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[Continued on next page]

(54) Title: PROCESSING OF PALM KERNEL WASTE USING MANNANASE AND PECTINASE

(57) Abstract: Described are compositions and methods for processing palm kernel waste (PKW) using a combination of mannanase and pectinase to significantly improve mannose yield.

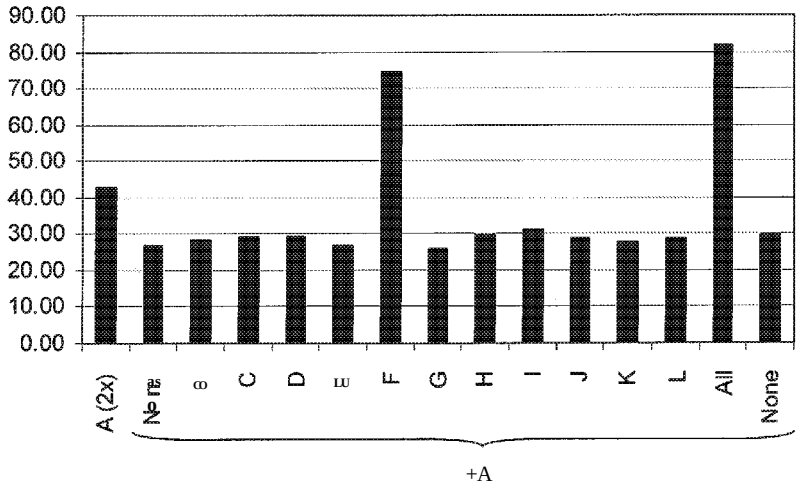

Figure 1

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PROCESSING OF PALM KERNEL WASTE USING MANNANASE AND PECTINASE

PRIORITY

[01] The present application claims priority to U.S. Provisional Application Serial No. 61/390,846, filed October 7, 2010, which is incorporated by reference in its entirety.

TECHNICAL FIELD

[02] Described are compositions and methods for processing palm kernel waste (PKW) using a combination of mannanase and pectinase.

BACKGROUND

[03] Palm kernel waste (PKW) is a by-product from the process of extracting palm oil from oil palm kernels. PKW is typically a ground or pulverized composition containing, *e.g.,* palm kernel shells, mesocarp fibers, and empty fruit bunches. PKW can be used as biomass, either directly as a fuel source *(i.e.,* to burn and produce energy) or via enzymatic processing to produce more environmentally friendly forms of fuel.

[04] PKW is rich in galacto-mannans (typically 30-50% by weight), and can be processed using mannanases to yield significant quantities of mannose. Mannose has a variety of uses, *e.g.,* in the food and beverage industries, and represents a higher-value product than biomass.

SUMMARY

[05] Described are compositions and methods for preparing mannose from palm kernel waste (PKW), involving contacting the PKW with a combination of a mannanase and a pectinase.

[06] In one aspect, a method of preparing mannose from palm kernel waste is provided, comprising: contacting the palm kernel waste with a pectinase and a mannanase, wherein the amount of mannose produced by contacting the palm kernel waste with the pectinase and the mannanase is greater than the amount of mannose produced by contacting an equivalent amount of palm

kernel waste with an equivalent amount of the mannanase in the absence of the pectinase.

[07] In some embodiments, the palm kernel waste is contacted with the pectinase and the mannanase in the same reaction vessel. In some embodiments, the palm kernel waste is contacted with the pectinase and the mannanase simultaneously. In some embodiments, the palm kernel waste is contacted with the pectinase and the mannanase sequentially. In some embodiments, the palm kernel waste is first contacted with the pectinase and then contacted with the mannanase.

[08] In some embodiments, the pectinase and the mannanase are present in an amount sufficient to produce a mannanase to pectinase activity ratio of from about 10,000:1 to about 200,000:1 , based on activity defined in U/g. In some embodiments, the pectinase and the mannanase are present in an amount sufficient to produce a mannanase to pectinase activity ratio of from about 35,000:1 to about 140,000:1 , based on activity defined in U/g.

[09] In some embodiments, the amount of pectinase is sufficient to produce a pectinase activity of from about 0.1 8 to about 3.6 U/g PKW. In some embodiments, the amount of mannanase is sufficient to produce a mannanase activity of from about 12,250 to about 250,000 U/g PKW.

[10] In some embodiments, the pectinase is a plurality of pectinases. In some embodiments, the mannanase is a plurality of mannanases.

