Abstract: An agent for use in the case of disorders of blood sugar metabolism, including diabetes, is described, which reduces the glucose content of food and other substances with the help of 5-D-fructose dehydrogenase and glucose isomerase.
Agent for use in the case of disorders of blood sugar metabolism, including diabetes

The present invention relates to the use of an agent in the case of diabetes, which reduces the content of glucose in food and other substances consumed. In the context of this application, the term "diabetes" means all forms of disorders of blood sugar metabolism, also of the mild type, including all forms of diabetes, such as diabetes type I, including LADA diabetes (latent autoimmune diabetes in adults), diabetes type II, pregnancy-induced diabetes and impaired glucose tolerance. Accordingly, the invention is also suitable for persons who only have minor disorders of blood sugar metabolism and, therefore, are not (yet) referred to as diabetics in the medical sense.

According the invention the term "agent" includes a pharmaceutical composition, a medical device, a foodstuff and a special foodstuff.

According to the present invention, the terms food and foodstuff are used as synonyms. They mean to also include feed in the sense of animal feed. In the context of this application special foodstuffs are foodstuffs for particular nutritional uses, foods for special medical purposes, medical foods, food supplements, dietary supplements, dietetic food supplements, health foods, nutraceuticals and food additives. In the context of this application the term foodstuff means to include special foodstuffs as used herein, where applicable.

In the context of this application the term "glucose containing" refers to all substances and foodstuffs that either contain glucose in pure form or from which glucose can be released in the digestive tract. The glucose content of substances and foodstuffs refers to all the glucose in a glucose containing food or substance in whatever form (e.g. also as part of sucrose) it is contained in such
a food or substance. From sucrose, glucose and fructose are released in equal parts by enzymatic cleavage in the intestines. In the context of this application the term "fructose containing" refers to all substances and foodstuffs that either contain fructose in pure form or from which fructose can be released in the digestive tract. The fructose content of substances and foodstuffs refers to all the fructose in a fructose containing food or substance in whatever form (e.g. also as part of sucrose) it is contained in such a food or substance.

Diabetes is an extremely wide-spread problem and the incidence of diabetes has constantly increased in Western Europe and North America. The two most important forms are diabetes type I (approx. 5 to 10 %) and diabetes type II (approx. 90 %). In the Federal Republic of Germany, for example, more than 9.9 million people suffer from diabetes of which approx. 3.9 million people suffer from the so-called impaired glucose tolerance. The consequences of diabetes are an increase in the level of blood glucose, glucosuria and later sequelae of the increased level of blood glucose in different organ systems, disorders of lipid metabolism, etc. De-pending on whether it is a question of the various damage caused by average blood sugar values being too high over many years, such as to the eyes, kidneys and nerves, or damage caused by short-term blood sugar peaks, such as premature damage to the walls of the large arterial vessels, one refers to micro- or macro-angiopathic sequelae. The list of possible complications and late damage caused by diabetes is long and they are described in detail in the specialist literature.

Until now, therapies for disorders of blood sugar metabolism have basically been limited to increasing the content of insulin in the blood (by administering insulin or by enhancing the excretion of insulin) and enhancing the efficiency of insulin. An alternative approach is to delay the uptake of carbohydrates (agent for delaying absorption), but this leads to abdominal pain due to the bacterial degradation of carbohydrates that accumulate in the lower sections of the intes-
tines. Despite all methods found to date for treating diabetes, there is still a need for agents that prevent or reduce the increase in the level of blood sugar as a result of the intake of glucose containing foods and substances, without placing a burden on the body.

An agent that would reduce the absorbed amount of glucose would thus satisfy an extremely wide-spread and pressing need which has existed for decades, since sufferers have considerable difficulties with "individual adjustment", i.e. with the exact adjustment of the dose of insulin in relation to the intake of carbohydrates. These difficulties remain, despite the variety of available medicaments and glucose free dietetic foodstuffs especially for diabetics, who are already very limited in the choice of foodstuffs they can eat. Such an agent would overcome the prejudice widely held in the specialist world and among those suffering from diabetes that a low-carbohydrate diet has to be maintained and that a considerable change in nutritional habits is necessary, and would also mean a significant improvement and dramatic development in the therapeutic and nutritional options in diabetes. Such an agent would also put an end to the as yet fruitless efforts of the specialist world to find an agent to treat diabetes which can be administered broadly, easily, and long-term, without causing side effects. This would apply all the more to an agent which, in addition, has no negative effects on health.

Thus, it is an object of the present invention to provide an agent that significantly reduces the usable content of glucose in food, in particular for facilitating the intake of foodstuffs that normally contain glucose also in the case of diabetes, without resulting in negative effects on health. Further, it is an object of the invention to make it possible for diabetics to eat foodstuffs which until now were not allowed to them due to their glucose content or the eating of which was associated with negative effects for the sufferers' health. Moreover, the objective is to provide an agent that can reduce or prevent the associated negative effects
on health after the intake of glucose in diabetes. Another object of the invention is to provide an agent that can reduce or prevent an increase in the level of blood glucose in diabetes after the intake of glucose containing food.

These objects are solved by the subject matter as described in claims 1 to 230. Therefore, the subject matter of the invention is an agent that can solve all of the problems described above. The invention relates to the use of an agent for the curative or prophylactic treatment of diabetes, for diagnosis of diabetes, for reducing the bioavailability of glucose and/or fructose in the human or animal body, for reducing the glucose content and/or the fructose content in a foodstuff, and for preventing or at least reducing an increase of the level of blood glucose after the intake of glucose containing food.

The agent contains 5-D-fructose dehydrogenase (syn. fructose 5-dehydrogenase) and glucose isomerase. The enzyme 5-D-fructose dehydrogenase effects the conversion of fructose into 5-keto-D-fructose. Glucose isomerase has the property of converting glucose into fructose and vice versa with an equilibrium concentration of approximately 50% glucose and 50% fructose. In the context of this application, a 5-D-fructose dehydrogenase is an enzyme that can catalyze the dehydrogenation of fructose to 5-keto-D-fructose. A glucose isomerase, in the context of this application, is an enzyme that is able to transform glucose into fructose. This conversion can also be brought about, for example, by a xylose isomerase. Thus, such a xylose isomerase is, in the sense of this invention, also a glucose isomerase. A possible method for the production of a xylose isomerase is, for example, described in Yamanaka, Biochimica et Biophysica Acta, Volume 151 (3), 1968, 670-680, "Purification, Crystallization and Properties of the D-Xylose Isomerase from Lactobacillus brevis" and in Yamanaka, Methods in Enzymology, Volume 41, 1971, 466-471, "D-Xylose Isomerase from Lactobacillus brevis".
The effect of the enzyme combination according to the present invention will be explained using starch as an example. Since in particular glucose is released from starch as a monosaccharide during digestion, this is transformed by the glucose isomerase into fructose, which is then converted by the 5-D-fructose dehydrogenase into 5-keto-D-fructose, which cannot be metabolized by the body. Thus, the 5-D-fructose dehydrogenase prevents the establishment of the above-mentioned equilibrium. Therefore, said glucose isomerase will convert glucose into fructose, which itself will be dehydrogenated into 5-keto-D-fructose by the 5-D-fructose dehydrogenase, until no further glucose is present in the food or food pulp.

Also in the case of sucrose, a reduction of glucose can be achieved with this combination agent. Fructose which is released from sucrose during digestion is converted into 5-keto-D-fructose by the 5-D-fructose dehydrogenase, as described above. The Glucose isomerase then tries to balance out the resulting "disequilibrium" by converting glucose into fructose. As in the case of starch, this conversion process continues until no further glucose is present in the food pulp. It is possible that part of the glucose has already been absorbed by then. However, the total amount of glucose that will be absorbed from a sugar containing meal will be significantly reduced with the help of the invention disclosed herein. Depending on the level of enzyme activity per dose unit and the amount of the glucose content of the respective meal, it is possible to influence the amount of glucose absorbed by the body. If desired, according to the present invention, it is also possible to achieve a complete or virtually complete elimination of glucose.

Thus, the invention is based on the fact that the glucose contained in the consumed carbohydrates, such as sucrose, or released from them in the intestines, is no longer available for the undesired absorption from the intestines and release into the bloodstream. This is achieved by consuming a mixture of the two
enzymes glucose isomerase and 5-D-fructose dehydrogenase before, shortly before, with, shortly after or after consumption of glucose-containing-foods, by conversion of glucose to fructose and its subsequent dehydrogenation to 5-keto-D-fructose. The enzymes transform glucose into fructose and the fructose into 5-keto-D-fructose, until no further glucose is present. The dosage of the enzymes added may be selected in such a way that, also in the case of an intake of larger amounts of glucose, the reaction can take place at an appropriate rate.

