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Optimization of cationic amino starch synthesis using biogenic amines

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ABSTRACT

Harvesting microalgae presents a challenge in selecting the most economical method for low cost algal bioproducts. Previous studies have shown coagulation–flocculation to be the most efficient method for large scale microalgae harvesting. This study focused on modifying native potato starch with biogenic amines and optimizing the reaction parameters. Such modification rendered the starch cationic, with an ability to destabilize microalgae suspensions or colloids. The effect of time, temperature, and reactant concentrations on the zeta potential of the cationic amino starch was studied. Biogenic amines including putrescine, histamine, cadaverine, and tyramine were selected for study based on the number of nitrogen groups in their structure. Zeta potential for histamine cationic amino starch was significantly higher ($+9.0 \pm 2.0$ mV) at lower reaction temperatures, regardless of the amine to starch ratio and reaction time intervals. Putrescine, cadaverine, and tyramine cationic amino starches exhibited significantly higher zeta potential values (13.76 ± 3.60, 6.81 ± 1.64, and 5.68 ± 1.60 mV, respectively) with amine to starch ratio higher than reaction stoichiometry, irrespective of reaction temperature or time intervals. This optimization study has presented a basis for designing reaction conditions for the synthesis of cationic amino starch from an inhomogeneous mix of biogenic amines derived from waste sources.

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1. Introduction

Starch is the most abundant natural polymer, which is stored as a major source of carbohydrate reserve in the stem, roots, and grains of all plants. Due to its flexibility as a feedstock, it has been exploited for numerous industrial applications (Tharanathan, 2005). Starch primarily consists of a mixture of amylose and amylopectin. Amylose is a linear polymer of 1-4 linked α-D-glucopyranosyl linkages and constitutes 20–40% weight of the starch. Amylopectin, which constitutes about 60–80% of the total weight exhibits a highly branched structure with 1-4 linked α-D-glucopyranosyl linkages branched at 1–6 bonds (Pal, Mal, & Singh, 2005). Starches in their native form are often unsuitable for most applications and hence need to be modified either chemically and/or physically to improve their properties. Chemical modification of starch is more stable and consists of esterification, etherification, grafting, or oxidation of the available hydroxyl groups on the anhydrous glucose unit of starch to add the desired functional groups (Chiu & Solarek, 2009). One such modification, which incorporates cationic groups to the starch backbone is known as cationization and is discussed here.[1]

Cationization of starch, which is the attachment of cationic groups such as amino, ammonium, sulfonium, imino, or phosphonium to the starch molecule, can be performed by any of the modification methods listed (Chiu & Solarek, 2009). Conventionally, cationic starches are prepared by the reaction of quaternary ammonium on the starch backbone, which provides the necessary cationic charge. Literature is abundant with studies on cationic starch synthesis with quaternary ammonium using all the known starch modification methods (Carr & Bagby, 1981; Ellis, Utah, Abiola, & Ogedengbe, 1982; Hunt & Hunt, 1974; You, Lu, Li, Qiao, & Yeping, 2009). Phosphonium cationic starches have been synthesized by Aszalos (1963) to exhibit specific properties such as "viscosity-stability" and cationicity. Similarly, sulfonium cationic starch synthesized by etherification with 2-chloroethyl-methyl-ethyl sulfonium iodide resulted in improved viscoelastic properties and cationicity (Rutenberg, Plainfield, Volpe, & New Brunswick, 1961). Cationic starches have been traditionally used in the paper industry as wet-end additives for dry strength and as a sizing agent. However, they have been used in wastewater treatment (Ellis et al., 1982) and microalgae harvesting (Vandamme, Foubert, Meesschaert, & Muylaert, 2009) to a certain extent. In wastewater treatment and microalgae harvesting, cationic starch acts by coagulation or charge neutralization of the particles in suspension after which the inherent polymeric structure of the starch aids in bridging the neutralized particles to form flocs, which are separated by gravity settling.

Although effective, the use of cationic starch synthesized from ammonium, sulfonium, and phosphonium groups in harvesting microalgae have detrimental impact on the environment and downstream processes including toxicity and antibacterial

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[1] Biogenic amines (BA), cationic amino starch (CAS), degree of substitution (DS), statistical analysis software (SAS), and anhydrous glucose unit (AGU).

