



De-icers derived from corn steep water [☆]

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Abstract

Corn steep water (CSW) and other byproducts derived from fermentations and sugar productions are presently forming the base of compositions for de-icing and anti-icing materials. Since the de-icing and anti-icing values are in part a colligative property, increase in the molar concentration of ionic species has been frequently necessary to decrease further the freezing point of this byproducts stream. In the present study this has been achieved by the generation of biodegradable organic acid salts in situ, without the use of chloride or other inorganic salts, by the alkaline degradation of reducing sugars added to corn steep water, which alone is not an efficient de-icer. Reducing sugars, such as glucose, react with alkali metal hydroxides to produce principally hydroxy carboxylic acids that react with the alkali metal hydroxide to form a mixture of organic acid salts. The ionic strength of the resulting solution is increased since each sugar molecule produces nearly two acid molecules upon degradation. The ionic strength necessary to achieve the desired freezing point depression is determined by the amount and concentration of the alkali metal hydroxide used, with the necessary counter anions being derived from the degradation of the reducing sugar. The amount of the sugar used is that required to result in a near to neutral final solution. The well-known anti-corrosive property of CSW is used in the de-icer preparations, either by conducting the alkaline degradation of the sugar in this medium, or by using water for the degradation of the sugar followed by dilution of the resulting solution with CSW to adjust the viscosity of the final solution to meet the requirements for spraying. The monovalent metal hydroxides are more efficient in producing de-icer solutions than the divalent metal hydroxides.

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1. Introduction

Inclement weather, with the deposition of ice and snow on roads, airports and many other surfaces, calls for relief, which often involves the application of products for de-icing or the prevention of ice formation. Sodium chloride is an effective compound for road de-icing and is inexpensive. Solid calcium and magnesium chlorides work better than sodium chloride, in that they are exothermic upon dissolution, which helps to melt ice at low temperatures. However, massive use of such chloride salts for roadway de-icing has been questioned because of residual effects on the environment, such as the pollution of aquatic habitats, ground water and roadside vegetation and also the serious problems of

corrosion or deterioration of concrete and metals used in the nation's infrastructure. Calcium and magnesium chlorides are hygroscopic, which can cause them to clump and even liquefy on storage. Also they can leave a slippery residue that is difficult to clean.

Alternative materials for road anti-icing and de-icing have been the continuing subjects of scientific and industrial interest. Calcium magnesium acetate (CMA) and methanol were found to be acceptable and non-corrosive de-icing chemicals (Schenk, 1991; Chollar and Virmani, 1988; Dunn and Schenk, 1980). CMA competes well with sodium chloride, particularly in biodegradability and negligible environmental impact, but it still has limited uses (such as on bridge decks) due to its relatively high cost of production. Similarly, potassium acetate is expensive, but has advantages in airport application.

Some amelioration of the adverse effects of chlorides has been noted by adding carbohydrates in the de-icing of highways, as reflected by numerous US and European

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patent applications. Each patent is diverse, depending upon the origin of the sugars (Bloomer, 2000; Johnson and Pratt, 1999; Janke et al., 1997). Native or modified polysaccharides derived from either starch or cellulose have been reported to be useful to treat ice when mixed with chloride salts and sprayed on highways (Gambino et al., 1998; Kuhajek and Waatti, 1992), where the polysaccharide increased the solution viscosity to form a water gel structure that remains in contact with the road surface or anti-skid particles. Low molecular weight (<1000) sugars have a significant effect upon freezing point depression, when concentrated solutions (50% solution) are applied (Hartley and Wood, 2001). The presence of glucose or fructose in the de-icing or anti-icing solution synergistically inhibited or reduced the corrosive effect of added salts. Methyl glucoside and other methyl oligo-glycosides were effective when combined with salts to provide a catalytic effect that speeds melting and helps other chemicals work at lower temperatures (Sapienza, 2001; Connor and Tratnyek, 1990).

The desirable properties of de-icing preparations include cost-effectiveness; ease of application to road or other surfaces; low freezing temperature; prevention of ice formation and reduction of the bonding of snow to the surface of the road; non-corrosive both in the short term, such as for trucks, and in the long term, such as reinforcing rods in cement; biodegradable and having a low impact on the environment; an ability to penetrate ice and snow on roads; rapid solution after application of the solid mixture; persistence on the surface after application; stable composition on storage; low or no conductivity on surfaces, such that electrical systems are not shorted out, as in airports.

Glucose or fructose monosaccharides under alkaline conditions are completely degraded within 30 min at 100 °C into smaller molecular weight acids, consisting primarily of lactic acid together with other hydroxy acids (Yang and Montgomery, 1996a,b). Preliminary results during this study had indicated that the mixture of alkaline degradation products was a potential candidate for road de-icing or anti-icing purposes, as assessed by freezing point, ice melting and the undercutting of annular ice. This study has now been extended to improve the de-icing properties of corn steep water (CSW) by treatment with alkali in the presence of glucose. The desirable properties are compared with those of de-icers presently in use.