Furthermore, the enzyme combination according to the present invention also has the effect of reducing the intake of calories, which is desired in the case of diabetes, since the enzyme combination, as described above, converts carbohydrates contained in the food into 5-keto-D-fructose, which is significantly less caloric than fructose and glucose. In particular, this is desired in the case of diabetes type II, since diabetics often suffer from obesity, hypertension and disorders of lipid metabolism. In addition, the enzyme combination according to the present invention has the effect that it can convert fructose originating from fructose containing substances and foodstuffs into 5-keto-D-fructose, which is desired in the case of diabetes. Although fructose is used in large amounts as a sweetener in food for diabetics, and fructose is generally regarded as being well-tolerated and harmless for diabetics, there is an ongoing debate among specialists that the intake of fructose is also undesirable and contraindicated in diabetes and should be limited, in particular due to the utilizable calorie content of fructose. In contrast to glucose, fructose is metabolized independently of insulin. Since insulin influences the occurrence of the sensation of satiation indirectly, fructose does not eliminate the appetite, obesity may easily occur as a result of the extensive use of fructose as a sweetener. Free fructose in large amounts may also favour hypertension. It also influences the lipid profile (blood lipids) in an unfavourable way, since in larger amounts it promotes the synthesis of lipids and thus increases the postprandial serum triglycerides. Therefore, especially larger amounts of fructose should not be consumed by diabetics.
Patients with metabolic syndrome are advised in particular not to consume beverages that are sweetened with HFCS (high fructose corn syrup) or sucrose.

Therefore, a subject matter of the invention is an agent for use in the case of diabetes which contains a glucose isomerase and a 5-D-fructose dehydrogenase.

Further, a subject matter of the invention is an agent that reduces the bioavailability of glucose in the human or animal body with the help of a glucose isomerase in combination with a 5-D-fructose dehydrogenase.

Also, a subject matter of the invention is an agent for reducing the utilizable content of glucose of food, which contains a glucose isomerase in combination with a 5-D-fructose dehydrogenase.

Also, a subject matter of the invention is an agent for preventing or reducing an increase of the level of blood glucose in the case of diabetes after the intake of glucose containing food.

A further subject matter of the invention is the use of a glucose isomerase in combination with a 5-D-fructose dehydrogenase in the case of diabetes and in the case of health problems and diseases associated with diabetes.

According to the present invention, a 5-D-fructose dehydrogenase in combination with glucose isomerase can also be used for reducing the utilizable content of glucose in a foodstuff.

In a particularly easy way, the invention facilitates the transformation of glucose in a foodstuff into a form that does not result in an increase in the level of blood
glucose. Thus, the invention enables diabetics to consume foodstuffs that they have had to avoid up till now, due to their glucose content.

According to the present invention, 5-D-fructose dehydrogenase in combination with glucose isomerase is further mentioned for use in medicine, for example, as a pharmaceutical composition. Accordingly, a subject matter of the invention is also a product that consists of 5-D-fructose dehydrogenase in combination with glucose isomerase or contains 5-D-fructose dehydrogenase in combination with glucose isomerase, beside one or more other active ingredients, for use in a medical method, especially in a method for the therapeutic treatment of the human or animal body. In the context of this application, a pharmaceutical composition is a product, especially a substance or a substance mixture, for use in a method for surgical or therapeutic treatment of the human or animal body and in diagnostic methods that are performed on the human or animal body. Thus, in the sense of the invention, pharmaceutical compositions are also products, in particular substances or substance mixtures, that are meant or suitable for curing, alleviating, preventing or determining diabetes.

The term "treating" when used in connection with the foregoing disorders includes amelioration, prevention or relief from the symptoms and/or effects associated with these disorders and includes the prophylactic administration of an enzyme or a mixture thereof to diminish the likelihood or seriousness of the conditions.

According to a further aspect, according to the present invention, a foodstuff is provided that contains glucose isomerase in combination with 5-D-fructose dehydrogenase. Further, according to the present invention, a foodstuff is provided that contains 5-D-fructose dehydrogenase in combination with glucose isomerase in an amount which is effective for converting fructose into 5-keto-D-fructose and glucose isomerase in an amount which is effective for transforming
glucose into fructose. Such a foodstuff may be produced advantageously using a method for treating a foodstuff in which the foodstuff is placed in contact with a 5-D-fructose dehydrogenase in combination with glucose isomerase under such conditions under which the 5-D-fructose dehydrogenase can dehydrogenate fructose to 5-keto-D-fructose and the glucose isomerase can convert glucose into fructose. In contrast to otherwise untreated foodstuffs, such a foodstuff has a reduced content of glucose and therefore, for the first time, is suitable to be eaten by diabetics. Particularly advantageously, a foodstuff may be prepared by a method in which a glucose isomerase in combination with a 5-D-fructose dehydrogenase is added to the foodstuff in a manner in which the action of the two enzymes does not start until after the foodstuff has been consumed. Such a foodstuff that contains 5-D-fructose dehydrogenase and glucose isomerase has the same taste as an untreated food-stuff and is, for the first time, suitable to be eaten by diabetics, due to the reduced content of glucose which is established after consumption.

According to a further aspect, according to the present invention, 5-D-fructose dehydrogenase in combination with glucose isomerase is provided as a medical device. Accordingly, the subject matter of the invention is also a medical device that consists of 5-D-fructose dehydrogenase in combination with glucose isomerase or contains 5-D-fructose dehydrogenase in combination with glucose isomerase, be-side one or more other active ingredients.

In the following, the invention will be described further in its various aspects.

5-D-fructose dehydrogenase is a compound that has been known for nearly 40 years, but has only been used for analytical purposes to date. Glucose isomerase is a compound that has been known for more than 40 years and has only been used for starch saccharification to date. In the industry, it is used for the
conversion of glucose into fructose as well as for the conversion of fructose into glucose.

Until now, 5-D-fructose dehydrogenase has not been used in combination with glucose isomerase in the case of diabetes of humans or animals.

The agent according to the present invention can be taken orally prior to meals, immediately before meals, with meals or immediately after meals, so that it can exert its converting effect on glucose and dehydrogenating effect on fructose in the food pulp. The agent according to the present invention may contain the enzymes without further additives. However, it is preferable that the agent according to the present invention further contains additives that are pharmaceutically acceptable and/or acceptable for foodstuffs, such as for example extenders, binders, stabilizers, preservatives, flavourings, etc. Such additives are commonly used and well known for the production of pharmaceutical compositions, medical devices, food-stuffs, and special foodstuffs and the person skilled in the art knows which additives in which amounts are suitable for certain presentation forms. The agents according to the present invention may for example contain as additives dicalcium phosphate, lactose, modified starch, microcrystalline cellulose, maltodextrin and/or fibersol.

The agent according to the present invention can also be added to a foodstuff before its consumption. It can already be added to the foodstuff during production, with the aim that it exhibits its effect only after eating the foodstuff. This could also be achieved by microencapsulation, for example. With this, the utilizable glucose content of the foodstuff would be reduced without negatively affecting its taste. Therefore, preparations containing 5-D-fructose dehydrogenase and glucose isomerase are useful, which release this enzyme only in the digestive tract of a human or animal or let it become effective in another way, especially in the stomach or small intestine. Therefore, the invention can be used, for
example, in the production of desserts, fruit preparations (e.g. apple sauce), jam, honey, chocolate and chocolate products, bakery products (e.g. biscuits and cakes), breads, pastas, vegetable dishes, potato dishes, ice cream, cereals, dairy products (e.g. fruit yogurt and pudding), fructose- and/or glucose-containing beverages, fructose- and/or glucose-containing sauces (e.g. tomato ketchup) and fructose- and/or glucose-containing sweeteners. For dishes that are boiled or baked, the agent according to the present invention could e.g. be mixed into or sprinkled onto them after cooling.

The agent according to the present invention can also be added to a foodstuff, to exert its effect after eating on the glucose originating from another foodstuff. An example of this would be the addition of the agent according to the present invention to a spread so that the reduction of the glucose that is contained in the bread and that can be used by the body occurs after the intake of the bread, without impairing its taste. Further examples would be mixed spices and mayonnaise for use with french fries.

The agent according to the present invention may also be used in immobilized form. This is especially useful for the treatment of liquid foodstuffs. For example, the enzymes can be embedded in a matrix which is permeable for glucose. If a glucose containing liquid foodstuff is allowed to flow along the enzyme containing matrix, then glucose is extracted from the foodstuff by the action of the enzymes and converted to 5-keto-D-fructose.

A subject matter of the present invention are also agents that, in addition to other active ingredients, also contain glucose isomerase in combination with 5-D-fructose dehydrogenase.

The agent may be formulated in any form which is suitable for the intended route of administration. A preferred route of administration is oral administration.
For oral administration, the agent may be formulated for example in the form of capsules (coated or non-coated) containing powder, coated or non-coated pellets, granules or micro-/mini-tablets or in the form of tablets (coated or non-coated) pressed from powder, coated or non-coated pellets, dragees or micro-/mini-tablets. The agent may also be formulated for example in the form of gel caps or in liquid form as solution, drops, suspension or gel. The agent may also be formulated e.g. as dried or moist oral supplement. The formulation of the agent according to the present invention as powder is particularly suitable for admixing with foodstuff. The powder may be sprinkled onto a meal or mixed into a pulp or beverage. It is particularly beneficial, if the agent offered as bulk powder is packaged in single dosage amounts, such as in single bags or capsules, or if it is provided in a dosing dispenser.

For oral administration, the 5-D-fructose dehydrogenase in combination with glucose isomerase may be used with acceptable excipients and/or carriers.

The total amount of the carrier and/or excipient of an agent containing 5-D-fructose dehydrogenase and glucose isomerase is preferably between 5 and 99.9 % by weight, more preferably between 10 and 80 % by weight and even more preferably between 25 and 60 % by weight of the composition.

