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(54) Titre : UTILISATION D'HYDROLASE DE GLYCOSIDE DE FAMILLE 61 POUR L'AMELIORATION DU DRAINAGE DE LA PULPE DE PAPIER

(54) Title: USE OF GLYCOSIDE HYDROLASE FAMILY 61 FOR IMPROVING DRAINAGE OF PAPER PULP

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The use of GH61 polypeptides in the treatment of pulp, for improving the freeness of the pulp, and/or for improving the short span compression strength of paper materials made from the pulp, such as paper, linerboard, corrugated paperboard, tissue, towels, corrugated containers and boxes.

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(54) Title: IMPROVING DRAINAGE OF PAPER PULP

(57) Abstract: The use of GH61 polypeptides in the treatment of pulp, for improving the freeness of the pulp, and/or for improving the short span compression strength of paper materials made from the pulp, such as paper, linerboard, corrugated paperboard, tissue, towels, corrugated containers and boxes.

USE OF GLYCOSIDE HYDROLASE FAMILY 61 FOR IMPROVING DRAINAGE OF PAPER PULP

BACKGROUND OF THE INVENTION

5

Field of the Invention

The present invention relates to improving properties of paper or packaging pulp and/or paper or packaging materials made from the paper or packaging pulp, by treatment with glycoside hydrolase Family 61 polypeptides.

10

Description of the Related Art

It is well-known to use enzymes in the manufacture of paper and packaging materials. Examples of enzymes used for this purpose are proteases, lipases, xylanases, amylases, cellulases, as well as various oxidizing enzymes such as laccases and peroxidases.

15 The effects of these enzymes are wide-spread, *e.g.*, control of various deposits such as pitch, strength-improvement, de-inking, drainage improvement, tissue softening, bleaching etc.

Summary of the Invention

The present inventors surprisingly found that certain properties of pulp, such as the

20 freeness, and/or properties of paper materials made from the pulp, such as short span compression strength, can be modified by treating or contacting the pulp with a glycoside hydrolase Family 61 (GH61) polypeptide during the paper or board making process.

DETAILED DESCRIPTION OF THE INVENTION

25 The current invention describes the application of GH61 polypeptides, alone or in concert with other enzymes, onto virgin or recycled pulp as a means to improve the freeness of paper, board or molded package-making furnishes (fiber, filler, water and functional additives) in order to reduce manufacturing costs, improve productivity and/or impart value within final products. Without being bound by theory, the mechanism appears to include enzymatic dissolution of

30 colloidal substances and fibrils that tend to restrict the free drainage of water from the consolidating fibrous web (of the pulp) on the wire and the extraction of water from the consolidated fibrous web during pressing (*e.g.*, within the press section). Moreover, the reduction of the fraction of such colloidal substances within the fibrous web will reduce the amount of bound water within the web thereby reducing the amount of steam necessary for

35 drying the web within the dryer section.

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When applied to and incubated with virgin and recycled pulp commonly used to manufacture paper or board, GH61 polypeptides significantly improve the standard "freeness" values of the resultant furnishes. A provisional correlation exists between freeness and paper/board machine drainage and/or dewatering. In light of the correlation, the pre-treatment
5 of the fibrous slurries with GH61 polypeptides, alone or in concert with one or more additional enzymes, most preferably selected from the glycosyl hydrolases, may improve the on-machine performance of the furnishes containing 1-100% of the enzymatically treated pulp. "On-machine" performance is a cumulative response dependent upon several factors including furnish type & composition, furnish drainage/dewatering rates & degrees, chemical additives
10 (*e.g.*, coagulants and polymeric retention & drainage aids), head box consistency, basis weight and machine design and operation. By directly impacting one or more of these factors, the current invention is assumed to improve the overall "on-machine" performance of furnishes containing GH61 polypeptides conditioned pulp.

The present invention thus imparts several advantages, which include:
15 a) a novel enzymatic means to improve on-machine performance of virgin and recovered pulp in the absence of glycosyl hydrolases.
b) a means to extend the on-machine benefits of certain traditional enzymatic applications beyond levels and degrees obtainable through application of the traditional enzymatic applications alone.
20 Accordingly, the invention relates to the use of GH61 polypeptides in the treatment of pulp, for improving the freeness of the pulp, and/or for improving the short span compression strength of the paper, board or packaging materials made from the pulp, such as paper, linerboard, corrugated paperboard, tissue, towels, molded packaging, corrugated containers and boxes.

25

Paper and Pulp

The term "paper and packaging material" refers to products, which can be made out of pulp, such as paper, linerboard, corrugated paperboard, tissue, towels, packaging materials, corrugated containers or boxes.
30 The term "pulp" means any pulp which can be used for the production of a paper and packaging material. Pulp is a lignocellulosic fibrous material prepared by chemically or mechanically separating cellulose fibres from wood, fibre crops or waste paper. For example, the pulp can be supplied as a virgin pulp, or can be derived from a recycled source. The pulp may be a wood pulp, a non-wood pulp or a pulp made from waste paper. A wood pulp may be
35 made from softwood such as pine, redwood, fir, spruce, cedar and hemlock or from hardwood

such as maple, alder, birch, hickory, beech, aspen, acacia and eucalyptus. A non-wood pulp may be made, *e.g.*, from flax, hemp, bagasse, bamboo, cotton or kenaf. A waste paper pulp may be made by re-pulping waste paper such as newspaper, mixed office waste, computer print-out, white ledger, magazines, milk cartons, paper cups etc.

5 In a particular embodiment, the pulp to be treated comprises both hardwood pulp and softwood pulp.

The wood pulp to be treated may be mechanical pulp (such as ground wood pulp, GP), chemical pulp (such as Kraft pulp or sulfite pulp), semichemical pulp (SCP), thermomechanical pulp (TMP), chemithermomechanical pulp (CTMP), or bleached chemithermomechanical pulp

10 (BCTMP).

Mechanical pulp is manufactured by the grinding and refining methods, wherein the raw material is subjected to periodical pressure impulses. TMP is thermomechanical pulp, GW is groundwood pulp, PGW is pressurized groundwood pulp, RMP is refiner mechanical pulp, PRMP is pressurized refiner mechanical pulp and CTMP is chemithermimechanical pulp.

