Rapid Determination of Some Nonsucrose Components of Beet Juices

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Received for publication March 8, 1967

Introduction

Analytical procedures are basic to any study of cause and effect relationships that are operative in the processing of beet juices. The purpose for which a particular analysis is made determines the accuracy that is required, but speed and simplicity are always desirable.

It is the purpose of this paper to describe, briefly, some variations we have imposed upon existing methods.

Colorimetric Methods

I. Iron

1,10-phenanthroline and 4,7-diphenyl - 1,10-phenanthroline (bathophenanthroline) are today reagents of choice in the determination of iron (1)[2]. Both reagents react with the ferrous ion in slightly acidic media to give an orange or red color. Although much more sensitive, bathophenanthroline is rather more expensive and seems to adhere to Beer's law only at very low concentrations. 1,10-phenanthroline can be adapted to all situations.

Because of color interference, some inaccuracy results when the method is applied directly to diluted juices.

Ashing or digestion are cumbersome preparations for the test. Decolorizing with iron-free activated carbon has been used successfully (2). Our choice is the use of cation exchange columns.

A strong acid type catex resin of low cross-linkage, such as Dowex 50-WX4, can be rendered iron-free by treating it with a small amount of 20% v/v HCl. Iron can be concentrated on the resin to detect very low concentrations. Interfering color passes through with a small amount of washing, while acidity on the columns is sufficient to free any complexed iron. After rinsing, the iron is simply eluted into test tubes containing buffer, reducing agent and phenanthroline. Batteries of a dozen or so columns can be operated simultaneously.

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[2] Numbers in parentheses refer to literature cited.

Equipment

Test tubes, 20 × 150 mm

Column - pyrex tubing 10 mm dia. × 200 mm long

Resin - Dowex 50-WX4 (100-150 mesh), a bed depth of 2 cm is sufficient.

Reagents

Buffer - Conc. ammonium hydroxide, glacial acetic acid mixed 1.8:1. The final pH should be about 9.0.

Hydroxlamine hydrochloride

0-phenanthroline - 0.2% w/v in distilled water.

20% v/v HCl in distilled water

Working solution - 400 ml buffer, 100 ml 0.2% 0-phenanthroline, and 2.5 g hydroxylamine hydrochloride.

Procedure

Pass a sample of juice containing between 2 and 10 micrograms of iron over the column. Wash with two 5 ml portions of distilled water, allowing the column to drain thoroughly between rinsings (the small mesh resin prevents the bed from going dry). Place a test tube containing 8 ml of working solution under the column. Elute the iron with five 2 ml portions of 20% v/v HCl. Mix thoroughly and allow to stand at room temperature for at least 10 minutes. Read on a colorimeter at 505 μ against distilled water as blank.

The standard curve is similarly prepared, using the same amount of rinse, eluate and reagent. The plot of micrograms of Fe vs. % transmission is a perfectly straight line on two cycle semi-log graph paper.

If a colorless blank is desired, the reagents can be rendered essentially iron-free. A 10% solution of hydroxylamine hydrochloride is of proper pH to allow iron contaminants to react with bathophenanthroline. The colored iron complex may then be extracted with iso-amyl alcohol and the proper amount of hydroxylamine added to the working solution as an aliquot. The proportions of NH_4OH and acetic acid to give a pH of 3.5 to 4.0 pH can be similarly freed of iron, then enough NH_4OH added to bring the pH to 9.0.

If it is necessary to use large amounts of sample—for example, to concentrate the iron in white sugar—it is expedient to have columns expanded at the top as a large reservoir. For example, we use 50 mm lengths of 10 mm diameter tubing sealed to the bottom of large test tubes for columns. Vacuum can be applied to speed the flow of sugar solutions. When these large volumes are used, the standard curve must be set up using as much water over the columns as was used with the sugar.

II. Nitrite

Bacterial infections on the beet end have been shown to be accompanied by nitrite formation which correlates with lactic acid formation (3,4). Most workers now use N-1-napthylethylenediamine dihydrochloride and sulfanilamide in acidic medium for the determination. Sulfanilamide is added first to the acidic sample and a few minutes allowed for diazotization. Ammonium sulfamate and napthylethylenediamine are then added for color formation via coupling with the diazotized sulfanilamide.

We found that if a-napthylamine is substituted for napthylethylenediamine, all reagents can be mixed for a single addition and the reaction still goes to completion. This might also be considered a substitution of sulfanilamide for sulfanilic acid in the classical Griess reaction.

