

PATENT SPECIFICATION

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(54) DEXTROSE PRODUCTION WITH IMMOBILISED GLUCOAMYLASE

(71) We, CPC INTERNATIONAL, INC., a Corporation organized under the laws of the State of Delaware, United States of America, of International Plaza, Englewood Cliffs, New Jersey 07632, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to the production of dextrose from starch through the use of an immobilized glucoamylase enzyme.

Processes for conversion of starch to dextrose have long been known in the art. Glucoamylase is an enzyme capable of converting starch to dextrose. The use of glucoamylase for producing dextrose and dextrose-containing syrups is well known in the art. Processes using glucoamylase generally fall into three categories. These are the acid-liquefaction-enzyme conversion process, the enzyme-liquefaction-enzyme conversion process, and the enzyme-solubilization-enzyme conversion process (the granular starch hydrolysis process as disclosed and claimed in U.S. Patent Nos. 3,922,197; 3,922,198; 3,922,199, and 3,922,200).

In the acid-enzyme process, starch is liquefied and hydrolyzed in an aqueous suspension containing 20 to 40 percent starch and an acid, such as hydrochloric acid. The suspension is then heated to a high temperature, i.e., a temperature between about 70°C. and about 160°C. and at a pH between about 1 and 4.5 to liquefy and partially hydrolyze the starch. The liquefied and partially hydrolyzed starch will generally have a dextrose equivalent (D.E.) value up to about 20 and preferably up to about 15. Typical acid-enzyme processes are disclosed in U.S. Patent Nos. 2,305,168; 2,531,999; 2,893,921; 3,012,944 and 3,042,584.

In the enzyme-enzyme process, starch is liquefied and partially hydrolyzed in an aqueous suspension containing 20 to 40 percent starch and a liquefying enzyme such as bacterial *alpha*-amylase enzyme at a temperature

of from about 85°C. to about 105°C. The dextrose equivalent value of the liquefied and partially hydrolyzed starch is generally less than about 20 and preferably less than about 15. A process for preparing a low dextrose equivalent partial hydrolysate suitable for converting starch to dextrose and dextrose-containing syrups comprises liquefying starch in water with a bacterial *alpha*-amylase enzyme preparation to a dextrose equivalent value of from about 2 to about 15, heat treating the slurry containing the liquefied starch to a temperature greater than about 95°C., and thereafter converting the liquefied starch with a bacterial *alpha*-amylase enzyme preparation to a D.E. of up to about 20. This process is disclosed and claimed in U.S. Patent No. 3,853,706. In the enzyme-liquefaction-enzyme process, dextrose equivalent values of about 97 are regularly obtained.

In the enzyme-solubilization-enzyme process, a slurry of granular starch is digested by the action of bacterial *alpha*-amylase (preferably a bacterial *alpha*-amylase enzyme preparation derived from the microorganism *Bacillus licheniformis*) under conditions such that some granular starch is present during digestion. The digested starch may be thereafter converted to dextrose or dextrose-containing syrups by other enzymes such as glucoamylase.

The low dextrose equivalent liquefied starch hydrolysates prepared by any one of the three processes mentioned above can then be treated with soluble glucoamylase enzyme preparations to convert the low dextrose equivalent starch hydrolysate to dextrose or dextrose containing syrups.

Glucoamylase preparations are produced from certain fungi strains such as those of genus *Aspergillus*, for example, *Aspergillus phoenicis*, *Aspergillus niger*, *Aspergillus awamori*, and certain strains from the *Rhizopus* species and certain *Endomyces* species. Glucoamylase effects the hydrolysis of starch proceeding from the non-reducing end of the starch molecule to split off single glucose units

at the α -1,4 or at the α -1,6 branch points. Commercial glucoamylase enzyme preparations comprise several enzymes in addition to the predominating glucoamylase, for example, traces of proteinases, cellulases, α -amylases, and transglucosidases.

α -Amylase enzyme is produced from many types of microorganisms, for example by certain *Aspergillus* species and *Bacillus subtilis*. α -Amylase is an endo enzyme capable of randomly splitting the starch molecule into smaller chain units and is used in the enzyme-enzyme process as liquefying enzyme. α -Amylase does not selectively split off dextrose units and breaks only the α -1,4 chain link. Debranching enzymes or α -1,6-glucosidases have recently been used for their ability to break the α -1,6 linkages which cannot be hydrolyzed or broken by the action of α -amylase.

