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ENZYMATIC CONVERSION PROCESS

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This invention relates to an enzymatic conversion process and has particular reference to a process for removing glucose from an aqueous medium involving the use of an enzyme system having glucose oxidase and catalase activity. This invention is in certain respects an improvement over the disclosures contained in Baker application Serial No. 179,651, U. S. Patent 2,651,592 and Baldwin application Serial No. 179,652, both filed on August 15, 1950.

In the above identified Baldwin application it is disclosed that glucose may be effectively removed from egg constituents by utilizing the enzyme glucose oxidase and added oxygen. In accordance with the disclosure in that application, catalase is preferably used in conjunction with the glucose oxidase and the oxygen is added in the form of hydrogen peroxide which is decomposed by the catalase. In one embodiment of the process disclosed in the Baldwin application, the hydrogen peroxide is added in increments to the wet eggs undergoing conversion in order to insure that adequate peroxide is present during the time that the conversion of glucose to gluconic acid is taking place. Thus Baldwin suggests that hydrogen peroxide be added in equal increments to effect this desired result. However, it has been found in certain commercial operations that the successive addition of equal increments of hydrogen peroxide may give rise to certain foaming difficulties because of the large amount of unused molecular oxygen that may be evolved, particularly in the latter stages of the desugarizing step. Accordingly it is an object of this invention to provide a process for desugarizing an aqueous medium such as aqueous egg constituents utilizing an enzyme system having glucose oxidase and catalase activity and hydrogen peroxide under conditions such that the amount of foaming in the process may be substantially reduced.

A further object of this invention is the provision of an enzymatic process of converting glucose to gluconic acid in which the total amount of hydrogen peroxide utilized is substantially reduced.

A still further object of this invention is the provision of means for reducing the amount of peroxide necessary for the production of a desugarized dehydrated egg product, particularly where the product is dried over a period of time by pan-drying techniques.

Further and additional objects will appear from the following description and the appended claims.

It has now been discovered that the conversion of glucose to gluconic acid by means of an enzyme system containing glucose oxidase and catalase in the presence of an excess of hydrogen peroxide is a first order chemical reaction wherein the graph obtained by plotting time against the logarithm of the concentration of the residual glucose is substantially a straight line. Thus in this process the conversion of the glucose to gluconic acid occurs rather rapidly in the early stages of the reaction but proceeds more slowly thereafter, thus giving a substantially straight line when the logarithm of

the sugar concentration of the medium is plotted against the time of the reaction. This first order chemical reaction may be represented by the following equation:

$$K=\frac{1}{T_1-T_2}\cdot \log \frac{S_1}{S_2}$$

wherein K is a constant, S_1 is the glucose concentration at time T_1, and S_2 is the glucose concentration at a later time T_2. The above indicated relationship appears to hold only when there is an excess of hydrogen peroxide in the medium undergoing conversion. The value of K within certain limits is dependent upon temperature of and the concentration of glucose oxidase in the medium being treated.

By utilization of the above indicated discovery it has now been found that in commercial operations the desugarizing of aqueous media, particularly media containing egg constituents, may be more economically carried out if the hydrogen peroxide is added in increments to the medium during the conversion step, the successive increments being in decreasing amounts which correspond to between about one and two times the stoichiometric equivalent of the amount of glucose converted in the reaction to gluconic acid during the period between the addition of the successive increments of peroxide. Expressed in another way, the hydrogen peroxide is added to the medium at a rate to insure an excess thereof in the medium but in increments containing successively decreasing amounts of hydrogen peroxide which is proportional to the rate of conversion of the glucose at the time that the peroxide is added. Thus by successively decreasing the rate of addition of the peroxide, there is less free oxygen liberated from the medium undergoing treatment and the tendency of the medium to foam is markedly reduced. Also by decreasing the amount of peroxide added substantial economies in the process are effected.

It has also been discovered in accordance with one embodiment of this invention that peroxide economies and, in some instances, enzyme economies may be effected by using peroxide as the oxidizing agent only in the early stages of the conversion process and thereafter using gaseous oxygen from an external source for this purpose. This modification of the invention has particular applicability in processes for preparing dehydrated eggs in which the dehydrating step is effected over a substantial interval of time in a drier such as a pan drier. Thus in accordance with one modification, wet egg constituents are treated with an enzyme system having catalase and glucose oxidase activities in the presence of hydrogen peroxide which is preferably added in increments as above indicated. However, before all of the sugar has been converted to gluconic acid, the material is then placed in a pan drier wherein the eggs are dehydrated. These pan driers are ordinarily constructed so that an abundance of atmospheric oxygen comes into contact with the material being dried and it is this oxygen which oxidizes the glucose to gluconic acid by virtue of the glucose oxidase activity retained in the eggs undergoing treatment. This modification has advantages not only in reducing the amount of peroxide that may be necessary, but also results in certain economies in the amount of enzyme that may be necessary for the desired treatment. Also this modification is desirable since a portion of the desugarizing step and the dehydration step may be effected simultaneously, thus resulting in time economies.