[11] In some embodiments, the amount of mannose produced by contacting the palm kernel waste with the pectinase and the mannanase is greater than the amount of mannose produced by contacting an equivalent amount of palm kernel waste with twice the equivalent amount of the mannanase in the absence of the pectinase.

[12] In some embodiments, the amount of mannose produced by contacting the palm kernel waste with the pectinase and the mannanase is twice the amount of mannose produced by contacting an equivalent amount of palm kernel waste with the equivalent amount of the mannanase in the absence of the pectinase. In some embodiments, the amount of mannose produced by contacting the palm kernel waste with the pectinase and the mannanase is three times the amount of mannose produced by contacting an equivalent

amount of palm kernel waste with the equivalent amount of the mannanase in the absence of the pectinase.

[13] In another aspect, mannose produced by any of the described methods is provided.

[14] In another aspect, a composition for use in preparing mannose from palm kernel waste is provided, comprising: (a) a pectinase, and (b) a mannanase.

[15] In some embodiments, the pectinase and the mannanase are present in an amount sufficient to produce a mannanase to pectinase activity ratio of from about 10,000:1 to about 200,000:1 , based on activity defined in U/g. In some embodiments, the pectinase and the mannanase are present in an amount sufficient to produce a mannanase to pectinase activity ratio of from about 35,000:1 to about 140,000:1 , based on activity defined in U/g.

[16] In some embodiments, the amount of pectinase is sufficient to produce a pectinase activity of from about 0.1 8 to about 3.6 U/g PKW. In some embodiments, the amount of mannanase is sufficient to produce a mannanase activity of from about 12,250 to about 250,000 U/g PKW.

[17] In some embodiments, the pectinase is a plurality of pectinases. In some embodiments, the mannanase is a plurality of mannanases

[18] These and other aspects and embodiments of the present compositions and method will be apparent in view of the following description.

BRIEF DESCRIPTION OF THE DRAWINGS

[19] Figure 1 is a graph showing the amount of mannose produced (expressed as mg mannose/g PKW) from PKW treated with 1.0% mannanase (first bar, labeled "A (2x)") or 0.5% mannanase plus 0.5% of a second indicated carbohydrase, (all subsequent bars). The reference codes (A-L) for Figure 1 are given in Table 1.

DETAILED DESCRIPTION

I. Overview

[20] Described are compositions and methods for preparing mannose from palm kernel waste (PKW), involving contacting the PKW with a combination of a mannanase and a pectinase. This combination of enzymes produces more

mannose from PKW that is produced by either enzyme alone. In some cases, the increase in mannose yield that results from the use of pectinase is about 2-fold, or more.

II. Definitions

[21] Prior to describing the present compositions and methods in detail, the following terms are defined for clarity. Terms not defined should be accorded their ordinary meanings as used in the relevant art.

[22] As used herein, a "pectinase" is an enzyme capable of hydrolyzing the substrates pectin, pectate, and/or derivatives, thereof, and/or is encompassed by the enzyme classifications EC 4.2.2.2, EC 4.2.2.10, or EC 4.2.2.22.

[23] As used herein, a "mannanase" is an enzyme capable of hydrolyzing the substrate mannan, and/or derivatives, thereof, and/or is encompassed by the enzyme classifications EC 3.2.1.25, EC 3.2.1.78, or EC 3.2.1.113. Preferred mannanases release free mannose, either alone or in combination with other enzymes.

[24] As used herein, "mannose" refers to a-D-mannopyranose, β-D-mannopyranose, a-D-mannofuranose, β-D-mannofuranose, and mixtures or blends, thereof, any one or more of which may, in various embodiments, be expressly included or excluded from the definition.

[25] As used herein, "pectinase activity" is defined in units/gram (U/g)), and may be determined using the following assay: A mixture of apples (50% Golden Delicious, 25% Cox Orange and 25% Lobo) are ground in a meat grinder, pressed and pasteurized, to obtain a juice having an outlet time of approximately 60 seconds (compared to 20 seconds for water). Cold juice (approximately 3°C) is pipetted into glass containers in about 25 ml portions and 1 ml enzyme solution (of known concentration) is added per glass and mixed to treat the juice. The juice and enzyme mixtures are incubated for about 1 hr at 50 °C. The concentration of enzyme solution should be adjusted so that juice treated with 100% enzyme solution has an outlet time of 1-2 seconds more than a completely depectinised juice. There should also be at least a 5-second difference in the outlet time between juice treated with a 25% enzyme solution and a 100% enzyme solution. Following pasteurization and sifting through a nylon cloth, the viscosity of the treated juice is measured at

25 °C with a capillary viscosimeter. Viscosity (in seconds) is plotted as a function of the dilution factors 1.00, 0.75, 0.50, and 0.25 *(i.e.,* 100%, 75%, 50%, and 25% enzyme solution). The plotted data can be compared to a standard curve to determine pectinase activity in a sample. The exemplary pectinase composition (MULTIFECT® Pectinase FE) has known pectinase activity and can be used as a standard.

