optimum ratio obtained. The CMC production by all ingredients mixing method of this experiment, the optimum ratio of nata cellulose powder to sodiumhydroxide was 1.0 : 3.0 which was chosen for followed experiment.

4.2.4 Effect of ratio of nata cellulose powder to sodiumchloroacetate on CMC production

The ratio of nata cellulose powder to distilled water of 1.0 : 2.0 (w/v) and the ratio of nata cellulose powder to sodiumhydroxide of 1.0 : 3.0 (w/w) were chosen. Various ratio of nata cellulose powder to sodiumchloroacetate were applied ; 1.0 : 0.36, 1.0 : 0.72, 1.0 : 1.08, 1.0 : 1.44 and 1.0 : 1.8, respectively. Other ingredients and methods followed as described in previous experiment.

To improve the DS and viscosity of CMC, the effect of ratio of cellulose powder to sodiumchloroacetate was considered (McLaughlin and Herbst, 1950). The results of CMC from nata cellulose powder reacted with various amount of sodiumchloroacetate were as followed ; the maximum amount of CMC product was 10.87g which obtained from the ratio of 1.0 : 1.8. The higher amount of sodiumchloroacetate. the weight of CMC increased. The L-value, moisture content and pH of CMC from all ratio were slightly different.

The properties of CMC were as followed. The highest viscosity of CMC was 288 cP which obtained from the ratio of 1.0 : 1.44. The lowest viscosity of CMC (32 cP) obtained from the ratio of 1.0 : 0.36. The viscosity of CMC from the ratio of 1.0 : 0.72, 1.0 : 1.08 and 1.0 : 1.8 were closely. The maximum DS of CMC was 0.462 which obtained from the ratio of 1.0 : 1.8. It was closed to the DS of CMC from the ratio of 1.0 : 1.44 (0.460 DS). The greater amount of sodiumchloroacetate, the DS improved. The lowest impurities were 1.13% sodiumchloride and 2.56%

sodiumglycolate which obtained from the ratio of 1.0 : 0.36. The ratio of 1.0 : 1.8 gave the highest impurities , 3.88% sodiumchloride and 4.34% sodiumglycolate. The quantity of sodiumchloroacetate increased, more impurities were detected.

Table 4.13 Weight ,moisture content ,L-value and pH of CMC produced by different ratio of nata cellulose powder to sodiumchloroacetate (w/w).

nata cellulose powder to ClH_2COONa	1.0:0.36	1.0:0.72	1.0:1.08	1.0:1.44	1.0:1.80
weight of CMC (g)	8.94	9.04	9.75	10.62	10.87
moisture content (%)	8.13	8.27	8.06	7.98	8.01
L-value	79.52	80.16	79.94	78.43	79.82
pH (1%CMC Solution)	8.11	7.95	8.05	7.88	7.84

Table 4.14 Viscosity, DS and impurities of CMC produced by different ratio of nata cellulose powder to sodiumchloroacetate (w/w)

nata cellulose powder to $ClCH_2COONa$	1.0:0.36	1.0:0.72	1.0:1.08	1.0:1.44	1.0:1.80
viscosity (cP) of 1% CMC	32	228	242	288	216
DS	0.167	0.256	0.384	0.460	0.462
NaCl (%)	1.13	1.98	2.34	2.89	3.88
Na-glycolate (%)	2.56	2.87	2.94	3.01	4.34

The unreacted sodiumchloroacetate was analysed to determine the completion of the etherification reaction of the alkali cellulose and sodiumchloroacetate. Green(1963) mentioned that as the unreacted sodiumchloroacetate was detected in the mixture, the reaction was not completed. It was found that in the mixture of the ratio of 1.0 : 0.36, 1.0 : 0.72, unreacted sodiumchloroacetate were not detected. In the mixture of the ratio of 1.0 : 1.08, 1.0 : 1.44 and 1.0 : 1.80, unreacted sodiumchloroacetate were found. It could be assumed that the reaction in the mixture of

the ratio of 1.0 : 1.08, 1.0 : 1.44 and 1.0 : 1.80, which contained unreacted sodiumchloroactetate, were not completely reaction. However, the etherification reaction could be completed by extending the mixing time and the incubation time (Ganz, 1973).

