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Fortsættes ...

DESCRIPTION

Description

Field of the invention

[0001] The present invention relates to a solid substrate, bio-conversion method for the production of a valuable solid transformation product of the substrate wherein the bio-conversion is carried out by the use of one or more suitable enzyme preparations by a continuous plug flow process in a vertical, non-agitated tank where the transport is mediated by gravitational force.

Background of the invention

[0002] There is a need for bio-products that primarily can be used as food or feed or as ingredients in food or feed. The basic constituents in such products are proteins, fats, and carbohydrates. Suitable biomasses for such products are oil bearing crops such as oilseeds, cereals, and legumes. Cereals have a protein content up to 15 % e.g. in wheat, and legumes have a protein content of up to 40 % e.g. in soya beans, based on dry matter. Soya beans are primarily an industrial crop, cultivated for oil and protein. It has a relatively low oil content of the seed, but still soya beans are a large single source of edible oil.

[0003] The nutritional quality of protein, measured by its chemical score as essential amino acid composition, and the palatability of protein products are important and essential parameters for nutritional purposes in feed and food products and nutritional supplements. Applications of the protein products in pharma products and cosmetics may sometimes also require high palatability, and/or specific functional properties.

[0004] A general problem especially related to pulses and fruits and seeds from legumes which comprise carbohydrates and proteinaceous matter as sources of bio-product (in particular protein products) are the content of indigestible oligosaccharides, such as stachyose and raffinose, causing flatulence and diarrhoea when fermented in the colon.

[0005] The approximate average chemical composition of soya bean, taken as an example, measured on moisture-free basis, is 40 % protein; 20 % fat, mostly triglycerides and some phospholipids; 35 % carbohydrate in the form of soluble oligosaccharides (sucrose, raffinose, stachyose, verbascose) and insoluble fibre; and 5 % ash comprising the minerals, in particular potassium, calcium and magnesium.

[0006] Proteins from pulses, seeds, cereals, and grasses, including soya bean proteins, such

as trypsin inhibitors, allergens and lectins, are known as anti-nutritional factors. They exert specific physiological effects. Trypsin inhibitors impair protein digestion by inactivating trypsin and are considered harmful for the nutritional value of soya bean and are suggested to be responsible for impaired growth in chickens. β -conglycinin is a soy allergen inducing intestinal inflammation and dysfunction.

[0007] CN 103 875 982 discloses a solid-state anaerobic fermentation of a feed blend comprising soybean meal with protease at a water content of 32 wt%, at 34 °C for 72 hours.

[0008] The object of the present invention is to provide a method for the production of a transformation product of a biomass substrate in a vertical, plug flow bio-conversion process carried out in the presence of one or more suitable enzymes.

[0009] Another object is to provide a method, which can be performed in a large and simple reactor design and thereby at low costs.

[0010] Yet an object is to provide an efficient method for bio-conversion of biomasses, in particular soya bean or rape seed or mixtures thereof, so as to produce bio-products with desirable properties, such as a high protein content, and/or a modified sugar profile, and/or improved nutritional value, and/or reduced anti-nutritional factors, and/or improved palatability, and/or enhanced organoleptic properties, and/or improved functional properties.

[0011] A final object of the invention is to provide an improved method for the production of bio-products comprising a considerable reduced amount of indigestible carbohydrates, and/or anti-nutritional factors.

[0012] These objects are fulfilled with the method of the present invention.

Summary of the invention

[0013] Accordingly, in one aspect of the present invention it relates to a method as defined in claim 1 for producing a solid transformation product of a substrate.

[0014] The present method for treatment of biomass uses gravitational force to transport/move the biomass during bio-conversion. Although the use of gravity for transportation in general is straightforward, it requires careful selection of reaction conditions for the specific purpose, such as in the case of the present plug-flow process.

[0015] Normally, when the water content is increased, an incubation mixture tends to compact, by the reduction of void volume, so that the transportation behaviour is affected negatively. When a certain water content is reached the mixture is compacted to an extent so that the transportation by gravitational force is stopped. The material will stick to the walls of the reactor, and the uniform plug-flow is disrupted resulting in uneven retention time of the biomass.

[0016] The solution according to the present invention to the problem connected with transportation by gravitational force of the incubation mixture is to make use of a tank as defined in the claims for incubation wherein the flow of material can be kept so high and uniform that plug-flow conditions are achieved and maintained. The flow rate is regulated by the inlet and outlet means and by the dimensions (width to height ratio) of the tank.

[0017] Furthermore, the solution according to the invention must secure balancing of the water content in the incubation mixture so that the water activity on the particle surface is sufficient for the reaction process. This is achieved by keeping the ratio wet bulk density to dry bulk density of the substrate low and within certain limits as defined in claim 1.

[0018] More specifically, the present inventors have found that the necessary uniform process can be achieved by using an initial incubation mixture having a water content from 30 % to 70 % by weight, and a ratio of wet bulk density to dry bulk density from 0.60 to 1.45. In combination with the present, vertical design for the plug-flow process it is possible to secure a uniform plug-flow and ensure the same processing time for the incubation mixture. Furthermore, the method of the present invention is conducted without agitation. If the water content exceeds approximately 70 % by weight, the biomass cannot hold the water, and the incubation mixture becomes a slurry having a water phase and a solid phase. These two phases will not flow with the same flow rates, uniform plug flow will not be obtained, and the incubation mixture may stick to the incubator walls. A water content of more than approximately 70 % will result in a ratio of wet bulk density to dry bulk density, exceeding 1.45 that is the upper limit according to the invention.

[0019] The vertical design is less expensive in investment than a horizontal design due to its larger capacity in a single production line. It is also less expensive to maintain due to less mechanical movements. The use of a non-agitated tank further contributes to reduced operational costs.

[0020] The present method is, in particular, efficient if the substrate of biomass has been pretreated before it is mixed with the enzyme preparation or a combination of enzyme preparations, because the pre-treatment improves the access of the enzymes to the components in the biomass which are to be transformed. The pre-treatment is typically carried out by chemical or physical pre-treatment, e.g. by means of disintegration, milling, flaking, heat treatment, pressure treatment, ultrasonic treatment, hydrothermal treatment, or acid or alkaline treatment.

[0021] The method of the invention provides a solid transformation product of the substrate which is a product of the transformation of proteinaceous matter, and/or carbohydrates originating from said biomass.

[0022] Such solid transformation products can be used e.g. in a processed food product or as an ingredient in a food or feed product or as an ingredient of a cosmetic or a pharmaceutical product or a nutritional supplement. The solid transformation of the substrate may e.g. be included in a food, feed, cosmetic or pharmaceutical product or a nutritional supplement

containing from 1 % to 99% by weight of a solid transformation product.

Definitions

[0023] In the context of the current invention, the following terms are meant to comprise the following, unless defined elsewhere in the description.

[0024] The terms "about", "around", "approximately", or "~" are meant to indicate e.g. the measuring uncertainty commonly experienced in the art, which can be in the order of magnitude of e.g. +/- 1, 2, 5, 10, 20, or even 50 %.

[0025] The term "comprising" is to be interpreted as specifying the presence of the stated part(s), step(s), feature(s), composition(s), chemical(s), or component(s), but does not exclude the presence of one or more additional parts, steps, features, compositions, chemicals or components. E.g., a composition comprising a chemical compound may thus comprise additional chemical compounds, etc.