[26] As used herein, "mannanase activity" is defined in units/gram (U/g), as determined using the following assay: 20 g 3,5-dinitrosalicylic acid (DNS) is added to 1 L deionized water in a 2 L beaker. 300 ml 10.67% sodium hydroxide is added and the suspension is heated on a stir plate (not to exceed 50°C) until clear. 600 g potassium sodium tartrate tetrahydrate is gradually added to the solution with continual mixing and the solution is allowed to reach room temperature, diluted to 2 L, and optionally filtered through a course sintered glass filter. The solution is stored in a dark amber bottle at room temperature.

[27] 1.4 g locust bean gum is gradually added to 500 ml Tris-HCI buffer (15.67 g Tris-HCI adjusted to pH 7.5 with ammonium hydroxide (1.5%) in a 2 L volume) in a 1 L beaker at 60°C for 60 minutes, and then cooled to room temperature and adjusted to 500 ml with deionized water. Cleared supernatant (subjected to centrifugation at 3,500 rpm for 10 minutes) is used the as a substrate for a known amount *(i.e.,* g or g/L) of mannanase standard and unknown mannanase sample. The amount of standard and sample used are selected to be within the linear range of the assay, where the change in absorbance (ΔA) is from about 0.17 to about 0.52. Standard and sample concentrations between 0.050-0.140 mannanase units/L typically fall within the linear range of this assay. Where necessary, samples can be diluted in the Tris-HCI buffer.

[28] To perform the assay, 2 ml locust bean gum substrate is equilibrated in 16x100 mm glass test tubes for 20 minutes at 40 °C. 0.5 ml of enzyme sample dilution is added, mixed, and incubate for 10 minutes. The reaction is stopped by the addition of 3.0 ml of the DNS-solution, mixed, boiled for 15 min in a covered test tube to prevent evaporation, cooled in an ice water bath for 50 min, and allowed to equilibrate to room temperature for 10 minutes prior to

reading the absorbance at 540 nm. The measurement is compared to a deionized water blank.

[29] The reaction control (i.e., reagent/enzyme blank) is the amount of reducing sugars present in the locust bean gum substrate and/or present in the enzyme sample. As before, 2 ml locust bean gum substrate is equilibrated in 16x100 mm glass test tubes for 20 minutes at 40°C. However, 3.0 ml of DNS-solution is added and mixed prior to the addition of 0.5 ml of enzyme dilution, and then boiled, cooled, equilibrated, and read at 540 nm against a deionized water blank.

[30] ΔA is determined by subtracting the average absorbance values for the reagent/enzyme blanks from the absorbance readings of reactions in which enzyme is present. A standard curve is prepared using linear regression, where net absorbance is plotted on the y-axis and concentration (mannanase units/liter) is plotted on the x-axis, and the mannanase activity of each sample is determined based on the standard curve. The exemplary mannanase composition (GC266) has known mannanase activity and can be used as a standard.

[31] As used herein, "palm kernel waste (PKW)" refers to by-products from the process of extracting palm oil or other materials from oil palm kernels. PKW may include palm kernel shells, mesocarp fibers, empty fruit bunches, and/or other materials. PKW may be in a ground or pulverized form.

[32] As used herein, the term "contacting" refers to bringing specified components, *e.g.,* an enzyme and a substrate, into physical contact. Contacting includes mixing dry compositions and mixing liquid compositions, or combinations, thereof.

[33] As used herein, the term "equivalent amount," with reference to a substrate, enzyme, or other specified component, refers to the same or equal amount (in terms of, *e.g.,* units, grams, or moles) with reference to an antecedent composition and amount. For example, with reference to a composition comprising 10 U of mannanase activity and 20 U of pectinase activity, a composition having an equivalent amount of mannanase activity has 10 U of mannanase activity.

[34] As used herein, the phrase "substantially free of an activity" (or similar phrases) means that a specified activity is either undetectable in an admixture

of polypeptides, or present in an amount that would not interfere with the intended purpose of the admixture.

[35] As used herein, the singular articles "a," "an," and "the" encompass the plural referents unless the context clearly dictates otherwise.