The appropriate ratio of nata cellulose powder to sodiumchloroacetate of 1: 1.44 (w/w) which provided the highest viscosity (288 cP) and DS (0.46). Paton (1974) reported the ratio of cellulose (from apple tissue) to sodiumchloroacetate was 1:0.9 (w/w). The properties of CMC from apple tissue were 0.42 DS and 1% viscosity range in 5,000-138,000 cP. The CMC from apple tissue provided the high quality than CMC from nata cellulose powder because of difference treatment conditions. The CMC from apple tissue was treated by using water and organic solvent as reaction media in order to increase the efficiency of the reaction. The CMC from nata cellulose powder was only treated by water as reaction medium. In addition, the experiment of the CMC production using the different conditions, temperature and time of incubation . In order to produce high quality of CMC product, the ratio of nata cellulose powder to sodiumchloroacetate used in next step was 1.0 : 1.44.

4.2.5 Effect of mixing time on CMC production

The optimum ingredients of CMC production were as followed ; the ratio of nata cellulose powder to distilled water of 1.0 : 2.0 (w/v), the ratio of nata cellulose powder to sodiumhydroxide to sodiumchloroacteate of (w/w) 1.0 : 3.0 : 1.44. Various mixing time were used ; 4, 5, 6, 7 and 8 hours. Other experimental procedures followed as performed in previous experiment.

The data showed that the yield of CMC from various mixing time were closely. The lowest amount of CMC was 9.15 g which got from the mixing time of 4 hours. The moisture content, L-value and pH of CMC from all mixing time were slightly different.

Table 4.15 Weight ,moisture content ,L-value and pH of CMC produced by various mixing time

mixing time (hr)	4	5	6	7	8
weight of CMC (g)	9.15	10.03	10.17	10.12	10.05
moisture content (%)	7.92	7.88	7.98	7.87	7.81
L-value	79.92	78.82	78.65	76.17	78.48
pH (1%CMC Solution)	7.85	8.02	7.96	8.01	8.17

The CMC from 4 hours of mixing time provided the lowest DS of 0.425 and viscosity of 288 cP. The longer period of mixing time, the DS and viscosity increased. As the time of mixing extended from 4 to 6 hours, the DS and the viscosity improved from 0.425 to 0.462 and 288 to 332 cP, respectively. The more period of mixing did not gave an improvement on the DS and viscosity. As the CMC from the 6, 7 and 8 hours of mixing, the DS and viscosity from these mixing period were closely. The highest impurities of CMC, 2.93% sodiumchloride and 4.02% sodiumglycolate, obatained from the mixing time of 8 hours. It was found that the more contaminants in CMC were detected at the longer period of mixing time.

Table 4.16 Viscosity, DS and impurities of CMC produced by various mixing time.

mixing time (hr)	4	5	6	7	8
viscosity(cP) of 1%CMC solution	288	304	332	336	334
DS	0.425	0.458	0.462	0.464	0.465
NaCl (%)	1.65	2.12	2.54	2.88	2.92
Na-glycolate (%)	2.74	3.04	3.24	3.87	4.02

The unreacted sodiumchloroacetate was detected. The reaction masses mixed for 4 and 5 hours were found the unreacted sodiumchloroacetate. It was found that the reaction mixtures mixed longer, the unreacted sodiumchloroacetate decreased.

Waldeck and Smith(1952) the ingredients were mixed at the temperature of 35°C to 45°C for 2 hours, the DS and viscosity of CMC were 0.86 and 600 cP, respectively. In this experiment of CMC production from nata cellulose powder, the mixing time and incubation period were treated at room temperature (28-32°C) which used the lowest operation cost. The DS of 0.462 and viscosity of 332 cP of CMC were obtained. That might be due to the mixing time was depended upon the production level, type and size of raw materials, operating temperature during mixing and incubating, type and number of spindle of mixer including spindle speed (Ganz, 1973). The high DS and viscosity of CMC could be obtained by using and treating as followed. The raw materials with the smallest size used because the more reaction size caused the DS and viscosity of CMC improved. The optimum mixing and incubating temperature which were depend upon the type of cellulose and the rate of reaction designed, as the low and high temperature used, 10-40° C and 40-100 °C, respectively, the mixtures were reacted slowly and quickly, respectively. The number and speed of spindle which were also depend upon the type and size of raw materials and the rate of reaction desired.

The optimum time of mixing of CMC production from nata cellulose powder which presented the higher DS and viscosity and the lower impurities was 6 hours of mixing. It was found that the unreacted sodiumchloroactate was detected in the reaction mixture of 6 hours of mixing time. To get rid of unreacted sodiumchloroacetate, improve DS and viscosity and decrease of impurities, the incubation time was studied in the next experiment. The 6 hours of mixing period was chosen for the followed experiment.

