Sucrose Extraction From Beet By Methanolic Calcium Chloride

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ABSTRACT

The use of methanolic $CaCl_2$ for the non-aqueous extraction of sucrose from dried sugar beet (*Beta vulgaris* L.) cossettes was investigated, with emphasis on the effects of $CaCl_2$ concentration, time of extraction, and operating temperature. Solubility of sucrose from dried cossettes was optimal in range of 10-15% $CaCl_2$ in methanol and increased by a factor of four as the temperature was raised from 1C to 60C. Nearly complete extraction (98%) was achieved in batch experiments with four successive extractions. The methanolic $CaCl_2$ extract was found to have both higher purity and greater sucrose stability compared with aqueous diffusion juice. The sucrose could be precipitated completely from the solution by the addition of 5 volumes of acetone at 20C, leaving 90% of the $CaCl_2$ in solution. Steady state conditions could not be reached in simulated continuous column experiments, probably because of calcium exchange with other cations in the beet pectin. Pure sucrose was precipitated from these effluent solutions on cooling. The amount of sucrose recovered was proportional to the decrease in calcium content on passage through the column. From this, the molar ratio of calcium to sucrose in the methanolic $CaCl_2$-sucrose complex was calculated to be 2:1.

Additional Key Words: beet sugar, beet cossette, non-aqueous extraction, calcium-sucrose complex.

Although aqueous diffusion of sucrose from sugar beets has long been the standard of the industry, the possibility of non-aqueous extraction and recovery of sucrose from sugar beets using various organic solvents has been the subject of research by many investigators (Moye, 1972). Liquid ammonia and ammonia dissolved in solvents were used to extract sucrose from beet cossettes (Hingst, 1957; Kishihara, 1970; Palzer et al., 1963). Various aliphatic alcohols at elevated temperatures under high pressure have been suggested as alternatives to water (Haury,1960). Alcohols also have been extensively studied for non-aqueous affination of raw sugar (Othmer, 1978). The use of acetic acid, a carboxylic acid, has been suggested for refining sugars (Othmer, 1963). Although none of these aforementioned processes have been implemented in industrial practice, there is continuing interest in the use of solvents as a method for improving the separation of sucrose from the non-sucrose compounds in the beet.

The formation of soluble complexes with calcium chloride in methanol has been reported for a wide range of carbohydrates (Domovs and Freud, 1960), although sucrose was not included. In our recent study, addition of $CaCl_2$ to methanol was found to increase the solubility of sucrose 40-50 fold, to as high as 45%(w/v); the effects of various parameters on the solubility of sucrose in methanolic $CaCl_2$ solution and the recovery of sucrose from the calcium-sucrose complex were also reported (Wong et al.,1986).

The present study examined the effectiveness of methanolic $CaCl_2$ solution for the extraction of sucrose from dried beet cossettes, and methods for the recovery of sucrose from solution. Results of extraction with methanolic $CaCl_2$ were compared with those of aqueous extraction.

MATERIALS AND METHODS

Preparation of Dried Beet Cossettes

Fresh beets were washed, dipped for 5 min in a sodium hypochlorite solution containing 1000 ppm free chlorine, surface dried at 40C for 10 min and stored at 1C. The beets were cut into cossettes, the standard beet form used in the beet sugar industry, using a pilot plant cossette cutter (29 division Konigsfeld cutter blades mounted 2.25 mm back and 2.5 mm up). The cossettes were dried on screen trays at 80-90C for 24 hr in a tunnel dryer. The dried cossette weight was 22.8% of the original weight of the fresh beet; moisture content was 6.4%. Some dried cossettes were later ground, and particles were classified by passing the material through sieves between 25-60 mesh.

Extraction

Methanolic $CaCl_2$ solutions of various concentrations (up to 20% w/v) were prepared by diluting a saturated stock solution. Methanolic calcium chloride stock solution was prepared by saturating methanol (0.02% moisture) with excess anhydrous $CaCl_2$ by shaking for 1 hr at 60C. The dried cossettes were extracted with methanolic $CaCl_2$ solution in capped 25 ml vials, clamped to a mechanical shaker, and immersed in an oil or water bath kept at constant temperature. In control experiments, to determine total extractable sucrose, the cossettes were extracted in 80% ethanol by the standard AOAC method (AOAC, 1980) using cossettes/solvent ratios ranging from 1:5 to 1:8 (w/v). For comparison, the cossettes were also extracted with water at 80C using one or more successive 1 hr extractions. Sucrose in the aqueous extracts was determined by HPLC (refer to "carbohydrate analysis").