[36] The following abbreviations/acronyms have the following meanings unless otherwise specified:

EC	enzyme commission
kDa	kiloDalton
kb	kilobase
MW	molecular weight
w/v	weight/volume
w/w	weight/weight
v/v	volume/volume
wt%	weight percent
℃	degrees Centigrade
H_2O	water
dH_2O or DI	deionized water
dIH_2O	deionized water, Milli-Q filtration
g or gm	gram
µg	microgram
mg	milligram
kg	kilogram
lb	pound
µL and µl	microliter
mL and ml	milliliter
mm	millimeter
µm	micrometer
M	molar
mM	millimolar
µM	micromolar
U	unit
ppm	parts per million
hr	hour
EtOH	ethanol
eq.	equivalent
N	normal
PCR	polymerase chain reaction
DNA	deoxyribonucleic acid
UV	ultraviolet
rpm	revolutions per minute

[37] All references cited herein are hereby incorporated by reference in their entirety.

III. Compositions and methods for preparing mannose from PKW

[38] Described are compositions and methods for preparing mannose from palm kernel waste (PKW), involving contacting the PKW with a combination of a mannanase and a pectinase. The combination of these two enzymes results in a significantly higher yield of mannose that can be obtained using either enzyme alone.

[39] Preferably, PKW is contacted with the mannanase and the pectinase in the same reaction vessel, avoiding the need to transfer PKW to different reaction vessels for different portions of the enzymatic treatment. In some cases, PKW is contacted with the mannanase and the pectinase simultaneously. In other cases, the PKC is contacted with the mannanase and the pectinase sequentially. Where PKC is contacted with the mannanase and the pectinase sequentially, PKC is preferably first contacted with the pectinase and then the mannanase, although PKC may also be contacted first with the mannanase and then with the pectinase. Where mannanase and pectinase are contacted with PKW simultaneously, they are ideally included together in a single composition.

[40] Examples of pectinases suitable for use as described include enzymes encompassed by the enzyme classifications EC 4.2.2.2 *(i.e.,* pectate lyase enzymes that favors pectate, the anion, over pectin, the methyl ester); EC 4.2.2.1 0 *(i.e.,* pectin lyase enzymes that favors pectin, the methyl ester, over pectate, the anion); and EC 4.2.2.22 *(i.e.,* pectate trisaccharide-lyase or exopectate-lyase).

[41] In some embodiments, the pectinase is derived from a fungal organism, such as an *Aspergillus* spp., a *Penicillium* spp., or a *Trichoderma* spp. In some embodiments, the pectinase is derived from a bacterium, such as an *Erwinia* spp., a *Pseudomonas* spp., a *Klebsiella* spp., a *Xanthomonas* spp., a *Bacillus spp. (e.g.,* Nasser *et al.* (1993) *FEBS Letts.* 335:31 9-26; Kim ef *al.* (1994) *Biosci. Biotech. Biochem.* 58:947-49; Dave and Vaughn (1971) *J. Bacterid.* 108:1 66-74; Nagel and Vaughn (1961) *Arch. Biochem. Biophys.* 93:344-52; Karbassi and Vaughn (1980) *Can. J. Microbiol.* 26:377-84, Hasegawa and Nagel (1966) *J. Food Sci.* 3 1:838-45; and Kelly and Fogarty

(1978) *Can. J. Microbiol.* 24:1 164-72). Particular pectinases can be obtained from *Bacillus subtilis.*

[42] Suitable pectinases may be divalent cation-independent and/or thermostable. In particular embodiments, the pectinase is as described in Heffron ef *al.* (1995) *Mol. Plant-Microbe Interact.* 8:331 -34; Henrissat ef *al.* (1995) *Plant Physiol.* 107: 963-76; WO 99/27083; WO 99/27084; WO 02/006442; or U.S. Pat. No. 6,284,524.

[43] Specific examples of suitable commercially available pectinase products include CELLULOSIN™ PC5, PE60, PEL, and ME (HBI Products), SUMIZYME™ AP2, PX, PMAC, PCLA, MC, and SPG (Shin Nihon), and MULTIFECT® Pectinase FE (Genencor), each of which includes a pectinase from *Aspergillus niger,* PECTINASE™ G, GL, and PL (Amano), which includes a pectinase(s) from *Aspergillus niger* and/or *Apregillus pulverulentes,* PECTINEX™ (Novozymes), which includes a pectinase from *Aspergillus niger* and/or *Aspergillus aculeatus,* PRIMAGREEN® EcoScour (Genencor), SCOURZYME™ (Novozymes), and PECTINASE™ XP-534 (Nagase ChemteX), each of which includes a pectinase from *Bacillus,* as well as GAMMAPECT™ PCL and ROHAPECT® (AB Enzymes), RAPIDASE® X-Press and C80L (DSM Food specialties), SUKULASE™ N and S (Sankyo Lifetech), PECTINASE-GODO™ (Godo Syusei), and BIOPREP™, CITRAZYME™ and VINOZYME™ (Novozymes).

