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Carboxymethylation of Cassava Starch in Different Solvents and Solvent-Water Mixtures: Optimization of Reaction Conditions

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Abstract: The influence of reaction medium on carboxymethylation process was investigated by treating cassava starch with sodium monochloroacetate in different solvents and solvent-water mixtures under alkaline conditions. The amount of carboxyl groups introduced into the starch moiety was determined titrimetrically and used to calculate the Degree of Substitution (DS) and Reaction Efficiency (RE). The results showed that carboxymethylation is significantly affected by the nature of reaction medium at $p<0.05$. Carboxymethylation in different solvent-water mixtures showed that aqueous 80% n-propanol offered the best medium for carboxymethylation. Optimization of reaction conditions in aqueous 80% n-propanol showed that the best condition for carboxymethylation was at starch-liquor ratio of 1:3, NaOH/reagent molar ratio of 4.0 and reagent-starch molar ratio of 0.35. An increase in temperature was required to effect the reaction at shorter time. At 55°C the highest values of DS and RE achieved in 0.5 h would require three hours to achieve the same values of DS and RE at 45°C.

Key words: Cassava starch, carboxymethylation, solvent-water mixtures, optimization, degree of substitution, reaction efficiency

INTRODUCTION

Starches are used in large quantities in various industrial applications to provide body and consistency to solutions (Ragheb *et al.*, 1995). However, natural starches often do not match the properties required for a particular application (BeMiller, 1997). The inherent objectionable characteristics of native starches include poor aqueous dispersion and poor freeze thaw stability.

Carboxymethylation is used to improve aqueous dispersibility and cold storage stability of starch pastes. In carboxymethylation, the hydroxyl groups of linear amylose and branched amylopectin molecules of starch granule are derivatized to form carboxymethyl starch ethers. This derivatization interferes with the side-by-side alignment of starch hydroxyl groups such that when the starch is pasted in water, the granules disrupt and the carboxymethyl groups stabilize the aqueous dispersion through improved starch-water interaction. In addition, since carboxymethylation occurs without degradation of starch molecules, they are excellent thickeners and have found application in many industrial formulations (Nabais *et al.*, 2007; Kittipongpatana and Sirithunyalug, 2006; Kim and Lim, 1999; Ragheb *et al.*, 1997). The numerous applications of carboxymethyl starch require large-scale production of it to match demand. Hence the

need to study conditions which optimize the carboxymethylation process. A few of these works have been reported by Khalil *et al.* (1990), Raval *et al.* (1994) and Tijsen *et al.* (2001). This study was carried out in continuation of the search for the best medium and the reaction conditions which favor carboxymethylation process.

MATERIALS AND METHODS

Materials: The cassava starch sample used in the carboxymethylation reactions was prepared in our laboratory as described elsewhere (Ogunmola *et al.*, 2001). The sodium monochloroacetate was prepared from monochloroacetic acid according to the method of Green (1963). All other chemicals used were products of British Drug House and of laboratory grade.

Carboxymethylation of starch in different solvent-water mixtures: Starch (5.0 g (dry basis, d,b)) was weighed into large heating tubes, to each was added 10 mL of appropriate solvent-water mixtures (0-80% solvent range) containing 28 mmol of sodium hydroxide. This was followed by addition of 1.08 g of sodium monochloroacetate and the dispersions thoroughly mixed. The dispersions were set up in a thermostatic Clifton

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water bath maintained at 45°C for 1 h. The dispersions were kept agitated throughout the reaction. At the end of reaction time, the samples were brought down and recovered by washing with excess aqueous 80% methanol and finally with methanol. Plasticized samples were granulated by grinding in excess aqueous 80% methanol and finally washed with methanol. The modified starch materials were dried in the oven at 100°C for 1 h and stored. The solvents used were xylene, n-propanol, methanol and water.