Continuous extraction was done by pumping the methanolic $CaCl_2$ solution at a 1 ml/min flow rate through a column (2.5 cm x 25 cm) packed with 45-60 mesh ground dried cossettes. A constant temperature of 65C was maintained by circulating heated water through the column jacket and through a condenser used for preheating. Flow from the top outlet was diverted to a fraction collector, usually with 15 ml fractions. The amount of sucrose in the fraction(s) was analyzed by the colorimetric method of Dubois et al. (1956) or HPLC.

Precipitation of Sucrose with Acetone

Various amounts of acetone were added with rapid mixing to the methanolic $CaCl_2$ extract. The white precipitate formed was recovered by centrifugation and the supernatant was analyzed for sucrose, calcium and chloride.

Carbohydrate Analysis

The sugar content in the extracts normally was analyzed by a phenol-sulfuric acid method (Dubois et al., 1956). This colorimetric method determines only the amount of total sugars, but since sucrose normally exceeds 98% of the total sugar present in beets, the results obtained by these procedures provide a good approximation of the amount of sucrose in the samples analyzed.

In experiments where exact quantitation of sucrose was required, analysis was performed using HPLC [Spectra Physics SP6700 solvent delivery system with SP6040 differential refractometer, SP4270 integrator, Aminex HPX87C carbohydratecolumn (250 mm x 4 mm), eluate water, flow rate 0.3ml/min, column temperature 85C]. Sample aliquots were dried under nitrogen and redissolved in water to the appropriate concentration for analysis. All calculations were based on external stan-

dards. The two carbohydrate analyses gave similar results, agreeing within 3-5%.

Other Analyses

In general, for calcium determination, samples were dried under nitrogen and redissolved in hydroxynaphthol blue (HNB) buffer solution (0.5% w/v of thioacetamide in 1 N KOH, pH 13.0). Water was added to bring the final volume to 50 ml. The solution was titrated with standardized titraver solution (1/28 N EDTA, pH 5.0) to a permanent blue end point (Zaragosa et al., 1982). Analysis for sodium and potassium, and for calcium in a few instances where K^+ and Na^+ were also being analyzed, was done by atomic absorption (Perkin Elmer 303 AA) with samples prepared by dry ashing (Anon, 1982). The two methods of calcium analysis gave equivalent results. Chloride concentration was determined by the Mohr method by titrating with $AgNO_3$ to a light brown end point with K_2CrO_4 as an indicator (Johnson and Ulrich, 1959).

Purity and Color

The purity and color of aqueous and methanolic $CaCl_2$ extracts were determined as follows. Ground cossettes suspended in water or methanolic $CaCl_2$ (1/10 w/v) were heated at 65C for 1hr. The methanolic $CaCl_2$ extracts were dried under nitrogen at 50C and brought to volume with HPLC grade water. Extracts were then filtered, and refractometer readings were taken with a Bausch and Lamb Precision Model refractometer. The amount of sucrose in the filtrate was analyzed by HPLC. Juice purity was calculated as (% sucrose/rds) x 100. For the methanolic $CaCl_2$ extract, the Brix reading of the aqueous solution was corrected by subtracting the Brix reading contributed by the $CaCl_2$. Color values in ICUMSA (International Commission for Uniform Methods of Sugar Analysis) units were calculated as 1000 [-log $T_{420\ nm}$ (rds x apparent density x cell path in cm)] (Schneider, 1979).

RESULTS AND DISCUSSION

Methanolic $CaCl_2$ Extraction

The extractibility of sucrose in methanolic $CaCl_2$ solution from cossettes was quite different from the solubility of pure sucrose in the equivalent solutions. With pure sucrose, solubility always increased with increasing concentration of up to 30% $CaCl_2$, but with dried cossettes, the sucrose extracted rose rapidly as the concentration of $CaCl_2$ increased up to 15% $CaCl_2$, and then began to drop (Fig. 1). Three separate experiments confirmed the optimal range of 10-15% $CaCl_2$ in which the highest extraction yield was obtained.

