US 20150147786A1

(19) **United States**

(12) **Patent Application Publication**

CLARKSON et al.

(10) **Pub. No.: US 2015/0147786 A1**

(43) **Pub. Date: May 28, 2015**

(54) **HIGH FORCE AND HIGH STRESS DESTRUCTURING FOR STARCH BIOMASS PROCESSING**

(71) Applicant: **E I DU PONT DE NEMOURS AND COMPANY**, Wilmington, DE (US)

(72) Inventors: **KATHLEEN A CLARKSON**, San Francisco, CA (US); **F Glenn Gallagher**, Wilmington, DE (US); **Aaron Perelman**, Wilmington, DE (US); **Luis Fernando Romero Millan**, Swindon (GB); **Vivek Sharma**, Fremont, CA (US); **Jayarama K. Shetty**, Pleasonton, CA (US); **Daniel A. Slanac**, Greenville, DE (US); **Paula Johanna Maria Teunissen**, Saratoga, CA (US)

(21) Appl. No.: **14/546,250**

(22) Filed: **Nov. 18, 2014**

Related U.S. Application Data

(60) Provisional application No. 62/014,313, filed on Jun. 19, 2014, provisional application No. 61/908,158, filed on Nov. 24, 2013.

Publication Classification

(51) **Int. Cl.**

C08B 30/02	(2006.01)
C12P 19/02	(2006.01)
A23K 1/165	(2006.01)
C12P 7/14	(2006.01)
C08B 30/12	(2006.01)
A23K 1/14	(2006.01)
C12P 19/14	(2006.01)
C12P 19/20	(2006.01)

(52) **U.S. Cl.**

CPC ***C08B 30/02*** (2013.01); ***C12P 19/14*** (2013.01); ***C12P 19/02*** (2013.01); ***C12P 19/20*** (2013.01); ***C12P 7/14*** (2013.01); ***C08B 30/12*** (2013.01); ***A23K 1/14*** (2013.01); ***A23K 1/1653*** (2013.01); *C12P 2201/00* (2013.01)

(57) **ABSTRACT**

A process for mechanical destructuring of starch-based biomass was developed that makes use of a short application of high compression, impact, and shearing forces. The biomass may be destructured using a specific energy input that is less than 40% of the total combustible energy of the biomass. The destructured starch-based biomass, with or without saccharification and/or in-feed glycosyl hydrolase enzymes, may be used in feed applications. The destructured starch-based may saccharified to produce syrups and fermentable sugars, and for production of products including ethanol using a biocatalyst.

Fig. 1

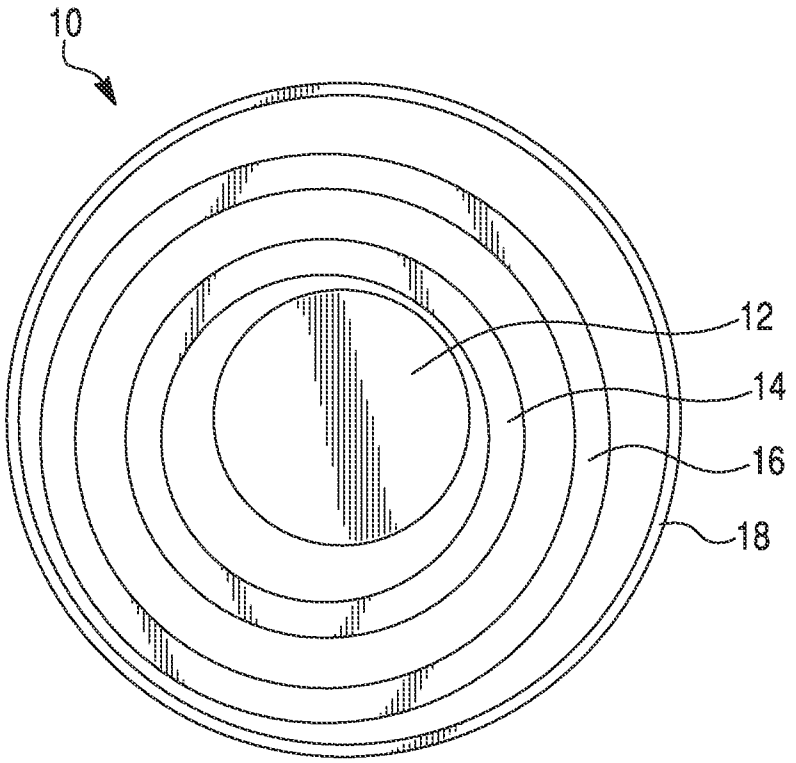


Fig. 2A

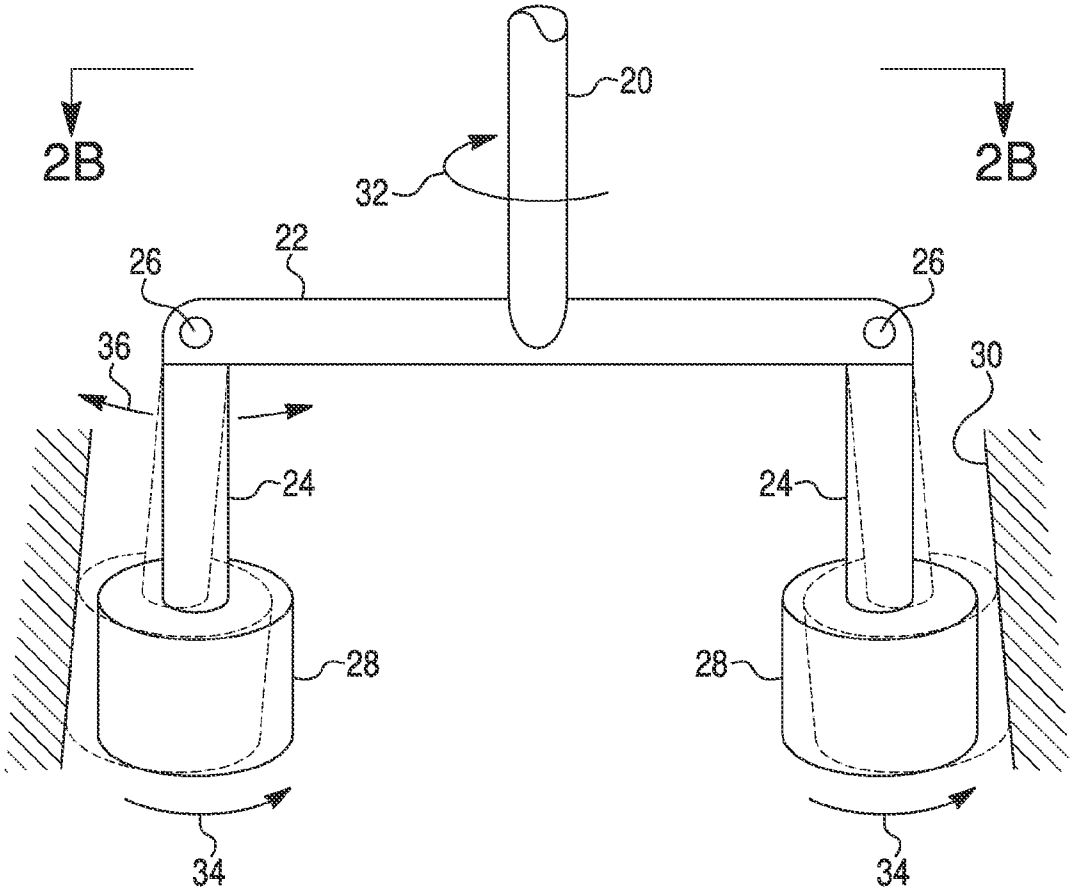
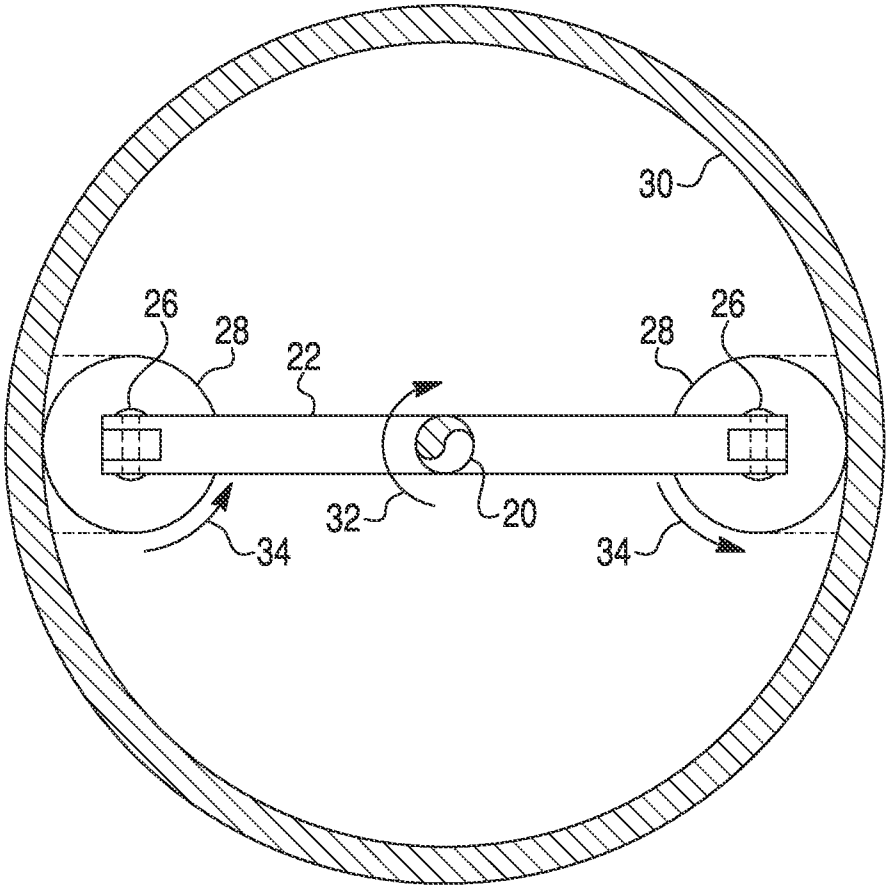


Fig. 2B



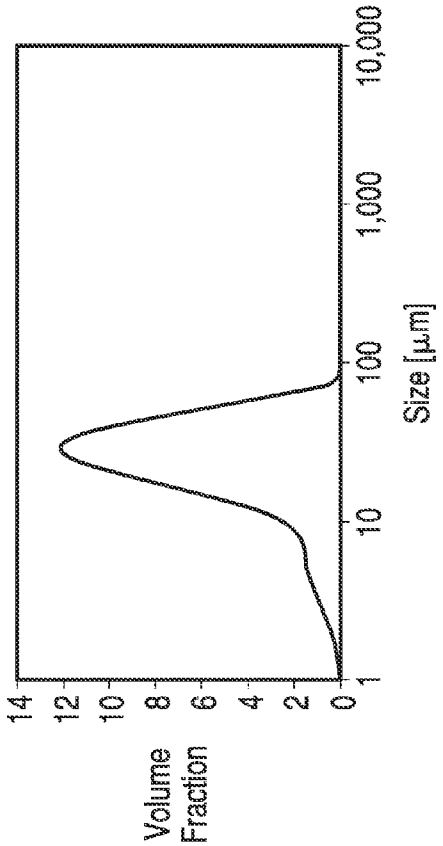

Volume Fraction
14
12
10
8
6
4
2
0
1
10
100
1,000
10,000
Size [µm]

Fig. 3A

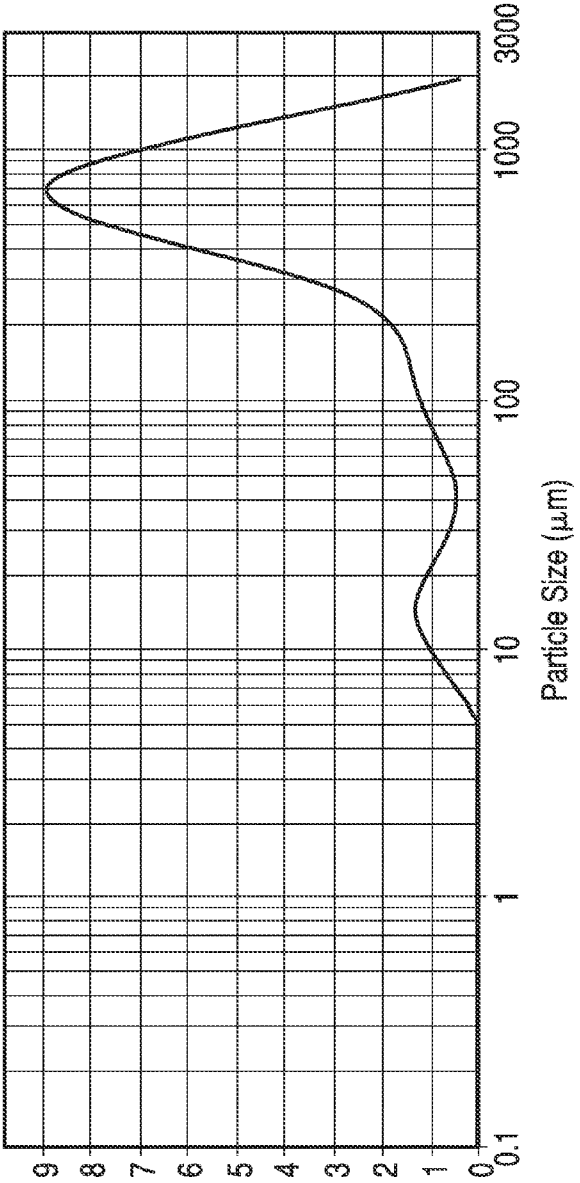
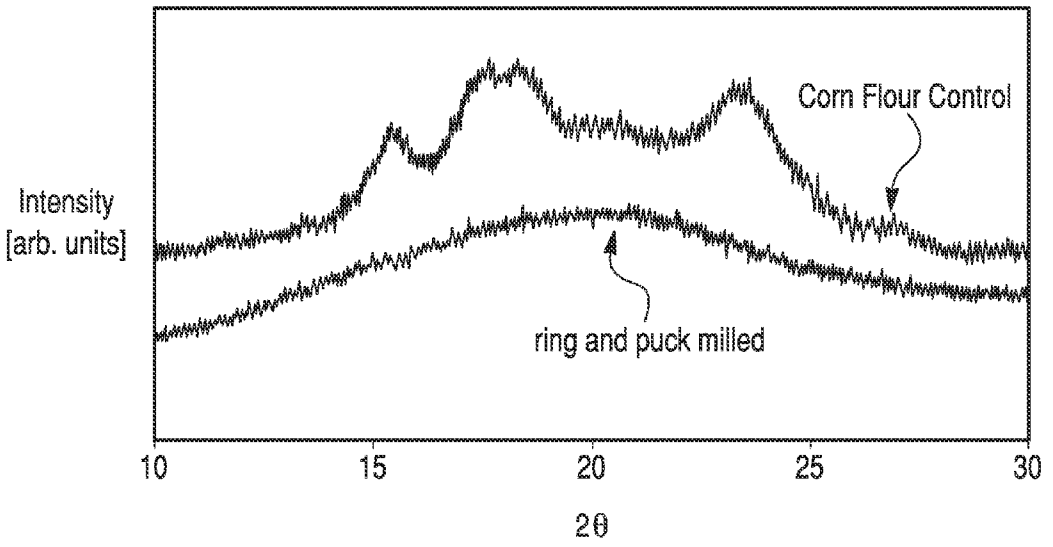

Volume (%)
9
8
7
6
5
4
3
2
1
0
0.1
1
10
100
1000
3000
Particle Size (µm)

Fig. 3B

Fig. 4


Intensity
[arb. units]
Corn Flour Control
ring and puck milled
10
15
20
25
30
2θ

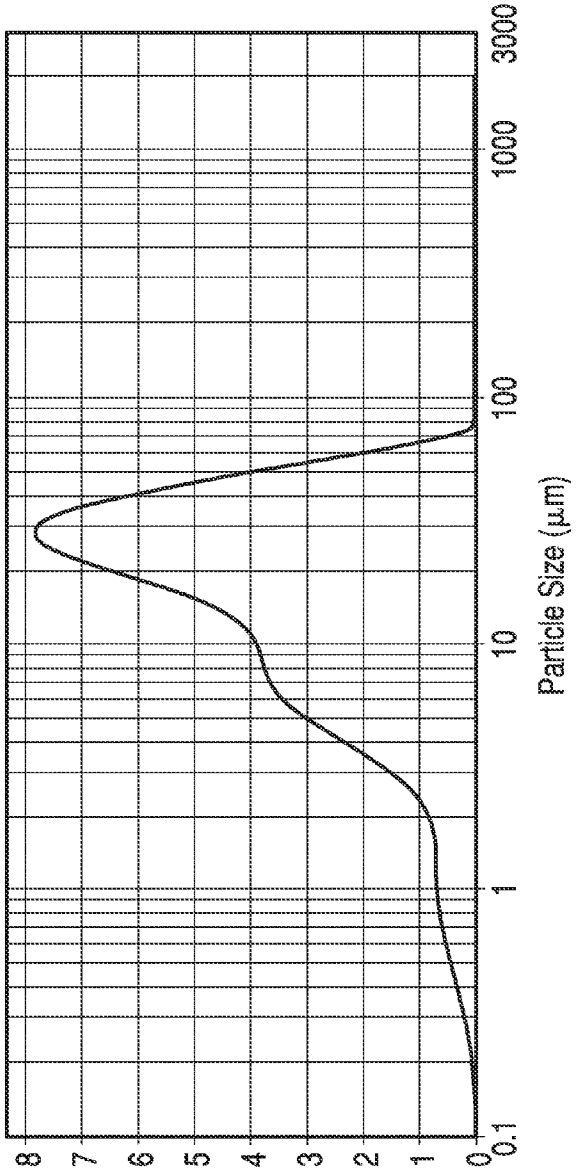

Volume (%)
0
1
2
3
4
5
6
7
8
0.1
1
10
100
1000
3000
Particle Size (µm)

Fig. 5A

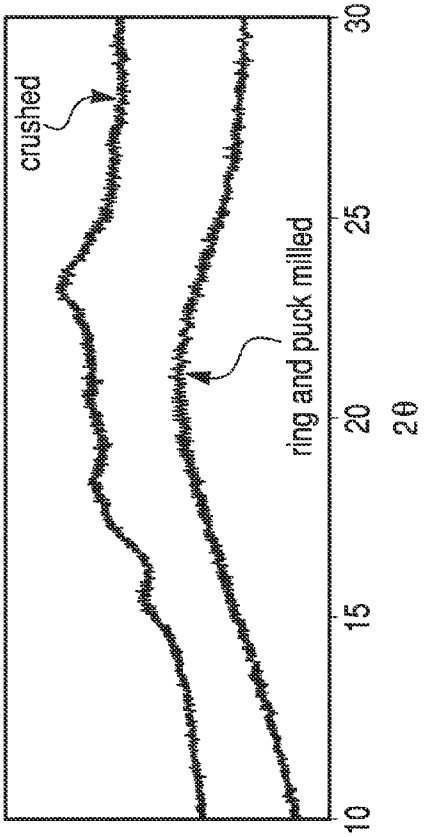

crushed
ring and puck milled
Intensity
[arb. units]
10
15
20
25
30
2θ

Fig. 5B

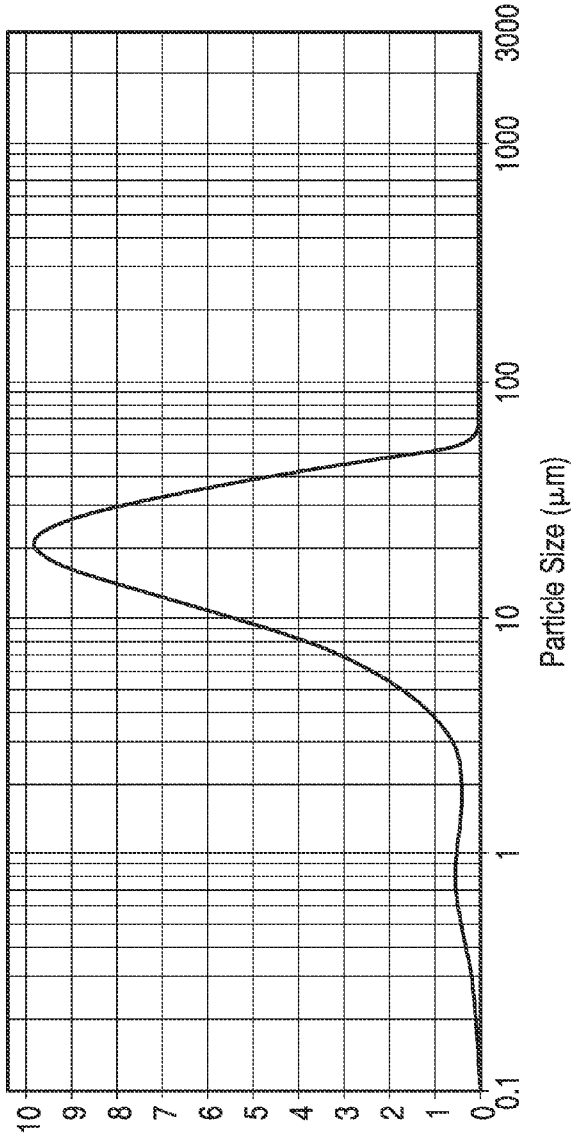

Volume (%)
0
1
2
3
4
5
6
7
8
9
10
0.1
1
10
100
1000
3000
Particle Size (µm)

Fig. 6A

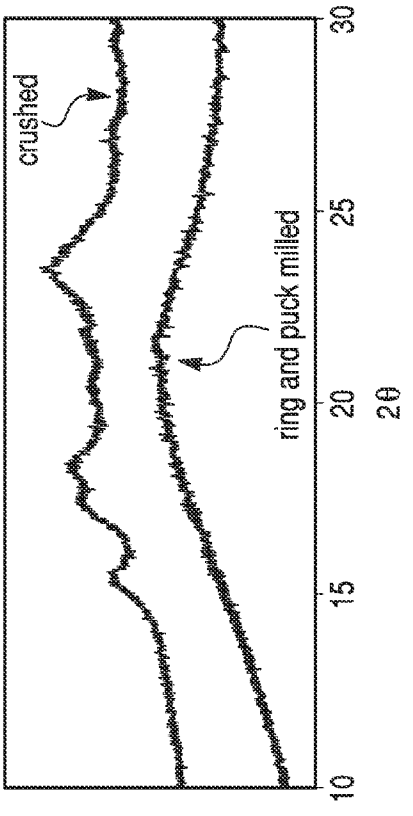

crushed
ring and puck milled
Intensity
[arb. units]
10
15
20
25
30
2θ

Fig. 6B

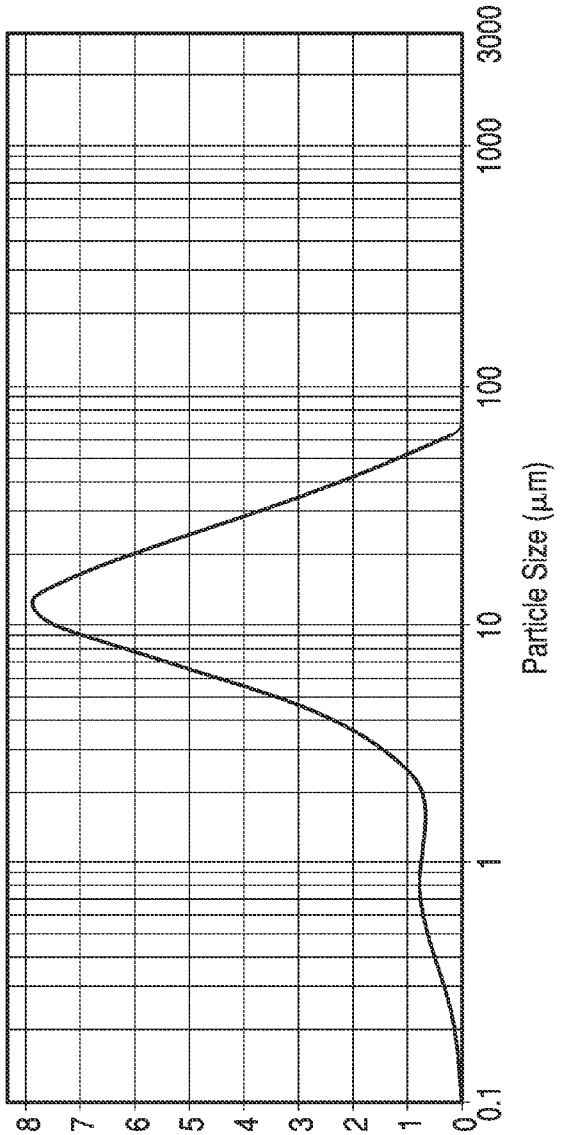

Volume (%)
0
1
2
3
4
5
6
7
8
0.1
1
10
100
1000
3000
Particle Size (µm)

Fig. 7A

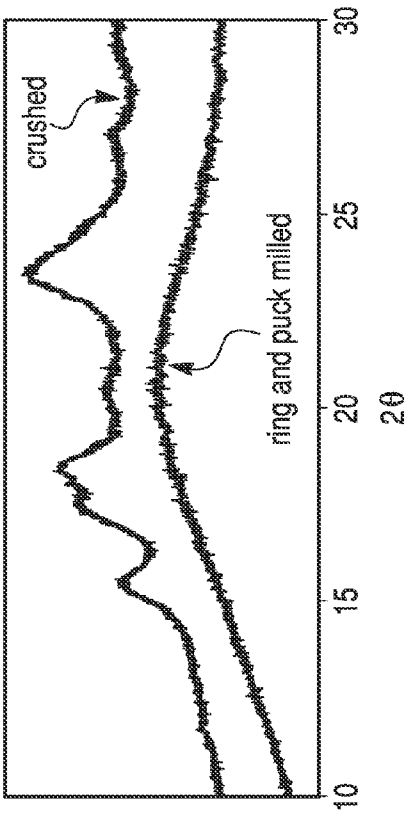

crushed
ring and puck milled
Intensity
[arb. units]
10
15
20
25
30
2θ

Fig. 7B

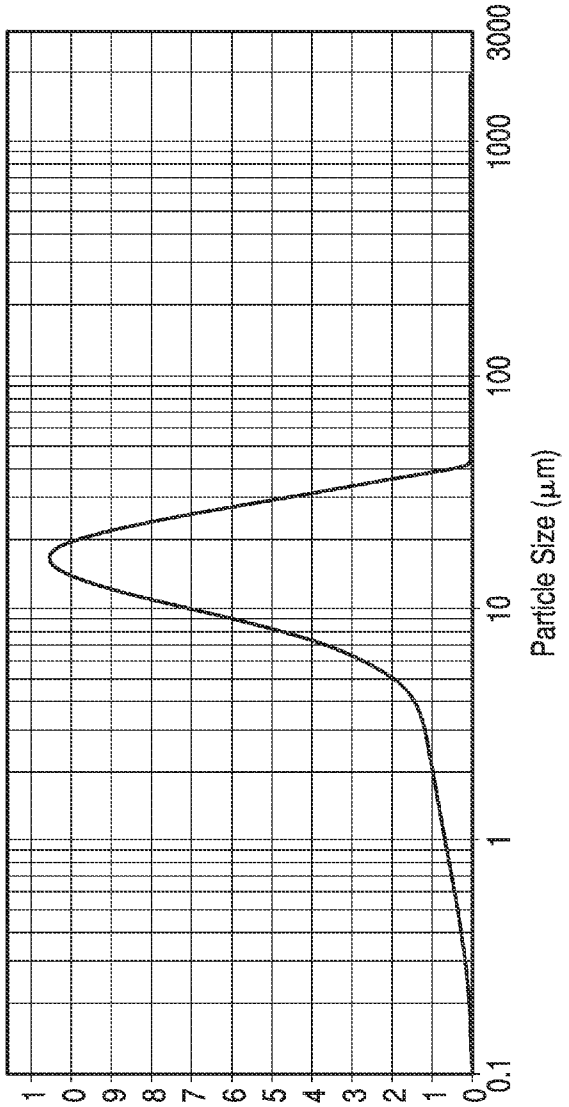

Volume (%)
0
1
2
3
4
5
6
7
8
9
10
11
0.1
1
10
100
1000
3000
Particle Size (µm)

