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A Colorimetric Method for the Quantitation of Galacturonic Acid

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ABSTRACT

A simple, colorimetric assay that is specific for galacturonic acid is presented. The assay uses sulfuric acid, carbazole, a controlled heat water bath, and a spectrophotometer. Galacturonic acid can be detected as free sugar or in polymer forms, such as pectin.

Index Entries: Galacturonic acid; pectin; colorimetric assay.

INTRODUCTION

In the last few years, sugar analysis has been carried out, in many laboratories, by High-Performance Liquid Chromatography (HPLC). Although this analysis is good if a number of sugars are being quantified at once, it is an inefficient way of measuring large sample sets when there is only one sugar of interest. The cost of obtaining and running an HPLC may also be of concern to some laboratories that may not have the funds, expertise, or need for the equipment.

For the analysis of certain sugars, for instance, galacturonic acid, a simple and fast colorimetric assay may be preferable to the more expensive and time-consuming practice of HPLC analysis. A method that will quantify galacturonic acid both in its free form and in such complexes as pectin would be of real benefit to the large-scale analysis of human and animal feedstuffs.

The method presented here (*1*) is based on a test developed by Dische (*2,3*) using carbazole in sulfuric acid. The reaction was quite specific for uronic acids in both monomer and polymer form. By manipulating temperature and acid concentration carefully, galacturonic acid could be distinguished from glucuronic and other acids.

Dekker and Richards (*4*) found that they had to use a correction formula to adjust their values because of increasing absorbance values while reading the assays. McComb and McCready (*5*) and Bitter and Muir (*6*) made further modifications to the assay in an attempt to improve specificity and reflect accurately uronic acid values in biological samples.

The methods all required precise timing or temperature control, and they also suffered to some degree from interference by neutral sugars in nonspecific browning reactions. The method described below is greatly simplified with regard to time, temperature, and reagents. It also shows an improved specificity for galacturonic acid. The assay is very simple, involving only the mixing of two reagents and a short incubation in a water bath. Hundreds of samples per day can be screened for galacturonic acid content. The chromagen is stable at room temperature for at least 21 h, so no correction factors are needed because of delays in reading the first and last of a set of assays.

MATERIALS

1. Concentrated sulfuric acid (96%) is used as received from Fisher Scientific.
2. Carbazole is obtained from Sigma and is also used as received. It is approx 95% pure and should be tested if a different lot is being used for the first time. Carbazole is listed as a possible carcinogen, so appropriate precautions should be taken. Carbazole reagent is a 0.1% w/v solution made up in 100% ethanol. This reagent is kept at room temperature in a lightless cupboard, but no other precautions during storage are taken. The reagent seems to be stable for many weeks.

METHOD

1. Sample, in 200 μL of water, is placed in a 16×150 mm borosilicate tube. Concentrated sulfuric acid (3 mL) is added. Carbazole reagent (100 μL) is then added, and the tube contents are mixed.
2. The tube is then incubated at 60°C in a water bath for 1 h and cooled to room temperature in another water bath.

3. The absorbance of the pink- to red-colored samples is then read on a spectrophotometer at 530 nm against a water blank. (Spec zeroed on water, not reagent blank. Value of reagent blank later subtracted from absorbance value.) If maximum discrimin ation between the uronic acids is required, the incubation can be carried out at 40°C for 4 h.

   For a standard curve, 0–100 μg of galacturonic acid, polygalacturonic acid, or pectin are used.

NOTES

1. Acid concentration: All of the uronic acid methods previously described have involved adding the sugar in a fairly large volume of water to concentrated sulfuric acid. Unless this addition is carefully controlled, the rapid and extreme rise in temperature that results causes highly variable assay results. This might have been the result of incomplete and variable hydrolysis or decarboxylation of the uronic acids. High temperatures also cause the nonspecific destruction of neutral sugars in acid to yield brown residues that can cause high background absorbance. Another problem with assays that contain significant volumes of water is the precipitation of carbazole, which is insoluble in water. This can cause variable results.

   In the present method, there is not enough water in the sample (200 μL) to cause any noticeable heating of the solution. It is, of course, important that all samples are in the same volume of water or buffer when the acid is added, not just for the final concentration of chromagen, but also to equalize any variation owing to the small changes in acid concentration between tubes. No more than 200 μL sample volume should be used or the assay loses specificity.

   The sulfuric acid can be dispensed using a pump attached to the bottle. The whole assembly can be stored on the bench if care is taken to empty the pump chamber before beginning to add acid to the tubes. No other precautions are taken to prevent atmospheric water from entering the acid bottle, but this may become a problem in humid areas.
2. Order of reagent addition: The acid must be added before the carbazole reagent to prevent precipitation of the carbazole on contact with the water. If this precipitate forms, the assay becomes variable, presumably because of erratic dissolution of the chemical or its destruction by acid hydrolysis.

3. Incubation temperature: Although the best response to galacturonic acid was obtained at 60°C, a slightly more specific reaction occurs at lower temperatures. At higher temperatures, other sugars begin to show reactions of more than 10% of the galacturonic acid value, whereas the galacturonic acid itself shows a drop in absorbance. Nevertheless, if the assay is incubated in a boiling water bath, maximum and stable absorbance values are reached in just 5 min; 60°C for 1 h is a good and useful incubation combination for good discrimination.
4. Interfering substances: None of the neutral sugars tested so far showed a reaction of over 10% of the galacturonic acid value in this assay. Most of the buffers we have tested so far show no reaction, but sodium citrate does show a repression of galacturonic acid absorbance. Borate ions will destroy the selectivity of the assay to glucuronic acid, allowing it to react similarly to galacturonic acid, which gives a somewhat lower absorbance. Some neutral sugars also show increased absorbances in the assay with borate ions.

   Glyceraldehyde, formaldehyde, and glutaraldehyde all react, and will cause an overestimation of galacturonic acid if present in the sample. Aldehydes do however make the assay green, so these substances should be easy to spot.
5. Polymer sugars and esterified galacturonic acid: The assay seems to give values for polymers, such as pectin and polygalacturonic acid, which are close to the stated nonesterified galacturonic acid contents. Pectin shows a maximum absorbance at 1.5 h of incubation, which indicates that some polymers may be more resistant to hydrolysis than others. Each sample type should be checked to ensure that complete reaction has been achieved.

   Esterified galacturonic acid may be deesterified by incubation in 0.05% NaOH for 30 min at room temperature. This can be done in the sample tube prior to addition of the acid. The saponification has no effect on galacturonic acid values.

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