Carboxymethylation of starch in aqueous 80% n-propanol

Effect of Starch-Liquor Ratio (SLR): Starch (5.0 g d,b) was weighed into large heating tubes, each containing 28 mmol of sodium hydroxide in different volumes of aqueous 80% n-propanol to give different SLRs. This was followed by the addition of 1.08 g of sodium monochloroacetate and the dispersions thoroughly mixed. The samples were set up in a thermostatic Clifton water bath maintained at 45°C for 1 h. At the end of reaction time, the modified starches were processed as described earlier.

Effect of sodium hydroxide-reagent molar ratio (NaOH/RMR): The effect of NaOH/RMRs at SLR of 1:3 was investigated by treating starch (5.0 g, d.b.) in different large heating tubes with 15 mL of aqueous 80% n-propanol containing different amounts of sodium hydroxide (9.25-65.25 mmol). This was followed by the addition of 1.08 g sodium monochloroacetate and the dispersions thoroughly mixed. The samples were kept in a thermostatic Clifton water bath maintained at 45°C for 1 h. At the end of reaction time, the modified starch samples were processed as earlier described and stored.

Effect of Reagent-Starch Molar Ratio (RSMR): The effect of RSMR was investigated at NaOH/RMR of 4.0 and SLR of 1:3 in aqueous 80% n-propanol containing 37.25 mmol of sodium hydroxide. Thus to 5.0 g starch (d,b) in large heating tubes containing the stated reaction mixture were added different amounts of sodium monochloroacetate to give different RSMRs (0.1- 0.7). The dispersions were kept in a Clifton water bath maintained at 45°C for 1 h and resulting product processed as earlier described.

Effect of duration: The effect of duration was investigated at NaOH/RMR of 4.0, RSMR of 0.35 and SLR of 1:3, in aqueous 80% n-propanol at different reaction times of 0.5-5.0 h in a thermostatic Clifton water bath at 45°C. The modified starch samples were processed as earlier described and stored.

Effect of temperature: Starch was treated at the same reaction conditions as used in effect of duration except that the reaction temperature was maintained at 55°C. The resulting modified starch samples were processed as earlier described and stored.

Analysis by reaction parameters

Determination of percentage substitution: The percentage substitution was determined according to the method of Wing (1996) with some modification. 1.0 g of carboxymethyl starch was converted to the H-form by treating with excess 0.1 M aqueous 80% methanolic HCl in a 100 mL beaker with occasional stirring for 1 h. This was filtered and washed under suction in a sintered glass funnel with aqueous 80% methanol until free from acid (filtrate had no effect on blue litmus paper). The resulting sample was dried in the oven at 100°C for 1 h and cooled in a desiccator. 0.25 g of the sample was weighed into a 250 mL conical flask and 100 mL distilled water added, followed by 10 mL of standard NaOH solution. This was heated over a boiling water bath for 20 min when a clear solution resulted. The hot solution was titrated with standard HCl solution to a phenolphthalein endpoint. Native starch processed similarly as above was used as correction factor for the blank. Each sample analysis was carried out in triplicate and values averaged.

The percentage carboxyl was calculated as follows:

$$\text{Carboxyl (\%)} = \left[\frac{\text{Titre for sample}}{\text{Weight of sample}}\right] - \left[\frac{\text{Titre for native starch}}{\text{Weight of native starch}}\right] \times$$
$$\text{Molarity of NaOH} \times 0.045 \times 100$$

Determination of degree of substitution and Reaction Efficiency (RE): The DS was calculated according to the method of Khalil *et al.* (1990) and the RE by the method of Kweon *et al.* (1996).

$$DS = \frac{162 \times \%\text{Carboxyl}}{4500 - 58 \times \%\text{Carboxyl}}$$

$$RE (\%) = \frac{DS \times 100}{RSMR}$$

Statistical analysis: One-way Analysis of Variance (ANOVA) was conducted on each of the variables. Duncan multiple range test at $p < 0.05$ was performed using SPSS/11 software for windows to compare the differences among means. The results were expressed as the means±standard deviation of three separate readings. Non-linear regression was performed using Microsoft

Excel 2003 software to generate the regression models and the corresponding R^2 values.