For a more complete understanding of this invention, reference is now made to several specific examples which are, however, to be in no wise limitative of the claims appended to this specification.

EXAMPLE 1

Three batches of frozen egg whites having a pH of 9.1 were warmed to 30° C. and the pH of each was adjusted to 7.5 with lactic acid. Thereafter equal quantities of an enzyme system containing glucose oxidase and catalase were added to each of these batches. The enzyme system was prepared susbtantially in accordance with the disclosure contained in Reissue Patent No. 23,523 of Dwight L. Baker, dated July 22, 1952, and the amount of enzyme added to each batch was sufficient to provide a concentration of glucose oxidase of about 75 Sarett units and a concentration of catalase of about 3.5 units per pound of wet albumen, these units being determined susbtantially in accordance with the procedures outlined in said Baker application Serial No. 179,651. Hydrogen peroxide was then added to each of these batches with continuous stirring in the amounts indicated at the several times in the following table:

Table

Time of actual additions	Cc. of 35% H_2O_2 added per 1,000 lbs. of egg albumen		
	A	B	C
9:00 a. m.	216	216	216
9:15	72	100	72
9:30	65	90	72
9:45	60	84	72
10:07	55	76	72
10:15	50	70	72
10:30	46	64	72
10:45	43	59	72
11:00	39	54	72
11:15	36	50	72
11:32	33	45	72
11:45	30	42	72
12:00 noon	28	38	72
12:15 p. m.	25	35	72
12:30	23	32	72
12:45	22	30	72
1:00	20	28	72
1:15	18	25	72
1:30	17	23	72
1:45	15	21	72
2:00	14	20	72
2:25	13	18	72
2:35	12	16	72
2:50	11	15	72
3:05	10	14	72
3:15	9.2	13	72
3:30	8.6	12	72
3:45	7.8	11	72
4:00	7.2	10	72
Total cc. added over entire time	1,005.8	1,311	2,232

In batch A of the above table the peroxide (except for the first addition) was added at substantially the rate at which the glucose was being converted to gluconic acid in the medium under the selected conditions. In batch B the peroxide (except for the first addition) was added at a rate roughly 1.5 times the rate that it was added to batch A. In batch C the peroxide (except for the first addition) was added in equal increments.

It was found that batch C foamed considerably while both batches A and B were substantially free of foam throughout the conversion process. In each case desugarizing of the finished egg albumen was substantially complete. It will be noted from the last line of the table that substantial economies were effected in adding the hydrogen peroxide in accordance with the disclosure of this invention. Generally speaking, the amount of foam produced is minimized if the peroxide is added at a rate less than twice as fast as it is being consumed by the glucose oxidation reaction at the time of addition, it being borne in mind that it is desirable always to have an excess of peroxide present.

EXAMPLE 2

A batch of egg albumen was treated with an enzyme containing glucose oxidase and catalase under the conditions indicated in Example 1 and subjected to treatment with hydrogen peroxide for a period of about 6½ hours in the manner indicated above in accordance with the time schedule set forth in column B of the table of Example 1. The initial concentration of the glucose in the albumen was 3.44 per cent (dry basis). After the 6½ hour period, the amount of glucose was reduced to about 0.32 per cent (dry basis). Thereafter the composition was placed in a commercial pan drier, the addition of further hydrogen peroxide being discontinued. The temperature of the albumen in the pan drier was maintained at about 35° C. In this pan drier and during the drying operation, the oxygen of the air by reason of the presence of glucose oxidase further oxidized glucose to gluconic acid, whereby the glucose content of the material was reduced to about 0.07 per cent (dry basis) when the drying had been completed. This example indicates that certain economies may be effected in a pan drying process if a portion of the enzymatic glucose oxidation step is permitted to occur in the pan driers.

It will, of course, be apparent that this invention may be modified so as to depart from the specific examples given in the foregoing. Reference is again made to the copending Baldwin application in which conditions of time, temperature and concentrations are set forth in somewhat more detail for efficient practice of an enzymatic converting process employing catalase and glucose oxidase enzyme systems. For example, the temperature in the process employing the enzyme system and hydrogen peroxide may be between about 4° and 40° C., the pH may be between 3.5 and 9.0, preferably between 6.3 and 8.0, and the concentration of glucose oxidase is preferably in excess of 10 Sarett units per pound of wet eggs.

The invention also has been described above as being particularly useful for the removal of glucose from egg constituents wherein foaming may constitute a problem. It is particularly useful for removing sugar from egg whites but may also be used for removing sugar from egg yolks or whole eggs. Also in its broader aspects, the invention finds applicability in the removal of sugar from other food products or other aqueous media.