Plug-flow process:

[0026] In this type of continuous process, the reaction mixture flows through e.g. a tubular or polyhedral reactor with limited back mixing. The flow is a laminar flow where the composition of the reaction mixture changes along the axial direction of the reactor, or a uniform mass flow.

Biomass:

[0027] Comprises biological material, as produced by the photosynthesis and that can be used as raw material in industrial production. In this context, biomass refers to plant matter in the form of seeds, cereals, pulses, e.g. beans and peas, etc., and mixtures thereof, and in particular fruits and seeds of legumes. Furthermore, a biomass comprising pulses is specifically applicable due to the protein content and composition.

[0028] The substrate of biomass may be disintegrated by pre-treatment, such as chemical or physical pre-treatment, e.g. by means of disintegration, milling, flaking, heat treatment, pressure treatment, ultrasonic treatment, hydrothermal treatment, or acid or alkaline treatment.

Bio-conversion

[0029] Is the process to incubate enzymes on a substrate for a specific purpose, e.g. incubating a protease on a protein to produce peptides or single amino acids.

Solid transformation product of the substrate:

[0030] In the present context, solid transformation product of the substrate refers to a product resulting from incubation of the selected biomass with an enzyme preparation, or combination of enzyme preparations, which can convert matter of the substrate to a desirable product, and optionally processing aids.

Bulk density:

[0031] Bulk density is a parameter important for the physical behaviour of a biomass which has the form of powder, granules, and the like. The parameter is defined as weight per volume, and may be measured in, e.g., g/ml. It is not an intrinsic property, but can change depending on handling, and can be used as an index of structural changes. The density of a material is determined by placing a fixed volume of the material in a measuring cup and determining the weight or by determining the weight of a measured volume of a material. By this test the following features can be determined:

Bulk density (also known as pour density) = mass/untapped dry volume in g/mL or kg/m³;

Wet bulk density (also known as total density) = the ratio of the total mass ($M_s + M_l$) to its total volume;

M_s = mass of solids and M_l = mass of liquids.

[0032] Thus, in the context of the present invention, "dry bulk density" is the measured bulk density of the biomass without addition of water, viz. the bulk density/pour density. "Wet bulk density" is the bulk density measured after addition of a certain amount of water. Normally, the bulk density is determined in accordance with International Standards ISO 697 and ISO 60, but due to the nature of the substances this was not applicable in the present context. The individual method used is described in the examples.

Oligosaccharides and polysaccharides:

[0033] An oligosaccharide is a saccharide polymer containing at least two component monomer sugars. Polysaccharides are saccharide polymers containing many component monomer sugars, also known as complex carbohydrates. Examples include storage polysaccharides such as starch and structural polysaccharides such as cellulose.

Carbohydrates:

[0034] Comprise mono-, di-, oligo- and polysaccharides.

Proteinaceous materials:

[0035] Comprise organic compounds with a substantial content of proteins made of amino acids arranged in one or more chains. At a chain length of up to approximately 50 amino acids the compound is called a peptide; at higher molecular weight the organic compound is called a polypeptide or a protein.

Fats:

[0036] Comprise esters between fatty acids and glycerol. One molecule of glycerol can be esterified to one, two and three fatty acid molecules resulting in a monoglyceride, a diglyceride or a triglyceride respectively. Usually fats consist of mainly triglycerides and minor amounts of lecithins, sterols, etc. If the fat is liquid at room temperature it is normally called oil. With respect to oils, fats, and related products in this context, reference is made to "Physical and Chemical Characteristics of Oils, Fats and Waxes", AOCS, 1996, as well as "Lipid Glossary 2", F.D. Gunstone, The Oily Press, 2004.

Glycerides:

[0037] Comprise mono-, di-, and triglycerides.

Enzymes:

[0038] Enzyme(s) is a very large class of protein substances with the ability to act as catalysts. Commonly, and according to the Enzyme Nomenclature Committee Recommendations, they are divided in six classes.

[0039] Typical examples in the context of the invention can comprise, but are not limited to, protease(s), peptidase(s), phytase(s), carbohydrase(s), lipase(s), amylase(s), glucosidase(s), amyloglucosidase(s), galactosidase(s), decarboxydase(s), glucanase(s), pectinase(s), cellulase(s), hemicellulase(s), phospholipase(s), transferase(s), and oxidoreductase(s).

Processing aids:**1. Plant components and organic processing agents**

[0040] Some of the functional properties that are important in this context are: Antioxidant,

antibacterial action, wetting properties and stimulation of enzyme activity.

[0041] The list of plant-based components is huge, but the most important are the following: Rosemary, thyme, oregano, flavonoids, phenolic acids, saponins, and α - and β - acids from hops e.g. α -lupulic acid for the modulation of soluble carbohydrates.

[0042] Furthermore, organic acids e.g. sorbic-, propionic-, lactic-, citric-, and ascorbic acid, and their salts for the adjustment of the pH-value, preservation and chelating properties is part of this group of processing aids.

2. Inorganic processing agents

[0043] Comprise inorganic compositions that can preserve against bacterial attack during processing, e.g. sodium bisulphite, etc.; anticaking agents, and flow improving agents in the final product, e.g. potassium aluminium silicate, etc.

[0044] Comprise inorganic acids e.g. hydrochloric acid or sulphuric acid.

Processed food products:

[0045] Comprise dairy products, processed meat products, sweets, desserts, ice cream desserts, canned products, freeze dried meals, dressings, soups, convenience food, bread, cakes, etc.

Processed feed products:

[0046] Comprise ready-to-use feed for animals such as piglets, calves, poultry, furred animals, sheep, cats, dogs, fish, and crustaceans, etc.

Pharmaceutical products:

[0047] Comprise products, typically in the form of a tablet or in granulated form, containing one or more biologically active ingredients intended for curing and/or alleviating the symptoms of a disease or a condition. Pharmaceutical products furthermore comprise pharmaceutically acceptable excipients and/or carriers. The solid bio products herein disclosed are very well suited for use as a pharmaceutically acceptable ingredient in a tablet or granulate.

Cosmetic products:

[0048] Comprise products intended for personal hygiene as well as improved appearance such as conditioners and bath preparations.

Brief description of drawings

[0049]

Figure 1 shows: in lane 1: Marker; Lane 2: Protease treated sample from example 2; Lane 3: Reference sample for both examples 2 and 3; Lane 4: Protease treated sample from example 3.

Figure 2 shows composition of soluble sugars and oligosaccharides. Lane 1: Carbohydrase-treated sample; Lane 2: Reference (soya bean meal).

Figure 3 shows thin layer chromatography showing soluble sugars and oligosaccharides. Lane 1: carbohydrase-treated product; lane 2: reference.

Detailed description of the invention

[0050] At least 20 % by weight of the biomass comprises carbohydrates and proteinaceous matter originating from soya bean seeds, seed of rape seed, or mixtures thereof.

[0051] In a first embodiment of the method of the invention at least 30 % by weight of the biomass, such as at least 40 %, at least 50 %, at least 60 %, at least 70 %, at least 80 %, or at least 90 % by weight, comprises proteinaceous matter originating from optionally defatted soya. The soya may also be dehulled.