2. Methods

Sugar beet molasses were provided by American Crystal Sugar Co. (Moorhead, MN, USA). Industrial-grade enzymes were gifts from Mr. M.B. Utter (Bio-Cat Inc., Troy, VA, USA): proteases derived from the fungus *Aspergillus oryzae* var. (Lot no. FP400-MK17) or *A.*

niger (Lot no. ASP05-NK02); amylases derived from the fungus *A. oryzae* var. (Lot no. FA100-MK17) or bacterium *Bacillus subtilis* (Lot no. BA200-MK17). These enzyme preparations contained carbohydrate, 35% (w/w) in fungal protease, 48% (w/w) in fungal amylase and 23% (w/w) in bacterial amylase, each as determined by the phenol-sulfuric acid method (Dubois et al., 1956). Amyloglucosidase derived from *A. niger* (Lot no. 40801; concentrated form, 200 U/ml) was obtained from Megazyme, Ireland. Gluconolactone (Sigma, St. Louis, MO, USA) was hydrolyzed in boiling water to a constant pH 9 by adding NaOH and the resulting sodium gluconate was dried in vacuo (60 °C, 48 h). CSW for this study was obtained from a local industry; it was taken from the first holding tank derived from the last steeping tank. All other chemicals used were of reagent grade.

2.1. Preparation of alkaline degradation products of glucose in water

The desired concentration of glucose in water was prepared together with either an equimolar amount of divalent alkali (calcium oxide only or a mixture of calcium oxide and magnesium oxide) or with twice the molar equivalent of monovalent alkali (potassium hydroxide or sodium hydroxide) and heated with stirring for selected temperatures and times as summarized in Table 1, unless otherwise indicated. The resulting reaction solution was freeze-dried and pulverized to a fine powder for ice-melting and undercutting experiments. The products from the alkaline reactions with monovalent alkalis were further dried in a high vacuum desiccator (60 °C, 24 h) for subsequent experiments before pulverization.

2.2. Modification of corn steep water

Protein was precipitated when the CSW, pH 4, was neutralized with alkali. Two approaches were made to study this observation; (i) the protein from CSW (100 ml) was precipitated by adjusting pH to 7.0 or 8.5 with NaOH, giving 1.44 or 1.80 g, respectively. The supernatants (CSW 1) were recovered by centrifugation (9800g, 30 min) and used for de-icer studies; (ii) CSW (100 ml) was mixed with water-soluble proteases (1.0 g) of *A. oryzae* or *A. niger* and incubated at 50 °C for 24 h. The resulting solution was adjusted to pH 6 with 10 M KOH (2.5 ml) and incubated further at 50 °C for 24 h with and without amylases (0.2 mg) and amyloglucosidase (100 µl, 20 U). The resulting solution (CSW 2) was used for de-icer studies.

2.3. Alkaline degradation of modified corn steep water

Several procedures were followed to study the de-icer properties of the CSW. (i) CSW 2 (100 ml) was adjusted

Table 1
Alkaline degradation condition of glucose and molasses

Sample ^a	Glucose (g)	Alkali (g)				Water (ml)	Temp. (°C)	Time (h)
		NaOH	KOH	CaO	MgO			
1.8Ca	5.0			1.56		280	100	1.5
1.8CaMg	5.0			0.52	0.80	280	100	1.5
50Ca	5.0			1.56		5.0	100	1.5
50CaMg	5.0			0.52	0.80	5.0	100	1.5
83.3CaMg	5.0			0.52	0.80	1.0	100	1.5
100CaMg ^b	5.0			0.52	0.80	0	100	1.0
						0	125	2.0
MB ^c	73.39			20.05		23.0	200	0.3
MS ^d	17.32			8.19		15.5	100	1.5
22Ca	44.0			12.4		200	100	2.0
22KOH	44.0		27.4			200	100	2.0
22NaOH	44.0	19.55				200	100	2.0

^a Number indicates percentage of initial glucose in the reaction solution (w/w).

^b Solid materials were stirred with a magnetic stirring bar overnight. The resulting mixture was heated at 100 °C for 1 h and then at 125 °C for a further 2 h.

^c Molasses (73.39 g) reaction performed in a high pressure-high temperature autoclave (Autoclave Engineers, Inc., Erie, PA, USA).

^d Molasses (17.32 g) were hydrolyzed in sulfuric acid (15.5 ml of 1 M), at 100 °C for 1 h. The resulting solution at room temperature was alkalized with CaO and heated as indicated.

to pH 12.5 with 10 M KOH (9.2 ml) and heated at 100 °C for 2 h, or 85 °C for 45 min or 16 h, with *n*-octanol (100 µl) as a de-foaming agent. The resulting solutions when cooled to room temperature showed a pH 10.8 at the higher reaction temperature (100 °C) or longer reaction time (16 h) and pH 11.3 after 85 °C for 45 min, representing a small generation of acidic products. The freezing point of the reaction solution was determined either directly or after its concentration following removal of any insoluble material; (ii) procedure (i) was repeated using, however, equivalent amounts of NaOH,

CaO, or Ca(OH)₂ instead of KOH; (iii) glucose (20 g: 0.11 mol) and NaOH or KOH in a 1:1 molar ratio were added to the native or modified CSWs (100 ml) and heated under various conditions as summarized in Tables 2 and 3; (iv) glucose (100 g: 0.56 mol) was dissolved in CSW (50 ml) at 60 °C. *n*-Octanol (500 µl) was added, followed by solid KOH (34.36 g: 0.53 mol) of 87% reagent grade (Fisher Scientific, New Jersey, USA) in three portions and with stirring. The resulting solution was maintained at 60 °C for 16 h. The resulting reaction solution when cooled to room temperature showed a pH