[44] In some embodiments, a single pectinase is used. In some embodiments, a plurality of pectinases is used.

[45] Examples of mannanases suitable for use as described include enzymes encompassed by the enzyme classifications EC 3.2.1 .25 *(i.e.,* mannase; β-D-mannosidase; β-mannoside mannohydrolase; exo-β-D-mannanase; or β-D-mannoside mannohydrolase); EC 3.2.1 .78 *(i.e.,* endo-1 ,4-β-mannanase; β-mannanase); and EC 3.2.1 .113 *(i.e.,* mannosidase; 1,2-a-mannosidase; exo-a-1 ,2-mannanase; mannose-9 processing α-mannosidase).

[46] The mannanase enzymes may be of bacterial or fungal origin. In some embodiments, the mannanase is derived from a strain of filamentous fungus, such as an *Aspergillus* spp. (WO 94/25576) or *Trichoderma* spp. (e.g., WO 93/24622). In some embodiments, the mannanase is derived from a

bacterium, such as a *Bacillus* spp. (e.g., Talbot *et al.* (1990) *Appl. Environ. Microbiol.* 56:3505-10; Mendoza *et al.* (1994) *World J. Microbiol. Biotech.* 10:551-55; JP-A-03047076; JP-A-63056289; JP-A-63036775; JP-A-08051975; WO 97/11164; or WO 99/64619) or a *Humicola* spp. (e.g., WO 99/64619).

[47] Specific examples of suitable commercially available mannanase products include MANNASTAR® 375 (Genencor), GC 266 (Genencor), ECONASE® MP 1000 (AB Enzymes), and ROHALASE® GMP (AB Enzymes), each of which includes a mannanase from *Trichoderma reesei,* CELLULOSINTM GM5 (HBI Products) and SUMIZYME™ ACH (Shin Nihon), each of which includes a mannanases from *Aspergillus niger,* and MANNAWAY® (Novozymes).

[48] In some embodiments, a single mannanase is used. In some embodiments, a plurality of mannanases is used.

[49] On an activity basis, varying amounts of pectinase and mannanase enzymes can be used, where pectinase and mannanase activity can be measured as described, herein. In some embodiments, the amount of pectinase used is at least about 0.1 pectinase units per gram of palm kernel extruder waste (U/g PKW). In some embodiments, the amount of pectinase used is at least about 0.1, at least about 0.2, at least about 0.3, at least about 0.4, at least about 0.5, at least about 0.6, at least about 0.7, at least about 0.8, at least about 0.9, at least about 1.0, at least about 1.1, at least about 1.2, at least about 1.3, at least about 1.4, at least about 1.8, at least about 1.9, or even at least about 2 U/g PKW. Exemplary ranges are from about 0.18 to about 3.6, from about 0.36 to about 3.24, from about 0.54 to about 3.06, from about 0.72 to about 2.88, from about 0.90 to about 2.70, and from about 0.90 to about 1.80 U/g PKW.

[50] In some embodiments, the amount of mannanase used is at least about 12,000 mannanase units per gram of palm kernel extruder waste (U/g PKW). In some embodiments, the amount of mannanase used is at least about 21,250, at least about 25,000, at least about 41,250, at least about 50,000, at least about 62,500, at least about 75,000 U/g PKW. Exemplary ranges are from about 12,250 to about 250,000, from about 25,000 to about 225,000, from about 35,000:1 to about 140,000:1, from about 37,500 to about

212,500, from about 41,250 to about 212,500, from about 50,000 to about 200,000, from about 62,500 to about 187,500, and from about 62,500 to about 125,000 U/g PKW.

[51] On an activity ratio basis, varying ratios of pectinase and mannanase enzymes can be used as described. In some embodiments, the ratio of mannanase activity to pectinase activity (each in U/g PKW) is from about 10,000:1 to about 200,000:1 , from about 20,000:1 to about 150,000:1 , from about 30,000:1 to about 120,000:1 , or even from about 60,000:1 to about 70,000:1 .