[0052] In a second embodiment of the method of the invention at least 30 % by weight of the biomass, such as at least 40 %, at least 50 %, at least 60 %, at least 70 %, at least 80 %, or at least 90 % by weight, comprises proteinaceous matter originating from optionally defatted rape seeds.

[0053] In a third embodiment of the method of the invention the biomass comprises proteinaceous matter originating from defatted soya flakes in an amount of from 5 % to 95 % by weight in mixture with proteinaceous matter originating from optionally defatted rape seed in an amount of from 95 % to 5 % by weight optionally in further mixture with proteinaceous matter originating from fava beans, peas, sunflower seeds and/or cereals in amounts to make up a total amount of the proteinaceous matter of 100 % by weight.

[0054] In any of the embodiments of the invention the biomass comprising proteinaceous matter may further comprise oligosaccharides, and/or polysaccharides, and/or further comprises oils and fats, e.g. from seeds of oil bearing plants.

[0055] In any of the embodiments of the invention the solid transformation product of the substrate may be a product of the transformation of proteinaceous matter and/or carbohydrates originating from said biomass, such as a transformation product of pulses, such as soya, pea, lupine, sunflower, and/or cereals, such as wheat, or maize, or from seeds of oil bearing plants, e.g. rape seed.

[0056] In any of the embodiments of the invention the substrate, or the substrate after mixing with an enzyme preparation or a combination of enzyme preparations and water, may not comprise any live baker's yeast, and/or it may not comprise any live yeast selected among *Saccharomyces cerevisiae* strains, including spent brewer's yeast and spent distiller's yeast and baker's yeast and spent yeast from wine production, or does not comprise any live yeast; and/or in particular it may not comprise baker's yeast when the enzyme is α -galactosidase.

[0057] In any of the embodiments of the invention the solid transformation product of the substrate may be a product of the transformation of proteinaceous matter and/or carbohydrates originating from said biomass.

[0058] In any of the embodiments of the invention the enzyme preparation or mixture of enzyme preparations comprises one or more enzymes selected from proteases, peptidases, phytases, carbohydrases, such as α -galactosidase, amylase, amyloglucosidase, cellulase, pectinase, and hemi-cellulases, e.g. xylanase, mannanase, or glucanase; and lipase, and oxidoreductase.

[0059] In any of the embodiments of the invention the dry matter ratio of said substrate of biomass to said enzyme preparation, or said combination of enzyme preparations, may be from 2:1 to 100,000,000:1, such as 1,000:1, 10,000:1, 50,000:1, 100,000:1, 500,000:1, 1,000,000:1, 5,000,000:1, 10,000,000:1, 50,000,000:1, or 100,000,000:1. The skilled person will appreciate that the ratio can be selected in dependence of parameters, such as process conditions, the activity of the enzyme, and the desired product, and the skilled person will be able to optimise the ratio in accordance with these parameters.

[0060] In any of the embodiments of the invention water may be added to the substrate in an amount to provide a ratio of wet bulk density to dry bulk density from about 0.60 to 1.45 in the substrate, such as from about 0.65 to about 1.40, e.g. 0.70, 0.75, 0.80, 0.85, 0.90, 0.95, 1.00, 1.10, 1.15, 1.20, 1.25, 1.30, or 1.35.

[0061] In any of the embodiments of the invention at least 40 % by weight of the biomass, such as at least 50 %, at least 60 %, at least 70 %, at least 80 % or at least 90 % by weight, may comprise proteinaceous matter originating from optionally defatted rape seeds, whereas water may be added to the substrate in an amount to provide a ratio of wet bulk density to dry bulk density from about 0.65 to about 1.10, such as 0.75, 0.80, 0.85, 0.90, 0.95, 1.00, or 1.05.

[0062] In any of the embodiments of the invention one or more processing aids selected from enzymes, plant components, and organic and inorganic processing agents may be added to the substrate before or during incubation.

[0063] In any of the embodiments of the invention an α -galactosidase preparation may be added to the substrate of biomass and/or to the initial incubation mixture in an amount of from 0.05 to 50 α -galactosidase units pr. g. dry matter of substrate of biomass, such as from 0.5 to 25 α -galactosidase units pr. g. dry matter of substrate of biomass, e.g. from 1 to 10, from 2 to 8, from 3 to 6, or from 4 to 5 α -galactosidase units pr. g. dry matter of substrate of biomass.

[0064] In any of the embodiments of the invention the filling degree of said incubation tank may be kept constant. This will result in a uniform flow.

[0065] In any of the embodiments of the invention the incubation can be carried out under anaerobic conditions.

[0066] In any of the embodiments of the invention the water content in the incubation mixture may be from 35 % to 70 % by weight, such as 40 %, 45 %, 50 %, 55 %, 60 %, or 65 % by weight.

[0067] Thus, the water content in the initial mixture does not exceed 70 % by weight and it may vary from e.g. from 40 % to 65 %, from 45 % to 60 %, from 48 % to 52 %, or 50 % to 55 %, such as 49, 50, 51, 52, 53, or 54 %.

[0068] In any of the embodiments of the invention the mixture may be incubated for 1-240 hours at 20-70 °C. The skilled person will know how to optimise the reaction time and the reaction temperature in view of the other reaction conditions, such as the selection of enzyme(s). Thus, the temperature may vary as e.g. 20-65 °C, 25-60 °C, 30-55 °C, 35-50 °C, or 40-45 °C; and the reaction time may be selected as e.g. 1 to 180 hours, such as 2 to 150 hours, 3 to 120 hours, 5 to 90 hours, 8 to 72 hours, or 12 to 48 hours, at each and every one of the here mentioned temperature intervals.

[0069] In any of the embodiments of the invention the solid transformation product of the substrate may be dried, optionally followed by milling.

[0070] In any of the embodiments of the invention the substrate mixture may be incubated at a time and a temperature sufficient to inactivate the enzyme(s), any anti-nutritional factors, and any processing aids, and if used partly or totally, and if desired.

[0071] In any of the embodiments of the invention the vertical, non-agitated incubation tank may be closed.

[0072] In any of the embodiments of the invention the non-agitated incubation tank can be of a vertical, oblong cylindrical or polyhedral type. The advantage of using this type is that it is space-saving and as it is non-agitated the operating costs and maintenance costs for mixing equipment are avoided.

[0073] In any of the embodiments of the invention the area in the upper part of said non-agitated incubation tank may be less than the area in the lower part i.e. the tank is of conical

shape. The advantage of this is that the slip effect is increased so that biomasses with a reduced flowability can be used.

[0074] In any of the embodiments of the invention the non-agitated incubation tank may have insulating matting or a thermal dimple jacket and means to control the temperature inside the incubation tank.

[0075] The solid transformation product of the substrate of the invention may be dried to a water content of not more than 15 %, 13 %, 10 %, 6 %, 4 %, or 2 % by weight and optionally be in milled form.

[0076] The solid product of the invention can be a product of the transformation of proteinaceous matter, oligosaccharides, and/or polysaccharides originating from said biomass. The solid transformation product may have reduced content of anti-nutritional factors, such as trypsin inhibitors, antigens, flatulence-producing oligosaccharides, e.g. stachyose and raffinose; phytic acid, and lectin.