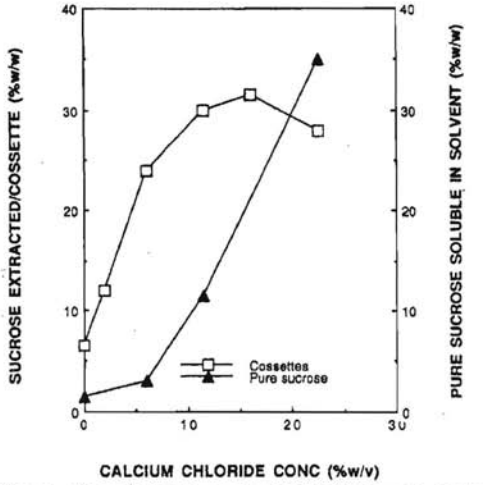

Figure 1. Effect of calcium concentration on extraction of sucrose from dried beet cossettes by methanolic CaCl2. Conditions: Dried cossettes: CH_3OH-$CaCl_2$ = 1:5 (w/v), extracted for 40 min at 60C. The results are compared with the solubility curve of sucrose in methanolic $CaCl_2$ solution.

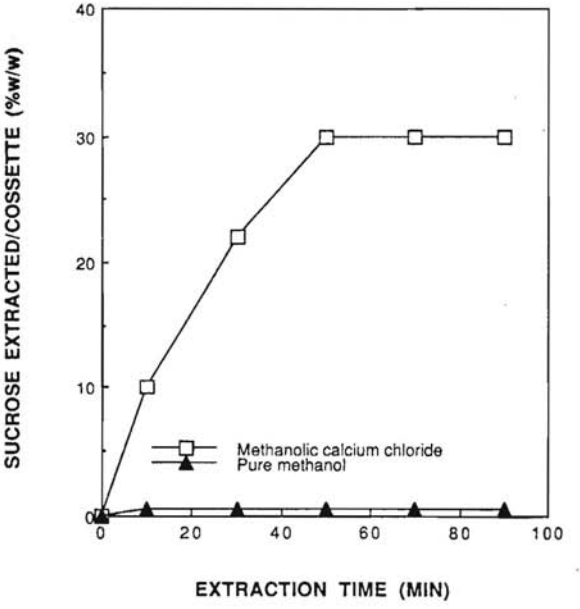

Figure 2. Effect of time on extraction of sucrose from beet cossettes by methanolic $CaCl_2$ solution. Conditions: Dried cossettes: CH_3OH-$CaCl_2$ (12.5%) = 1:8 (w/v); extracted for various times at 60C.

The extraction at 60C reached equilibrium after 50 min (Fig. 2). Increasing time of extraction beyond 50 min did not increase the yield significantly. Pure methanol did not extract any sucrose under the same conditions.

The effect of temperature on the extraction is shown in Fig. 3. Using 12.5% $CaCl_2$ in methanol, the amount of sucrose extracted increased approximately 4 fold when the temperature was raised from 1C to 60C. The result corresponds to the increase in the solubility of sucrose in methanolic $CaCl_2$ solution with temperature. Previous work showed a 4.5 fold increase in pure sucrose solubility in the same temperature range (Wong et al., 1986).

Batch experiments in which sucrose was successively extracted from ground dried cossettes at 65C showed that nearly complete extraction of the sucrose in the cossettes was achieved after four sequential extractions (Fig. 4). Analysis of the combined extracts from four successive extractions of a single sample indicated that 72.2 g of sucrose were extracted from 100 g of cossettes. Based on the total amount of sucrose in the cossettes, as determined by the standard AOAC extraction method followed by HPLC analysis, the yield was calculated to be 98%. A higher efficiency of extraction could be expected if the cossettes were extracted in a continuous countercurrent operation.