[52] The temperature and time of incubation of the mannanase and pectinase with the PKW is not believed to be critical, long as the conditions do not prematurely inactivate one or both of the enzymes. In some embodiments, the time of incubation using mannanase and pectinase, together, is less the time of incubation using either mannanase or pectinase, alone. The selection of particular incubation temperatures and times of incubation depend on the particular mannanase and pectinase used and the desired results. Lower temperatures *(i.e.,* close to ambient temperature) are generally preferred for cost and environmental reasons, while elevated temperatures *(i.e.,* significantly above ambient temperature) are generally preferred to increase enzyme activity. Shorter incubation times are generally preferred to increase throughput, although overnight or longer incubations may allow the use of less enzyme.

[53] Compositions containing mannanase and/or pectinase may further include any number of buffers, salts, stabilizing agents, formulation agents, surfactants, polymers, dyes, or additional enzymes. Exemplary additional enzymes include but are not limited to cellulases, xylanases, amylases, and proteases. However, in some embodiments, mannanase and/or pectinase compositions are substantially free of other enzymatic activities, such as cellulase, xylanase, amylase, and protease activities.

[54] These and other features of the present compositions and methods will be apparent from the description and appended Examples.

EXAMPLES

[55] An initial screen of mannanase in combination with selected carbohydrase enzymes was performed to identify enzyme combinations that produced a beneficial effect.

[56] Briefly, a series of palm kernel waste (PKW) samples was prepared by adding 15 mL of 50 mM sodium citrate (pH 3.5-4.5) to 15 grams of PKW. 1.0% mannanase product (w/w) or 0.5% mannanase product plus 0.5% of a second carbohydrase (w/w) (or no second carbohydrase, as a control) was then added to each sample, followed by incubation at 55°C for 23 hours. The identity of the enzymes tested is shown in Table 1, wherein "ID" corresponds to the identifier used in the graph. The first bar of the graph represents a double dose (1%) of mannanase (i.e. component "A"), while the all subsequent bars represent 0.5% mannanase ("A") plus the indicated second carbohydrase ("B" through "L" or no second carbohydrase ("none").

Table 1. Enzymes tested.

ID	Commercial name	Enzyme
A	GC266	Non-genetically-modified Mannanase composition derived from *Trichoderma reesei*
B	MULTIFECT® CX B	Beta-glucanase composition derived from *Trichoderma reesei*
C	MULTIFECT® CX GC	Cellulase composition derived from *Trichoderma reesei*
D	MULTIFECT® CX 12L	Xylanase composition derived from *Trichoderma reesei*
E	MULTIFECT® CX 2000L	Cellulase composition derived from *Penicillium funiculosum*.
F	MULTIFECT® Pectinase FE	Pectinase composition derived from *Aspergillis niger*
G	OPTIMASE® CX 55L	Engineered bacterial neutral cellulase derived from *Streptomyces lividans*.
H	ACCELERASE™ 1000	Cellulase/beta-glucanase composition from *Trichoderma reesei*.
I	Beta-glucanase 1000L	Beta-glucanase derived from *Trichoderma reesei*.
J	Y5 xylanase	Xylanase composition derived from *Trichoderma reesei*
K	GC260	Xylanase composition from *Bacillus licheniformis*.
L	TrTG	Engineered transglucosidase derived from *Aspergillus niger*.

[57] The particular mannanase composition (GC 266) used had a minimum activity of 12,500,000 mannanase units/gram, as measured by mannanase assay described, herein. 1.0% mannanase is equivalent to 125,000 mannanase units per gram of palm kernel extruder waste, and 0.5% mannanase is equivalent to 62,500 mannanase units per gram of palm kernel extruder waste. The level of pectinase activity in the mannanase preparation was assumed to be nominal.

[58] The particular pectinase composition (MULTIFECT® Pectinase FE) used had a minimum activity of 180 pectinase units/gram, as measured pectinase assay described, herein. 1.0% pectinase is equivalent to 1.8 pectinase units per gram of palm kernel extruder waste and 0.5% pectinase is equivalent to 0.9 pectinase units per gram of palm kernel extruder waste. The level of mannanase activity in the pectinase preparation was assumed to be nominal.

[59] Following incubation, the samples were diluted to 100 grams nominal weight by the addition of 70 grams water. The samples were mixed thoroughly and a small aliquot was removed and subjected to centrifugation at 13,000 x g.

[60] The supernatants were loaded onto an Agilent 1100 series HPLC (Santa Clara, CA, USA), and separated using a Bio-Rad Aminex HPX-87C (Hercules, CA, USA) column at 80°C. Mannose peaks were detected based on refractive index, and the peaks were integrated using full baseline. The resulting mannose concentration was calculated using linear regression based on a mannose standard curve prepared using 1 g/L, 5 g/L and 10 g/L mannose.