RESULTS AND DISCUSSION

Sodium monochloroacetate reacts with starch in the presence of sodium hydroxide to form carboxymethyl starch. The process involves two main reactions:

- The carboxymethylation reaction which proceeds through the following steps:

$$\underset{\text{Starch}}{St-OH} + NaOH \xrightarrow{\text{alkalization}} \underset{\text{sodium starch}}{St-ONa} + H_2O \qquad (1)$$

$$St-ONa + Cl-CH_2COONa \xrightarrow{\text{carboxymethylation}} \underset{\text{sodium carboxymethylstarch}}{St-OCH_2COONa} + NaCl \qquad (2)$$

Overall,

$$St-OH + Cl-CH_2COONa \xrightarrow{\text{NaOH}} St-OCH_2COONa + H_2O + NaCl \qquad (3)$$

- The hydrolysis reaction leads to loss of sodium monochloroacetate in side reactions with formation of sodium glycolate.

$$Cl-CH_2COONa + NaOH \xrightarrow{\text{Hydrolysis}} HOCH_2COONa + NaCl \qquad (4)$$

The extent of carboxymethylation depends on which of the two reactions-carboxymethylation and hydrolysis-prevails under a set of reaction conditions.

Since the main objective of this study was to establish the conditions which favor carboxymethylation, the effect of nature of reaction medium, SLR, NaOH/RMR, RSMR, duration and temperature were monitored by following the DS and RE to optimize the reaction conditions.

Effect of nature of reaction medium: Table 1 shows that DS and RE varied significantly ($p < 0.05$) with percentage solvent in solvent-water mixtures. With a given solvent,

the highest DS was obtained in a reaction medium consisting aqueous 80% solvent. Khalil *et al.* (1990) have reported similar observation. Aqueous 80% n-propanol gave the highest DS and therefore provides the best medium for carboxymethylation reaction. Figure 1 shows the effect of solvent dielectric constant on the carboxymethylation reaction. The DS was not significantly different in xylene, methanol and water, but different in n-propanol. Among the hydroxy solvents, that is, n-propanol, methanol and water, the DS decreased with increase in solvent dielectric constant (Marsden and Mann, 1963), hence hydrolytic loss of monochloroacetate was favored by increasing solvent polarity. The low DS in xylene may be attributed to its large molecular size (Patel *et al.*, 1993).

Since aqueous 80% n-propanol provided the best medium for reaction, further carboxymethylation studies were carried out in it.

Optimization of carboxymethylation reaction in aqueous 80% n-propanol

Effect of Starch-Liquor Ratio (SLR) on extent of carboxymethylation of starch: Figure 2 shows the effect of SLR on extent of carboxymethylation. An increase in DS was obtained from SLR 1:1 to 1:3 and decreased again as SLR increased to 1:5. It is worth noting the limiting effect of SLR on extent of carboxymethylation. The results show that there is a critical amount (volume) of liquor required for swelling of the starch granule and dissolving

Fig. 1: Effect of solvent dielectric constant on degree of substitution of starch

Table 1: Effect of percentage solvent in solvent-water mixture on extent of carboxymethylation of starch

Solvent (%) in solvent-water mixture	Xylene		Methanol		N-propanol	
	DS	RE (%)	DS	RE (%)	DS	RE (%)
0	0.072 ± 0.004^a	24.0 ± 1.3^a	0.072 ± 0.004^a	24.0 ± 1.3^a	0.072 ± 0.004^a	24.0 ± 1.3^a
20	0.073 ± 0.000^a	24.3 ± 0.0^a	0.072 ± 0.004^a	24.0 ± 1.3^a	0.081 ± 0.008^b	27.0 ± 2.7^b
40	0.100 ± 0.002^b	33.3 ± 0.5^b	0.073 ± 0.000^a	24.3 ± 0.0^a	$0.076 \pm 0.000^{a,b}$	$25.3 \pm 0.0^{a,b}$
60	0.112 ± 0.002^b	37.3 ± 0.5^c	0.085 ± 0.000^b	28.3 ± 0.0^b	0.134 ± 0.005^c	44.7 ± 1.7^c
80	0.150 ± 0.000^c	50.0 ± 0.0^d	0.160 ± 0.002^c	53.3 ± 0.5^c	0.194 ± 0.002^e	64.7 ± 0.5^e
100	0.070 ± 0.000^a	23.3 ± 0.0^a	0.085 ± 0.002^b	28.3 ± 0.5^b	0.180 ± 0.001^d	60.0 ± 0.4^d

