INVERSION CONTROL IN SUGARCANE JUICE
BY SODIUM META-SILICATE

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ABSTRACT

Sucrose inversion in sugarcane juice samples was delayed for several days by adding sodium meta-silicate immediately after milling. Effective meta-silicate levels, of the order of 40-60 µ moles/ml, gave complete inversion control for 48-60 hr and partial control up to 96 hr. The quantity of inhibitor required was lowered to 2-3 µ moles/ml by prior filtration and treatment of the juice with zinc sulfate and barium hydroxide. Although the deterioration rate for untreated samples varied greatly among different batches of juice, the relative inversion control of meta-silicate remained essentially constant. The principal effect of meta-silicate appears to be the inhibition of endogenous invertases released from stalk tissue by the grinding process. The presence of fructose or glucose in fresh juice leads to more rapid juice deterioration. This suggests that the meta-silicate has no immediate effect upon bacterial invertase other than to deny the microbes their source of carbon for rapid growth. Chromatographic evidence suggests that at low levels meta-silicate forms a physical complex with sucrose which prevents the union of invertase with its substrate. At higher levels a chemical complex is formed specifically at the fructose end of sucrose. The hypothetical fructose-silicate configuration is retained even after sucrose is inverted, thereby preventing fructose from being metabolized by microorganisms. Fructose appears to be the preferential hexose for microbial growth, i.e., the most suitable carbon source. The effective preservation of fructose by silicate may, therefore, constitute a bacterial repression operating in addition to the invertase-inhibitory action.

INTRODUCTION

Since Hartt (1933) first described invertase as a major factor governing sucrose yield, increasing attention has been given to its roles in the intact sugarcane plant. Recent studies have implicated invertase in the sugar accumulation cycle (Glasziou and Waldron, 1964a, 1964b), in growth activity (Glasziou and Bull, 1967; Hatch and Glasziou, 1963), as a diurnal factor of extreme variability (Slack, 1965), and as a useful subject for examining enzyme synthesis mechanisms (Glasziou and Waldron, 1964a, 1964b). There is also reason to believe that sugarcane ripening involves major changes in the levels or behavior of acid and neutral invertases (Hawker and Hatch, 1965). Efforts to control these enzymes in vivo have met with only limited success owing in part to the enormous complexity of an intact physiological system (Alexander, 1966). However, the control of invertase in milled juice—essentially an in vitro environment and hence
a much simpler system for enzyme regulation—has received practically no attention from sugarcane physiologists or biochemists.

Sucrose inversion in milled juice is accomplished by 2 systems: endogenous invertases released from the crushed stalks, and bacterial invertases providing hexoses as carbon and energy sources for the rapid growth of microbial populations. Tilbury (1968) has shown that post-harvest deterioration of cane stalks and juice, i.e., stale and sour cane, is caused principally by the lactic acid bacterium *Leuconostoc mesenteroides*. One consequence of this microbe's presence is an increased production of slime in the juice, which seriously restricts filtration processes and sugar recovery. Tilbury (1969) has further shown that the gelatinous slime masses, comprised mainly of dextran-like polysaccharides, can be removed by treatment of deteriorating juice with the enzyme dextranase. However, the very presence of these deterioration products implies that much sucrose has already been inverted and reconstituted in a useless carbohydrate form. In effect, the problem of preventing the initial sucrose inversion remains unsolved.

In recent years the element silicon (Si) has shown promise as an in vitro invertase inhibitor when provided in the meta-silicate form (Alexander, 1968). The present report describes the use of sodium meta-silicate to delay sucrose inversion in milled juice and summarizes evidence appertaining to the mode of silicate action in preventing sucrose breakdown.

**MATERIALS AND METHODS**

Typical inversion-control experiments employed juice from field-grown stalks of the varieties PR 980 and CP 52-43. Unclarified batches of juice were incubated with sodium meta-silicate or other additives in a water bath at 30 C or 37 C. Samples were withdrawn at intervals of 8, 24, or 48 hr and frozen for subsequent analyses. In some experiments low-quality juice from immature PR 980 stalks was employed; in others, glucose or fructose was added to simulate lower quality. All experiments were initiated within 1 hr after the juice was milled, and incubation periods ranged from 48-332 hr.

