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(54) IMPROVEMENTS IN OR RELATING
TO ENZYME PRODUCTS

(71) We, NOVO INDUSTRI A/S, a Company organized under the laws of Denmark, of Novo Allé, DK-2880 Bagsvaerd, Denmark, 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:-

5 This invention relates to insolubilized enzymes and to a process for preparing 5 insolubilized enzyme products.

More particularly, this invention relates to particulate physically stabilized forms of 10 insolubilized saccharifying enzymes selected from amyloglucosidase and maltogenic alpha-amylase, both usually of fungal origin, and to a process for the preparation of such insolubilized enzyme products.

15 Insolubilization (or immobilization) of extracellular and of intracellular water soluble enzymes, that is, the fixation of catalytically active (native) enzymes in an insolubilized, solid or quasisolid, shaped or shapable structure has become of increasing technological and economic importance, as a means of rendering a particular enzyme product reusable in 20 batch-type processes or suitable for a continuous mode of operation in a so-called enzyme reactor.

25 During recent years efforts have been devoted to the development of immobilization technology in the starch processing industry in connection with the manufacture of such products as high dextrose and high fructose syrups. Lately, the relative importance of the latter has been increasing in food and related industries, high fructose syrup being an 20 advantageous substitute for sucrose and for (the less sweet) high dextrose syrup. Two additional types of sugar syrup based on starch hydrolysis and having a substantial content of maltose, namely high maltose and high conversion syrup are being used to an increasing extent, particularly in the confectionary (hard candy) and canning industries, respectively.

30 The overall industrial process of converting starch via high dextrose syrup into high fructose syrup comprises three consecutive steps, namely the starch thinning or liquefaction process to dextrans, catalyzed by acid and/or by a bacterial alpha-amylase, followed by saccharification to a high dextrose syrup and then the conversion of the latter into high fructose syrup, each process catalyzed by its specific enzyme, viz. amyloglucosidase and glucose isomerase, respectively. Whereas the most recent technological development has 30 made continuous processes available for both the first and the third step of the above production sequence, industrial saccharification is still predominantly a batch-type process.

35 The starting material of a high maltose type of syrup is also liquefied starch produced by either one of the methods just described. However, in this case the subsequent saccharification step resulting in a syrup of high maltose content (and usually containing relatively little glucose) is effected by means of a maltogenic amylase, preferably a fungal alpha-amylase. Like glucogenic saccharification, the corresponding maltogenic process is usually conducted batch-wise using the soluble enzyme.

40 Although the convenience of having at one's disposal a totally continuous production sequence is evident, the development of industrial enzyme reactor techniques involving the

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use of insolubilized amyloglucosidase in the saccharification step is only at a preliminary stage.

The prior art contains a number of references to the insolubilization of amyloglucosidase, for example by bonding the enzyme to insoluble inorganic or organic carriers. However, only few of these methods appear to have been developed beyond the laboratory bench scale. Among the methods that apparently have passed that stage specific mention should be made of the immobilization of amyloglucosidase by covalent bonding to porous particles of glass or ceramic material. In this connection reference is made to a recent article published by D.D. Lee et al. in "Die Stärke", Vol. 27 (1975) pp 384-387.

In a continuous process in which a pilot plant size column was packed with this particular enzyme product and fed with solutions of commercially available dextrins, degrees of conversion to dextrose were attained which approached the minimal conversion of about 92 percent generally required in a batch saccharification process with soluble amyloglucosidase although a conversion of above 93 percent is of course preferred. However, product analyses indicated that reversion reactions, i.e. amyloglucosidase catalyzed repolymerization of glucose into maltose, isomaltose and higher oligomers, occurs to a greater extent in the column process than in the batch-type, free enzyme process.

The phenomenon of increased reversion encountered with an immobilized enzyme product of this type appears at least partly to be an inherent disadvantage of using porous enzyme carrier materials. Clearly, the porous structure contributes substantially to the total surface area lending itself to enzyme bonding and, consequently, to the maximal enzyme activity obtainable from the immobilized enzyme product.

However, the high concentration of amyloglucosidase attached to the carrier pore surface combined with a reduced diffusion rate within the pores inevitably results in locally high glucose concentrations, thus constituting favourable conditions for the promotion of enzyme processes having high K_m -values. This is exactly the case with the undesirable reversion reactions, and particularly those in which isomaltose and isomaltotriose are formed.