15 Chemical pulp is manufactured by alkaline cooking whereby most of the lignin and hemicellulose components are removed. In Kraft pulping or sulphate cooking sodium sulphide or sodium hydroxide are used as principal cooking chemicals.

The Kraft pulp to be treated may be a bleached Kraft pulp, which may consist of softwood bleached Kraft (SWBK, also called NBKP (Nadel Holz Bleached Kraft Pulp)),

20 hardwood bleached Kraft (HWBK, also called LBKP (Laub Holz Bleached Kraft Pulp and)) or a mixture of these.

The pulp to be used in the process of the invention is a suspension of mechanical or chemical pulp or a combination thereof. For example, the pulp to be used in the process of the invention may comprise 0%, 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%,

25 80-90%, or 90-100% of chemical pulp. In a particular embodiment, a chemical pulp forms part of the pulp being used for manufacturing the paper material. In the present context, the expression "forms part of" means that in the pulp to be used in the process of the invention, the percentage of chemical pulp lies within the range of 1-99%. In particular embodiments, the percentage of chemical pulp lies within the range of 2-98%, 3-97%, 4-96%, 5-95%, 6-94%, 7-

30 93%, 8-92%, 9-91%, 10-90%, 15-85%, 20-80%, 25-75%, 30-70%, 40-60%, or 45-55%.

In a particular embodiment of the use and the process of the invention, the chemical pulp is a Kraft pulp, a sulfite pulp, a semichemical pulp (SCP), a thermomechanical pulp (TMP), a chemithermomechanical pulp (CTMP), a bleached chemithermomechanical pulp (BCTMP). In particular embodiments the Kraft pulp is bleached Kraft pulp, for example softwood bleached

Kraft (SWBK, also called NBKP (Nadel Holz Bleached Kraft Pulp)), hardwood bleached Kraft (HWBK, also called LBKP (Laub Holz Bleached Kraft Pulp and)) or a mixture thereof.

Freeness

5 The freeness of pulp is designed to give a measure of the rate at which a dilute suspension of pulp (*e.g.*, 3 g of pulp in 1 L of water) drains within standardized testing equipment. The freeness, or drainage rate (see *e.g.*, TAPPI test method T 221 "Drainage Time of Pulp"), has been shown to be related to the surface conditions and swelling of the fibers. Besides these factors, the result is dependent also on conditions under which the test is
10 carried out, such as stock preparation, temperature, and water quality.

In many cases there is a correlation between freeness values and either (a) a target level of refining of pulp, or (b) the ease of drainage of white water from the wet web, especially in the early sections of a Fourdrinier former. Standard tests of freeness are based on gravity dewatering through a screen. The devices are designed so that an operator can judge the
15 speed of dewatering by observing the volume of liquid collected in a graduated cylinder. Freeness tends to be decreased by refining and by increases in the level of fines in the furnish. Freeness can be increased by use of drainage aids, removal of fines, or enzymatic treatments to convert mucilaginous materials into sugars.

Freeness, as defined in the methods, compositions and uses of the present invention, is
20 measured as Canadian Standard Freeness, which is reflected in TAPPI test method T 227 "Freeness of pulp (Canadian standard method)", as published by the Technical Association of the Pulp and Paper Industry (TAPPI).

Compression strength

25 Compression strength, as defined in the methods, compositions and uses of the present invention, is measured according to TAPPI test method T 826 "Short span compressive strength of containerboard".

Short span compressive strength index is the recommended measure of Short span compressive strength for comparing papers of differing grammages. Short span compressive
30 strength index is obtained by dividing the Short span compressive strength measured in units of newtons per meter (N/m) by the grammage of the paper in units of grams per square meter (g/m^2).

GH61 polypeptides

The term "GH61 polypeptide" means a polypeptide falling into the glycoside hydrolase Family 61 according to Henrissat, 1991, A classification of glycosyl hydrolases based on amino-acid sequence similarities, *Biochem. J.* 280: 309-316, and Henrissat and Bairoch, 1996, Updating the sequence-based classification of glycosyl hydrolases, *Biochem. J.* 316: 695-696.

5 In the compositions and methods of the present invention, any GH61 polypeptide can be used.

In a first aspect, the GH61 polypeptide comprises the following motifs:
[ILMV]-P-x(4,5)-G-x-Y-[ILMV]-x-R-x-[EQ]-x(4)-[HNQ] and [FW]-[TF]-K-[AIV],
wherein x is any amino acid, x(4,5) is any four or five contiguous amino acids, and x(4) is any
10 four contiguous amino acids.

The GH61 polypeptide comprising the above-noted motifs may further comprise:
H-x(1,2)-G-P-x(3)-[YW]-[AILMV],
[EQ]-x-Y-x(2)-C-x-[EHQN]-[FILV]-x-[ILV], or
H-x(1,2)-G-P-x(3)-[YW]-[AILMV] and [EQ]-x-Y-x(2)-C-x-[EHQN]-[FILV]-x-[ILV],
15 wherein x is any amino acid, x(1,2) is any one or two contiguous amino acids, x(3) is any three contiguous amino acids, and x(2) is any two contiguous amino acids.

In a preferred aspect, the GH61 polypeptide further comprises H-x(1,2)-G-P-x(3)-[YW]-[AILMV]. In another preferred aspect, the GH61 polypeptide further comprises [EQ]-x-Y-x(2)-C-x-[EHQN]-[FILV]-x-[ILV]. In another preferred aspect, the GH61 polypeptide further comprises
20 H-x(1,2)-G-P-x(3)-[YW]-[AILMV] and [EQ]-x-Y-x(2)-C-x-[EHQN]-[FILV]-x-[ILV].

In a second aspect, the GH61 polypeptide comprises the following motif:
[ILMV]-P-x(4,5)-G-x-Y-[ILMV]-x-R-x-[EQ]-x(3)-A-[HNQ],
wherein x is any amino acid, x(4,5) is any 4 or 5 contiguous amino acids, and x(3) is any 3 contiguous amino acids. In the above motif, the accepted IUPAC single letter amino acid
25 abbreviation is employed.