Considerable interest has been developed in the use of immobilized enzyme technology to continuously produce dextrose from starch. Various procedures have been described for the immobilization of glucoamylase, α -amylase, and amylolytic enzyme combinations.

In the art of enzyme immobilization, glucoamylase immobilization has received considerable attention. Many methods of glucoamylase immobilization are available, for example, in U.S. Patent Nos. 2,717,852; 3,519,538; 3,619,371; 3,627,638; 3,672,955; 3,715,277; 3,783,101; and 3,950,222.

Processes using immobilized glucoamylase treatment of starch hydrolysates have recently been reported from Iowa State University. Examples of these processes are the following: Weetall and Suzuki *Immobilized Enzyme Technology: Research and Applications*, Plenum Press, New York, New York (1975) pp. 269—297; Lee et al. *Die Starke* 27 (1975) No. 11 pp. 384—387; Lee et al. *Biotechnology and Bioengineering*, Vol. XVII (1976) pp. 253—267; Lee et al. Paper 601, 10th ACS Midwest Meeting, Nov. 7, 1974.

In the Iowa State work, glucoamylase was covalently immobilized on Corning porous silica ceramic carrier. Low dextrose equivalent starch hydrolysates produced by the previously described processes were continuously passed over the immobilized glucoamylase. The maximum dextrose concentration in the product (based on dissolved solids) varied from 87 to 93 percent depending on the dextrose equivalent and the amount of reversion products in the feed. In all examples, yields of dextrose using immobilized glucoamylase were lower than that obtained using soluble glucoamylase on the same substrate.

High dextrose equivalent hydrolysates produced using α -amylase resulted in lower dextrose yields than obtained with low dextrose equivalent hydrolysates. For example, in the Die Starke report, when higher dextrose equivalent substrates were substituted for the 24

D.E. material initially employed, dextrose concentration as percent of total dissolved solids decreased from 90.1% to 87.0% for 34 D.E. substrate and 86.6% for 42 D.E. substrate.

German Patent Specification OLS 25 38 322 discloses a process for the conversion of starch to dextrose through the use of a combination enzyme system consisting of immobilized glucoamylase and α -amylase. The latter enzyme can be soluble α -amylase or immobilized α -amylase. It is important to note that, during the immobilization of the glucoamylase preparation, the α -amylase inherently present therewith becomes essentially inactive. Thus, in order to provide maximum utilization of the immobilized glucoamylase in the conversion of starch to dextrose, it is taught that additional soluble and/or immobilized α -amylase must be made available during the conversion in addition to the glucoamylase.

In accordance with this invention a process is provided for the production of dextrose from starch which comprises the steps of:

- providing a low D.E. starch hydrolysate having a dextrose equivalent from 2 to 20 and treating the low D.E. starch hydrolysate with a soluble glucoamylase preparation to produce a starch hydrolysate having a dextrose equivalent less than 85,
- reacting the soluble glucoamylase treated starch hydrolysate with an effective amount of an enzyme consisting essentially of immobilized glucoamylase and;
- recovering a dextrose product having a dextrose content of at least 90 percent dry weight basis.

Preferably the starting starch hydrolysate is produced by reacting starch with hydrolytic enzymes or acid.

It has unexpectedly been discovered that through practice of this invention, high dextrose yields can be obtained by continuous treatment of high dextrose equivalent (D.E.) starch hydrolysates utilizing an enzyme process differing from the prior art processes in that the process of this invention consists essentially of an immobilized glucoamylase (devoid of free of immobilized active α -amylase). In contrast to previous methods, dextrose contents found using an immobilized glucoamylase closely parallel those obtained using soluble glucoamylase. Furthermore, dextrose content was essentially constant throughout the range of 30 D.E. to 85 D.E. In addition, improved enzyme utilization was obtained as shall be demonstrated more fully hereinafter. The combination of high dextrose yield and improved enzyme utilization in a continuous process using an immobilized glucoamylase as the sole enzyme is a commercially significant development for conversion of starch to dextrose.