Reagents

A. Dissolve 100 mg a-napthylamine in ca. 1 ml glacial acetic acid. Dilute to 100 ml with distilled water. This is stable for days if refrigerated.

B. Dissolve 1.0 gram sulfanilamide in 100 ml glacial acetic acid (this concentration can be varied considerably without affecting the reaction). This reagent is stable.

C. Working solution. Mix one part A with 3 parts B. This is stable several days under refrigeration.

Procedure

Use clarified sample unless the juice is light in color. Add an aliquot containing 0 to 20 micrograms of nitrite to a test tube and make up to 15 ml total volume. Add 1 ml (or 2 if desired) of working solution and mix thoroughly. Set aside for at least 10 minutes to allow full color development. Read at 520 μ. The standard curve is prepared from $NaNO_2$ standards in the same manner.

Papergram Methods

This approach is not new. It involves the assessment of materials in a spot of sample on filter paper, without chromatographing.

The general method is to spot a series of standards on filter paper. Apply as many unknown spots as desired. React with the proper reagent and compare color intensities of samples and standards.

Spots of standards or samples are applied to the paper with an applicator rod fashioned by drawing out a piece of 5 mm glass rod so that it can be cut cleanly at a position where it is about 1.5 mm in diameter. Slightly fire polish this tip. Test

the applicator to be sure it gives fairly uniform spots about 3-5 mm in diameter. To apply the spot: place the paper on a clean hard surface; barely touch the applicator to the surface of the sample; and tap it lightly to the surface of the paper to deposit the sample. Wiping the tip immediately with a piece of tissue is all the cleaning required before going to the next sample.

I. Nitrite

For this determination, the papers can be pre-impregnated with the nitrite reagents. For this it is necessary to substitute a non-volatile acid for acetic acid; oxalic acid seems to be the most suitable. Higher excesses of sulfanilamide gives more uniform spots, faster reaction and less discoloration of the paper.

Preparation of Indicator Papers

Dissolve about 2 grams of sulfanilamide, 1 gram of oxalic acid, and 20 - 40 mg of a-napthylamine in 100 ml of methanol.

Dip filter paper into this reagent, and blot off the excess between clean towels.

Spread the paper to air dry.

Store in a sealed, light-proof container. Papers are stable for weeks when so handled. Continued exposure to light and/or laboratory fumes will cause deterioration in a few days.

Use of Papers

The papers can be used with the spotting technique described, using standards from 0 to 80 ppm (of NO_2-from $NaN0_2$). Nitrite in raw juice can be estimated to within about 5 ppm.

We find these papers most useful when cut into strips and used as ordinary indicator paper. Simply dipping the paper into suspect juice gives an almost instantaneous appraisal of its nitrite (hence, infection) status. Consequently they are useful for on-site monitoring for infection along diffusers and heaters. Because nitrite build-up is fairly rapid and reaches high levels, no standards are required to determine if nitrite accumulation has begun.

The importance of preventing nitrite accumulation has been amply studied and discussed by Carruthers et al. (4,5).

II. Invert

In a previous study of the TTZ (2, 3, 5-triphenyltetrazolium) reaction for invert, it was noted that, though caustic is necessary to the reaction, excesses caused spontaneous production of the red formazan. Concentration of caustic from the regular TTZ reagent, when dried on paper, would consequently lead to heavy background color.

This is circumvented by substituting an organic base, 2-ethanolamine, for the caustic.

Though not necessary, a little copper in the reagent can serve as a modifying agent to further retard background color and alter the color of the spots from red toward blue. This gives somewhat sharper visual color gradation between different invert concentrations.

Reagents

A. 0.3% w/v solution of TTZ in isopropanol (methanol or 2-methoxyethanol may be used).

B. 0.75% w/v solution of $CuCl_2$ in distilled water.

C. 2-ethanolamine.

D. Working Solution: mix 100 ml of A; 10 ml of B; 2 ml of C.

Procedure

Standards can be any series containing 0 to 2.0 mg of invert/ml; 0.4 mg/ml increments allows rather close evaluation over the range of invert levels normally encountered in press juice. Sample is a drop or two of juice squeezed from a bit of rasped tare sample through a suitable tissue.

Spot standards and samples, as described, on untreated filter paper.

Dip paper through the working solution.

Blot off excess reagent between clean towels and dry at room temperature.

Develop color in a 110°C oven for 3 minutes.

Compare color intensity of samples against standards.

Discussion

Heavy process juices need be diluted to prevent sugar interference (1:4 is satisfactory for thick juice or molasses). If the dilution is too much for the described standards, a lower range of standards can be used and developing time in the oven extended.