In simulated continuous column extractions, the expected steady state extraction phase in which the solvent leaving the column is saturated with sucrose, was not achieved. Instead, the sucrose concentration in the eluate was found to gradually decrease from the start (Fig. 5), indicating that there was insufficient packing of ground beet for equilibrium to be achieved. However, for the experiment shown in Fig. 5, the initial sucrose in solution was 30%, almost the same as the maximum for batch runs (Fig. 1).

Methanolic $CaCl_2$ extraction of sucrose from beet tissue undoubtedly involves the formation of a complex similar to that in aqueous solutions, and possibly adsorption and dissociation. This complicates the simple model of extraction of a solute (sucrose) from the substrate by a solvent (methanolic $CaCl_2$). Precipitates were observed in the first few collected fractions after cooling to ambient temperature and were found to be pure sucrose. Also, the concentration of calcium ion in the early fractions was lower than in the feed. Apparently, calcium ion preferentially adsorbed onto or reacted with the beet tissue,resulting in dissolution of the sucrose-methanolic $CaCl_2$ complex and the release and precipitation of free sucrose. In support of this, sugar beet pectin has been shown to behave as a weak carboxylic cation exchanger with a high affinity for calcium ion, which will actually crosslink the pectin polymers and change the mechanical properties (Camirand et al., 1981).

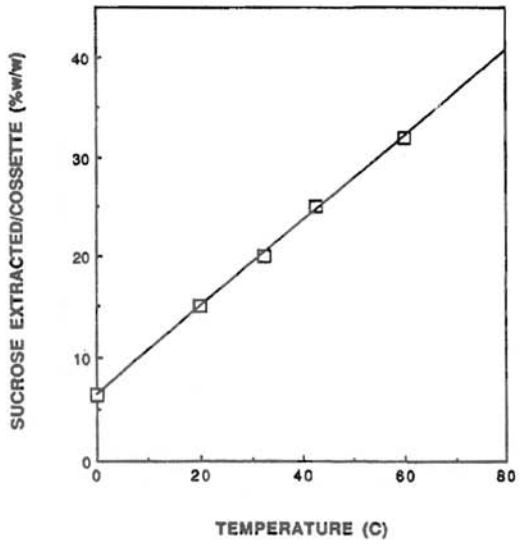

Figure 3. Effect of temperature on extraction of sucrose from beet cossettes by methanolic $CaCl_2$ solution. Conditions: Dried cossettes: CH_3OH-$CaCl_2$ (12.5%) = 1.8 (w/v); extracted for 1 hr.

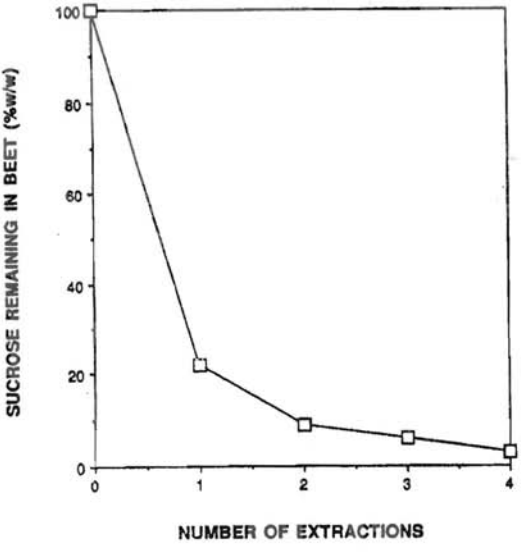

Figure 4. Successive extraction of sucrose from beet cossettes in methanolic $CaCl_2$ solution. Conditions: Dried cossettes: CH_3OH-$CaCl_2$ (11%) = 1:5 (w/v); extracted for 1 hr at 65C four times. Percent (w/w) sucrose remaining in beet calculated based on comparing controls using 80% ethanol as the extractant under same conditions.