Table 2
The effect of each sequential treatment on the freezing temperatures of CSW

Treatment in order	Freezing temperature (°C)			
	CSW ^a	CSW ^a	CSW ^b	CSW ^b
Native	-1.1	-1.1	-1.1	-1.2
Protein removed at pH 7	N/A	-2.0	N/A	N/A
Protease (<i>A. oryzae</i>) digestion (1 g)	-1.8	N/A	1.6	-2.0
Glucose added	-6.6	-5.3	Nd	-3.8
NaOH added (8.8 g)		N/A		
Before heating	-14.5	-11.4		Nd
After heating at 81 °C				
For 2 h	-10.9	-10.9		
For 1 h	-10.4	Nd		
After heating at 100 °C				
For 2 h				-5.3 (5.9) ^f
KOH (7.85 g) added	N/A	N/A		N/A
Before heating			-4.8	
After heating (100 °C, 2 h)			-4.8	

The freezing point of the resulting solution was monitored by thermistor and the resulting voltage output (*V*) was converted into temperature (*T*) using the calibration equation ($T = 23.211V + 84.682$; $R^2 = 0.9992$) obtained from three different temperatures of 0.0, -8.7 and -13.5 °C.

N/A: not applicable; Nd: not determined.

^a 20 g of glucose added to 100 ml CSW.

^b 10 g of glucose added to 100 ml CSW.

^c Glucose degradation product with NaOH (100 °C, 2 h) independently prepared and mixed with protease digested CSW to give the same reaction concentration.

Table 3

The freezing temperatures of CSW mixed with the alkaline degradation products of glucose

Conc. % (w/v)	Freezing temperature (°C)			
	CSW 2		CSW 1	
	Glc-NaOH	NaCl	Glc-NaOH	NaCl
10	-5.6 ± 0.4	-10.3 ± 0.1	-5.6 ± 0.2	-11.3 ± 1.4
15	Nd	-13.3 ± 1.1	-7.4 ± 0.1	-15.5 ± 1.0
20	-8.8 ± 0.6	-18	-9.8 ± 0.3	-19
23	Nd	-21	Nd	-21
25	-12.1 ± 1.2	Nd	-11.4 ± 0.0	Nd
30	-12.9 ± 0.9	Nd	-13.4 ± 1.0	Nd

The alkaline degradation products of 22% (w/w) initial glucose in NaOH solution were prepared as described in Table 1. The dried materials (Glc-NaOH) or NaCl (for comparison) were mixed with the supernatant CSW at pH 7.0 (CSW 1) or protease-digested (*A. oryzae*) corn steep water (CSW 2). The freezing point of the solution was monitored by thermistor and the resulting voltage output was converted into temperature using the same calibration equation as described in Table 2. The data are expressed as an average of three measurements with the standard deviation derived by Microsoft Excel 2000 computer program.

Nd: not determined.

8.8 and was appropriately diluted with water, followed by storage at -20 °C for 48 h. Samples that had not frozen at that temperature were analyzed for freezing temperature, viscosity and specific gravity (Table 4). A weighed aliquot of these samples was taken and dried in vacuo (60 °C, 24 h) to determine the soluble solid materials. Another aliquot of the solution (3 ml, containing 2.586 g of dry solid) was dialyzed against water (10 K MWCO dialysis tubing, Pierce, Rockford, IL, USA) and the insoluble materials from the retentate were recovered by centrifugation and freeze-dried (0.002 g).

2.4. Determination of freezing temperature

The freezing temperature equipment consisted of a cooling bath at either -18 °C or -45 °C with a digital thermometer and a positive temperature coefficient thermistor. The digital thermometer could read to 0.1 °C

from -15 to 5 °C. Below -15 °C the accuracy was limited to whole degree increments. The thermistor voltage was calibrated against the digital thermometer in the two ranges, -15 to 0 °C and -15 to -35 °C, each calibration resulting in a linear relationship that was slightly different over the two ranges. The voltage output from the thermistor was fed into the computer so that readings during the freezing were recorded every second. The sample (20–25 ml) was cooled with stirring in either the -18 or -45 °C bath. The cooling curves were generally seen with a supercooling minimum from which the temperature increased to a constant voltage due to the heat of crystallization. The freezing temperature of the solution was expressed as the constant temperature reached immediately after crystal formation. The rate of stirring during the freezing had no effect on the freezing point, just the rate of freezing. Aqueous solutions of NaCl, CaCl₂ and sodium gluconate were included for

Table 4

The freezing temperature, viscosity and specific gravity of alkaline degradation products of glucose by KOH

Percentage of the reaction products (v/v)	Dry weight ^a % (w/w)	Freezing temperature (°C)	Viscosity ^b (cp)			Specific gravity (g/ml) 25 °C
			20 °C	5 °C	-8 °C	
75	52.8	-26	13.4	18.2	54.2	1.273
81	56.2	-29	20.4	43.1	90.8	1.298
88	59.3	-29	31.5	72.5	91.7	1.315
100 ^c	65.7	nc	62.8	237.7	615.5 ^d	1.360
Ethylene glycol			18.9 (19.9) ^e	40.9	76.1	1.112 (1.113) ^f

Glucose (100.0 g) and KOH (34.36 g) was reacted in CSW (50 ml), 60 °C, 16 h and the resulting solution was diluted with water to 75%, 81% and 88% of the starting solution by volume. The freezing point of the resulting solution was monitored by thermistor and the resulting voltage output (V) was converted into temperature (T) using the calibration ($T = 37.695V + 146.76$; $R^2 = 0.9995$) obtained from three different temperatures of -17, -23 and -35 °C. Ethylene glycol included for comparison.

nc: no crystallization observed.