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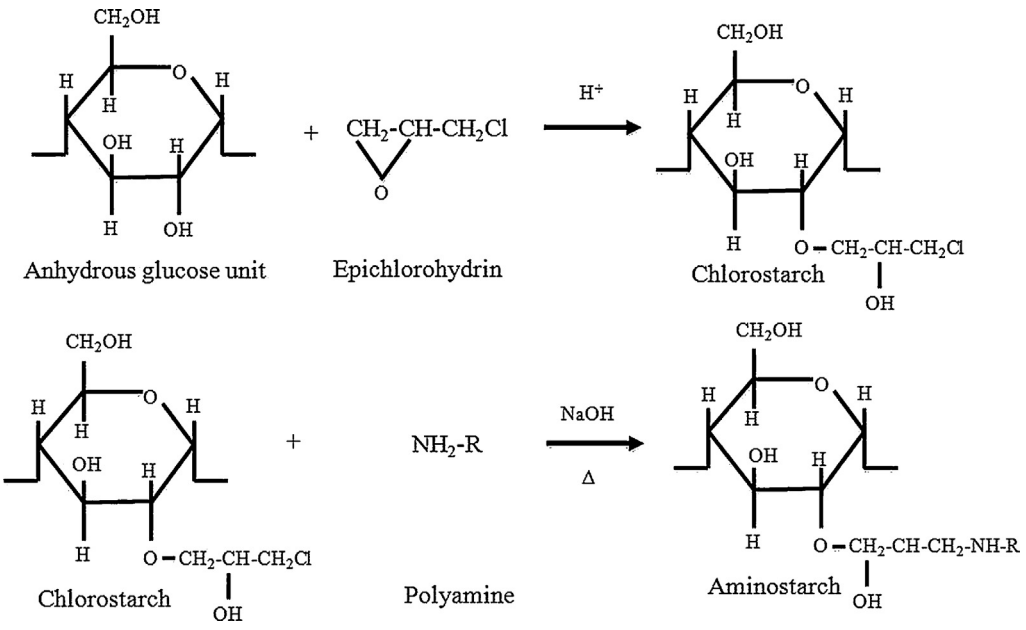

Fig. 1. General reaction scheme for the synthesis of cationic amino starch.

properties (Ohta, Kondo, Kawada, Teranaka, & Yoshino, 2008). These properties may potentially inhibit downstream processes such as anaerobic digestion or fermentation using the cationic starch harvested biomass as feedstock (Cathey, 1964; García, Campos, Sanchez-Leal, & Ribosa, 1999). Besides, the reagents containing these functional groups are obtained from non-renewable sources and thus not sustainable for large scale microalgae harvesting.

This study focused on identifying an inexpensive, renewable compound to replace the traditional cationic functional groups. Biogenic amines (BA1), which are naturally occurring amines formed by microbial decarboxylation of amino acids (Santos, 1996; Visciano, Schirone, Tofalo, & Suzzi, 2012), proved to be the most suitable alternative due to their abundance and renewability. BAs have been reported in variety of foods, such as fish, meat, cheese, vegetables, or any product that contains proteins and/or amino acids (Naila, Flint, Fletcher, Bremer, & Gerrit, 2010). BAs are indicators of toxicity in foods and are sometimes found in high concentrations (100 mg/kg) in meat products (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994). BAs are classified as aromatic amines, which are histamine and tyramine, aliphatic diamines, which are putrescine and cadaverine, and aliphatic polyamine, agmatine (Ruiz-Capillas & Jiménez-Colmenero, 2004).The abundance of BAs in the waste streams of meat processing industries can be utilized by extracting these amines and synthesizing cationic amino starch (CAS).