The present invention can utilize starch or a low dextrose equivalent starch hydrolysate of

2 to 20 D.E. as starting material. A significant distinction over prior art processes is that the starch or low dextrose equivalent starch hydrolysate must be treated with a soluble glucoamylase preparation to produce a high dextrose equivalent starch hydrolysate prior to reaction with the immobilized glucoamylase. The high dextrose equivalent hydrolysate has a dextrose equivalent in the range of 30 to 85 D.E. and most preferably has a dextrose equivalent in the range of 45 to 85 D.E.

The low dextrose equivalent starch hydrolysate can be produced as previously described, for example, the acid-liquefaction-enzyme process, the enzyme-liquefaction-enzyme conversion process, or the enzyme-solubilization-enzyme conversion process. If a low dextrose equivalent starch hydrolysate is the starting material, the dextrose equivalent must be increased to the desired high dextrose equivalent level through treatment with a soluble glucoamylase preparation either alone or in combination with other hydrolytic enzymes such as *alpha*-1,6-glucosidases.

According to this invention, the high dextrose equivalent starch hydrolysate of less than about 85 D.E. is treated solely with an immobilized glucoamylase preparation (i.e. devoid of free or immobilized active *alpha*-amylase) to produce the dextrose. The immobilized glucoamylase preparation is used alone without the addition or combination of other soluble or immobilized *alpha*-amylase enzymes, and no further steps or enzymatic treatment are required to produce the dextrose level desired in the product. Dextrose products containing about 95 percent dextrose were produced in column operation using immobilized glucoamylase preparations at 25 percent solids and 45°C. when the starch hydrolysate feed was greater than 30 D.E. Dilution of the starch hydrolysate feed to 10 percent solids gave an effluent containing about 97 percent dextrose.

Immobilized glucoamylase preparations are well known and can be produced, for example, by any of the methods previously discussed, during which the *alpha*-amylase portion of the glucoamylase becomes inactivated and can therefore be present as the inactive form of *alpha*-amylase. It is preferred to use the immobilized glucoamylase preparation produced according to U.S. Patent No. 3,783,101 in which the glucoamylase preparation is covalently coupled to silica.

Glucoamylase activity units are determined as follows: The substrate is a 10–20 D.E. *alpha*-amylase thinned hydrolysate of waxy maize starch dissolved in water and diluted to 4.0 grams of dry substance per 100 ml. of solution. Exactly 50 ml. of the solution is pipetted into a 100 ml. volumetric flask. To the flask is added 5.0 ml. of 1.0 molar sodium acetate-acetic acid buffer (pH: 4.3). The flask is placed in a water bath at 60°C. and after 10

minutes the proper amount of enzyme preparation is added. At exactly 120 minutes after addition of the enzyme preparation the solution is adjusted to a phenolphthalein end-point with 0.5 normal sodium hydroxide. The strength of the sodium hydroxide solution must be adjusted so that the total volume after neutralization is less than 100 ml. The solution is then cooled to room temperature, and diluted to volume. A reducing sugar value, calculated as dextrose, is determined on the diluted sample and on a control with no enzyme preparation added. Glucoamylase activity is calculated as follows:

$$A = \frac{S - B}{2 \times E}$$

where

A = glucoamylase activity units per ml. (or per gram) of enzyme preparation.

S = reducing sugars in enzyme converted sample, grams per 100 ml.

B = reducing sugars in control, grams per 100 ml.

E = amount of enzyme preparation used, ml. (or grams).

S should not exceed 1.0 grams per 100 ml.

The term dextrose equivalent or "D.E." value used herein refers to the reducing sugars content of the dissolved solids in a starch hydrolysate expressed as percent dextrose as measured by the Schoorl method (*Encyclopedia of Industrial Chemical Analysis*, Vol. II, pp. 41–42).

Dextrose was determined by the procedure of Scobell Tai and Hill as reported in *Advances in Automated Analysis, Technicon International Congress*, Vol. II, p. 70 (1969). In this colorimetric procedure, dextrose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide reacts with potassium ferrocyanide to give the yellow ferricyanide with a color intensity proportional to the original dextrose concentration.

The following examples demonstrate the conversion of a high D.E. starch hydrolysate to high dextrose containing products with immobilized glucoamylase.

EXAMPLE I.

