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PREPARATION OF SODIUM STRONTIUM GALACTURONATE FROM CITRUS PRODUCTS

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ABSTRACT

The present paper describes the convenient separation of galacturonic acid in the form of sodium strontium galacturonate from the hydrolyzates of citrus products, namely pectic acid, pectin, and the peel of oranges and of grapefruit. The methods are suitable for the preparation of large or small quantities of the salt, and it is believed that the preparation from pectic acid is commercially practicable.

Sodium strontium galacturonate is obtained from pectic acid in about 90-percent yield without the use of alcohol or other organic solvent. By treatment of the salt with an equivalent quantity of aqueous sulfuric acid, a solution is obtained from which about 65 percent of the galacturonic acid may be crystallized in one crop, substantially pure. Additional crops obtained by the use of an organic solvent make the yield nearly quantitative.

Pectin is not quite so satisfactory in the process as pectic acid, but because of its educational value, the preparation of sodium strontium galacturonate from pectin might be included in a course of laboratory instruction to demonstrate the presence of galacturonic acid in pectic substances. Both orange peel and grapefruit peel may be used for the preparation of sodium strontium galacturonate, but the industrial use of these materials does not seem feasible at present on account of the large quantities of relatively expensive enzyme required for hydrolysis.

CONTENTS

	Page
I. Introduction.....	401
II. Experimental procedure.....	402
1. Preparation of sodium strontium galacturonate from pectic acid.....	402
2. Preparation of sodium strontium galacturonate from citrus pectin.....	404
3. Preparation of sodium strontium galacturonate from orange peel.....	404
4. Preparation of sodium strontium galacturonate from grapefruit peel.....	405
5. Preparation of galacturonic acid from sodium strontium galacturonate.....	406
III. References.....	406

I. INTRODUCTION

The possibility of preparing vitamin C from galacturonates [1]¹ makes important the examination of various methods and raw materials for the production of salts of galacturonic acid. In previous papers from this laboratory it has been shown that galacturonic acid can be separated directly from the hydrolyzates of pectic substances in the form of certain new salts, particularly calcium galacturonate, sodium calcium galacturonate and sodium strontium galacturonate [2]. Some of the studies on the preparation of galacturonic acid have been conducted on hydrolyzates of beet pulp [3]. In the course of the work it was found that sodium strontium galacturonate crystal-

¹ Figures in brackets indicate the literature references at the end of this paper.

lizes from crude hydrolyzates in higher yield than the other aforementioned salts. The present paper describes the convenient separation of galacturonic acid in the form of sodium strontium galacturonate from the hydrolyzates of citrus products, namely, pectic acid, pectin, and the peel of oranges and of grapefruit. The methods are suitable for the preparation of large or small quantities of the salt, and it is believed that the process described here is commercially practicable.

The use of pectic acid for the preparation of galacturonic acid was studied by Ehrlich [4] and by Link and coworkers [5]. Mottern and Cole [6] improved the process by employing a commercial pectic enzyme for the hydrolysis of pectic acid, and Rietz and Maclay [7] showed that the method was also applicable to the preparation of galacturonic acid from pectin. In the preparation of sodium strontium galacturonate reported here, pectic acid is the most convenient raw material for laboratory use, and the most economical at the present price of the commercial enzyme. Pectin is not quite so satisfactory in the process as pectic acid; however, the preparation of sodium strontium galacturonate from citrus pectin is described, because its educational value and simplicity are such that it might well be included in a course of laboratory instruction to demonstrate the presence of galacturonic acid as a constituent of pectic substances.

Both orange peel and grapefruit peel may be used for the preparation of crystalline sodium strontium galacturonate. Although a considerable part of the residues from oranges and lemons is used industrially in the preparation of pectin, the residues from grapefruit have at present little use, aside from a small portion, from which naringin, the bitter glycoside of the peel, is extracted [8]. The convenient preparation of sodium strontium galacturonate from grapefruit peel is therefore significant in that it indicates a possible use for this waste product. The hydrolysis of the citrus fruit residues requires a somewhat larger amount of the enzyme than the hydrolysis of purified pectic acid or pectin. Hence the practicability of the direct utilization of the residues depends upon the cost of the enzyme in large quantities, a factor unknown at present. As mentioned in connection with the utilization of beet pulp [3], the culture media from molds grown for the production of penicillin or other substances may ultimately provide an inexpensive source of pectic enzymes.