Biogenic amines in food are found due to the breakdown of the 20 naturally occurring amino acids present therein (Hornback, 2005). The nitrogen in these amines is the cationic group that plays the most important role in providing the desired cationicity to the cationic amino starch. In terms of the number of nitrogen groups present, the 20 proteinogenic amino acids were divided into four categories that included arginine containing four nitrogen atoms, histidine containing 3 nitrogen atoms, lysine, asparagine, glutamine, and tryptophan containing 2 nitrogen atoms, and tyrosine and others with one nitrogen atom. This study focused on synthesizing cationic amino starch (CAS) with four different amines representing each of the four groups of amino acids classified previously. The four amines including putrescine, histamine, cadaverine, and tyramine were chosen for synthesizing cationic amino starch using potato starch as the substrate. Putrescine is the resultant polyamine generated by the decarboxylation of arginine and was chosen to represent one of the four amino acid groups (Lawrence, 2004). Cationic amino starch was prepared by a two step process, which involved halogenation of starch and subsequent alkylation with amines. In addition to the reagents, reaction parameters such as time and temperature were optimized using the zeta potential of CAS as the performance indicator. The objectives of this research were to optimize time, temperature, and amine to starch mass ratio in the synthesis of cationic amino starch independently with putrescine, histamine, cadaverine, and tyramine.

2. Materials and methods

Potato starch, histamine, tyramine, putrescine, cadaverine, and epichlorohydrin were obtained from Sigma–Aldrich (St. Louis, MO). All reagents were used as received. In the preparation of CAS, the first step was to halogenate starch by reacting 1.0 g of starch with 1.8 mL of epichlorohydrin and 50 μl of hydrochloric acid for 1 h at 110 °C. The halogenated starch was then alkylated by adding biogenic amines in varying ratio of amine to starch and reacted for 4, 8, and 12 h in 0.16 N NaOH solution at 60, 80 and 100 °C (Fig. 1). After completion of the reaction, the CAS was precipitated out of solution using ethanol as needed and washed with ethanol in a Soxhlet apparatus for 4 h. After washing, the CAS was dried of ethanol, pulverized, and stored until further use.

The zeta potential of the starch was measured using Brookhaven ZetaPlus zeta meter (Holtsville, NY) to determine the extent of cationization of CAS. Zeta potential is a measure of the average surface charge of the particles in a colloidal suspension, measured in millivolts. Surface charges on particles arise mainly due to ionization of surface groups, adsorption of charged species and differential loss of ions from crystal lattice (Hubbard, 2002). The zeta potential for colloidal systems is measured by the electrophoresis phenomenon of dispersions that cause movement of charged particles within an electric field (Dukhin & Goetz, 2002). For cationic amino starch to be used as a coagulant, the highest possible positive zeta potential was desired. The magnitude of zeta potential depends on the degree of substitution of the cationic starch. The degree of substitution (DS) is the average number of hydroxyl

Table 1
Experimental matrix for the effect of time, temperature, and mass ratio of starch to amines on zeta potential values of cationic amino starch.

Ratio amine: starch		Putrescine CAS			Histamine CAS			Cadaverine CAS			Tyramine CAS			Time (h)
		Temperature (°C)			Temperature (°C)			Temperature (°C)			Temperature (°C)			
		60 C1	80 C2	100 C3	60 C1	80 C2	100 C3	60 C1	80 C2	100 C3	60 C1	80 C2	100 C3	
R1 0.5	i	5.89	7.42	5.86	9.32	1.33	2.06	0.89	3.33	0.15	0.03	−0.06	−3.17	4
	ii	4.70	8.45	7.82	13.76	2.35	1.11	3.25	2.61	1.45	−0.09	−0.85	−1.47	8
	iii	6.55	6.41	1.70	10.26	0.62	4.02	−1.4	2.21	3.53	−1.19	−1.20	−9.91	12
R2 1.0	i	7.06	13.48	10.31	6.9	1.53	2.58	3.68	6.78	4.72	1.81	1.53	−0.87	4
	ii	7.77	13.10	19.56	2.29	4.73	4.7	7.31	3.03	1.68	2.22	2.40	5.26	8
	iii	14.43	11.48	11.32	11.48	3.3	4.84	7.72	3.43	4.77	1.38	1.79	3.12	12
R3 2	i	12.45	19.45	2.71	9.44	3.47	6.39	5.73	4.08	0.48	1.05	2.65	2.75	4
	ii	14.76	18.25	0.82	14.01	6.01	1.53	11.97	8.97	6.3	4.92	4.57	9.80	8
	iii	20.56	17.47	17.35	3.55	5.96	5.89	8.16	6.6	8.97	7.86	9.29	8.24	12