[a-e]: Means in the same column with different superscripts are significantly different at $p < 0.05$, Values are means of triplicates±standard deviation

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the reagents as well as facilitating dissociation, diffusion and adsorption of the etherifying agent on starch granule for reaction to occur. The SLR 1:3 constitute that optimum for aqueous 80% n-propanol. Khalil *et al.* (1990) have reported maximum DS at SLR 1:2.5 in water. The gradual decrease in DS above SLR 1:3 shows the effect of increasing dilution on extent of carboxymethylation.

Effect of sodium hydroxide-reagent molar ratio (NaOH/RMR): The result in Fig. 3 shows that the DS and RE increased to a maximum at NaOH/RMR of 4.0 and then decreased sharply again with increasing NaOH/RMR. This observation points to the role of sodium hydroxide in the carboxymethylation reaction. Sodium hydroxide is needed to swell the starch granule to facilitate the carboxymethylation process. Sodium hydroxide attacks the starch molecule to form sodium starchate, which then reacts with the sodium monochloroacetate in a nucleophilic attack to form the carboxymethyl starch (Eq. 1 and 2). For a high DS to take place, sufficient alkali must be present to generate enough starchate ions for the reactions. This optimal alkali concentration was obtained at NaOH/RMR of 4.0. This is why the DS and RE increased gradually up till NaOH/RMR of 4.0. The highest DS obtained was 0.205 and the corresponding RE was 68.3%. Above this NaOH/RMR, excess alkali was present in the reaction medium, the resultant effect was the hydrolytic loss of the sodium monochloroacetate in side reactions and the formation of sodium glycolate (Eq. 4). Thus carboxymethylation and hydrolysis were competing reactions in the process. Carboxymethylation prevailed up till NaOH/RMR of 4.0 but above this point hydrolysis of the monochloroacetate predominated. This explains why both the DS and RE decreased at higher NaOH/RMR.

Effect of Reagent-Starch Molar Ratio (RSMR): From Fig. 4, the increase in DS against RSMR obeyed a second-order polynomial (R^2 = 0.94), conversely the RE decreased polynomially (R^2 = 0.79) against RSMR. The highest RE was 98.0% at RSMR of 0.1. The intercept of the R.E and DS curves was at RSMR of 0.35. Thus RSMR of 0.35 corresponds to the optimum RSMR for carboxymethylation of starch with best DS and RE under the stated conditions in aqueous 80% n-propanol. The DS under this condition was 0.230 and its corresponding RE was 65.0%. The enhancement of DS by increasing the RSMR is equivocally due to greater availability of the sodium monochloroacetate in the proximity of the starch molecules, since the sites of carboxymethylation are the starch hydroxyl groups, which are immobile. On the other hand, the decrease in RE is attributed to the increasing
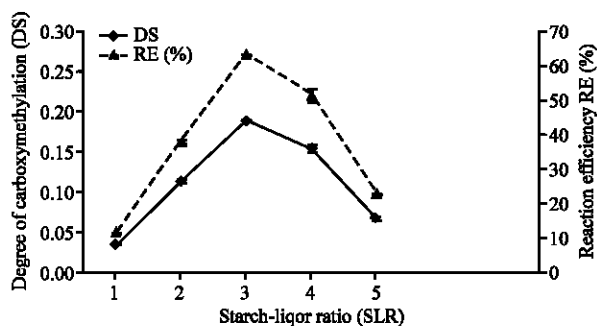