4.2.6 Effect of incubation time on CMC production

The suitable ingredients of CMC production were as followed ; the ratio of nata cellulose powder to distilled water of 1.0 : 2.0 (w/v), the ratio of nata cellulose powder to sodiumhydroxide to sodiumchloroacteate of (w/w) 1.0 : 3.0 : 1.44. The optimum mixing time was 6 hours. Various incubation time were applied ; 0, 12, 24, 36, 48 and 60 hours. Other experimental details followed as described in previous experiment.

The highest quantity of CMC was 11.95 g which obtained from the 60 hours of incubation time. As the experiment carried on without the incubation time, the

lowest amount of CMC was detected. The moisture content and pH of CMC from all incubation time were closely. The L-value of CMC from all incubation time were slightly different, except the CMC from 0 hour gave the lowest L-value of 62.13.

The maximum viscosity of CMC (340 cP) was obtained from which incubated at 36 hours. The lowest viscosity (84 cP) was obtained from 0 hour of incubation. The longer incubation time, the viscosity of CMC improved. As the increasing of incubation period from 0 to 36 hours, the viscosity raised from 84 cP to 340 cP. The more incubation time, as increasing from 36 to 60 hours, the viscosity decreased from 340 cP to 292 cP. The lowest DS of CMC was 0.102 which obtained from the 0 hour of incubation time. The DS increased from 0.102 to 0.469 since the incubation time was extended from 0 to 24 hours. As the longer incubation period of 24, 36, 48 and 60 hours, the DS of these CMC were closely. The highest impurities, 2.38% sodiumchloride and 4.23% sodiumglycolate, were obtained from 60 hours of incubation time. It was found that the longer incubation time, the higher amount of impurities, as sodiumchloride and sodiumglycolate, were detected.

Table 4.17 Weight ,moisture content ,L-value and pH of CMC produced by various incubation time

incubation time (hr)	weight of CMC (g)	moisture content (%)	L-value	pH of 1%CMC solution
0	4.39	8.64	62.13	8.12
12	8.55	7.76	77.55	8.03
24	10.86	7.75	79.04	7.96
36	11.10	7.82	80.12	7.96
48	11.32	7.79	80.06	7.84
60	11.95	7.86	79.72	7.91

Table 4.18 Viscosity, DS and impurities of CMC produced by various incubation time.

incubation time (hr)	viscosity* (cP) of 1%CMC	DS	NaCl (%)	Na-glycolate (%)
0	84	0.102	0	0
12	220	0.238	0.24	2.76
24	332	0.469	2.54	3.24
36	340	0.465	2.48	3.46
48	280	0.488	2.35	3.88
60	292	0.490	2.38	4.23

* 1% CMC solution

The incubation time or aging time affected to the reaction. The etherification reaction between alkali cellulose and sodiumchloroacetate could not complete in the short time (Green, 1963). In contrast, the longer incubation time caused the viscosity reducing. As the incubation time extended, the twisted cellulose fibers were configured to the untwisted fibers. These untwisted fibers caused water absorption ability of fiber decreased so, the viscosity of CMC was lessen (McLaughlin and Herbst, 1950). Not only the incubation time affected to the quality of product, but the incubation temperature also affected. Green (1963) revealed that to produce CMC from wood with the high DS, the optimum temperature and time for aging the reaction mixture were 55 ^{o}C for 3.5 hours. The product of DS 0.89 obatined. Suwannapruek and Siripaisanpipat (1984) reported the appropiate temperature and time for incubation reaction mass of CMC from hyacinth cellulose were the room temperature(28 - 32 ^{o}C) for 7 days. The CMC product provided DS of 0.84 which was higher than DS of CMC from nata cellulose powder. That might be due to the CMC production from the hyacinth was treated by 3% alcoholic HNO_3 and bleached by hypochlorite to get rid of the impurities before it was reacted with the ingredients of CMC production. The CMC production from nata cellulose powder had not the step of purification of the raw material as the method of all ingredients mixing. The optimum incubation time which provided the best quality of CMC product of 36 hours was used in next experiments.

4.2.7 Effect of ratio of reaction mixture to hot water on CMC production

The nata cellulose powder were added with distilled water by the ratio of cellulose powder to water, 1.0 : 2.0(w/v), the ratio of cellulose powder to sodiumhydroxide to sodiumchloroacetate (w/w) was 1.0 : 3.0 : 1.44. The reaction mixture was mixed for 6 hours and incubated for 36 hours. Various ratio of wet reaction mass to hot water (w/v) used were as followed ; 1.0 : 5.0, 1.0 : 7.5, 1.0 : 10.0 and 1.0 : 12.5. Other experimental details followed as decribed in previous experiment.