Sodium meta-silicate has been the most effective form of Si employed to date in the inversion-control experiments. An unrefined calcium meta-silicate (Hawaiian Cement Corporation) and a Ludox colloidal silica (DuPont) produced little effect at physiological levels. The calcium meta-silicate was almost totally insoluble. At the close of the incubation phase, all samples were thawed, clarified by low-speed centrifugation, and analyzed for sucrose and reducing sugars. Sucrose was determined by the resorcinol method, as modified by Cardini, et al. (1955), and reducing sugars were estimated by the dinitrosalicylic acid method of Sumner (1921). Other juice samples were chromatographed on Whatman no. 1 filter paper using the solvent butanol-pyridine-water (6:4:3, v/v) in one dimension. Authentic and unknown sugar spots were developed by the silver nitrate method of Dube and Nordrin (1961).

Sugar-silicate complexes were examined with a Perkin Elmer gas-liquid chromatography unit (model 154) equipped with flame-ionization and thermal-conductivity detectors. Aqueous solutions of sucrose, fructose, and glucose, with and without added meta-silicate, were lyophilized to dryness and reacted with trimethylsilyl reagent in accordance with published procedures. An acid-washed SE-30 column (3% w/w), coated on Chromosorb W, was employed at 170 C.
RESULTS AND DISCUSSION

Inversion Delay by Sodium Meta-silicate

Preliminary experiments indicated that at least partial preservation of sucrose occurred when sodium meta-silicate was added to the crude juice at rates greater than 20 μ moles/ml. Treated juice immediately assumed a slightly darker color, flocculation of suspended matter occurred, pH rose to about 10.5, and sucrose was retained intact for at least 36 hr. Untreated juice deteriorated rapidly. Within 8 hr the latter showed a lighter color, increased acidity, increased reducing sugars, lower sucrose content, and a rapidly developing odor of fermentation.

Raw juice incubated for 48 hr was found to require some 40-60 μ moles/ml of meta-silicate for complete sucrose preservation (Fig. 1). Partial inversion con-

![Fig. 1. Paper chromatogram illustrating sucrose, fructose, and glucose changes in raw juice incubated with variable amounts of sodium meta-silicate. Dark spots represent heavy reducing sugar concentrations. Grey spots, corresponding to Su, represent sucrose. Authentic sugars are identified along the left-hand side as follows: Ri = ribose; F = fructose; G = glucose; Su = sucrose; Me = melezitose; Ra = raffinose; St = stachyose.](image-url)

control was obtained with 20 μ moles/ml. Subsequent experiments usually employed Si at the rate of 50 μ moles/ml. At this level, initial inversion began after 48-60 hr had elapsed (Fig. 2), although much of the sucrose substrate remained intact at 60 hr. The paper chromatogram illustrated in Fig. 2 suggests that, by 60 hr, inversion had progressed in treated juice to approximately the same degree attained in untreated juice by 8 hr.
Fig. 2. Paper chromatogram illustrating sugar transformations in raw juice incubated for 60 hr with variable amounts of sodium metasilicate. The headings Si 0 and Si 50 appertain to silicate levels of 0 and 50 μ moles/ml, respectively. The numbers 0-60 refer to incubation time in hr. Authentic sugars on the left-hand margin are identical to those defined in Fig. 1.

Both the sucrose decline and its preservation by meta-silicate were reflected in direct sucrose and polarization analyses (Fig. 3A and 3B, respectively).

Fig. 3. A. Preservation of sucrose in raw juice with 50 μ moles/ml of sodium meta-silicate; B. Retention of pol values for sugarcane juice incubated with 50 μ moles/ml of sodium meta-silicate.

Neither sucrose nor pol values changed appreciably during 48 hr when Si was present, but both values declined enormously in the absence of Si.

Silicate-pH Relationships

Because sodium meta-silicate produces a strongly basic pH in aqueous
solution, pH changes were examined in deteriorating juice treated with variable amounts of Si. As shown in Fig. 4, 10 μ moles/ml of Si gave an initial pH value of 8.0 in the crude juice; 100 μ moles/ml gave a value of 11.2. During a 60-hr incubation period all juice samples became increasingly acid, but those having higher Si levels resisted the pH change more effectively. A Si level of 50 μ moles/ml thus retained a pH value of 7.0 or higher for about 36 hr (Fig. 4).

The possibility that high pH is itself restrictive against inversion was examined. Juice samples treated with Si and adjusted to pH 7.0 and 5.6 with 0.1 N HCl deteriorated at rates comparable to unadjusted juice samples. Inversion was delayed in all Si-treated samples, indicating that the silicate's preservative action directly involved the enzyme (invertase) or its substrate (sucrose) rather than the medium's pH.