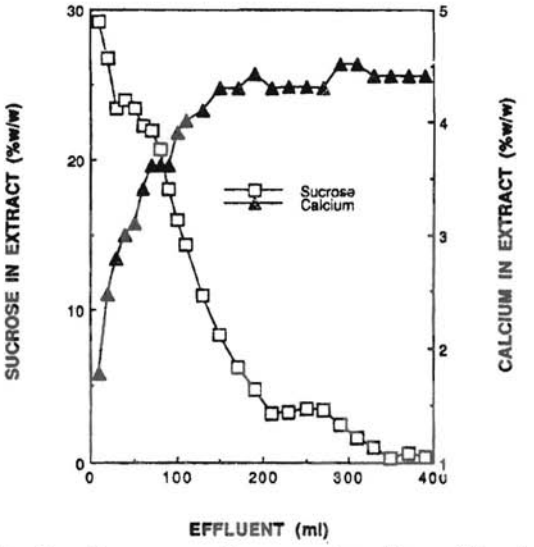

Figure 5. Continuous column extraction of beet cossettes. Conditions: 70 g dried cossettes extracted with methanolic $CaCl_2$ (13%) solution at 65C, constant flow rate of 60 ml/hr.

The amount of sucrose recovered in the precipitates was proportional to the decrease of calcium ion in the eluate fractions from the column. The observed correlation between the loss of calcium and the simultaneous precipitation of sucrose from solution provides a convenient means of estimating the molar ratio of calcium to sucrose in the complex. Calcium and sucrose in each 15 ml effluent sample were analyzed before and after sucrose precipitation. Comparing the weights of sucrose in the precipitates and the differences in calcium content between the feed and eluate fractions, the molar ratio of calcium to sucrose in the complex was found to be 2:1. Variations were observed depending on the original concentrations of calcium and sucrose in solution. Similar variations in stoichiometry and combining ratio have been shown to exist in the interaction of alkali metal complexes of various carbohydrates, depending on cation radius, concentration of the metal salt, and carbohydrate configurations (Rendleman, 1966). Crystalline complexes of glucose-calcium chloride and lactose-calcium chloride formed in methanol were shown to have molar ratios of unity, while fructose complexes were 2:1 (Domovs and Freund, 1960).

Recovery of Sucrose

Recovery of sucrose from solution was effected by adding acetone to the medium, precipitating the sucrose and leaving

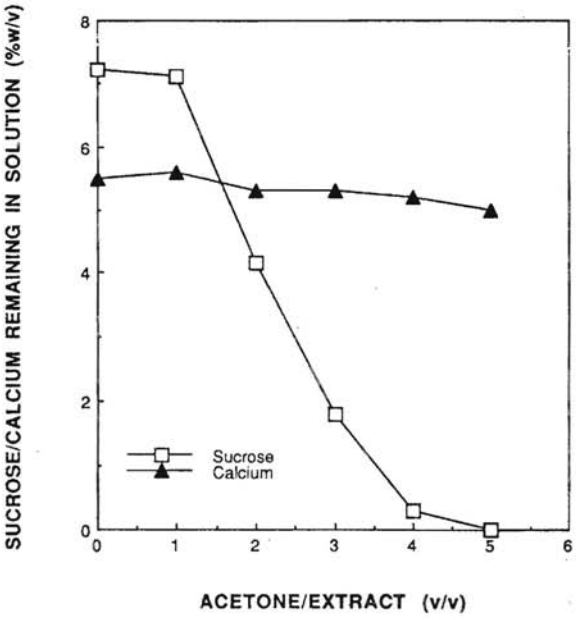

Figure 6. Recovery of sucrose from methanolic $CaCl_2$ extract by acetone precipitation at 25C.

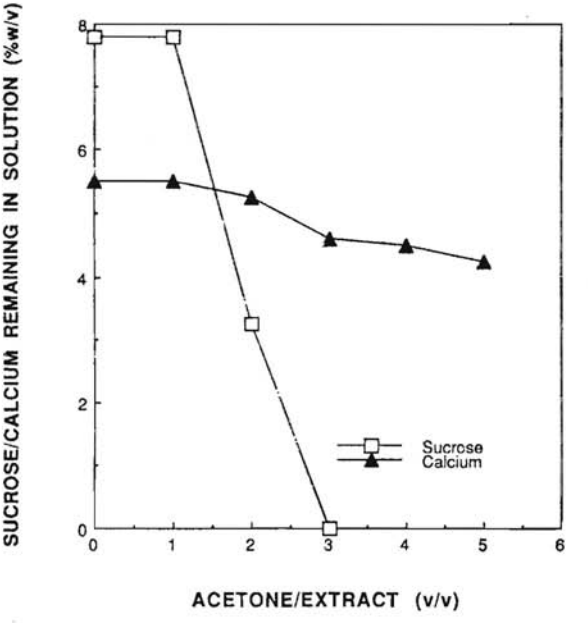

Figure 7. Recovery of sucrose from methanolic $CaCl_2$ extract by acetone precipitation at 60C.