A starch hydrolysate having a dextrose equivalent level of 11 D.E. was incubated at 60°C. and pH 4.3 at 30 percent solids by dry weight basis for 16 hours using a soluble glucoamylase preparation at 14 units per 100 g. dry weight basis to produce an 84 D.E. hydrolysate product.

Immobilized glucoamylase was prepared by binding glucoamylase to silanized porous silica at pH 7.0 with glutaraldehyde according to the procedure presented in U.S. Patent No. 3,783,101.

After incubation, the 84 D.E. hydrolysate was heated to inactivate any soluble enzymes and adjusted to a feed liquor solids level of 25 percent and fed through a column (inside diameter 30 mm, length 190 mm) containing a 100 ml. bed of immobilized glucoamylase on porous silica (55 g. dry weight basis or 14 units of glucoamylase per g. dry weight basis) at a rate of 2.0 bed volumes per hour.

A dextrose level of 95.8 percent dry weight basis was initially obtained. Increasing the flow rate to 5.1 bed volumes per hour and reducing the solids level to 10 percent resulted in a dextrose level of 97.2 percent dry basis weight.

glucoamylase at 60°C. and pH 4.3 for exactly 16 hours at 25 percent solids by dry weight basis. Glucoamylase dosage and the dextrose equivalent attained are shown below:

Glucoamylase Dosage Units/100g Dry Substance	Dextrose Equivalent Attained
1.4	30
3.1	47
6.4	65
13.4	80

The reaction was stopped by heating at 95°C. for 15 minutes. Each substrate was re-adjusted to 25 percent solids by dry weight basis. Saccharide distribution for each hydrolysate feed is as follows:

EXAMPLE II.

A low dextrose equivalent starch hydrolysate of 11 D.E. was treated with soluble

Feed Composition

% d.b. by Paper Chromatography

Feed D.E.	Dextrose(a)	Maltose	Isomaltose	Maltulose	DP-3	DP-4+(b)
11	0.8		3.2(c)		4.7	91.3
30	26.0	5.6	0.1	0	7.9	60.4
47	44.0	8.6	0	0.1	6.3	41.0
65	59.9	10.6	0	0.6	0.7	28.2
80	76.6	3.9	0.5	0.6	0.7	17.7

(a) Dextrose by difference (i.e. 100%—Sum of percent non-dextrose)

(b) Degree of Polymerization of "4" and greater as total percent

(c) Total Degree Polymerization of "2" components

A column containing a 100 ml. bed of glucoamylase preparation immobilized on porous silica was operated at 1 bed volume per hour at 45°C. and pH 4.3 using the prepared feeds at 25% solids and also the 11 D.E. starch hydrolysate. The procedure used for binding

glucoamylase was essentially the same as that presented in U.S. Patent No. 3,783,101. Composition of the dextrose containing product obtained at the various D.E. levels is as follows:

Product Composition

% d.b. by Paper Chromatography(a)

Feed D.E.	Dextrose(b)	Maltose	Isomaltose	Maltulose	DP-3	DP-4+(c)
11	93.7	0.8	2.4	0.9	0.3	1.9
30	94.1	1.0	2.3	0.9	0.3	1.4
47	94.2	0.8	2.6	0.9	0.4	1.1
65	94.7	0.7	2.6	0.8	0.4	0.8
80	94.6	0.8	2.8	0.8	0.5	0.5

(a) Normalized

(b) Glucose Oxidase Method

(c) Degree of Polymerization of "4" and Greater as total percent.

EXAMPLE III.

Example II was repeated with the exception that the column of immobilized glucoamylase was operated at 1.5 bed volume per hour.

Composition of the dextrose containing product obtained at the various D.E. levels is as follows:

Feed D.E.	Dextrose(b)	% d.b. by Paper Chromatography(a)				
		Maltose	Isomaltose	Maltulose	DP-3	DP-4 ⁺ (c)
11	92.6	0.9	1.8	0.9	0.4	3.4
30	93.2	0.9	1.5	0.7	0.3	3.4
47	93.6	0.9	1.7	0.9	0.3	2.6
65	93.5	0.8	2.2	1.0	0.5	2.0
80	94.6	0.8	2.2	0.8	0.4	1.2

(a) Normalized

(b) Glucose Oxidase Method

(c) Degree of Polymerization of "4" or Greater as total percent.