Fig. 2: Effect of starch-liquor ratio on extent of degree of substitution in aqueous 80% n-propanol
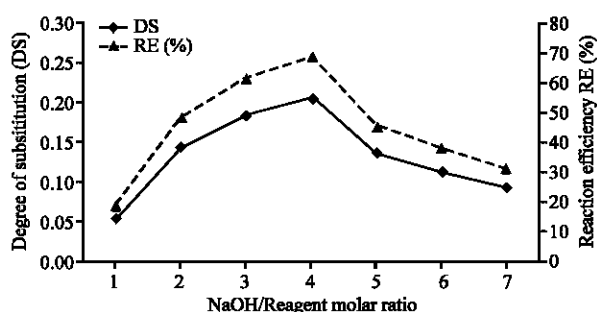

Fig. 3: Effect of NaOH/reagent molar ratio on degree of substitution in aqueous 80% n-propanol
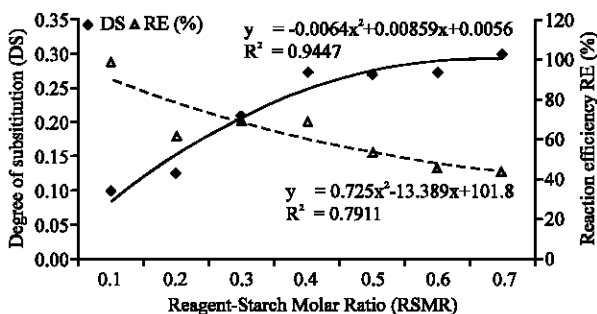

Fig. 4: Effect of reagent starch molar ratio on degree of substitution in aqueous 80% n-propanol

concentration of sodium monochloroacetate in the alkaline reaction medium, which favored glycolate formation (Khalil *et al.*, 1990).

Effect of time and temperature: From Fig. 5, the DS and RE varied polynomially with time of reaction at both 45°C and 55°C. There was better correlation between DS and RE with reaction time at 45°C (R^2 = 0.98) than at 55°C (R^2 = 0.78). At 45°C, the maximum DS and RE of 0.297 and 84.8%, respectively were achieved after 3 h treatment, whereas at 55°C under the same conditions, the highest DS and RE of 0.296 and 84.6%, respectively were obtained
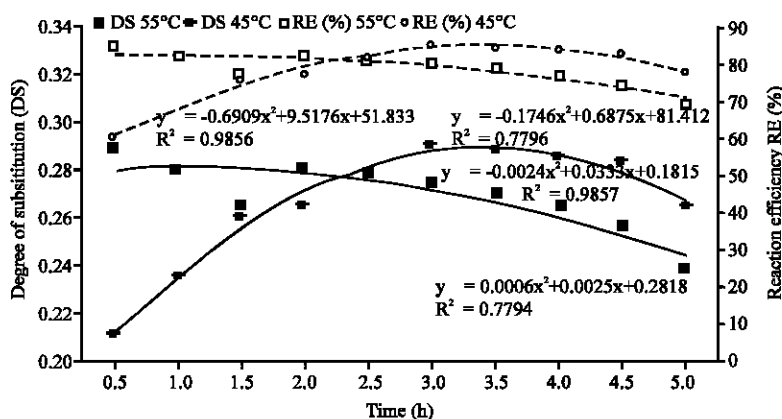
Fig. 5: Effect of duration and temperature on degree of substitution in aqueous 80% n-propanol

after 0.5 h. This shows that the rate of carboxymethylation is greatly increased with rise in temperature.

CONCLUSION

Cassava starch was carboxymethylated in different solvents and solvent-water mixture. The results showed that the extent of carboxymethylation is a function of nature of solvent used. In all reaction media, aqueous 80% solvent gave the highest degree of carboxymethylation, with aqueous 80% n-propanol providing the best medium for caboxymethylation.

Optimization studies carried out in aqueous 80% n-propanol showed that SLR of 1:3, NaOH/RMR of 4.0 and RSMR of 0.35 gave the best result in terms of DS and RE. A higher temperature was required to effect the reaction at shorter time as indicated by the highest values of DS and RE being achieved at 55°C in 0.5 h against 3.0 h required to attain the same values of DS and RE at 45°C.

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