**Deterioration Rate as a Function of Juice Quality**

The use of raw juice from immature stalks or greenhouse-grown cane invariably led to a more rapid deterioration which Si was less effective in delaying. In these instances paper chromatograms showed a relative abundance of reducing sugars already present in the juice at zero hr. By comparison, juice from well-ripened stalks had contained mere traces of reducing sugar and silicate was highly effective in delaying inversion. It was thought that microbial populations had multiplied using the free hexoses as their carbon source, and these in turn contributed toward sucrose breakdown.

Occasionally, batches of juice were obtained which deteriorated much more slowly than indicated above. In one experiment large amounts of sucrose were still intact after 96 hr of incubation in the absence of Si (Fig. 5). In these instances the presence of Si still served to retard inversion by at least 48 hr. The cause of slow deterioration is not immediately clear, although it did not appear to be a varietal feature. In addition to the ripening status of the milled stalks, the condition of the stalk relative to invertase content, endogenous enzyme inhibitor content, and the degree of microbial contamination during harvest and
pre-milling operations are all factors capable of affecting subsequent juice deterioration rates.

Possible Mode of Silicate Action

It has been proposed that the mode of Si action against invertase is essentially physical; that is, a silicic acid gel might entrap the enzyme-substrate complex or water involved in the hydrolytic reaction. This thesis, which has never been proven, rests on the following evidence: a) Silicate becomes inhibitory over an extremely narrow concentration range, in the order of 2.4-2.9 μ moles/ml for both yeast and cane invertase types. This implies a rather abrupt physical change within the silicate solution, most probably a shift of the silicic acid from a sol to gel form, rather than random encounter of enzyme and inhibitor molecules. b) Silicic acid gels are known to develop in aqueous solutions as a function of pH and temperature (Vlasov and Morgen, 1964; Moulik and Ghosh, 1963). Protein-silicate inhibitory action has shown a particular affinity for hydrolytic enzymes, i.e., cane amylase, phosphatase, ATP-ase, and yeast and cane invertase (Alexander, 1968).

Additional evidence of a silicate-substrate complex was found in the present study during the post-inversion phases of microbial hexose metabolism. In particular, the possibility that Si had formed a linkage with some portion of the fructose end of sucrose became apparent. Paper chromatography of juice samples incubated over a 4-day period showed that all sucrose was inverted by 48 hr unless Si had been added (Fig. 6). Moreover, the microbes which metabolized the newly liberated hexose showed a clear preference for fructose rather than glucose. By 96 hr all fructose was utilized whereas much glucose remained in the deteriorating medium. The phenomenon might be explained by an in-
Fig. 6. Paper chromatogram illustrating sugar transformations in raw juice over a 4-day period. Sugar spots and authentic sugar definitions are identical to those given for Fig. 1.

The preference for fructose is verified in Fig. 7, which illustrates sugar changes during a 6-day experiment in both treated and untreated juice. However, in the Si-treated juice complete inversion was appreciably delayed, and sufficiency of glucokinase or a greater abundance of fructokinase, i.e., enzymes required to phosphorylate the respective sugars prior to their entry into metabolic pathways.
glucose rather than fructose was the product utilized by microbes. Fructose appeared to remain unaffected during the entire 6-day period.

A possible explanation of the Si effect is that silicate formed a chemical linkage with sucrose at the fructose end, thereby rendering the beta-fructosidic bond inaccessible to invertase, i.e., inaccessible to beta-fructosidase. Under these conditions hydrolysis would have to occur at the glucose end through the mediation of an alpha-glucosidase. An invertase of this type, taka invertase, is found in small amounts in immature storage tissue (Alexander, 1965). Traces of the catalyst are possibly present in milled juice. This in turn would explain the slowness of inversion in raw juice.

Once the hydrolysis by alpha-glucosidase had occurred, a Si-fructose configuration would remain which prevents fructose from being utilized by microorganisms. Presumably, the Si-fructose complex would effectively block the C-6 atom so that phosphorylation to fructose-6-phosphate could not occur, or it would block the C-1 atom so that conversion to fructose-1,6-diphosphate could not occur. Bacterial populations would now be forced to metabolize glucose. Phosphorylation of glucose to glucose-6-phosphate (via glucokinase), and conversion of the latter to fructose-6-phosphate (via phosphohexose isomerase) could be a very slow process if either of the enzymes exists in rate-limiting supply.