The procedure can also be applied to the Sachs Le Docte filtrate in the tare lab. Since lead retards the reaction somewhat, the standards are prepared to contain about the same amount of lead as the samples: a series of 2 to 10 mg/ml invert standards are diluted 1:20 with 4° Brix lead to give spotting standards of 0.1 to 0.5 mg per ml.

III. Amino Acids

Here, again, integration with tare lab operations is easily accomplished. Just as for invert, two slightly different techniques allow the determination to be made on the Sachs Le Docte filtrate or on press juice.

A. Sachs Le Docte Filtrate

Standard

Standards, for spotting, contain 0.1 to 0.5 mg/ml of a 1:1 mixture of glutamic and aspartic acids.

Reagents

A. 3.5-4% aqueous solution of sodium or potassium oxalate, for deleading.

B. 0.2% ninhydrin (1, 2, 3-triketohydrindene) solution in methanol.

Procedure

With medicine droppers, mix one drop of filtrate with 1 drop of oxalate solution on wax paper or a spot-test plate (oxalate strength should be sufficient for complete deleading, but a large excess must be avoided). Final juice dilution is about 1/20.

Sample is taken from the deleaded drop of juice with the applicator tip and applied to filter paper along with the standards.

After the last sample application is dry, dip the paper through the ninhydrin reagent, blot off the excess reagent between clean towels, and air dry.

Develop color in a 105-110°C oven for one minute or until lowest standard is visible.

The evaluation $\times$ 20 is the approximate amino acid content per ml of press juice.

B. Press Juice

A controlled inhibition of the reaction is required for direct application of the test to juice squeezed from rasped tare samples. This is accomplished with copper and isopropanol.

Standards

Should be 2, 4, 6 and 8 mg/ml of amino acid. Juice standards are more desirable than synthetic standards of the glutamic aspartic acid mixture, because of the effects on hue, and sometimes intensity, of color caused by different juice components. It is satisfactory to select a juice of high amino acid content and make suitable water dilutions of this. Actual amino acid content can be closely approximated by the determination of total amino nitrogen and multiplying this value by 10 (7).

Reagents

Inhibitor - 0.5% w/v $CuCl_2$ in 80-90% isopropanol

Stock ninhydrin - 5% w/v in 2-methoxyethanol

Working Solution - 96 ml of inhibitor plus 4 ml of stock ninhydrin

Alternate Working Solution - 97 ml of Fehling #1 solution plus 3ml of 0.2% ninhydrin in methanol

Procedure

A bit of rasped pulp, from the tare sample, is placed on a Kimwipe (or other tissue) and a drop of juice is ssueezed onto wax paper. A spot of the juice is applied to filter paper previously spotted with standards, using the applicator rod previously described. As many additional spots as desired may be spotted on the same filter paper.

After the last spot is completely dry, dip the paper into the working solution, blot the excess reagent off between clean towels and air dry.

Place the paper in an oven at 105-110°C for 3 to 5 minutes, or until the lowest standard is visible. Evaluate the unknown spots against standards.

Literature Cited

(1) DIEHL, HARVEY and G. FREDERICK SMITH. 1960. The iron reagents: bathophenanthroline, 2, 4, 6-tripyridyl - S - triazine, phenyl - 2 - pyridyl Ketoxime. The G. Frederick Smith Co., 867 McKinley Ave., Columbus, Ohio.

(2) STACHENKO, S. and H. EBELL. 1964. Decolorizing beet juices for iron determination. Unpublished.

(3) COX, L. and M. TVEIT. 1964. Assessment of nitrite level in diffusion juices as a tool for measurement of microbial activity. Socker. 19 (4): 47-50.

(4) CARRUTHERS, A., P. J. GALLAGHER and J. F. T. OLDFIELD. 1958. Nitrate reduction by thermophilic bacteria in beet diffusion systems. 11th Ann. Tech. Conf. British Sugar Corp.

(5) CARRUTHERS, A., J. F. T. OLDFIELD, J. V. DUTTON, L. C. MANNING and M. SHORE. 1961. Juice components in relation to sugar quality. 14th Ann. Tech. Conf. British Sugar Corp.

(6) HARRIS, W. A. 1964. The TTZ (Triphenyltetrazolium chloride) reaction for invert sugars. Presented to 13th Gen. Meeting, Am. Soc. Sugar Beet Technol. Unpublished.

(7) HARRIS, W. A. 1962. Determination of amino nitrogen, pyrrolidone carboxylic acid nitrogen and total nitrogen with ninhydrin. J. Am. Soc. Sugar Beet Technol. 12 (3): 200-209.