In a third aspect, the GH61 polypeptide comprises an amino acid sequence that has a sequence identity to the mature polypeptide of SEQ ID NO: 1 (*Acremonium alcalophilum*), SEQ ID NO: 2 (*Acremonium alcalophilum*), SEQ ID NO: 3 (*Acremonium alcalophilum*), SEQ ID NO: 4 (*Thielavia terrestris*), SEQ ID NO: 5 (*Thielavia terrestris*), SEQ ID NO: 6 (*Thielavia*
30 *terrestris*), SEQ ID NO: 7 (*Thielavia terrestris*), SEQ ID NO: 8 (*Thielavia terrestris*), SEQ ID NO: 9 (*Thielavia terrestris*), SEQ ID NO: 10 (*Thermoascus aurantiacus*), SEQ ID NO: 11 (*Trichoderma reesei*), SEQ ID NO: 12 (*Myceliophthora thermophila*), SEQ ID NO: 13 (*Myceliophthora thermophila*), SEQ ID NO: 14 (*Myceliophthora thermophila*), SEQ ID NO: 15 (*Myceliophthora thermophila*), SEQ ID NO: 16 (*Myceliophthora thermophila*), SEQ ID NO: 17
35 (*Thermoascus aurantiacus*), SEQ ID NO: 18 (*Aspergillus fumigatus*), SEQ ID NO: 19

(*Penicillium pinophilum*), SEQ ID NO: 20 (*Thermoascus sp.*), SEQ ID NO: 21 (*Penicillium sp.*), SEQ ID NO: 22 (*Thielavia terrestris*), SEQ ID NO: 23 (*Thielavia terrestris*), SEQ ID NO: 24 (*Thielavia terrestris*), SEQ ID NO: 25 (*Thielavia terrestris*), SEQ ID NO: 26 (*Thielavia terrestris*), SEQ ID NO: 27 (*Thielavia terrestris*), SEQ ID NO: 28 (*Thielavia terrestris*), SEQ ID
5 NO: 29 (*Thielavia terrestris*), SEQ ID NO: 30 (*Thielavia terrestris*), SEQ ID NO: 31 (*Thielavia terrestris*), SEQ ID NO: 32 (*Thielavia terrestris*), SEQ ID NO: 33 (*Thermoascus crustaceus*), SEQ ID NO: 34 (*Thermoascus crustaceus*), or SEQ ID NO: 35 (*Thermoascus crustaceus*) of at least 50%, *e.g.*, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, or at
10 least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100%.

Sequence identity: The relatedness between two amino acid sequences or between two nucleotide sequences is described by the parameter "sequence identity".
For purposes of the present invention, the sequence identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch,
15 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16: 276-277), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labeled "longest identity" (obtained
20 using the –nobrief option) is used as the percent identity and is calculated as follows:
(Identical Residues x 100)/(Length of Alignment – Total Number of Gaps in Alignment)

For purposes of the present invention, the sequence identity between two deoxyribonucleotide sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *supra*) as implemented in the Needle program of the
25 EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *supra*), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EDNAFULL (EMBOSS version of NCBI NUC4.4) substitution matrix. The output of Needle labeled "longest identity" (obtained using the –nobrief option) is used as the percent identity and is calculated as follows:
30 (Identical Deoxyribonucleotides x 100)/(Length of Alignment – Total Number of Gaps in Alignment)

In a sixth aspect, the GH61 polypeptide is an artificial variant comprising a substitution, deletion, and/or insertion of one or more (or several) amino acids of the mature polypeptide of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6,
35 SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID

NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID
5 NO: 34, or SEQ ID NO: 35; or a homologous sequence thereof.

Preferably, amino acid changes are of a minor nature, that is conservative amino acid substitutions or insertions that do not significantly affect the folding and/or activity of the protein; small deletions, typically of one to about 30 amino acids; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue; a small linker peptide of
10 up to about 20-25 residues; or a small extension that facilitates purification by changing net charge or another function, such as a poly-histidine tract, an antigenic epitope or a binding domain.

Examples of conservative substitutions are within the group of basic amino acids (arginine, lysine and histidine), acidic amino acids (glutamic acid and aspartic acid), polar
15 amino acids (glutamine and asparagine), hydrophobic amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tryptophan and tyrosine), and small amino acids (glycine, alanine, serine, threonine and methionine). Amino acid substitutions that do not generally alter specific activity are known in the art and are described, for example, by H. Neurath and R.L. Hill, 1979, *In*, *The Proteins*, Academic Press, New York. The most commonly
20 occurring exchanges are Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, and Asp/Gly.

Alternatively, the amino acid changes are of such a nature that the physico-chemical properties of the polypeptides are altered. For example, amino acid changes may improve the thermal stability of the polypeptide, alter the substrate specificity, change the pH optimum, and
25 the like.

Essential amino acids in a parent polypeptide can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, 1989, *Science* 244: 1081-1085). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant
30 molecules are tested for cellulolytic enhancing activity to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al.*, 1996, *J. Biol. Chem.* 271: 4699-4708. The active site of the enzyme or other biological interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction, or photoaffinity labeling, in conjunction with
35 mutation of putative contact site amino acids. See, for example, de Vos *et al.*, 1992, *Science*

255: 306-312; Smith *et al.*, 1992, *J. Mol. Biol.* 224: 899-904; Wlodaver *et al.*, 1992, *FEBS Lett.* 309: 59-64. The identities of essential amino acids can also be inferred from analysis of identities with polypeptides that are related to the parent polypeptide.

Single or multiple amino acid substitutions, deletions, and/or insertions can be made and 5 tested using known methods of mutagenesis, recombination, and/or shuffling, followed by a relevant screening procedure, such as those disclosed by Reidhaar-Olson and Sauer, 1988, *Science* 241: 53-57; Bowie and Sauer, 1989, *Proc. Natl. Acad. Sci. USA* 86: 2152-2156; WO 95/17413; or WO 95/22625. Other methods that can be used include error-prone PCR, phage display (*e.g.*, Lowman *et al.*, 1991, *Biochemistry* 30: 10832-10837; U.S. Patent No. 5,223,409; 10 WO 92/06204), and region-directed mutagenesis (Derbyshire *et al.*, 1986, *Gene* 46: 145; Ner *et al.*, 1988, *DNA* 7: 127).

