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(54) Title: PROCESS FOR MAKING GLUCOSONE (57) Abstract <p>Methods of producing glucosone which comprises enzymatically oxidizing glucose with glucose-2-oxidase in a first zone and separating the concomitantly produced hydrogen peroxide from said first zone through a semi-permeable membrane into a second zone wherein an alkene is reacted with said hydrogen peroxide to form oxygenated products of said alkene, said membrane being permeable only to compounds of a molecular weight of less than about 100.</p>		

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PROCESS FOR MAKING GLUCOSONE

This invention is concerned with a new and useful process for the production of glucosone and more particularly for the production glucosone from which food-grade fructose
5 can be obtained.

Commercial methods for the production of fructose, a commercially important sweetner, primarily involve a two-step process, the first, hydrolysis of a polysaccharide such as starch to produce glucose and the second, isomerization of the
10 so-produced glucose to form fructose. The latter step, as is well-known, produces a mixture of glucose and fructose from which it is difficult to separate the desired product, fructose. The commercial separation method involves the use of crystallization techniques which are costly and time-consuming.
15 More detailed description of the various methods of isomerizing glucose can be found in the literature, e.g. U.S. Patent 3,788,945, and 3,616,221.

Glucose can also be converted to fructose by the action of an enzyme, designated glucose-2-oxidase, to form
20 glucosone (D-arabino-2-hexosulose) which in turn can be reduced to fructose with zinc and acetic acid [Folia Microbiol. 23, 292-298 (1978) and Czechoslovakian Patent No. 175897 to Volc et al.].

The reaction of glucose-2-oxidase with glucose to
25 produce glucosone also yields hydrogen peroxide in equimolar amount. The use of the so-produced hydrogen peroxide in the conversion of alkenes to corresponding halohydrins and epoxides has been proposed in European Patent Application 7176. In the published application, the in situ formation of hydrogen
30 peroxide is proposed by inclusion of glucose-2-oxidase and glucose in the reaction mixture which includes a halogenating



1 enzyme and a source of inorganic halide into which the selected
alkene is to be introduced. The disclosure of the European
patent application further indicates that the glucosone pro-
duct of the enzymatic oxidation of glucose can be converted
5 to fructose by simple chemical hydrogenation.

However, fructose produced by the said process can
be contaminated with significant amounts of by-products from
both the enzymatic conversion of glucose and the alkene con-
version reaction. In particular, the latter reaction produces
10 halohydrins and alkylene oxides, e.g. ethylene oxide, which
are highly toxic material even at levels in the region of
parts per million. Thus, fructose produced by such a process
will require careful and costly purification to attain food-
grade purity. Further, the potential for contamination of .
15 fructose by virtue of secondary reactions during the initial
processing state is quite high due to the highly reactive
products, halohydrins and alkyleneoxides, and substantial
purification procedures are required to assure the high level
purity required for food grade fructose.

20 SUMMARY OF THE INVENTION

This invention provides a method for the production
of glucosone by enzymatic oxidation of glucose to glucosone in
a reaction zone from which hydrogen peroxide is removed by
use of a hydrogen peroxide-permeable membrane into a second
25 reaction zone where the hydrogen peroxide is reacted with
an alkene to convert the alkene to an oxidation product.

In accordance with one embodiment of the invention,
the alkene is converted to a glycol by reaction with hydrogen
peroxide. This reaction is catalyzed by osmium, vanadium or
30 chromium oxide or by ultraviolet light in accordance with the
procedure described J.A.C.S. 58, 1302 (1936); 59, 543, 2342,
2345 (1937), incorporated herein by reference.



1 In accordance with a second embodiment of the invention, the alkene is converted to a halohydrin and then to an alkylene oxide or glycol corresponding to the original alkene reactant by reaction with hydrogen peroxide, a halogenating enzyme and a halide ion source, to form the halohydrin which is then converted to an epoxide or glycol, by the methods described in European patent application 7176.

 The membranes employed in the present process are for the purpose of establishing two separate zones and permitting migration of hydrogen peroxide from the first to the second zone. The membranes therefore should be of suitable pore size to selectively permit hydrogen peroxide migration, but to preclude passage of larger molecules in the first reaction zone. Such membranes are readily available commercially and can be defined in terms of the molecular weight of solute particles to pass through the membrane. In the present invention, membranes which permit substances of a molecular weight of less than about 100 are to be used, and preferably less than 50.

20 The migration or passage of hydrogen peroxide through the aforesaid membrane is accomplished through establishment of an equilibrium predicated on the relative concentrations of H_2O_2 on each side of the membrane. As the concentration of hydrogen peroxide in the first zone increases, the H_2O_2 tends to migrate to the second zone until equilibrium is reestablished. The reaction with an alkene in the second zone increases the rate of flow of hydrogen peroxide through the membrane by offsetting the equilibrium in the direction of the second zone.