II. EXPERIMENTAL PROCEDURE

1. PREPARATION OF SODIUM STRONTIUM GALACTURONATE FROM PECTIC ACID

The pectic acid used in the present investigation was purchased from the California Fruit Growers' Exchange, Ontario, Calif. It contained 98 percent of galacturonic acid as determined by the carbon-dioxide method [9], but analyses of material purchased at various times have differed considerably. The method given here is suitable for a pectic acid having a galacturonic acid content of 95 percent or above. For a pectic acid of lower galacturonic acid content, the amounts of sodium bicarbonate and strontium carbonate should be proportionately adjusted.

A dry mixture consisting of 1,000 g of pectic acid, 150 g of sodium bicarbonate, and 100 g of Pectinol-46AP² was added in small por-

² The pectic enzyme is manufactured by Rohm and Haas, Philadelphia, who have standardized it by dilution with diatomaceous earth to an activity arbitrarily designated 100D.

tions, with continuous stirring, to 10 liters of water. Excessive foaming was prevented by the addition of a commercial antifoam agent. Sufficient toluene was added to cover the surface of the liquid, and the mixture was allowed to stand at 40° C. After 1 day, 125 g of strontium carbonate was added in portions, and the mixture was seeded with crystals of sodium strontium galacturonate. During the period of hydrolysis the salt crystallized from the hydrolyzate. After a total of 9 days, an additional quantity of 125 g of strontium carbonate was added, and the mixture was allowed to stand in the refrigerator for a day. The salt was then separated by filtration, washed with cold water and dried. The crude product weighed 1,300 g and contained 128 g of water-insoluble material. To crystallize the sodium strontium galacturonate in the filtrate, 25 g of strontium acetate was added and the solution was evaporated under reduced pressure at a temperature below 50° C to a volume of about 500 ml. The concentrated solution was allowed to stand for 1 day in the refrigerator and the crystals which formed (152 g) were separated by filtration. An additional quantity of 54 g was obtained by concentration of the mother liquor. The three crops of crude sodium strontium galacturonate were combined and recrystallized by dissolving the salt in 12 liters of boiling water, filtering the hot solution with the aid of a decolorizing carbon, and allowing the mixture to cool. The crystals which formed were separated, washed with cold water, and dried. The filtrate was concentrated in a vacuum still, and additional crystals were thus obtained. The yield of sodium strontium galacturonate, $\text{Na Sr}(\text{C}_6\text{H}_5\text{O}_7)_3 \cdot 6\text{H}_2\text{O}$, obtained without the use of an organic solvent, was 1,190 g, or 88.6 percent of the galacturonic acid content of the pectic acid used.

The mother liquor from the first crop of crude sodium strontium galacturonate contains the residual pectic enzymes and may be used for the preparation of additional salt without the use of a new supply of the enzyme preparation. In an experiment similar to that just described, the solution which remained after separation of 1,085 g of sodium strontium galacturonate from the hydrolyzate of 1 kg of pectic acid was mixed with a second kilogram of pectic acid and 150 g of sodium bicarbonate. The mixture was allowed to stand, with occasional stirring, at 40° C. After 2 days, 125 g of strontium carbonate was added, and after a total of 10 days, an additional quantity of 125 g. The mixture was placed in a refrigerator for 1 day, and the crystals, which formed, were separated, washed with cold water, and air-dried. They weighed 1,400 g. Thus the total yield of sodium strontium galacturonate obtained from 2 kg of pectic acid without an evaporation step, or the addition of a second quantity of enzyme, was 2,485 g, or 92.5 percent of the galacturonic acid content of the pectic acid used. When additional pectic acid and sodium bicarbonate were added to the mother liquor, hydrolysis of the pectic acid was very slow, and it thus appeared that the enzyme had become more or less inactive. Upon the addition of a second quantity of Pectinol (50 g), the hydrolysis proceeded as before. Strontium carbonate was added as in the previous hydrolysis, and the resulting sodium strontium galacturonate was separated. Although in continuous operation the enzyme loses its activity and the impurities accumulate in the mother liquor, the crystallizing properties of sodium strontium galacturonate are such that the process may be continued for a considerable period before it is necessary to discard the mother liquors.