The CMC powder obtained composed of the CMC and the impurities, as sodiumchloride and sodiumglycolate. These compositions could be effectively dissolved in hot water. Then, the alcohol aqueus solution was added into the reaction mixture to precipitate the CMC product whereas, the impurities could not be precipitated (Waldeck and Smith, 1952).

The ratio of reaction mixture to hot water of 1.0 : 5.0 (w/v) provided the CMC with the highest yield of 11.18 g. The lowest quantity of CMC was 9.98 g which got from the ratio of reaction mixture to hot water of 1.0 : 5.0. There was no different in the value of pH and moisture content from all treatments. Among all treatments, the CMC from the ratio of reaction mixture to hot water of 1.0 : 5.0 showed the lowest L-value.

Table 4.19 Weight ,moisture content ,L-value and pH of CMC produced by different ratio of reaction mixture to hot water (w/v).

reaction mixture : hot water	1.0 : 5.0	1.0 : 7.5	1.0 : 10.0	1.0:12.5	1.0 :15.0
weight of CMC (g)	11.18	10.63	10.17	10.08	9.88
moisture content (%)	7.82	7.85	7.74	7.78	7.76
L-value	69.03	79.12	80.02	80.34	81.59
pH (1%CMC Solution)	8.15	8.11	8.07	7.89	7.90

The CMC with the highest viscosity and DS were obtained from the ratio of 1.0 : 15.0. The more volume of hot water, the higher viscosity and DS of CMC obtained. As the volume of hot water increased from 1.0 : 5.0 to 1.0 : 15.0 (w/v), the viscosity and DS improved from 268 to 350 cP and 0.468 to 0.478 DS, respectively. The viscosity of CMC from the ratio of 1.0 : 12.5 and 1.0 : 15.0 ,348 cP and 350 cP, were closely. The data showed that the impurities cut down as the higher amount of hot water.

Table 4.20 Viscosity, DS and impurities of CMC produced by different ratio of reaction mixture to hot water (w/v).

ratio of reaction mixture to hot water	1.0 : 5.0	1.0 : 7.5	1.0 : 10.0	1.0:12.5	1.0 :15.0
viscosity (cP) of 1% CMC	268	304	338	348	350
DS	0.468	0.475	0.474	0.475	0.478
NaCl (%)	2.90	2.76	2.21	1.98	1.22
Na-glycolate (%)	4.06	3.97	3.20	2.81	2.10

The treatment of carboxymethylation reaction mass depend upon the purity of product desired. If a technical unpurified product was needed, the wet reaction mass may neutralized with acid and no need to dissolve in hot water to get rid of impurities (Toyoshima, 1993). For the purified product, the CMC could be purified by several methods ; by thorough washing with aqueous ethanol or methanol (Green, 1963) or by washing with hot water (Waldeck and Smith,1952). The purified CMC by washing in hot water, which was more profitable(Toyoshima, 1993), was chosen in this experiment. It was found that the optimum ratio of reaction mixture to hot water (w/ v) of 1.0 : 12.5 gave the best quality of CMC. This ratio was used in next experiment.

4.2.8 Effect of final concentration of ethanol on CMC precipitation

The ingredients of CMC production as mentioned in previous experiments, were mixed for 6 hours and incubated for 36 hours. Washing the wet reaction mass with hot water by the ratio of 1.0 : 12.5. This experiment conducted to

50%, 60%, 70% and 80% of final concentration of ethanol for CMC precipitation.

Alcohol was mostly chosen for polysaccharide precipitation which the ability of polysaccharide in water was lessen by alcohol. The CMC was the one of polysaccharides, the alcohol was to precipitate CMC. The ethanol activity in CMC precipitation was either decreasing dissolving ability of CMC or declining bonding force or inducing anion in solution (Na^+ or K^+) to combine with cation of CMC (Smith and Pace, 1982).

The yield of CMC from various final concentration of ethanol were closely. The lowest amount of CMC (8.57 g) was obtained from the 50 %ethanol final concentration. The moisture content, L-value and pH of CMC solution were similarly. In addition, it was found that the final concentration of ethanol for CMC precipitation was not affecting to the moisture content, powder colour and pH of CMC.