Mutagenesis/shuffling methods can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides expressed by host cells (Ness *et al.*, 1999, *Nature Biotechnology* 17: 893-896). Mutagenized DNA molecules that 15 encode active polypeptides can be recovered from the host cells and rapidly sequenced using standard methods in the art. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide.

The total number of amino acid substitutions, deletions and/or insertions of the mature GH61 polypeptide of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID 20 NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID 25 NO: 33, SEQ ID NO: 34, or SEQ ID NO: 35 is not more than 4, *e.g.*, 1, 2, 3, or 4.

In another aspect, the GH61 polypeptide is used in the presence of a soluble activating divalent metal cation as described in WO 2008/151043, *e.g.*, manganese ions or copper ions.

In one aspect, the GH61 polypeptide is used in the presence of a dioxy compound, a bicylic compound, a heterocyclic compound, a nitrogen-containing compound, a quinone 30 compound, or a sulfur-containing compound.

The dioxy compound may include any suitable compound containing two or more oxygen atoms. In some aspects, the dioxy compounds contain a substituted aryl moiety as described herein. The dioxy compounds may comprise one or more (several) hydroxyl and/or hydroxyl derivatives, but also include substituted aryl moieties lacking hydroxyl and hydroxyl derivatives. 35 Non-limiting examples of dioxy compounds include pyrocatechol or catechol; caffeic acid; 3,4-

dihydroxybenzoic acid; 4-tert-butyl-5-methoxy-1,2-benzenediol; pyrogallol; gallic acid; methyl-3,4,5-trihydroxybenzoate; 2,3,4-trihydroxybenzophenone; 2,6-dimethoxyphenol; sinapinic acid; 3,5-dihydroxybenzoic acid; 4-chloro-1,2-benzenediol; 4-nitro-1,2-benzenediol; tannic acid; ethyl gallate; methyl glycolate; dihydroxyfumaric acid; 2-butyne-1,4-diol; (croconic acid; 1,3-propanediol; tartaric acid; 2,4-pentanediol; 3-ethyoxy-1,2-propanediol; 2,4,4'-trihydroxybenzophenone; cis-2-butene-1,4-diol; 3,4-dihydroxy-3-cyclobutene-1,2-dione; dihydroxyacetone; acrolein acetal; methyl-4-hydroxybenzoate; 4-hydroxybenzoic acid; and methyl-3,5-dimethoxy-4-hydroxybenzoate; or a salt or solvate thereof.

The bicyclic compound may include any suitable substituted fused ring system as described herein. The compounds may comprise one or more (several) additional rings, and are not limited to a specific number of rings unless otherwise stated. In one aspect, the bicyclic compound is a flavonoid. In another aspect, the bicyclic compound is an optionally subsituted isoflavonoid. In another aspect, the bicyclic compound is an optionally substituted flavylium ion, such as an optionally substituted anthocyanidin or optionally substituted anthocyanin, or derivative thereof. Non-limiting examples of bicyclic compounds include epicatechin; quercetin; myricetin; taxifolin; kaempferol; morin; acacetin; naringenin; isorhamnetin; apigenin; cyanidin; cyanin; kuromanin; (keracyanin; or a salt or solvate thereof.

The heterocyclic compound may be any suitable compound, such as an optionally substituted aromatic or non-aromatic ring comprising a heteroatom, as described herein. In one aspect, the heterocyclic is a compound comprising an optionally substituted heterocycloalkyl moiety or an optionally substituted heteroaryl moiety. In another aspect, the optionally substituted heterocycloalkyl moiety or optionally substituted heteroaryl moiety is an optionally substituted 5-membered heterocycloalkyl or an optionally substituted 5-membered heteroaryl moiety. In another aspect, the optionally substituted heterocycloalkyl or optionally substituted heteroaryl moiety is an optionally substituted moiety selected from pyrazolyl, furanyl, imidazolyl, isoxazolyl, oxadiazolyl, oxazolyl, pyrrolyl, pyridyl, pyrimidyl, pyridazinyl, thiazolyl, triazolyl, thienyl, dihydrothieno-pyrazolyl, thianaphthenyl, carbazolyl, benzimidazolyl, benzothienyl, benzofuranyl, indolyl, quinolinyl, benzotriazolyl, benzothiazolyl, benzooxazolyl, benzimidazolyl, isoquinolinyl, isoindolyl, acridinyl, benzoisazolyl, dimethylhydantoin, pyrazinyl, tetrahydrofuranyl, pyrrolinyl, pyrrolidinyl, morpholinyl, indolyl, diazepinyl, azepinyl, thiepinyl, piperidinyl, and oxepinyl. In another aspect, the optionally substituted heterocycloalkyl moiety or optionally substituted heteroaryl moiety is an optionally substituted furanyl. Non-limiting examples of heterocyclic compounds include (1,2-dihydroxyethyl)-3,4-dihydroxyfuran-2(5H)-one; 4-hydroxy-5-methyl-3-furanone; 5-hydroxy-2(5H)-furanone; [1,2-dihydroxyethyl]furan-2,3,4(5H)-trione; α-hydroxy-γ-butyrolactone; ribonic γ-lactone; aldohexuronicaldohexuronic

acid γ-lactone; gluconic acid δ-lactone; 4-hydroxycoumarin; dihydrobenzofuran; 5-(hydroxymethyl)furfural; furoin; 2(5H)-furanone; 5,6-dihydro-2H-pyran-2-one; and 5,6-dihydro-4-hydroxy-6-methyl-2H-pyran-2-one; or a salt or solvate thereof.

The nitrogen-containing compound may be any suitable compound with one or more
5 nitrogen atoms. In one aspect, the nitrogen-containing compound comprises an amine, imine, hydroxylamine, or nitroxide moiety. Non-limiting examples of nitrogen-containing compounds include acetone oxime; violuric acid; pyridine-2-aldoxime; 2-aminophenol; 1,2-benzenediamine; 2,2,6,6-tetramethyl-1-piperidinyloxy; 5,6,7,8-tetrahydrobiopterin; 6,7-dimethyl-5,6,7,8-tetrahydropterine; and maleamic acid; or a salt or solvate thereof.