An insolubilized maltogenic alpha-amylase product, prepared by bonding the soluble enzyme to aminoethylcellulose by means of glutaraldehyde, is disclosed in U.S. Patent No. 4,011,137 (Thompson et al). However, according to this reference the intended use of the immobilized enzyme is limited to increasing the degree of conversion of dextrinized starch to glucose by means of a similarly immobilized amyloglucosidase preparation, the saccharification process being conducted by means of a mixture of the two immobilized enzymes. There is no demonstration whatsoever in the patent of the use of the immobilized maltogenic alpha-amylase for the production of a high maltose syrup.

An additional disadvantage of using a particulate immobilized saccharifying enzyme, in which the enzyme is bonded to a porous or reticular material which is essentially homogeneous throughout the particulate structure can be foreseen when taking into account the heterogeneity in molecular size of the dextrinized starch serving as substrate. All dextrins, whether produced by acid or by enzymatic hydrolysis of starch, contain substantial fractions of different glucose oligomers. Normally, the starch thinning process is stopped when the average chain length of the hydrolysate is in the range of from 6 to 10 glucose residues, and it is conceivable that steric hindrance may impede the diffusion of the larger dextrin molecules to enzyme sites embedded beyond a certain depth within the porous or reticular particle structure.

It is one object of the present invention to overcome or at least to mitigate the principal disadvantages encountered with the prior art immobilized saccharifying enzyme products described above, by providing an insolubilized product having process characteristics essentially similar to those of the soluble enzyme, irrespective of whether the immobilized product is used in a batch process or in a continuous mode of operation.

In the case of immobilized amyloglucosidase this requirement normally entails that a continuous saccharification process can be conducted under industrial process conditions (e.g. in terms of composition and flow rate of the dextrin feed) as to yield a glucose concentration of at least 92 percent, the total concentration of disaccharides (maltose and isomaltose) and trisaccharides (mainly panose and isomaltotriose) not exceeding about 4 and 1 percent, respectively.

In the case of the immobilized maltogenic alpha-amylase the corresponding conversion parameters required would be: approximately 40-60 percent maltose, 25-35 percent maltotriose and, preferably, less than 10 percent glucose.

Since commercially available soluble saccharifying enzymes are relatively cheap and highly active, another preferable but not essential object of the present invention is the provision of economically favourable, insolubilized products. The pursuance of this goal necessitates the utilization of comparatively inexpensive, commercially available carrier and other auxiliary materials and, furthermore, the achievements of substantial recoveries of enzyme activities in the immobilization process. In addition, from a toxicological point of

view all materials used should be acceptable for food processing purposes.

Briefly, these objects are attained according to the present invention which provides an immobilized saccharifying enzyme product in particulate form in which enzymatically inert, water-insoluble carrier particles are essentially coated with a liquid-permeable, proteinaceous layer in which the saccharifying enzyme is immobilized by cross-linking with glutaraldehyde.

Attempts to prepare such insolubilized products in which the proteinaceous layer is composed of the glutaraldehyde cross-linked saccharifying enzyme alone proved unsuccessful in the sense that, irrespective of the choice of core material, they resulted in incompletely immobilized products from which the enzyme would rapidly leak away. Similar problems were encountered with a variety of water-insoluble particulate carrier materials of inorganic (for example mineral or ceramic) origin and with certain types of proteinaceous core material (such as granular soy protein), apparently because of an insufficient number of cross-link bonding sites at the surface of such particles.

However, it has now been found that the physical and enzymatic properties of the immobilized enzyme coated products can be greatly improved, provided that granular casein is used as core material and, furthermore, the glutaraldehyde cross-linking of the enzyme in the coating layer is effected in the presence of a water-soluble, non-enzymatic binder protein, egg albumen, such materials evidently constituting a favourable combination for creating a sufficient number of inter-protein enzyme stabilizing cross-links.

Thus, more specifically, according to one aspect of the present invention there is provided an immobilized saccharifying enzyme product in particulate form, the saccharifying enzyme being selected from amyloglucosidase and maltogenic alpha-amylase, which product comprises carrier particles of granular casein, having a coating of a liquid-permeable proteinaceous layer comprising the saccharifying enzyme and egg albumen cross-linked by reaction with glutaraldehyde.

The invention also provides a process for the preparation of an immobilized saccharifying enzyme product, the saccharifying enzyme being selected from amyloglucosidase and maltogenic alpha-amylase, which process comprises coating carrier particles of granular casein with a reaction mixture of the saccharifying enzyme, egg albumen and glutaraldehyde, whereby the granular casein becomes coated by a liquid-permeable proteinaceous layer, in which the saccharifying enzyme and egg albumen are cross-linked by reaction with the glutaraldehyde and, in a process of saccharifying dextrin, the improvement which comprises using a product in accordance with the present invention.