The viscosity and DS of CMC precipitated with 60%, 70% and 80% of final concentration of ethanol were nearly related. The CMC precipitated with 50% of ethanol concentration presented the lowest viscosity (292 cP) and DS (0.461). The product impurities, sodiumglycolate and sodiumchloride, trended to decline as the concentration of ethanol increased.

Table 4.21 Weight, moisture content, L-value and pH of CMC produced by different final concentration of ethanol

final concentration of ethanol (%)	50	60	70	80
weight of CMC (g)	8.57	10.02	10.28	10.36
moisture content (%)	7.78	7.78	7.75	7.88
L-value	78.92	80.15	80.11	80.24
pH (1%CMC Solution)	7.96	7.97	7.89	7.88

Table 4.22 Viscosity, DS and impurities of CMC produced by different final concentration of ethanol.

final concentration of ethanol (%)	50	60	70	80
viscosity (cP) of 1%CMC solution	292	332	334	326
DS	0.461	0.478	0.480	0.483
NaCl (%)	2.24	2.20	2.19	1.62
Na-glycolate (%)	2.97	2.52	2.48	2.35

The purification methods involved either the use of alcohol- water mixtures to extract the sodiumchloride and the sodiumglycolate fromthe mixture, or conversion to the insoluble acid form or to an insoluble salt. It was found that the concentration of 70% ethanol precipitated the CMC with the highest viscosity and DS. It was no need to use higher concentration of ethanol than 70% because this concentration was adequate for precipitation. In addition, the impurities, especially sodiumglycolate which was toxic substant (Food chemical codex,1981), detected from the CMC of 70 and 80% were closely. It could be assumed that the suitable final concentration of ethanol for CMC precipitation was 70%.

4.3 Properties of CMC produced from cellulosic gel by *Acetobacter xylinum*

4.3.1 Effect of temperature on viscosity of CMC solution

The CMC powder obtained from the optimum condition of previous section was used in this study. The CMC from nata cellulose powder and two types of CMC; low and high viscosity, of commercial (Hycel CMC) were prepared to 1%CMC solution. These three types of CMC were determined the viscosity as the temperature of 20, 30, 40, 50, 60 , 70 and 80°C, respectively. The result were presented in Figure 4.3.