^a An aliquot of each solution was taken, weighed and freeze dried, followed by drying in vacuo (60 °C, 24 h).

^b Viscosities in the range of shear rate 100–400 (s⁻¹).

^c Initial reaction preparation.

^d Viscosity in the range of shear rate 100–200 (s⁻¹).

^e CRC Handbook of Chemistry and Physics, 60th ed. and temperature is not known.

^f Nominal value of ethylene glycol.

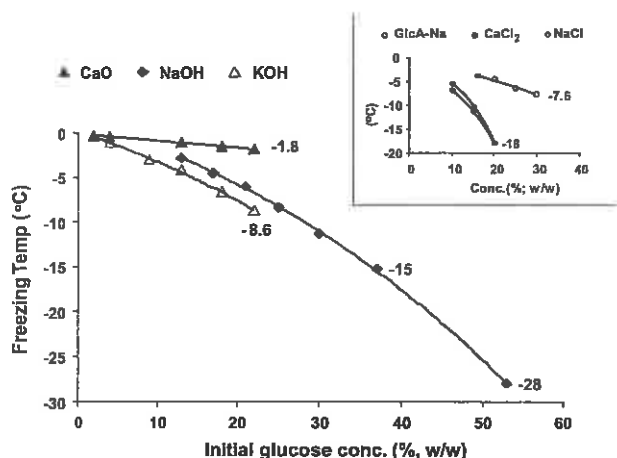


Fig. 1. Freezing temperature of alkaline degradation products of glucose in water and the effect of their concentration. Degradation products of 22% (w/w) initial glucose concentration was prepared as described in Table 1 and diluted with water or concentrated in vacuo by rotary evaporation (43 °C). The freezing point of the resulting solution was monitored by thermistor and the resulting voltage output (V) was converted into temperature (T) using the calibration equations of the desired temperature ranges: $T = 23.211V + 84.682$ ($R^2 = 0.9992$) obtained from three different temperatures of 0.0, -8.7 and -13.5 °C and $T = 37.695V + 146.76$ ($R^2 = 0.9995$) obtained from three different temperatures of -17 , -23 and -35 °C. Sodium gluconate (GlcA-Na), NaCl and CaCl_2 were included for comparison (Inset).

comparison (Fig. 1). The freezing point determinations in some cases were repeated three times.

2.5. Determination of viscosity and specific gravity

Selected reaction products were filtered (0.45 μm , Syringe Filter, Gelman Sciences, Ann Arbor, MI, USA) and the viscosity of the resulting filtrates were determined at the range of shear rates (100–400 s^{-1}) at 20, 5, and -8 °C using a plate-and-cone viscometer (DV-III V3.3 RV, Brookfield Engineering Laboratories Inc., Middleboro, MA, USA). The specific gravity of these products was determined by weight of a known volume. Ethylene glycol (Fisher Scientific, New Jersey, USA) in these experiments was included for comparison.

2.6. De-icing tests

De-icing tests (Chappelow et al., 1992) were performed by the melting of packed ice or undercutting of annular ice with powdered reaction products: (i) after the alkaline reaction products and equipment were equilibrated at the selected temperature ($+2$ °C) for 2 h, the degradation products (1.0 g) were sprinkled on top of packed ice (20 g) held in a filter funnel. The volume of water resulting from the melting after 30 min was measured (Fig. 2); (ii) annular ice (0.8 cm high, 1.0 cm inner diameter, and 4.8 cm outer diameter) was prepared

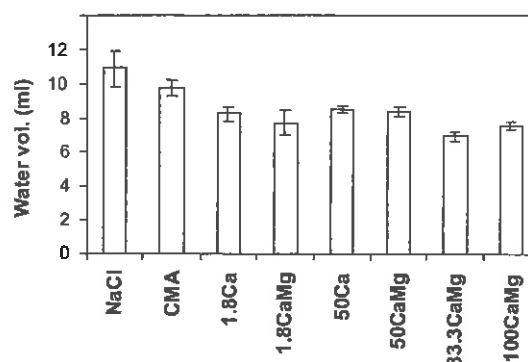


Fig. 2. The water volume (ml) of melted ice collected from the spreading of dried reaction products (1.0 g) over the surface of ice (20.0 g) at 2 °C. The number of the sample indicates the initial glucose concentration in the alkaline reaction solution (w/w %); CMA for calcium magnesium acetate; Ca for CaO; CaMg for a mixture of CaO and MgO; 100 represents the mixture of solids with no water added. Reaction conditions are given in Table 1. The error bars represent the standard deviation of four determinations as generated by Microsoft Excel 2000.

by freezing deionized water (15 ml) in a Styrofoam cup that had a plastic syringe inserted through the bottom (Trost et al., 1987). Cold water (0 °C) was sprinkled on concrete or asphalt block surfaces, on which the annular ice was immediately placed and then re-frozen at -1 , -5 , or -8 °C, followed by inclusion of a couple of drops of dye solution in the hole. The annular ice was brought to thermal equilibrium at the selected temperature overnight, following which a known amount of de-icer (0.4 g) was introduced onto the hole. The increase of internal diameter was measured at intervals (Figs. 3 and 4). NaCl and commercial CMA (ICE-B-GON Deicer, Chevron Deicing Technology, Ft. Madison, IA, USA) were used for comparison in all the experiments. A fine powder of CMA was prepared similar to the glucose reaction products.