Suitable excipients and/or carriers include maltodextrin, calcium carbonate, dicalcium phosphate, tricalcium phosphate, microcrystalline cellulose, dextrose, rice flour, magnesium stearate, stearic acid, croscarmellose sodium, sodium starch glycolate, crospovidone, sucrose, vegetable gums, lactose, methylcellulose, povidone, carboxymethyl cellulose, corn starch, modified starch, fibersol, gelatine, hydroxypropylmethyl cellulose and the like (including mixtures thereof).
Preferable carriers include calcium carbonate, magnesium stearate, maltodextrin, dicalcium phosphate, modified starch, microcrystalline cellulose, fibersol, gelatine, hydroxypropylmethyl cellulose and mixtures thereof.

The various ingredients and the excipient and/or carrier may be mixed and formed into the desired form using common methods well known to the skilled person. The administration form according to the present invention which is suited for the oral route, such as e.g. tablet or capsule, may be coated with a coating which is resistant against low pH values (approximately pH 1 to 2.5) and which dissolves at a pH value of approximately 3.0 to 8.0, preferably at a pH value of 3.0 to 6.5 and particularly preferable at a pH value of 4.0 to 6.0. An optionally used coating should be in accordance with the pH optimum of the enzyme used and its stability at pH values to which the formulation will be exposed. Also a coating may be used which is not resistant to low pH values but which delays the release of the enzyme at low pH values. It is also possible to prepare the agent according to the present invention as coated (see above) pellets, granules or micro-/mini-tablets which can be filled into coated or non-coated capsules or which can be pressed into coated or non-coated tablets. Suitable coatings are, for example, cellulose acetate phthalate, cellulose derivatives, shellac, polyvinylpyrrolidone derivates, acrylic acid, poly-acrylic acid derivatives and polymethyl methacrylate (PMMA), such as e.g. Eudragit® (from Rohm GmbH, Darmstadt, Germany), in particular Eudragit® L30D-55. The coating Eudragit® L30D-55 is dissolved, for example, at a pH value of 5.5 and higher. If it is desired to release the enzyme already at a lower pH value, this may be achieved e.g. by the addition of sodium hydroxide solution to the coating agent Eudragit® L30D-55, because in this case carboxyl groups of the methacrylate would be neutralised. Therefore, this coating will be dissolved, for example, already at a pH value of 4.0 provided that 5 % of the carboxyl groups are neutralised. The addition of about 100 g of 4 % sodium hydroxide solution to 1 kg of Eudragit® L30D-55 would result in a neutralisation of about 6 % of the

Other suitable acceptable carriers or adjuvants for use in the present invention include, but are not restricted to water, mineral oil, ethylene glycol, propylene glycol, lanolin, glycercyl stearate, sorbitan stearate, isopropyl myristate, isopropyl palmitate, acetone, glycerine, phosphatidylcholine, sodium cholate or ethanol.

The compositions for use in the present invention may also comprise at least one co-emulsifying agent which includes but is not limited to oxyethylenated sorbitan monostearate, fatty alcohols, such as stearyl alcohol or cetyl alcohol, or esters of fatty acids and polyols, such as glycercyl stearate.

The agents according to the present invention may be provided in a stabilized form. Generally, stabilization methods and procedures which may be used according to the present invention include any and all methods for the stabilization of chemical or biological material which are known in the art, comprising e.g. the addition of chemical agents, methods which are based on temperature modulation, methods which are based on irradiation or combinations thereof. Chemical agents that may be used according to the present invention include, among others, preservatives, acids, bases, salts, antioxidants, viscosity enhancers, emulsifying agents, gelatinizers, and mixtures thereof.
Usually, the industrial production of enzymes is performed in a technical fermentation way using suitable microorganisms (bacteria, moulds, fungi). Usually the strains are recovered from natural ecosystems according to a special screening protocol, isolated as pure cultures as well as improved in their properties with respect to the enzyme spectrum and biosynthesis performance (volume/time yield). Enzyme production may also be carried out by methods developed in the future.

5-D-fructose dehydrogenase is commercially available (e.g. Sigma-Aldrich or Toyobo Enzymes, Japan) and is usually prepared in a microbiological way with the help of the microorganism *Glucobacter industrius*. Glucose isomerase is also commercially available (e.g. Sigma-Aldrich or Novozymes A/S, Denmark) and usually prepared in a microbiological way with the help of the microorganism *Streptomyces murinus*. However, the invention is not limited to the enzymes that are commercially available at the moment, but generally relates to enzymes that can catalyze the conversion of fructose - specifically or non-specifically - to 5-keto-D-fructose, and of glucose - specifically or non-specifically - to fructose. A person skilled in the art can prepare suitable further enzymes by conventional methods, for example by mutagenesis of the gene encoding 5-D-fructose dehydrogenase which is present in *Glucobacter industrius* or by mutagenesis of the gene encoding glucose isomerase in *Streptomyces murinus*. The enzymes may also be prepared with the help of other microorganisms, such as fungi, in sufficient amounts and the required purities, also by the use of the genetic engineering methods which are presently known or may be developed in the future. For example, if it is desired to produce the enzymes with other microorganisms, then the genetic information of a microorganism which has been found initially by extensive screening and which has been proven to be a suitable source of the enzyme with the desired properties can be transferred to a microorganism which is normally used for the production of enzymes. Also the modification of the enzymes and the production of the enzymes by means of methods which are
presently known or may be developed in the future in the area of industrial enzyme development and enzyme production, such as genetic engineering, is possible. The use and the manner of performing all these methods for developing and producing the enzyme(s) with the desired purities and activities and with the desired properties, in particular with respect to the stability of the enzyme(s) at various pH values, regarding the optimum of the pH value, the stability at various temperatures and temperature optimum, are well known to a person skilled in the art. The explanations in chapter 2 (page 82 to page 130) of the textbook "Lebensmittel-Biotechnologie und Ernährung" of Heinz Ruttlloff, Jürgen Proll and Andreas Leuchtenberger, published by Springer Verlag 1997 (ISBN 3-540-61 135-5) describe these methods in detail. These methods are also described in "Advances in Fungal Biotechnology for Industry, Agriculture, and Medicine" by Jan S. Tkacz, Lene Langeand (published in 2004, ISBN 0-306-47866-8), in "Enzymes in Industry: Production and Applications" by Wolfgang Aehle (Editor), published in 2004, ISBN 3527295925 and in "Microbial Enzymes and Biotransformations" by Jose-Luis Barredo (Humana Press 2005, ISBN 1588292533). These documents are herewith incorporated into the patent application by reference. All this also applies to the enzymes mentioned below that can optionally be added to the agent according to the present invention.

The activity of 5-D-fructose dehydrogenase is defined in units (assay available e.g. from Sigma-Aldrich), whereby one unit is the amount of 5-D-fructose dehydrogenase that converts one micromole of D-fructose to 5-keto-D-fructose per minute at pH 4.5 and 37°C. Generally, the activity of 5-D-fructose dehydrogenase per dose unit should be between 10 and 5 million units, preferably between 25 and 2.5 million units and particularly preferably between 50 and 1 million units.

The activity of glucose isomerase should generally be between 0.01 and 100,000 GIU, preferably between 0.05 and 10,000 GIU and particularly prefera-
bly between 0.1 and 1,000 GIU per dose unit. One unit of this enzyme is defined as a glucose isomerase unit (GIU). One GIU converts 1 g of glucose into fructose at a pH value of 6.0 and at a temperature of 37°C from a solution of initially 10% (percent by weight, i.e. 10 g of glucose + 90 g of water) in 5 minutes.

The wide range of the above mentioned dosages may be explained by the fact that the agent according to the present invention can be applied in completely different types of diabetes in the whole range of different severities. Furthermore, the different dosages also result from the fact that strongly varying amounts of glucose are supplied, depending on the food in question.

The agent according to the present invention may comprise one or more additional enzymes, such as invertase (syn. beta-fructofuranosidase or beta-fructosidase), lactase (syn. beta-galactosidase), maltase (syn. alpha-glucosidase), alpha-amylase, beta-amylase, glucoamylase, pullulanase, isoamylase, amyloglucosidase, cyclomaltodextrin glucantransferase (CGTase). These enzymes have the property of releasing fructose and/or glucose from fructose and/or glucose containing substances and foodstuffs - alone or in combination with one or more of these enzymes -, whereby the enzymes pullulanase and isoamylase also increase the efficiency of glucoamylase and beta-amylase. All these enzymes are commercially available (e.g. BioCat Inc., Troy, USA or Novozymes A/S, Denmark or Amano Enzymes Inc., Japan or Sigma-Aldrich) and, up to now, have never been used in combination with 5-D-fructose dehydrogenase and glucose isomerase in the case of diabetes. Examples for agents according to the present invention include:

5-D-fructose dehydrogenase in combination with invertase, or 5-D-fructose dehydrogenase in combination with glucose isomerase and invertase, or 5-D-fructose dehydrogenase in combination with glucose isomerase and lactase and invertase, still further 5-D-fructose dehydrogenase in combination with glucose
isomerase, Invertase, alpha amylase, beta amylase, glucoamylase, maltase, isoamylase and pullulanase (combination of 9 enzymes), or 5-D-fructose dehydrogenase in combination with glucose isomerase, alpha amylase, beta amylase, glucoamylase, maltase, isoamylase and pullulanase as well as invertase and lactase (combination of 10 enzymes).