While particular embodiments of this invention are shown above, it will be understood, of course, that the invention is not to be limited thereto, since many modifications may be made, and it is contemplated, therefore, by the appended claims, to cover any such modifications as fall within the true spirit and scope of this invention.

I claim:

1. In a process for converting glucose to gluconic acid in an aqueous medium in the presence of hydrogen peroxide and an enzyme system having glucose oxidase and catalase activity wherein the rate of conversion of glucose in the presence of excess hydrogen peroxide is represented by the equation

$$K=\frac{1}{T_1-T_2}\cdot \log \frac{S_1}{S_2}$$

in which K is a constant, S_1 is the glucose concentration in said medium at time T_1, and S_2 is the glucose concentration in said medium at a later time T_2, the improvement of adding successive increments of hydrogen peroxide to the medium during conversion in decreasing amounts corresponding to between about one and two times the stoichiometric equivalent of S_1—S_2.

2. In a process of removing glucose from egg constituents in the presence of hydrogen peroxide, water and an enzyme system having glucose oxidase and catalase activity wherein the rate of conversion of glucose in the presence of excess hydrogen peroxide is represented by the equation

$$K=\frac{1}{T_1-T_2}\cdot \log \frac{S_1}{S_2}$$

in which K is a constant, S_1 is the glucose concentration at time T_1, and S_2 is the glucose concentration at a later time T_2, the improvement of adding hydrogen peroxide at spaced time intervals in successive increments, the

amount of hydrogen peroxide in each increment being between about one and two times the stoichiometric equivalent of S_1—S_2 during the corresponding interval.

3. In a process of removing glucose from wet egg constituents in the presence of hydrogen peroxide and an enzyme system having catalase and glucose oxidase activity wherein the rate of conversion of glucose in the presence of excess hydrogen peroxide is represented by the equation

$$K=\frac{1}{T_1-T_2}\cdot \log \frac{S_1}{S_2}$$

in which K is a constant, S_1 is the glucose concentration at time T_1 and S_2 is the glucose concentration at a later time T_2, the improvement of maintaining an excess of hydrogen peroxide in the constituents undergoing treatment by the periodic addition of hydrogen peroxide thereto in successive increments of successively decreasing amounts, each amount being sufficient to maintain an excess of hydrogen peroxide but less than an amount which will cause substantial liberation of gaseous oxygen from the reaction mixture.

4. A process for removing glucose from eggs which comprises successively adding increments of hydrogen peroxide to an aqueous medium containing egg constituents and an enzyme system having glucose oxidase and catalase activity whereby at least a portion of the glucose occurring in said constituents is converted to gluconic acid by a first order chemical reaction, the rate of addition of said hydrogen peroxide being sufficient to insure an excess thereof in said medium, said increments containing successively decreasing amounts of hydrogen peroxide proportional to the rate of conversion of said glucose at the time when added.

5. A process of preparing dehydrated eggs which comprises successively adding increments of hydrogen peroxide to an aqueous medium containing egg constituents and an enzyme system having glucose oxidase and catalase activity whereby a substantial portion of the glucose naturally occurring in said constituents is converted to gluconic acid by a first order chemical reaction, the rate of addition of said hydrogen peroxide being sufficient to insure an excess thereof in said medium, said increments containing successively decreasing amounts of hydrogen peroxide proportional to the rate of conversion of said glucose at the time when added, and then, before said conversion is complete, drying said medium over an

extended period of time in the presence of gaseous oxygen whereby a substantial proportion of the unconverted glucose is then converted to gluconic acid.

6. In a process of desugarizing egg constituents in a body of an aqueous medium and in the presence of water, hydrogen peroxide and a nonviable enzyme system having catalase and glucose oxidase activity in which the rate of the desugarizing reaction is represented by the formula

$$K=\frac{1}{T_1-T_2}\cdot \log \frac{S_1}{S_2}$$

wherein K is a constant, S_1 is the glucose concentration in said medium at time T_1 and S_2 is the glucose concentration in said medium at a later time T_2, the improvement which comprises adding hydrogen peroxide to said body in successive increments, the amount of hydrogen peroxide added at the several times being less than twice the stoichiometric equivalent of S_1—S_2.

7. In a process of removing glucose from egg constituents in the presence of hydrogen peroxide, water and an enzyme system having glucose oxidase and catalase activity wherein the rate of conversion of glucose in the presence of excess hydrogen peroxide is represented by the equation

$$K=\frac{1}{T_1-T_2}\cdot \log \frac{S_1}{S_2}$$

in which K is a constant, S_1 is the glucose concentration at time T_1, and S_2 is the glucose concentration at a later time T_2, the improvement of adding hydrogen peroxide at a successively diminishing rate, the amount of hydrogen peroxide added between T_1 and T_2 being between about one to two times the stoichiometric equivalent of S_1—S_2.

References Cited in the file of this patent

UNITED STATES PATENTS

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OTHER REFERENCES

Baldwin et al.: Food Technology, July 1953, pages 275 to 282.