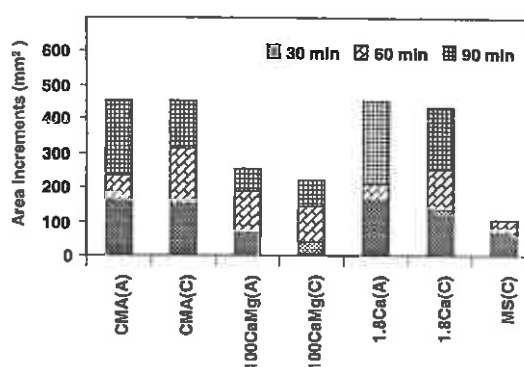


Fig. 3. Undercutting of annular ice (15 ml of water frozen) at -1 °C by alkaline degradation products (0.4 g). (A) For asphalt block; (C) for concrete block; MS for alkaline degradation products of acid hydrolyzed molasses as described in Table 1 and other notations are as in the Fig. 1. The results are the average of three measurements.

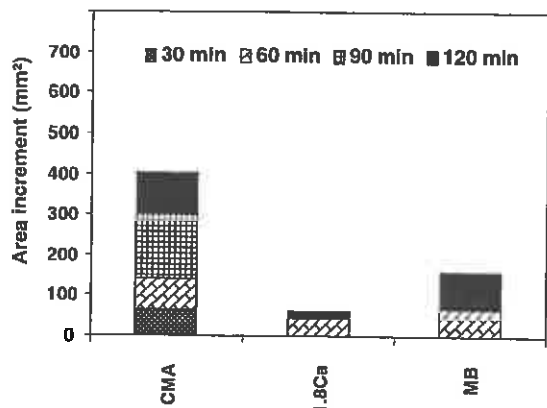


Fig. 4. Undercutting of annular ice (15 ml of water frozen) at -5°C concrete block by alkaline degradation products (0.4 g). MB: alkaline degradation products of molasses in $\text{Ca}(\text{OH})_2$ solution at 200°C (see Table 1). The results are the average of three measurements.

3. Results

3.1. Alkaline degradation of glucose in water

Extensive research of the products from the alkaline degradation of glucose and fructose in $\text{Ca}(\text{OH})_2$ solution demonstrated a complex mixture of hydroxy organic acids (Fig. 5). The relative distribution of the acids in the degradation products varies with the initial concentration of sugar (Fig. 6a and b). Glucose is completely degraded in $\text{Ca}(\text{OH})_2$ solution within 30 min at 100°C and similar results, as determined by the phenol sulfuric acid method (Dubois et al., 1956), were also achieved at lower reaction temperatures (81 $^{\circ}\text{C}$ for 1 h or 50°C for 16 h) with KOH or NaOH. The reaction solution of glucose with NaOH or KOH was clear without any insoluble material. However the degradation products of glucose with CaO generated significant precipitates at the end of reaction, particularly when stored in the cold

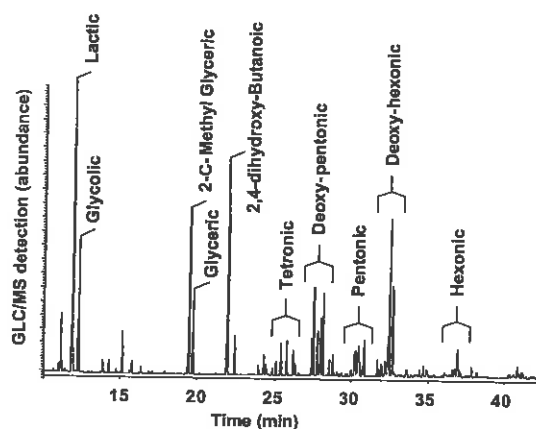
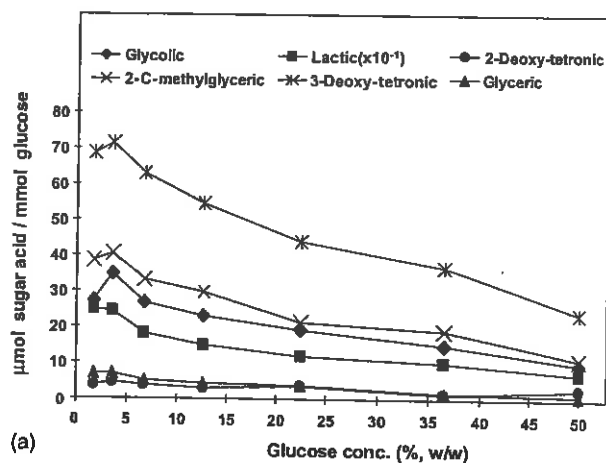
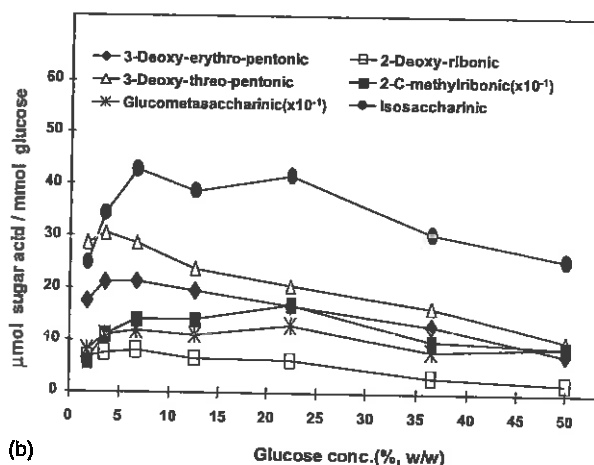