For example, said invertase can release glucose from e.g. sucrose and lactase can release glucose from lactose. Beta-amylase breaks down e.g. 1,4-alpha-bonds in starch, starting at the non-reducing end of the polysaccharide chain with cleaving of maltose, and glucose is released by the action of maltase on maltose. By the addition of one or more of these enzymes to the agent according to the present invention, the endogenic release of glucose from glucose containing substances or foodstuffs, in particular from sucrose and starch, may also be promoted and accelerated, so that the conversion of glucose into fructose effected by the glucose isomerase and the conversion of fructose into 5-keto-D-fructose which is catalyzed by the 5-D-fructose dehydrogenase may occur earlier. Therefore, the addition of one or more of these enzymes to the agent according to the present invention may have the benefit of reducing the required amount of 5-D-fructose dehydrogenase and glucose isomerase.

The activity of invertase is measured in Sumner units (SU, assay available e.g. from Bio-Cat Inc., Troy, Virginia, USA). An SU is defined as the amount of the enzyme which converts 1 mg of sucrose into glucose and fructose under standard test conditions within 5 minutes at 20°C and a pH value of 4.5. If the agent according to the present invention also contains invertase, the activity of the invertase per dose unit should be between 50 and 250,000 SU, preferably between 100 and 150,000 SU and particularly preferably between 150 and 100,000 SU per dose unit.
The activity of lactase is given in Food Chemical Codex (FCC) units (assay is published in the Food Chemical Codex, fifth edition, and also available e.g. from Bio Cat Inc. Troy, Virginia or Amano Enzymes, Japan or from Sigma Aldrich). If the agent according to the present invention also contains lactase, the activity of the lactase per dose unit should be between 50 and 200,000 FCC units, preferably between 100 and 100,000 FFC units and particularly preferably between 150 and 50,000 FCC units.

The activity of maltase is defined in units, wherein one unit is the amount of maltase which will convert maltose to D-glucose at a rate of one milligram per minute at 37°C and a pH of 4.0 in a 10% maltose solution by weight.

Where the agent according to the present invention also contains maltase, the activity per dose unit should be between 100 and 100,000 units, preferably between 200 and 50,000 units and particularly preferably between 500 and 20,000 units.

Also for the other enzymes mentioned, the standard test conditions and the way in which the enzyme activities are to be determined are known and can be read up by specialists in the field.

Insofar as one or more of the optional enzymes are added to the agent according to the present invention, they - as is the case for the 5-D-fructose dehydrogenase and the glucose isomerase - should be used in sufficient amounts so that they can develop a sufficient enzyme activity for the intended purpose, e.g. sufficient invertase, so that an amount of sucrose usually ingested with a normal meal (e.g. 15 g) can be cleaved, and/or lactase, so that an amount of lactose usually ingested with a normal meal (e.g. 10 g) can be cleaved.
The enzymes used can be for example in solid form, e.g. as crystalline or amorphous granules or powders, as a paste or as a liquid, as well as in other forms. In some embodiments, the enzyme is a free enzyme. In other embodiments, the enzyme may e.g. be immobilized on substrate, which can be powderized if necessary before the enzyme is used in accordance with the invention.

If the agent according to the present invention is added to a foodstuff before consumption or during production, the activity of 5-D-fructose dehydrogenase should be between 10 and 250,000 units, preferably between 25 and 150,000 units and particularly preferably between 50 and 100,000 units per gram of fructose and glucose combined contained in the foodstuff and the activity of the glucose isomerase should be between 0.01 and 20,000 GIU, preferably between 0.05 and 10,000 GIU and particularly preferably between 0.1 and 1000 GIU per gram of glucose in the foodstuff.

It may be advantageous to add an electron acceptor to the agent according to the present invention at e.g. a ratio (acceptor : substrate) of 1:1 to 1:1,000, preferably at a ratio of 1:2 to 1:200, particularly preferably at a ratio of 1:10 to 1:50. Examples of suitable acceptors which may be used include NAD+, NADP+, FAD+, vitamins, such as vitamin C, vitamin E or vitamin A, ferricyanide, ketones, aldehydes, 2,6-dichlorophenolindophenol, phenazine methosulfate, nitroblue tetrazolium (including mixtures thereof), but are not limited thereto.

The physiologically present electrolytes should be sufficient for the function of glucose isomerase. But it may also be advantageous to add electrolytes to the agent according to the present invention, e.g. in an amount of 0.0001 % to 0.1 % of the substrate (glucose). Examples of electrolytes include, but are not limited to, MgSO₄, Na₂CO₃, NaHCO₃, NaOH, Na₂SO₄, MgCO₃, H₂SO₄, NaS₂O₃, NaS₂O₅ (including mixtures thereof).
It may also be advantageous to add metal ions, especially cations, such as \( \text{Mn}^{2+} \), \( \text{Mg}^{2+} \), \( \text{Ca}^{2+} \), \( \text{Zn}^{2+} \), \( \text{Fe}^{2+} \), \( \text{Co}^{2+} \) or \( \text{Cu}^{2+} \), including mixtures thereof, to the agent according to the present invention, namely preferably in a molar ratio of \( 10^{-6} \) to \( 10^{-2} \). For the above mentioned (xylose) glucose isomerase which is described by Yamanaka, especially \( \text{Mn}^{2+} \) is a suitable cation.

Capsule sizes mentioned below refer to the size definitions used by Capsugel Belgium BVBA, Bornem, Belgium. The size of the capsules should be chosen according to the specific formulation of the agent.

A composition according to the present invention for the production of capsules (e.g. of size 3) may consist of 55 mg of 5-D-fructose dehydrogenase with an activity of 1000 units/mg, 50 mg of glucose isomerase with an activity 1 GIU/mg and 55 mg of dicalcium phosphate per capsule.

A further example for a dosage form according to the present invention consists of capsules (size 00) that contain 165 mg of 5-D-Fructose dehydrogenase with an activity of 1000 units/mg, 150 mg of glucose isomerase with an activity of 1 GIU/mg and 155 mg of dicalcium phosphate per capsule.

In a further composition example, a capsule of size 0 may contain 250 mg of 5-D-fructose dehydrogenase with an activity of 90 units/mg and 20 mg of glucose isomerase with an activity of 1 GIU/mg and 50 mg of dicalcium phosphate.

A further example for the production of capsules of size 00 may contain 370 mg of 5-D-fructose dehydrogenase with an activity of 90 units/mg, 30 mg of glucose isomerase with an activity of 1 GIU/mg and 70 mg of dicalcium phosphate.
Another example for the dosage form according to the present invention consists of capsules of size 00 which contain 110 mg of 5-D-fructose dehydrogenase with an activity of 500 units/mg, 50 mg of glucose isomerase with an activity of 1 GIU/mg, 100 mg of invertase with an activity of 200 SU units/mg, 90 mg of lactase with an activity of 100 FCC units/mg and 120 mg of dicalcium phosphate.

The invention may for example contain between 10 and 5 million units of 5-D-fructose dehydrogenase and between 0.01 and 100,000 GIU (= glucose isomerase units) of glucose isomerase per dose unit. In addition, suitable additives may be used in the required amount.

The invention may be provided for medical purposes and non-medical purposes, e.g. as a pharmaceutical composition, medical device, foodstuff or special foodstuff.

With the agent according to the present invention, afflictions and impairments of health that are caused by diabetes in its various degrees of severity can be significantly reduced or eliminated. The invention disclosed herein is especially suitable for the therapeutic treatment of diabetes. It is also suitable for use in methods for the therapeutic treatment of the human or animal body in which an uncontrolled increase in the level of blood sugar is to be prevented or a decrease in the level of blood sugar is intended.

In the following claims, the term "glucose equivalent-containing" refers to all substances and foodstuffs that contain glucose (a) as glucose per se, (b) in a form from which glucose can be released in the digestive tract (e.g. by cleavage as glucose from a saccharide chain containing at least two saccharide monomers, such as sucrose), (c) in a form that can be converted to glucose, e.g. as fructose per se, or (d) in a form that can be released in the digestive tract and
converted to glucose, e.g. as a saccharide chain containing at least two saccharide monomers, at least one of which can be cleaved from the saccharide chain as fructose.

In the following claims, the term "total glucose" refers to the total content of glucose in a foodstuff (a) as glucose per se, (b) in a form from which glucose can be released in the digestive tract (e.g. by cleavage as glucose from a saccharide chain containing at least two saccharide monomers, such as sucrose), (c) in a form that can be converted to glucose, e.g. as fructose per se, or (d) in a form that can be released in the digestive tract and converted to glucose, e.g. a saccharide chain containing at least two saccharide monomers, at least one of which can be cleaved from the saccharide chain as fructose.

In the following claims, the term "effective glucose content" of an item refers to the effective amount of total glucose in that item, taking into account the prior action of glucose and fructose converting enzymes that have been added and the future action of glucose and fructose converting enzymes that have been added to the item. Thus, for example, a foodstuff having a given glucose content and having microencapsulated glucose isomerase and microencapsulated 5-D-fructose dehydrogenase incorporated therein will have a lower effective glucose content than a foodstuff which lacks the microencapsulated glucose isomerase and 5-D-fructose dehydrogenase but is otherwise identical, since release of the glucose isomerase and 5-D-fructose dehydrogenase after ingestion will result in at least a portion of the glucose in the foodstuff being converted to 5-keto D-fructose.