Fig. 8A

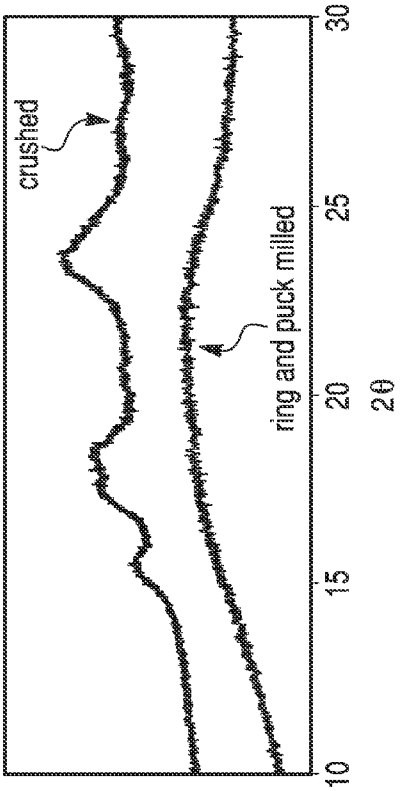
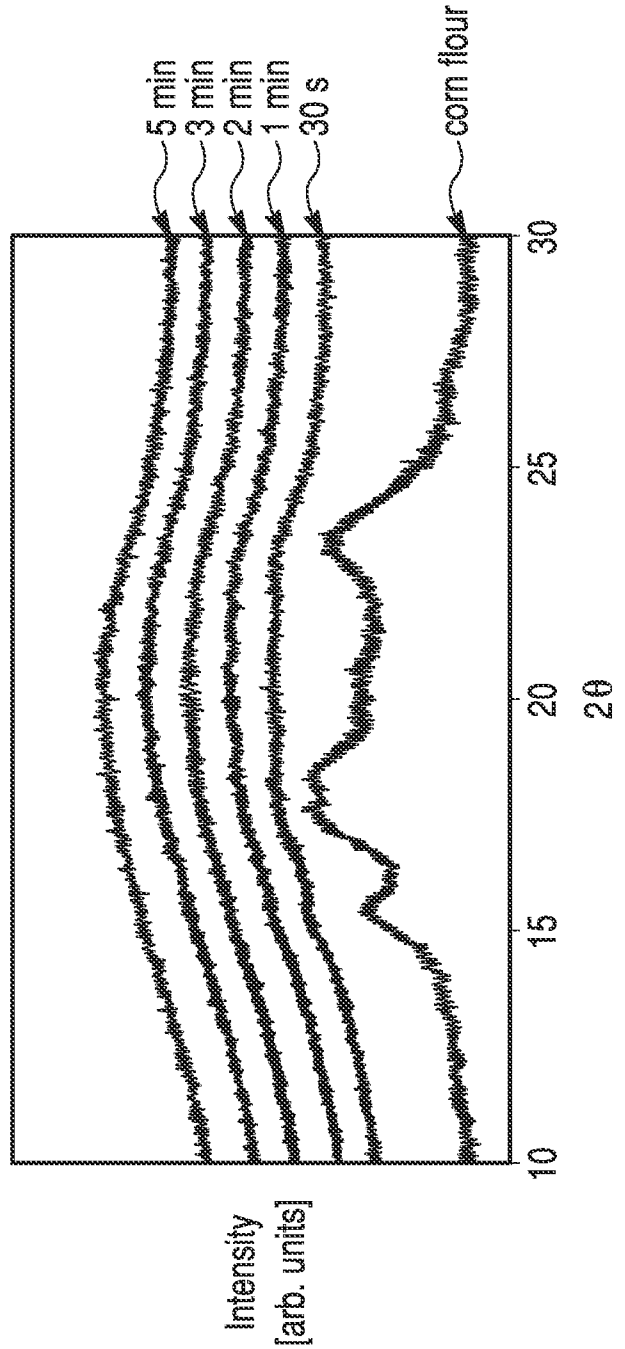

crushed
ring and puck milled
Intensity
[arb. units]
10
15
20
25
30
2θ

Fig. 8B

Fig. 9


5 min
3 min
2 min
1 min
30 s
corn flour
Intensity
[arb. units]
10
15
20
25
30
2θ

HIGH FORCE AND HIGH STRESS DESTRUCTURING FOR STARCH BIOMASS PROCESSING

[0001] This application claims the benefit of U.S. Provisional Applications 61/908,158 and 62/014,313, filed Nov. 24, 2013 and Jun. 19, 2014, respectively, now pending, which are incorporated in their entirety herein by reference.

FIELD OF THE INVENTION

[0002] Processes for destructuring biomass that is high in starch content are provided. Specifically, starch-based biomass is processed using high compression, impact, and shearing forces thereby producing a destructured starch-based biomass product that is digestible and from which sugars can be obtained.

BACKGROUND OF THE INVENTION

[0003] In a typical bio-ethanol production process corn kernels are used as a source of starch to provide sugars utilized by a biocatalyst for fermentative production of ethanol. Typically the starch in corn kernels is made available by dry grinding, where the corn is ground into flour which is prepared in a slurry of insoluble starch granules, which is then heat treated at temperatures between 80° C. and 100° C. in the presence of thermostable alpha-amylases producing low viscosity mash in a process called liquefaction. During liquefaction, the heat causes the starch granules to swell and burst releasing long-chained starch molecules and causing a dramatic increase in viscosity, which is reduced by enzymatic degradation of the long-chained starch molecules by alpha-amylase into dextrins, which are shorter chains of glucose. Saccharification enzymes, typically various amylases, glucosidases, and/or glucanases, are then added to break down the dextrins producing sugars that can be fermented by a biocatalyst. Fermentation may be a separate step, or combined with saccharification in a simultaneous saccharification and fermentation (SSF) step. Other grains such as wheat, barley, sorghum, millet, and rye, as well as tubers such as cassava and potato may provide starch in this manner as well.

[0004] No-cook processes for saccharification of corn have been used as in U.S. Pat. No. 7,303,899, but typically much higher amounts of enzymes are required than when using a process that includes a heating step.

[0005] There remains a need for an improved process for treating sources of starch-based biomass to provide material that is readily digestible, and for simplified processes that make use of the material.

SUMMARY OF THE INVENTION

[0006] The invention provides a process for treating starch-based biomass by which the starch and other components of the biomass are made available for further processing.

[0007] Accordingly, the invention provides a process for producing destructured starch-based biomass comprising:

[0008] a) providing a starch-based biomass; and

[0009] b) applying to the biomass of (a) at least one set of compression and impact forces of at least 5,000 N combined with shearing forces;

[0010] wherein contact stress of greater than 5,000 psi is applied to the biomass and wherein a destructured starch-based biomass is produced.

[0011] In another aspect the invention provides a process for producing destructured starch-based biomass comprising:

[0012] a) providing a portion of starch-based biomass; and

[0013] b) applying to the biomass of (a) at least one set of compression and impact forces of at least 1,500 N combined with shearing forces, and contact stress of greater than 5,000 psi;

[0014] wherein specific energy input in the process is less than 40% of the total combustible energy of the portion of biomass being treated and wherein a destructured starch-based biomass is produced.

[0015] In another aspect the invention provides a destructured starch-based biomass obtainable by the above process comprising starch having at least 25% reduction in crystallinity as compared to provided starch-based biomass.

[0016] In other aspects the invention provides a feed additive composition, a feed additive kit, a feed or feedstuff, and a premix comprising the destructured starch-based biomass.

[0017] In other aspects the invention provides a method for improving a biophysical characteristic of an animal which method comprises administering to an animal said feed additive composition, use of said feed additive composition for improving a biophysical characteristic of an animal, and a method of preparing a feedstuff comprising contacting a feed component with said feed additive composition.

[0018] In yet another aspect the invention provides a method of saccharifying a composition comprising destructured starch-based biomass to produce a composition comprising glucose, wherein said method comprises:

[0019] (i) contacting a destructured starch-based biomass composition with a glucoamylase; and

[0020] (ii) saccharifying the composition to produce said glucose composition.

[0021] In yet another aspect the invention provides a method of saccharifying a composition comprising destructured starch-based biomass to produce a composition comprising maltose, wherein said method comprises:

[0022] (i) contacting a destructured starch-based biomass composition with a maltogenic alpha-amylase; and

[0023] (ii) saccharifying the composition to produce said maltose composition.

[0024] In yet another aspect the invention provides a method of saccharifying a composition comprising destructured starch-based biomass to produce a composition comprising maltotriose, wherein said method comprises:

[0025] (i) contacting a destructured starch-based biomass composition with a beta-amylase and optionally a pullulanase; and

[0026] (ii) saccharifying the composition to produce said maltotriose composition.

[0027] In yet another aspect the invention provides a method of saccharifying a composition comprising destructured starch-based biomass to produce a composition comprising maltotetraose, wherein said method comprises:

[0028] (i) contacting a destructured starch-based biomass composition with a DP4 producing alpha-amylase and optionally a pullulanase; and

[0029] (ii) saccharifying the composition to produce said maltotetraose composition.

[0030] In yet another aspect the invention provides a method of saccharifying a composition comprising destructured starch-based biomass to produce a composition comprising maltopentaose, wherein said method comprises:

[0031] (i) contacting a destructured starch-based biomass composition with a DP5 producing alpha-amylase and optionally a pullulanase; and

[0032] (ii) saccharifying the composition to produce said maltopentaose composition.

[0033] In yet another aspect the invention provides a method of producing alcohol from destructured starch-based biomass by simultaneous saccharification and fermentation (SSF) comprising,

[0034] (i) contacting a slurry comprising destructured starch-based biomass with an alpha-amylase, a glucoamylase, a yeast and optionally an acid stable protease, at a temperature below the starch gelatinization temperature of the destructured starch-based biomass to produce oligosaccharides fermentable by the yeast;

[0035] (ii) fermenting the oligosaccharides to produce alcohol.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] FIG. **1** is a diagram of an apparatus for imparting compression and impact forces using centrifugal motion.

[0037] FIG. **2**A is a diagram of a side view of a ring-roller mill that employs centrifugal force between revolving milling media and a grinding surface.

[0038] FIG. **2**B is a diagram of a top view of a ring-roller mill that employs centrifugal force between revolving milling media and a grinding surface.

[0039] FIG. **3**A shows a graph of particle size distribution (PSD) for ring and puck milled whole corn kernels.

[0040] FIG. **3**B shows a graph of PSD for hammer milled whole corn kernels.

[0041] FIG. **4** shows a graph of XRD Spectra for whole corn kernels either hammer milled (corn flour; top curve) or ring and puck milled (bottom curve).

[0042] FIG. **5**A shows a graph of PSD for ring and puck milled whole white wheat kernels.

[0043] FIG. **5**B shows a graph of XRD Spectra for whole white wheat kernels either crushed with a mortar and pestle (top curve) or ring and puck milled (bottom curve).

[0044] FIG. **6**A shows a graph of PSD for ring puck milled whole sorghum.

[0045] FIG. **6**B shows a graph of XRD Spectra for whole sorghum either crushed with a mortar and pestle (top curve) or ring and puck milled (bottom curve).

[0046] FIG. **7**A shows a graph of PSD for ring and puck milled whole brown rice.

[0047] FIG. **7**B shows a graph of XRD Spectra for whole brown rice either crushed with a mortar and pestle (top curve) or ring and puck milled (bottom curve).

[0048] FIG. **8**A shows a graph of PSD for ring and puck milled dried cassava.

[0049] FIG. **8**B shows a graph of XRD Spectra for dried cassava either crushed with a mortar and pestle (top curve) or ring and puck milled (bottom curve).

[0050] FIG. **9** shows a graph of XRD spectra of corn kernels that were ring and puck milled for various times.

DETAILED DESCRIPTION

[0051] The invention relates to a process for pretreating starch-based biomass with high force and stress wherein a destructured starch-based biomass product is made. The destructured biomass product may be saccharified producing sugars which may be used in production of desired target products, such as through fermentation using a biocatalyst, or by reacting with a biochemical (enzyme) or chemical catalyst.

[0052] Alternatively the destructured starch-based biomass product or saccharified destructed biomass (also referred to herein as starch-based biomass hydrolysate) may be used as an animal feed or as a feed additive composition. The destructured starch-based biomass product or the saccharified destructured starch-based biomass product (e.g the starch-based biomass hydrolysate) may be admixed or combined with at least one in-feed enzyme prior to feeding to an animal.

[0053] The following definitions and abbreviations are to be used for the interpretation of the claims and the specification.

[0054] As used herein, the terms "comprises," "comprising," "includes," "including," "has," "having," "contains" or "containing," or any other variation thereof, are intended to cover a non-exclusive inclusion. For example, a composition, a mixture, process, method, article, or apparatus that comprises a list of elements is not necessarily limited to only those elements but may include other elements not expressly listed or inherent to such composition, mixture, process, method, article, or apparatus. Further, unless expressly stated to the contrary, "or" refers to an inclusive or and not to an exclusive or. For example, a condition A or B is satisfied by any one of the following: A is true (or present) and B is false (or not present), A is false (or not present) and B is true (or present), and both A and B are true (or present).

[0055] Also, the indefinite articles "a" and "an" preceding an element or component of the invention are intended to be nonrestrictive regarding the number of instances (i.e. occurrences) of the element or component. Therefore "a" or "an" should be read to include one or at least one, and the singular word form of the element or component also includes the plural unless the number is obviously meant to be singular.

[0056] The term "invention" or "present invention" as used herein is a non-limiting term and is not intended to refer to any single embodiment of the particular invention but encompasses all possible embodiments as described in the specification and the claims.

[0057] As used herein, the term "about" modifying the quantity of an ingredient or reactant of the invention employed refers to variation in the numerical quantity that can occur, for example, through typical measuring and liquid handling procedures used for making concentrates or use solutions in the real world; through inadvertent error in these procedures; through differences in the manufacture, source, or purity of the ingredients employed to make the compositions or carry out the methods; and the like. The term "about" also encompasses amounts that differ due to different equilibrium conditions for a composition resulting from a particular initial mixture. Whether or not modified by the term "about", the claims include equivalents to the quantities. In one embodiment, the term "about" means within 10% of the reported numerical value, preferably within 5% of the reported numerical value.

[0058] The term "fermentable sugar" refers to oligosaccharides and monosaccharides that can be used as a carbon source by a microorganism in a fermentation process.

[0059] The term "lignocellulosic" describes to a composition comprising both lignin and at least 10 wt % cellulose. Lignocellulosic material may also comprise hemicellulose. Examples of lignocellulosic biomass include, but are not limited to, corn cobs, crop residues such as corn husks, corn

stover, corn grain fiber, grasses, wheat, wheat straw, barley, barley straw, hay, rice straw, switchgrass, waste paper, sugar cane bagasse, sorghum, sweet sorghum, soy, components obtained from milling of grains or from using grains in production processes (such as corn fiber fraction, DDGS: dried distillers grains with solubles), trees, branches, roots, leaves, wood chips, sawdust, shrubs and bushes, vegetables, fruits, flowers, and animal manure or a combination thereof.

[0060] The term "cellulosic" describes a composition comprising at least 10 wt % cellulose or cellulose plus hemicellulose. Cellulosic material may also include lignin. Thus lignocellulosic biomass is a type of cellulosic biomass. Cellulosic also refers to compositions enriched in cellulose and/or hemicellulose obtained from processing of grains, including but not limited to grain fractions (for example a fiber fraction), by-products (for example wet cake, distillers dry grains (DDG) or distillers dry grains with solubles (DDGS)), biomass post any liquefaction, saccharification, fermentation, SSF and/or other process or treatment.

[0061] The term "starch-based biomass" refers to a biomaterial that is at least about 10% starch in composition by weight. Examples of starch-based biomass include grains such as corn, wheat, barley, rye, millet, milo, sorghum, sweet sorghum, and rice, as well as starch storage root biomass such as potato, sweet potato, yam, taro, carrot, turnip, triticale, sago and cassava. Starch-based biomass may in addition contain other components such as protein, cellulose, hemicellulose, and lignin. "Starch-based biomass" also refers to compositions obtained from processing of starch-based biomass wherein the processed starch-based biomass is at least about 1% starch in composition by weight. For example, starch-based biomass obtained from processing of grains may include grain fractions and by-products such as fiber fractions, endosperm fractions, wet cake, distillers dry grains (DDG) or distillers dry grains with solubles (DDGS)), and biomass post any liquefaction, saccharification, fermentation, SSF process and/or other process or treatment of starch-based biomass.

[0062] The term "starchy root" refers to starch storage root biomass.

[0063] The term "saccharification" refers to the production of sugars from polysaccharides. Saccharification may be by any method including enzymatic digestion and chemical treatment.

[0064] The term "destructured" refers to a biomass that has at least a 25% reduction in crystallinity that is measured as described in General Methods herein.

[0065] As used herein the term "compression force" refers to the application of inward ("pushing") forces to different points on a material or structure, that is, forces with no net sum or torque directed so as to reduce its size in one or more directions. Compression force is applied between two surfaces where equal and opposite force vectors are generated normal to the curvature of the surfaces.

[0066] As used herein an "impact force" is a high force or shock applied over a short time period when two or more bodies collide. For example, when a compressive force is applied over a short period of time, this is considered as an impact force. As used herein, the impact force is a force resulting from a fast compression between surfaces. It is a mode of applying an impact force that is different from a mode where force is generated by only one surface effectively applying a force on a material (e.g. a swinging hammer hitting material in a hammer mill). Hammer milling or jet milling are types of milling that are used herein for comparative purposes and do not result in destructured cellulosic biomass in accordance with the present invention. These are used as control treatments in the examples referred to herein.

[0067] As used herein the term "milling media" refers to any grinding surface inside the confines of a mill's grinding chamber, that does not include the chamber itself, including but not limited to balls in a ball mill, rods in a rod mill, the rollers in a ring-roller mill employing centrifugal force, the rollers in a ring-roller mill employing spring and/or hydraulic force, rings and puck in a puck mill, and gears in a gear-type grinding media mill.

[0068] The term "G-force" as used herein refers to the ratio of the actual acceleration to the gravitational acceleration, where the gravitational acceleration is ~9.8 m/s^2. For example, if the acceleration of the milling media is calculated to be ~60 m/s^2, then the corresponding "G-force" is ~6 G.

[0069] "Enzyme consortium" or "saccharification enzyme consortium" is a collection of enzymes, usually secreted by microorganisms, which in the present case will typically contain one or more amylases, glucoamylases, cellulases, xylanases, glycosidases, ligninases, other hemicellulases, esterases, and other glycosyl hydrolysas. In addition, other enzymes may be included in an enzyme consortium to enhance access to the various carbohydrates present, help stabilize one or more enzymes, improve saccharification and/or SSF process, including but not limited to proteases, cutinases, lipases, phytases, and transferases.

[0070] The term "target compound" refers to any product that is produced by a microbial production host cell in a fermentation. Target compounds may be the result of genetically engineered enzymatic pathways in host cells or may be produced by endogenous pathways. In addition, target compounds may be produced via chemical reaction with or without a chemical catalyst. Typical target compounds include but are not limited to acids, alcohols, alkanes, alkenes, aromatics, aldehydes, ketones, biopolymers, proteins, peptides, amino acids, vitamins, antibiotics, and pharmaceuticals.

[0071] The term "coherent domain size" refers to the average distance (length) over which crystalline order is observed in cellulose, that is, the cellulose crystalline size that is free of structural defects.

[0072] The term "crystalline fraction" or "crystallinity fraction" refers to volume ratio of the crystalline portion of cellulose to the total volume of cellulose (including both amorphous and crystalline regions).

[0073] The term "yield" with respect to sugars refers to the amount obtained as a percentage of the total calculated yield.

[0074] The term "processed energy equivalency" refers to an energy percentage that is determined by the ratio of the difference of the combustible energy of the input material minus the energy used in processing of the material to the combustible energy of the input material.

[0075] The phrase "simultaneous saccharification and fermentation" or "SSF" refers to a process in the production of biochemicals in which a microbial organism, such as an ethanologenic microorganism, and at least one enzyme, such as one or more glucoamylases, are present during the same process step. SSF includes the contemporaneous hydrolysis of starch substrates to saccharides, including glucose, and the fermentation of the saccharides into alcohol or other biochemical or biomaterial in the same reactor vessel.

[0076] The term "liquefaction" refers to a stage in starch conversion in which gelatinized starch is hydrolyzed to give low molecular weight soluble dextrins.

Pretreatment using High Force and High Stress Milling

[0077] In the present process, starch-based biomass is destructured using mechanical forces in preparation for saccharification or use in animal feed. In addition, biomass derived from starch-based biomass processing, which may contain cellulosic biomass (such as corn grain fiber) and/or contain starch, is treated using mechanical forces for destructuring. The present process performs mechanical destructuring of the starch-based biomass such that it produces a destructured biomass product. The present mechanical destructuring is effective with a short period of application for the required power, thereby providing a process that requires relatively low energy input in support of a commercially effective starch-based biomass pretreatment process.

[0078] Starch-based biomass refers to any biomaterial that contains at least about 10% starch by weight, as well as compositions obtained from processing of starch-based biomass wherein the processed starch-based biomass is at least about 1% starch in composition by weight. Examples of starch-based biomass are grains and starchy roots of plants. Grains include but are not limited to corn, wheat, barley, sorghum, and rice. Starchy roots are starch storage root biomass such as potato, sweet potato, yam, taro, carrot, turnip, and cassava. Examples of compositions obtained from processing of starch-based biomass may include grain fractions and by-products such as fiber fractions, endosperm fractions, wet cake, distillers dry grains (DDG) or distillers dry grains with solubles (DDGS)), and biomass post any liquefaction, saccharification, fermentation, SSF process and/or other process or treatment of starch-based biomass.

[0079] SSF process and/or other process or treatment of starch-based biomass.

[0080] In various embodiments the starch-based biomass may be size reduced, such as by hammer milling, shredding, chopping, chipping, disc refining, and/or cutting. Particles of starch-based biomass used may be from very small, in the micron range (i.e. 1-999 microns), to much larger, with at least one dimension on the order of centimeters. In various embodiments the starch-based biomass may be dried, such as air dried or dried with heat and optionally with vacuum. The moisture content of starch-based biomass used in the present process is generally less than 30%. In various embodiments the moisture content of starch-based biomass used in the present process is less than at least about 30%, 25%, 20%, 15%, 10%, or less. Typical moisture content of dried starch-based biomass is less than 15%. The moisture content is low enough to provide a friablilty to the biomass. Techniques such as freezing may be employed to increase the brittleness/friability of the starch-based biomass to allow for milling of high moisture content biomass.

[0081] The mechanical forces applied to the starch-based biomass are compression, and impact forces that result in normal and shear stresses. Stress may be generated by rolling action against a surface wherein forces are applied by rolling a mass over material against a surface, where sandwiching the material between the mass and the surface creates an anisotropic stress distribution (i.e. shearing, sudden compression (impact) and decompression. The mass is generally referred to as a media. Types of media that may be used to apply compression and impact forces include, but are not limited to, a puck, a ring, a roller, a rod, a disc, and a sphere.

[0082] The particles that are ground via these mechanisms may also shear against one another for additional destructuring. In one embodiment at least one force applied is of at least about 5,000 N, with stresses of at least 5,000 psi. There may be one level of force applied, or more than one level of force which may be applied concurrently. For example, there may be multiple media that apply forces of different strengths, as described below and shown in FIG. **1**. In the case of multiple forces, included is at least one force of at least about 1,500 N with stresses of at least 5,000 psi. There may be additional lower forces (as well as stresses) applied concurrently. In one embodiment, whether a single level or multiple levels of forces are applied, the forces include a level of at least about 7,000 N combined with stresses of at least 5,000 psi. In other embodiments with single or multiple forces, the forces include a level of at least about 7,000, 10,000, 12,000, 15,000, 20,000, 25,000, 50,000, 100,000 N or greater combined with stresses of at least about 5,000, 8,000, 13,000, 15,000, 18,000, 20,000, 22,000, or 25,000 psi. In the present process these forces are imparted via a non-vibratory apparatus which does not contain any free flowing media. Media of an apparatus used in the present process are attached to a support, and the described forces are generated using centrifugal, hydraulic, or spring mechanisms and applied to the media and/or a grinding surface.

[0083] For illustrative purposes, a diagram of a small scale apparatus for imparting compression, impact and shear forces using centrifugal motion is shown in FIG. **1**. This apparatus is a vibratory mill which has free flowing media. A top view of the apparatus is shown (**10**). A puck (**12**) is centrally located in a chamber (**18**), which is attached to an eccentric motor. Two rings (**14** and **16**) surround the puck, one inside the other, in the chamber (**18**). When driven by the eccentric motor, the puck and rings produce centrifugal motion, applying compression and impact forces to biomass contained within the chamber.

[0084] An example of a non-vibratory apparatus without free flowing media for imparting compression, impact and shear forces that may be used in the present process is a ring-roller mill (Perry's Chemical Engineers' Handbook, 8^{th} Edition, p 21-60). One mill of this type which generates centrifugal force is a centrifugal force roller mill.

[0085] In one embodiment, force is applied through centrifugal motion in a centrifugal force roller mill using at least one grinding medium that is attached to a rotating shaft. The grinding medium revolves around the rotating shaft with an angular frequency to create a centrifugal force and swings against a grinding surface thereby applying compression, impact, and shearing forces to starch-based biomass which is between the medium and grinding surface in the apparatus. In one embodiment the medium is an object with a mass of at least 100 kilograms.