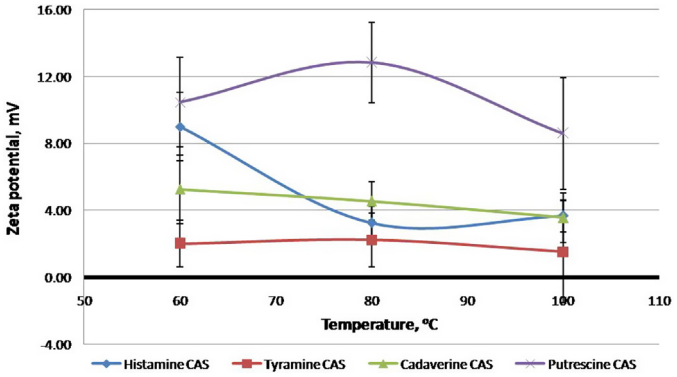

Fig. 2. Temperature effect on the zeta potential of the cationic amino starch synthesized.

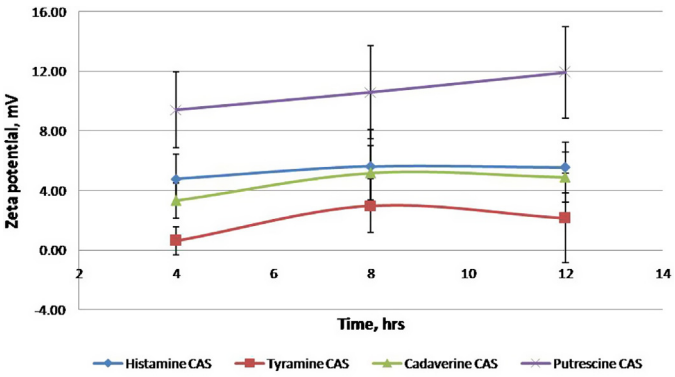

Fig. 4. Effect of reaction time on the zeta potential of the cationic amino starch synthesized.

groups that have been substituted in one anhydrous glucose unit (AGU) of starch. The degree of substitution was calculated using Equation 1 by measuring the total nitrogen content of the CAS using Lachat QuikChem 8500 (Loveland, CO) employing the 4500-N B Standard Methods (Clescerl, Greenberg, & Eaton 1999).

$$\text{Degree of substitution (DS)} = \frac{162 \times N\%}{1400 \times n - (M \times N\%)}$$

Where, 162 = molecular weight of one anhydrous glucose unit of starch, M = molecular weight of the biogenic amine in consideration, $N\%$ = wt% of nitrogen in starch, and n = number of nitrogen atoms in the biogenic amines in consideration (n for tyramine = 1, n for putrescine and cadaverine = 2, n for histamine = 3). The DS can range between 0 and 3.

After identifying the optimum reaction conditions, cationic amino starches were synthesized independently with the biogenic amines. A zeta potential titration curve (pH 5–10) was developed for each cationic amino starch in order to evaluate the CAS zeta potential with respect to pH and to identify the iso-electric point of the CAS. ^{13}C NMR was performed on the cationic amino starches using Jeol ECX-300 in D_2O at 298 K. The spectra from ^{13}C NMR provided verification of attachment of the biogenic amines to starch.

3. Results and discussion

The data collection matrix is shown in Table 1. The matrix is divided into four repeating sections for the four different amines. The rightmost column represents the reaction time intervals and the leftmost column represents the amine to starch mass ratios. Reaction temperatures are represented at the top of each section

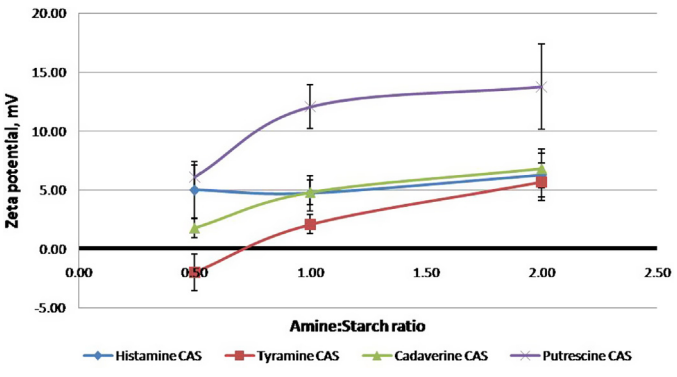

Fig. 3. Effect of amine to starch ratio on the zeta potential of the cationic amino starch synthesized.