10 The quinone compound may be any suitable compound comprising a quinone moiety as described herein. Non-limiting examples of quinone compounds include 1,4-benzoquinone; 1,4-naphthoquinone; 2-hydroxy-1,4-naphthoquinone; 2,3-dimethoxy-5-methyl-1,4-benzoquinone or coenzyme Q_0; 2,3,5,6-tetramethyl-1,4-benzoquinone or duroquinone; 1,4-dihydroxyanthraquinone; 3-hydroxy-1-methyl-5,6-indolinedione or adrenochrome; 4-tert-butyl-
15 5-methoxy-1,2-benzoquinone; pyrroloquinoline quinone; or a salt or solvate thereof.

The sulfur-containing compound may be any suitable compound comprising one or more sulfur atoms. In one aspect, the sulfur-containing comprises a moiety selected from thionyl, thioether, sulfinyl, sulfonyl, sulfamide, sulfonamide, sulfonic acid, and sulfonic ester. Non-limiting examples of sulfur-containing compounds include ethanethiol; 2-propanethiol; 2-
20 propene-1-thiol; 2-mercaptoethanesulfonic acid; benzenethiol; benzene-1,2-dithiol; cysteine; methionine; glutathione; cystine; or a salt or solvate thereof.

In an embodiment, the GH61 polypeptide is present in the amount of 2-1000 micrograms/g dry solids (DS), *e.g.*, 5-100, 10-40, or 20-40 micrograms/g DS.

25 Compositions, Methods and Uses

In a first aspect, the present invention provides a method for increasing (improving) the freeness of a pulp, and/or increasing the short span compression strength of a paper or packaging material made from the pulp, comprising treating the pulp with a GH61 polypeptide.

Optionally, a paper or packaging material is subsequently made from the treated pulp.

30 In an embodiment, the short span compression strength is measured according to TAPPI test method T 826, and the freeness is measured according to TAPPI test method T 227.

The method of the invention conveys improved properties of the paper or packaging material. The improved properties of the paper or packaging material are improved as compared to a paper or packaging material, which is made without contacting the pulp with a
35 GH61 polypeptide. The treated pulp may also exhibit improved drainage/dewatering.

In an embodiment, the amino acid sequence of the GH61 polypeptide comprises the motif(s):
[ILMV]-P-x(4,5)-G-x-Y-[ILMV]-x-R-x-[EQ]-x(4)-[HNQ] and/or
[FW]-[TF]-K-[AIV] and/or
5 H-x(1,2)-G-P-x(3)-[YW]-[AILMV] and/or
[EQ]-x-Y-x(2)-C-x-[EHQN]-[FILV]-x-[ILV].

In another embodiment, the amino acid sequence of the GH61 polypeptide comprises or consists of an amino acid sequence that has at least 50% sequence identity to the mature polypeptide of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3; preferably at least 55%
10 sequence identity, more preferably at least 60% sequence identity, more preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably at least 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, and most preferably at least 95% sequence identity to the mature polypeptide of SEQ ID NO: 1, SEQ ID
15 NO: 2, or SEQ ID NO: 3.

In another embodiment, the amino acid sequence of the GH61 polypeptide comprises or consists of an amino acid sequence that has up to 10, up to 9, up to 8, up to 7, up to 6, up to 5, up to 4, up to 3, up to 2, or up to 1 substitution(s) as compared to the mature polypeptide of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

20 In another embodiment, the amino acid sequence of the GH61 polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 1, SEQ ID NO: 2, and/or SEQ ID NO: 3.

In another embodiment, the amino acid sequence of the GH61 polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID
25 NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, or SEQ ID NO: 35; or a homologous sequence
30 thereof.

In another embodiment, the GH61 polypeptide is used in the presence of manganese sulphate (or manganese ions), copper sulphate (or copper ions) and/or ascorbic acid. Other suitable compounds, such as pyrogallol (1,2,3-trihydroxybenzene), which may be used with the GH61 polypeptide, are mentioned above.

In another embodiment, the pulp is also treated (or contacted) with an endoglucanase, such as the endoglucanase of SEQ ID NO: 36.

In another embodiment, the paper or packaging material is paper, linerboard, corrugated paperboard, tissue, towels, molded packaging materials, or corrugated containers or boxes.

5 In another embodiment, the pulp is a recovered, recycled or secondary pulp.

In another embodiment, the pulp is a chemical pulp. Preferably, the pulp is Kraft pulp or sulphite pulp. The pulp may be a wood pulp, such as a hardwood pulp (for example eucalyptus pulp) or softwood pulp (for example pine pulp).

The present invention also provides a paper or packaging material made from a pulp,
10 wherein the pulp has been subjected to the methods of the invention.

In a second aspect, the present invention provides a composition for making paper or packaging materials, comprising a pulp and a GH61 polypeptide. The pulp and the GH61 polypeptide are the same components as described in the methods of the invention.

15 In an embodiment, the amino acid sequence of the GH61 polypeptide comprises the motif(s):

[ILMV]-P-x(4,5)-G-x-Y-[ILMV]-x-R-x-[EQ]-x(4)-[HNQ] and/or

[FW]-[TF]-K-[AIV] and/or

H-x(1,2)-G-P-x(3)-[YW]-[AILMV] and/or

20 [EQ]-x-Y-x(2)-C-x-[EHQN]-[FILV]-x-[ILV].

In another embodiment, the amino acid sequence of the GH61 polypeptide comprises or consists of an amino acid sequence that has at least 50% sequence identity to the mature polypeptide of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3; preferably at least 55% sequence identity, more preferably at least 60% sequence identity, more preferably at least
25 65% sequence identity, more preferably at least 70% sequence identity, more preferably at least 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, and most preferably at least 95% sequence identity to the mature polypeptide of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

30 In another embodiment, the amino acid sequence of the GH61 polypeptide comprises or consists of an amino acid sequence that has up to 10, up to 9, up to 8, up to 7, up to 6, up to 5, up to 4, up to 3, up to 2, or up to 1 substitution(s) as compared to the mature polypeptide of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In another embodiment, the amino acid sequence of the GH61 polypeptide comprises or
35 consists of the mature polypeptide of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In another embodiment, the amino acid sequence of the GH61 polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15,
5 SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, or SEQ ID NO: 35; or a homologous sequence thereof.