most of the $CaCl_2$ in solution. At 20C-25C, a 5:1 ratio of acetone to cossette extract was necessary to precipitate all the sucrose, while 90% of the $CaCl_2$ remained in the supernatant (Fig. 6). But when the acetone was added to the extract while the temperature was maintained at 60C, the sucrose could be precipitated completely at a 3:1 ratio of acetone to extract (Fig. 7). However, in this case, 15% of the calcium chloride coprecipitated with the sucrose. Precipitation at ambient temperature retained more $CaCl_2$ in the solution, but required larger volumes of acetone to achieve the same yield of sucrose obtained by hot precipitation.

Methanol-acetone azeotrope mixtures have been shown to exhibit nonazeotropic behavior below 200 or above 15,000 mm Hg (Hutting and Horsley, 1947). Theoretically, it is therefore possible to recover the methanol and acetone after sucrose is recovered as a precipitate.

Addition of methanolic NaOH to the extracts precipitated sucrose, but calcium was also precipitated out of the solution. The same result was observed with the addition of aqueous NaOH at low concentrations. High concentrations of aqueous NaOH allowed the sucrose-$CaCl_2$ complex to separate (Wong et al., 1986). Phosphoric acid also interacted with calcium to form phosphate precipitates. However, the sucrose remaining in solution was subject to hydrolysis and degradation. Attempts to recover sucrose from extract were not pursued in the present study, since these methods appeared to be technologically complicated and not economically feasible.

Characteristics of Methanolic $CaCl_2$ Extract

Sucrose is known to form soluble complexes with $CaCl_2$ in methanol, which explains in part the exceedingly high stability of sucrose in methanol-$CaCl_2$ extract. Chromatographic peak profiles of the methanolic solutions showed no degradative loss of sucrose after storage at room temperature for 7 days, while water extracts stored under the same conditions showed significant degradation (Table 1). Losses of 8, 32 and 85% sucrose occurred in samples of water extracts stored for 2, 4 and 7 days, respectively. Similar results were obtained in experiments employing different extraction and storage conditions. The extensive degradation observed in the aqueous extracts could have been caused by microbial actions.

Sucrose purity determinations for both methanolic $CaCl_2$ and water extracts from the same experimental conditions showed that the purity of the former was markedly high (97.10 ± 0.28 vs 92.95 ± 2.05), and the color value was one-half that of the latter.

Table 1. Sugar profiles of extracts stored at 20C.

Sample	Storage Time (Days)	Sucrose	Glucose (g/100 ml)	Fructose
Water Extract †	0	13.60	0	0
	2	12.53	0.55	0.35
	4	9.30	1.93	0.83
	7	2.05	3.65	1.05
Methanolic $CaCl_2$ Extract ‡	0	12.48	0	0
	2	12.30	0	0
	4	12.45	0	0
	7	12.35	0	0

†Extractions were carried out once with ground cossettes, for 1 hr at 80C.

‡Extractions were carried out once with ground cossettes, for 50 min at 60C. (Experiments were not designed to obtain optimum extraction with methanolic $CaCl_2$.)

The advantages of non-aqueous solvent extraction over conventional water extraction include: (1) less colloidal material and colorants that must be removed from the sugar juice, and (2) more recoverable sucrose because of the higher juice purity. Recovery of sucrose from the methanolic $CaCl_2$ system has been shown to be difficult. Precipitation with acetone results in a mixture containing ~5% $CaCl_2$ at optimum conditions. However, the unexpected precipitation of pure sucrose in the extract after cooling in the continuous extraction experiment raises the possibility of employing a suitable ion exchange system to remove calcium ion from the methanolic $CaCl_2$ extract, breaking the calcium saccharate complex. The liberated sucrose would then precipitate as a fairly pure product as the solution cooled.

ACKNOWLEDGEMENT

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Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

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