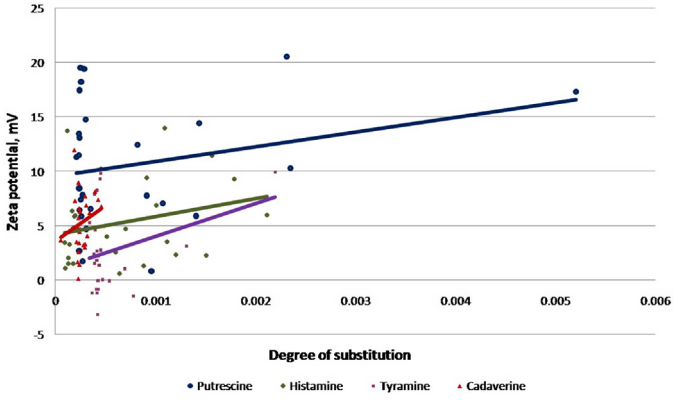

Fig. 5. Correlation between zeta potential and degree of substitution for cationic amino starch.

Table 2
Multiple regression analysis result for the four CAS with *P*-values and beta coefficients.

	Putrescine CAS		Histamine CAS		Cadaverine CAS		Tyramine CAS	
	Pr > |t|	β	Pr > |t|	β	Pr > |t|	β	Pr > |t|	β
Ratio	0.0068	0.51289	0.354	0.15704	0.0003	0.63646	<.0001	0.75428
Time	0.3011	0.18261	0.6204	0.08334	0.1739	0.20729	0.2455	0.15792
Temperature	0.4426	−0.13486	0.002	−0.57843	0.1439	−0.22355	0.7252	−0.04715

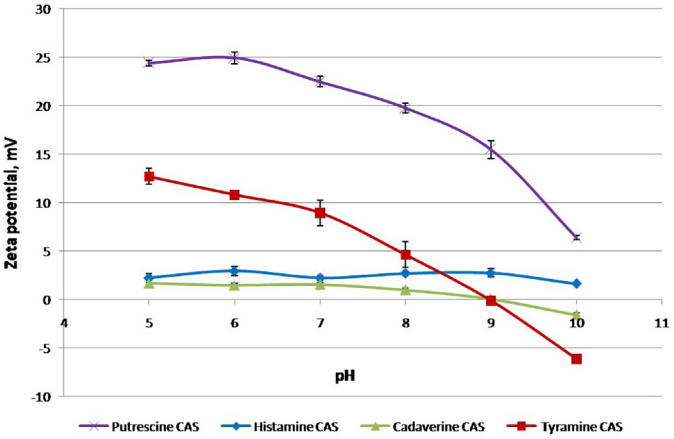

Fig. 6. Zeta potential titration curves across pH 5–10 for the four cationic amino starch synthesized.

and zeta potential of the cationic amino starch is the dependent variable measured.

3.1. Effect of reaction temperature on zeta potential of the cationic amino starch

To independently analyze the effect of temperature on the zeta potential of cationic amino starch synthesized with putrescine, histamine, cadaverine, and tyramine, zeta potential readings for the samples of CAS were lumped based solely on different reaction temperatures, irrespective of the ratios and reaction times. Zeta potential readings from columns C1, C2, and C3 in Table 1 for the individual amine CAS was averaged to represent the temperature effect on the cationicity of the CAS synthesized (Fig. 2). Histamine CAS showed a significant temperature effect on the zeta potential based on standard deviation suggesting higher zeta potential attainment with lower reaction temperature for the range tested. At 60 °C, histamine CAS achieved a zeta potential of +9.0 ± 2.0 mV. No significant difference was observed based on standard deviation among the zeta potential values