In the following claims, the term "total fructose" refers to the total content of fructose in a foodstuff (a) as fructose per se, (b) in a form from which fructose can be released in the digestive tract (e.g. by cleavage as fructose from a saccharide chain containing at least two saccharide monomers), (c) in a form that
can be converted to fructose, e.g. as glucose per se, or (d) in a form that can be released in the digestive tract and converted to fructose, e.g. a saccharide chain containing at least two saccharide monomers, at least one of which can be cleaved from the saccharide chain as glucose.
Claims:

1. A use of glucose isomerase in combination with 5-D-fructose dehydrogenase and optionally in combination with one or more enzyme(s) selected from invertase, lactase, maltase, alpha-amylase, beta-amylase, glucoamylase, pullulanase, isoamylase, amyloglucosidase and cyclomaltodextrin glucanotransferase for production of an agent, preferably a pharmaceutical composition, for the curative or prophylactic treatment of diabetes.

2. A use of glucose isomerase in combination with 5-D-fructose dehydrogenase and optionally in combination with one or more enzyme(s) selected from invertase, lactase, maltase, alpha-amylase, beta-amylase, glucoamylase, pullulanase, isoamylase, amyloglucosidase and cyclomaltodextrin glucanotransferase for production of an agent, preferably a pharmaceutical composition, for diagnosis of diabetes.

3. A use of glucose isomerase in combination with 5-D-fructose dehydrogenase and optionally in combination with one or more enzyme(s) selected from invertase, lactase, maltase, alpha-amylase, beta-amylase, glucoamylase, pullulanase, isoamylase, amyloglucosidase and cyclomaltodextrin glucanotransferase for production of an agent, preferably a pharmaceutical composition, for reducing the bioavailability of glucose and/or fructose in the human or animal body.

4. A use of glucose isomerase in combination with 5-D-fructose dehydrogenase and optionally in combination with one or more enzyme(s) selected from invertase, lactase, maltase, alpha-amylase, beta-amylase, glucoamylase, pullulanase, isoamylase, amyloglucosidase and cyclomaltodextrin glucanotransferase for production of an agent, preferably a pharmaceutical composition, for reducing the glucose content and/or the fructose content in a foodstuff.
5. A use of glucose isomerase in combination with 5-D-fructose dehydro¬
genase and optionally in combination with one or more enzyme(s) selected from invertase, lactase, maltase, alpha-amylase, beta-amylase, glucoamylase, pullu¬
lanase, isoamylase, amyloglucosidase and cyclomaltodextrin glucanotrans¬
ferase for production of an agent, preferably a pharmaceutical composition, for preventing or at least reducing an increase of the level of blood glucose after the intake of glucose containing food.

6. The use according to one or more of the preceding claims, wherein the agent is selected from a pharmaceutical composition, a medical device, a food¬
stuff or a special foodstuff.

7. The use according to one or more of the preceding claims, wherein the agent is in a form for oral use.

8. The use according to one or more of the preceding claims, wherein the enzymes are protected by a coating to be stable at pH values of less than 4, preferably less than 3.

9. The use according to one or more of the preceding claims, wherein the agent is suited to be added to food at the production stage of the same and/or before eating.

10. The use according to one or more of the preceding claims, wherein the agent in a form for use in immobilised form.

11. A process for the treatment of a foodstuff, comprising the steps of con¬tacting the foodstuff with glucose isomerase in combination with 5-D-fructose dehydrogenase and optionally in combination with one or more enzyme(s) se-
lected from invertase, lactase, maltase, alpha-amylase, beta-amylase, glucoamylose, pullulanase, isoamylase, amyloglucosidase and cyclomaltodextrin glucanotransferase and initiating the reduction of the glucose content and/or fructose content of the foodstuff.

12. The process according to claim 11, wherein as a further step prior to the initiation ingestion of the foodstuff takes place.

13. A mammalian-ingestible composition of matter which comprises a plurality of enzymes that collectively convert D-glucose into a first form that is at least one of (a) biologically inactive in a chosen mammalian body, (b) not digestible in the digestive tract of said mammalian body and (c) not metabolizable in said mammalian body.

14. A mammalian-ingestible composition of matter according to claim 13 wherein said plurality of enzymes collectively converts D-glucose into a second non-glucose form and converts said non-glucose form into said first form.

15. A composition of matter according to claim 14 wherein said second non-glucose form is D-fructose.

16. A mammalian-ingestible composition of matter according to any of claims 13 to 15 wherein said chosen mammalian body is a human body.

17. A mammalian-ingestible composition of matter according to any of claims 13 to 15 wherein said chosen mammalian body is a non-human body.

18. A composition of matter according to any of claims 13 to 17 wherein said plurality of enzymes collectively converts D-glucose to 5-keto-D-fructose.
19. A composition of matter according to any of claims 13 to 17 wherein said first form is 5-keto-D-fructose.

20. A composition of matter according to any of claims 13 to 19 wherein at least one of said plurality of enzymes is a glucose isomerase.

21. A composition according to claim 20 wherein said glucose isomerase is a xylose isomerase.

22. A composition of matter according to any of claims 13 to 20 wherein at least one of said plurality of enzymes is 5-D-fructose dehydrogenase.

23. A composition of matter according to any of claims 13 to 22 which is a human dietary supplement or a pharmaceutical composition.

24. A composition of matter according to any of claims 13 to 22 which is an animal dietary supplement or a veterinary composition.

25. A composition of matter according to any of claims 13 to 22 which is a special foodstuff.

26. A composition of matter according to claim 23 or 25 which further comprises at least one pharmaceutically or dietarily acceptable carrier or excipient.

27. A composition of matter according to claim 24 which further comprises at least one veterinarily acceptable carrier or excipient.

28. A composition of matter according to any of claims 23 to 27 wherein said composition of matter contains each enzyme of said plurality of enzymes in microencapsulated form.
29. A composition of matter according to any of claims 23 to 28 wherein said composition of matter is in the form of a capsule or tablet.

30. A composition of matter according to any of claims 23 to 28 wherein said composition of matter is in the form of granules or pellets.

31. A composition of matter according to any of claims 23 to 27 wherein said composition of matter is in the form of a solution.

32. A composition of matter according to any of claims 23 to 28 wherein said composition of matter is in the form of a liquid.

33. A composition of matter according to any of claims 23 to 28 wherein said composition of matter is in the form of a gel or suspension.

34. A composition of matter according to claim 33 wherein said composition of matter is in the form of a gelcap.

35. A composition of matter according to any of claims 23 to 28 wherein said composition of matter is in the form of a powder.

36. A composition of matter which is microencapsulated enzyme glucose isomerase.

37. A composition of matter which is a mixture of microencapsulated glucose isomerase and microencapsulated 5-D-fructose dehydrogenase.

38. A composition of matter according to claim 37 wherein said glucose isomerase and said 5-D-fructose dehydrogenase are microencapsulated together.
39. A composition of matter according to claim 37 wherein said glucose isomerase and said 5-D-fructose dehydrogenase are separately microencapsulated.

40. A composition of matter according to any of claims 13 to 39 wherein said composition is adapted to be mixed with a food.

41. A composition of matter comprising the enzymes glucose isomerase and 5-D-fructose dehydrogenase admixed with a mammalian-ingestible substance.

42. A composition of matter according to claim 41 wherein said mammalian-ingestible substance is a human-ingestible substance.

43. A composition of matter according to claim 41 wherein said mammalian-ingestible substance is an animal-ingestible substance.

44. A composition of matter according to claim 41 or 42 wherein said mammalian-ingestible substance is a pharmaceutically or dietarily acceptable carrier or excipient.

45. A composition of matter according to claim 41 or 43 wherein said mammalian-ingestible substance is a veterinarily acceptable carrier or excipient.

46. A composition of matter according to any of claims 41 to 45 wherein at least one of said glucose isomerase and said 5-D-fructose dehydrogenase is microencapsulated.

47. A composition of matter according to claim 46 wherein both said glucose isomerase and said 5-D-fructose dehydrogenase are microencapsulated.
48. A composition of matter according to any of claims 13 to 47 wherein said plurality of enzymes constitutes between 5 and 99.9% by weight of the composition of matter.

49. A composition of matter according to claim 48 wherein said plurality of enzymes constitutes between 10 and 80% by weight of the composition of matter.

50. A composition of matter according to claim 48 wherein said plurality of enzymes constitutes between 25 and 60% by weight of the composition of matter.

51. A composition of matter according to any of claims 13 to 50, wherein said composition of matter is in unit dosage form and said unit dosage contains between 10 and 5 million units of 5-D-fructose dehydrogenase activity.

52. A composition of matter according to claim 51 wherein said unit dosage contains between 25 and 2.5 million units of 5-D-fructose dehydrogenase activity.

53. A composition of matter according to claim 51 wherein said unit dosage contains between 50 and 1 million units of 5-D-fructose dehydrogenase activity.

54. A composition of matter according to any of claims 13 to 53 wherein said composition of matter is in unit dosage form and each dosage unit contains 0.01 to 100,000 units of glucose isomerase activity per dose unit.

55. A composition of matter according to claim 54 wherein each dosage unit contains 0.05 to 10,000 units of glucose isomerase activity per dose unit.
56. A composition of matter according to claim 54 wherein each dosage unit contains 0.1 to 1,000 units of glucose isomerase activity per dose unit.