[0086] An example of this apparatus is shown in FIG. **2**, with a side view in A and a top view in B (the two arrows labeled **2**B in A show the region of the top view). A drive shaft (**20**) that rotates (**32**) is attached to a cross bar (**22**) that has a hinge (**26**) at each end. The hinge at each end attaches a rod (**24**) to the cross bar. Attached to the rod is a roller (**28**) which rotates (**34**). As the shaft turns, the rod swings (**36**) at the hinge and the roller contacts the grinding surface (**30**). In FIG. **2**B is shown that the grinding surface (**30**) is a continuous ring that the rollers continuously make contact with as the drive shaft (**20**) rotates. The shape of the roller can be tapered in any manner and the grinding surface can be angled in any manner

such that when the drive shaft rotates and the rollers swing out, the roller makes contact with the grinding surface. Contact may be either over the total surface of the roller, or over a substantial portion of the surface of the roller. Preferably, the contact is substantially uniform along the length of the roller.

[0087] The centrifugal force of this type of mill is related to the mass of the medium, the center-of-mass radius of the medium, and the angular frequency of rotation, as described in Equation 1, and will vary depending on these aspects of an apparatus used in the present process. The magnitude of centrifugal force [N] is described by:

$$F=mr\omega^2 \qquad \text{[Equation 1]}$$

where m represents the mass [kg] of the media, r represents the center-of-mass radius of the media [m], and ω represents the angular frequency [rad/s] of rotation.

[0088] From the Hertzian contact equations, the contact stress formed by contact of two cylinders is calculable. For a concave cylinder (ring) and a convex cylinder (roller) the calculation of the Hertzian contact half-width, b, and stress, σ_H, is calculated by the following equations:

$$b=\sqrt{\frac{2F}{\pi i}}\times\sqrt{\frac{\frac{(1-\nu_1^2)}{E_1}+\frac{(1-\nu_2^2)}{E_2}}{\frac{1}{d_1}-\frac{1}{d_2}}} \qquad \text{Equation 2}$$

$$\sigma_H=\sqrt{\frac{2F}{\pi i}}\times\sqrt{\frac{\frac{1}{d_1}\frac{1}{d_2}}{\frac{(1-\nu_1^2)}{E_1}+\frac{(1-\nu_2^2)}{E_2}}} \qquad \text{Equation 3}$$

[0089] Where F is the force between the two cylinders (centrifugal force in this case), E_1 and E_2 are the elastic moduli for the roller and ring, respectively, d_1 and d_2 are the diameters of the convex and concave surfaces (e.g. roller and ring (or chamber)), respectively, l is the contact length along the cylinders, and v_1 and v_2 are the Poisson ratios for the roller and ring, respectively.

[0090] In an apparatus with low masses of rollers and low center of mass radii, the G-forces required to achieve a desired force are high, (for example, greater than 15 G) such as a combination of ~1,500 N and G-forces of 21.5 G. However lower G-force is desired to reduce the power requirement. In one embodiment an apparatus applies to the starch-based biomass greater than 5,000 N and the G-force is less than 10 G. In another embodiment an apparatus applies to the starch-based biomass at least about 10,000 N and the G-force is about 5 G.

[0091] A centrifugal force roller mill can contain a wide variety of dimensions based on the scale of the operation, such as a ring diameter of between about 12 and120 inches, roller diameters of between about 4 and 40 inches, and heights of the ring and roller of between about 3 and 30 inches. Mills with different dimensions can be operated with rpm values from about 50 to about 500 rpm. A mill used in the present process additionally may be equipped with a gas conveying system with a recycle classifier and cyclone for product recovery. The gas composition and feed rate may be adjusted to prevent dust explosions. One skilled in the art can choose the mill size, rpm, and required motors to drive the mill to obtain the desired force, which is governed by Equation 1 above. The residence time in the mill can be tuned by modifying classifier and air handling systems, to ensure that the starch-based material that exits the mill contains destructured starch. One of skill in the art can modify the system to achieve the required force, residence time, and power input to achieve energy input of less than 40% of the starting total combustible energy of the input starch-based biomass.

[0092] To achieve processed energy equivalency greater than 60% for starch-based biomass, mill dimensions are tailored to reach high performance in a centrifugal force roller mill. The ratio between the radius of the media and radius of the ring or chamber, herein referred to as Radii Ratio, is a defining parameter in stress calculations (Equation 4).

$$\text{RadiiRatio}=\text{radius}_{media}/\text{radius}_{chamber} \qquad \text{Equation 4}$$

[0093] When operating the roller mill with high centrifugal force, a preferred geometric arrangement for any force magnitude is when the Radii Ratio equals 1:3 (0.33). At a Radii Ratio of 0.33 the pressure reaches a maximum. For a non-vibratory mill with a central driving shaft, the maximum Radii Ratio is ~1:2 (0.5), defined by the distance between the chamber radius and the central shaft. Thus in the present process wherein centrifugal motion is used to impart the described forces, the Radii Ratio is less than 0.5. In one embodiment the Radii Ratio is less than 0.4. In one embodiment the Radii Ratio is about 0.33, which is used to achieve maximal pressure.

[0094] Mill dimensions that approximately represent a Radii Ratio of 1:3 are desired to achieve an energy equivalency greater than 60% for processed starch-based biomass. In one embodiment, the mill cfRRM-1 in Table 1 represents a mill with a chamber diameter of 40 inches, roller height of 10 inches, and operates at 90 rpm. For this fixed rpm, roller height, and chamber diameter, the optimal roller radius to achieve a peak pressure would be 6.67 inches (16.94 cm) (Radii Ratio=1/3 or 0.33). The minimum Radii Ratio for the cfRRM-1's fixed ring diameter, roller height, and rpm would be 0.3129 to achieve a minimum of 5,000 N and 5,000 psi (34.47 MPa). The centrifugal force on the system can be changed by increasing or decreasing the contact length of the mass-ring interface (i.e. making the roller and grinding surface longer). For example, increasing the contact length effectively increases the mass of the roller and hence increases the centrifugal force (assuming all else is constant, see Equation 1). Changing of the contact length while all else is kept constant will not affect the Hertzian stress calculation.

[0095] In another embodiment, the mill cfRRM-4 in Table 1 represents a mill with a chamber diameter of 100 inches, roller height of 29.2 inches, and operates at 100 rpm. For the fixed rpm, roller height, and chamber diameter, the optimal roller radius to achieve a peak pressure would be 16.67 inches (42.34 cm) (Radii Ratio=1/3 or 0.33). The minimum Radii Ratio for the cfRRM-4's fixed ring diameter, roller height, and rpm would be 0.0610 to achieve a minimum of 5,000 N and 5,000 psi (34.47 MPa).

[0096] With an optimized Radii Ratio, such as those in Table 1, much higher forces and stresses can be achieved. In various embodiments the stress is greater than about 5,000, 8,000, 13,000, 15,000, 18,000, 20,000, 22,000, or 25,000 psi (34.37, 55.16, 89.63, 103.42, 124.11, 137.90, 151.68, 172.37 MPa).

[0097] Equation 6 shows a modified Hertzian equation from Equation 3, which illustrates the embodiments mentioned above. Equation 6 is derived from combining Equa-

tions 4 and 5 with Equation 3, to yield a simplified equation relating Hertzian contact theory to the Radii Ratio that defines a mill.

$$F = \pi * (radius_{mass})^2 * l * (radius_{chamber} - radius_{mass}) * \omega^2 \quad \text{Equation 2}$$

$$\text{Pressure} = radius_{chamber} * \omega * \sqrt{\frac{\rho * \text{ratio} * (\text{ratio} - 1)^2}{\frac{(1 - \nu_1^2)}{E_1} + \frac{(1 - \nu_2^2)}{E_2}}} \quad \text{Equation 6}$$

[0098] In another embodiment, the force is applied using spring and/or hydraulic mechanisms. An apparatus for applying force in this manner typically has one or more milling media (e.g. rollers) that are forced against a grinding surface (e.g. a ring). The apparatus may function by moving the grinding surface (such as by rotating a shaft to which the ring is attached) while the grinding media remain motionless, which causes material to be fed through and ground between the media and the surface. An apparatus of this type is available commercially such as from FLSmidth (Bethlehem, Pa.), ALSTOM Power Inc. (Windsor, Conn.), and Babcock & Wilcox (Charlotte, N.C.). Alternatively, this type of apparatus may function by moving grinding media attached to a central rotating shaft and keeping the grinding ring stationary. Further, both the media and the surface may move, but motion relative to each other is required to cause grinding.

[0099] In one embodiment each individual application of force occurs in less than ten milliseconds. More typically, each individual application of force occurs in between 1 and 200 microseconds. In one embodiment the compression, impact, and shearing forces are applied with a specific energy input that is less than 40% of the total combustible energy of the starch-based biomass being treated, as further described below. A low energy input is achieved by application of the described high forces together with a throughput of starch-based biomass that achieves a power draw that is less than 40% of the total combustible energy of the starch-based biomass being treated. The high forces (>5,000 N) are used to achieve a stress of at least 5,000 psi. This process performs mechanical destructuring of the starch-based biomass such that it produces a destructured biomass product. At constant power, for lower time periods over which forces are applied, the specific energy requirement is lowered compared to methods requiring longer times, thereby providing a more cost-effective, energy-efficient pretreatment process.

[0100] In one embodiment the compression and impact forces applied to the starch-based biomass are applied by surfaces which are not intentionally textured as in a cog ring or gear type media. Thus the surfaces of the media and the grinding surface are considered to be smooth, although there may be imperfections in these surfaces. Owing to the isotropic form of a smooth surface of a circular ring, the wear resistance is expected to be higher.

[0101] The specific energy input [kJ/g of dry biomass] required to convert an amount of starch-based biomass to a destructured product may be calculated by dividing the steady state power output [kW] of the apparatus used by the throughput [g/s]. The power output for any auxiliary equipment required for the operation of the apparatus, such as a blower and classifier, is added to the power output of the mill. The specific energy input is reduced for a high force centrifugal apparatus as compared to other processes.

[0102] In the present process the destructured starch-based biomass is produced with high compression and impact forces in combination with some shear from the rolling action of the media against a surface. The mechanical energy input that is required to achieve starch-based biomass destructuring can be compared to the total combustible energy of the biomass. The total combustible energy of the biomass, or higher heating value (HHV) or Gross Calorific Value (GCV), can be measured according to the American Society for Testing and Materials procedure ASTM-D2015. For example, the total combustible energy value for corn is generally 18,000 to 20,000 kj/kg. The present process is performed such that the energy input for obtaining destructured corn is less than 40% of this value, such that the destructured starch-based biomass has a processed energy equivalency of greater than 60%. Energy input may be less than 40%, 35%, 30%, 25% or less, which gives 60%, 65%, 70%, 75% or greater energy equivalency.

[0103] In one embodiment energy input of less than 40% of total combustible energy of the biomass feed is used to produce a destructured biomass in a ring and puck mill, wherein a force of at least 1,500N creates a stress of greater than 5,000 psi. The force may be at least 1,500, 3,000, 5,000, 10,000, 12,000, 15,000, 20,000, 25,000, 50,000, 100,000 N or greater. The stress may be greater than 5,000, 8,000, 13,000, 15,000, 18,000, 20,000, 22,000, or 25,000 psi. Energy input may be less than 40%, 35%, 30%, 25% or less which gives 60%, 65%, 70%, 75% or greater energy equivalency, respectively.

[0104] Starch-based biomass treated using the present process contains destructured starch. The destructured starch-based biomass has crystallinity that is reduced, as compared to the crystallinity of the starch-based biomass which is not treated, by at least about 25%. In various embodiments, crystallinity of starch-based biomass may be reduced by at least about 25%, 30%, 35%, 40%, 45%, 50%, or greater.

[0105] Starch-based biomass may contain a fiber component that contains any of cellulose, hemicellulose, and lignin. This component is cellulosic biomass, and occurs for example in the embryo or germ of a grain. The forces used in the present process also act to destructure cellulosic (including lignocellulosic) biomass that is present in a starch-based biomass. In one embodiment is a destructured starch-based biomass that includes destructured starch and a destructured cellulosic biomass component. Sugars may be released from the destructured cellulosic biomass component using saccharification enzymes that degrade cellulose and/or hemicellulose. Enzymes that may be used include saccharification enzyme preparations that are commercially available such as Spezyme® CP cellulase, Multifect® xylanase, Accelerase® 1500, Accellerase® DUET, and Accellerase® Trio™ (Dupont™/Genencor®, Wilmington, Del.), and Novozyme-188 (Novozymes, 2880 Bagsvaerd, Denmark). In addition, the saccharification enzymes may be unpurified and provided as a cell extract or a whole cell preparation. The enzymes may be engineered and may be produced using recombinant microorganisms that have been engineered to express one or more saccharifying enzymes.

[0106] Starch-based biomass may contain an oil component, such as in the embryo or germ of a grain. In destructured starch-based biomass more oil is substantially free, as compared to oil in starch-based biomass that is not destructured as described herein. Free oil is defined as oil that is obtained by

a gentle hexane extraction, such as oil obtained by following the method of "Oil analysis" as described in General Methods herein.

Animal Feed or Feed Additive

[0107] In some embodiments the destructured starch-based biomass obtained in accordance with the present invention and/or the starch-based biomass hydrolysate (which refers to a product resulting from saccharification (suitably enzyme saccharification) of the destructured starch-based biomass) is used in feed (e.g. animal feed) applications, e.g. as a feed additive composition, a premix, or a feedstuff or a part of any one of a feed additive composition, a premix, or a feedstuff.

[0108] The destructed starch-based biomass and/or the starch-based biomass hydrolysate may be used in combination with at least one in-feed enzyme.

[0109] When the destructured starch-based biomass or the starch-based biomass hydrolysate is used in combination with the at least one in-feed enzyme, the combination may be fed to an animal immediately after admixing the destructured starch-based biomass or the starch-based biomass hydrolysate with the at least one in-feed enzyme.

[0110] In other embodiments the in-feed enzyme is maintained in an inactive form on the destructured starch-based biomass or the starch-based biomass hydrolysate, until such time that the enzyme enters the gastrointestinal tract (GIT) of an animal. In such instances, when the enzyme enters the GIT of the animal the enzyme is activated (e.g. becomes active).

[0111] In other embodiments, the in-feed enzyme is selected from the group consisting of α-amylases (E.G. 3.2.1.1), β-amylases (E.G. 3.2.1.2), γ-amylases (E.G. 3.2.1.3), glucoamylases, cellulases, in particular endo-1,4-β-glucanases (EC 3.2.1.4) or endo-1,3-β-glucanases (3.2.1.6), xylanases, in particular endo-1,4-β-glucanases (EC 3.2.1.8) or xylan-endo-1,3-β-xylosidases (EC 3.2.1.32), hemicellulases, α-galactosidases (EC 3.2.1.22), polygalacturonases (EC 3.2.1.15), cellulose-1,4-β-cellobiosidase (EC 3.2.1.91), endoglucanases, in particular endo-1,6-6-glucanases (EC 3.2.1.75), endo-1,2-β-glucanases (EC 3.2.1.71), endo-1,3-β-glucanases (EC 3.2.1.39) or endo-1,3-α-glucanases (EC 3.2.1.59), lipases, proteases, in particular subtilisins (E.G. 3.4.21.62), bacillolysins (E.G. 3.4.24.28), alkaline serine proteases (E.G. 3.4.21.x), keratinases (E.G. 3.4.x.x) or metalloproteases, mannanases, in particular endo-beta-mannanases, esterases, exo-mannanases, galactanases, xylanases, transferases, alpha-galactosidases, arabinosidases, aryl esterases, beta-galactosidases, catalases, cellobiohydrolases, cutinases, keratinases, laccases, lactases, ligninases, lipoxygenases, oxidases, pectate lyases, pectin acetyl esterases, pectinases, pentosanases, peroxidases, phenoloxidases, phosphatases, phospholipases, polygalacturonases, pullulanases, rhamnogalacturonases, tannases, transglutaminases, xylan acetyl-esterases, xyloglucanases, xylosidases, additional phytases, and combinations thereof.

[0112] In a preferred embodiment, the in-feed enzyme is a glucoamylase.

[0113] The term "in-feed" as used herein means that the enzyme is functional, preferably primarily functional, more preferably solely functional, in the GIT of the animal. In other words, the term "in-feed" as used herein means that the enzyme is substantially inactive (or is inactive) in the feed additive composition and/or on the destructured starch-based biomass or the starch-based biomass hydrolysate prior to feeding the feed additive composition or feedstuff comprising same to an animal.

[0114] The term "primarily functional" means that the enzyme mainly functions on its substrate once it enters the GIT. In other words, prior to entering the GIT the level of enzyme activity defined as the amount of solubilisation of biomass material to oligosaccharides and monosaccharides is less than 20%, suitably less than 10%, preferably less than 5%, of the level of enzyme activity after it enters the GIT (particularly, after it enters the small intestine of the GIT).

[0115] The term "solely functional" as used herein means that the enzyme is inactive before entering the GIT and is activated upon entering the GIT.

[0116] The term "inactive" as used herein means that the enzyme is not active. This may mean that the enzyme's activity is somehow inhibited or that the enzyme is in an environment in which it is inactive or that the enzyme is presented to its substrate immediately prior to feeding to the animal such that there is not enough time to be active. The "inactivity" of the enzyme is in any event reversible once it enters the GIT of an animal.

[0117] The term "substantially inactive" as used herein means that the enzyme has low activity compared with its activity once it has entered the GIT (e.g. in the small intestine of the animal). For instance, substantially inactive may mean that the enzyme in the feed additive composition and/or on the lignocellulosic biomass has less than 10% of its activity when compared with its activity in the GIT (particularly, in the small intestine of the GIT).

[0118] Maintaining the "in-feed" enzyme in an inactive or substantially inactive state in the feed additive composition and/or on the destructured starch-based biomass or the starch-based biomass hydrolysate can be achieved in a number of ways known to one skilled in the art.

[0119] By way of example only maintaining the water content (wt %) of the destructured starch-based biomass or the starch-based biomass hydrolysate and/or of the in-feed enzyme and/or of the feed additive composition at less than 15%, preferably less than 10%, is sufficient to ensure that the in-feed enzyme is inactive or substantially inactive in the feed additive composition and/or on the destructured starch-based biomass or on the starch-based biomass hydrolysate.

[0120] Therefore in one embodiment the in-feed enzyme may be admixed with the destructured starch-based biomass or the starch-based biomass hydrolysate when the destructured starch-based biomass or the starch-based biomass hydrolysate, the in-feed enzyme or both are in a dry state or a substantially dry state.

[0121] In one embodiment the destructured starch-based biomass or the starch-based biomass hydrolysate and/or the in-feed enzyme and/or the feed additive composition, post-admixing the destructured starch-based biomass or the starch-based biomass hydrolysate and the in-feed enzyme, are (maintained and/or stored) in a dry state or substantially dry state.

[0122] The term "dry state" as used herein means that the destructured starch-based biomass or the starch-based biomass hydrolysate and/or the in-feed enzyme and/or the feed additive composition contains no or only a very low amount of water. In other words the term "dry state" as used herein may mean that the destructured starch-based biomass or the starch-based biomass hydrolysate and/or the in-feed enzyme

and/or the feed additive composition comprises less than 5%, preferably less than 1%, water content (wt %).

[0123] The term "substantially dry state" as used herein means that the destructured starch-based biomass or the starch-based biomass hydrolysate and/or the in-feed enzyme and/or the feed additive composition contains only a very low amount of water. In other words the term "substantially dry state" as used herein may mean that the destructured starch-based biomass or the starch-based biomass hydrolysate and/or the in-feed enzyme and/or the feed additive composition comprises less than 15%, preferably less than 10%, water content (wt %).

[0124] In one embodiment, the method according to the present invention may comprise drying the destructured starch-based biomass or the starch-based biomass hydrolysate prior to, during or after (preferably prior to) admixing the biomass with at least one in-feed enzyme.

[0125] In another embodiment the destructured starch-based biomass or the starch-based biomass hydrolysate either before or after adding the at least one in-feed enzyme comprises less than 15 wt % moisture content.

[0126] In another embodiment the destructured starch-based biomass or the starch-based biomass hydrolysate either before or after adding the at least one in-feed enzyme comprises less than 10 wt % moisture content.

[0127] In another embodiment the destructured starch-based biomass or the starch-based biomass hydrolysate either before or after adding the at least one in-feed enzyme comprises less than 5 wt % moisture content.

[0128] In another embodiment the destructured starch-based biomass or the starch-based biomass hydrolysate either before or after adding the at least one in-feed enzyme comprises less than 1 wt % moisture content.

[0129] The "in-feed" enzyme may be maintained in an inactive or substantially inactive state in the feed additive composition and/or on the destructured starch-based biomass or the starch-based biomass hydrolysate by physically preventing the enzyme from interacting with its substrate. For example the in-feed enzyme may be encapsulated prior to admixing with the destructured starch-based biomass or the starch-based biomass hydrolysate.

[0130] When the in-feed enzyme is physically prevented from interacting with its substrate in the destructured starch-based biomass or the starch-based biomass hydrolysate, then once in the GIT the physical barrier is removed thus allowing the interaction of the in-feed enzyme with its substrate.

[0131] By way of example only, the encapsulation may be removed by passage of the encapsulated enzyme through the stomach of an animal. The stomach of an animal is at very low (acidic) pH (e.g. pH 2-4). This acidity can be used to activate encapsulated enzymes.

[0132] In one embodiment the enzyme may be encapsulated by a polymer, such as chitin or chitosans, gelatin, gum arabic or wax for example. By way of example only the polymer may be a gelatin or gum arabic as taught in Xue et al Food Funct. 2013, Apr. 25; 6 February (epub); 4 (4) 610-7 (which is incorporated herein by reference). Alternatively, the polymer may a chitosan-based hydrogel as taught in Zhang et al Biomacromolecules 2011, 12,2894-2901 (which is incorporated herein by reference).

[0133] In one embodiment the at least one in-feed enzyme may be activated by feeding the at least one in-feed enzyme to an animal.

[0134] The term "inactive" as used herein may mean that the enzyme is presented to its substrate immediately prior to feeding to the animal such that there is not enough time to be active before it enters the GIT of the animal.

[0135] In one embodiment the at least one in-feed enzyme may be admixed with the destructured starch-based biomass or the starch-based biomass hydrolysate immediately prior to feeding the feed additive composition or feedstuff comprising the destructured starch-based biomass or the starch-based biomass hydrolysate to an animal.

[0136] In one preferred embodiment the in-feed enzyme is maintained in an inactive or substantially inactive state by maintaining the feed additive composition in a dry state or substantially dry state. This has additional benefits in that the handling, e.g. processing, packaging, storage and transport of a dry composition is easier than non-dry formulations (e.g. liquids).

[0137] However, it can also be envisaged that a kit where the destructured starch-based biomass or the starch-based biomass hydrolysate is physically separated from the in-feed enzyme (e.g. by being in separate containers) would enable the end user (e.g. farmer) to admix the destructured starch-based biomass or the starch-based biomass hydrolysate with the in-feed enzyme immediately prior to feeding the mixture to the animal. In such a situation the destructured starch-based biomass or the starch-based biomass hydrolysate and/or the in-feed enzyme may be in any formulation, e.g. solid, semi-solid or liquid for example.

[0138] The destructured starch-based biomass or the starch-based biomass hydrolysate can have even more nutritional value for use as a feed if one combines this biomass with in-feed enzymes.

In-Feed Enzymes

[0139] In one embodiment the enzyme composition comprises at least two (suitably at least three) enzymes. The enzyme composition comprising the at least two (suitably at least three) enzymes preferably has at least two (suitably at least three) of the following: an endoglucanase, an endoxylanase or a β-glucosidase.

[0140] In one embodiment the at least one in-feed enzyme (s) may be a single enzyme or a combination of enzymes (e.g. an enzyme mix).

[0141] In one preferred embodiment the at least one in-feed enzyme is an enzyme mixture.

[0142] Preferably the in-feed enzyme in accordance with the present invention is stable and active in the gastrointestinal tract (GIT) of an animal. In one embodiment the in-feed enzyme is resistant to pepsin. In one embodiment the in-feed enzyme is tolerant to bile salts. In one embodiment the in-feed enzyme is resistant to low pH. In one embodiment the in-feed enzyme can withstand pelleting temperatures (70-95° C.). In one embodiment the in-feed enzyme is active in the range of 37-40° C.

Biophysical Characteristics

[0143] The present invention also relates to uses and methods for improving the biophysical characteristics of an animal by administering to an animal an effective amount of a feed additive composition or a feedstuff according to the present invention.

[0144] As used herein the term "biophysical characteristics" as used herein means one or more of the group selected

from the following: performance of an animal, growth performance of an animal, feed conversion ratio (FCR), ability to digest a raw material (e.g. nutrient digestibility, including starch , fat, protein, fibre digestibility), nitrogen retention, carcass yield, growth rate, weight gain, body weight, mass, feed efficiency, body fat percentage, body fat distribution, growth, egg size, egg weight, egg mass, egg laying rate and environmental impact, e.g. manure output and/or nitrogen excretion.

[0145] In one embodiment the biophysical characteristic of the animal means the performance of the animal.

Performance

[0146] As used herein, "performance of the animal" may be determined by the feed efficiency and/or weight gain of the animal and/or by the feed conversion ratio and/or by the digestibility of a nutrient in a feed (e.g. amino acid digestibility) and/or digestible energy or metabolizable energy in a feed and/or by nitrogen retention.

[0147] Preferably "performance of the animal" is determined by feed efficiency and/or weight gain of the animal and/or by the feed conversion ratio (FCR).

[0148] By "improved performance of the animal" it is meant that there is increased feed efficiency, and/or increased weight gain and/or reduced feed conversion ratio and/or improved digestibility of nutrients or energy in a feed and/or by improved nitrogen retention in the animal resulting from the use of feed additive composition of the present invention compared with feeding the animal the lignocellulosic biomass which has not been treated in accordance with the present invention.

[0149] Preferably, by "improved animal performance" it is meant that there is increased feed efficiency and/or increased weight gain and/or reduced feed conversion ratio.

[0150] As used herein, the term "feed efficiency" refers to the amount of weight gain in an animal that occurs when the animal is fed ad-libitum or a specified amount of food during a period of time.

[0151] By "increased feed efficiency" it is meant that the use of a feed additive composition according the present invention in feed results in an increased weight gain per unit of feed intake compared with an animal fed with the lignocellulosic biomass which has not been treated in accordance with the present invention.

Feed Conversion Ration (FCR)

[0152] As used herein, the term "feed conversion ratio" refers to the amount of feed fed to an animal to increase the weight of the animal by a specified amount.

[0153] An improved feed conversion ratio means a lower feed conversion ratio.

[0154] By "lower feed conversion ratio" or "improved feed conversion ratio" it is meant that the use of a feed additive composition in feed results in a lower amount of feed being required to be fed to an animal to increase the weight of the animal by a specified amount compared to the amount of feed required to increase the weight of the animal by the same amount when the lignocellulosic biomass which has not been treated in accordance with the present invention is used in or as the feed.