EXAMPLE IV.

Example II was repeated with the exception that the column of immobilized glucoamylase was operated at 0.7 bed volume per hour.

Composition of the dextrose containing product obtained at various D.E. levels is as follows:

Feed D.E.	Dextrose(b)	% d.b. by Paper Chromatography(a)				
		Maltose	Isomaltose	Maltulose	DP-3	DP-4 ⁺ (c)
11(d)	93.2	0.7	4.4	0.8	0.6	0.3
30	94.4	0.8	3.0	0.8	0.4	0.6
47	94.3	0.7	3.3	0.8	0.4	0.5
65	94.1	0.9	3.5	0.8	0.4	0.3
80	93.6	0.7	4.1	0.9	0.5	0.2

(a) Normalized

(b) Glucose Oxidase Method

(c) Degree of Polymerization of "4" or Greater as total percent

(d) This sample (only) run at 0.5 bed volume per hour.

EXAMPLE V.

This example shows the effect of flow rate expressed as bed volume per hour (BVH) on the product from the immobilized glucoamylase column using the 80 D.E. hydrolysate prepared as in Example II and further diluted

with water to 10% solids (dry weight basis). The column containing a 100 ml. bed of glucoamylase preparation immobilized on porous silica was operated at 45°C. and pH 4.3. Composition of the dextrose containing product is as follows:

Flow Rate BVH	Dextrose(b)	% d.b. by Paper Chromatography(a)				
		Maltose	Isomaltose	Maltulose	DP-3	DP-4 ⁺ (c)
2.0	94.9	0.6	2.7	1.2	0.3	0.3
2.4	95.4	0.6	2.3	1.1	0.3	0.3
3.0	95.1	0.6	2.2	1.2	0.3	0.6
4.0	95.7	0.5	1.5	1.0	0.3	1.0

(a) Normalized

(b) Glucose Oxidase Method

(c) Degree of Polymerization of "4" or Greater as total percent.

EXAMPLE VI.

This example demonstrates the effect of solids (dry weight basis) on the conversion of a high dextrose equivalent starch hydrolysate to a high dextrose product with an immobilized glucoamylase.

An 11 D.E. starch hydrolysate was converted

to an 80 D.E. starch hydrolysate as shown in Example II. The 80 D.E. starch hydrolysate was diluted to different solids levels by dry weight basis and treated with immobilized glucoamylase as shown in Example II. The results for the 80 D.E. starch hydrolysate are as follows:

Solids % w/w	Flow Rate BVH	% d.b. by Paper Chromatography(a)					
		Dextrose(b)	Maltose	Isomaltose	Maltulose	DP-3	DP-4 ⁺ (c)
25	1.0	94.6	0.8	2.8	0.8	0.5	0.5
10	4.0	95.7	0.5	1.5	1.0	0.3	1.0
5	5.5	97.1	0.2	1.1	0.9	0.1	0.6

(a) Normalized

(b) Glucose Oxidase Method

(c) Degree of Polymerization of "4" and Greater as total percent.

As seen from the above data, dextrose yields using immobilized glucoamylase according to this invention are essentially constant throughout the entire range of about 30 D.E. to about 85 D.E. In contrast with the prior art, the above data also show high dextrose levels produced from high dextrose equivalent starch hydrolysates using solely an immobilized glucoamylase.

Although the foregoing examples demonstrate a preferred immobilized glucoamylase, it is to be understood that other types of immobilized glucoamylases can be employed, so long as the α -amylase portion is in the inactive form and the high D.E. hydrolysate feed was produced using soluble glucoamylase.

WHAT WE CLAIM IS:—

1. A process for the production of dextrose from starch which method comprises:

- providing a low D.E. starch hydrolysate having a dextrose equivalent from 2 to 20 and treating the low D.E. starch hydrolysate with a soluble glucoamylase preparation to produce a starch hydrolysate

- having a dextrose equivalent less than 85;
- reacting the soluble glucoamylase treated starch hydrolysate with an effective amount of an enzyme consisting essentially of immobilized glucoamylase and;

- recovering a dextrose product having a dextrose content of at least 90 percent dry weight basis.

2. A process according to claim 1 wherein there is produced a soluble glucoamylase treated starch hydrolysate which has a dextrose equivalent from 30 to 85.