1 Employing the present process results in considerable
advantage particularly in the further processing of glucosone
to fructose. The migration of hydrogen peroxide from the first
reaction zone of course affects the rate of the enzymatic oxi-
5 dation of glucose so that the reaction tends to be more complete
and the reaction times can be shorter than normally required.
Further, the first reaction zone is essentially free of con-
taminants that will accumulate primarily in the second reaction
zone where the so produced hydrogen peroxide is reacted. The
10 glucosone solution produced in the first reaction zone can be
used as such in the hydrogenation step or can be concentrated
or otherwise processed as desired. The glucosone solution is
substantially free of contaminants other than some unreacted
glucose, or glucose dimer or trimer, and whatever contaminants
15 that may have been introduced in the original glucose charge.
Usually, the glucose charge will be a hydrolysate of a natural
product containing glucose units, most commonly starch, which
will contain soluble contaminants such as other carbohydrates,
e.g. maltose, formed in the starch hydrolysis.

20 Accordingly, the reduction of the reaction product
of the first zone will provide a product, fructose, which will
be comparatively free of contaminants that effect food grade
status for the product, the contaminants being derived only
from the glucose natural sources, e.g. starches such as corn
25 starch.

The alkene reaction zone also is cleaner than attain-
able when both reactions are conducted in the same reactor.

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¹PREFERRED EMBODIMENTS

The membranes to be used in the present process are any of those commonly employed in aqueous systems and include a wide variety. Most commonly, the membranes will be
⁵comprised of nylon, a styrene polymer, usually polystyrene, teflon, or a cellulose ester such as cellulose acetate or propionate. In a first embodiment, the membrane is fitted into a reactor to provide two zones in a manner to preclude unintended mixing of the contents of the two zones. In a
¹⁰second embodiment, separate reactors can be coupled with the selected membrane providing the requisite interface in the coupling. For maximum migration of hydrogen peroxide from the first zone to the second zone, membranes of significant exposed surface area are of course preferred for which reason
¹⁵the first embodiment is more preferable.

The glucose-2-oxidase enzyme can be provided in the form of the enzyme solution in water, immobilized enzyme or immobilized cells or mycelium or the free cells or mycelium. Most commonly since the enzyme is intracellular, the cells or
²⁰mycelium of the selected microorganism are used by merely suspending them in the reaction solution. Promoters and protectors for the enzyme can also be present. For example, as described in the aforesaid Folia Microbiol. 23, 292-298 (1978), the presence of fluoride ion promotes the enzymatic oxidation
²⁵of glucose with O. mucida. Protectors for enzymes can also be used, e.g. Co, Mn and Mg salts.

1 The enzymatic oxidation reaction is carried out until
substantially complete as can be determined by monitoring the
mixture using aliquots to test for glucose content, or by
colorimetric determination of glucosone or by determination of
5 of hydrogen peroxide. Usually, reaction periods of about 24-
48 hours are sufficient, depending on enzyme potency or activity.

A wide variety of microorganisms can be used to pro-
duce the glucose-2-oxidase employed in the present process.
For example, the following organisms are described in the
10 literature for this purpose:

- I *Aspergillus parasiticus* [Biochem. J. 31, 1033 (1937)]
- II *Iridophycus flaccidum* [Science 124, 171 (1956)]
- III *Oudemansiella mucida* [Folia Microbiol. 13, 334 (1968)
 ibid. 23, 292-298 (1978)]
- 15 IV *Gluconobacter roseus* [J. Gen Appl. Microbiol. 1, 152 (1955)]
- V *Polyporus obtusus* [Biochem. Biophys. Acta 167, 501 (1968)]
- VI *Corticium caeruleum* [Phytochemistry 1977 Vol. 16, p 1895-7]

The temperature for the enzymatic oxidation reaction
20 is not critical. The reaction can be conducted at room tempera-
ture, or even somewhat higher than room temperature where the
enzyme system employed is of reasonable heat stability. In
particular, it is preferable to operate at 50°C. and above.
with heat stable enzyme systems in which range bacterial infecti
25 of the reaction mixture is minimized. Alternatively, the enzy-
matic reaction mixture can contain antibacterial agents to pre-
clude extensive bacterial growth.

The first reaction zone of course should contain no
significant amounts of a reducing agent for hydrogen peroxide
30 so that the beneficial results of the present process can be
realised. Thus, the system should be substantially free of
reducing agents for H_2O_2 , i.e. a non reducing system.



1 During the course of the present process, it is
possible for some diffusion of material from the second reaction
zone into the first zone, especially where anions, cations or
low molecular weight compounds are present in the second zone,
5 but such diffusion is not significant under the present conditions.

The procedures employed for the conversion of alkenes
to oxygenated products are those described in the hereinbefore
described references which are incorporated herein by reference
for the said disclosure.

10 The reduction of glucosone to fructose is accomplished
by known procedures including chemical reduction as with zinc
and acetic acid as well as catalytic hydrogenation, with the
usual metal catalysts. Of these, the preferred metal catalyst
is Raney Ni since its use is compatible with the desired food
15 grade of fructose, i.e. no residues or contaminants are left
by this catalyst.