The relative amounts of the constituent saccharifying enzyme, casein and albumen may vary within rather wide limits, depending *inter alia* on the unit activity (as hereinafter defined) of the soluble saccharifying enzyme starting material. However, in a preferred embodiment of this invention the weight ratio of saccharifying enzyme : casein is in the range of from 1:200 to 1:2, preferably 1:25 to 1:5, the amount of amyloglucosidase and maltogenic alpha-amylase being calculated on the basis of products having activities of 10,000 and 3,000 units per gram, respectively. Likewise, a preferred range of the ratio of saccharifying enzyme: egg albumen is from 1:5 to 1:0.05 and still more preferred is the range from 1:2 to 1:0.2.

According to a further aspect of this invention there is provided a process for the preparation of an immobilized saccharifying enzyme product in particular form, said saccharifying enzyme being selected from amyloglucosidase and maltogenic alpha-amylase, which process comprises coating carrier particles, consisting mainly of granular casein, with a proteinaceous layer in which the saccharifying enzyme is co-crosslinked with egg albumen by means of glutaraldehyde.

In further detail, this process comprises the step of wetting a dry mixture of granular casein and egg albumen powder with an aqueous mixture consisting of the saccharifying enzyme and glutaraldehyde dissolved at pH 4-7 with vigorous stirring; followed by the step of maintaining the resulting wetted mixture in a quiescent state, usually at ambient temperature, to complete the combined carrier coating and protein cross-linking process.

Wetting may be conducted either manually, for example by thorough mixing and kneading in a mortar, or mechanically, for example in a plough type horizontal mixer obtainable from Gebr. Lödige Maschinenbau G.m.b.H., Paderborn, West Germany, or a similar type of industrial mixing apparatus. Optionally, the albumen may be dissolved together with the enzyme and glutaraldehyde being mixed in the dry state with the casein.

The weight ratio of glutaraldehyde (usually added as a 50 percent (w/w) aqueous solution) to the protein in the coating layer (enzyme and albumen) may vary considerably, the preferred range being 0.1:1 to 1:1 on a w/w dry basis. The water content of the aqueous solution is usually adjusted to be in the range of from 30 to 60 w/w percent of the total mixture.

The properties of the granular casein product selected as carrier material are significant

for attaining the objects of this invention. Thus, the casein granules should possess sufficient physical stability to resist substantial deformation upon soaking under packed bed column conditions. In addition, the degree of swelling in water should be reasonably low, preferably not exceeding 150 percent. Exemplary of a product meeting such requirements is acid precipitated granular casein. Food grade products of this type, preferably having particle diameters in the range of from 100 to 500 microns are obtainable from various sources, for example from the French company Scerma S.A. Scanning electron microscope examination reveals that the surface of such particles has a very uneven and irregularly folded structure, bearing some resemblance to the macroscopic appearance of pumice. It is believed that a surface microstructure of this type exposes a large number of glutaraldehyde binding sites.

The preferred grade of albumen is a generally available spray-dried product, which is almost instantly soluble in water.

The mixing process may entail a certain degree of agglomeration of the coated particles, resulting upon completion of the cross-linking reaction in the formation of a coarse and lumpy product. This product may be subjected to disintegration, for example by granulation, to form a particulate product with particle sizes within a preferred range. Granulation may be effected on an oscillatory granulator of the type supplied by several companies (Diaf, Copenhagen or Manosty, Liverpool). The granulate product passing through the granulator screen, for example one with holes of 1-2 mm, may be freed of fine material by conventional means. The product so obtained may optionally be washed, followed by drying to a desired water content.

Prior to being used for a saccharification process, the dried enzyme product is usually conditioned by pre-soaking in an aqueous solution. At this stage and also subsequently during the initial phase of the use of the product, some leakage of enzyme activity may be observed. It has been found that inclusion of an additional cross-linking step, optionally in connection with the soaking process, may substantially reduce this loss of enzyme activity. Hence, according to one embodiment of this invention pre-treatment of the enzyme product is performed in an aqueous solution containing a suitable amount, preferably 5 to 0.5 percent of glutaraldehyde, followed by removal of excess reagent by washing and, optionally, recovery of the dried product. It has been found that pre-treatment of the enzyme product with a salt of sulphurous acid may effect a significant increase in the degree of conversion (as expressed in percent of dextrose equivalent or DE-value) of the saccharified product. A theory of the sulphite action is an "opening up" of certain reticular structures involving the proteinaceous enzymatically active layer but apparently not affecting the bonding of the enzyme, thus facilitating the access of higher oligosaccharides to enzyme active sites. Accordingly, a further embodiment of this invention comprises treating the enzyme product with a dilute aqueous solution of sodium sulphite, preferably a 0.1-2 percent of sodium sulphite solution of pH about 4.5. The sulphite treatment is preferably conducted at ambient temperature and terminated after 120 minutes by washing with water, optionally followed by recovery of the dried product. After the sulphite treatment, enzyme leakage does not usually reappear. Furthermore, after treatment with an aqueous solution of glutaraldehyde, the enzyme product may be treated with an aqueous solution of a salt of sulphurous acid.