57. A composition of matter according to any of claims 54 to 56 wherein said glucose isomerase is a xylose isomerase.

58. A composition of matter according to any of claims 13 to 57 wherein said composition further comprises at least one of an electrolyte and a metal ion.

59. A composition of matter according to claim 58 wherein said electrolyte is selected from the group consisting of MgSO₄, Na₂CO₃, NaHCO₃, NaOH, Na₂SO₄, MgCO₃, H₂SO₄, NaS₂O₃, NaS₂O₅ and mixtures thereof.

60. A composition of matter according to claim 58 or 59 wherein said metal ion is selected from the group consisting of Mn²⁺, Mg²⁺, Ca²⁺, Zn²⁺, Fe²⁺, Co²⁺, Cu²⁺ and mixtures thereof.

61. A composition of matter according to any of claims 13 to 60 wherein said composition of matter comprises a coating which dissolves in an aqueous medium at a pH of between 3.0 and 8.0.

62. A composition of matter according to claim 61 wherein said coating does not dissolve in an aqueous medium at a pH of below 3.0.

63. A composition of matter according to claim 61 wherein said coating does not dissolve in an aqueous medium at a pH below 4.0.

64. A composition of matter according to any of claims 61 to 63 wherein said coating does not dissolve in an aqueous medium at a pH above 6.5.
65. A composition of matter according to claim 64 wherein said coating does not dissolve in an aqueous medium at a pH above 6.0.

66. A composition of matter according to any of claims 13 to 60 wherein said composition of matter is a slow-release or extended-release formulation.

67. A composition of matter according to claim 66 wherein said slow-release or extended-release formulation comprises a slow-release or extended-release coating.

68. A composition of matter according to any of claims 13 to 67 wherein said composition of matter further comprises at least a third enzyme.

69. A composition of matter according to claim 68 wherein said third enzyme is capable of cleaving fructose or glucose from a sugar that contains at least two saccharide monomers.

70. A composition of matter according to claim 69 wherein said third enzyme is invertase or maltase.

71. A composition of matter according to claim 70 wherein said third enzyme is invertase, said composition of matter is in unit dosage form, and each unit dosage contains between 50 and 250,000 Sumner units of invertase activity.

72. A composition of matter according to claim 71 wherein each unit dosage contains between 100 and 150,000 Sumner units of invertase activity.

73. A composition of matter according to claim 71 wherein each unit dosage contains between 150 and 100,000 Sumner units of invertase activity.
74. A composition of matter according to claim 70 wherein said third enzyme is maltase, said composition of matter is in unit dosage form, and each unit dosage contains between 100 and 100,000 units of maltase activity.

75. A composition of matter according to claim 74 wherein each unit dosage contains between 200 and 50,000 units of maltase activity.

76. A composition of matter according to claim 74 wherein each unit dosage contains between 500 and 20,000 units of maltase activity.

77. A composition of matter according to any of claims 70 to 76 wherein said composition of matter comprises both invertase and maltase.

78. A composition of matter according to any of claims 13 to 77 wherein said composition further comprises one or more members of the group consisting of lactase, alpha-amylase, beta-amylase, glucoamylase, pullulanase, isoamylase, amyloglucosidase and cyclomaltodextrin glucantransferase (CGTase).

79. A composition of matter according to claim 78 wherein said member is lactase, said composition of matter is in unit dosage form and each dosage unit contains 50 to 200,000 FCC units of lactase activity per dose unit.

80. A composition of matter according to claim 79 wherein each dosage unit contains 100 to 100,000 FCC units of lactase activity per dose unit.

81. A composition of matter according to claim 80 wherein each dosage unit contains 150 to 50,000 units of lactase activity per dose unit.

82. A composition of matter according to any of claims 13 to 22 which is a foodstuff, and said plurality of enzymes is in active form.
83. A composition of matter according to claim 82 wherein the amount or concentration of at least one enzyme in said plurality of enzymes in said foodstuff is greater than the naturally occurring concentration or amount of said enzyme in said foodstuff.

84. A composition according to claim 82 or 83 wherein said foodstuff is a glucose equivalent-containing foodstuff.

85. A composition according to claim 84 wherein said foodstuff is a fructose-containing foodstuff.

86. A composition according to claim 84 wherein said foodstuff is a glucose-containing foodstuff.

87. A composition of matter according to any of claims 82 to 84 wherein said foodstuff is a glucose-containing foodstuff and contains glucose isomerase in an amount of 0.01 to 20,000 units of activity per gram of total glucose in said foodstuff.

88. A composition of matter according to claim 87 wherein said glucose isomerase is present in an amount of 0.05 to 10,000 units of activity per gram of total glucose in said foodstuff.

89. A composition of matter according to claim 88 wherein said glucose isomerase is present in an amount of 0.1 to 1,000 units of activity per gram of total glucose in said foodstuff.

90. A composition of matter according to any of claims 87 to 89 wherein at least one of said enzymes is 5-D-fructose dehydrogenase which is present in an
amount of 10 to 250,000 units of activity per gram of total fructose in the foodstuff.

91. A composition of matter according to claim 90 wherein said 5-D-fructose dehydrogenase is present in an amount of 25 to 150,000 units of activity per gram of total fructose in said foodstuff.

92. A composition of matter according to claim 91 wherein said 5-D-fructose dehydrogenase is present in an amount of 50 to 100,000 units of activity per gram of total fructose in said foodstuff.

93. A composition of matter according to any of claims 82 to 92, wherein said foodstuff is a foodstuff which has been baked.

94. A composition of matter according to any of claims 82 to 92, wherein said foodstuff is a foodstuff which has been cooked.

95. A composition of matter according to any of claims 82 to 92, wherein said foodstuff is a liquid, paste or broth.

96. A composition of matter according to any of claims 82 to 95, wherein said plurality of enzymes is present in microencapsulated form.

97. A composition of matter according to any of claims 82 to 96, wherein at least one of said plurality of enzymes is 5-D-fructose dehydrogenase.

98. A composition of matter according to claim 97, wherein the concentration or amount of said 5-D-fructose dehydrogenase in said foodstuff is greater than the naturally occurring concentration or amount of said 5-D-fructose dehydrogenase in said foodstuff.
99. A composition of matter according to any of claims 82 to 98, wherein at least one of said enzymes of said plurality of enzymes is a glucose isomerase.

100. A composition of matter according to claim 99, wherein the concentration or amount of said glucose isomerase in said foodstuff is greater than the naturally occurring concentration or amount of said glucose isomerase in said foodstuff.

101. A composition of matter according to claim 99 or 100, wherein said glucose isomerase is a xylose isomerase.

102. A composition of matter according to any of claims 82 to 101, wherein said foodstuff further contains at least one third enzyme in active form.

103. A composition of matter according to claim 102, wherein the concentration or amount of said third enzyme in said foodstuff is greater than the naturally occurring concentration or amount of said third enzyme in said foodstuff.

104. A composition of matter according to claim 103, wherein said third enzyme is selected from the group consisting of invertase, maltase, lactase, alpha-amylase, beta-amylase, glucoamylase, pullulanase, isoamylase, amyloglucosidase and cyclomaltodextrin glucantransferase (CGTase) or a mixture thereof.

105. A composition of matter according to claim 104 wherein said third enzyme is invertase.

106. A composition of matter according to claim 104, wherein said third enzyme is maltase.
107. A composition of matter according to claim 104 wherein said third enzyme is lactase.

108. A composition of matter according to claim 104 wherein said third enzyme is alpha-amylase.

109. A composition of matter according to claim 104 wherein said third enzyme is beta-amylase.

110. A composition of matter according to claim 104 wherein said third enzyme is glucoamylase.

111. A composition of matter according to claim 104 wherein said third enzyme is pullulanase.

112. A composition of matter according to claim 104 wherein said third enzyme is isoamylase.

113. A composition of matter according to claim 104 wherein said third enzyme is amyloglucosidase.

114. A composition of matter according to claim 104 wherein said third enzyme is CGTase.

115. A composition of matter according to claim 99 to 101 wherein said composition further comprises at least one of an electrolyte and a metal ion.

116. A composition of matter according to claim 115 wherein said electrolyte is selected from the group consisting of MgSO₄, Na₂CO₃, NaHCO₃, NaOH, Na₂SO₄, MgCO₃, H₂SO₄, Na₂S₂O₃, NaS₂O₅ and mixtures thereof.
117. A composition of matter according to claim 115 or 116 wherein said metal ion is selected from the group consisting of Mn$^{2+}$, Mg$^{2+}$, Ca$^{2+}$, Zn$^{2+}$, Fe$^{2+}$, Co$^{2+}$, Cu$^{2+}$ and mixtures thereof.

118. A composition of matter according to any of claims 103 to 117, wherein said third enzyme is a mixture of at least invertase and maltase.

119. A composition of matter according to any of claims 103 to 117, wherein said third enzyme is a mixture of at least two of the group of invertase, maltase, lactase, amylase, beta-amylase, glucoamylase, pullulanase, isoamylase, amyloglucosidase and CGTase.

120. A composition of matter according to any of claims 103 to 119, wherein said third enzyme is in microencapsulated form.

121. A composition of matter according any of claims 82 to 120, wherein said composition of matter is a foodstuff which is not a dough.

122. A composition of matter according to any of claims 13 to 121, wherein none of the enzymes in said plurality of enzymes is contained in an inorganic-based sol-gel biocompatible matrix.