Fig. 5. GLC/MS chromatogram of anionic fraction, as trimethylsilyl derivatives, from the degradation of glucose in $\text{Ca}(\text{OH})_2$ solution at 100°C , 1.5 h (Yang and Montgomery, 1996a).



(a)



(b)

Fig. 6. (a) The formation of C_2 , C_3 and C_4 saccharinic acids at the different initial glucose concentrations (the reaction conditions given in Table 1) (Yang and Montgomery, 1996a). (b) The formation of C_5 and C_6 saccharinic acids at the different initial glucose concentrations (Yang and Montgomery, 1996a).

room. These precipitates were complexes of the calcium ion with saccharinic acids present in the reaction solution. Under comparable conditions these alkaline degradation products of glucose with CaO are not as good as NaCl or CMA (Figs. 1–3) in either melting of ice at 2°C or undercutting of ice at -1°C or -5°C . This is reasonable because the molar masses of hydroxy acids derived from glucose degradation are larger than those of chloride and acetate present in NaCl and CMA, consequently more alkaline degradation material would be needed to achieve the same results. As noted in Fig. 6a and b the concentration of organic acids decreased as the starting concentration of glucose in the reaction solution increased, in particular, above 20–25%. As the concentration of the initial glucose increased so the freezing point depression of the resulting solution increased. The complex formation of hydroxy acids by calcium ion reduces the ionic strength of the solution and thus the freezing point (Fig. 1).

3.2. Alkaline degradation of corn steep water

A detailed analysis of the CSW from four different plants at different stages of the steeping process showed the presence of 10–15% (w/w) dry solids composed of protein and polypeptides, polysaccharides (mostly starch) and some lower molecular weight carbohydrates, lactic acid, inorganic salts and a little lipid (Hull et al., 1996). The various steeping procedures of the four wet milling plants resulted in a difference in the quantity of these components at each stage of the steeping, but within any plant the results were similar.

CSW for this study was obtained from the first holding tank of the late steeping process (the last steep tank) in the local industry and contained 3.2% (w/v) lactic acid equivalent (as determined by titration with NaOH) and 1.2% (w/v) carbohydrate (glucose equivalent of 11.8 g l^{-1} determined by the phenol sulfuric acid method). This carbohydrate was mainly polysaccharide in nature and would therefore add little to the alkaline degradation reactions (Whistler and BeMiller, 1958). When the steep water was neutralized with NaOH, insoluble precipitates of 28.0 g l^{-1} at pH 7.0 and of 41.4 g l^{-1} at pH 8.5 (dried in high vacuum desiccator at 60°C , 24 h) were formed, which contained small amounts of carbohydrate (4%, w/w, based on the insoluble precipitates). The insoluble materials following protease digestion and addition of KOH were significantly reduced (7.8 g l^{-1} at pH 7.6). When the protease-digested steep water (CSW 2) was neutralized with CaO, the insoluble materials (49.7 g l^{-1}) were sixfold more than those with KOH due principally to the precipitation of calcium protein complexes.

The same reaction conditions (pH 12.5, 100°C , 2 h) were applied to the CSW as were used for glucose degradation in water in the earlier studies, using mono- and divalent alkali. These reactions were further extended to corn steep water (CSW 2) that had been treated with proteases and/or amylases and amyloglucosidase to study the effect on the freezing point depression before and after such treatments. CSW had a freezing point of -2°C , that was lowered (-2.7°C) after proteolytic digestion due to the generation of smaller peptides (Fig. 7). Treatment with KOH lowered the freezing point of CSW further (-3.8°C), principally due to the increase in ionic strength from the potassium. Some further reduction in freezing point was seen (-4.0 to -4.8°C) after the combined treatments of proteolysis and alkaline degradation of the small amount of carbohydrate in the CSW that is not increased by amylase treatment. The CSW following all the reactions contained 19% dry weight soluble materials (188 g l^{-1} , freeze dried), which comprised carbohydrate-derived component (1%), lactic acid (3%), KOH (5%) added for alkaline reaction and the rest (10%) being modified proteins and other materials originally present in the CSW. The