2. PREPARATION OF SODIUM STRONTIUM GALACTURONATE FROM CITRUS PECTIN

A mixture of 10 g of dry citrus pectin,³ 1.25 g of sodium bicarbonate, and 1 g of Pectinol was added in portions with stirring to 100 ml of water contained in a 200-ml Erlenmeyer flask. The mixture was protected from mold growth by the addition of 5 ml of toluene and allowed to stand in a warm place (25° to 40° C). After 1 week, the mixture was neutralized by the addition of strontium carbonate (3 g) and heated to boiling to insure complete solution of the product. The hot solution was filtered with the aid of about 1 g of diatomaceous earth. The filtrate was concentrated in a vacuum still, at a temperature below 50° C, to a volume of about 30 ml and was then kept overnight in the refrigerator. The crystals of sodium strontium galacturonate, which formed, were collected on a filter, washed with cold water, and dried. The product obtained from 10 g of citrus pectin weighed 8.7 g, corresponding to approximately 75 percent of the galacturonic acid content of the pectin.⁴

3. PREPARATION OF SODIUM STRONTIUM GALACTURONATE FROM ORANGE PEEL

The peels from Florida oranges were coarsely ground, and washed with cold water to remove the water-soluble constituents. The washed, moist peel contained 71.3 g of galacturonic acid per kilogram, as determined by the CO₂ method. One kilogram of the moist peel was heated to boiling with 2 liters of water, and the mixture was then cooled to 40° C. After the addition of 50 g of Pectinol and 20 ml of toluene, the mixture was kept at a temperature of 40° C and stirred occasionally. In the course of the first day, the acidity increased to about pH 3.2 and then remained substantially constant for the remainder of the hydrolysis period. Since this value does not differ widely from the optimum for the enzyme, no adjustment of acidity was made. After 7 days, the material was transferred to a filter, and the hydrolyzate was separated from the residue.⁵

An analysis of the hydrolyzate gave the following results:⁶

Soluble solids	131 g/kg of moist peel.
Volatile acid	0.048 equivalent/kg of moist peel.
Free acid	0.338 equivalent/kg of moist peel.
Copper-reducing substances	115 g/kg of moist peel.
Galacturonic acid (CO ₂ method)	64.3 g/kg of moist peel.
Arabinose	28 g/kg of moist peel.

The hydrolyzate from 1 kg of orange peel, containing 64.3 g of galacturonic acid, was heated to boiling, and treated with 16.4 g of strontium carbonate. After the strontium carbonate had reacted, the solution was cooled, and 9.3 g of sodium bicarbonate was added. The solution was acidified with acetic acid, and filtered with the aid of a decoloriz-

³ The sample of pectin contained 84 percent of galacturonic acid by analysis. After the first day, the acidity of the mixture should be within the pH range 3.5 to 4.5. With most pectins, the proportions given here are satisfactory.

⁴ For student instruction, the presence of the galacturonate radical may be readily demonstrated by the following reaction discovered by Ehrlich [10]: A few milligrams of the salt, dissolved in 2 ml of water, is treated with 5 ml of a saturated solution of basic lead acetate. A white precipitate forms, which is soluble in an excess of the reagent. When the solution is boiled, the precipitate becomes orange, and finally brick red.

⁵ The addition of the pectic enzyme to citrus fruit residues causes a softening or degradation of the cell walls, which facilitates the separation of the oil in the peels. Although removal of the oil is not necessary in the preparation of sodium strontium galacturonate, it may nevertheless be advantageous to separate it prior to filtration, or to use as a raw material, citrus fruit residues from which the oil has been removed.

⁶ The analytical methods are described in [3].

ing carbon. The filtrate was concentrated in a vacuum still at 40° C to a volume of 110 ml. During the evaporation, crystals of sodium strontium galacturonate formed. The material was allowed to stand overnight in a refrigerator, and the salt was then collected on a filter, washed with a small quantity of cold water, and dried. The product, substantially pure sodium strontium galacturonate, weighed 58.8 g. The amount corresponds to a yield of 66.7 percent, based on the galacturonic acid content of the hydrolyzate.