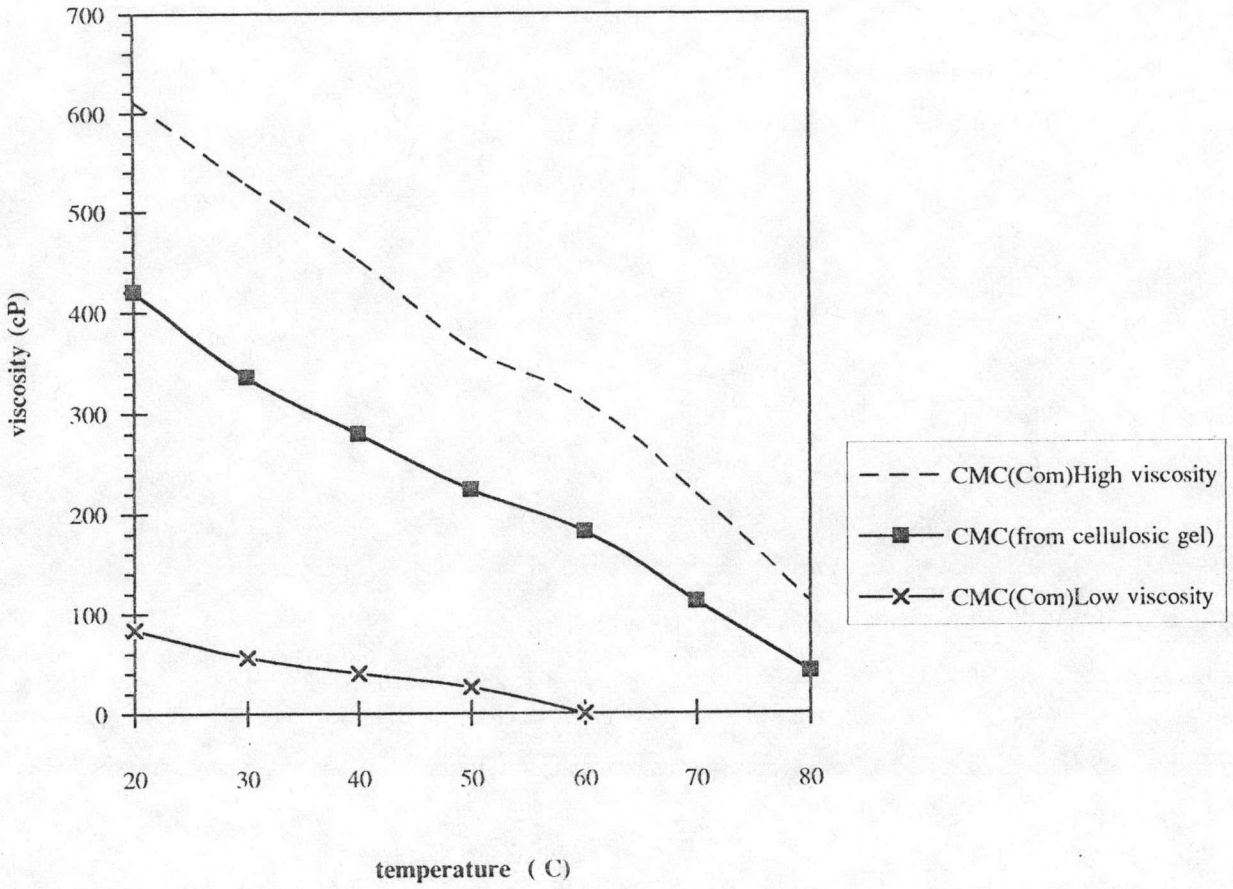

Figure 4.3 Relationship between temperature and viscosity of CMC solution

4.3.2 Effect of pH on viscosity of CMC solution

The CMC from nata cellulose powder and two types of CMC ; low and high viscosity, of commercial (Hycel CMC) were prepared to 1%. CMC solution. Adjusted pH of CMC solution to 2, 3, 4, 5, 6, 7, 8, 9 and 10, respectively. Determined its viscosity by Brookfield viscometer and the results, shown in Figure 4.4, were as followed ;