The particulate enzyme products prepared according to the above procedure may be used in batch-type saccharification process with separation and reuse of the enzyme product or in a continuous type of process in an enzyme reactor.

A preferred commercial amyloglucosidase product for the practice of this invention is obtained by cultivating strains belonging to the genera of *Aspergillus* or *Rizopus*, e.g. *Aspergillus niger* or *Rizopus delemar*. Likewise, preferred maltogenic alpha-amylases may be obtained from strains of *Aspergillus oryzae*.

Determination of amyloglucosidase activity

The unit activity of soluble amyloglucosidase as well as that of the immobilized enzyme, defined as the amount of enzyme or enzyme product which hydrolyses an aqueous solution containing 30 percent (w/w) of maltose, at an initial rate of 1 micromole of maltose per minute, is assayed under standard conditions, which are pH 4.5 and a temperature of 55.0°C. The activity of the immobilized enzyme is assayed batch-wise on a sample which is kept suspended by means of a shaking device.

Determination of fungal alpha-amylase activity

The unit activity of soluble fungal alpha-amylase as well as that of the immobilized enzyme, defined as the amount of enzyme or enzyme product which hydrolyses an aqueous solution containing soluble starch (Merck Amylum soluble, DAB, Erg. B VI), (6.95 g per 1000 ml) at an initial rate of 5.26 mg of starch per hour, is assayed under standard

conditions, which are pH 5.6-5.7, a temperature of $37.0^{\circ}\text{C} \pm 0.05^{\circ}\text{C}$ and in the presence of 4.3 mmolar Ca^{++} ion. The formation of a blue colour with iodine is used to follow the reaction. During breakdown of the starch this colour becomes weaker and changes gradually to red-brown. The colour change is checked visually by comparison with coloured glass standards. The activity of the immobilized enzyme is assayed under conditions analogous to those indicated for amyloglucosidase. The word "Merck" is a Trade Mark.

5 Soluble saccharifying enzymes are commercially available. Exemplary of such products are AMYLOGLUCOSIDASE NOVO 150 (an aqueous solution) and FUNGAMYL 1600 (a powder). Aqueous solution enzyme sources are usually converted into a spray-dried powder prior to being used for the purpose of this invention. Optionally, the drying process 0 may be preceded by ultrafiltration, usually combined with a washing process to remove low-molecular contaminants. By using such concentration and purification processes, all well known to the skilled worker in the art, a solid amyloglucosidase product with an activity of 5000 units per gram or higher is readily obtained. The words "Novo" and 5 "Fungamyl" are Trade Marks.

10 FUNGAMYL 1600 has an activity of 1600 units per gram. The commercial product as such may serve as a satisfactory starting material. If desired, purification and concentration procedures may be used to increase enzyme unit activity.

15 The overall recovery of activity in the insolubilized saccharifying enzyme product will 0 vary with the detailed parameters of the immobilization process, but is usually not less than 40 percent, and frequently higher.

20 The immobilized enzyme products of this invention may be employed on any 5 oligosaccharide undergoing saccharification in the presence of the corresponding soluble enzyme. Exemplary of preferred substrates are dextrans obtained by acid and/or enzyme liquefaction of starch and having DE-values in the range of from 5 to 40; high maltose 15 syrups (DE-values from 35 to 60); and residual oligosaccharides occurring in mother liquors from dextrose crystallisation or in raffinates obtained from fructose-glucose fractionation. The immobilized amyloglucosidase is particularly applicable to saccharification of dextrin 25 solutions having a dry solids content in the range of from 20 to 55 percent (w/v).