In the usual procedure employed, the glucosone is
hydrogenated at elevated pressure and temperature over the
selected metal catalyst until the desired degree of hydrogenation
20 has been achieved. Pressures can range from 100 to 700 atmos-
phere and even higher while the temperature can range up to
about 200°C. Preferred is 100° to 150°C. and a pressure of
about 500 atmospheres.

The following example further illustrates the
25 invention.



EXAMPLE

1

Mycelium of *O. mucida* are grown in accordance with Example 1 of Czechoslovakian patent 175897 and the equivalent of 15 g. (dry weight) of the mycelium is suspended in 3 L. of 2.5% glucose solution 0.05M NaF in one zone of a 10 L. reactor fitted with a hydrogen peroxide-permeable membrane to form two zones. In the second zone, ethylene gas is bubbled through an aqueous solution of chloroperoxidase and halide ion buffered with a phosphate buffer (0.1M potassium phosphate) as described in European patent application 7176 (Examples 1-18).

10

The suspension in the first zone is mixed at 25°C. and aerated with oxygen. After 24 hours the mycelium is then separated from the solution in the first zone and resulting clear solution is then hydrogenated over Raney Ni at 500 atmospheres hydrogen gas and 100°C. The aqueous mixture is filtered clear of the catalyst, decolorized with carbon, deionized with ion-exchange (anionic and cationic) and concentrated to a fructose syrup at reduced pressure. Alternatively, the aqueous mixture is concentrated and fructose allowed to crystallize.

15

The fructose obtained as either syrup or crystalline product is of food grade quality.

20

The halohydrins obtained by the reaction in the second zone are converted to the corresponding epoxides by treatment with sodium hydroxide.

25

Essentially the same results are obtained when *O. mucida* is replaced with the following organisms:

30

Polyporus obtusus
Radulum casearium
Lenzites Trabea
Irpex flanus
Polyporus versicolor
Pellicularia filamentosa
Armillaria mellea
Schizophyllum commune
Corticium caeruleum

35



1 WHAT IS CLAIMED IS:

1. A method of producing glucosone which comprises enzymatically oxidizing glucose with glucose-2-oxidase in a first zone and separating the concomitantly produced hydrogen peroxide from said first zone through a semi-permeable membrane into a second zone wherein an alkene is reacted with said hydrogen peroxide to form oxygenated products of said alkene, said membrane being permeable only to compounds of a molecular weight of less than about 100.
2. The method according to Claim 1 wherein the product of said reaction of an alkene with hydrogen peroxide is a glycol.
3. The method according to Claim 1 wherein the product of said reaction of an alkene with hydrogen peroxide is the corresponding alkylene oxide.
4. The method according to Claim 1 where said membrane is permeable only to compounds of a molecular weight of less than about 50.
5. A method of producing fructose which comprises the steps of
 - a) producing glucosone which comprises enzymatically oxidizing glucose with glucose-2-oxidase in a first zone and separating the concomitantly produced hydrogen peroxide from said first zone through a semi-permeable membrane into a second zone wherein an alkene is reacted with said hydrogen peroxide to form oxygenated products of said alkene, said membrane being permeable only to compounds of a molecular weight of less than about 100, and
 - b) reducing the so-produced glucosone to obtain fructose.
6. The method according to Claim 5 wherein the reduction is effected by catalytic hydrogenation.



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1 7. The method according to Claim 6 wherein the catalyst is Raney Ni.

8. The method according to Claim 5 wherein the filtered reaction mixture obtained in Step a is employed in Step b.

nonthermochemical method of producing nonthermochemical products from nonthermochemical reactants

1. The method of claim 1, wherein the reactants are nonthermochemical reactants and the products are nonthermochemical products.

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INTERNATIONAL SEARCH REPORT

International Application No PCT/US81/00822

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC		
INT. CL. C12P 7/26,19/02; C12N 11/04		
U.S. CL. 435/105,148,182		
II. FIELDS SEARCHED		
Minimum Documentation Searched *		
Classification System	Classification Symbols	
U.S.	435/72,105,147,148,155,157,158,182,190,813,819	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *		
III. DOCUMENTS CONSIDERED TO BE RELEVANT 14		
Category *	Citation of Document, 16 with indication, where appropriate, of the relevant passages 17	Relevant to Claim No. 18
A,P	US,A, 4,246,347, PUBLISHED 20 JANUARY 1981, NEIDLEMAN ET AL.	1-8
A	US,A, 3,911,140, PUBLISHED 07 OCTOBER 1975, OSBORNE ET AL.	1-8
A,P	US,A, 4,247,641, PUBLISHED 27 JANUARY 1981, NEIDLEMAN ET AL.	2
A	CS,A, 175,897, PUBLISHED 27 AUGUST 1976, VOLC ET AL.	1-8
<p>* Special categories of cited documents: 15</p> <p>"A" document defining the general state of the art</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document cited for special reason other than those referred to in the other categories</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but on or after the priority date claimed</p> <p>"T" later document published on or after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search 2	Date of Mailing of this International Search Report 2	
11 SEPTEMBER 1981	06 OCT 1981	
International Searching Authority 1	Signature of Authorizing Officer 20	
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