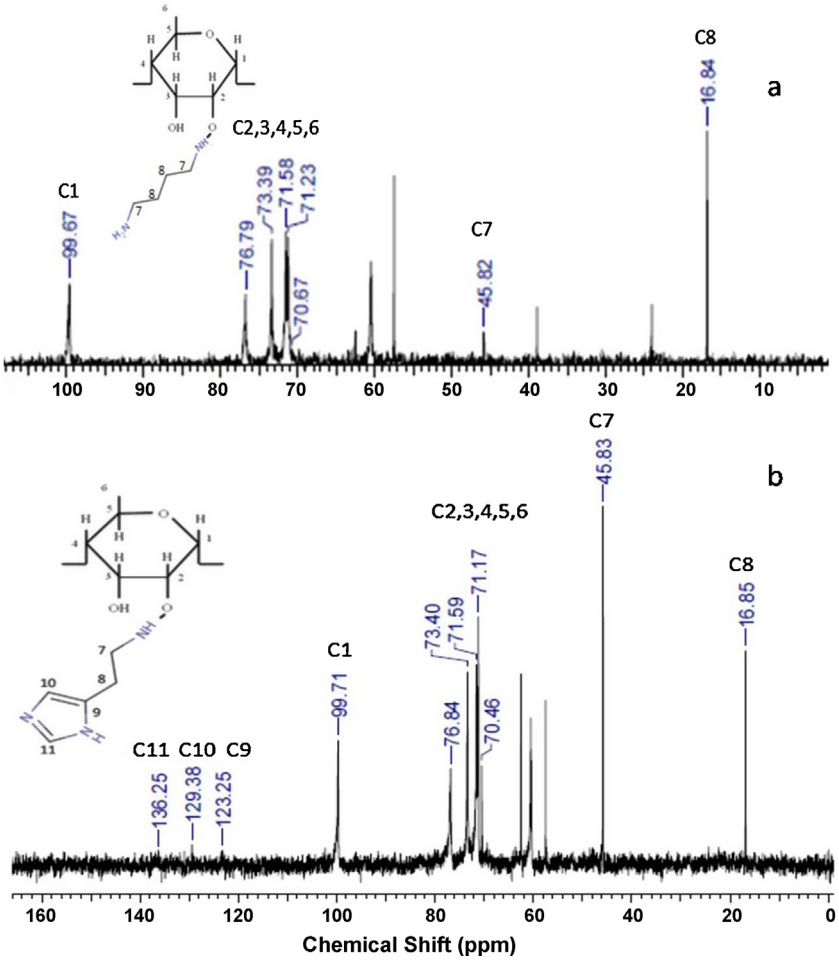

Fig. 7. ^{13}C NMR spectra for cationic amino starch (a) putrescine CAS and (b) histamine CAS.