123. A composition of matter according to claim 122, wherein none of the enzymes in said composition of matter is contained in an inorganic-based sol-gel biocompatible matrix.

124. A composition of matter according to any of claims 13 to 123 wherein said composition is substantially free of substances which are not approved for oral human ingestion.
125. A composition of matter according to any of claims 13 to 124 wherein said composition is substantially free of substances which are not approved for oral non-human mammal ingestion.

126. A composition of matter according to any of claims 13 to 125 wherein said composition of matter is adapted for oral ingestion.

127. A composition of matter according to any of claims 13 to 126, wherein said composition comprises an electron acceptor.

128. A composition of matter according to claim 127 wherein said electron acceptor is selected from the group consisting of Nicotinamide Adenine Dinucleotide $^+$ (NAD$^+$), nicotinamide adenine dinucleotide phosphate$^+$ (NADP$^+$), flavin adenine dinucleotide$^*$ (FAD$^*$), vitamin C, E or A, ferricyanide, ketones, aldehydes, 2,6-di-chloro-phenolindophenol, phenazine methsulfate and mixtures thereof.

129. A composition of matter according to claims 127 or 128 wherein the molar ratio of electron acceptor to total fructose is from 1:1 to 1:1000.

130. A composition of matter according to claim 129 wherein the molar ratio of electron acceptor to total fructose is from 1:2 to 1:200.

131. A composition of matter according to claim 129 wherein the molar ratio of electron acceptor to total fructose is from 1:10 to 1:50.

132. A composition of matter according to claims 127 or 128 wherein the molar ratio of electron acceptor to fructose is from 1:1 to 1:1000.
133. A composition of matter according to claim 132 wherein the molar ratio of electron acceptor to fructose is from 1:2 to 1:200.

134. A composition of matter according to claim 132 wherein the molar ratio of electron acceptor to fructose is from 1:10 to 1:50.

135. A composition of matter according to any of claims 13 to 134 which further comprises one or more enzyme stabilizers.

136. A composition according to claim 135 wherein said enzyme stabilizer stabilizes 5-D-fructose dehydrogenase.

137. A composition according to claim 135 wherein said enzyme stabilizer stabilizes a glucose isomerase.

138. A method of reducing the effect of diabetes on a mammalian subject body, comprising administering to a mammalian subject an efficacious amount of a plurality of enzymes that collectively converts D-glucose to a first form that is at least one of (a) biologically inactive in the subject body, (b) not digestible in the subject digestive tract and (c) not metabolizable in the subject body.

139. A method of reducing the effect of D-glucose on a mammalian subject body, comprising administering to a mammalian subject an efficacious amount of a plurality of enzymes that collectively converts D-glucose to a first form that is at least one of (a) biologically inactive in the subject body, (b) not digestible in the subject digestive tract and (c) not metabolizable in the subject body.

140. A method of reducing the effect of total glucose on a mammalian subject body, comprising administering to a mammalian subject an efficacious amount of a plurality of enzymes that collectively converts D-glucose to a first form that
is at least one of (a) biologically inactive in the subject body, (b) not digestible in
the subject digestive tract and (c) not metabolizable in the subject body.

141. A method according to claim 138 or 140 wherein said plurality of enzymes
collectively converts D-glucose to a second non-glucose form and converts said
second non-glucose form to said first form.

142. A method according to any of claims 138 to 141 wherein said plurality of
enzymes includes a glucose isomerase.

143. A method according to claim 142 wherein said glucose isomerase is a
xylose isomerase.

144. A method according to any of claims 138 to 142 wherein said plurality of
enzymes includes 5-D-fructose dehydrogenase.

145. A method according to any of claims 138 to 144 wherein said mammalian
subject is a human subject.

146. A method according to any of claims 138 to 144 wherein said mammalian
subject is a non-human subject.

147. A method according to any of claims 138 to 146, wherein said administer¬
ing comprises administering a mammalian-ingestible composition of matter
according to any one of claims 13 to 137.

148. A method according to any of claims 138 to 147 wherein said effect of D-
glucose or total glucose is a deleterious effect on the health of said mammal.
149. A method according to any of claims 138 to 147 wherein said effect of D-glucose or total glucose is hyperglycemia.

150. A method according to any of claims 138 to 147 wherein said effect of D-glucose or total glucose is diabetic coma.

151. A method according to any of claims 138 to 147 wherein said effect of D-glucose or total glucose is a complication of diabetes.

152. A method according to claim 151 wherein said complication of diabetes is selected from the group consisting of diabetic angiopathy, macroangiopathy, coronary heart disease, microangiopathy, retinopathy diabetica, maculopathy, nephropathy diabetica, gangrene, Gastroparesis diabeticorum, impaired function of the small intestine, constipation, stool incontinence, diabetic neuropathy, diabetic autonome neuropathy and diabetic foot.

153. A method according to any of claims 138 to 152, wherein said composition of matter is administered prior to eating.

154. A method according to claim 153, wherein said composition of matter is administered immediately prior to eating.

155. A method according to any of claims 138 to 152, wherein said composition of matter is administered concurrently with a meal.

156. A method according to any of claims 138 to 152, wherein said composition of matter is administered after eating.

157. A method according to any of claims 138 to 152, wherein said composition of matter is administered immediately after eating.
158. A method according to any of claims 138 to 157, wherein said second non-glucose form is D-fructose.

159. A method according to any of claims 138 to 158 wherein said first form is 5-keto-D-fructose.

160. A method according to any of claims 138 to 159 wherein said method is part of a program of therapeutic treatment or management of diabetes.

161. A method according to claim 160, wherein said diabetes is diabetes type I.

162. A method according to claim 160, wherein said diabetes is LADA diabetes (latent autoimmune diabetes in adults).

163. A method according to claim 160, wherein said diabetes is diabetes type II.

164. A method according to claim 160, wherein said diabetes is pregnancy-induced diabetes

165. A method according to claim 160, wherein said diabetes is impaired glucose tolerance.

166. A method according to claim 160, wherein said diabetes is a minor disorder of blood sugar metabolism.

167. A method according to any of claims 138 to 166, further comprising administering to said subject at least one third enzyme selected from the group
consisting of invertase, maltase, lactase, alpha-amylase, beta-amylase, glucoamylase, pullulanase, isoamylase, amylglucosidase and cyclomaltodextrin glucantransferase (CGTase) or a mixture thereof.

168. A kit comprising a plurality of enzymes that collectively converts D-glucose into a first form that is at least one of (a) biologically inactive in a chosen mammalian body, (b) not digestible in the digestive tract of said mammalian body and (c) not metabolizable in said mammalian body, and instructions explaining how to use said plurality of enzymes to reduce the effects of glucose in said mammalian body.

169. A kit according to claim 168 wherein said plurality of enzymes collectively converts D-glucose into a second non-glucose form and converts said second non-glucose form to said first form.

170. A kit according to claim 168 or 169 wherein said instructions explain how to use said plurality of enzymes to reduce the effects of total glucose in said mammalian body.

171. A kit according to any of claims 168 to 170 wherein said mammalian body is the human body.

172. A kit according to any of claims 168 to 170 wherein said mammalian body is a non-human body.

173. A kit according to any of claims 168 to 172 wherein said plurality of enzymes is present as a composition of matter according to any of claims 13 to 137.
174. A kit according to any of claims 168 to 173 wherein said plurality of enzymes collectively converts D-glucose into 5-keto-D-fructose.

175. A kit according to any of claims 168 to 174 wherein one of said plurality of enzymes is 5-D-fructose dehydrogenase.

176. A kit according to any of claims 168 to 175 wherein one of said plurality of enzymes is a glucose isomerase.

177. A kit according to claim 176 wherein said glucose isomerase is a xylose isomerase.

178. A kit according to any of claims 168 to 177 wherein said kit further comprises at least one third enzyme.

179. A kit according to claim 178 wherein said third enzyme is selected from the group consisting of invertase, maltase, lactase, alpha-amylase, beta-amylase, glucoamylase, pullulanase, isoamylase, amyloglucosidase and cyclo-maltodextrin glucantransferase (CGTase) or a mixture thereof.

180. A kit according to claim 179 wherein said instructions further explain how to use said third enzyme or mixture thereof in conjunction with said plurality of enzymes.

181. A reduced-total glucose foodstuff.

182. A foodstuff according to claim 181 which is a reduced-fructose foodstuff.

183. A foodstuff according to claim 181 or 182 which is a reduced-glucose foodstuff.
184. A method for preparing a reduced-total glucose foodstuff, comprising contacting a foodstuff or foodstuff precursor with a plurality of enzymes that collectively converts D-glucose into a first form that is at least one of (a) biologically inactive in a chosen mammalian body, (b) not digestible in the digestive tract of said mammalian body and (c) not metabolizable in said mammalian body, and completing any additional steps necessary to prepare the foodstuff.

185. A method according to claim 184 wherein said plurality of enzymes collectively converts D-glucose to a second non-glucose form and converts said non-glucose form to said first form.

186. A method according to claim 185 wherein said second non-glucose form is D-fructose.

187. A method according to claim 185 or 186 wherein said first form is 5-D-ketofructose.

188. A method according to any of claims 184 to 187 wherein said mammalian body is a human body.

189. A method according to any of claims 184 to 187 wherein said mammalian body is a non-human body.

190. A method according to any of claims 184 to 189, wherein one of said plurality of enzymes is a glucose isomerase.

191. A method according to any of claims 184 to 190, wherein one of said plurality of enzymes is 5-D-fructose dehydrogenase.
192. A method according to any of claims 184 to 191, wherein said foodstuff is not a baked foodstuff.