[0077] The solid product of the invention may comprise at least 40 % proteinaceous matter by weight of dry matter originating from soya.

[0078] The solid product of the invention may comprise at least 40 % proteinaceous matter by weight of dry matter originating from rape seed.

[0079] The solid product of the invention may comprise proteins in an amount of 30-65 % by weight on dry matter basis originating from plant parts of soya, rape seed, or sun flower, or mixtures thereof.

[0080] The solid product of the invention may comprise a total amount of raffinose, stachyose, and verbascose of 3 % by weight or less, such as 2 % or less, 1 % or less, 0.5 % or less, or 0.4 % or less.

[0081] Finally, the invention provides a food, feed, cosmetic or pharmaceutical product or a nutritional supplement containing from 1 % to 99 % by weight of a solid transformation product provided according to the invention.

EXAMPLES

DENSITY RATIO

Example 1:

Ratio of wet bulk density / dry bulk density for preferred substrates based on various

biomasses

1.1 Biomasses used in the procedure:

Soya

[0082] The soya used was defatted Soya Bean Meal (SBM).

Maize

[0083] The maize used was whole maize, ground on a hammer mill through a 3.5 mm sieve.

Wheat

[0084] The wheat used was whole wheat, ground on a hammer mill through a 3.5 mm sieve.

Sunflower

[0085] The sunflower used was defatted Sunflower Seed Meal (SSM).

Rapeseed

[0086] The rapeseed used was defatted Rape Seed Meal (RSM).

Fava Beans

[0087] The beans used were whole fava beans.

Pea protein

[0088] The pea protein used was a pea protein concentrate.

1.2 Description of the procedure:

[0089] The amount(s) of biomass and water tabulated in the following was mixed for ten minutes followed by fifty minutes of equilibration in a closed container.

[0090] After this the material was poured into a measuring cup of 500 mL and its mass determined by weighing the cup and subtracting the tare of the cup.

[0091] The bulk density was calculated as mass/untapped volume in kg/m^3 .

[0092] The dry bulk density used was the measured bulk density of the biomass without addition of water.

[0093] The wet bulk density was the bulk density of the biomass with added water.

[0094] The ratio was calculated as wet bulk density divided by the dry bulk density.

[0095] The moisture content of the biomasses was determined by drying to constant weight.

[0096] After addition of water the moisture in the mixture was determined by calculation.

1.3 Results:

[0097] The results for 100 % soya and 80 % mixtures with soya are tabulated in the following:

Soya	Maize	Wheat	Sunflower	Rapeseed	Fava bean	Pea	Water In g	Moisture In %	Bulk Density kg/m^3	Ratio
1000 g							0	10.9	665	-
1000 g							100	19.0	638	0.96
1000 g							250	28.7	500	0.75
1000 g							450	38.6	476	0.72
1000 g							750	49.1	470	0.71
1000 g							900	53.1	572	0.86
1000 g							1100	57.6	655	0.98
1000 g							1400	62.9	715	1.07

Soya	Maize	Wheat	Sunflower	Rapeseed	Fava bean	Pea	Water In g	Moisture In %	Bulk Density kg/m ³	Ratio
1000 g							1900	69.3	889	1.34
800 g	200 g						0	11.4	703	-
800 g	200 g						450	38.9	617	0.88
800 g	200 g						900	53.4	634	0.90
800 g	200 g						1900	69.4	1008	1.43
800 g		200 g					0	11.7	694	
800 g		200 g					450	39.1	580	0.84
800 g		200 g					900	53.5	623	0.90
800 g		200 g					1900	69.5	960	1.38
800 g			200 g				0	10.4	683	-
800 g			200 g				450	38.2	554	0.81
800 g			200 g				900	52.9	598	0.88
800 g			200 g				1900	69.1	926	1.36
800 g				200 g			0	11.3	711	
800 g				200 g			100	19.4	576	0.81
800 g				200 g			250	29.0	514	0.72
800 g				200 g			450	38.8	483	0.68
800 g				200 g			750	49.3	490	0.69

Soya	Maize	Wheat	Sunflower	Rapeseed	Fava bean	Pea	Water In g	Moisture In %	Bulk Density kg/m ³	Ratio
800 g				200 g			900	53.3	597	0.84
800 g				200 g			1100	57.8	528	0.74
800 g				200 g			1900	69.4	908	1.28
800 g					200 g		0	11.1	691	-
800 g					200 g		450	38.7	569	0.82
800 g					200 g		900	53.2	605	0.88
800 g					200 g		1900	69.3	941	1.36
800 g						200 g	0	11.2	703	
800 g						200 g	450	38.7	488	0.69
800 g						200 g	900	53.2	728	1.04
800 g						200 g	1900	69.4	964	1.37

[0098] The results for 60 % and 40 % of soya mixtures with maize, sunflower and rapeseed as well as 100 % rapeseed are tabulated in the following:

Soya	Maize	Sunflower	Rapeseed	Water	Moisture In %	Bulk Density kg/m ³	Ratio
600 g	400 g			0 g	11.8	703	-
600 g	400 g			250 g	29.5	651	0.93
600 g	400 g			450 g	39.2	626	0.89
600 g	400 g			750 g	49.6	631	0.90
600 g	400 g			900 g	53.6	666	0.95
600 g	400 g			1100 g	58.0	723	1.03
600 g	400 g			1400 g	63.3	796	1.13

Soya	Maize	Sunflower	Rapeseed	Water	Moisture	Bulk Density	Ratio
					In %	kg/m ³	
600 g		400 g		0 g	10.0	644	-
600 g		400 g		100 g	18.2	530	0.82
600 g		400 g		250 g	28.0	435	0.68
600 g		400 g		450 g	37.9	433	0.67
600 g		400 g		750 g	48.6	436	0.68
600 g		400 g		900 g	52.6	480	0.75
600 g		400 g		1100 g	57.1	449	0.70
600 g		400 g		1400 g	62.5	616	0.96
600 g			400 g	0 g	11.7	643	-
600 g			400 g	100 g	19.7	560	0.82
600 g			400 g	250 g	29.4	502	0.78
600 g			400 g	450 g	39.1	503	0.78
600 g			400 g	750 g	49.5	492	0.77
600 g			400 g	900 g	53.5	516	0.80
600 g			400 g	1100 g	57.9	545	0.85
600 g			400 g	1400 g	63.2	655	1.02
400 g	600 g			0 g	12.3	718	-
400 g	600 g			250 g	29.9	636	0.89
400 g	600 g			450 g	39.5	638	0.89
400 g	600 g			750 g	49.9	666	0.93
400 g	600 g			900 g	53.8	721	1.00
400 g	600 g			1100 g	58.2	802	1.12
400 g	600 g			1400 g	63.5	988	1.38
400 g		600 g		0 g	9.5	654	-
400 g		600 g		100 g	17.7	535	0.82
400 g		600 g		250 g	27.6	422	0.65
400 g		600 g		450 g	37.6	487	0.74
400 g		600 g		750 g	48.3	491	0.75
400 g		600 g		900 g	52.4	512	0.78
400 g		600 g		1100 g	56.9	585	0.89
400 g		600 g		1400 g	62.3	612	0.94