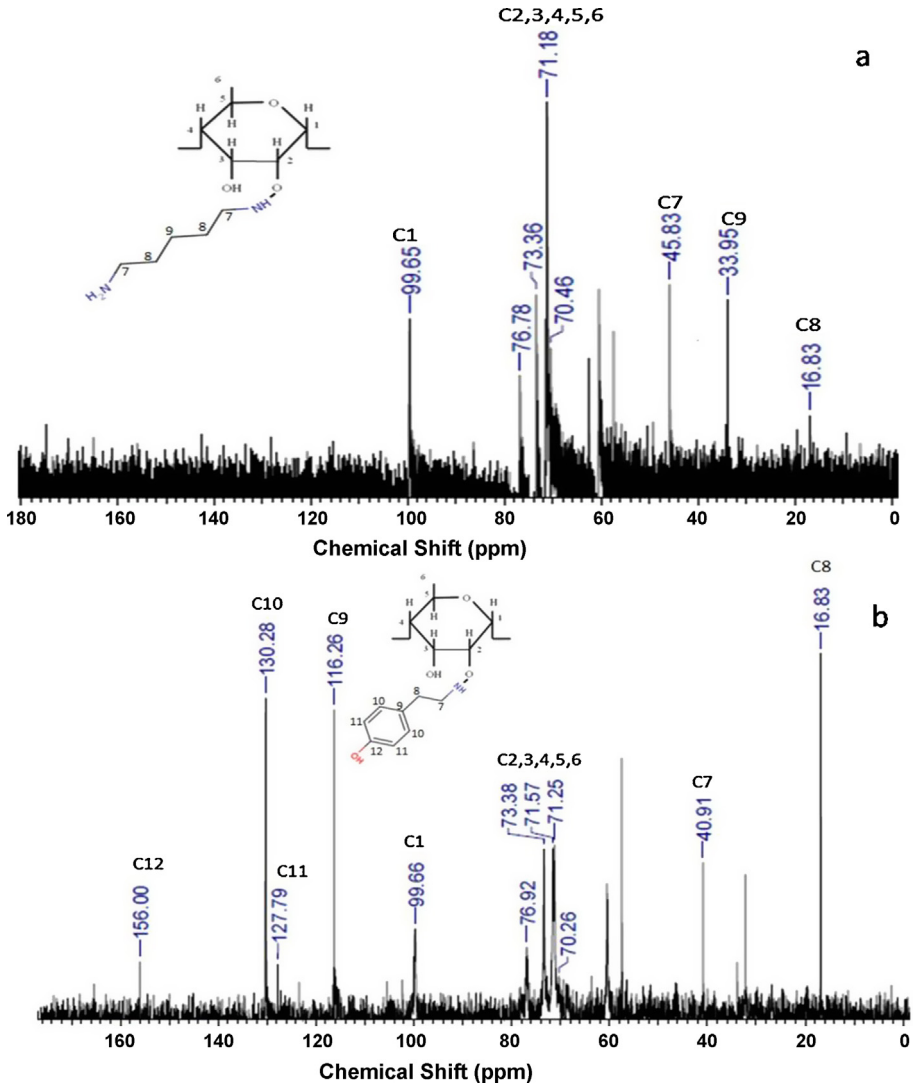

Fig. 8. ^{13}C NMR spectra for cationic amino starch (a) cadaverine CAS and (b) tyramine CAS.

+3.26 ± 0.99 mV and +3.68 ± 0.96 mV for histamine CAS at 80 and 100 °C, respectively suggesting lower temperature for higher zeta potential histamine CAS. This may be due to partial degradation of histamine at higher temperatures. The zeta potential of the cationic amino starch synthesized from the other three amine did not show a significant temperature dependence based on the standard deviations shown in Fig. 2.

3.2. Effect of amine to starch ratio on zeta potential of the cationic amino starch

The effect of amine to starch ratio on the zeta potential of CAS was independently studied by averaging the zeta potential readings in rows R1i,ii,iii; R2i,ii,iii; R3i,ii,iii in Table 1 to obtain the three data points for respective amine CAS (Fig. 3). Putrescine CAS showed significantly higher zeta potential based on standard deviation with higher ratio with +12.06 ± 1.87 mV at 1:1 amine to starch ratio and increasing. No statistically significant increase in zeta potential was observed with a further increase in the amine concentration. Similarly, cadaverine CAS and tyramine CAS showed significant high values of zeta potential with an increasing amine concentration. However, an increase in amine concentration above stoichiometry resulted in statistically insignificant increase in zeta potential based on the standard deviation shown in Fig. 3. Increasing zeta potential with increase in amine concentration could be the result of greater availability of nitrogen sites for alkylation, thus increasing the probability of reaction. Histamine CAS exhibited no statistically significant effect with change in the amine to starch ratio. Higher number of nitrogen sites in the histamine molecule could explain attainment of equivalent zeta potential even at low histamine concentrations in the reaction.

3.3. Effect of reaction time on zeta potential of the cationic amino starch

The effect of reaction time on the zeta potential of CAS was studied by averaging rows R1i,R2i,R3i; R1ii,R2ii,R3ii; R1iii,R2iii,R3iii from Table 1 to obtain the three data points for each of the individual amine CAS (Fig. 4). The standard deviation of the plots indicated that there is no statistically significant difference between the zeta potential for any of the amine CAS reacted at different time intervals. This result suggested no correlation between reaction time and zeta potential of the CAS.

3.4. Multi-variable regression analysis of reaction parameters for cationic amino starch synthesis

The effect of time, temperature, and amine to starch ratio on the magnitude of zeta potential of the cationic amino starch for all the four amines was statistically analyzed using multiple

variable regression with the help of the Statistical Analysis Software (SAS version 9.1.3). Table 2 presents the *P*-values and the standardized beta coefficients of the three independent variables namely, ratio, time and temperature for the dependent variable, zeta potential. The standardized beta coefficients were calculated in order to evaluate the effect the independent variables had on the zeta potential of the CAS. The results of the analysis were in agreement with our previous discussion and suggested ratio as a variable that significantly (*P*-value < 0.05) affected the zeta potential of the CAS synthesized with putrescine, cadaverine, and tyramine. Zeta potential for histamine CAS was significantly affected by temperature and a negative beta coefficient indicated a inverse relationship between the two variables.