0 The following Examples further illustrate the present invention.

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Example 1

A relatively coarse grade granular hydrochloric-acid-precipitated casein (9 g of "caséine alimentaire" (Scerma S.A., France), consisting of 100-500 micron particles, the 300-500 5 micron fraction being about 60 percent) was mixed with commercial spray-dried egg albumen (1.2 g). To this mixture was added a pre-mixed solution of amyloglucosidase powder (6.5 ml of 18.5 percent (w/v) aqueous solution, containing a total of 12,500 0 amyloglucosidase units) and glutaraldehyde (1.2 ml of 50 percent (w/w) aqueous solution). The amyloglucosidase powder was an ultrafiltrated, spray-dried product prepared from 5 AMYLOGLUCOSIDASE NOVO (NOVO INDUSTRI A/S, Denmark). The mixture was kneaded carefully in a mortar and then allowed to stand at room temperature for one hour. The resulting aggregate was disintegrated by mortaring to form a granular product which was allowed to dry at room temperature for one day.

The dried granular product (11.4 g) had an activity of 425 units per g, thus representing a 5 recovery after immobilization of 38.8. percent.

Example 2

Granular acid-precipitated casein (1,500 g of type M60 (Scerma S.A.), consisting of 100-500 micron particles, the 150-350 micron fraction being about 70 percent) was mixed in 0 the dry state with commercial spray-dried albumen (120 g) in a 20 litre plough-type horizontal mixer (Gebr. Lüdige Maschinenbau, G.m.b.H., West Germany).

An ultrafiltrated concentrate of amyloglucosidase, prepared from AMYLOGLUCOSIDASE NOVO (650 g of a solution having a dry matter content of about 25 percent and containing ca. 3200 units of soluble amyloglucosidase per g of concentrate) was mixed 5 thoroughly with glutaraldehyde (180 ml of a 50 percent (w/w) aqueous solution) at pH 4.9 and a temperature of 18°C , whereafter the resulting solution was poured into the mixing apparatus. Vigorous mixing was continued for an additional 0.5 to 1 minute, followed by removal of the wetted particulate product from the mixer. The moist product was left 0 quiescent for about 45 minutes to complete the cross-linking process, whereby lumps of aggregated particles were formed. Granulation of the product was conducted on an oscillatory granulator (for example of the type supplied by Diaf A/S, Copenhagen) provided with a 1.5 mm diameter screen.

The granulate was washed with de-ionized water (10 l) for 10 minutes, recovered by filtration and then subjected to fluid-bed drying. The dried product (1,600 g, 450 5 amyloglucosidase units per g) was freed of fines by sieving.

Example 3

A solution of spray-dried amyloglucosidase powder (105 g, activity: 11,400 units per g, prepared as described in Example 1) and spray-dried albumen (105 g) was prepared in tap water (400 ml) and left overnight in the refrigerator at 6-7°C. The pH of the solution was 5.4.

Glutaraldehyde (100 ml of 50 percent (w/w) aqueous solution) was then added and the resulting solution was transferred in the course of 3 minutes to a vigorously stirred batch of granular hydrochloric-acid-precipitated casein (725 g of the same grade as that used in Example 1). Mixing was conducted in an industrial mixer of the same type as that of

Example 2. Vigorous stirring of the reaction mixture was continued for an additional few minutes, whereafter it was left quiescent until the cross-linking reaction was completed after about 1 hour. The resulting aggregated and lumpy product was granulated on an oscillatory granulator through a 2 mm diameter screen.

The granulate was washed with an aqueous solution of sodium acetate (pH 4.2), followed by vacuum drying at 35°C.

The dried granular product (925 g) had an activity of 800 units per g, representing a recovery after immobilization of 67.8 percent.

Example 4

The immobilized amyloglucosidase product prepared according to Example 2 (20 g) was soaked for one hour at ambient temperature in an aqueous solution of glutaraldehyde (500 ml of 1 percent solution adjusted to pH 7), followed by recovery of the dried product (20 g).

The activity of this product was 625 units per g, thus representing a recovery of 78 percent from the second immobilization step and an overall recovery from soluble amyloglucosidase of 53 percent.

Example 5

An aqueous solution of maltogenic alpha-amylase (2 ml of 5 percent (w/v) solution of FUNGAMYL 1600 (NOVO INDUSTRI A/S, Denmark) in de-ionized water at pH 6.2) was mixed with an aqueous solution of glutaraldehyde (0.3 ml of 50 percent (w/w) solution) and then quickly added to a dry mixture of granular acid-precipitated casein (4.0 g of type M60) and commercial spray-dried egg albumen (0.5 g). After thorough kneading in a mortar in the resulting product was left for 1 hour at room temperature. Lumps were disintegrated by mortaring whereafter the product was allowed to dry at room temperature for 1 day.

Example 6

A 15 mm inner diam. jacketed column, maintained at 55°C., was loaded with the immobilized amyloglucosidase product (5.7 g) prepared according to Example 1.