Soya	Maize	Sunflower	Rapeseed	Water	Moisture	Bulk Density	Ratio
					In %	kg/m ³	
400 g			600 g	0 g	12.1	658	-
400 g			600 g	100 g	20.1	556	0.84
400 g			600 g	250 g	29.7	471	0.72
400 g			600 g	450 g	39.4	458	0.70
400 g			600 g	750 g	49.8	486	0.74
400 g			600 g	900 g	53.7	486	0.74
400 g			600 g	1100 g	58.1	531	0.81
400 g			600 g	1400 g	63.4	605	0.92
0 g			1000 g	0 g	12.9	616	-
0 g			1000 g	100 g	20.8	484	0.79
0 g			1000 g	250 g	30.3	438	0.71
0 g			1000 g	450 g	39.9	457	0.74
0 g			1000 g	750 g	50.2	507	0.82
0 g			1000 g	900 g	54.1	535	0.87
0 g			1000 g	1100 g	58.5	585	0.95
0 g			1000 g	1400 g	63.7	688	1.12

PILOT-SCALE BIO-CONVERSION

Materials and methods:

Materials

[0099] Biomasses: Soya Bean Meal (SBM), Soya Flakes, Rape Seed Meal (RSM) and Sunflower Seed Meal (SSM).

Water: Normal tap water

Enzymes:

[0100]

Protease: papain from Enzybel; Ronozyme Pro Act from DSM; Acid protease from Suntaq International Ltd;

α -galactosidase: from Bio-Cat (12,500 U/g);

Phytase: Natuphos from BASF

Other carbohydrases: Viscozyme L from Novozymes, Ronozyme VP from Novozymes

Example 2:**[0101] Bio-conversion with protease on SBM and RSM (50:50 ratio)****2.1 Incubator:**

[0102] The pilot incubator used was an insulated, cylindrical oblong stainless-steel tube with an effective operating volume of 2 m³ and in- and outlets. Furthermore, the incubator was equipped with a temperature probe at the inlet as well as at the outlet.

2.2 Method:

[0103] In a pilot scale vertical reactor with a total volume of 2.0 m², a continuous inlet amount of 250 kg/h soya bean meal (SBM), 250 kg/h rapeseed meal (RSM), 967 kg/h water at 25°C, 0.5 kg/h protease (papain from Enzybel), and 0.5 kg/h protease (Ronozyme ProAct from DSM) was applied. The ratio wet bulk density/dry bulk density of the incubation mixture was 1.10.

[0104] The outlet amount was adjusted to keep a constant level of filling inside the reactor, and the level of filling was set to yield a total processing time of 1.0 hour. Immediately after leaving the vertical reactor, the product was heat treated at 99°C for 15 min followed by air drying.

2.3 Test procedure for product:

[0105] The enzyme-treated product and its untreated reference, a 50:50 mixture of SBM and RSM, were analysed for soluble peptides by SDS-PAGE using the following method:

5.0 g of product was suspended in water, pH adjusted to 8.5, and adjusted with water to a total weight of 50.0 g. The suspension was heated to 90°C for 15 min followed by centrifugation at 3000 RCF for 15 min. The supernatant was mixed 1+5 with Laemmli sample buffer + 50 mM DTT (Laemmli, 1970) and heated to 90°C for 15 min. 15 μ L of each sample was loaded onto a

TGX Any kD gel and run following the manufacturer's instructions. The gel was stained with colloid coomassie (Kang et al., 2002).

References:

[0106]

Kang D, Gho YS, Suh M, and Kang C, Bull. Korean Chem. Soc., 2002, Vol. 23, No. 11, pp. 1511-1512.

Laemmli UK, Nature, 1970, Vol. 227, pp. 680-685.

2.4 Results:

[0107] Results of the SDS-PAGE are shown in figure 1, lanes 2 and 3.

[0108] It is clear from the SDS-PAGE that most of the distinct protein bands in the untreated SBM/ RSM mixture (lane 3) have been hydrolysed to peptides by the protease treatment in the vertical reactor used according to the invention (lane 2).

Example 3:

Bio-conversion with protease on SBM and RSM (50:50 ratio)

3.1 Incubator:

[0109] The pilot incubator used was an insulated, cylindrical oblong stainless-steel tube with an effective operating volume of 2 m³ and in- and outlets. Furthermore, the incubator was equipped with a temperature probe at the inlet as well as at the outlet.

3.2 Method:

[0110] In a pilot scale vertical reactor with a total volume of 2.0 m², a continuous inlet amount of 15.6 kg/h soya bean meal (SBM), 15.6 kg/h rapeseed meal (RSM), 24 kg/h water at 40°C, 0.03 kg/h protease (Acid protease from Suntaq International Ltd.), and 0.88 kg/h H₂SO₄ was applied. The ratio wet bulk density/dry bulk density of the incubation mixture was 0.75.

[0111] The outlet amount was adjusted to keep a constant level of filling inside the reactor, and the level of filling was set to yield a total processing time of 16 hour. Immediately after leaving the vertical reactor, the product was heat treated at 99°C for 15 min followed by air drying.

3.3 Test procedure for product:

[0112] The enzyme-treated product and its untreated reference, a 50:50 mixture of SBM and RSM, were analysed for soluble peptides by SDS-PAGE using the following method:

5.0 g of product was suspended in water, pH adjusted to 8.5, and adjusted with water to a total weight of 50.0 g. The suspension was heated to 90°C for 15 min followed by centrifugation at 3000 RCF for 15 min. The supernatant was mixed 1+5 with Laemmli sample buffer + 50 mM DTT (Laemmli, 1970) and heated to 90°C for 15 min. 15 µL of each sample was loaded onto a TGX Any kD gel and run following the manufacturer's instructions. The gel was stained with colloid coomassie (Kang et al., 2002).

References:

[0113] Kang D, Gho YS, Suh M, and Kang C, Bull. Korean Chem. Soc., 2002, Vol. 23, No. 11, pp. 1511-1512.

[0114] Laemmli UK, Nature, 1970, Vol. 227, pp. 680-685.

3.4 Results:

[0115] Results of the SDS-PAGE are shown in figure 1, lanes 3 and 4.

[0116] It is clear from the SDS-PAGE that almost all of the distinct protein bands in the untreated SBM / RSM mixture (lane 3) have been hydrolysed to peptides by the 16 hour protease treatment in the vertical reactor (lane 4).

Example 4:

Bio-conversion with α -galactosidase on soya flakes

[0117] This biomass comprises polysaccharides and proteins from pulses

4.1 Incubator

[0118] The incubator used was an insulated, cylindrical oblong stainless-steel tube with an internal diameter of 1.55 m and a total height of 4.75 m. In the upper part, there was an array of three rotating paddle type level monitors to regulate the inlet and distribution system to a level at 4.25 m. This gives the incubator an effective operating volume of 8 m³. Furthermore, the incubator was equipped with a temperature probe at the inlet as well as at the outlet.

4.2 Method

[0119] A mixture of dehulled, defatted and desolventised soya flakes, sulfuric acid, α - galactosidase, and water at 60°C was prepared continuously in amounts to reach a dry matter content of 50 % by weight in the mixture, a pH of 4.7, and an alpha-galactosidase concentration of 1.1 kg/ton soya. The ratio wet bulk density / dry bulk density of the incubation mixture was 0.73.