3.5. Correlation between zeta potential and degree of substitution

Degree of substitution (DS) was calculated for CAS synthesized by individual amines using Equation 1. With the help of DS, for all the 27 samples of each of the amine CAS, a correlation was established between the degree of substitution and zeta potential in Fig. 5. This correlation confirmed the importance of the nitrogen groups on the CAS to provide the required cationicity. The interaction between the two variables was analyzed using the Statistical Analysis Software (SAS version 9.1.3).

The analysis showed statistically significant correlations between the degree of substitution and zeta potential for all four amine CAS. The correlation showed an increase in zeta potential with an increase in the degree of substitution. This relationship verified the observation that high cationicity was achieved with an increase in nitrogen attachment to the cationic amino starch.

3.6. Zeta potential titration curve

Zeta potential titration was performed to establish the iso-electric point and the working pH range of the CAS (Fig. 6). In order to be an effective polyelectrolyte, the zeta potential of the CAS must remain constant or change very little with pH. However, since cationization in tertiary, secondary, and primary amines is due to protonation of the nitrogen atom, lower zeta potential was observed at pH > 7.

Tyramine CAS and cadaverine CAS showed an iso-electric point (≈pH 9), which was due to the pK_as of the two biogenic amines ranging from 9.0 to 11.0. However, histamine CAS and putrescine CAS showed no iso-electric point, which could be due to uncontrolled multiple alkylations taking place resulting in quaternary ammonium cation formation of the amines (Carey & Sundberg, 2007).

3.7. ^{13}C NMR of cationic amino starches

^{13}C NMR spectra for putrescine CAS and histamine CAS is presented in Fig. 7. The cationic amino starches synthesized were dissolved in D_2O and analyzed at 298 K. The peaks for the carbons on the anhydrous glucose unit (AGU) of starch appears between 60 and 100 ppm (Heinze, Haack, & Rensing, 2004). For putrescine CAS, the peaks at 45.82 ppm and 16.84 ppm were attributed to carbons C7 and C8, respectively as shown on the structure. For histamine CAS, the peaks at 45.83 ppm and 16. 85 ppm were attributed to carbons C7 and C8, respectively as shown on the structure. The peaks for the carbons on the imidazole ring of histamine appear at 123.25 ppm, 129.38 ppm and 136.25 ppm for C9, C10 and C11, respectively as shown on the structure.

^{13}C NMR spectra for cadaverine CAS and tyramine CAS are presented in Fig. 8. For cadaverine CAS, the peaks at 45.83 ppm and 16. 83 ppm were attributed to carbons C7 and C8, respectively. The peak for C9 as shown on the structure, appears at 33.95 ppm. For tyramine CAS, the peaks at 40.91 ppm and 16.83 ppm were attributed to carbons C7 and C8, respectively. The peaks for C9, C10, C11 and C12 were observed at 116.26 ppm, 127.79 ppm, 130.26 ppm and 156.00 ppm, respectively.

4. Conclusions

This research was conducted to understand the effect of time, temperature, and reactant concentrations on the synthesis of cationic amino starch using biogenic amines. Cationic amino starch was successfully synthesized using putrescine, histamine, cadaverine and tyramine as the biogenic amines. The reaction parameters were optimized for each amine for attainment of high zeta potential of the cationic amino starch. For practical large scale synthesis of cationic amino starch, the biogenic amines could be derived from a mix of amino acids obtained from waste streams of meat processing industries. Results presented in this optimization study will help to identify a range of design process parameters for the synthesis of high zeta potential cationic amino starch depending upon the composition of the amino acids in the feedstock.

As future work, the application of cationic amino starch in the precipitation and harvesting of microalgae and treatment of wastewater will be studied. Currently, inorganic metal coagulants such as aluminum sulfate, ferric chloride, and more recently used organic coagulants namely cationic starch prepared by quaternary ammonium salts cost $250/ton of metal coagulants and about $1000/ton of cationic starch (Vandamme et al., 2009), respectively. However, using cationic amino starch is predicted to considerably reduce operating costs in microalgae harvesting and wastewater treatment. Besides reducing overall process costs, the carbohydrate nature of starch and the presence of amines in the cationic amino starch would provide an additional source of carbon and nitrogen for anaerobic digestion or fermentation of the harvested biomass to produce biogas and biosolvents, respectively.Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.07.043.

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