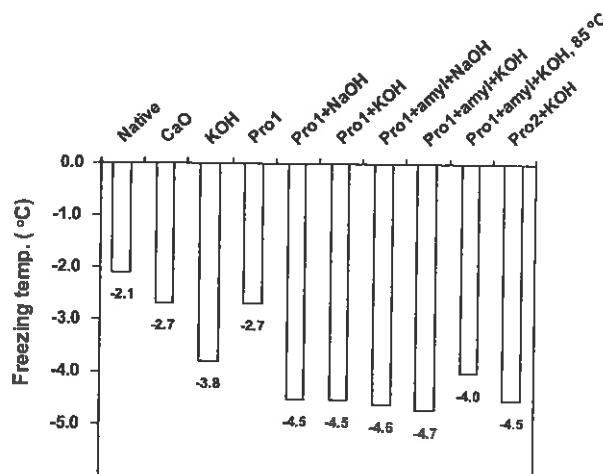


Fig. 7. Freezing temperatures of various CSWs treated with enzyme and alkali (100°C , 2 h and 80°C , 45 min). Pro1 and Pro2: proteases from *A. oryzae* and *A. niger*; amy: bacterial amylase from *B. subtilis*. The freezing point of the solution was monitored by thermistor. The resulting voltage output was converted into temperature using the same calibration equation as described in Fig. 1.

molar concentrations of high molecular weight constituents, such as polysaccharides and proteins or peptides, were relatively low and the resulting degraded products by alkali of the negligible amount of reducing sugars in the CSW have minimal effect on depressing the freezing point of the solution. The impact on this property was mostly due to the increased ionic strength of the alkali added in order to degrade the carbohydrates, as seen in Fig. 7 by improvement of freezing temperature with KOH alone added to CSW. A concentration of the products as shown in Fig. 8, showed substantial reduction in freezing points. The CSW digested with protease

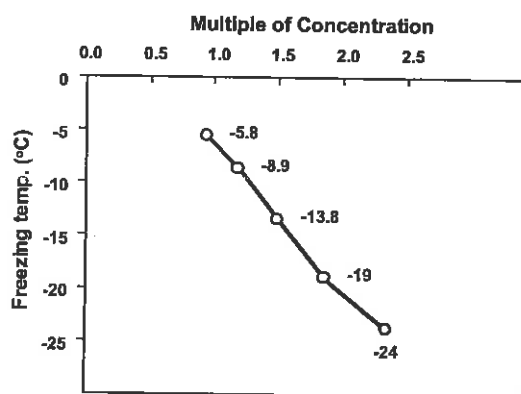


Fig. 8. The effect of concentration on the freezing temperature depression of protease-digested (*A. oryzae* var) CSW by alkali degradation with KOH (100°C , 2 h). The reacted solution was appropriately reduced in vacuo by rotary evaporation (43°C). The freezing point of the solution was monitored by thermistor. The resulting voltage output was converted into temperature using the calibration equations as described in Fig. 1.

alone had freezing temperature of $-15\text{ }^{\circ}\text{C}$ after fourfold concentration and the subsequent alkali-treatment of the protease-digested solution gave the freezing temperature of about $-20\text{ }^{\circ}\text{C}$ in a twice concentrated form. The solution of ≥ 3 times concentration did not have a clear crystallization temperature plateau during cooling, but was present as a viscous liquid even at $-30\text{ }^{\circ}\text{C}$.

Unreacted carbohydrates ($2.1\text{--}2.4\text{ g l}^{-1}$) from the CSW after the alkaline reaction at $100\text{ }^{\circ}\text{C}$ were similar regardless of the amylase and amyloglucosidase treatments due to the nature of the carbohydrate that was not a substrate for the enzymes. At the lower reaction temperature of $85\text{ }^{\circ}\text{C}$ the remaining carbohydrates were slightly higher (3.5 g l^{-1}) in 45 min, and a similar result was attained after 16 h as in the reaction at $100\text{ }^{\circ}\text{C}$. The undegraded carbohydrates following the alkaline reaction are either oligosaccharidic acids (Whistler and BeMiller, 1958), derived by alkaline peeling of carbohydrate polymers originally present in enzyme preparations and the CSW, or glycated proteins and/or polypeptides produced by the condensation of the glucose with the free amino groups of the proteins or peptides in the Maillard reaction (Wrodnigg and Eder, 2001). The glycated products are stable in alkaline conditions. CSW heated with CaO or $\text{Ca}(\text{OH})_2$ generated substantial amounts of insoluble materials compared with the use of either NaOH or KOH.

3.3. Alkaline degradation of glucose in CSW or modified CSW

The alkaline degradation of glucose was carried out in CSW or modified CSW, to which the sugar (10% or 20%, w/w) was added before alkaline degradation (Table 2). A substantial decrease in freezing point of the resulting solution was seen when glucose was added and a further decrease with the addition of NaOH. However, the solution after heating did not improve its freezing point depression and an adverse effect was seen when glucose was available to enhance the formation of glycated proteins and/or peptides by Maillard reactions, consequently reducing the number of molecules present in the solution. This adverse effect can be minimized or prevented when alkaline degradation products of glucose in water were prepared separately and subsequently mixed with CSW (Table 3).