Nutrient Digestibility

[0155] Nutrient digestibility as used herein means the fraction of a nutrient that disappears from the gastro-intestinal tract or a specified segment of the gastro-intestinal tract, e.g. the small intestine. Nutrient digestibility may be measured as the difference between what is administered to the animal and what comes out in the faeces of the animal, or between what is administered to the animal and what remains in the digesta on a specified segment of the gastro intestinal tract, e.g. the ileum.

[0156] Nutrient digestibility as used herein may be measured by the difference between the intake of a nutrient and the excreted nutrient by means of the total collection of excreta during a period of time; or with the use of an inert marker that is not absorbed by the animal, and allows the researcher calculating the amount of nutrient that disappeared in the entire gastro-intestinal tract or a segment of the gastro-intestinal tract. Such an inert marker may be titanium dioxide, chromic oxide or acid insoluble ash. Digestibility may be expressed as a percentage of the nutrient in the feed, or as mass units of digestible nutrient per mass units of nutrient in the feed.

[0157] Nutrient digestibility as used herein encompasses starch digestibility, fat digestibility, protein digestibility, fiber digestibility and amino acid digestibility.

[0158] Energy digestibility as used herein means the gross energy of the feed consumed minus the gross energy of the faeces or the gross energy of the feed consumed minus the gross energy of the remaining digesta on a specified segment of the gastro-intestinal tract of the animal, e.g. the ileum. Metabolizable energy as used herein refers to apparent metabolizable energy and means the gross energy of the feed consumed minus the gross energy contained in the faeces, urine, and gaseous products of digestion. Energy digestibility and metabolizable energy may be measured as the difference between the intake of gross energy and the gross energy excreted in the faeces or the digesta present in specified segment of the gastro-intestinal tract using the same methods to measure the digestibility of nutrients, with appropriate corrections for nitrogen excretion to calculate metabolizable energy of feed.

Nitrogen Retention

[0159] Nitrogen retention as used herein means an animal's ability to retain nitrogen from the diet as body mass. A negative nitrogen balance occurs when the excretion of nitrogen exceeds the daily intake and is often seen when the muscle is being lost. A positive nitrogen balance is often associated with muscle growth, particularly in growing animals.

[0160] Nitrogen retention may be measured as the difference between the intake of nitrogen and the excreted nitrogen by means of the total collection of excreta and urine during a period of time. It is understood that excreted nitrogen includes undigested protein from the feed, endogenous proteinaceous secretions, microbial protein, and urinary nitrogen.

Carcass Yield and Meat Yield

[0161] The term carcass yield as used herein means the amount of carcass as a proportion of the live body weight, after a commercial or experimental process of slaughter. The term carcass means the body of an animal that has been slaughtered for food, with the head, entrails, part of the limbs,

and feathers or skin removed. The term meat yield as used herein means the amount of edible meat as a proportion of the live body weight, or the amount of a specified meat cut as a proportion of the live body weight.

Weight Gain

[0162] The present invention further provides a method of increasing weight gain in an animal, e.g. poultry or swine, comprising feeding said animal a feedstuff comprising a feed additive composition according to the present invention.

[0163] An "increased weight gain" refers to an animal having increased body weight on being fed feed comprising a feed additive composition compared with an animal being fed a feed comprising or consisting of lignocellulosic biomass which has not been treated in accordance with the present invention.

Improving

[0164] The term "improving" as used herein means improved compared with feeding animal the lignocellulosic biomass which has not been treated in accordance with the present invention.

Admixing

[0165] In one embodiment "admixing" as used herein includes any method for admixing, such as mixing, combining, spraying etc.).

Animal

[0166] The term "animal", as used herein, means an animal that is to be or has been administered with a feed additive composition according to the present invention or a feedstuff comprising said feed additive composition according to the present invention.

[0167] Preferably, the animal is a mammal (e.g. a non-human mammal), bird, fish or crustacean including for example livestock or a domesticated animal (e.g. a pet).

[0168] In one embodiment the "animal" is livestock.

[0169] The term "livestock", as used herein refers to any farmed animal. Preferably, livestock is one or more of cows or bulls (including calves), pigs (including piglets, swine), poultry (including broilers, layers, chickens and turkeys), birds, fish (including freshwater fish, such as salmon, cod, trout and carp, e.g. koi carp, and marine fish, such as sea bass), crustaceans (such as shrimps, mussels and scallops), horses (including race horses), sheep (including lambs).

[0170] In one embodiment the animal is poultry (including broilers, layers, chickens and turkeys).

[0171] In another embodiment the "animal" is a domesticated animal or pet or an animal maintained in a zoological environment.

[0172] The term "domesticated animal or pet or animal maintained in a zoological environment" as used herein refers to any relevant animal including canines (e.g. dogs), felines (e.g. cats), rodents (e.g. guinea pigs, rats, mice), birds, fish (including freshwater fish and marine fish), and horses.

[0173] In one embodiment the animal is a monogastric animal. In a preferred embodiment the monogastric animal may be poultry or pig (or a combination thereof).

[0174] In another embodiment the animal is a ruminant animal.

Packaging

[0175] In one embodiment the enzyme composition and/or feed additive composition and/or premix and/or feed or feedstuff according to the present invention is packaged.

[0176] In one preferred embodiment feed additive composition and/or feed ingredient and/or premix and/or feed or feedstuff is packaged in a bag, such as a paper bag.

[0177] In an alternative embodiment the enzyme composition and/or feed additive composition and/or feed ingredient and/or premix and/or feed or feedstuff may be sealed in a container. Any suitable container may be used.

Feed

[0178] The destructured starch-based biomass or the starch-based biomass hydrolysate (with or without in feed enzyme(s)) may be used as or in the preparation of a feed additive composition or a feed.

[0179] The feed additive composition of the present invention may be used as—or in the preparation of—a feed.

[0180] The term "feed" is used synonymously herein with "feedstuff".

[0181] The feed may be in the form of a solution or as a solid—depending on the use and/or the mode of application and/or the mode of administration.

[0182] When used as—or in the preparation of—a feed—such as functional feed—the destructured starch-based biomass or the starch-based biomass hydrolysate (with or without in feed enzyme(s)) of the present invention may be used in conjunction with one or more of: a nutritionally acceptable carrier, a nutritionally acceptable diluent, a nutritionally acceptable excipient, a nutritionally acceptable adjuvant, a nutritionally active ingredient.

[0183] In a preferred embodiment the destructured starch-based biomass or the starch-based biomass hydrolysate (with or without in feed enzyme(s)) or the feed additive composition of the present invention is admixed with a feed component to form a feedstuff.

[0184] The term "feed component" as used herein means all or part of the feedstuff. Part of the feedstuff may mean one constituent of the feedstuff or more than one constituent of the feedstuff, e.g. 2 or 3 or 4. In one embodiment the term "feed component" encompasses a premix or premix constituents.

[0185] Preferably the feed may be a fodder, or a premix thereof, a compound feed, or a premix thereof. In one embodiment the feed additive composition according to the present invention may be admixed with a compound feed, a compound feed component or to a premix of a compound feed or to a fodder, a fodder component, or a premix of a fodder.

[0186] The term fodder as used herein means any food which is provided to an animal (rather than the animal having to forage for it themselves). Fodder encompasses plants that have been cut. The term fodder includes hay, straw, silage, compressed and pelleted feeds, oils and mixed rations, and also sprouted grains and legumes.

[0187] Fodder may be obtained from one or more of the plants selected from: alfalfa (Lucerne), barley, birdsfoot trefoil, brassicas, Chau moellier, kale, rapeseed (canola), rutabaga (swede), turnip, clover, alsike clover, red clover, subterranean clover, white clover, grass, false oat grass, fescue, Bermuda grass, brome, heath grass, meadow grasses (from naturally mixed grassland swards, orchard grass, rye grass,

Timothy-grass, corn (maize), millet, oats, sorghum, soybeans, trees (pollard tree shoots for tree-hay), wheat, and legumes.

[0188] The term "compound feed" means a commercial feed in the form of a meal, a pellet, nuts, cake or a crumble. Compound feeds may be blended from various raw materials and additives. These blends are formulated according to the specific requirements of the target animal.

[0189] Compound feeds can be complete feeds that provide all the daily required nutrients, concentrates that provide a part of the ration (protein, energy) or supplements that only provide additional micronutrients, such as minerals and vitamins.

[0190] The main ingredients used in compound feed are the feed grains, which include corn, soybeans, sorghum, oats, and barley.

[0191] Suitably a premix as referred to herein may be a composition composed of microingredients such as vitamins, minerals, chemical preservatives, antibiotics, fermentation products, and other essential ingredients. Premixes are usually compositions suitable for blending into commercial rations.

[0192] Any feedstuff of the present invention may comprise one or more feed materials selected from the group comprising a) cereals, such as small grains (e.g., wheat, barley, rye, oats and combinations thereof) and/or large grains such as maize or sorghum; b) by products from plants (e.g. cereals), such as wet-cake, distillers dried grain (DDG), and distillers dried grain solubles (DDGS), corn fibre, corn germ meal, corn bran, Hominy feed, corn gluten feed, wheat shorts, wheat middlings or combinations thereof (preferably by products of methods according to the present invention); c) protein obtained from sources such as soya, sunflower, peanut, lupin, peas, fava beans, cotton, canola, fish meal, dried plasma protein, meat and bone meal, potato protein, whey, copra, sesame; d) oils and fats obtained from vegetable and animal sources; e) minerals and vitamins.

[0193] A feedstuff of the present invention may contain at least 30%, at least 40%, at least 50% or at least 60% by weight corn and soybean meal or corn and full fat soy, or wheat meal or sunflower meal.

[0194] In addition or in the alternative, a feedstuff of the present invention may comprise at least one high fibre feed material and/or at least one by-product of the at least one high fibre feed material to provide a high fibre feedstuff. Examples of high fibre feed materials include: wheat, barley, rye, oats, by products from plants (e.g. cereals), such as wet-cake, distillers dried grain (DDG), and distillers dried grain solubles (DDGS), corn fibre, corn germ meal, corn bran, Hominy feed, corn gluten feed, wheat shorts, wheat middlings or combinations thereof. Some protein sources may also be regarded as high fibre: protein obtained from sources such as sunflower, lupin, fava beans and cotton.

[0195] In the present invention the feed may be one or more of the following: a compound feed and premix, including pellets, nuts or (cattle) cake; a crop or crop residue: corn, soybeans, sorghum, oats, barley, corn stover, copra, straw, chaff, sugar beet waste; fish meal; freshly cut grass and other forage plants; meat and bone meal; molasses; oil cake and press cake; oligosaccharides; conserved forage plants: hay and silage; seaweed; seeds and grains, either whole or prepared by crushing, milling etc.; sprouted grains and legumes; yeast extract.

[0196] The term "feed" in the present invention also encompasses in some embodiments pet food. A pet food is plant or animal material intended for consumption by pets, such as dog food or cat food. Pet food, such as dog and cat food, may be either in a dry form, such as kibble for dogs, or wet canned form. Cat food may contain the amino acid taurine.

[0197] The term "feed" in the present invention also encompasses in some embodiments fish food. A fish food normally contains macro nutrients, trace elements and vitamins necessary to keep captive fish in good health. Fish food may be in the form of a flake, pellet or tablet. Pelleted forms, some of which sink rapidly, are often used for larger fish or bottom feeding species. Some fish foods also contain additives, such as beta carotene or sex hormones, to artificially enhance the colour of ornamental fish.

[0198] The term "feed" in the present invention also encompasses in some embodiment bird food. Bird food includes food that is used both in birdfeeders and to feed pet birds. Typically bird food comprises of a variety of seeds, but may also encompass suet (beef or mutton fat).

[0199] As used herein the term "contacting" refers to the indirect or direct application of the destructured starch-based biomass or the starch-based biomass hydrolysate (with or without in feed enzyme(s)) of the present invention to the product (e.g. the feed). Examples of the application methods which may be used, include, but are not limited to, treating the product in a material comprising the feed additive composition, direct application by mixing the feed additive composition with the product, spraying the feed additive composition onto the product surface or dipping the product into a preparation of the feed additive composition.

[0200] In one embodiment the destructured starch-based biomass or the starch-based biomass hydrolysate (with or without in feed enzyme(s)) or the feed additive composition of the present invention is preferably admixed with the product (e.g. feedstuff). Alternatively, the destructured starch-based biomass or the starch-based biomass hydrolysate (with or without in feed enzyme(s)) or the feed additive composition may be included in the emulsion or raw ingredients of a feedstuff.

[0201] For some applications, it is important that the in feed enzyme(s) is made available on or to the surface of a product to be affected/treated. This allows the composition to impart one or more of the following favourable characteristics: biophysical characteristics, e.g. wherein the biophysical characteristic is selected from the group consisting of one or more of the following: performance of an animal, growth performance of an animal, feed conversion ratio (FCR), ability to digest a raw material (e.g. nutrient digestibility, including starch , fat, protein, fibre digestibility), nitrogen retention, carcass yield, growth rate, weight gain, body weight, mass, feed efficiency, body fat percentage, body fat distribution, growth, egg size, egg weight, egg mass, egg laying rate and environmental impact, e.g. manure output and/or nitrogen excretion.

[0202] The feed additive compositions of the present invention may be applied to intersperse, coat and/or impregnate a product (e.g. feedstuff or raw ingredients of a feedstuff) with a controlled amount of enzyme(s).

[0203] Preferably, the enzyme composition and/or feed additive composition of the present invention will be thermally stable to heat treatment up to about 70° C.; up to about 85° C.; or up to about 95° C. The heat treatment may be

performed for up to about 1 minute; up to about 5 minutes; up to about 10 minutes; up to about 30 minutes; up to about 60 minutes. The term thermally stable means that at least about 75% of the enzyme components that were present/active in the additive before heating to the specified temperature are still present/active after it cools to room temperature. Preferably, at least about 80% of the enzyme components that were present and active in the additive before heating to the specified temperature are still present and active after it cools to room temperature.

[0204] In a particularly preferred embodiment the in-feed enzymes may be formulated into an enzyme composition.

[0205] The enzyme composition and/or feed additive composition may be homogenized to produce a powder.

[0206] In an alternative preferred embodiment, the enzyme composition and/or feed additive composition may be formulated to granules as described in WO2007/044968 (referred to as TPT granules) incorporated herein by reference.

[0207] In another preferred embodiment when the enzyme composition and/or feed additive composition is formulated into granules the granules comprise a hydrated barrier salt coated over the protein core. The advantage of such salt coating is improved thermo-tolerance, improved storage stability and protection against other feed additives otherwise having adverse effect on the enzyme.

[0208] Preferably, the salt used for the salt coating has a water activity greater than 0.25 or constant humidity greater than 60% at 20° C.

[0209] Preferably, the salt coating comprises a Na_2SO_4.

[0210] The method of preparing a feed additive composition may also comprise the further step of pelleting the powder. The powder may be mixed with other components known in the art. The powder, or mixture comprising the powder, may be forced through a die and the resulting strands are cut into suitable pellets of variable length.

[0211] Optionally, the pelleting step may include a steam treatment, or conditioning stage, prior to formation of the pellets. The mixture comprising the powder may be placed in a conditioner, e.g. a mixer with steam injection. The mixture is heated in the conditioner up to a specified temperature, such as from 60-100° C., typical temperatures would be 70° C., 80° C., 85° C., 90° C. or 95° C. The residence time can be variable from seconds to minutes and even hours. Such as 5 seconds, 10 seconds, 15 seconds, 30 seconds, 1 minutes 2 minutes., 5 minutes, 10 minutes, 15 minutes, 30 minutes and 1 hour.

[0212] It will be understood that the feed additive composition of the present invention is suitable for addition to any appropriate feed material.

[0213] As used herein, the term feed material refers to the basic feed material to be consumed by an animal. It will be further understood that this may comprise, for example, at least one or more unprocessed grains, and/or processed plant and/or animal material such as soybean meal or bone meal.

[0214] As used herein, the term "feedstuff" refers to a feed material to which one or more feed additive compositions or destructured starch-based biomass or the starch-based biomass hydrolysate (with or without in feed enzyme(s)) have been added.

[0215] It will be understood by the skilled person that different animals require different feedstuffs, and even the same animal may require different feedstuffs, depending upon the purpose for which the animal is reared.

[0216] Preferably, the feedstuff may comprise feed materials comprising maize or corn, wheat, barley, triticale, rye, rice, tapioca, sorghum, and/or any of the by-products, as well as protein rich components like soybean mean, rape seed meal, canola meal, cotton seed meal, sunflower seed mean, animal-by-product meals and mixtures thereof. More preferably, the feedstuff may comprise animal fats and/or vegetable oils.

[0217] Optionally, the feedstuff may also contain additional minerals such as, for example, calcium and/or additional vitamins.

[0218] Preferably, the feedstuff is a corn soybean meal mix.

[0219] In another aspect there is provided a method for producing a feedstuff. Feedstuff is typically produced in feed mills in which raw materials are first ground to a suitable particle size and then mixed with appropriate additives. The feedstuff may then be produced as a mash or pellets; the later typically involves a method by which the temperature is raised to a target level and then the feed is passed through a die to produce pellets of a particular size. The pellets are allowed to cool. Subsequently liquid additives such as fat and enzyme may be added. Production of feedstuff may also involve an additional step that includes extrusion or expansion prior to pelleting—in particular by suitable techniques that may include at least the use of steam.

[0220] The feedstuff may be a feedstuff for a monogastric animal, such as poultry (for example, broiler, layer, broiler breeders, turkey, duck, geese, water fowl), swine (all age categories), a pet (for example dogs, cats) or fish, preferably the feedstuff is for poultry.

Formulation

[0221] In one embodiment the destructured starch-based biomass or the starch-based biomass hydrolysate (with or without in feed enzyme(s)) and/or feed additive composition and/or premix and/or feedstuff in accordance with the present invention are dried.

[0222] The term "drying" means that the water content (wt %) of the destructured starch-based biomass product (with or without in feed enzyme(s)) or starch-based biomass hydrolysate product (with or without in feed enzyme(s)) and/or feed additive composition and/or premix and/or feedstuff in accordance with the present invention is reduced to at less than 15%, preferably less than 10%.

[0223] Therefore the present invention yet further provides a dried or substantially dried destructured starch-based biomass product (with or without in feed enzyme(s)) or starch-based biomass hydrolysate product (with or without in feed enzyme(s)) or feed additive composition or premix or feedstuff.

[0224] The term substantially-dried in accordance with the present invention means that the water content (wt %) of the product/feed additve composition/premix/feedstuff is less than 30%, preferably less than 15%, preferably less than 10%, preferably less than 5%.

[0225] In one embodiment the in-feed enzyme composition may be in a dry enzyme formulation (e.g. in the form of granules or on a carrier (such as a wheat carrier)) prior to admixing with the destructured starch-based biomass or starch-based biomass hydrolysate.

[0226] In one embodiment the saccharification enzyme composition may be in a liquid enzyme formulation prior to admixing with the destructured starch-based biomass.

[0227] In another embodiment the in-feed enzyme composition may be in a liquid formulation prior to admixing with the destructured starch-based biomass or starch-based biomass hydrolysate.

[0228] In another embodiment the saccharification enzyme composition may be in a liquid formulation prior to admixing with the destructured starch-based biomass.

[0229] In some embodiments the present invention may provide a semi-liquid product or slurry product. A semi-liquid product or slurry product in accordance with the present invention is a product the water content (wt %) of which is at less than 90%, preferably less than 80%, preferably less than 70% or more preferably less than 60%.

[0230] When the enzyme is in a liquid formulation prior to admixing with the the destructured starch-based biomass or starch-based biomass hydrolysate, the enzyme may be admixed by spraying the enzyme formulation or dipping the destructured starch-based biomass or starch-based biomass hydrolysate into the enzyme formulation for example.

[0231] The destructured starch-based biomass or starch-based biomass hydrolysate (before or after enzyme treatment) and/or the dried solid fraction may be milled and/or powdered and/or formed into a meal.

[0232] In one embodiment the product and/or feed additive composition and/or premix and/or feedstuff in accordance with the present invention, are packaged and/or stored in a dry state or substantially dry state.

[0233] The terms "dried" or "dry state" as used herein means that the product and/or feed additive composition and/or premix and/or feedstuff in accordance with the present invention contain no or only a very low amount of water. In other words the term "dried" or "dry state" as used herein may mean that the product and/or feed additive composition and/or premix and/or feedstuff in accordance with the present invention comprises less than 5%, preferably less than 1%, water content (wt %).

[0234] The term "substantially dry state" as used herein means that the product and/or feed additive composition and/or premix and/or feedstuff in accordance with the present invention contains only a very low amount of water. In other words the term "substantially dry state" as used herein may mean that the and/or feed additive composition and/or premix and/or feedstuff in accordance with the present invention comprises less than 30%, preferably less than 15%, preferably less than 10%, water content (wt %).

[0235] In one embodiment the product and/or feed additive composition and/or premix and/or feedstuff in accordance with the present invention comprises less than 20 wt % moisture content.

[0236] In another embodiment the product on and/or feed additive composition and/or premix and/or feedstuff in accordance with the present invention comprises less than 15 wt % moisture content.

[0237] In another embodiment the product and/or feed additive composition and/or premix and/or feedstuff in accordance with the present invention comprises less than 10 wt % moisture content.

[0238] In another embodiment the product and/or feed additive composition and/or premix and/or feedstuff in accordance with the present invention comprises less than 5 wt % moisture content.

Sugar Production using High Force Milled Starch-Based Biomass

[0239] In some embodiments the destructured starch-based biomass obtained in accordance with the present process is saccharified to produce sugar-containing compositions. These compositions may be called syrups. Different types of syrups may be produced with use of enzymes having different hydrolytic activities. The exact composition of the products of saccharification depends on the combination of enzymes used, as well as the type of destructured starch-based biomass processed. In various embodiments syrups are produced by processes that omit at least one of: 1) liquefaction and 2) additional alpha-amylase. Destructured starch-based biomass produced using the present method provides a material that is a better substrate for saccharification than a starch-based biomass that is not destructured using the present process.

[0240] In one embodiment the syrup is a composition comprising glucose (a glucose composition) produced by contacting destructured starch-based biomass with glucoamylase and optionally at least one of an alpha-amylase and a phytase. In various embodiments the produced glucose composition has a DP1 concentration higher than would result when saccharifying a whole ground corn liquefact composition in place of the destructured starch-based biomass composition; wherein the higher DP1 concentration is at least 0.1%, 0.2%, 0.4%, or 0.5% higher. In one embodiment the glucose composition has a DP2 concentration lower than would result when saccharifying a whole ground corn liquefact composition in place of the destructured starch-based biomass; wherein DP2 concentration at least 0.1% lower.

[0241] In some embodiments a glucose composition is referred to as a composition comprising fermentable sugars. In addition to contact with a glucoamylase, for production of a composition comprising fermentable sugars, destructured starch-based biomass may be contacted with at least one of an alpha-amylase and a phytase. The glucose composition, or composition comprising fermentable sugars, produced by methods described herein may be used in a fermentation process to produce a product, called herein an End of Fermentation (EOF) product. The glucose composition or composition comprising fermentable sugars may also be called mash.

[0242] In one embodiment the syrup is a composition comprising maltose (maltose composition) produced by contacting destructured starch-based biomass with a maltogenic alpha-amylase. In various embodiments the produced maltose composition has a DP2 concentration higher than would result when saccharifying a whole ground corn liquefact composition in place of the destructured starch-based biomass composition; wherein the higher DP2 concentration is at least 0.1% higher.

[0243] In one embodiment the syrup is a composition comprising maltotriose (maltotriose composition) produced by contacting destructured starch-based biomass with a beta-amylase and optionally a pullulanase. In various embodiments the produced maltotriose composition has lower DP1 and DP2 concentrations than would result when saccharifying a whole ground corn liquefact composition in place of the destructured starch-based biomass composition; wherein the DP1 and DP2 concentrations are at least 0.1% lower.

[0244] In one embodiment the syrup is a composition comprising maltotetraose (maltotetraose composition) produced by contacting destructured starch-based biomass with a DP4

producing alpha-amylase and optionally a pullulanase. In various embodiments the produced maltotetraose composition has a DP4 concentration higher than would result when saccharifying a whole ground corn liquefact composition in place of the destructured starch-based biomass composition; wherein the higher DP4 concentration is at least 0.1%, 0.2%, 0.4%, or 0.5% higher.

[0245] In one embodiment the syrup is a composition comprising maltopentaose (maltopentaose composition) produced by contacting destructured starch-based biomass with a DP5 producing alpha-amylase and optionally a pullulanase. In various embodiments the produced maltopentaose composition has a DP5 concentration higher than would result when saccharifying a whole ground corn liquefact composition in place of the destructured starch-based biomass composition; wherein the higher DP5 concentration is at least 0.1%, 0.2%, 0.4%, 0.5%, 1.0%, 1.5%, or 2.0% higher.

[0246] In some embodiments the high force milled starch-based biomass is processed for saccharification by mixing with an aqueous solution to obtain a slurry. The aqueous solution may be obtained, for example from water, thin stillage and/or backset. A destructured starch-based biomass composition comprises destructured starch-based biomass and optionally other components such as the aqueous solution used to obtain a slurry. A slurry may have dry solids (DS) of between 5-60%; 10-50%; 15-45%; 15-30%; 20-45%; 20-30% and also 25-40%.

[0247] Saccharification is often conducted as a batch process. Saccharification typically is most effective at temperatures of about 55°-75° C. and a pH of about 3.5-7.0, e.g., pH 5.0, preferably at a pH range of 4.0 to 6.0 and more preferably at a pH range of 4.5 to 5.5. Saccharification may be performed, for example, at a temperature between about 40° C., about 55° C., or about 65° C. to about 70° C., about 75° C., or about 80° C. In some embodiments, the temperature is held between 45° C. and 70° C.; in other embodiments, the temperature is held between 50° C. and 70° C.; between 55° C. and 70° C.; between 60° C. and 70° C., between 60° C. and 65° C.; between 55° C. and 65° C. and between 55° C. and 68° C. In further embodiments, the temperature is at least 45° C., 48° C., 50° C., 53° C., 55° C., 58° C., 60° C., 63° C., 65° C. and 68° C. In other embodiments, the temperature is not greater than 65° C., 68° C., 70° C., 73° C., 75° C. and 80° C.

[0248] In various embodiments the destructured starch-based biomass is held in contact with at least one saccharifying enzyme for a period of 5 minutes to 48 hours; or for a period of 5 minutes to 24 hours. In some embodiments the period of time is between 15 minutes and 12 hours, 15 minutes and 6 hours, 15 minutes and 4 hours and also 30 minutes and 2 hours.