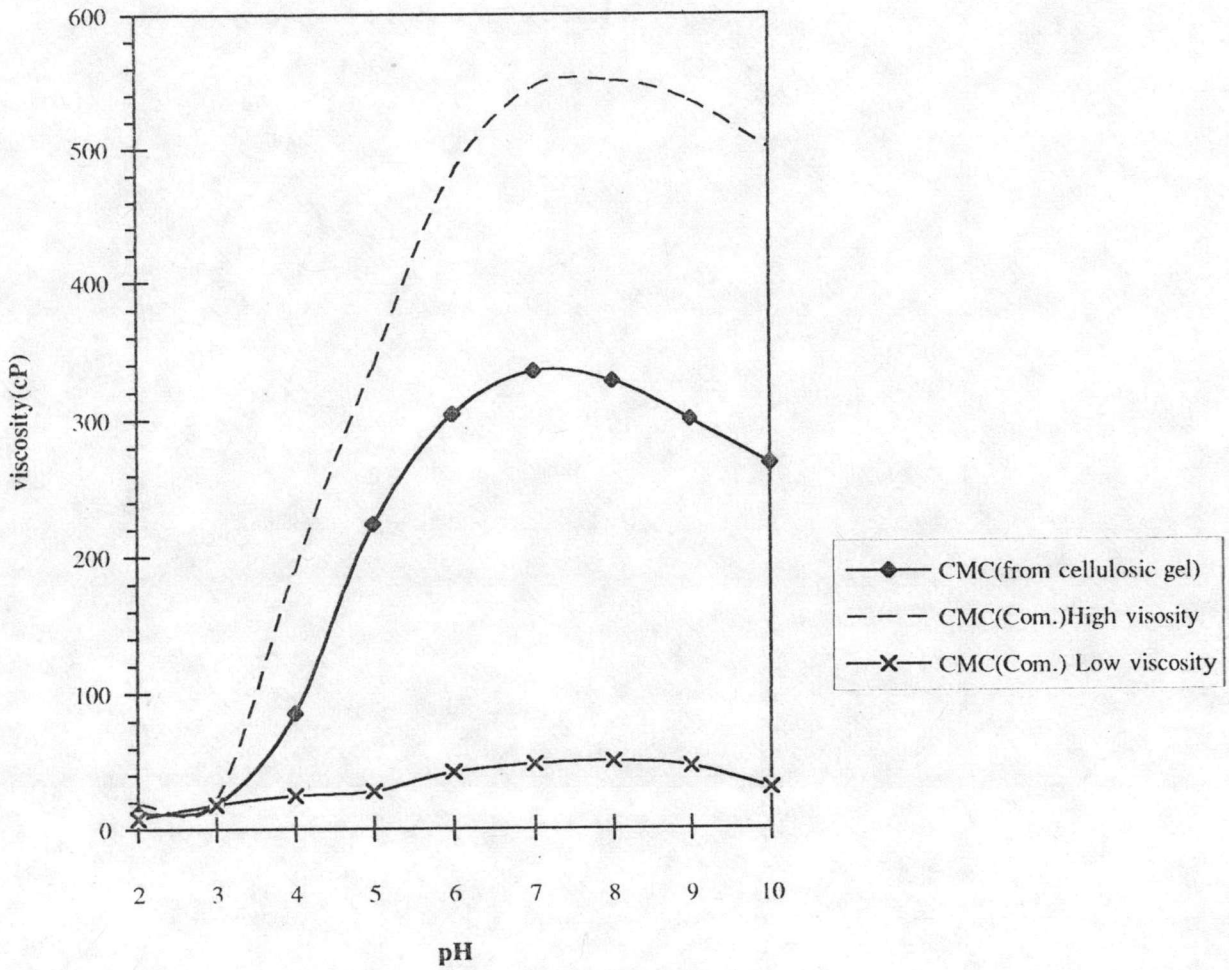

Figure 4.4 Relationship between viscosity (cP) and pH of CMC solution

4.3.3 Relationship between CMC concentration and its viscosity

The CMC from nata cellulose powder and two types of CMC ; low and high viscosity, of commercial (Hycel CMC) were used. Prepared each type of CMC to make concentration of 1, 2, 3, 4, 5 and 6%, respectively. Determined their viscosity by Brookfield viscometer (Figure 4.5).