[61] As shown in Figure 1, of the combinations tested, only mannanase combined with a pectinase (labeled "F"), or mannanase combined with all the tested carbohydrases including the pectinase (labeled "ALL"), demonstrated increased mannose yield, even compared to twice the dose of mannanase alone [labeled "A (2X)"].

[62] In a further effort to optimize the amount of mannanase required to maximize mannanase yield, the mannanase:pectinase ratio was varied and a similar experiment was performed. The results are shown in Table 2.

Table 2. Mannose yields using different amounts of mannanase and pectinase.

% mannanase	% pectinase	mg mannose/g PKW
0.50	0.50	88.99
0.33	0.50	78.09
0.17	0.50	67.56
0.00	0.50	20.01
0.33	0.33	68.17
0.33	0.17	56.06
0.50	0.00	25.67

[63] As shown in the table, the best mannose yields *(i.e.,* about 8.9% release) were obtained using about 0.5% mannanase and about 0.5% pectinase. By comparison, using mannanase alone resulted in a 2.6% release and using pectinase alone resulted in a 2.0% release. The increase in mannose release obtained using the combination of mannanase and

pectinase was, therefore, greater than 3-fold compared to either mannanase or pectinase, alone.

[64] Different levels of mannose can be released using different ratios and amount of mannanase and pectinase, depending on the desired results.

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CLAIMS

What is claimed is:

1. A method of preparing mannose from palm kernel waste, comprising: contacting the palm kernel waste with a pectinase and a mannanase, wherein the amount of mannose produced by contacting the palm kernel waste with the pectinase and the mannanase is greater than the amount of mannose produced by contacting an equivalent amount of palm kernel waste with an equivalent amount of the mannanase in the absence of the pectinase.

2. The method of claim 1, wherein the palm kernel waste is contacted with the pectinase and the mannanase in the same reaction vessel.

3. The method of claim 1, wherein the palm kernel waste is contacted with the pectinase and the mannanase simultaneously.

4. The method of claim 1, wherein the palm kernel waste is contacted with the pectinase and the mannanase sequentially.

5. The method of claim 4, wherein the palm kernel waste is first contacted with the pectinase and then contacted with the mannanase.

6. The method of any of the preceding claims, wherein the pectinase and the mannanase are present in an amount sufficient to produce a mannanase to pectinase activity ratio of from about 10,000:1 to about 200,000:1 , based on activity defined in U/g.

7. The method of any of the preceding claims, wherein the pectinase and the mannanase are present in an amount sufficient to produce a mannanase to pectinase activity ratio of from about 35,000:1 to about 140,000:1 , based on activity defined in U/g.

8. The method of any of the preceding claims, wherein the amount of pectinase is sufficient to produce a pectinase activity of from about 0.1 8 to about 3.6 U/g PKW.

9. The method of any of the preceding claims, wherein the amount of mannanase is sufficient to produce a mannanase activity of from about 12,250 to about 250,000 U/g PKW.

10. The method of any of the preceding claims, wherein the pectinase is a plurality of pectinases.

11. The method of any of the preceding claims, wherein the mannanase is a plurality of mannanases.

12. The method of any of the preceding claims, wherein the amount of mannose produced by contacting the palm kernel waste with the pectinase and the mannanase is greater than the amount of mannose produced by contacting an equivalent amount of palm kernel waste with twice the equivalent amount of the mannanase in the absence of the pectinase.

13. The method of any of the preceding claims, wherein the amount of mannose produced by contacting the palm kernel waste with the pectinase and the mannanase is twice the amount of mannose produced by contacting an equivalent amount of palm kernel waste with the equivalent amount of the mannanase in the absence of the pectinase.

14. The method of any of the preceding claims, wherein the amount of mannose produced by contacting the palm kernel waste with the pectinase and the mannanase is three times the amount of mannose produced by contacting an equivalent amount of palm kernel waste with the equivalent amount of the mannanase in the absence of the pectinase.

15. Mannose produced by the method of any of the preceding claims.

16. A composition for use in preparing mannose from palm kernel waste, comprising:

(a) a pectinase, and

(b) a mannanase.

17. The composition of claim 16, wherein the pectinase and the mannanase are present in an amount sufficient to produce a mannanase to pectinase activity ratio of from about 10,000:1 to about 200,000:1, based on activity defined in U/g.