[0249] Saccharification is normally conducted in stirred tanks, which may take several hours to fill or empty. Enzymes typically are added either at a fixed ratio to dried solids as the tanks are filled or added as a single dose at the commencement of the filling stage. A saccharification reaction to make a syrup typically is run over about 24-72 hours, for example, 24-48 hours. When a maximum or desired dextrose equivalents (DE) has been attained, the reaction is stopped by heating to 85° C. for 5 min., for example. Further incubation will result in a lower DE, eventually to about 90 DE, as accumulated glucose re-polymerizes to isomaltose and/or other reversion products via an enzymatic reversion reaction and/or with the approach of thermodynamic equilibrium.

[0250] An effective concentration of enzyme used in the contacting step for saccharification will vary according to the specific process conditions and destructured starch-based biomass used. Saccharification may be for any process such as to produce a syrup, to produce fermentable sugars prior to fermentation, or to produce fermentable sugars in a simultaneous saccharification and fermentation process (SSF). Thus in some embodiments the contacting step may occur prior to fermentation or during fermentation.

[0251] In some embodiments, the effective dose of a glucoamylase for the contacting step will be in the range of 0.01 to 10 GAU/g DS; also 0.05 to 10 GAU/g DS; also 0.1 to 5 GAU/g DS.

[0252] In some embodiments, alpha-amylase is included in the contacting step. In some embodiments, the effective dose of alpha-amylase for the contacting step will be in the range of 0.01 to 20 AAU/g DS, 0.01 to 10 AAU/g DS, 0.05 to 10.0 AAU/g DS, 0.5 to10.0 AAU/g DS, and also 0.5 to 2.0 AAU/g DS.

[0253] In various embodiments different types of alpha-amylase are used including maltogenic alpha-amylase, DP4 producing alpha-amylase, and DP5 producing alpha-amylase. In some embodiments, the effective dose of maltogenic alpha-amylase to be used in the contacting step will be in the range of 0.01 to 20 BU/g DS, 0.01 to 15 BU/g DS, 0.05 to 15 BU/g DS, 0.5 to 10 BU/g DS. In some embodiments, the effective dose of DP4 producing alpha-amylase to be used in the contacting step will be in the range of 0.01-4 BMK/g DS, 0.01-2 BMK/g DS. In some embodiments, the effective dose of DP3 producing alpha-amylase to be used in the contacting step will be in the range of 0.01 to 10 SSU/g DS, 0.05-10 SSU/g DS, 0.5 to 10 SSU/g DS and 0.5 to 5 SSU/g DS. In some embodiments, the effective dose of DP5 producing alpha-amylase to be used in the contacting step will be in the range of 0.01-10 μg/g ds, 0.05-10 μg/g ds, 0.1-5 μg/g DS.

[0254] In some embodiments, phytase is included in the contacting step. In some embodiments, the effective dose of phytase to be used in the contacting step will be in the range of 0.001 to 10 FTU/g DS; also 0.005 to 10 FTU/g DS; and also 0.05 to 5 FTU/g DS. One phytase unit (FTU) is the amount of enzyme, which liberates 1 micromole inorganic phosphorus per minute from sodium phytate, 0.0051 moles/liter, at 37° C. and at pH 5.0.

[0255] In some embodiments, a protease is included in the contacting step. In some embodiments, the effective dose of a protease to be used in the contacting step will be in the range of 0.01 to 10 SAPU/g DS; 0.05 to 5 SAPU/g DS, and also 0.1 to 5 SAPU/g DS. SAPU refers a spectrophotometric acid protease unit, wherein 1 SAPU is the amount of protease enzyme activity that liberates one micromole of tyrosine per minute from a casein substrate under conditions of the assay.

[0256] In some embodiments, a pullulanase is included in the contacting step. In some embodiments, the effective dose of a pullulanase to be used in the contacting step will be in the range of 0.01 to 10 ASPU/g DS, also 0.05 to 10 ASPU/g DS; also 0.1 to 5 ASPU/g DS.

[0257] In some embodiments, a beta-amylase is included in the contacting step. In some embodiments, the effective dose of a beta-amylase to be used in the contacting step will be in the range of 0.01 to 15 DP/g DS, 0.05 to 10 DP/g DS, and also 0.1 to 5 DP/g DS.

[0258] In some embodiments, during the contacting step between 25-90% of the destructured starch-based biomass is solubilized to produce oligosaccharides and monosaccha-

rides. In some embodiments, greater than 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% and 90% of the destructured starch is solubilized.

[0259] After contacting the destructured starch-based biomass with glucoamylase, and optionally with at least one of alpha-amylase and phytase, for a period of time as indicated above, a soluble starch substrate (mash) is obtained which comprises greater than 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% or 80% glucose. The soluble starch substrate or mash is also referred to herein as the glucose composition, or the composition comprising fermentable sugars.

[0260] Prior to subjecting the mash including at least 10% glucose to fermentation, the mash may be further exposed to an aqueous solution comprising, for example, backset and/or corn steep (in the case of corn grain) and adjusted to a pH in the range of pH 3.0 to 6.0; pH 3.5 to 5.5, or pH 4.0 to 5.5. In this embodiment of the invention, the % DS of the mash may be diluted. For example, the DS of the diluted mash maybe between 5 to 35%; 5 to 30%; 5 to 25%; 5 to 20%; 5 to 20%; 5 to 15%; and 5 to 10% less than the % DS of the slurry in the contacting step. In one non-limiting example, if the % DS of the slurry in the contacting step is approximately 32% and the mash is further exposed to a diluting aqueous solution which dilutes the DS between 5 to 10%, the DS of the mash to be fermented will be between 22% and 27%. In some specific embodiments, if the DS of the contacting slurry is between 30 to 35%, the DS of the diluted slurry will be about 20 to 30%.

Fermentation

[0261] The composition comprising fermentable sugars (mash) may be used in fermentation using a biocatalyst to produce a product, herein called an End of Fermentation (EOF) product. An EOF product may be any product made by the biocatalyst used in fermentation, such as a metabolite that is naturally produced by the biocatalyst or that the biocatalyst is genetically engineered to produce. Examples of metabolite EOF products include but are not limited to citric acid, lactic acid, succinic acid, monosodium glutamate, gluconic acid, sodium gluconate, calcium gluconate, potassium gluconate, glucono delta-lactone, sodium erythorbate, omega 3 fatty acid, butanol, an amino acid, lysine, itaconic acid, 1,3-propanediol, and isoprene. In one embodiment, the EOF product is ethanol.

[0262] Ethanologenic microorganisms include yeast, such as *Saccharomyces cerevisiae* and bacteria, e.g., *Zymomonas moblis,* expressing alcohol dehydrogenase and pyruvate decarboxylase. The ethanologenic microorganism can express xylose reductase and xylitol dehydrogenase, which convert xylose to xylulose. Improved strains of ethanologenic microorganisms, which can withstand higher temperatures, for example, are known in the art and can be used. See Liu et al. (2011) *Sheng Wu Gong Cheng Xue Bao* 27(7): 1049-56. Commercial sources of yeast include ETHANOL RED® (LeSaffre); Thermosacc® (Lallemand); RED STAR® (Red Star); FERMIOL® (DSM Specialties); and SUPERSTART® (Alltech).

[0263] Microorganisms that produce other metabolites, such as citric acid and lactic acid, by fermentation are also known in the art. See, e.g., Papagianni (2007) "Advances in citric acid fermentation by *Aspergillus niger:* biochemical aspects, membrane transport and modeling," *Biotechnol. Adv.* 25(3): 244-63; John et al. (2009) "Direct lactic acid fermentation: focus on simultaneous saccharification and lactic acid production," *Biotechnol. Adv.* 27(2): 145-52.

[0264] In one embodiment, the mash comprising at least 10% glucose is subjected to fermentation processes using fermenting microorganisms as described above. These fermentation processes are described in The Alcohol Textbook 3rd ED, A Reference for the Beverage, Fuel and Industrial Alcohol Industries, Eds Jacques et al., (1999) Nottingham University Press, UK.

[0265] In some embodiments, the mash is fermented with a yeast at temperatures in the range of 15 to 40° C. and also 25 to 35° C.; at a pH range of pH 3.0 to 6.5; also pH 3.0 to 6.0; pH 3.0 to 5.5, pH 3.5 to 5.0 and also pH 3.5 to 4.5 for a period of time of 12 to 240 hours, preferably 12 to 120 and more preferably from 24 to 90 hours to produce an alcohol product, preferably ethanol.

[0266] Yeast cells are generally supplied in amounts of 10^4 to 10^{12}, and preferably from 10^7 to 10^{10} viable yeast count per ml of fermentation broth. The fermentation will include in addition to a fermenting microorganisms (e.g. yeast) nutrients, optionally acid and additional enzymes.

[0267] In some embodiments, in addition to the raw materials described above, fermentation media will contain supplements including but not limited to vitamins (e.g. biotin, folic acid, nicotinic acid, riboflavin), cofactors, and macro and micro-nutrients and salts (e.g. $(NH4)_2SO_4$; K_2HPO_4; NaCl; $MgSO_4$; H_3BO_3; $ZnCl_2$; and $CaCl_2$).

[0268] In one embodiment, the contacting step is conducted in a separate vessel from the fermenting step. It is also contemplated that the contacting step and fermenting step may be conducted in a simultaneous saccharification and fermentation (SSF) process in the same vessel.

[0269] The SSF process may further comprise the addition of enzymes to the process, including but not limited to: glucoamylase, trehalase, pullulanase, isoamylase, hexokinase, xylanase, glucose isomerase, xylose isomerase, phosphatase, phytase, β amylase, α-amylase, protease, cellulase, hemicellulase, lipase, cutinase, isoamylase, redox enzyme, esterase, transferase, pectinase, alpha-glucosidase, beta-glucosidase, lyase or other hydrolases, or a combination thereof.

[0270] Additional enzymes that may be included in the fermentation step may be the same or different from the enzymes used in the contacting step. In some embodiments, the enzyme will include alpha-amylases and glucoamylases, including destructured starch hydrolyzing enzymes. In some embodiments, the glucoamylase and alpha-amylase may occur in a blend. Some specific enzyme blends include STARGEN™ 001 (DuPont Industrial Biosciences), which is a blend of an alpha-amylase from *A. kawachi* and a glucoamylase from *A. niger.* In some embodiments, the glucoamylase will be derived from a *Trichoderma* glucoamylase, *Humicola* glucoamlyase, a *Athelia* glucoamylase, a *Talaromyces* glucoamylase, a *Aspergillus* glucoamylase, a *Penicillium* glucoamylase, a *Trametes* glucoamylase, a *Thermomyces* glucoamylase and hybrid and variants glucoamylase derived there from. In some preferred embodiments, the enzyme is selected from a xylanase, a cellulase, a phytase and a protease.

[0271] During fermentation for production of ethanol as the EOF product the ethanol content of the broth or "beer" may reach about 8-18% v/v, e.g., 14-15% v/v. The broth may be distilled to produce enriched, e.g., 96% pure, solutions of ethanol. Further, CO_2 generated by fermentation may be collected with a CO_2 scrubber, compressed, and marketed for

other uses, e.g., carbonating beverage or dry ice production. Solid waste from the fermentation process may be used as protein-rich products, e.g., livestock feed.

[0272] Continuous or fed-batch fermentation may be used. In a fed-batch fermentation process, fermentation substrate is added in increments as the fermentation progresses. Fed-batch systems are useful when catabolite repression may inhibit the metabolism of the cells and where it is desirable to have limited amounts of substrate in the medium. The actual substrate concentration in fed-batch systems is estimated by the changes of measurable factors such as pH, dissolved oxygen and the partial pressure of waste gases, such as CO_2. Batch and fed-batch fermentations are common and well known in the art.

[0273] Continuous fermentation is an open system where a defined fermentation medium is added continuously to a bioreactor, and an equal amount of conditioned medium is removed simultaneously for processing. Continuous fermentation generally maintains the culture at a constant high density where cells are primarily in log phase growth. Continuous fermentation permits modulation of cell growth and/or product concentration. For example, a limiting nutrient such as the carbon source or nitrogen source is maintained at a fixed rate and all other parameters are allowed to moderate. Because growth is maintained at a steady state, cell loss due to medium being drawn off should be balanced against the cell growth rate in the fermentation. Methods of optimizing continuous fermentation processes and maximizing the rate of product formation are well known in the art of industrial microbiology.

[0274] In one embodiment the present destructured starch-based biomass is used to produce an alcohol in SSF by contacting a slurry comprising destructured starch-based biomass with an alpha-amylase, a glucoamylase, a yeast and optionally an acid stable protease, at a temperature below the starch gelatinization temperature of the destructured starch-based biomass to produce oligosaccharides fermentable by the yeast; and fermenting the oligosaccharides to produce alcohol. In one embodiment the initial percentage of alcohol production over the initial phase of SSF is a higher percentage than would result when using a milled corn flour or whole ground corn composition in place of the destructured starch-based biomass composition. In one embodiment the initial phase of SSF is the first 15 minutes of SSF. In one embodiment the higher percentage of alcohol resulting in the initial phase of SSF is a fold higher % selected from the list consisting of at least 2 fold higher % vs. corn flour; and at least 4 fold higher % vs. whole ground corn. In one embodiment the end of SSF total alcohol yield requires 3 fold less glucoamylase dosage as when using a milled corn flour or whole ground corn composition in place of the destructured starch-based biomass composition. In one embodiment up to 10 fold less glucoamylase is required to produce a comparable end of SSF alcohol yield for destructured starch-based biomass composition than would be required for corn flour. In one embodiment the alcohol is ethanol.

Recovery of Alcohol and Other End Products

[0275] One end product of the instant fermentation process is an alcohol product, preferably ethanol. The end product produced according to the process may be separated and/or purified from the fermentation media. Methods for separation and purification are known, for example by subjecting the media to extraction, distillation and column chromatography. In some embodiments, the end product is identified directly by submitting the media to high-pressure liquid chromatography (HPLC) analysis.

[0276] In further embodiments, the fermentation broth may be separated by for example centrifugation into the liquid phase and solids phase and end products such as alcohol and solids recovered. The alcohol may be recovered by means such as distillation and molecular sieve dehydration or ultra filtration.

[0277] In some embodiments, the yield of ethanol will be greater than 8%, 10%, 12%, 14%, 16% and 18% by volume. The ethanol obtained according to process of the invention may be used as a fuel ethanol, potable ethanol or industrial ethanol.

[0278] In further embodiments, the end product may include the fermentation co-products such as distillers dried grains (DDG) and distiller's dried grain plus solubles (DDGS), which may be used as an animal feed.

Sugar Syrups

[0279] Maltose, the major sugar in maltose syrup, is widely used in many industrial and consumers' applications, for example, as a sweetener and in the preparation of maltitol, a low calorie sweetener. Maltose is less sweet than high fructose corn syrup, with a relative sweetness compared to sucrose of 30-40%. Maltose is also used in brewing to increase the through-put and reduce haze caused by varying quality of wort. Since maltose has a low freezing point, high maltose syrup is useful in frozen desserts. Maltose solutions exhibit lower viscosity and less humictantancy than equal concentrations of glucose syrups and therefore is used a in candy formulations to reduce stickiness. Maltitol is also used as a sugar substitute, does not promote tooth decay, and has somewhat lesser effect on blood sugar.

[0280] Maltotriose, maltotetrose and maltopentose are classified as specialty syrups which are mainly used in the food industry.

EXAMPLES

[0281] The present invention is further defined in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various uses and conditions.

[0282] The meaning of abbreviations is as follows: "h" or "hr" means hour(s), "min" means minute(s), "s" or "sec" means second(s), "d" means day(s), "L" means liter(s), "mL" means milliliter(s), "μL" means microliter(s), "kg" means kilograms, "g" means grams, "μg" means microgram(s), "ng" means nanogram(s), "mg" means milligrams, "g/L" means grams per liter, "mM" means millimolar, "μM" means micromolar, "nm" means nanometer(s), "mm" means millimeter, "cm" means centimeter(s), "μmol" means micromole(s), "pmol" means picomole(s), "N" means newton(s), "G-force" means force of gravity, "MPa" means megapascals, "kPa" means kilopascals, "psi" means pounds per square inch, "CF" means crystalline fraction, "CDS" means coherent domain size, "F" means force, "" or "in" means inch(s), DS is dry solids; GAU is Glucoamylase Units; BU is units of OPTI-

MALT® 2G activity; DE is Dextrose Equivalent(s); AAU is alpha amylase activity units; gds means grams of dry solids; MT=metric ton; H means hour(s); DP is activity units of OPTIMALT® BBA; ASPU is activity units of OPTIMAX® L-1000; SSU is activity units of fungal alpha-amylase; BMK is activity units of OPTIMALT® 4G; % HS is % higher sugars, meaning DP4 and higher DPs (DP5, DP6 etc.); SAPU is units of protease activity; AAU is units of bacterial alpha amylase activity, FTU is units of phytase activity, SAPU refers a spectrophotometric acid protease unit.

General Methods

Non High Force Milling

[0283] Milling was performed on the whole grains: wheat, sorghum, rice, and vacuum dried cassava to generate control materials for XRD, particle size, and fermentation using a model ZM 200 12 toothed rotor mill (Retsch) and a 120 micron screen. The mill was run at 12000 rpm. Samples were milled in 200 g batches.

[0284] Course ground corn is hammer milled corn.

[0285] Fine ground corn is purchased flour (Azure Farm).

Centrifugal Ring and Puck Milling or High Force Milling

[0286] High starch content biomass was treated in a vibratory pulverizer (model VP-1989: 3 phase Vibratory Pulverizer; Bico Inc., Burbank, Calif.); also called a ring and puck mill. The pulverizer consists of a hardened chrome alloy steel bowl (8.125" (20.64 cm) inner diameter), which contains two rings of ½" (1.27 cm) thick hardened steel, one with an inner diameter of 3.06" (7.77 cm) and the other with an inner diameter of 2.05" (5.21 cm) and a solid puck (i.e. disc) with diameter of 1.64" (4.17 cm). The lengths of the rings and puck and corresponding depth of the bowl are 2" (5.08 cm). The bowl containing the rings, puck, and sample were vibrated with a one horsepower eccentric motor at 900 rpm. The radius of eccentricity of the mill is 4.5 mm, which is the radius of the circle that is drawn out by the vibration of the mill. The power of the mill was determined by the using the measured current, applied voltage, and a power factor of 0.8 in the standard equation for a three phase motor.

Moisture Assay

[0287] The moisture content of the biomass was measured using a Mettler-Toledo HR73 Halogen Moisture Analyzer. An amount of at least 0.5 g (but less than 3 g) of starch based feedstock was used for each measurement. Each sample was weighed out into an aluminum pan and heated according to the standard drying program to at temperature of 105° C. Each sample was held at 105° C. until the measured mass loss was <1 mg/50 s, at which point the weight was recorded and the moisture content determined by the ratio of the difference between the initial and final weights to the initial weight of the starch based feedstocks.

Particle Size Assay

[0288] Particle size distribution (PSD) was determined via laser diffraction (reference ISO 13320-1:1999) using a Malvern Mastersizer 2000 (Malvern Instruments, Malvern UK). Sample particles were dispersed in air using a Malvern Scirocco 2000 dry disperser unit with the dispersion jet pressure set to about 60 psi (0.41 MPa) and the feed rate set to about 45% of maximum. The dispersed particles were pneumatically conveyed through the Mastersizer's flow cell, which was outfitted with quartz windows for optical access. Laser light entering the flow cell was diffracted by the particles, and the diffraction pattern was imaged onto an array of detectors. The PSD was calculated by analyzing the recorded diffraction pattern using the Fraunhofer scattering model (described in ISO 13320-1:1999, Annex A). A standard reference for PSD measurement and terminology is T. Allen, Particle Size Measurement, Vol. 1, 5th Ed. (Chapman & Hall 1997).

XRD Technique and Analysis for Crystallinity Assessment

[0289] X-ray diffraction data were obtained with a Philips X'PERT automated powder diffractometer, Model 3040. The diffractometer is equipped with automatic variable anti-scatter and divergence slits, X'Celerator RTMS detector, and Ni filter. The radiation is CuK(alpha) (45 kV, 40 mA). Data were collected at room temperature from 4 to 80 degrees 2-theta; using a continuous scan with an equivalent step size of 0.02 degrees; and a count time of 80 seconds per step in theta-theta geometry. Samples were packed into an aluminum sample holder and run with no additional grinding. MDI/Jade software version 9.1 was used to convert the data to text format for further processing. Microsoft® Excel® (2010) software was used for all additional processing.

[0290] The degree of crystallinity is calculated from the X-ray diffraction data by using a crystalline area integration method based on Cheetham and Leping (Carbohydrate Polymers 36:277-284 (1998)), Nara et al. (Starch 35,12:407-410 (1983)), and Benedetti et al. (Journal of Material Science 18.4:1039-1048 (1983)). The intensities are first normalized over a limited range of data (10-30 2-theta). The normalization occurs by creating a baseline connecting the upper and lower bounds of 10 and 30 2-theta and then dividing the intensities by the integrated area below the intensities curve and above the baseline. After normalization, the data is smoothed by using a Savitzky-Golay filter. The crystalline and amorphous regions are then separated by a function which connects peak baselines. The upper region is the crystalline portion, and the lower region is the amorphous portion. The crystalline portion area and the total diffraction area are integrated. The degree of crystallinity is taken to be the ratio of the crystalline area over the total diffraction area.

$$\text{Degree of Crystallinity} = \frac{\text{Crystalline Area}}{\text{Total Diffraction Area}}$$

Sugar Composition and Concentration Analysis by HPLC

[0291] 166.67 μL of sample was diluted in 1.5 mL ultrapure water and incubated in a thermo-mixer at 99° C. for 15 minutes. Diluted samples were cooled and then filtered using a 96-well filtration plate (Millipore Multiscreen HTS, 0.22 μm Durapore membrane) prior to HPLC analysis.

[0292] RHM column: Phenomenex #00A0132K0 s/n 356583222 300×7.8 mm; Column

[0293] Temperature: 85° C.; Mobile Phase: DI H_2O HPLC grade; Flow Rate: 0.6 ml/min;

[0294] Detector: RI detection; Detector Temperature: 40° C.; Injection Volume: 1 μL

[0295] A designation of DP1 is a monosaccharide, which is substantially glucose; a designation of DP2 is a disaccharide, which is substantially maltose and may include trehalose; a

designation of DP3 is a trisaccharide, which is substantially maltotriose; a designation of DP4 is a tetrasaccharide, which is substantially maltotetraose; and the designation "DPn" refers to those components which elute together, containing among others an oligosaccharide having a degree of polymerization (DP) of 5 or greater, but also components like sulfate, chloride, and non-starch polysaccharides.

[0296] Area percentages of the different sugars (DPn, DP4, DP3, DP2, and DP1) were calculated by dividing the area of that specific sugar peak by the area of all five peaks. The combined area percentages of DPn, DP4, DP3, DP2 and DP1 add up to 100%. Concentrations of the different sugars (DP3, DP2, and DP1) were calculated by multiplying the peak area of that specific sugar with the response factor, which is determined by linear regression of known concentrations of the specific sugar (y-axis) and the obtained peak areas (x-axis).

Preparation of Reference Corn Liquefact

[0297] Corn kernels were ground with a hammer mill with a 2 mm sieve at 10,000 rpm. The resulting corn meal was mixed with water producing a slurry with up to 30% DS and the pH was adjusted to 5.5 with 2 M H_2SO_4. 0.155 kg/MT SPEZYME® CL was added and the mixture was kept under constant stirring at 60° C. for 30 minutes before being brought to 85° C. for 90 minutes. Then the mixture slurry was cooled down to room temperature and the pH was brought to 4.5 with 2 M H_2SO_4 resulting in a corn liquefact with 31.6% DS.

Enzymes

[0298] A fungal alpha-amylase from *Aspergillus clavatus* having an activity of 301 U/mg on amylopectin, and optimal activity at temperature of 66° C.

[0299] A bacterial alpha-amylase from *Cytophaga* sp. having an activity of 2459 U/mg on amylopectin.

[0300] A fungal gluco-amylase from *Aspergillus fumigatus* (AfGA) having an activity of 242.9 U/mg on DP7.

[0301] A fungal alpha-amylase from *Aspergillus kawachii* (AkAA) having an activity of 58.9 U/mg on amylopectin. This enzyme is commercially available as GC626 from Genencor/Danisco/Dupont.

[0302] Acid stable fungal gluco-amylase from *Trichoderma reesei* (TrGA) which is described in U.S. Pat. No. 7,037,704.

[0303] The following enzymes are commercially available from Genencor/Danisco/Dupont:

[0304] OPTIMAX® 4060 VHP

[0305] OPTIMAX® HDS

[0306] OPTIMALT® 2G

[0307] SPEZYME® XTRA

[0308] OPTIMALT® BBA

[0309] OPTIMAX® L-1000

[0310] OPTIMALT® 4G

[0311] SPEZYME® RSL

[0312] FERMGEN™ 2.5x

[0313] SPEZYME® CL

Dextrose Equivalents Measurement

[0314] The term "DE," or "dextrose equivalent," is defined as the percentage of reducing sugar, i.e., D-glucose, as a fraction of total carbohydrate in a syrup. Dextrose equivalents (DE) were measured using the Schoorl method (Schoorl, N., Zurjodometrischen Zukerbestimmung mittles Fehlingscher Losung., *Zeitschr. F. agnew. Chem.*, 12, 633 (1899); Hodge, J. E & Davis, H. A. Selected Methods for Determining Reducing Sugars. *United States Department of Agriculture Technical Bulletin A1C333*. (1952); Schenck, F. W. & Hebeda, R. E., *Starch Hydrolysis Products, Worldwide Technology Production and Applications*, p 379, VCH Publishers (1992))

True Solubility

[0315] True solubility is the actual solubility in a dry sample which is calculated from the measured solubility through RI.

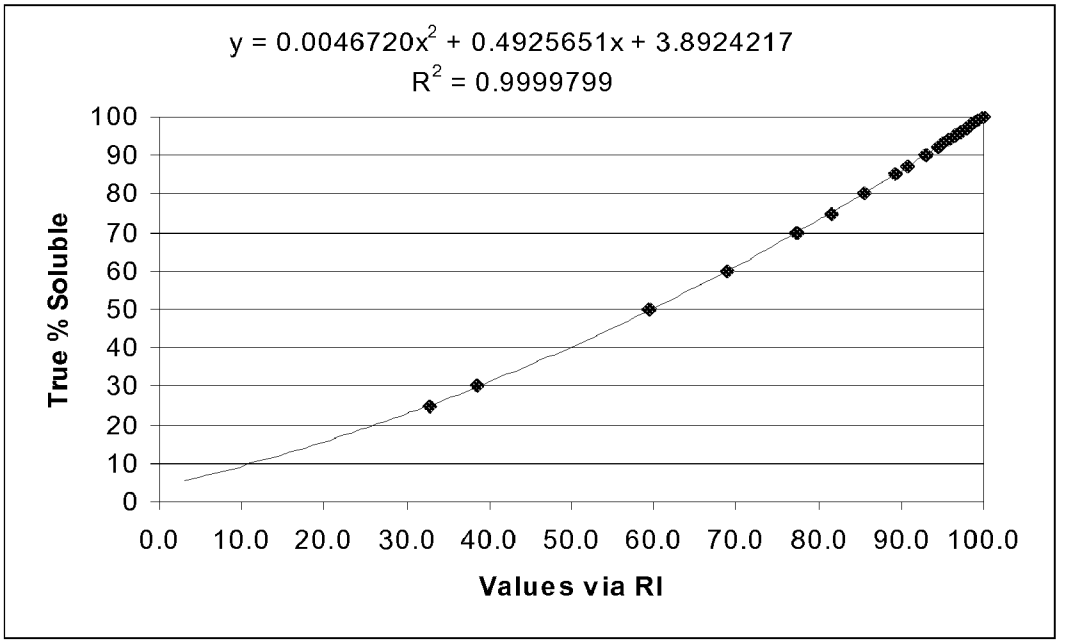
[0316] Determination of the actual solubility in a dry sample which is calculated from the measured solubility through RI using the following formula:

$$Y=0.0046720x^2+0.4925651x+3.8924217$$

[0317] Where Y is the true soluble and x is the solubility determined with RI.