4. PREPARATION OF SODIUM STRONTIUM GALACTURONATE FROM GRAPEFRUIT PEEL

The peel of thick-skinned, seedless western grapefruit was coarsely ground in a food chopper and thoroughly washed in a bag suspended for 18 hours in running water. The moist, washed peel, containing 12.7 percent of solids, was heated to boiling with 2 parts of water. The mixture was cooled, treated with one-twentieth part of Pectinol and a small quantity of toluene, and allowed to stand at 40° C. After 10 days, the material was filtered on a Büchner funnel, and the residue was washed with water. The combined filtrate and washings were used for analysis and for the preparation of sodium strontium galacturonate.

An analysis of the hydrolyzate gave the following results:

Soluble solids	79 g/kg of moist peel.
Volatile acid	0.036 equivalent/kg of moist peel.
Free acid	0.172 equivalent/kg of moist peel.
Copper-reducing substances	62 g/kg of moist peel.
Galacturonic acid (CO ₂ method)	44.9 g/kg of moist peel.
Arabinose	11 g/kg of moist peel.

The hydrolyzate corresponding to 1 kg of moist peel was heated to boiling and neutralized with 11.5 g of strontium carbonate and 6.5 g of sodium bicarbonate. By filtration and concentration of the solution, 50.2 g of crystalline sodium strontium galacturonate was obtained. The amount corresponds to a yield of 81.6 percent, based on the galacturonic acid content of the hydrolyzate.

A similar experiment conducted on the unwashed peel, containing 19.3 percent of total solids, yielded a hydrolyzate that gave the following results:

Soluble solids	148 g/kg of moist peel.
Volatile acid	0.027 equivalent/kg of moist peel.
Free acid	0.170 equivalent/kg of moist peel.
Copper-reducing substances	136 g/kg of moist peel.
Galacturonic acid (CO ₂ method)	29.8 g/kg of moist peel.
Arabinose	6 g/kg of moist peel.

The hydrolyzate corresponding to 1 kg of the moist peel was treated with 7.5 g of strontium carbonate and 4.3 g of sodium bicarbonate. By concentration of the solution, 15 g of sodium strontium galacturonate was obtained. This amount corresponds to 36.7 percent of the galacturonic acid content of the hydrolyzate. The mother liquors contained a large amount of reducing substances which require further study.

5. PREPARATION OF GALACTURONIC ACID FROM SODIUM STRONTIUM GALACTURONATE

One mole of sodium strontium galacturonate (798.2 g) was added with stirring to 1,500 ml of aqueous 2 *N* sulfuric acid.⁷ The solution was filtered to remove the strontium sulfate, and was then concentrated to a volume of 900 ml in a vacuum still at a temperature of 40° C. The strontium sulfate that separated during the evaporation was removed by filtration, and the residue on the filter was washed with 100 ml of hot water. The clear filtrate was seeded with crystalline galacturonic acid hydrate and was allowed to cool to room temperature with constant stirring. After crystallization at room temperature was substantially complete, the mixture was placed in a refrigerator for 18 hours. The crystals of galacturonic acid hydrate which formed were collected on a filter and washed with ice water. The crop of galacturonic acid monohydrate, obtained without the use of an organic solvent, weighed 407 g and was substantially pure. The mother liquor was concentrated in a vacuum still to a volume of 400 ml and was then diluted with 1 liter of methyl alcohol. The alcohol was added portion-wise over the space of several hours in order to facilitate crystallization of sodium sulfate. Crystallization was induced by seeding with $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$. The salt was separated by filtration, washed with methyl alcohol, and discarded. The alcohol filtrate and wash liquor were transferred to a vacuum still and the methyl alcohol was recovered. The sirupy residue (200 ml) from the still was seeded with galacturonic acid hydrate, and the crystals which formed in the course of 1 day were separated by filtration. By concentrating the mother liquor, additional material was obtained. The total yield of crystalline galacturonic acid was 607 g, or 95.4 percent of the theoretical. The acid may be recrystallized readily from water or from aqueous acetic acid, as described in a previous publication [11]. The yield of crystalline acid obtained from the sodium strontium salt is considerably higher than that reported by Link and Nedden from barium galacturonate [5(b)].

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WASHINGTON, October 2, 1944.

⁷ For satisfactory results an excess of sulfuric acid must be avoided; a small excess of the salt is not objectionable.

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