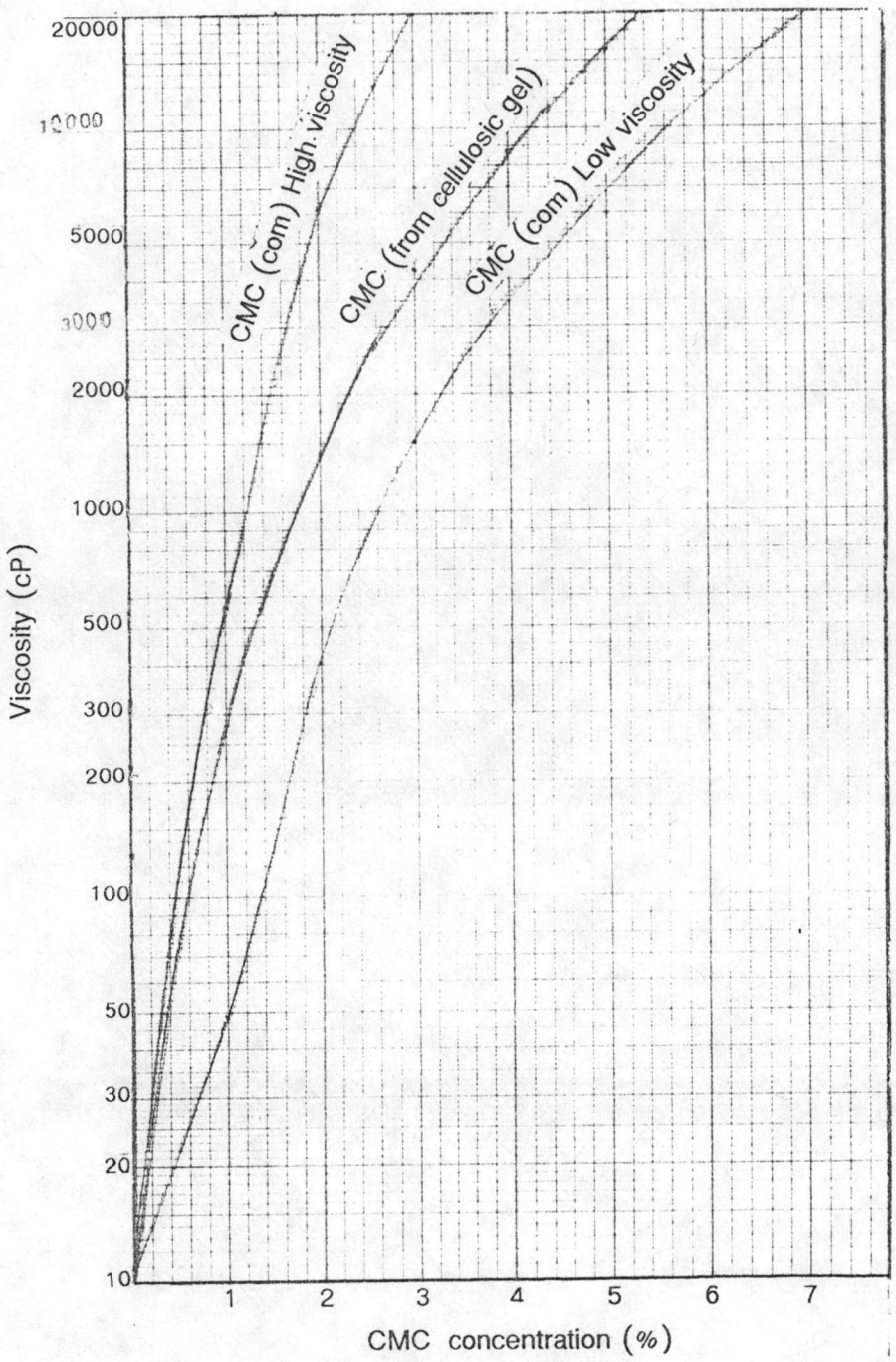

Figure 4.5 Relationship between CMC concentration and its viscosity

The CMC from nata cellulose powder and two grades of CMC of commercial, low and high viscosity, were determined the relationship of temperature, pH and concentration of on the viscosity of CMC solution.

The viscosity of three kinds of CMC ; CMC from nata cellulose powder, CMC of commercial, high and low viscosity, trended to decrease with increasing temperature (Figure 4.3). It could be assumed that temperaure affected to the viscosity of CMC solution. As the temperature increased, the viscosity of CMC solution decreased. Presumably, as the thermal energy of the water molecule increased, hydrogen bonds were broken, and less water was attached to the polymer molecule at the higher temperature. Also, the viscosity of the water phase decreased with increasing temperature (Thai cellulose products Ltd, 1994). This results had the same trend of the other experiments of various gum such as xanthan gum (Pawinee, 1981) and gellose (Puangpetch, 1979).

The effect of pH on viscosity of 1% CMC solution of three types of CMC, it was found in Figure 4.4 that the viscosity was decreased at low pH. That might be due to the polysaccharide ; CMC, could be hydrolyzed by acid (Rock ,1971) so, its viscosity was lowered. The all three kinds of CMC had the highest of their viscosity in the pH 6-9 range. It could be assumed that CMC should be used in low acid food such as ice cream and not suitable for acid food , juices , jam or jelly.

The relationship between the concentration and the viscosity of CMC was investigated. The increase in viscosity of CMC solution was directly proportional to changes in CMC concentration as shown in Figure 4.5. It might be due to as the lower concentration, the molecule could be completely expanded and there is no competition between polymers chains for available water. Also, the water attached to the molecule may be serving as a lubricant, permitting the polymer chains to slide past one another (Graban,1977). As the concentration of polymer increased, there was not enough to permit complete expansion of the chains or sliding; the molecule could become entangled or aggregated, water can be trapped, and larger kinetic units of flow result. Consequently, the viscosity rised rapidly. So, it was more accurate to think of gum concentration in a formulation in term of the water present, than of total formula weight (Ganz, 1977).

With reference of viscosity of CMC solution, the following classification exists ; low viscosity ,25-30 cP in 2% solution, medium viscosity, 400-600 cP in 2%solution, and high viscosity, 1500 cP or above in 1% solution. Consequently, CMC from nata cellulose powder(348 cP in 1% solution) of this experiment could be classified in the type of medium viscosity.

The reference of purity, the following classification exists ; technical grades (NaCMC content 50-72%), purified grades (NaCMC content 98%) and food, pharmaceutical grades (NaCMC content 99.5%). The CMC produced in this experiment which contained NaCMC of 95.67%, the impurity of which was in between the technical and purified grades.

The CMC produced from nata cellulose powder of this study presented low DS value and viscosity and also contained more impurities(sodiumglycolate and sodiumchloride), when compared with CMC of commercial grade with high viscosity. That because of method for CMC producing from nata cellulose powder was the easiest procedure ; all ingredients mixing, which consumed the lowest energy and also more profitable. It could be assumed that the DS value and viscosity of CMC from nata cellulose powder including yield and L-value could be improved by using other CMC production method such as pre-acid hydrolysis method or using under pressure method. The CMC production from wood which used the modified all-ingredients mixing method, multisteps of all-ingredients mixing, the DS value could be improved to 2.76 after passing five steps (Ranee,1976). Therefore, it was so interesting to study the other method of CMC production from nata cellulose powder for improving either its properties or yield.