10 In another embodiment, the composition includes manganese sulphate, copper sulphate and/or ascorbic acid. Other suitable compounds, such as pyrogallol (1,2,3-trihydroxybenzene), which may be included in the composition and used with the GH61 polypeptide, are mentioned above.

In another embodiment, the composition includes an endoglucanase, such as the
15 endoglucanase of SEQ ID NO: 36, or an endoglucanase having an amino acid sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identical to the amino acid sequence of SEQ ID NO: 36. In an embodiment, the number of amino acid changes (substitutions) introduced into the endoglucanase of SEQ ID NO: 36 is not more than 10, e.g., 1, 2, 3, 4, 5, 6, 7, 8 or 9. The amino acid changes may be of a minor
20 nature, that is conservative amino acid substitutions or insertions that do not significantly affect the folding and/or activity of the protein.

In another embodiment, the paper or packaging material is paper, linerboard, corrugated paperboard, tissue, towels, molded packaging materials, or corrugated containers or boxes.

In another embodiment, the pulp is a recovered, recycled or secondary pulp.

25 In another embodiment, the pulp is a chemical pulp. Preferably, the pulp is Kraft pulp or sulphite pulp. The pulp may be a wood pulp, such as a hardwood pulp (for example eucalyptus pulp) or softwood pulp (for example pine pulp).

In another embodiment, the composition is an aqueous composition with a pH of about 4 to about 8, preferably the composition has a pH of about 5 to about 7.

30 The composition of the second aspect may be used for making a paper or packaging material with increased short span compression strength, as compared to a paper or packaging material made from the composition without a GH61 polypeptide.

Process Conditions

The process of the invention is particularly applicable to the improvement of certain properties, such as improvement of the freeness of a pulp, or improvement of short span compression strength of paper or packaging materials made from the pulp.

In the case of paper or packaging, and pulp processing, the process according to the invention can be carried out at any pulp production stage. The GH61 polypeptide can be added to any holding tank, *e.g.*, to a pulp storing container (storage chest), storage tower, mixing chest or metering chest. The GH61 polypeptide treatment can be performed before the bleaching of pulp, in connection with the pulp bleaching process or after the bleaching. The GH61 polypeptide can also be added to the circulated process water (white water) originating from bleaching and process water (brown water) originating from the mechanical or chemimechanical pulping process.

In the present context, the term "process water" comprises i.a. 1) water added as a raw material to the paper manufacturing process; 2) intermediate water products resulting from any step of the process for manufacturing the paper material; as well as 3) waste water as an output or by-product of the process. In a particular embodiment, the process water is, has been, is being, or is intended for being circulated (re-circulated), *i.e.*, re-used in another step of the process. The term "water" in turn means any aqueous medium, solution, suspension, *e.g.*, ordinary tap water, and tap water in admixture with various additives and adjuvants commonly used in paper manufacturing processes. In a particular embodiment the process water has a low content of solid (dry) matter, *e.g.*, below 20%, 18%, 16%, 14%, 12%, 10%, 8%, 7%, 6%, 5%, 4%, 3%, 20% or below 1% dry matter.

The process of the invention may be carried out at conventional conditions in the paper and pulp processing. The process conditions will be a function of the polypeptide(s) applied, the reaction time and the conditions given.

The GH61 polypeptide of the invention should be added in an effective amount. By the term "effective amount" is meant the amount sufficient to achieve the desired and expected effect, such as improving pulp freeness and/or paper or packaging material strength.

In a particular embodiment, the dosage of the GH61 polypeptide and additional enzymes, if any, is from about 0.1 mg enzyme protein to about 100,000 mg enzyme protein (of each polypeptide) per ton of paper pulp.

In further particular embodiments, the amount of the GH61 polypeptide and additional enzymes, if any, is in the range of 0.00001-20; or 0.0001-20 mg of polypeptide (calculated as pure protein) per gram (dry weight) of pulp material, such as 0.0001-10 mg/g, 0.0001-1 mg/g, 0.001-1 mg/g, 0.001-0.1, or 0.01-0.1 mg of polypeptide per gram of pulp material. Again, these amounts refer to the amount of each polypeptide.

The GH61 polypeptide treatment can be done at conventional consistency, *e.g.*, 0.1-10% dry substance. In particular embodiments, the consistency is within the range of 0.1-45%; 0.1-40%; 0.1-35%; 0.1-30%; 0.1-25%; 0.1-20%; 0.1-15%; 0.1-10%; 0.1-8%; 0.1-6%; or 0.1-5% dry substance.

5 The GH61 polypeptide treatment may be carried out at a temperature of from about 10°C to about 100°C. Further examples of temperature ranges (all "from about" and "to about") are the following: 20-120°C, 30-120°C, 35-120°C, 37-120°C, 40-120°C, 50-120°C, 60-120°C, 70-120°C, 10-100°C, 10-90°C, 10-80°C, 10-70°C, 10-60°C, and 30-60°C, as well as any combination of the upper and lower values here indicated. A typical temperature is from about
10 20 to 90°C, or 20 to 95°C, preferably from about 40 to 70°C, or 40 to 75°C. Usually, the GH61 polypeptide treatment is carried out at atmospheric pressure. But when the temperature exceeds 100°C, the treatment is carried out at a pressure of 1-2 bar (up to 1 bar above atmospheric pressure).

The GH61 polypeptide treatment is carried out at a pH of from about 3 to about 10,
15 preferably at a pH from about 3.5 to about 9, more preferably at a pH from about 4 to about 8, and most preferably at a pH from about 5 to about 7.

A suitable duration of the GH61 polypeptide treatment may be in the range from a few seconds to several hours, *e.g.*, from about 30 seconds to about 48 hours, or from about 1 minute to about 24 hours, or from about 1 minute to about 18 hours, or from about 1 minute to
20 about 12 hours, or from about 1 minute to 5 hours, or from about 1 minute to about 2 hours, or from about 1 minute to about 1 hour, or from about 1 minute to about 30 minutes. A typical reaction time is from about 10 minutes to 3 hours, 10 minutes to 10 hours, preferably 15 minutes to 1 hour, or 15 minutes to 2 hours.

Molecular oxygen from the atmosphere will usually be present in sufficient quantity, if
25 required. Therefore, the reaction may conveniently be carried out in an open reactor, *i.e.*, at atmospheric pressure.