A down-flow feed consisting of a 30 percent (w/v) commercial acid/enzyme-hydrolyzed dextrin (CPC Snowflake Maltodextrin 01915 of D.E. 20) having the following approximate composition:

		percent	
45	glucose (DP ₁):	4-5	45
	disaccharides (DP ₂):	8-9	
	trisaccharides (DP ₃):	6-7	
	tetra- and oligosaccharides (DP ₄₊):	79-82	

50 to which was added 0.2 percent (w/v) sodium sulphite followed by adjustment of pH to 4.5, was applied to the column at a constant flow rate of 15 ml per hour. The word "Snowflake" is a Trade Mark.

55 The effluent was analyzed by high pressure liquid chromatography (HPLC) to give the following results:

		Days	DP ₁ percent	DP ₂ percent	DP ₃ percent	DP ₄ ⁺ percent	Calculated D.E.	
		1	92.0	5.6	0.7	1.7	95.3	
5	4	93.2		4.2	0.6	2.0	95.8	5
	5	92.9		3.9	0.7	2.5	95.4	
10	7	93.6		3.2	0.6	2.5	95.8	10
	9	93.3		3.1	0.7	2.9	95.5	
	11	92.6		3.2	0.8	3.3	94.9	
15	13	92.6		2.9	0.9	3.6	94.9	15

Example 7

A 25 mm inner diam. jacketed column, maintained at 55°C., was loaded with the immobilized amyloglucosidase product (20 g) prepared according to Example 2.

20 An up-flow substrate feed having the same concentration, composition and pH as that used in Example 6 was applied to the column at a constant flow rate of 50 ml per hour. The glucose content (percent DP₁) was analyzed by the hexokinase method to give the following results:

	Days	percent DP ₁	
	1	89.0	
	4	93.6	
30	6	93.6	30
	8	93.1	

Example 8

35 A 25 mm inner diam. jacketed column, maintained at 55°C, was loaded with the immobilized amyloglucosidase product (25 g) prepared according to Example 2. An up-flow substrate feed of the same composition as that used in Example 6 was applied to the column. The feed (pH 4.5) had a dry solids content of 30 percent (w/w) and potassium 40 sorbate (2 g per litre) was added as a preservative. The flow-rate was adjusted so as to maintain an approximately constant degree of conversion.

The effluent was analyzed by means of HPLC to give the following conversion to glucose (DP₁) with time:

	Days	DP ₁ percent	Flow rate ml/hour	
	1	92.6	115	
	2	-	112	
50	3	92.4	101	50
	4	92.0	82	
55	5	92.2	75	55
	6	92.3	70	
60	7	92.1	66	60
	8	92.6	64	
	9	-	60	
65	10	92.0	57	65

After completion of the run no changes were observed in the hardness or other physical properties of the column material.

Example 9

5 By using the procedure of Example 7, but substituting the immobilized amyloglucosidase product of Example 2 with that prepared according to Example 4, the following results were obtained:

	Days	percent DP_1	
10	1	90.3	10
	2	92.9	
15	4	93.7	15
	13	93.3	

Example 10

20 The immobilized maltogenic alpha-amylase prepared according to the procedure of Example 5 (4 g) was packed into a jacketed column maintained at 45°C, and a down-flow feed of commercial dextrin (CPC Snowflake Maltodextrin 01913) containing 30 percent (w/v) dry solids and sodium sulphite (0.2 percent) as a preservative was applied. The pH of the feed adjusted to 4.5 and the flow-rate to 15 ml per hour. The following table shows the 25 composition of the column effluent: DP_2 mainly representing the maltose content: 25

	Days	DP_1 percent	DP_2 percent	DP_3 percent	DP_{4+} percent	Calculated D.E.	
30	1	5.3	45.4	21.0	28.3	42.5	30
	2	5.0	44.9	21.6	28.5	42.1	
	3	4.7	44.1	22.0	29.2	41.7	
35	4	4.4	43.5	22.7	29.4	41.2	35

Example 11

A.

40 A 15 mm inner diam. jacketed column, maintained at 55°C was loaded with immobilized amyloglucosidase (12 g) prepared according to the method of Example 2. 40

40 A down-flow feed of dextrin (31 percent (w/v) based on dry matter content), prepared by liquefaction of starch with TERMAMYL L60 (NOVO INDUSTRI A/S, Denmark) and having the following composition:

		percent	
45	glucose (DP_1):	1	45
	disaccharides (DP_2):	7-8	
	trisaccharides (DP_3):	10-12	
50	tetra- and oligosaccharides (DP_{4+}):	79-82	50
	D.E.	21	

was applied to the column. The word "Termamyl" is a Trade Mark.