[0318] This is due to the remaining starch not participating in the RI determination. This diminishes as greater solubility is achieved.

[0319] The solubility as obtained from the described procedure can then be inserted as x in the equation extracted from the graph below.


y = 0.0046720x2 + 0.4925651x + 3.8924217
R2 = 0.9999799
True % Soluble
100
90
80
70
60
50
40
30
20
10
0
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
Values via RI

EXAMPLE

[0320] Start material: 28% ds (starch)

[0321] RI_{sup}: 1.3784=>equals 29.49% ds retrieved from the DE 95 conversion table.

29.49% ds/(28*1.1)*100=95.7

[0322] RI_{total}: 1.3836=>equals 32.45% ds retrieved from DE 95.7 conversion table

% starch solubility=29.49/32.45=90.9

[0323] Corrected % starch solubility:

$0.0046720*90.9^2+0.4925651*90.9+3.8924217=87.3$

Oil Analysis

[0324] A sample of 20 g in 50 ml centrifuge tube is heated to 80° C. for 30 min.

[0325] The tube is then centrifuged at 10000 rpm for 2 min.

[0326] 2 ml of hexane is added carefully to the top of the centrifuged sample and gently swirled, then slowly shaken for 5 min.

[0327] Let the tubes sit for 15 min total.

[0328] 200 μl of the oil containing hexane is siphoned off and centrifuged to break any emulsion.

[0329] The sample is then diluted further with hexane and 10 μl is injected into the LC.

[0330] This is free oil.

[0331] Then take the hexane containing tubes and shake them vigorously for a few minutes and re-centrifuge.

[0332] This recovers the total oil.

Example 1

Destructuring in a Centrifugal Force Ring-Roller Mill

[0333] Centrifugal force ring-roller mills (cfRRMs) have been described in Perry's Chemical Engineers' Handbook (8^{th} Edition, ch 21 p 60), and can contain a wide variety of dimensions based on the scale of the operation. Dimensions for representative scales of cfRRMs were chosen and the forces and stresses generated for these examples of commercial scale cfRRMs were calculated based on their dimensions and masses. The dimensions, masses, and results are given in Table 1. The mill designs have at least two rollers, each attached to a swinging arm, with the arms attached to a drive shaft. The rollers revolve within a chamber, which causes the rollers to press radially outward against a ring.

TABLE 1

Centrifugal Force Ring-Roller Mill Specifications and Forces

Parameter	cfRRM-1	cfRRM-2	cfRRM-3	cfRRM-4
RPM	90	150	100	100
Chamber Diameter [in]	40	40	80	100
Roller Diameter [in]	13.3	13.3	26.7	33.3
Roller/Ring Height [in]	10	10	20	29.2
Roller Density [g/cm3]	8	8	8	8
Mass of Roller [kg]	183	183	1,464	3,337
Centrifugal Force [N]	5512	15311	108,770	309,810
Hertzian Stress (w/o material) [psi]	8140	13566	18,046	22,557
G Force	3.07	8.53	7.57	9.47

[0334] Equation 1 was used to calculate the centrifugal forces in each apparatus. The center-of-mass radii for the rollers were found by first setting the axis along the central drive shaft as the origin. The distances from the center of each roller to the central drive shaft are the center-of-mass radii. They are positioned so that they are against the ring, as well as equidistant from each other along the circumference of the ring (such that the center of mass of the all of the rollers considered together, would align with the central shaft on which they are attached).

[0335] The "g-forces" in the centrifugal force roller mill were calculated by dividing the centripetal acceleration (i.e. F/m) by the acceleration owing to gravity (~9.8 m/s^2).

[0336] Thus biomass treated in the described apparatuses would be subjected to forces of >5000 N and "G-forces" of ~3 G, or forces >15,000 N and "G-forces" <9 G, or forces of >100,00 N and "G-forces" of <8 G, or forces >300,000 N and "G-forces" <10 G.

[0337] Equation 3 was used to calculate Hertzian stress. The number used for poison's ratio (v) was 0.3 and the number used for elastic modulus (E) was $2.1*10^{11}$ Pa. These values were chosen based on assumptions that are generally applied to common metals. These same numbers were used for v_1 as well as v_2, and for E_1 as well as E_2. The centrifugal forces calculated and given in Table 1 were used as F. The Ring/Roller Heights given in Table 1 were used as I. For purposes of the calculation, an assumption was made that the roller and ring surfaces were perfect cylinders, while in reality the roller becomes concave and does not present an even line contact with the ring.

[0338] For a combined milling system (mill and auxiliary equipment such as blower or classifier), the hp can vary dramatically based on the size of the milling system. The full power capabilities for typical cfRRM systems can be anywhere from ~20 hp up to <5000 hp. Once the mill is operated in the desired force and stress range (>5000 N and >5,000 psi (34.47 MPa)), then the air handling system and classifier are modified to achieve a throughput such that the power draw on the machine divided by the throughput yields a specific energy that is <40% of the total combustible energy of the feed material. All of these running modifications are routine to one of skill in the art. The starch-based biomass product is expected to have crystallinity that is reduced, as compared to the crystallinity of the starch-based biomass which is not treated, by at least about 25% and efficient saccharification without using a heating step. In the case of corn kernels the specific energy input is expected to be <7,200 kJ/kg, which is <40% of the combustible energy of corn kernel biomass (~18,000 to 20,000 kJ/kg of biomass).

Example 2

Destructuring Effect of Ring and Puck Milling of Whole Corn Kernels

[0339] 5 g of whole corn kernels (11.1% moisture) were divided equally in the three spaces between the media in the bowl (i.e. between the bowl and larger ring, between the larger ring and smaller ring, and between the smaller ring and puck) of the vibratory pulverizer (ring and puck mill) described in General Methods for centrifugal milling. The ring and puck mill was run for 5 min. The throughput for the milling was ~1 g/min or 1.67×10^{-5} kg/s, which gives a specific energy input of 0.134 hp/(1.67×10^{-5} kg/s) or ~1.68 kWh/kg or ~6.03 kJ/g.

[0340] The corn kernels were subjected to forces ranging from 646 to 1,511 N and "G-forces" ranging from 21.5 to 32.3 as calculated and described below.

[0341] Dimensions and mass of the media in the ring and puck mill are:

[0342] Bowl: 8.125" (20.64 cm) inner diameter, 2" (5.08 cm) depth, 18.80 lbs (8.53 kg)

[0343] Large Ring: 6.125" (15.56 cm) inner diameter, 2" (5.08 cm) depth, 0.6" (1.52 cm) thickness, 6.60 lbs (2.99 kg)

[0344] Small Ring: 4.1" (10.41 cm) inner diameter, 2" (5.08 cm) depth, 0.6" (1.52 cm) thickness, 4.69 lbs (2.13 kg)

[0345] Puck: 3.270" (8.31 cm) diameter, 2" (5.08 cm) depth, 4.49 lbs (2.04 kg)

The ring and puck mill operates at a constant 900 rpm angular frequency. The circle of eccentricity of the motor (i.e. the circumference of the circle drawn out by the motion of the oscillating stage) is 1.11". Equation 1 can be used to calculate the centrifugal force at the interface between any of the media in the mill. The center-of-mass radius is calculated according to Equation 7.

$$r = \frac{\sum_{i}^{n} m_i r_i}{m_{total}} \qquad \text{Equation 7}$$

where n is the number of media, i is the specific index identifying each unique medium, m_i is the specific mass of an indexed medium, r_i is the specific center-of-mass radius of an indexed medium, and m_{tot} is the total or combined mass of all the media. The total mass of the medial is 15.78 lbs (7.16 kg). The center-of-mass radii of the media are found first by setting the center of the milling stage as the origin. Next, the large ring, small ring, and puck are placed into the bowl and pushed to one side, causing the center-of-mass radius for each medium to be offset from the center of the milling stage, as well as the bowl. The circle of eccentricity of the motor (i.e. the circumference of the circle drawn out by the motion of the oscillating stage) is 1.11", which further offsets the center of mass from the center of the milling stage by the radius of the circle (0.18"). The specific center-of-mass radius, r_i, for each medium is the distance from the bowl center to the center of each medium, with the addition of 0.18" to account for the motion of the stage. For example, the center-of-mass radius of the puck is 1.40" (5.44 cm), which is found by subtracting the large ring thickness (0.6"; 1.52 cm), small ring thickness (0.6"; 1.52 cm), and puck radius (1.64") from the bowl inner radius (4.0625") and adding 0.18". Lastly, the "g-forces" in the ring and puck mill can be calculated by dividing the centripetal acceleration (i.e. F/m) by the acceleration due to gravity (~9.8 m/s^2). Table 2 summarizes the masses, center-of-mass radii, centrifugal forces at the interfaces between the media, and the "g-forces" in the ring and puck mill.

TABLE 2

Parameters of the apparatus used for centrifugal milling

Interface	Mass [kg]	Center-of-mass radius [m]	Force [N]	"G-force"	Pressure [psi]
Bowl-Large Ring	7.16	0.024	1,511	21.5	4,934
Large Ring-Small Ring	4.17	0.030	1,121	27.4	5,843
Small Ring-Puck	2.04	0.036	646	32.3	6923

After milling, the whole corn kernels were reduced to a fine powder with a d_{50} of ~27 μm. The PSD is shown in FIG. **3**A.

[0346] A comparison was made with conventional hammer milled corn kernels which showed a d_{50} of 543.2 μm, as well as a biomodal distribution (FIG. **3**B). The XRD patterns indicating the destructured nature of the starch granules in corn kernels that were conventionally processed to corn flour or ring and puck milled are shown in FIG. **4**. The crystallinity of the ring and puck milled corn kernels, determined as described in General Methods, decreased from 24.4% to 0.9%. Thus the crystalline features that are present for hammer milled whole corn kernels were virtually eliminated in the ring and puck milled sample, indicating destructuring of the whole corn kernels. The ring and puck milling process described in this example was performed multiple times to generate enough destructured material for use in later examples. The material is called high force milled corn or ring and puck milled corn.

Example 3

Destructuring Effect of Ring and Puck Milling Whole White Wheat Kernels

[0347] 5 g of whole white wheat kernels (0.84% moisture) were divided equally in the three spaces between the media in the bowl (i.e. between the bowl and larger ring, between the larger ring and smaller ring, and between the smaller ring and puck) of the vibratory pulverizer (ring and puck mill) described in General Methods for centrifugal milling. The ring and puck mill was run for 5 min. The throughput for the milling was ~1 g/min or 1.67×10^{-5} kg/s, which gives a specific energy input of 0.134 hp/(1.67×10^{-5} kg/s) or ~1.68 kWh/kg or ~6.03 kJ/g.

[0348] After milling, the whole white wheat kernels were reduced to a fine powder with a d_{50} of 17.7 μm. The PSD is shown in FIG. **5**A. The corresponding XRD pattern indicating the destructured nature of the starch granules is shown in FIG. **5**B. The crystallinity, determined as described in General Methods, decreased from 12.9% to 0.1%. Control wheat kernels were gently crushed with a mortar and pestle into a fine powder, and used for XRD measurement (FIG. **5**B). The crystalline features that are present for whole white wheat kernels crushed with a mortar and pestle were virtually eliminated after ring and puck milling. The milling process described in this example was performed multiple times to generate enough destructured material for use in later examples.

Example 4

Destructuring Effect of Ring and Puck Milling Whole Sorghum

[0349] 5 g of whole sorghum (2.04% moisture) were divided equally in the three spaces between the media in the

bowl (i.e. between the bowl and larger ring, between the larger ring and smaller ring, and between the smaller ring and puck) of the vibratory pulverizer (ring and puck mill) described in General Methods for centrifugal milling. The ring and puck mill was run for 5 min. The throughput for the milling was ~1 g/min or 1.67×10^{-5} kg/s, which gives a specific energy input of 0.134 hp/(1.67×10^{-5} kg/s) or ~1.68 kWh/kg or ~6.03 kJ/g.

[0350] After milling, the whole sorghum was reduced to a fine powder with a d_{50} of 17.4 μm. The PSD is shown in FIG. **6**A. The corresponding XRD pattern indicating the destructured nature of the starch granules is shown in FIG. **6**B. The crystallinity, determined as described in General Methods, decreased from 28.0% to 0.2%. Control whole sorghum was gently crushed with a mortar and pestle into a fine powder, and used for XRD measurement (FIG. **6**B). The crystalline features that are present for whole sorghum crushed with a mortar and pestle were virtually eliminated after ring and puck milling. The milling process described in this example was performed multiple times to generate enough destructured material for use in later examples.

Example 5

Destructuring Effect of Ring and Puck Milling Whole Brown Rice

[0351] 5 g of whole brown rice (1.42% moisture) were divided equally in the three spaces between the media in the bowl (i.e. between the bowl and larger ring, between the larger ring and smaller ring, and between the smaller ring and puck) of the vibratory pulverizer (ring and puck mill) described in General Methods for centrifugal milling. The ring and puck mill was run for 5 min. The throughput for the milling was ~1 g/min or 1.67×10^{-5} kg/s, which gives a specific energy input of 0.134 hp/(1.67×10^{-5} kg/s) or ~1.68 kWh/kg or ~6.03 kJ/g.

[0352] After milling, the whole brown rice was reduced to a fine powder with a d_{50} of 11.5 μm. The PSD is shown in FIG. **7**A. The corresponding XRD pattern indicating the destructured nature of the starch granules is shown in FIG. **7**B. The crystallinity, determined as described in General Methods, decreased from 28.6% to 0.1%. Control whole brown rice was gently crushed with a mortar and pestle into a fine powder, and used for XRD measurement (FIG. **7**B). The crystalline features that are present for whole brown rice crushed with a mortar and pestle were virtually eliminated after ring and puck milling. The milling process described in this example was performed multiple times to generate enough destructured material for use in later examples.

Example 6

Destructuring Effect of Ring and Puck Milling Cassava

[0353] Frozen cassava pieces were cut into ~½" cubes and vacuum dried for 4 hours at 45° C. 5 g of dried cassava (7.05% moisture) were divided equally in the three spaces between the media in the bowl (i.e. between the bowl and larger ring, between the larger ring and smaller ring, and between the smaller ring and puck) of the vibratory pulverizer (ring and puck mill) described in General Methods for centrifugal milling. The ring and puck mill was run for 5 min. The throughput for the milling was ~1 g/min or 1.67×10^{-5} kg/s, which gives a specific energy input of 0.134 hp/(1.67×10^{-5} kg/s) or ~1.68 kWh/kg or ~6.03 kJ/g.

[0354] After milling, the dried cassava pieces were reduced to a fine powder with a d_{50} of ~14.0 μm. The PSD is shown in FIG. **8**A. The corresponding XRD pattern indicating the destructured nature of the starch granules is shown in FIG. **8**B. The crystallinity decreased from 48.4% to 4.0%. Control dried cassava pieces were gently crushed with a mortar and pestle into a fine powder, and used for XRD measurement (FIG. **8**B). The crystalline features that are present for dried cassava crushed with a mortar and pestle, determined as described in General Methods, were virtually eliminated after ring and puck milling. The milling process described in this example was performed multiple times to generate enough destructured material for use in later examples.

Example 7

Extent of Destructuring with Processing Time in Ring and Puck Mill

[0355] Approximately 5 g of whole corn kernels were ring and puck milled in the same manner as described in Example 2 except that the milling time was varied to generate corn with different levels of destructuring. It can be seen from FIG. **9** that the material was significantly destructured after only 30 seconds of milling. The crystallinity for each time sample was determined (see General Methods) giving results as follows: 30 s: 6.92%, 1 min: 5.22%, 2 min: 2.49%, 3 min: 1.91%, and 5 min: 0.86%. Corn flour (not ring and puck milled), with a crystallinity of about 26% in this experiment, is shown for reference.

[0356] The amount of energy used to produce each time sample was calculated by dividing the steady state power output [kW] of the apparatus used by the throughput [g/s]. Based on this calculation, the percent of the energy content of the corn used to produce the destructured sample was calculated for a corn energy value of 18,000 or 20,000 kj/kg and is shown in Table 3.

TABLE 3

Processing energy for destructured starch-based biomass samples.

		% of corn energy	
Time [s]	kJ/kg	18,000	20,000
30	603	3%	3%
60	1206	7%	6%
120	2412	13%	12%
180	3618	20%	18%
300	6030	34%	30%

Example 8

Glucose Production from High Force Milled Corn using Gluco-Amylases

[0357] In this experiment two commercial enzyme products were tested to see whether glucose syrup can be made directly from high force milled corn without application of liquefaction with alpha-amylase. The results were compared to those obtained with a traditional corn liquefact.

[0358] A 10% DS aqueous slurry of high force milled corn kernels, treated as described in Example 2, was incubated at

pH 4.4 and 60° C. with 0.16 GAU gds OPTIMAX® 4060 VHP (0.16 activity units are added per gram dry solids) or 0.10 GAU gds OPTIMAX® HDS.

[0359] As reference, corn liquefact prepared as described in General Methods was diluted to 10% DS and the slurry was also incubated at pH 4.4 at 60° C. with 0.16 GAU gds OPTIMAX® 4060 VHP, or 0.10 GAU gds OPTIMAX® HDS.

[0360] Each slurry was constantly shaken (orbital shaker incubator; 100 rpm) and samples were taken at different time intervals (t) for determination of sugar composition and concentration by HPLC (see General Methods). The results are shown in Table 4.

TABLE 4

Sugar compositions of high force milled corn and corn liquefact treated with different gluco-amylases

enzyme	substrate	t [h]	DPn %	DP4 %	DP3 %	DP2 %	DP1 %	DP3 (% w/V)	DP2 (% w/V)	DP1 (% w/V)
OPTIMAX ®	High	2	60.39	0.62	1.21	1.31	34.40	0.09	0.10	2.51
4060 VHP	force	4	36.95	0.51	1.38	3.23	55.80	0.11	0.27	4.30
0.16 GAU	milled	6	22.97	0.34	0.74	4.20	69.51	0.06	0.36	5.52
gds	corn	20	5.25	0.54	0.55	2.32	88.80	0.05	0.22	7.70
		25	4.43	0.50	0.41	2.25	89.91	0.04	0.22	8.00
		30	4.19	0.49	0.56	2.07	90.33	0.05	0.20	7.92
		45	3.87	0.47	0.59	2.04	90.58	0.06	0.20	8.09
		50	4.04	0.42	0.59	2.03	90.37	0.06	0.20	8.10
	Whole	2	31.88	1.61	7.46	13.63	44.55	0.54	1.00	3.02
	ground	4	20.03	0.75	1.82	16.39	60.09	0.14	1.25	4.21
	corn	6	13.37	1.01	1.19	12.21	71.04	0.09	0.93	4.96
	liquefact	20	4.01	0.58	1.20	2.42	90.25	0.10	0.20	6.93
		25	3.82	0.61	1.21	2.28	90.56	0.10	0.19	7.06
		30	3.65	0.52	1.17	2.16	91.22	0.10	0.18	7.13
		45	3.49	0.58	1.31	2.31	91.41	0.12	0.21	7.73
		50	3.48	0.53	1.36	2.27	90.79	0.11	0.19	7.11
OPTIMAX ®	High	2	72.10	0.51	1.01	0.68	23.60	0.07	0.05	1.60
HDS	force	4	53.49	0.59	1.45	1.85	40.56	0.12	0.15	3.08
0.10 GAU	milled	6	36.23	0.45	1.43	3.40	56.10	0.12	0.29	4.36
gds	corn	20	7.22	0.38	0.49	2.86	86.52	0.05	0.27	7.38
		25	6.07	0.38	0.49	2.29	88.32	0.05	0.22	7.91
		30	4.52	0.31	0.43	1.90	90.47	0.04	0.18	7.89
		45	4.43	0.24	0.60	1.36	91.04	0.06	0.13	8.09
		50	4.24	0.14	0.45	1.31	91.52	0.04	0.13	8.02
	Whole	2	46.51	3.93	10.15	8.96	29.63	0.74	0.66	2.01
	ground	4	31.74	1.75	7.93	13.55	44.10	0.58	1.00	3.01
	corn	6	19.75	0.87	3.50	17.23	57.76	0.26	1.30	4.02
	liquefact	20	4.88	0.85	1.18	6.50	85.07	0.09	0.53	6.37
		25	4.36	0.77	1.21	4.91	87.81	0.10	0.40	6.66
		30	3.94	0.70	1.15	3.91	88.85	0.10	0.35	7.44
		45	3.48	0.56	1.16	2.74	91.25	0.10	0.24	7.23
		50	3.50	0.45	1.17	2.52	90.89	0.10	0.22	7.14

[0361] Table 4 shows that saccharification of high force milled corn with OPTIMAX® 4060 VHP resulted in similar DP1 percentages as incubations with whole ground corn liquefact. For the high force milled corn the percentage of DPn was found to be higher, while DP4 and DP3 and DP2 were lower than the incubations with whole ground corn liquefact. The lower concentration of DP3 can be explained by limited exposure to alpha-amylase activity in the incubated high force milled corn, as this material has not been liquefied with enzymes like the reference liquefact.

[0362] The concentration of DP1 was found to be higher for the high force mill treated corn when compared to whole ground corn liquefact. This was also the case for the incubations with OPTIMAX® HDS. The results indicate that the high force mill treatment resulted in higher solubilisation of starch (shown in higher initial DPn %) than with traditional liquefaction treatment, meaning that more substrate was available for glucose production, resulting in higher concentrations of glucose.

[0363] The DP2 for the OPTIMAX® HDS incubation with high force milled corn was lower contributing to the higher concentration of DP1 when compared with the whole ground corn liquefact. This could also be related to limited exposure to alpha-amylase activity.

[0364] This example shows that glucose syrups were produced from corn without a liquefaction step by treatment of the corn with high force milling and incubating this material with gluco-amylase containing enzyme products. The high force mill treated corn resulted in highly soluble starch with a low DE resulting in higher glucose yield than possible with a conventional corn liquefact process. Thus high force milling resulted in a better substrate for glucose syrup production than can be obtained via traditional liquefaction.

Example 9

Maltose Production from High Force Milled Corn using a Maltogenic Alpha Amylase

[0365] In this experiment a maltogenic alpha-amylase with and without additional alpha-amylase was tested to see whether maltose syrup can be made directly from high force milled corn without application of liquefaction with alpha-amylase. The results were compared to those obtained with a traditional corn liquefact.

[0366] A 10% DS aqueous slurry of high force milled corn (prepared as described in Example 2) was incubated at pH 5.0 and 60° C. with 8 BU gds OPTIMALT® 2G with or without 0.5 AAU gds SPEZYME® XTRA.

[0367] As reference, corn liquefact (General Methods) was diluted to 10% DS and the slurry was also incubated at pH 4.4 at 60° C. with 8 BU gds OPTIMALT® 2G with or without 0.5 AAU gds SPEZYME® XTRA.

[0368] Each slurry was constantly shaken (orbital shaker incubator; 100 rpm) and samples were taken at different time intervals for determination of sugar composition and concentration. The results are shown in Table 5.

TABLE 5

Sugar compositions of high force milled corn and corn liquefact treated with different alpha-amylases

enzyme	substrate	t [h]	DPn %	DP4 %	DP3 %	DP2 %	DP1 %	DP3 (% w/V)	DP2 (% w/V)	DP1 (% w/V)
OPTIMALT ®	High	2	44.25	4.38	2.79	41.80	5.06	0.20	3.08	0.34
2G	force	4	42.06	3.18	2.35	44.56	6.27	0.18	3.51	0.46
8 BU	milled	6	38.54	2.61	2.21	47.87	7.20	0.18	3.86	0.53
gds	corn	20	30.25	1.63	1.70	55.83	9.02	0.14	4.71	0.70
		25	30.45	1.68	1.08	56.55	8.77	0.09	4.94	0.71
		30	28.57	1.45	1.58	57.65	9.25	0.13	4.97	0.73
		45	27.41	1.38	1.59	58.44	9.71	0.13	5.02	0.77
		50	27.05	1.36	1.54	58.68	9.91	0.13	5.06	0.78
	Whole	2	31.48	4.30	4.78	50.23	8.48	0.34	3.60	0.56
	ground	4	28.97	2.71	2.57	55.59	9.44	0.18	4.04	0.63
	corn	6	27.51	1.99	1.44	58.50	9.87	0.12	4.77	0.74
	liquefact	20	25.09	1.59	0.92	61.80	9.97	0.07	4.83	0.72
		25	25.24	1.53	0.81	62.03	9.78	0.07	5.34	0.78
		30	25.02	1.60	0.79	62.24	9.74	0.07	5.21	0.75
		45	24.69	1.65	0.82	62.53	9.72	0.07	5.21	0.74
		50	24.54	1.61	0.79	62.70	9.77	0.06	5.14	0.74
OPTIMALT ®	High	2	44.93	6.27	5.64	36.87	4.76	0.47	3.13	0.37
2G	force	4	32.26	4.44	5.30	49.29	7.20	0.44	4.18	0.56
8 BU	milled	6	27.75	2.98	3.44	55.54	8.79	0.29	4.70	0.68
gds +	corn	20	23.42	1.83	1.50	61.08	10.67	0.13	5.24	0.84
SPEZYME ®		25	21.52	1.61	1.61	63.51	10.35	0.15	6.02	0.90
XTRA		30	22.42	1.63	1.40	62.11	10.96	0.12	5.44	0.88
0.5 AAU		45	21.41	1.70	1.38	62.68	11.40	0.12	5.51	0.92
gds		50	22.89	1.80	1.43	61.20	11.25	0.13	5.57	0.94
	Whole	2	28.17	4.22	5.27	52.39	9.22	0.39	3.90	0.63
	ground	4	25.75	2.73	2.78	57.85	10.21	0.24	5.10	0.83
	corn	6	24.49	2.03	1.56	60.43	10.79	0.13	5.08	0.83
	liquefact	20	22.71	1.75	0.89	63.06	10.95	0.07	5.07	0.81
		25	23.09	1.77	0.82	63.00	10.71	0.07	5.47	0.86
		30	22.80	1.74	0.79	63.61	10.49	0.07	5.48	0.83
		45	22.44	1.89	0.85	63.82	10.44	0.07	5.27	0.79
		50	22.47	1.84	0.82	63.77	10.54	0.07	5.29	0.80

[0369] Table 5 shows that addition of OPTIMALT® 2G to high force milled corn resulted in more than 58% DP2, which is mainly maltose. With the reference liquefact a few percent more DP2 was found, but in absolute numbers similar amounts of DP2 were measured. The relative difference comes from a higher percentage of DPn and DP3 for the high force milled corn, which again indicates that high force milling results in a higher soluble substrate than conventional liquefaction.