Various additives over and above the GH61 polypeptide and additional enzymes, if any, can be used in the process or use of the invention. Surfactants and/or dispersants are often present in, and/or added to a pulp. Thus the process and use of the present invention may be
30 carried out in the presence of an anionic, non-ionic, cationic and/or zwitterionic surfactant and/or dispersant conventionally used in a pulp. Examples of anionic surfactants are carboxylates, sulphates, sulphonates or phosphates of alkyl, substituted alkyl or aryl. Examples of non-ionic surfactants are polyoxyethylene compounds, such as alcohol ethoxylates, propoxylates or mixed ethoxy-/propoxylates, poly-glycerols and other polyols, as
35 well as certain block-copolymers. Examples of cationic surfactants are water-soluble cationic

polymers, such as quartenary ammonium sulphates and certain amines, *e.g.*, epichlorohydrin/dimethylamine polymers (EPI-DMA) and cross-linked solutions thereof, polydiallyl dimethyl ammonium chloride (DADMAC), DADMAC/Acrylamide co-polymers, and ionene polymers, such as those disclosed in US patents nos. 5,681,862; and 5,575,993.
5 Examples of zwitterionic or amphoteric surfactants are betains, glycinates, amino propionates, imino propionates and various imidazolin-derivatives. Also the polymers disclosed in US patent no. 5,256,252 may be used.

Also according to the invention, surfactants such as the above, including any combination thereof, may be used in a paper making process together with a GH61
10 polypeptide as defined herein, and included in a composition together with such polypeptide. The amount of each surfactant in such composition may amount to from about 1 to about 1000 ppm of the composition. In particular embodiments the amount of each surfactant is from about 10 to about 1000 ppm, or from about 10 to about 500 ppm, or from about 50 to about 500 ppm.

In another particular embodiment, each of the above ranges refers to the total amount of
15 surfactants.

In further particular embodiments of the above method, and of the process of the invention, the GH61 polypeptide is used in an amount of 0.005-50 ppm (mg/L), or 0.01-40, 0.02-30, 0.03-25, 0.04-20, 0.05-15, 0.05-10, 0.05-5, 0.05-1, 0.05-0.8, 0.05-0.6, or 0.1-0.5 ppm. The amount of GH61 polypeptide refers to mg of a well-defined polypeptide preparation.

20 In the process of the invention, the GH61 polypeptide may be applied alone or together with an additional enzyme. The term "an additional enzyme" means at least one additional enzyme, *e.g.*, one, two, three, four, five, six, seven, eight, nine, ten or even more additional enzymes.

The term "applied together with" (or "used together with") means that the additional
25 enzyme may be applied in the same, or in another step of the process of the invention. The other process step may be upstream or downstream in the paper manufacturing process, as compared to the step in which the pulp is contacted with a GH61 polypeptide.

In particular embodiments the additional enzyme is an enzyme which has protease, lipase, xylanase, cutinase, oxidoreductase, glycosyl hydrolase cellulase, endoglucanase,
30 amylase, mannanase, steryl esterase, and/or cholesterol esterase activity. Examples of oxidoreductase enzymes are enzymes with laccase, and/or peroxidase activity. In a preferred embodiment, the additional enzyme is glycosyl hydrolase, such as endoglucanase.

The term "a step" of a process means at least one step, and it could be one, two, three, four, five or even more process steps. In other words the GH61 polypeptide of the invention
35 may be applied in at least one process step, and the additional enzyme(s) may also be applied

in at least one process step, which may be the same or a different process step as compared to the step where the GH61 polypeptide is used.

The term "polypeptide preparation" means a product containing at least one GH61 polypeptide. The polypeptide preparation may also comprise enzymes having other enzyme activities, preferably glycosyl hydrolytic enzymes, such as endoglucanase. In addition to the enzymatic activity, such a preparation may also contain at least one adjuvant. Examples of adjuvants, which are used in enzyme preparations for the paper and pulp industry are buffers, polymers, surfactants and stabilizing agents.

Additional enzymes

Any enzyme having protease, lipase, xylanase, cutinase, laccase, peroxidase, oxidase, cellulose, endoglucanase, amylase, mannanase, steryl esterase, and/or cholesterol esterase activity can be used as additional enzymes in the use and process of the invention. Below some non-limiting examples are listed of such additional enzymes. The enzymes written in capitals are commercial enzymes available from Novozymes A/S, Krogshoejvej 36, DK-2880 Bagsvaerd, Denmark. The activity of any of those additional enzymes can be analyzed using any method known in the art for the enzyme in question, including the methods mentioned in the references cited.

Examples of cutinases are thc) derived from *Humicola insolens* (US 5,827,719); from a strain of *Fusarium*, *e.g.*, *F. roseum culmorum*, or particularly *F. solani pisi* (WO 90/09446; WO 94/14964, WO 94/03578). The cutinase may also be derived from a strain of *Rhizoctonia*, *e.g.*, *R. solani*, or a strain of *Alternaria*, *e.g.*, *A. brassicicola* (WO 94/03578), or variants thereof such as those described in WO 00/34450, or WO 01/92502.

Examples of proteases are the ALCALASE, ESPERASE, SAVINASE, NEUTRASE and DURAZYM proteases. Other proteases are derived from *Nocardiopsis*, *Aspergillus*, *Rhizopus*, *Bacillus alcalophilus*, *B. cereus*, *B. natto*, *B. vulgatus*, *B. mycoide*, and subtilisins from *Bacillus*, especially proteases from the species *Nocardiopsis sp.* and *Nocardiopsis dassonvillei* such as those disclosed in WO 88/03947, and mutants thereof, *e.g.*, those disclosed in WO 91/00345 and EP 415296.

Examples of amylases are the BAN, AQUAZYM, TERMAMYL, and AQUAZYM Ultra amylases. An example of a lipase is the RESINASE A2X lipase. An example of a xylanase is the PULPZYME HC hemicellulase. Examples of endoglucanases are the NOVOZYM 613, 342, and 476, and NOVOZYM 51081 enzyme products.

Examples of mannanases are the *Trichoderma reesei* endo-beta-mannanases described in Ståhlbrand et al, J. Biotechnol. 29 (1993), 229-242.