The present theory thus depicts meta-silicate as having only an indirect effect on invertases per se. The main accomplishment of Si is to preserve fructose. Sucrose preservation is viewed in terms of inhibited beta-fructosidase activity, a very slow alpha-glucosidase reaction, and an inefficient utilization of glucose as a carbon source for microbe growth. This interpretation is supported by the fact that hexose-containing juice tends to deteriorate regardless of Si.

Sugar-silicate Relationships as a Function of Silicate Level

Gas-liquid chromatographic analyses indicated that no chemical reaction occurs between sugars and silicate in aqueous solution when silicate concentrations are less than 25 μ moles/ml (Fig. 8,A-D). At higher silicate levels, i.e., in the order of 50 μ moles/ml, atypical retention peaks were obtained with both sucrose and fructose. No conclusive evidence of a glucose-silicate complex was found. The appearance of multiple retention peaks in place of authentic sucrose (Fig. 8 D) and fructose (Fig. 9 B) is highly suggestive of chemical linkages having formed between these sugars and silicate. Since silicate does prohibit inversion at levels close to 2 μ moles/ml (Alexander, 1968), both in aqueous solution and in clarified juice, the lack of atypical retention patterns at low Si levels is taken as further evidence of a physical barrier forming between sucrose and invertase without chemical alteration of the substrate. Moreover, the fructose moiety rather than glucose is substantiated as the main site of silicate action.

Possible Sucrase-type Reactions in Milled Juice

A growing body of evidence indicates that certain bacterial enzymes (dextranumsucrase, levansucrase, amylonsucrase) catalyze the synthesis of dextrins, levans, and starch-glycogen polysaccharides from sucrose (Hehre, 1949, 1951; Hestrin et al., 1956). A new disaccharide, leucrose, has been synthesized from sucrose through the action of dextranumsucrase, an enzyme isolated from the bac-
A levansucrase from *Aerobacter levanicum* has been shown to produce a branched polysaccharide, or levan, from sucrose and other substrates having a terminal beta-fructofuranosidic group linked to the anomeric carbon of an aldose (Hestrin et al., 1956). During the present investigations with sugarcane juice, paper chromatograms have frequently shown evidence of sucrose derivatives other than fructose and glucose which appear to signify unidentified oligosaccharides, polysaccharides, and lower molecular weight products of hexose metabolism. Indications of chemical-type polymerization were also found in the presence of meta-silicate levels exceeding 50 μ moles/ml. However, there has been no evidence of sucrose derivatives having formed prior to the enzymic inversion of sucrose. This observation is consistent with the view that invertase control is the key to sucrose preservation in milled sugarcane juice.

Fig. 8. Gas-liquid chromatography of aqueous sucrose-silicate preparations. A-C. Normal sucrose retention peaks obtained in the presence of 0, 12.5, and 25 μ moles of silicate/ml, respectively. D. Atypical sucrose retention peaks obtained in the presence of 50 μ moles of silicate/ml.
Fig. 9. Gas-liquid chromatography of aqueous D-fructose solutions. A. Alpha and beta D-fructose retention peaks in the absence of silicate. B. Atypical retention peaks for D-fructose in the presence of 50 μ moles of silicate/ml.

Effects of Juice Clarification on Silicate Requirement.

Addition of meta-silicate to crude juice produces an almost immediate flocculation of suspended matter. It is believed that much of the Si is thereby diverted from invertase control owing to entrapment in the debris of unclarified juice. In 1 experiment, a single filtration through Whatman no. 1 filter paper gave a 5-fold reduction in the amount of Si needed to prevent inversion in the filtrate for 48 hr. Further clarification with zinc sulfate and barium hydroxide lowered the silicate requirement to about 3 μ moles/ml, very close to the level needed to inhibit aqueous solutions of cane and yeast invertase.

The relatively large amount of sodium meta-silicate needed to prevent sucrose inversion is itself evidence of a silicate relationship with the substrate rather than the enzyme. In another sense, such large quantities of Si may be prohibitive against its use in a commercial operation. No pilot tests with silicate have been performed in Puerto Rico at the factory level. In any event, meta-silicate does provide a useful tool for examining the problems of cane and microbial invertase control which must confront any suitable invertase inhibitor.

REFERENCES