[0370] Addition of SPEZYME® XTRA resulted in lower DPn for both samples, but the higher DP3, DP2, and DP1 concentrations (w/v %) in the high force milled corn incubation may indicate that the substrate was more accessible for OPTIMALT® 2G or that more starch was solubilized by the high force milling. With the extra alpha-amylase, higher percentages of DP2 were obtained for both samples.

[0371] This example shows that high maltose syrups were produced from corn without a liquefaction step by treating the corn with a high force milling and incubating this material with a maltogenic alpha-amylase with or without additional alpha-amylase. Also it shows that high force milling resulted in a better substrate for malto-saccharification than can be obtained via traditional liquefaction.

Example 10

Maltose Production from High Force Milled Corn with a Beta-Amylase

[0372] In this experiment a beta-amylase with pullulanase, with and without additional alpha-amylase, was tested to see whether maltose syrup can be made directly from high force milled corn without application of liquefaction with alpha-amylase.

[0373] A 10% DS aqueous slurry of high force milled corn (prepared as in Example 2) was incubated at pH 5.0 and 60° C. with 4 DP gds OPTIMALT® BBA with 1.5 ASPU gds OPTIMAX® L-1000, with or without 0.5 AAU gds SPEZYME® XTRA.

[0374] Each slurry was constantly shaken (orbital shaker incubator; 100 rpm) and samples were taken at different time intervals for determination of sugar composition and concentration. The results are shown in Table 6.

TABLE 6

Sugar compositions of high force milled corn with different combinations of beta-amlyase, pullinase, and alpha-amylase

enzyme	substrate	t [h]	DPn %	DP4 %	DP3 %	DP2 %	DP1 %	DP3 (% w/V)	DP2 (% w/V)	DP1 (% w/V)
OPTIMALT ® BBA 4 DP gds + OPTIMAX ® L-1000 1.5 ASPU gds	High force milled corn	2	42.87	2.02	3.15	45.87	4.24	0.21	3.15	0.27
		4	44.34	2.60	3.87	42.48	4.96	0.27	3.00	0.32
		6	44.19	3.05	4.30	41.25	5.49	0.32	3.06	0.37
		20	43.90	3.25	4.81	39.62	6.74	0.36	3.00	0.47
		25	44.15	3.47	5.36	38.84	6.60	0.42	3.10	0.49
		30	44.57	3.22	4.90	38.95	6.75	0.39	3.11	0.50
		45	42.53	3.58	5.32	39.68	7.23	0.39	2.96	0.50
OPTIMALT ® BBA 4 DP gds + OPTIMAX ® L-1000 1.5 ASPU gds + SPEZYME ® XTRA 0.5 AAU gds	High force milled corn	2	34.75	4.19	6.20	50.07	3.26	0.52	4.22	0.25
		4	27.31	6.00	8.64	52.83	3.71	0.71	4.37	0.28
		6	23.80	6.91	10.00	53.87	3.90	0.81	4.43	0.30
		20	19.34	7.95	11.71	55.19	4.28	0.96	4.60	0.33
		25	17.32	8.48	12.00	56.32	4.31	1.02	4.86	0.34
		30	18.17	8.36	11.85	55.76	4.31	0.99	4.71	0.34
		45	17.60	8.22	12.02	55.91	4.75	0.99	4.68	0.37

[0375] Table 6 shows that in a short time frame (<6 h) maltose syrup was made from incubating high force milled corn with beta-amylase and pullulanase.

[0376] Addition of alpha-amylase increased the percentage and concentration of DP2, but also helped to hydrolyse the corn further, as DP4% and DP3% were higher than without SPEZYME® XTRA

[0377] This example shows that maltose syrups were produced from corn without a liquefaction step by grinding the corn with a high force mill and incubating this material with a beta-amylase and pullulanase, with or without additional alpha-amylase.

Example 11

Maltotriose Production from High Force Milled Corn using an Alpha-Amylase

[0378] In this experiment an alpha-amylase with and without pullulanase was tested to see whether a syrup rich in DP3 can be made directly from high force milled corn without application of liquefaction with alpha-amylase. The results were compared to those obtained with a traditional corn liquefact.

[0379] A 10% DS aqueous slurry of high force milled corn (prepared as in Example 2) was incubated at pH 4.4 and 60° C. with 3 SSU gds *Aspergillus clavatus* alpha-amylase with or without 1.081 ASPU gds OPTIMAX® L-1000.

[0380] As reference, corn liquefact (see General Methods) was diluted to 10% DS and the slurry was incubated at pH 4.4 at 60° C. with 3 SSU gds *Aspergillus clavatus* alpha-amylase with or without 1.081 ASPU gds OPTIMAX® L-1000.

[0381] Each slurry was constantly shaken (orbital shaker incubator; 100 rpm) and samples were taken at different time intervals for determination of sugar composition and concentration. The results are shown in Table 7.

TABLE 7

Sugar compositions of high force milled corn and corn liquefact treated with different combinations of alpha-amylase and pullanase

enzyme	substrate	t [h]	DPn %	DP4 %	DP3 %	DP2 %	DP1 %	DP3 (% w/V)	DP2 (% w/V)	DP1 (% w/V)
AcAA 3 SSU gds	High force milled corn	2	57.69	11.32	18.42	8.47	2.54	1.55	0.72	0.20
		4	46.24	12.30	23.69	13.19	3.06	1.99	1.12	0.24
		6	40.88	11.84	26.00	16.32	3.38	2.19	1.39	0.27
		20	38.80	10.93	26.64	17.93	4.16	2.28	1.55	0.33
		25	38.97	10.74	26.51	17.97	4.30	2.32	1.59	0.35

TABLE 7-continued

Sugar compositions of high force milled corn and corn liquefact treated with different combinations of alpha-amylase and pullanase

enzyme	substrate	t [h]	DPn %	DP4 %	DP3 %	DP2 %	DP1 %	DP3 (% w/V)	DP2 (% w/V)	DP1 (% w/V)
		30	38.87	10.54	26.60	18.05	4.44	2.28	1.57	0.36
		45	38.88	10.11	26.35	18.26	4.92	2.27	1.60	0.40
		50	38.92	10.00	26.27	18.29	5.03	2.27	1.60	0.41
	Whole	2	36.20	11.45	29.24	17.75	4.61	2.19	1.35	0.32
	ground	4	33.96	10.05	30.14	20.55	4.60	2.35	1.62	0.33
	corn	6	33.42	9.32	30.32	21.64	4.60	2.16	1.56	0.31
	liquefact	20	33.06	8.89	30.29	22.36	4.73	2.22	1.66	0.32
		25	33.70	8.79	30.05	22.12	4.67	2.43	1.81	0.35
		30	33.78	8.69	29.99	22.21	4.69	2.27	1.70	0.33
		45	33.93	8.40	29.82	22.36	4.83	2.26	1.72	0.34
		50	33.87	8.52	29.79	22.34	4.82	2.22	1.68	0.33
AcAA	High	2	52.92	12.94	20.75	9.25	2.61	1.76	0.80	0.21
3 SSU	force	4	33.65	16.31	29.49	15.66	3.28	2.34	1.26	0.24
gds +	milled	6	29.36	15.76	31.66	18.28	3.39	2.62	1.53	0.26
OPTIMAX ®	corn	20	23.21	15.79	34.19	21.14	4.14	2.88	1.81	0.33
L-1000		25	23.38	15.64	33.95	21.22	4.31	2.98	1.89	0.35
1.081 ASPU		30	22.53	15.67	34.21	21.52	4.56	2.92	1.86	0.36
gds		45	22.90	15.17	33.71	21.55	5.17	2.88	1.87	0.41
		50	22.78	14.91	33.84	21.74	5.25	2.93	1.91	0.42
	Whole	2	29.24	15.08	32.48	17.91	4.58	2.55	1.42	0.34
	ground	4	21.98	14.58	35.86	22.19	4.71	2.55	1.60	0.31
	corn	6	17.90	14.84	37.23	24.41	4.93	2.84	1.89	0.35
	liquefact	20	15.39	13.24	37.87	26.49	6.34	2.78	1.97	0.43
		25	15.58	12.96	37.53	26.57	6.70	2.95	2.11	0.49
		30	14.68	13.14	37.53	26.93	7.10	2.82	2.05	0.50
		45	13.99	12.11	37.12	27.77	8.37	2.83	2.14	0.59
		50	14.54	11.34	37.02	27.93	8.55	2.97	2.27	0.63

[0382] Table 7 shows that a DP3 rich syrup was produced from high force milled corn by incubation with the *Aspergillus clavatus* alpha-amylase (AcAA). A higher percentage and concentration of DP3 was obtained by addition of pullulanase. The concentrations of DP3 in the syrups produced from high force milled corn and whole ground corn liquefact were similar, while the percentage of DP3 was lower in the former compared to the latter. The percentages of DPn and DP4 were higher for high force milled corn incubations than for the reference reactions. DP1 was higher for high force milled corn, than for the traditional liquefact.

[0383] Addition of OPTIMAX® L-1000 resulted for both substrates in a decrease of DPn % and an increase in DP4%. DP2 content was relatively and absolutely lower for high force milled corn incubations, while DP4% and DPn % were higher. DP1 was lower for the high force milled corn, than for the traditional liquefact. This may indicate that the whole ground corn liquefact contains limited dextrins, or similar structures, which result in more DP2 and DP1 upon hydrolysis by pullulanase, while the high force milled corn consists of larger starch fragments. Hydrolysis by pullulanase of these fragments resulted in more substrate for AcAA, so more DP3 was produced, but not more DP2 and DP1, as observed when comparing results with and without OPTIMAX® L-1000.

[0384] This example shows that syrups rich in DP3 were produced directly from corn without a liquefaction step by high force milling the substrate and incubating with a DP3 producing alpha-amylase, with or without a pullulanase. Also it was shown that incubations of high force milled corn with a DP3 producing alpha-amylase and pullulanase resulted in lower DP1 and DP2 than incubations of liquefaction of corn with the same enzymes.

Example 12

Maltotetraose Production from High Force Milled Corn using an Alpha-Amylase

[0385] In this experiment a DP4 producing alpha-amylase with and without pullulanase was tested to see whether a syrup rich in DP4 can be made directly from high force milled corn without application of liquefaction with alpha-amylase. The results were compared to those obtained with a traditional corn liquefact.

[0386] A 10% DS aqueous slurry of high force milled corn (prepared as described in Example 2) was incubated at pH 5.0 and 60° C. with 0.041 BMK gds OPTIMALT® 4G with or without 1.081 ASPU gds OPTIMAX® L-1000.

[0387] As reference, corn liquefact (see General Methods) was diluted to 10% DS and the slurry was incubated at pH 4.4 at 6° C. with 0.041 BMK gds OPTIMALT® 4G with or without 1.081 ASPU gds OPTIMAX® L-1000.

[0388] Each slurry was constantly shaken (orbital shaker incubator; 100 rpm) and samples were taken at different time intervals for determination of sugar composition and concentration (DP4% w/v was estimated by extrapolating DP3's calibration curve). The results are shown in Table 8.

TABLE 8

Sugar compositions of high force milled corn and corn liquefact treated with DP4 producing alpha-amylase with and without pullanase

enzyme	substrate	t [h]	DPn %	DP4 %	DP3 %	DP2 %	DP1 %	DP4 (% w/V)	DP3 (% w/V)	DP2 (% w/V)	DP1 (% w/V)
OPTIMALT ®	High	2	55.18	34.21	4.62	1.23	2.98	2.60	0.35	0.09	0.21
4G	force	4	47.77	38.69	6.36	1.88	3.65	3.03	0.50	0.15	0.27
0.041 BMK	milled	6	41.87	41.36	8.16	2.52	4.50	3.36	0.66	0.21	0.34
gds	corn	20	35.66	38.35	12.32	5.05	7.13	3.36	1.08	0.45	0.58
		25	35.23	37.22	13.02	5.49	7.62	3.31	1.16	0.49	0.63
		30	34.07	36.53	13.69	6.02	8.26	3.24	1.21	0.54	0.68
		45	33.19	33.48	15.23	7.05	9.64	2.96	1.35	0.63	0.80
		50	33.00	32.56	15.68	7.41	9.95	2.93	1.41	0.68	0.84
	Whole	2	35.24	39.41	10.73	8.11	5.79	3.08	0.84	0.64	0.42
	ground	4	33.74	40.43	10.75	8.48	5.93	3.00	0.80	0.64	0.41
	corn	6	32.64	41.47	10.99	8.15	6.05	3.15	0.83	0.63	0.43
	liquefact	20	30.49	40.09	12.98	8.62	7.20	3.16	1.02	0.69	0.53
		25	30.80	39.19	13.43	8.63	7.37	3.18	1.09	0.71	0.56
		30	30.25	38.65	13.94	8.83	7.74	3.16	1.14	0.73	0.59
		45	29.73	36.20	15.46	9.42	8.63	3.13	1.34	0.82	0.70
		50	29.82	35.53	15.77	9.44	8.89	3.12	1.38	0.84	0.73
OPTIMALT ®	High	2	48.32	39.50	5.53	1.67	3.25	3.14	0.44	0.13	0.24
4G	force	4	35.74	47.92	7.90	2.72	4.10	3.81	0.63	0.22	0.30
0.041 BMK	milled	6	25.99	53.58	10.29	3.53	5.08	4.39	0.84	0.29	0.39
gds +	corn	20	11.33	56.80	15.92	6.54	7.95	4.97	1.39	0.58	0.65
OPTIMAX ®		25	10.51	55.51	16.92	7.07	8.57	4.96	1.51	0.64	0.71
L-1000		30	8.61	54.77	18.16	7.68	9.34	4.83	1.60	0.69	0.77
1.081 ASPU		45	8.11	49.44	20.77	9.30	10.98	4.36	1.83	0.83	0.90
gds		50	8.88	47.93	20.98	9.42	11.39	4.26	1.86	0.85	0.94
	Whole	2	24.97	46.82	12.46	8.81	6.23	3.42	0.91	0.65	0.42
	ground	4	19.17	51.27	13.33	9.03	6.53	3.81	0.99	0.68	0.45
	corn	6	13.48	54.91	14.49	9.48	6.98	4.37	1.15	0.76	0.52
	liquefact	20	6.82	55.47	17.69	10.72	8.67	4.31	1.38	0.84	0.63
		25	7.46	53.62	18.43	11.22	8.73	4.61	1.59	0.98	0.70
		30	6.69	53.70	18.69	10.96	9.41	4.35	1.51	0.90	0.71
		45	5.50	50.06	21.20	11.81	10.92	4.32	1.83	1.03	0.88
		50	5.34	49.05	21.73	12.08	11.27	3.95	1.75	0.98	0.85

[0389] Table 8 shows that incubations with OPTIMALT® 4G with and without OPTIMAX® L-1000 resulted in similar DP4 percentages for the high force milled corn as for the whole ground corn liquefact.

[0390] With and without OPTIMAX® L-1000, the incubations with high force milled corn resulted in higher DP1, lower DP2 and again higher DP3 concentrations than incubations with corn liquefact. This indicates that the soluble substrate obtained by high force milling has a different structure than the material obtained by liquefaction of whole ground corn.

[0391] Without OPTIMAX® L-1000, DP4 was slightly lower for high force milled corn than for corn liquefact, but with OPTIMAX® L-1000 the experiment with high force milled corn resulted in a higher concentration of DP4 than with liquefact. This means that high force milling provided a better substrate than liquefaction.

[0392] This example shows that syrups rich in DP4 were produced directly from corn without a liquefaction step by high force milling the substrate and incubating with a DP4 producing alpha-amylase with or without a pullulanase. It also shows that the yield obtained when OPTIMALT® 4G and OPTIMAX® L-1000 were incubated with high force mill treated corn, was higher than when incubated with liquefact.

Example 13

Maltopentaose Production from High Force Milled Corn using an Alpha-Amylase

[0393] In this experiment a DP5 producing alpha-amylase with and without pullulanase was tested to see whether a syrup rich in DP5 can be made directly from high force milled corn without application of liquefaction with alpha-amylase. The results were compared to those obtained with a traditional corn liquefact.

[0394] A 10% DS aqueous slurry of high force milled corn (prepared as described in Example 2) was incubated at pH 5.0 and 60° C. with 3 μg gds *Cytophaga* sp. DP5 producing alpha-amylase (DP5AA) with or without 1.081 ASPU gds OPTIMAX® L-1000.

[0395] As reference corn liquefact (see General Methods) was diluted to 10% DS and the slurry was incubated at pH 4.4 at 60° C. with 3 μg gds DP5 producing alpha-amylase with or without 1.081 ASPU gds OPTIMAX® L-1000.

[0396] Each slurry was constantly shaken (orbital shaker incubator; 100 rpm) and samples were taken at different time intervals for determination of sugar composition and concentration. The results are shown in Table 9.

TABLE 9

Sugar compositions of high force milled corn and corn liquefact treated with a DP5 producing alpha-amylase with and without pullulanase

enzyme	substrate	t [h]	DPn %	DP10 %	DP9 %	DP8 %	DP7 %	DP6 %	DP5 %	DP4 %	DP3 %	DP2 %	DP1 %
DP5 AA	High	2	34.35	1.18	1.46	1.99	7.73	13.74	12.41	4.61	10.60	6.68	5.24
3 μg	force	4	32.20	1.19	1.33	1.54	6.16	13.12	13.94	5.23	11.81	8.05	5.40
gds	milled	20	23.63	1.85	1.99	1.96	2.49	12.04	21.21	5.43	13.32	9.32	6.76
	corn	25	23.19	1.73	1.98	1.94	2.45	11.72	21.50	5.61	13.46	9.47	6.92
		30	23.05	1.73	2.02	1.96	2.40	11.44	21.65	5.73	13.52	9.52	7.00
		45	22.92	1.71	2.05	2.00	2.39	10.94	21.48	6.01	13.60	9.61	7.29
		50	23.04	1.65	2.05	2.01	2.38	10.83	21.36	6.13	13.55	9.63	7.37
	Whole	2	55.05	1.63	2.11	3.05	7.25	7.81	7.76	2.73	7.07	2.94	2.61
	ground	4	39.68	1.40	1.67	2.25	6.93	10.81	12.93	4.20	10.74	5.35	4.03
	corn	20	21.83	1.95	2.16	2.11	2.40	8.65	20.57	7.23	13.33	10.73	9.04
	liquefact	25	20.97	1.90	2.11	2.06	2.33	8.17	20.46	7.55	13.55	11.24	9.67
		30	20.40	1.87	2.10	2.06	2.30	7.72	20.26	7.79	13.73	11.65	10.11
		45	19.84	1.72	2.04	2.04	2.27	6.92	19.19	8.28	14.20	12.58	10.93
		50	19.97	1.65	2.03	2.02	2.29	6.76	18.83	8.37	14.28	12.75	11.05
DP5 AA	High	2	28.01	1.56	2.33	3.44	9.97	14.47	11.91	5.52	11.40	6.39	5.02
3 μg	force	4	19.79	1.21	1.86	2.82	10.13	16.22	14.77	6.35	14.01	7.42	5.41
gds +	milled	20	4.45	0.35	0.73	1.51	4.42	17.69	25.43	8.35	18.85	11.32	6.89
OPTIMAX ®	corn	25	3.81	0.32	0.60	1.38	3.83	17.04	26.03	8.61	19.40	11.80	7.19
L-1000		30	3.64	0.30	0.49	1.24	3.49	17.19	26.82	8.53	19.28	11.85	7.18
1.081 ASPU		45	3.67	0.12	0.41	1.06	3.00	16.77	27.24	8.75	19.45	12.11	7.43
gds		50	3.53	0.33	0.39	1.05	2.90	16.66	27.18	8.84	19.45	12.21	7.46
	Whole	2	42.74	2.47	3.27	4.68	9.47	9.35	9.08	3.68	9.03	3.38	2.85
	ground	4	27.14	1.25	1.80	2.96	10.60	13.02	13.79	5.57	13.98	5.75	4.14
	corn	20	4.58	0.39	0.60	1.15	2.58	13.31	25.88	10.10	19.45	13.01	8.95
	liquefact	25	4.39	0.33	0.48	1.02	2.24	12.54	25.96	10.37	19.73	13.57	9.38
		30	3.48	0.30	0.42	0.95	2.03	11.99	25.97	10.72	20.13	14.16	9.83
		45	3.79	0.43	0.35	0.81	1.72	10.59	24.83	11.21	20.56	15.13	10.57
		50	3.95	0.33	0.35	0.78	1.66	10.34	24.45	11.35	20.69	15.37	10.74

[0397] Table 9 shows that from high force milled corn a DP5 rich syrup was produced by incubating the substrate with DP5 AA. It can be seen that a higher percentage of DP5 and DP6 was produced with high force milled corn in comparison with whole ground corn liquefact. The incubation with the latter resulted in higher DP1, DP2, DP3, and DP4. This indicates that the substrate available for hydrolysis had a larger polymer size than that found in liquefied corn, since the larger percentage of smaller sugars indicates more undesirable sugars (sugars other than maltopentaose) from hydrolysis. This resulted in a higher percentage of the desired maltodextrins; in this case DP5.

[0398] The results showed that addition of OPTIMAX® L-1000 increased the percentage of DP5 by hydrolysing DPn as well as reducing DP8-DP10. The differences between substrates can again be seen with addition of OPTIMAX® L-1000; incubation with high force milled corn resulted in lower DPn than with liquefact, DP6 and DP5 were higher, while DP1-DP4 were lower.

[0399] This example shows that syrups rich in DP5 were produced directly from corn without a liquefaction step by high force milling the substrate and incubating with a DP5 producing alpha-amylase with or without a pullulanase. It showed that corn treated by a high force mill resulted in a better substrate for this application than corn traditionally liquefied.

Example 14

Production of Fermentable Sugars During Hydrolysis of High Force Milled Corn

[0400] An experiment was performed to compare high force milled corn (prepared as described in Example 2) and corn flour in production of glucose during direct corn to fermentable sugar production at pH 4.5 and 60° C. The experiments were carried out using 20% dry solids, with DI water making up the 100% of the liquid phase of a slurry of each sample. The pH of each slurry was adjusted to pH 4.5. A 35 g aliquot of each corn slurry with 2 replications for each treatment was weighed into LABOMAT steel beakers. Following the weighing process the enzymes as given below in Table 10 were dispersed in each slurry. The metal beakers were fitted back into the LABOMAT for incubation at 60° C. for 48 hrs with clockwise and counter-clockwise constant mixing at 60 rpm. Samples were drawn at16, 24 and 48 hrs to analyze the percent solubility and saccharide profile (shown in Table 10).

[0401] The alpha-amylase used was SPEZYME® RSL (a blend of alpha-amylase and phytase), which was added at 8 AAUs/gds and the glucoamylase used was AfuGA, which was added at 0.2 GAUs/gds. Percent solubilization and saccharide profiles at various intervals are given in Table 10.

TABLE 10

Percent solubilization and saccharide profiles for high force milled corn and corn flour treated with alpha-amylase and glucoamylase

sample	enzyme	% sol.	hr	% HS	% DP3	% DP2	% DP1	g glucose/ liter
high	8 AAUs	97.5	16	3.95	1.46	4.09	90.49	152.1
force	0.2	98.1	24	2.40	1.23	4.09	92.28	156.2
milled	GAUs	98.4	48	2.78	0.85	4.25	92.12	156.4
corn								

TABLE 10-continued

Percent solubilization and saccharide profiles for high force milled corn and corn flour treated with alpha-amylase and glucoamylase

sample	enzyme	% sol.	hr	% HS	% DP3	% DP2	% DP1	g glucose/ liter
Corn flour	8 AAUs 0.2 GAUs	90.7 92.2 92.9	16 24 48	6.01 4.75 4.51	0.95 0.77 0.43	5.57 5.20 4.30	87.46 89.27 90.76	136.8 142.0 145.4

[0402] The DP1 production with increasing percent solubilization shown in Table 10 is evidence of ring and puck mill ground corn yielding higher solubility and providing a higher amount of glucose throughout the incubation process as compared to the ground corn.

Example 15

Use of High Force Milled Corn in a No-Cook Simultaneous Saccharification and Fermentation Process

[0403] High force milled (ring and puck milled) corn was evaluated for performance in a fuel ethanol production process using a no-cook simultaneous saccharification and fermentation (SSF) process. In this example high force milled corn (prepared as described in Example 2) was subjected to different gluco-amylase (GA) enzyme dosages and compared to a coarse whole ground corn (39%<500 μm) and a corn flour substrate (85%<500 μm). The experiments were carried out using a 22% dry solids corn slurry, solid urea was added to a final concentration of 800 ppm and the pH was adjusted to 4.5 using 6 N sulfuric acid. 50 grams (+/−0.2 g) of each slurry was weighed into replicate 125 ml Erlenmeyer flasks and 6 SSU/g ds *Aspergillus kawachii* alpha-amylase (AkAA) and 0.1 SAPU/g ds FERMGEN™ 2.5x was added to each flask. 0.1% w/w of FERMAX™ GOLD yeast (Martrex, Inc. Minnetonka, Minn.) was added to each flask as well. *Trichoderma reesei* GA (TrGA) was added to the flasks in the following dosages: 0.325-0.5-1.0 GAU/g ds. The control in this experiment was the fine milled corn flour with a TrGA dosage of 1.0 GAU/g ds and an AKAA dosage of 6 SSU/g ds. All flasks were incubated at 32° C. for up to 90 hours. Time point samples were collected throughout the saccharification and fermentation (SSF) at t=0, 4, 16, 30, 44, 55 and 72 hours.

[0404] Following SSF, each time point sample was prepared and analyzed via HPLC for ethanol, DP1, DP2, DP3, DP4+, and glycerol. Each sample was allowed to thaw at 4° C. and was centrifuged at 15,000 rpm for 5 minutes. 100 μl of each sample supernatant was incubated with 10 μl of 1.1 N sulfuric acid for 5 minutes at 100° C. Following incubation, 1 ml of Milli-Q water was added to each sample and all samples were filtered with a 0.22 μm filter. 20 μl of each sample was injected into an Agilent 1200 series HPLC and run on a Phenomenex Rezex-RFQ Fast Fruit column (cat #00D-0223-K0) with a Phenomenex Rezex ROA Organic Acid guard column (cat #03B-0138-K0), heated to 85° C., using a 9 minute isocractic elution in 0.01 N sulfuric acid at 1 ml/min. Component concentrations were determined from a five point calibration curve using a Supelco Fuel Ethanol standard (Sigma cat #48468-U).