193. A method according to any of claims 184 to 191, wherein said foodstuff is not bread.

194. A method according to any of claims 184 to 191, wherein said foodstuff is not a dough.

195. A method according to any of claims 184 to 194, wherein said plurality of enzymes collectively converts D-glucose to 5-keto-D-fructose.

196. A method according to any of claims 184 to 195, wherein said method also comprises contacting said foodstuff or foodstuff precursor with a third enzyme that cleaves glucose from saccharide chains having at least two saccharide monomers.

197. A method according to claim 196 wherein said third enzyme is invertase, maltase or a mixture thereof.

198. A method according to any of claims 184 to 197, wherein said method also comprises contacting said foodstuff or foodstuff precursor with an additional enzyme that cleaves fructose from saccharide chains having at least two saccharide monomers.

199. A method according to any of claims 184 to 198, wherein said method also comprises contacting said foodstuff or foodstuff precursor with a member of the group consisting of lactase, alpha-amylase, beta-amylase, glucoamylase, pullulanase, isoamylase, amyloglucosidase and cyclomaltodextrin glucantransferase (CGTase) or a mixture thereof.
200. A method for preparing a foodstuff or foodstuff precursor having a reduced effective glucose content, comprising incorporating into said foodstuff or foodstuff precursor a plurality of enzymes that collectively convert D-glucose into a first form that is at least one of (a) biologically inactive in a chosen mammalian body, (b) not digestible in the digestive tract of said mammalian body and (c) not metabolizable in said mammalian body, and completing any additional steps necessary to prepare the foodstuff, wherein said plurality of enzymes is incorporated in such manner that the plurality of enzymes will convert said D-glucose to said first form after ingestion of said foodstuff.

201. A method according to claim 200, wherein at least one of said plurality of enzymes is 5-D-fructose dehydrogenase.

202. A method according to claim 200 or 201, wherein at least one of said plurality of enzymes is a glucose isomerase.

203. A method according to claim 202 wherein said glucose isomerase is a xylose isomerase.

204. A method according to any of claims 200 to 202, wherein said foodstuff is not a baked foodstuff.

205. A method according to any of claims 200 to 202, wherein said foodstuff is not bread.

206. A method according to any of claims 200 to 202, wherein said foodstuff or foodstuff precursor is not a dough.
207. A method according to any of claims 200 to 206, wherein said plurality of enzymes collectively converts D-glucose to 5-keto-D-fructose.

208. A method according to any of claims 200 to 207 wherein said each enzyme in said plurality of enzymes is microencapsulated.

209. A method according to any of claims 200 to 208, further comprising incorporating into said foodstuff or foodstuff precursor at least one third enzyme in a manner that said third enzyme will be active in said mammalian body after ingestion therein.

210. A method according to claim 209 wherein said third enzyme is selected from the group consisting of invertase, maltase, lactase, alpha-amylase, beta-amylase, glucoamylase, pullulanase, isoamylase, amyloglucosidase and cyclo-maltodextrin glucantransferase (CGTase) or a mixture thereof.

211. A method according to claim 210 wherein said third enzyme is at least one of maltase and invertase.

212. A method according to claim 210 wherein said third enzyme is a mixture of maltase and invertase.

213. A method according to any of claims 210 wherein at least one said third enzyme cleaves glucose from saccharide chains having at least two saccharide monomers.

214. A method according to claim 210 or 213 wherein at least one said third enzyme cleaves fructose from saccharide chains having at least two saccharide monomers.
215. A method according to any of claims 209 to 214 wherein each said third enzyme is microencapsulated.

216. A method according to any of claims 184 to 215 wherein one of said plurality of enzymes is 5-D-fructose dehydrogenase which is present in an amount of 10 to 250,000 units of activity per gram of total fructose in the foodstuff or foodstuff precursor.

217. A method according to claim 216 wherein said 5-D-fructose dehydrogenase is present in an amount of 25 and 150,000 units of activity per gram of total fructose in said foodstuff or foodstuff precursor.

218. A method according to claim 217 wherein said 5-D-fructose dehydrogenase is present in an amount of 50 to 100,000 units of activity per gram of total fructose in said foodstuff or foodstuff precursor.

219. A method according to any of claims 184 to 218 wherein said foodstuff or foodstuff precursor is a glucose-containing foodstuff or foodstuff precursor and contains glucose isomerase in an amount of 0.01 to 20,000 units of activity per gram of total glucose in said foodstuff or foodstuff precursor.

220. A method according to claim 219 wherein said glucose isomerase is present in an amount of 0.05 to 10,000 units of activity per gram of total glucose in said foodstuff or foodstuff precursor.

221. A method according to claim 220 wherein said glucose isomerase is present in an amount of 0.1 to 1,000 units of activity per gram of total glucose in said foodstuff or foodstuff precursor.
222. A method according to any of claims 200 to 221, further comprising incorporating into said foodstuff or foodstuff precursor at least one further enzyme or mixture of enzymes in a manner that said further enzyme or mixture of enzymes will be active before ingestion of said foodstuff.

223. A method according to claim 222 wherein said further enzyme is selected from the group consisting of invertase, maltase, lactase, alpha-amylase, beta-amylase, glucoamylase, pullulanase, isoamylase, amyloglucosidase and cyclo-maltodextrin glucantransferase (CGTase) or a mixture thereof.

224. A method according to claim 223 wherein said further enzyme is at least one of invertase and maltase.

225. A method according to claim 224 wherein said further enzyme is a mixture of maltase and invertase.

226. A method according to any of claims 222 to 225, wherein said further enzyme or mixture of enzymes is the same as said third enzyme or mixture of enzymes.

227. A method according to any of claims 222 to 225, wherein said further enzyme or mixture of enzymes is different than said third enzyme or mixture of enzymes.

228. A method according to any of claims 222 to 227, wherein said further enzymes include a further group of enzymes which is the same as said plurality of enzymes.

229. A method according to any of claims 200 to 228 wherein said mammalian body is a human body.
230. A method according to any of claims 200 to 228 wherein said mammalian body is a non-human body.
INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/011232

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/34 A21D2/26 A23L1/00 A23L2/66

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A21D A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document with indication, where appropriate of the relevant passages</th>
<th>Relevant to claim No</th>
</tr>
</thead>
</table>

Further documents are listed in the continuation of Box C

See patent family annex

* Special categories of cited documents

'A' - document defining the general state of the art which is not considered to be of particular relevance
'E' - earlier document but published on or after the international filing date
'L' - document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
'O' - document referring to an oral disclosure, use, exhibition or other means
'P' - document published prior to the international filing date but later than the priority date claimed
'T' - later document published 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
'X' - document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
'Y' - document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
'S' - document member of the same patent family

Date of the actual completion of the international search 14 March 2007

Date of mailing of the international search report 29/03/2007

Name and mailing address of the ISA
European Patent Office, P B 5818 Patentlaan 2 NL - 2280 HV Rijswijk
Tel (+31-70) 340-2040, Tx 31 651 epo til,
Fax (+31-70) 340-3016

Authorized officer
Chavanne, Franz
## DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>wo 00/27215 Al (NOVONORDISK AS [DK]; GUTIERREZ MARTINEZ RAMIRO [ES]; SPENDLER TINA [DK]) 18 May 2000 (2000-05-18) abstract page 1, lines 8-33 page 2, lines 1-38 page 6, lines 1-20 page 10</td>
<td>181, 183</td>
</tr>
<tr>
<td>X</td>
<td>BHOSALE S H ET AL: &quot;Molecular and industrial aspects of glucose isomerase&quot; MEDLINE, 1996, XP002236676 the whole document</td>
<td>181, 183</td>
</tr>
<tr>
<td>X</td>
<td>US 6 663 903 B1 (NILESEN RUBY ILUM [DK]) 16 December 2003 (2003-12-16) abstract column 1, lines 14-16 column 12, lines 47-50</td>
<td>13-35, 41-137</td>
</tr>
<tr>
<td></td>
<td>column 13, lines 2,3</td>
<td></td>
</tr>
</tbody>
</table>
International Search Report

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos. because they relate to subject matter not required to be searched by this Authority, namely
   Although claims 138-167 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. Claims Nos. because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. Claims Nos. because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Observations where unity of invention is lacking

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims, it is covered by claims Nos/

Remark on Protest

- The additional search fees were accompanied by the applicant's protest
- No protest accompanied the payment of additional search fees

Form PGT/ISA/210 (continuation of first sheet (2)) (January 2004)
## INTERNATIONAL SEARCH REPORT

Information on patent family members

<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AU 3988499 A</td>
<td>29-11-1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 2003252858 A1</td>
<td>06-11-2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2331741 A1</td>
<td>18-11-1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 6306445 B1</td>
<td>23-10-2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 03051391 A1</td>
<td>26-06-2003</td>
</tr>
<tr>
<td>US 6663903</td>
<td>16-12-2003</td>
<td>NONE</td>
<td></td>
</tr>
<tr>
<td>US 4396602</td>
<td>NONE</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Form PCT/ISA/510 (patent family annex) (April 2005)