[0120] The incubator was filled with incubation mixture at a suitable rate per hour. After 16 hours the incubator was filled to operating level and the outlet means were set at a rate to keep the level of filling constant.

[0121] An aliquot volume of approx. 30 liters was taken after 18 hours of the test run and incubated at 100 °C with live steam for 25 min.

[0122] Subsequently, the wet solid transformation product of the biomass was flash dried and milled.

[0123] The overall incubation parameters were the following:

Incubation time - 16 hours

Temperature inlet - 45 °C

Temperature outlet - 45 °C

4.3 Results:

[0124] The solid transformation product of the biomass had a total crude protein (N x 6.25) content of 52.6 % and a water content of 5.6 % by weight, which corresponds to a protein of dry matter of 55.5 %. Furthermore, stachyose and raffinose in the dried, solid transformation product were significantly reduced as shown in table 1:

Table 1

	Soya flakes	Product
Protein of dry matter	56 % +/-1%	55.5 %

	Soya flakes	Product
Stachyose + raffinose	5-6 %	Absent

[0125] The solid transformation product is highly nutritious and palatable and thus suitable as an ingredient in a number of food and feed products or nutritional supplements. Furthermore, it can be used as an excipient in pharma products and in cosmetics e.g. bath formulations.

Example 5:

Bio-conversion with phytase on soya flakes

[0126] This biomass comprises polysaccharides and proteins from pulses

5.1 Incubator

[0127] The incubator used was an insulated, cylindrical oblong stainless-steel tube with an internal diameter of 1.55 m and a total height of 4.75 m. In the upper part, there was an array of three rotating paddle type level monitors to regulate the inlet and distribution system to a level at 4.25 m. This gives the incubator an effective operating volume of 8 m³. Furthermore, the incubator was equipped with a temperature probe at the inlet as well as at the outlet.

5.2 Method

[0128] A mixture of dehulled, defatted and desolventised soya flakes, thermostable phytase, and water at 95 °C was prepared continuously in amounts to reach a dry matter content of 46 % by weight in the mixture, and a phytase concentration of 250 g/ton soya.

[0129] The ratio wet bulk density/dry bulk density of the incubation mixture was 0.87.

[0130] The incubator was filled with incubation mixture at the rate of 750 liters per hour. After 12 hours the incubator was filled to operating level and the outlet means were set at a rate to keep the level of filling constant.

[0131] An aliquot volume of approx. 30 liters was taken after 14 hours of the test run and incubated at 100 °C with live steam for 25 min.

[0132] Subsequently, the wet solid transformation product of the biomass was flash dried and milled.

[0133] The overall incubation parameters were the following:

Incubation time - 12 hours

Temperature inlet - 67 °C

Temperature outlet - 66 °C

5.3: Results

[0134] The solid transformation product of the biomass had a total crude protein (N x 6.25) content of 51.0 % and a water content of 8.0 % by weight, which corresponds to a protein of dry matter of 55.4 %. Furthermore, phytic acid bound phosphor (an anti-nutrient) in the dried, solid transformation product were significantly reduced as shown in table 2:

Table 2

	Soya flakes	Product
Protein of dry matter	55 % +/- 1 %	55.4 %
Phytic acid bound phosphor	0.42 %	0.08 %

[0135] The solid transformation product is highly nutritious and palatable and thus suitable as an ingredient in a number of food and feed products or nutritional supplements. Furthermore, it can be used as an excipient in pharma products and in cosmetics e.g. bath formulations.

Example 6:

Bio-conversion with carbohydrase on soya bean meal (SBM)

[0136] This biomass comprises polysaccharides and proteins from pulses

6.1 Incubator

[0137] The incubator was a production scale vertical reactor with a total volume of 96 m².

6.2 Method

[0138] A continuous inlet amount of 1800 kg/h soya bean meal, 2800 kg/h water at 60°C, 6.5

kg/h Viscozyme L from Novozymes, and 2.8 kg/h Depol 679 from Biocatalysts was applied. The ratio wet bulk density/dry bulk density of the incubation mixture was 1.1.

[0139] The outlet amount was adjusted to keep a constant level of filling inside the reactor, and the level of filling was set to yield a total processing time of 16 hours. Immediately after leaving the vertical reactor, the product was heat treated at 99°C for 15 min followed by air drying.

6.3 Test procedure for product

[0140] Composition of soluble sugars and oligosaccharides was analysed by thin layer chromatography by extracting a watery suspension slurry of 10% DM for 30 min followed by centrifugation for 10 min at 3,000 x g and applying the supernatant onto TLC silica gel 60 plates (Merck). The different components were quantified by comparison to standards of known concentration (Chaplan and Kennedy, 1986).

References

[0141] Chaplan MF and Kennedy JF. Carbohydrate analysis - practical approach; I RL Press, Oxford, 1986

6.4 Results

[0142] Results of the thin layer chromatography are shown in figure 2. It is evident that the carbohydrase treatment (lane 1) liberates some soluble carbohydrates, visualised by the additional spot between sucrose and raffinose on the thin layer chromatography as well as a general smear throughout the lane compared to the reference (lane 2).

Example 7:

Bio-conversion with carbohydrase on soya bean meal (SBM), rapeseed meal (RSM) and wheat

[0143] This biomass comprises polysaccharides and proteins from pulses.

7.1 Incubator

[0144] The incubator was a laboratory scale plastic container.

7.2 Method

[0145] An amount of 60 g soya bean meal, 45 g rapeseed meal, 45 g ground wheat, 180 g water, 3.0 g H₂SO₄, 0.525 g Viscozyme L from Novozymes, 0.225 g Depol 679 from Biocatalysts, 0.30 g BAN 480 L from Novozymes, and 0.30 g AMG 300 L from Novozymes was mixed and left to incubate at 37 °C for 16 hours. The ratio wet bulk density/dry bulk density of the incubation mixture was 0.90.

[0146] Following incubation, the product was heat treated at 100 °C for 15 min followed by air drying and milling.

7.3 Test procedure for product

[0147] The enzyme-treated product and its untreated reference, 60 g soya bean meal + 45 g rapeseed meal + 45 g ground wheat, milled, were analysed for soluble/insoluble non-starch polysaccharides (NSP) using the method of Englyst et al. 1994. Content of carbohydrate content in watery extract was analysed by the phenol sulphuric acid method and composition of soluble sugars and oligosaccharides was analysed by thin layer chromatography by extracting a watery suspension slurry of 10% DM for 30 min followed by centrifugation for 10 min at 3,000 x g and applying the supernatant onto TLC silica gel 60 plates (Merck). The different components were quantified by comparison to standards of known concentration (Chaplan and Kennedy, 1986).

References

[0148]

Chaplan MF and Kennedy JF. Carbohydrate analysis - practical approach; I RL Press, Oxford, 1986

Englyst HN, Quigley ME, and Hudson GJ; Analyst, 1994, Vol 119, pp. 1497-1509.

7.4 Results

[0149] Results of the soluble and insoluble NSP as well as the contents of soluble carbohydrates are shown in table 3. Results of the thin layer chromatography are shown in figure 3.

[0150] It is evident that the carbohydrase treatment liberates some soluble carbohydrates,

visualised by a smear on the thin layer chromatography, and that the carbohydrase treatment changes the amounts of soluble carbohydrates and NSP present in the product compared to the reference.