[0405] Table 11 shows the yields for ethanol and glycerol during up to 44 hrs of SSF for the different substrates. The initial rate of ethanol production (over the first 15 min) was much higher for the ring and puck milled corn when compared to the coarse ground whole corn and the fine milled corn flour for all of the enzyme dosages tested. After 44 hrs no further increase in ethanol yield was observed. Maximum ethanol yield for the ring and puck milled corn was obtained within 44 hrs and was obtained with a 3× lower enzyme dosage (0.325 GAU/g ds) as compared to the control (corn flour treated with 1.0 GAU/g ds enzyme dosage. Coarse ground whole corn was not effectively hydrolyzed using the no-cook process. More glycerol was produced when more ethanol was produced.

TABLE 11

SSF yields during first 44 hr of fermentation for ethanol and glycerol for high force milled corn, corn flour and coarse whole ground corn

		EtOH conc (v/v %)			Glycerol conc (w/v %)		
TrGA dosage (GAU/ g ds)	Time	Ring and Puck Milled Corn	Corn Flour	Whole Ground Corn	Ring and Puck Milled Corn	Corn Flour	Whole Ground Corn
0.325	0	0	0	0	0.04	0.03	0.04
	4	0.53	0.61	0.59	0.15	0.11	0.12
	16	8.57	3.63	1.61	0.47	0.15	0.11
	30	10.18	4.99	1.98	0.44	0.09	0.05
	44	9.45	6.18	2.38	0.47	0.04	0.03
0.5	0	0	0	0	0.04	0.03	0.04
	4	0.52	0.61	0.57	0.15	0.12	0.11
	16	8.83	4.07	1.73	0.52	0.17	0.11
	30	10.12	5.75	2.31	0.50	0.10	0.06
	44	9.47	6.85	2.67	0.53	0.05	0.03
1.0	0	0	0	0	0.04	0.03	0.04
	4	0.49	0.61	0.71	0.15	0.13	0.13
	16	10.08	5.03	2.53	0.64	0.20	0.13
	30	10.02	6.89	3.57	0.63	0.13	0.08
	44	9.50	8.08	3.96	0.67	0.07	0.04

Example 16

Enzyme Dose Reduction for High Force Milled Corn in a No-Cook Simultaneous Saccharification and Fermentation Process (No-Cook SSF Process)

[0406] High force milled (ring and puck milled) corn was further evaluated after the initial observation that a 3× enzyme dose reduction as compared to the corn flour control was effective in the no-cook SSF process. A similar experimental set-up was used as described in Example 13 with the following deviation: *Trichoderma reesei* GA (TrGA) was added to the flasks in the following dosages for the ring and puck milled corn: 0.1-0.2-0.3 GAU/g ds; and for the corn flour control: 0.5 GAU/g ds and 1.0 GAU/g ds.

[0407] Table 12 shows the yields for ethanol for up to 60 hrs of fermentation for the two substrates and the different enzyme dosages applied. Initial ethanol formation rate was faster for ring and puck milled corn for all enzyme dosages tested compared to the corn flour control. The standard enzyme dosage in the no-cook process is 1.0 GAU/g ds and the data show that a 10× reduced dose (0.1 GAU/g ds) was successful when using the ring and puck milled corn, which has not been observed with any other milled sample. In addition, maximal amounts of ethanol were produced in less time for the ring and puck milled corn.

[0408] Table 13 shows the residual starch after 72 hrs of fermentation for the ring and puck milled corn and corn flour

at the different enzyme dosages applied. Residual starch data clearly show more complete hydrolysis of the ring and puck milled corn as compared to the corn flour and emphasizes the observation that this was obtained with a 10× lower enzyme dose when using ring and puck milled corn.

TABLE 12

SSF yields during first 60 hrs of fermentation for ethanol for 5 min ring and puck milled corn and corn flour at different enzyme dosages

	EtOH conc (v/v %)				
	Ring and Puck Milled Corn			Corn Flour	
Time	0.1 GAU/g ds	0.2 GAU/g ds	0.3 GAU/g ds	0.5 GAU/g ds	1.0 GAU/g ds
0	0	0	0	0.09	0.09
4	0.46	0.42	0.42	0.53	0.53
16	8.38	10.37	9.93	5.29	5.88
38	12.33	12.66	12.59	8.18	9.55
44	12.37	12.54	12.45	8.63	10.20
60	11.62	11.48	11.33	9.76	10.73

TABLE 13

Residual starch (% DW) after 72 hrs for puck mlled corn and corn flour at different enzyme dosages

Residual starch (% DW)				
Ring andPuck Milled Corn			Corn Flour	
0.1 GAU/g ds	0.2 GAU/g ds	0.3 GAU/g ds	0.5 GAU/g ds	1.0 GAU/g ds
6.14	5.36	5.08	45.15	22.23

Example 17

Effect of Milling Time in the Ring and Puck Mill on Required Enzyme Dosage in the No-Cook Simultaneous Saccharification and Fermentation Process (No-Cook SSF Process)

[0409] Corn kernels were treated in the ring and puck mill as described in example 2, except that the mill was run for 30 sec, 1 min and 5 min for different samples. These samples were evaluated in the no-cook SSF process to assess the enzyme dosage needed to convert the samples that were treated for different times. A similar experimental set-up was used as described in Example 13 with the following deviations: (1) 20% dry solids 30 sec, 1 min, and 5 min ring and puck milled corn substrates were used, (2) *Trichoderma reesei* GA (TrGA) was added to the flasks in the following dosages for the 30 sec, 1 min and 5 min ring and puck milled corn samples: 0.1-0.3-0.5 GAU/g ds, and for the corn flour control: 0.5 GAU/g ds and 1.0 GAU/g ds.

[0410] Table 14 shows the yields for ethanol up to 67 hrs of fermentation for the 30 sec, 1 min and 5 min ring and puck milled corn samples using different enzyme dosages; corn flour was used as the control. 30 sec ring and puck milled corn when dosed with 0.1-0.3 GAU/g ds of gluco-amylase did not reach the same final ethanol concentration as the 5 min ring and puck milled corn sample reached after 63 hrs. and clearly more enzyme is needed to finish the fermentation. Increasing the milling time from 30 sec to 1 min results in a faster fermentation with an enzyme dosage of 0.3 GAU/g ds of gluco-amylase for the 1 min ring and puck milled corn compared to the 30 sec ring and puck milled corn. Increasing the milling time from 1 min to 5 min shows that it is possible to obtain highest ethanol with only 0.1 GAU/g ds of gluco-amylase for the 5 min ring and puck milled corn whereas at least 0.3 GAU/g ds is needed for 1 min ring and puck milled corn. Clearly the time of milling can influence the needed enzyme dosage for an efficient fermentation.

[0411] Table 15 shows the residual starch after 97 hrs of fermentation for the 5 min ring and puck milled corn and 1 min ring and puck milled corn at the different enzyme dosages applied. Residual starch data clearly show the more complete hydrolysis of the 1 min and 5 min ring and puck milled corn compared to the corn flour and emphasizes the observation that this can be obtained with a 10× lower enzyme dose when using a 5 min ring and puck milled corn and a 3× lower enzyme dose when using 1 min ring and puck milled corn.

[0412] Table 16 shows the amount of free oil that was detected at the end of the fermentation. More free oil could be obtained from ring and puck milled corn than the control corn flour. This indicates that the high force milling has the potential to free up more oil from the corn than traditional milling.

TABLE 14

SSF yields during first 67 hrs of fermentation for ethanol for 30 sec, 1 min, and 5 min ring and puck milled corn and corn flour at different enzyme dosages

		EtOH conc (v/v %)							
Time	Enzyme dosage	30 sec Ring and Puck Milled Corn		1 min Ring and Puck Milled Corn		5 min Ring and Puck Milled Corn		Corn Flour	
(hrs)	(GAU/g ds)	0.1	0.3	0.3	0.5	0.1	0.3	0.5	1.0
0		0.07	0.07	0.10	0.10	0.07	0.07	0.05	0.05
7		1.79	1.67	2.03	1.87	1.31	1.54	1.68	1.78
14		5.63	6.43	—*	—	4.93	7.46	3.53	3.75
17		—	—	7.64	8.35	—	—	—	—
30		7.10	7.74	8.73	9.19	8.72	10.21	4.80	5.44
44		7.30	8.33	9.07	9.39	9.70	9.75	5.97	6.63
63		7.51	8.39	—	—	9.63	9.27	6.27	6.35
67		—	—	8.31	8.51	—	—	—	—

*sample was not taken

TABLE 15

Residual starch (% DW) after 97 hrs for 5 min ring and puck milled corn and 1 min ring and puck milled corn at different enzyme dosages. Corn flour is the control.

Residual starch (% DW)				
1 Min Ring and Puck Milled Corn		5 Min Ring and Puck Milled Corn	Corn Flour	
0.3 GAU/g ds	0.5 GAU/g ds	0.1 GAU/g ds	0.5 GAU/g ds	1.0 GAU/g ds
5.48	4.10	3.44	17.89	12.33

TABLE 16

Detected free oil after 97 hours of fermentation for 30 sec, 1 min, 5 min ring and puck milled corn and corn flour

Sample	µg free oil
Corn Flour/1.0 GAU	Below detection limit
30 sec ring and puck milled corn, 0.3 GAU	170
1 min ring and puck milled corn, 0.3 GAU	226
5 min ring and puck milled corn, 0.3 GAU	205

Example 18

Use of High Force Milled Starch Substrates in a No-Cook Simultaneous Saccharification and Fermentation (SSF) Process

[0413] High force milled (puck milled) cassava, rice, sorghum and wheat were evaluated for performance in a fuel ethanol production process using a no-cook simultaneous saccharification and fermentation (SSF) process. In this example high force milled starch containing substrates (prepared as described in Example 1) were compared to a conventionally milled substrate (equivalent to corn flour substrate (85%<500 µm)). The experiments were carried out using 20% dry solids slurry, solid urea was added to a final concentration of 800 ppm and the pH was adjusted to 4.5 using 6N sulfuric acid. 50 grams (+/−0.2 g) of each slurry was weighed into replicate 125 ml Erlenmeyer flasks and 6 SSU/g ds *Aspergillus kawachii* alpha-amylase (AkAA) and 0.1 SAPU/g ds FERMGEN™ 2.5x were added to each flask. A 0.1% w/w of FERMAX™ GOLD yeast (Martrex, Inc. Minnetonka, Minn.) was added to each flask as well. *Trichoderma reesei* GA (TrGA) was added to the flasks at the 0.3 GAUs/g ds for the high force milled substrate. The control in this experiment was the conventionally milled flour for each of the starch containing substrate with a TrGA dosage of 1.0 GAU/g ds and an AKAA dosage of 6 SSU/g ds. All flasks were incubated at 32° C. for up to 72 hours. Time point samples were collected throughout the saccharification and fermentation (SSF) at t=0, 11, 15, 23, 30, 45, 55 and 72 hours.

[0414] Following SSF, each time point sample was prepared and analyzed via HPLC for ethanol, DP1, DP2, DP3, DP4+, lactic acid, acetic acid and glycerol. Each sample was allowed to thaw at 4° C. and was centrifuged at 15,000 rpm for 5 minutes. 100 µl of each sample supernatant was incubated with 10 µl of 1.1 N sulfuric acid for 5 minutes at 100° C. Following incubation, 1 ml of Milli-Q water was added to each sample and all samples were filtered with a 0.22 µm filter. 20 µl of each sample was injected into an Agilent 1200 series HPLC and run on a Phenomenex Rezex-RFQ Fast Fruit column (cat #00D-0223-K0) with a Phenomenex Rezex ROA Organic Acid guard column (cat #03B-0138-K0), heated to 85° C., using a 9 minute isocractic elution in 0.01 N sulfuric acid at 1 ml/min. Component concentrations were determined from a five point calibration curve using a Supelco Fuel Ethanol standard (Sigma cat #48468-U).

[0415] Table 17 shows the yields for ethanol during up to 45 hrs of SSF for the different substrates. The initial rate of ethanol production (over the first 15 hr) was much higher for the puck milled substrate (except for rice) when compared to the conventionally milled substrate for the enzyme dosages tested. After 45 hrs no further increase in ethanol yield was observed. Maximum ethanol yield for the puck milled corn was obtained within 45 hrs and was obtained with a 3× lower enzyme dosage (0.3 GAU/g ds) as compared to the control (conventionally milled substrate treated with 1.0 GAU/g ds enzyme dosage.

TABLE 17

Cold cook SSF yields for various starch containing substrates during first 45 hr of fermentation for ethanol (% v/v)

Hrs	Puck milled Cassava 0.3 GAUs/g ds	Conventional milled Cassava 1 GAUs/g ds	Puck Milled Rice 0.3 GAUs/g ds	Conventional milled Rice 1 GAUs/g ds	Puck Milled *Sorghum* 0.3 GAUs/g ds	Conventional milled *Sorghum* 1 GAUs/g ds	Puck Milled Wheat 0.3 GAUs/g ds	Conventional milled Wheat 1 GAUs/g ds
11	2.39	2.04	1.46	1.92	1.71	2.44	2.07	2.02
15	5.52	2.85	3.70	4.95	4.45	3.67	5.04	2.47
23	7.47	3.78	6.30	6.19	6.54	4.75	6.74	3.03
30	7.98	4.58	7.67	6.80	7.53	5.35	7.03	3.49
45	8.21	5.58	8.36	7.52	7.67	6.09	7.05	4.05

What is claimed is:

1. A process for producing destructured starch-based biomass comprising:

a) providing a starch-based biomass; and

b) applying to the biomass of (a) at least one set of compression and impact forces of at least 5,000 N combined with shearing forces;

wherein contact stress of greater than 5,000 psi is applied to the biomass and wherein a destructured starch-based biomass is produced.

2. A process for producing destructured starch-based biomass comprising:

a) providing a portion of starch-based biomass; and

b) applying to the biomass of (a) at least one set of compression and impact forces of at least 1,500 N combined with shearing forces, and contact stress of greater than 5,000 psi;

wherein specific energy input in the process is less than 40% of the total combustible energy of the portion of biomass being treated and wherein a destructured starch-based biomass is produced.

3. The process of claim **2** wherein the compression and impact forces are at least 3,000 N.

4. The process of claim **1** or **2** wherein the stress is greater than about 8,000 psi.

5. The process of claim **4** wherein the stress is greater than about 13,000 psi.

6. The process of claim **1** wherein the forces are imparted using a non-vibratory apparatus which does not contain free flowing media.

7. The process of claim **1** or **2** wherein the forces are imparted by centrifugal motion, hydraulics, or springs.

8. The process of claim **7** wherein the forces are imparted by centrifugal motion and the Radii Ratio is less than 0.5.

9. The process of claim **8** wherein the forces are imparted by centrifugal motion and the Radii Ratio is less than 0.4.

10. The process of claim **9** wherein the forces are imparted by centrifugal motion and the Radii Ratio is about 0.33.

11. The process of claim **1** or **2** wherein the starch-based biomass is selected from the group consisting of grains and starchy roots or tubors.

12. The process of claim **11** wherein grains is selected from corn, rice, wheat, barley, sorghum, millet, rye, milo, triticale, and mixtures thereof.

13. The process of claim **11** wherein starchy roots are roots selected from potato, sweet potato, yam, taro, carrot, turnip, cassava, and mixtures thereof.

14. The process of claim **1** or **2** wherein the destructured starch-based biomass has crystallinity that is reduced by at least about 25% as compared to the starch-based biomass provided in (a).

15. The process of claim **1** or **2** wherein each individual application of force occurs in less than ten milliseconds.

16. The process of claim **1** or **2** wherein compression and impact forces applied to the biomass are applied by smooth surfaces.

17. The process of claim **1** wherein G-force is applied at less than 10 G.

18. The process of claim **1** or **2** wherein at least one set of compression and impact forces is at least about 10,000 newtons

19. The process of claim **18** wherein at least one set of compression and impact forces is at least about 50,000 newtons

20. The process of claim **19** wherein at least one set of compression and impact forces is at least about 100,000 newtons

21. The process of claim **7** wherein the compression and impact forces are imparted by centrifugal motion by moving an object with a mass of at least 100 kilograms.

22. The process of claim **1** or **2** further comprising contacting the destructured starch-based biomass with at least one saccharification enzyme under suitable conditions for saccharification wherein sugars are produced.

23. A destructured starch-based biomass obtainable by the process of claim **1** or **2** comprising starch having at least 25% reduction in crystallinity as compared to provided starch-based biomass.

24. The destructured starch-based biomass of claim **23** having a processed energy equivalency of greater than 60%.

25. The destructured starch-based biomass of claim **23** further comprising a destructured cellulosic biomass component.

26. The composition of claim **23** further comprising free oil.

27. A feed additive composition comprising a destructured starch-based biomass obtainable (e.g. obtained) by the process of any one of claims **1** to **22**.

28. A feed additive composition comprising a destructured starch-based biomass obtainable (e.g. obtained) by the process of any one of claims **1** to **22** in combination with at least one in-feed enzyme.

29. A feed additive kit comprising a first container comprising a destructured starch-based biomass obtainable (e.g. obtained) by the process of any one of claims **1** to **22** and a second container comprising at least one in-feed enzyme, and instructions for co-administering same.

30. A feed or feedstuff comprising a feed additive composition according to claim **27** or claim **28**.

31. A premix comprising a feed additive composition according to claim **27** or claim **28**, and at least one mineral and/or at least one vitamin.

32. A method for improving a biophysical characteristic of an animal which method comprises administering to an animal a feed additive composition according to claim **27** or claim **28** or a feed according to claim **30** or a premix according to claim **31**.

33. Use of a feed additive composition according to claim **27** or claim **28** or a feed according to claim **30** or a premix according to claim **31** for improving a biophysical characteristic of an animal.

34. A method of preparing a feedstuff comprising contacting a feed component with a feed additive composition according to claim **27** or claim **28**.

35. A method of saccharifying a composition comprising destructured starch-based biomass to produce a composition comprising glucose, wherein said method comprises:

(i) contacting a destructured starch-based biomass composition with a glucoamylase; and

(ii) saccharifying the composition to produce said glucose composition.

36. The method of claim **35**, wherein the destructured starch-based biomass is from a process of any one of claim **1-22**.

37. The method of claim **35** or claim **36**, wherein the glucose composition is produced without a liquefaction step.

38. The method of any one of claims **35-37**, wherein the glucose composition has a DP1 concentration higher than would result when saccharifying a whole ground corn liquefact composition in step (i) in place of the destructured starch-based biomass composition; wherein the higher DP1 concentration is selected from the list consisting of

(a) at least 0.1% higher DP1;

(b) at least 0.2% higher DP1;

(c) at least 0.4% higher DP1; and

(d) at least 0.5% higher DP1.

39. The method of any one of claims **35-38**, wherein the glucose composition has a DP2 concentration lower than would result when saccharifying a whole ground corn liquefact composition in step (i) in place of the destructured starch-based biomass composition; wherein DP2 concentration at least 0.1% lower.

40. A method of saccharifying a composition comprising destructured starch-based biomass to produce a composition comprising maltose, wherein said method comprises:

(i) contacting a destructured starch-based biomass composition with a maltogenic alpha-amylase; and

(ii) saccharifying the composition to produce said maltose composition.

41. The method of claim **40**, wherein the destructured starch-based biomass composition is from a process of any one of claim **1-22**.

42. The method of claim **40** or claim **41**, wherein the maltose composition is produced without one or both of a liquefaction step and addition of an additional alpha-amylase.

43. The method of any one of claims **40-42**, wherein the maltose composition has a DP2 concentration higher than would result when saccharifying a whole ground corn liquefact composition in step (i) in place of the destructured starch-based biomass composition; wherein the higher DP2 concentration is at least 0.1% higher.

44. A method of saccharifying a composition comprising destructured starch-based biomass to produce a composition comprising maltoriose, wherein said method comprises:

(i) contacting a destructured starch-based biomass composition with a beta-amylase and optionally a pullulanase; and

(ii) saccharifying the composition to produce said maltotriose composition.

45. The method of claim **44**, wherein the destructured starch-based biomass composition is from a process of any one of claim **1-22**.

46. The method of claim **44** or claim **45**, wherein the maltotriose composition is produced without one or both of a liquefaction step and addition of an additional alpha-amylase.

47. The method of any one of claims **44-46**, wherein the maltotriose composition has lower DP1 and DP2 concentrations than would result when saccharifying a whole ground corn liquefact composition in step (i) in place of the destructured starch-based biomass composition; wherein the DP1 and DP2 concentrations are at least 0.1% lower.

48. A method of saccharifying a composition comprising destructured starch-based biomass to produce a composition comprising maltotetraose, wherein said method comprises:

(i) contacting a destructured starch-based biomass composition with a DP4 producing alpha-amylase and optionally a pullulanase; and

(ii) saccharifying the composition to produce said maltotetraose composition.

49. The method of claim **48**, wherein the destructured starch-based biomass composition is from a process of any one of claim **1-22**.

50. The method of claim **48** or claim **49**, wherein the maltotetraose composition is produced without one or both of a liquefaction step and addition of an additional alpha-amylase.

51. The method of any one of claims **48-59**, wherein the maltotetraose composition has a DP4 concentration higher than would result when saccharifying a whole ground corn liquefact composition in step (i) in place of the destructured starch-based biomass composition; wherein the higher DP4 concentration is selected from the list consisting of

(a) at least 0.1% higher DP4;

(b) at least 0.2% higher DP4;

(c) at least 0.4% higher DP4; and

(d) at least 0.5% higher DP4.

52. A method of saccharifying a composition comprising destructured starch-based biomass to produce a composition comprising maltopentaose, wherein said method comprises:

(i) contacting a destructured starch-based biomass composition with a DP5 producing alpha-amylase and optionally a pullulanase; and

(ii) saccharifying the composition to produce said maltopentaose composition.

53. The method of claim **52**, wherein the destructured starch-based biomass composition is from a process of any one of claim **1-22**.

54. The method of claim **52** or claim **53**, wherein the maltopentaose composition is produced without one or both of a liquefaction step and addition of an additional alpha-amylase.

55. The method of any one of claims **52-54**, wherein the maltopentaose composition has a DP5 concentration higher than would result when saccharifying a whole ground corn liquefact composition in step (i) in place of the destructured starch-based biomass composition; wherein the higher DP5 concentration is selected from the list consisting of

(a) at least 0.1% higher DP5;

(b) at least 0.2% higher DP5;

(c) at least 0.4% higher DP5;

(d) at least 0.5% higher DP5;

(e) at least 1.0% higher DP5;

(f) at least 1.5% higher DP5; and

(g) at least 2.0% higher DP5.

56. The method of any one of claims **35-55**, wherein saccharifying is conducted at a temperature range of about 30° C. to about 65° C.

57. The method of claim **56**, wherein said temperature range is 47° C. to 60° C.

58. The method of any one of claims **35-57**, further comprising fermenting the produced composition to produce an End of Fermentation (EOF) product.

59. The method of claim **58**, wherein said fermentation is a simultaneous saccharification and fermentation (SSF) reaction.

60. The method of claim **58** or **59**, wherein said fermentation is conducted for 24-70 hours at pH 2-8 and in a temperature range of 25° C.-70° C.

62. The method of any one of claims **58-60**, wherein the EOF product comprises ethanol.

63. The method of any one of claims **58-61**, wherein the EOF product comprises 8%-18% (v/v) ethanol.

64. The method of any one of claims **58-60**, wherein the EOF product comprises a metabolite.

65. The method of claim **64**, wherein the metabolite is an organic acid, citric acid, lactic acid, succinic acid, monosodium glutamate, gluconic acid, sodium gluconate, calcium gluconate, potassium gluconate, glucono delta-lactone, sodium erythorbate, omega 3 fatty acid, butanol, an amino acid, lysine, itaconic acid, 1,3-propanediol, or isoprene.

66. The method of any one of claims **35-65**, further comprising adding in step (i) or step (ii) an additional glucoamylase, hexokinase, xylanase, glucose isomerase, xylose isomerase, phosphatase, phytase, protease, pullulanase, alpha-amylase, beta-amylase, protease, cellulase, hemicellulase, lipase, cutinase, trehalase, isoamylase, redox enzyme, esterase, transferase, pectinase, alpha-glucosidase, beta-glucosidase, lyase, hydrolase, or a combination thereof.

67. A method of producing alcohol from a destructured starch-based biomass substrate by simultaneous saccharification and fermentation (SSF) comprising,

(i) contacting a slurry comprising destructured starch-based biomass with an alpha-amylase, a glucoamylase, a yeast and optionally an acid stable protease, at a temperature below the starch gelatinization temperature of the destructured starch-based biomass to produce oligosaccharides fermentable by the yeast;

(ii) fermenting the oligosaccharides to produce alcohol.

68. The method of claim **67**, wherein the destructured starch-based biomass composition is from a process of any one of claim **1**-**22**.

69. The method of claim **67** or claim **68**, wherein the initial percentage of alcohol production over the initial phase of SSF is a higher percentage than would result when using a milled corn flour or whole ground corn composition in step (i) in place of the destructured starch-based biomass composition.

70. The method of any one of claims **67**-**69**, wherein the initial phase of SSF is the first 15 minutes of SSF.

71. The method of claim **69** or claim **70**, wherein the higher percentage of alcohol resulting in the initial phase of SSF is a fold higher % selected from the list consisting of

(a) at least 2 fold higher % vs. corn flour; and

(b) at least 4 fold higher % vs. whole ground corn.

72. The method of claim **67** or claim **68**, wherein the end of SSF total alcohol yield requires 3 fold less glucoamylase dosage as when using a milled corn flour or whole ground corn composition in step (i) in place of the destructured starch-based biomass composition.

73. The method of claim **67** or claim **68**, wherein up to 10 fold less glucoamylase is required to produce a comparable end of SSF alcohol yield for destructured starch-based biomass composition than would be required for corn flour.

74. The method of any one of claims **67**-**73**, wherein the alcohol produced is ethanol.

75. The method of any one of claims **35**-**70**, wherein the destructured starch-based biomass composition is selected from the group consisting of corn, barley, cassava, rice, rye, milo, millet, sorghum, triticale, wheat and any combination thereof.

76. The method of any one of claims **35**-**70**, wherein the destructured starch-based biomass composition is selected from the group consisting of corn, cassava, sorghum, wheat and any combination thereof.

77. The method of any one of claims **35**-**70**, wherein the destructured starch-based biomass composition is corn.

78. The method of any one of claims **35**-**70**, wherein the destructured starch-based biomass comprises a composition obtained from processed starch-based biomass.

* * * * *