When the molar ratio of glucose to KOH or NaOH was 0.5, unconsumed alkali remained after the reaction, as seen by high alkalinity (pH 11) of the resulting product. This was compensated for by increasing the relative concentration of glucose to alkali. When the molar ratio of glucose to KOH was increased to 0.9, 1.0 and 1.2, the degradation product of glucose in CSW at $100\text{ }^{\circ}\text{C}$ for 2 h approached to the neutral pH, 8.9, 8.0 and 7.4, respectively, from the initial pH 13.2 to 13.0. At lower reaction temperatures, equimolar reaction solution of glucose and

KOH exhibited slightly higher pHs than for $100\text{ }^{\circ}\text{C}$: pH 10.5 ($52\text{ }^{\circ}\text{C}$, 16 h) and pH 8.7 ($80\text{ }^{\circ}\text{C}$, 2 h).

The dissolution of alkali in water is exothermic. This heating was exploited to degrade glucose (100 g) in the practical minimum volume (50 ml) of CSW by adding KOH pellets. The temperature of the resulting solution, upon dissolution of solid KOH, increased to over $100\text{ }^{\circ}\text{C}$ from the initial $60\text{ }^{\circ}\text{C}$ of glucose solution. During this period (around 30 min) the viscosity of the solution drastically decreased and also the degradation of glucose was near to complete, as indicated by the pH 9.3 of the resulting solution when cooled to the room temperature. Further incubation at $60\text{ }^{\circ}\text{C}$ for 16 h did not significantly improve the generation of acid, as seen by the pH 8.8 of the resulting solution. The reaction solution was clear and virtually no insoluble materials ($<0.1\%$) were present. In the determination of freezing temperature the reaction product was very viscous around $-20\text{ }^{\circ}\text{C}$ and no crystallization was observed at $-30\text{ }^{\circ}\text{C}$. Diluted solutions with water (75–90% of the initial reaction solution by volume) comprised 50–60% (w/w) dry soluble materials and showed freezing temperatures approaching $-30\text{ }^{\circ}\text{C}$. A similar freezing point depression ($-28\text{ }^{\circ}\text{C}$) was also observed with the alkaline degradation products of glucose in water at the concentration of 53% (w/w) solid material, as shown in Fig. 1. The viscosities of the diluted samples, as determined at the range of 100–400 (s^{-1}) shear rate, were comparable to ethylene glycol. These analyses were summarized in Table 4.

4. Discussion

Calcium magnesium acetate (CMA) has been found to be an acceptable de-icer, without the addition of chloride salts, but its use has been limited by its expense. Our initial studies were directed toward providing a cheaper substitute for CMA by using the mixed organic acids generated by the alkaline degradation at elevated temperatures of glucose by a mixture of calcium and magnesium oxides. Although this met with partial successes as de-icing preparations (Figs. 2 and 3) it was found that a significant portion of the product was the poorly soluble CaMg salts, demonstrated (data not shown) by the acids released by ion exchange chromatography of the soluble and insoluble parts of the reaction mixture being the same. These preliminary experiments did indicate that the alkaline degradation of glucose with sodium or potassium hydroxide may provide solutions with de-icer properties.

CSW, either in concentrated form or mixed with chloride salts, is currently used as a de-icer (Ice Ban America Inc., Natural Solution Corporation of North Palm Beach, FL, USA) and is anti-corrosive (Highway Innovation Technology Center, 1998). Attempts to conduct the alkaline degradation of glucose in CSW

presented the initial problem of precipitation of materials, principally proteins, upon bringing the solution to pH 7–9. The alternatives to the next step were either to remove the precipitate, which was approximately one third of the 10–15% solids in the CSW, and could be used for other purposes, such as high-protein animal feed, or to reduce the precipitate by digesting the proteins with proteases before bring the solution to pH 7 to 9, or to continue the degradation of the glucose at elevated temperatures in the presence of the precipitate. As the results showed, the advantages of treating the CSW with proteases or amylases were minimal, the degradation reactions of glucose added to CSW with NaOH or KOH resulted in no insoluble products, and the resulting freezing point depressions were similar to using water.

Glucose added to native or protease-treated CSW lowered the freezing point (Table 2), which was further reduced by the addition of alkali, again all in keeping with the increase in ionic strength. Heating the resulting mixture brought the pH close to neutral but caused some reduction in ionic strength, particularly in the protease-treated CSW; the Maillard reaction of glucose with the proteins and peptides removed some of the glucose from alkaline degradation reactions, consequently reduced the formation of the organic acids that neutralized the NaOH. The effect was most noticeable when small peptides were present but less with proteins.

It may be concluded from these studies that the alkaline degradation of glucose in water or in CSW results in a product of similar freezing point depression (Table 2) that is dependent upon the initial concentration of the glucose. The ionic strength necessary to achieve the desired freezing point depression is determined by the amount and concentration of the alkali metal hydroxide used, with the necessary counter anions being derived from the degradation of the reducing sugar. The amount of the sugar used is that required to result in a final solution that is near to a neutral pH. The well-known anti-corrosive property of CSW (Highway Innovation Technology Center, 1998; Janke et al., 1997) is used in the de-icer preparations either by conducting the alkaline degradation of the sugar in this medium, or by using water for the degradation of the sugar. At the higher initial concentrations, the reaction solution is viscous (Table 4) so that CSW can be used as the diluent to adjust the viscosity of the final solution to meet the requirements for spraying. The CSW alone is not very efficient as a de-icer. The monovalent metal hydroxides are more efficient in producing de-icer solutions than the divalent metal hydroxides. The preferred alkali is either NaOH or KOH.

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