18. The composition of claim 16 or 17, wherein the pectinase and the mannanase are present in an amount sufficient to produce a mannanase to pectinase activity ratio of from about 35,000:1 to about 140,000:1, based on activity defined in U/g.

19. The composition of any of claims 16-18, wherein the amount of pectinase is sufficient to produce a pectinase activity of from about 0.18 to about 3.6 U/g PKW.

20. The composition of any of claims 16-19, wherein the amount of mannanase is sufficient to produce a mannanase activity of from about 12,250 to about 250,000 U/g PKW.

21. The composition of any of claims 16-20, wherein the pectinase is a plurality of pectinases.

22. The composition of any of claims 16-21, wherein the mannanase is a plurality of mannanases.

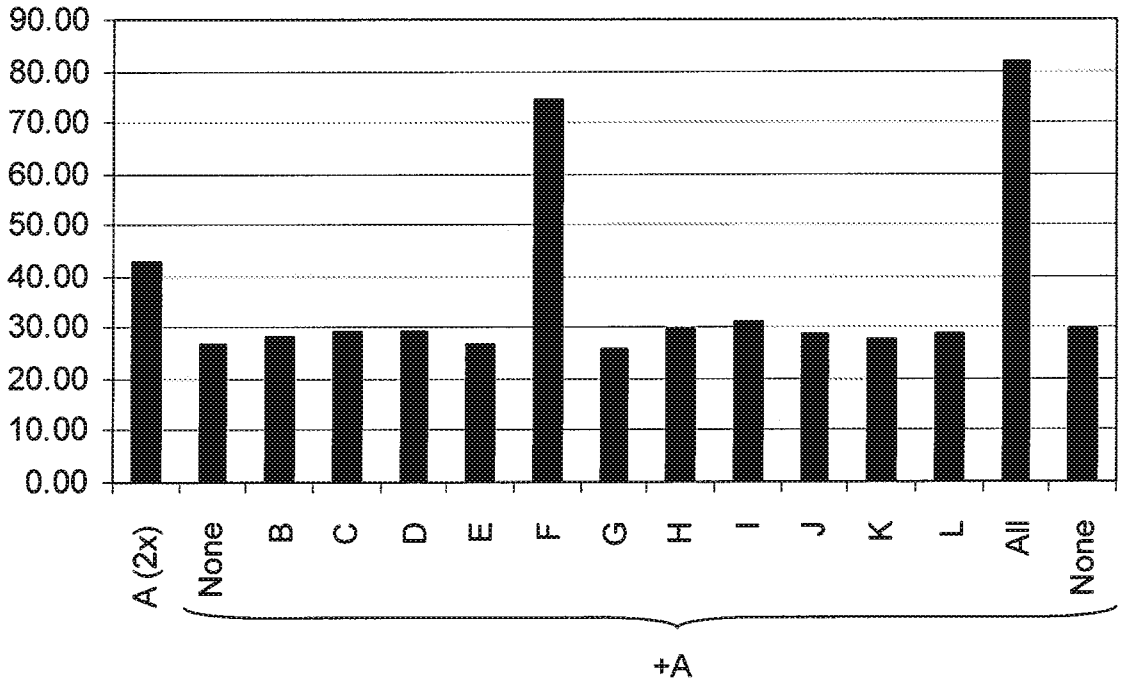
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
A (2x)
None
B
C
D
E
F
G
H
I
J
K
L
All
None
+A

Figure 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/050349

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12P19/02 C12P19/14 C12P19/20
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12P

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/074685 A1 (N0V0ZYMES AS [DK]; SOERENSEN HANNE RISBJERG [DK]; FELBY CLAUS [DK]; JO) 18 June 2009 (2009-06-18)	1,15,16
Y	page 1, line 25 - page 6, line 13 page 8, line 5 - page 11, line 8 example 1	2-14, 17-22

X	WO 2010/000858 A1 (N0V0ZYMES AS [DK]; 0LSEN HANS SEJR [DK]) 7 January 2010 (2010-01-07)	16
Y	page 25, line 10 - page 29, line 22	2-14, 17-22

X	CA 2 591 650 A1 (GR0BER INC [CA]) 15 December 2007 (2007-12-15)	15,16
Y	paragraph [0032] examples 1,2	2-14, 17-22

	-/-	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such docu¬ments, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 October 2011	07/11/2011

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/050349

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	page 14, line 18 - page 19, line 7; tables 1,2 page 29, line 28 - page 30, line 18 page 31, line 21 - line 34 -----	1,16

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2011/050349

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