3. A process according to claim 1 or claim 2 wherein there is produced a soluble glucoamylase treated starch hydrolysate which has a dextrose equivalent from 45 to 85.

4. A process according to any one of the preceding claims wherein there is employed immobilized glucoamylase which is covalently bound to porous silica.

5. A process according to any one of the preceding claims wherein there is produced a dextrose product which has a dextrose content of at least 93 percent dry weight basis.

6. A process according to any one of the

Flow Rate BVH	Dextrose(b)	% d.b. by Paper Chromatography(a)				
		Maltose	Isomaltose	Maltulose	DP-3	DP-4 ⁺ (c)
2.0	94.9	0.6	2.7	1.2	0.3	0.3
2.4	95.4	0.6	2.3	1.1	0.3	0.3
3.0	95.1	0.6	2.2	1.2	0.3	0.6
4.0	95.7	0.5	1.5	1.0	0.3	1.0

(a) Normalized

(b) Glucose Oxidase Method

(c) Degree of Polymerization of "4" or Greater as total percent.

EXAMPLE VI.

5 This example demonstrates the effect of solids (dry weight basis) on the conversion of a high dextrose equivalent starch hydrolysate to a high dextrose product with an immobilized glucoamylase.

An 11 D.E. starch hydrolysate was converted

to an 80 D.E. starch hydrolysate as shown in Example II. The 80 D.E. starch hydrolysate was diluted to different solids levels by dry weight basis and treated with immobilized glucoamylase as shown in Example II. The results for the 80 D.E. starch hydrolysate are as follows:

Solids % w/w	Flow Rate BVH	Dextrose(b)	% d.b. by Paper Chromatography(a)				
			Maltose	Isomaltose	Maltulose	DP-3	DP-4 ⁺ (c)
25	1.0	94.6	0.8	2.8	0.8	0.5	0.5
10	4.0	95.7	0.5	1.5	1.0	0.3	1.0
5	5.5	97.1	0.2	1.1	0.9	0.1	0.6

(a) Normalized

(b) Glucose Oxidase Method

(c) Degree of Polymerization of "4" and Greater as total percent.

20 As seen from the above data, dextrose yields using immobilized glucoamylase according to this invention are essentially constant throughout the entire range of about 30 D.E. to about 85 D.E. In contrast with the prior art, the above data also show high dextrose levels produced from high dextrose equivalent starch hydrolysates using solely an immobilized glucoamylase.

25 Although the foregoing examples demonstrate a preferred immobilized glucoamylase, it is to be understood that other types of immobilized glucoamylases can be employed, so long as the *alpha*-amylase portion is in the inactive form and the high D.E. hydrolysate feed was produced using soluble glucoamylase.

WHAT WE CLAIM IS:—

35 1. A process for the production of dextrose from starch which method comprises:

40 a) providing a low D.E. starch hydrolysate having a dextrose equivalent from 2 to 20 and treating the low D.E. starch hydrolysate with a soluble glucoamylase preparation to produce a starch hydrolysate

having a dextrose equivalent less than 85; b) reacting the soluble glucoamylase treated starch hydrolysate with an effective amount of an enzyme consisting essentially of immobilized glucoamylase and;

c) recovering a dextrose product having a dextrose content of at least 90 percent dry weight basis.

2. A process according to claim 1 wherein there is produced a soluble glucoamylase treated starch hydrolysate which has a dextrose equivalent from 30 to 85.

3. A process according to claim 1 or claim 2 wherein there is produced a soluble glucoamylase treated starch hydrolysate which has a dextrose equivalent from 45 to 85.

4. A process according to any one of the preceding claims wherein there is employed immobilized glucoamylase which is covalently bound to porous silica.

5. A process according to any one of the preceding claims wherein there is produced a dextrose product which has a dextrose content of at least 93 percent dry weight basis.

6. A process according to any one of the



preceding claims wherein the initial starch hydrolysate is produced by reacting starch with hydrolytic enzymes or acid.

5. 7. A process according to any one of the preceding claims wherein there is utilised a soluble glucoamylase treated starch hydrolysate which is free of active alpha-amylase enzyme.

8. Dextrose products produced in accord-

ance with the process of any one of the preceding claims. 10

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