55 The feed contained sodium sulphite (0.2 percent (w/v)) as a preservative and had a pH of 4.5. The flow was regulated to maintain a constant degree of conversion (in terms of percent DP_1). The effluent was analyzed by HPLC to give the following results: 55

	Days	DP ₁ percent	DP ₂ percent	DP ₃ percent	DP ₄₊ percent	Flow rate ml/hour	
	3	92.1	4.2	0.7	3.1	35	
5	7	92.3	3.6	0.8	3.3	33	5
	14	92.2	3.6	0.7	3.5	27	
10	28	92.4	3.1	0.9	3.6	18	10
	56	92.1	3.3	0.8	3.7	15	

B.

A dextrin substrate prepared according to the method described under (A) and having the following composition:

		percent	
	glucose (DP ₁):	1.1	
	disaccharides (DP ₂):	6.8	
20	trisaccharides (DP ₃):	11.0	20
	tetra- and oligosaccharides (DP ₄₊):	81.0	

was treated with 0.01 percent (based on total dry solids) of maltogenic alpha-amylase (FUNGAMYL 1600, NOVO INDUSTRY A/S) at pH of 5.0 and 50°C for two hours. After treatment the substrate had the following composition:

		percent	
	glucose (DP ₁):	1.3	
	disaccharides (DP ₂):	13.9	
30	trisaccharides (DP ₃):	22.3	30
	tetra- and oligosaccharides (DP ₄):	62.5	

Each of two 15 mm inner diam. jacketed columns, maintained at 55°C, were loaded with immobilized amyloglucosidase (10 g, prepared according to Example 2). Down-flow feeds consisting of 30 percent (w/v) of the two types of dextrin, prepared as described above, to which was added 0.2 percent (w/v) of potassium sorbate, followed by adjustment of pH to 4.5, were applied to the columns. The following results were obtained:

	Feed	Dextrin	
40	before FUNGAMYL treatment	after FUNGAMYL treatment	40
45	Maximum DX-value of effluent, percent	92.7	93.3

Example 12

Portions (10 g) of immobilized amyloglucosidase, prepared according to the method of Example 2, were soaked in 100 ml solutions containing different concentrations of sodium sulphite. The pH of the solution was adjusted to 4.5 by the addition of acetic acid. After soaking for 2 hours at ambient temperature the enzyme samples were washed with de-ionized water and packed into 15 mm inner diam. jacketed columns maintained at 55°C.

Down-flow feeds consisting of dextrin (30 percent (w/v)), prepared according to the method of Example 11 A to which was added potassium sorbate (0.2 percent (w/v)) followed by pH adjustment to 4.5 were applied to the columns.

By adjusting the flow rate of each column to give the maximum DX-value of the effluent the following results were obtained:

60	Sodium sulphite g/100 ml	0	0.01	0.1	0.2	0.5	1.0	60
	Maximum DX	90.3	90.2	91.2	91.7	91.9	92.4	

Example 13

The collected high maltose (D.E. about 42) effluent from the immobilized FUNGAMYL column of Example 10 was used as a feed for the immobilized amyloglucosidase column of Example 6. The flow rate was adjusted (to 73 ml per hour) to yield an effluent having the following composition of a high conversion (D.E. about 65) syrup:

		percent	
	glucose (DP ₁):	38.1	
10	disaccharides (DP ₂):	39.6	
	trisaccharides (DP ₃):	2.8	10
	tetra- and oligosaccharides (DP ₄₊):	19.6	

Example 14

A 25 mm inner diam. jacketed column, maintained at 55°C, was loaded with immobilized amyloglucosidase product (20 g) prepared according to Example 3.

An up-flow substrate feed consisting of the mother liquor (or raffinate) obtained from fructose-glucose fractionation and having the following composition:

		percent	
20	fructose	3.25	20
	glucose	85.71	
	disaccharides	9.17	
	trisaccharides	1.42	
25	higher saccharides	0.54	25
	and pH adjusted to 4.5 was applied to the column at a flow rate of 250 ml per hour at a concentration of 25 percent (w/w). The composition of the effluent, determined by HPLC was shown to be:		
30		percent	30
	fructose	3.25	
	glucose	91.16	
	disaccharides	4.14	
35	trisaccharides	1.15	35
	higher saccharides	0.30	

WHAT WE CLAIM IS:

1. An immobilized saccharifying enzyme product in particulate form, the saccharifying enzyme being selected from amyloglucosidase and maltogenic alpha-amylase, which product comprises carrier particles of granular casein, having a coating of a liquid-permeable proteinaceous layer comprising the saccharifying enzyme and egg albumen crosslinked by reaction with glutaraldehyde.
2. A product according to Claim 1, wherein the weight ratio of the amount saccharifying enzyme : casein is in the range of from 1 : 200 to 1 : 2.
3. A product according to Claim 1, wherein the weight ratio of the amount of saccharifying enzyme : casein is in the range of from 1 : 25 to 1 : 5.
4. A product according to Claim 1, 2 or 3, wherein the weight ratio of the amount of saccharifying enzyme : albumen is in the range of from 1 : 5 to 1 : 0.05.
5. A product according to Claim 1, 2 or 3, wherein the weight ratio of the amount of saccharifying enzyme : albumen is in the range of from 1 : 2 to 1 : 0.2.
6. A product according to any one of Claims 1 to 5, wherein the particle diameter of the granular casein is in the range of from 100 to 500 microns.
7. A product according to any one of Claims 1 to 6, wherein the granular casein is acid precipitated casein.
8. A product according to any one of the preceding claims, wherein the albumen is a spray-dried product.
9. A process for the preparation of an immobilized saccharifying enzyme product, the saccharifying enzyme being selected from amyloglucosidase and maltogenic alpha-amylase, which process comprises coating carrier particles of granular casein with a reaction mixture of the saccharifying enzyme, egg albumen and glutaraldehyde, whereby the granular casein becomes coated by a liquid-permeable proteinaceous layer, in which the saccharifying enzyme and egg albumen are crosslinked by reaction with the glutaraldehyde.
10. A process according to Claim 9, wherein the weight ratio of the amount saccharifying enzyme : casein is in the range of from 1 : 200 to 1 : 2.
11. A process according to Claim 9, wherein the weight ratio of the amount of

saccharifying enzyme : casein is in the range of from 1 : 25 to 1 : 5.

12. A process according to Claim 9, 10 or 11, wherein the weight ratio of the amount of saccharifying enzyme : albumen is in the range of from 1 : 5 to 1 : 0.05.

13. A process according to Claim 9, 10 or 11, wherein the weight ratio of the amount of saccharifying enzyme : albumen is in the range of from 1 : 2 to 1 : 0.2.

5 14. A process according to any one of Claims 9 to 13, wherein the particle diameter of the granular casein is in the range of from 100 to 500 microns.

15. A process according to any one of Claims 9 to 14, wherein the weight ratio of glutaraldehyde to the enzyme and albumen incorporated into the proteinaceous layer is in the range of from 0.1 : 1 to 1 : 1.

10 16. A process according to any one of Claims 9 to 15, wherein the immobilized saccharifying enzyme product is treated with an aqueous solution of glutaraldehyde.

17. A process according to any one of Claims 9 to 15, wherein the immobilized saccharifying enzyme product is treated with an aqueous solution of a salt of sulphurous acid.

15 18. A process according to Claim 16, wherein the immobilized saccharifying enzyme product is treated, after treatment with the aqueous solution of glutaraldehyde, with an aqueous solution of a salt of sulphurous acid.

19. A process according to any one of Claims 9 to 18, wherein the granular casein is acid precipitated casein.

20 20. A process according to any one of Claims 9 to 19, wherein the albumen is a spray-dried product.

21. An immobilized saccharifying enzyme product in particulate form, substantially as described in foregoing Example 1.

25 22. An immobilized saccharifying enzyme product in particulate form, substantially as described in foregoing Example 2.

23. An immobilized saccharifying enzyme product in particulate form, substantially as described in foregoing Example 3.

24. An immobilized saccharifying enzyme product in particulate form, substantially as described in foregoing Example 4.

30 25. An immobilized saccharifying enzyme product in particulate form, substantially as described in foregoing Example 5.

26. A process for the preparation of an immobilized saccharifying enzyme product, substantially as described in foregoing Example 1.

35 27. A process for the preparation of an immobilized saccharifying enzyme product, substantially as described in foregoing Example 2.

28. A process for the preparation of an immobilized saccharifying enzyme product, substantially as described in foregoing Example 3.

29. A process for the preparation of an immobilized saccharifying enzyme product, substantially as described in foregoing Example 4.

40 30. A process for the preparation of an immobilized saccharifying enzyme product, substantially as described in foregoing Example 5.

31. An immobilized saccharifying enzyme product whenever prepared by the process of any one of Claims 9 to 20 and 26 to 30.

45 32. A process of saccharifying dextrin, which comprises using a product in accordance with any one of Claims 1 to 8, 21 to 25 and 31.

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