Table 3: Contents of soluble/insoluble NSP as well as contents of soluble carbohydrates of enzyme-treated product and its untreated reference, respectively.

Product Name	Fraction	Total	
		g/100g	SD
Example 7, product	Soluble NSP	4.0	0.1
	Insoluble NSP	8.5	0.1
	Total NSP	12.5	0.3
	Soluble carbohydrates	28.7	4.8
Example 7, reference	Soluble NSP	2.8	0.2
	Insoluble NSP	12.0	0.2
	Total NSP	14.8	0.5
	Soluble carbohydrates	15.0	4.2

Example 8:

Bio-conversion with carbohydrase on soya bean meal (SBM) and sunflower

[0151] This biomass comprises polysaccharides and proteins from pulses

8.1 Incubator

[0152] The incubator was a pilot scale vertical reactor with a total volume of 2.0 m².

8.2 Method

[0153] A continuous inlet amount of 18.8 kg/h soya bean meal, 12.5 kg/h sunflower meal, 30 kg/h water at 40°C, 0.03 kg/h carbohydrase (Ronozyme VP from Novozymes), and 0.88 kg/h H₂SO₄ was applied. The ratio wet bulk density/dry bulk density of the incubation mixture was 0.80.

[0154] The outlet amount was adjusted to keep a constant level of filling inside the reactor, and the level of filling was set to yield a total processing time of 16 hour. Immediately after leaving the vertical reactor, the product was heat treated at 99°C for 15 min followed by air drying.

8.3 Test procedure for product

[0155] The enzyme-treated product and its untreated reference, 90 g soya bean meal + 60 g sunflower meal, were analysed for soluble/insoluble non-starch polysaccharides (NSP) using the method of Englyst et al. 1994.

[0156] *References:* Englyst HN, Quigley ME, and Hudson GJ; Analyst, 1994, Vol 119, pp. 1497-1509.

8.4 Results

[0157] The results of the NSP analysis are shown in table 4. It is evident that the processing changes the amounts of the NSP fractions.

Table 4: *Contents of soluble/insoluble NSP as well as contents of soluble carbohydrates of enzyme-treated product and its untreated reference, respectively.*

Product Name	Fraction	Total	
		g/100g	SD
Example 8, product	Soluble NSP	3.8	1.4
	Insoluble NSP	12.6	1.4
	Total NSP	16.3	0.2
Example 8, reference	Soluble NSP	3.4	1.0
	Insoluble NSP	14.8	1.0
	Total NSP	18.2	0.1

Example 9:

Large scale bioconversion

Incubator:

[0158] The reactor used was a vertical cylinder with an effective height of 7.3 m and a diameter of 4.3 m.

[0159] In the top of the vertical reactor, the feed mixture falls on position near the centre of the reactor. For even distribution, a scraper blade or level arm distributes the inlet feed mixture over the perimeter of reactor.

[0160] In the bottom of the reactor, the product was extracted by means to achieve a uniform residence time for any particle spread on the top of the reactor.

Testing uniform plug flow

[0161] The inlet and outlet means of the reactor were adjusted to achieve an expected residence time of 12 hours. For proving the uniform distribution time, an inert tracer substance was added to the feed mixture. The feed mixture used in the experiment had a natural content of iron of around 143 mg/kg dry matter (= off-set concentration); therefore, iron sulphate (FeSO_4) was used as a tracer in a concentration of 1167 mg FeSO_4 /kg feed mixture dry matter equal to a total iron content of 572 mg Fe/kg total dry matter. At time 0 hours, FeSO_4 was added to the feed mixture dosed to the reactor for a period of 60 minutes. Samples were drawn every 20 minutes, dried, and analysed for content of iron, and it was found that the FeSO_4 enriched product leaves the reactor 12-13 hours after dosing FeSO_4 to the inlet feed mixture, and a maximum concentration of 355 mg/kg Fe was found at 12.5 hours after start.

REFERENCES CITED IN THE DESCRIPTION

Cited references

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- **LAEMMLI UK**Nature, 1970, vol. 227, 680-685 [0106] [0114]
- **CHAPLAN MF**KENNEDY JF Carbohydrate analysis - practical approach I RL Press 1986 0000 [0141] [0148]
- **ENGLYST HN**QUIGLEY ME HUDSON GJ Analyst, 1994, vol. 119, 1497-1509 [0148] [0156]

P a t e n t k r a v

1. Fremgangsmåde til fremstilling af et fast transformationsprodukt af et substrat, hvor det faste transformationsprodukt er et produkt af transformationen af proteinholdigt materiale og/eller kulhydrater, der stammer fra en biomasse, omfattende følgende trin:

- fremstilling af et substrat af biomasse omfattende kulhydrater og proteinholdigt materiale, der stammer fra sojabønnefrø, rapsfrø eller blandinger deraf, hvor mindst 20 vægt% af biomassen omfatter kulhydrater og proteinholdigt materiale, der stammer fra sojabønnefrø, rapsfrø eller blandinger deraf, eventuelt i yderligere blanding med kulhydrater og proteinholdigt materiale, der stammer fra hestefrø, ærtefrø, solsikkefrø, lupinfrø og/eller kornfrø;

- blanding af substratet med et enzympræparat eller en kombination af enzympræparater og tilsætning af vand i en mængde, der giver en initial inkubationsblanding med et vandindhold fra 30 til 70 vægt% og et forhold mellem våd bulkdensitet og tør bulkdensitet fra 0,60 til 1,45, hvor tørbulkdensiteten er den målte bulkdensitet (vægt/volumen) af biomassen uden det tilsatte vand, og vådbulkdensiteten er den målte bulkdensitet (vægt/volumen) af biomassen med den tilsatte mængde vand;

- inkubering af den indledende inkubationsblanding i 0,15-240 timer ved en temperatur på 20-70 °C; og udvinding af et fast transformationsprodukt fra den inkuberede blanding;

yderligere omfattende, at inkubationstrinnet udføres som en ensartet, kontinuerlig plug-flow-proces i en vertikal, ikke-omrørt inkubationstank med indløbsmidler til blandingen og tilsætningsstoffer og udløbsmidler til det faste transformationsprodukt, hvor transport af biomassen inden i den vertikale, ikke-omrørte inkubationstank medieres af tyngdekraft.

2. Fremgangsmåde ifølge krav 1, der yderligere omfatter forbehandling af biomassesubstratet, før det blandes med enzympræparatet eller kombinationen af enzympræparater, såsom kemisk eller fysisk forbehandling, f.eks. ved hjælp

af desintegration, formalining, afskalning, varmebehandling, trykbehandling, ultralydsbehandling, hydrotermisk behandling eller syre- eller alkalisk behandling.

5 **3.** Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 2, hvor mindst 30 vægt% af biomassen, såsom mindst 40 vægt%, mindst 50 vægt%, mindst 60 vægt%, mindst 70 vægt%, mindst 80 vægt% eller mindst 90 vægt% af biomassen, omfatter kulhydrater og proteinholdigt materiale, der stammer fra eventuelt affedtede og/eller eventuelt afskallede sojabønnefrø, eventuelt affedtede rapsfrø eller blandinger deraf.

10 **4.** Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor biomassen omfatter oligosaccharider og/eller polysaccharider og eventuelt yderligere omfatter olier og fedtstoffer, f.eks. fra frø af olieholdige planter.

15 **5.** Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor biomassesubstratet eller den indledende inkubationsblanding ikke omfatter nogen levende gær, såsom nogen levende gær valgt blandt *Saccharomyces cerevisiae*-stammer, herunder brugt ølgær og brugt destillationsgær samt bagegær og brugt gær fra vinproduktion.

20 **6.** Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor det faste transformationsprodukt af biomassesubstratet er et produkt af transformationen af proteinholdigt stof, eller af transformationen af kulhydrater, eller af transformationen af proteinholdigt stof og kulhydrater, der stammer fra bælgfrugter, og/eller korn og/eller frø fra olieholdige planter.

25 **7.** Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor det faste transformationsprodukt af biomassesubstratet er et produkt af transformationen af proteinholdigt stof, eller af transformationen af kulhydrater, eller af transformationen af proteinholdigt stof og kulhydrater, der stammer fra frø af

soja, ærter, lupin, solsikke, hvede, majs eller rapsfrø.

5 **8.** Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor enzympræparatet eller kombinationen af enzympræparater omfatter et eller flere enzymer valgt blandt proteaser, peptidaser, fytaser, carbohydraser, lipase og oxidoreduktase, såsom et eller flere carbohydraser valgt blandt α -galactosidase, amylase, amyloglucosidase, pektinase, cellulase og hemi-cellulaser, f.eks. xylanase, mannanase eller glucanase.

10 **9.** Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor tørstofforholdet mellem biomassesubstratet og enzympræparatet eller kombinationen af enzympræparater er fra 2:1 til 100.000.000:1, såsom 1.000:1, 10.000:1, 50.000:1, 100.000:1, 500.000:1, 1.000.000:1, 5.000.000:1, 10.000.000:1, 50.000.000:1 eller 100.000.000:1.

15 **10.** Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor den indledende inkubationsblanding inkuberes i 1 til 180 timer, såsom 2 til 150 timer, 3 til 120 timer, 5 til 90 timer, 8 til 72 timer eller 12 til 48 timer.

20 **11.** Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor vand tilsættes biomassesubstratet i en mængde, der tilvejebringer en initial inkubationsblanding med et forhold mellem våd bulkdensitet og tør bulkdensitet fra 0,65 til 1,40, såsom 0,70, 0,75, 0,80, 0,85, 0,90, 0,95, 1,00, 1,10, 1,15, 1,20, 1,25, 1,30 eller 1,35.

25 **12.** Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor vandindholdet i den indledende inkubationsblanding er fra 35 til 70 vægt%, såsom 40 vægt%, 45 vægt%, 50 vægt%, 55 vægt%, 60 vægt% eller 65 vægt%.

30

13. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor et eller flere proceshjælpemidler valgt blandt plantekomponenter og organiske og uorganiske procesmidler, såsom α -galactosidase, tilsættes biomassesubstratet og/eller den indledende inkubationsblanding.

5

14. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor et α -galactosidasepræparat tilsættes biomassesubstratet og/eller den indledende inkubationsblanding i en mængde på fra 0,05 til 50 α -galactosidasenheder pr. g tørstof af biomassesubstrat, såsom fra 0,5 til 25 α -galactosidasenheder pr. g tørstof af biomassesubstrat, f.eks. fra 1 til 10, fra 2 til 8, fra 3 til 6 eller fra 4 til 5 α -galactosidasenheder pr. g tørstof af biomassesubstrat.

10

15. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, yderligere omfattende, at den vertikale, ikke-omrørte inkubationstank er lukket.

15

16. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor den ikke-omrørte inkubationstank er af en lodret, aflang cylindrisk eller polyedrisk type, eventuelt hvor arealet i den øvre del af den ikke-omrørte inkubationstank er mindre end arealet i den nedre del, dvs. tanken har en konisk form.

20

17. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor den ikke-omrørte inkubationstank har en isolerende måtte eller en termisk fordybningskappe og midler til at styre temperaturen i tanken, eventuelt hvor fyldningsgraden af den inkubationstanken holdes konstant.

DRAWINGS

Drawing

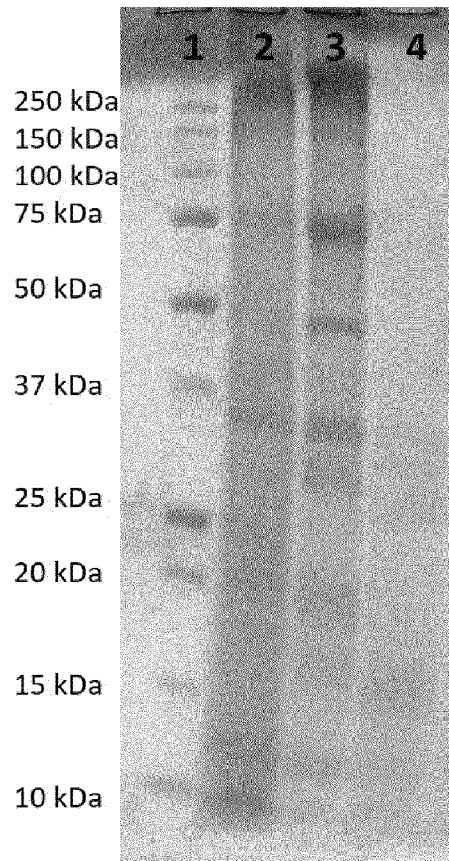


Figure 1

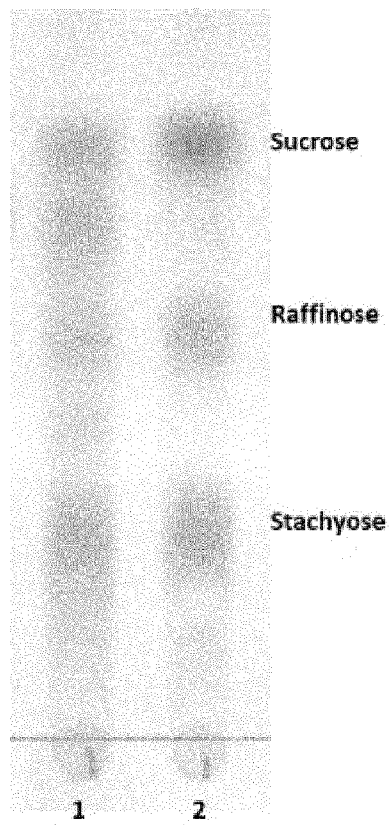


Figure 2

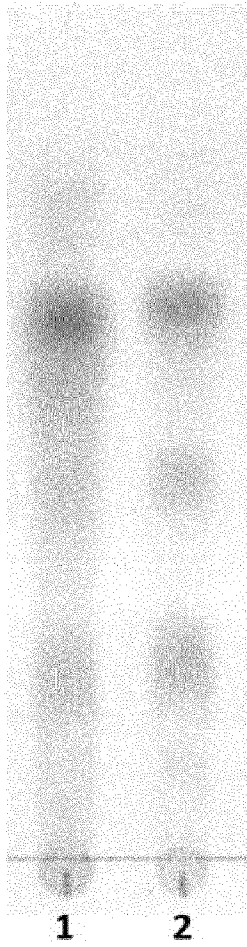


Figure 3