Examples of steryl esterases, peroxidases, laccases, and cholesterol esterases are disclosed in the references mentioned in the background art section hereof. Further examples of oxidoreductases are the peroxidases and laccases disclosed in EP 730641; WO 01/98469; EP 719337; EP 765394; EP 767836; EP 763115; and EP 788547. In the present context, whenever an oxidoreductase enzyme is mentioned that requires or benefits from the presence of acceptors (*e.g.*, oxygen or hydrogen peroxide), enhancers, mediators and/or activators, such compounds should be considered to be included. Examples of enhancers and mediators are disclosed in EP 705327; WO 98/56899; EP 677102; EP 781328; and EP 707637. If desired a distinction could be made by defining an oxidoreductase enzyme system (*e.g.*, a laccase, or a peroxidase enzyme system) as the combination of the enzyme in question and its acceptor, and optionally also an enhancer and/or mediator for the enzyme in question.

The invention described and claimed herein is not to be limited in scope by the specific embodiments herein disclosed, since these embodiments are intended as illustrations of several aspects of the invention. Any equivalent embodiments are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. In the case of conflict, the present disclosure including definitions will control.

EXAMPLES

EXAMPLE 1

Improving freeness of old corrugated container (OCC) fiber pulp with GH61 polypeptides

Pulp

Model old corrugated container (OCC) fiber pulp was prepared by soaking 100 oven dry grams (odg) of hand-torn corrugated cardboard box in 2 L of deionized water overnight at room temperature and then disintegrating the soaked material for 20,000 revolutions in a standard laboratory disintegrator. The resultant pulp was then vacuum filtered and the resultant cake 'scattered' to small pieces, transferred to a sealable plastic bag and stored for 24 hours at 4°C until use.

Reagents & Enzymes

Stock solutions of GH61 cofactors were prepared according to Table 1. Enzymes used in the trial are described in Table 2.

Table 1. Stock solutions

Reagent	**Concentration**	**Preparation**
Pyrogallol, 279KEM00218	3.17 M	Dissolve 4.0 g of pyrogallol in 10 ml MQ H_2O
$CuSO_4$, 279KEM00098	10 mM	Dissolve 0.5 g of $CuSO_4{\cdot}5\ H_2O$ in 200 ml of MQ H_2O

5

Table 2. Enzymes used in the trial

Enzyme	**Description**
Endoglucanase	Endoglucanase shown as SEQ ID NO: 36, which is a Q120H variant of the mature endoglucanase shown as SEQ ID NO: 9 of WO 96/29397. Also available as Renozyme™ from Novozymes A/S, Denmark.
GH61	1:1:1 mixture of three mature GH61s from *Acremonium alcalophilum* shown as SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3

Procedure

10 Aliquots of the model pulp, equivalent to 20 oven dry grams of solids, were placed in each of eight 1 L stainless steel Lab-O-Mat beakers and diluted to target consistency and pH with buffer according to Table 3. Enzymes and cofactors were added to each beaker according to the dosing schedule presented in Table 4. The beakers were sealed and placed in the Lab-O-Mat and incubation conducted according to the conditions described in Table 3. After

15 incubation, the contents of each beaker were diluted to 2 L with deionized water, disintegrated for 10,000 revolutions and diluted to 5 L (0.4% consistency). Duplicate 'freeness' values were determined from each diluted pulp sample and three 2.6 gram handsheets (equivalent to 130 g/m^2 basis weight), were prepared from each for drying and testing according to TAPPI standard procedure.

20

Table 3. Constant trial parameters

Parameter	**Value**
Substrate	Old corrugated container (OCC)
Oven Dry weight per trial	20 g
Consistency	3.4 % w/v
Buffer	Britton-Robinson 40 mM (pH 7)

Enzyme Dose	See Table 4
Temperature	50°C
pH	7
Retention time	240 min
Incubation vessel	Lab-O-Mat (20 rpm, 30 s left, 30 s right), no O_2 pressurization

Table 4. Enzyme and cofactor dosing schedule

#	Total fiber (odg)	GH61 (g EP/DT)	Endoglucanase (g EP/DT)	Pyrogallol (mM)	$CuSO_4$ (mM)
1	20	0	0	2.00	0.01
2	20	30	1.125	2.00	0.01
3	20	30	2.250	2.00	0.01
4	20	30	5.625	2.00	0.01
5	20	12	0	2.00	0.01
6	20	300	1.125	2.00	0.01
7	20	300	2.250	2.00	0.01
8	20	300	5.625	2.00	0.01

5 Results

Table 5 presents the freeness values obtained from 0.4% slurries of untreated fiber (*i.e.*, control) and enzymatically pre-treated fiber. While the application of endoglucanase alone increased the freeness, the addition of GH61 in concert with the endoglucanase boosted the improvement. Surprisingly, the addition of GH61 alone, at 12 grams of enzyme protein per dry
10 ton of pulp solids, improved freeness by 7% relative to the untreated control.

Interestingly, only the trial in which GH61 was added in the absence of endoglucanase was able to generate handsheets with improved short span compression strength relative to the untreated control (Table 6).

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Table 5. Canadian standard freeness (CSF) values obtained from pulp slurries (0.4%) prepared from fiber pre-treated with blends of endoglucanase and GH61. Endoglucanase and GH61 doses are given as grams of enzyme protein (EP) per DT of fiber.

#	GH61 (g EP/DT)	Endoglucanase (g EP/DT)	CSF	Delta
1	0	0	519.1	0%
2	30	1.125	573.7	11%
3	30	2.250	581.3	12%
4	30	5.625	591.6	14%
5	12	0	556.2	7%
6	100	1.125	584.6	13%
7	200	2.250	589.5	14%
8	500	5.625	615.6	19%

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Table 6. Short span compression strength (SCT) indices obtained from 130 g/m^2 handsheets prepared with fiber pre-treated with endoglucanase and GH61.

#	GH61 (g/DT)	Endoglucanase (g/DT)	Short Span Compressive Strength Index (Nm/g)	Delta
1	0	0	13.12	0%
2	30	1.125	12.51	-5%
3	30	2.250	13.14	0%
4	30	5.625	12.52	-5%
5	12	0	14.01	7%
6	100	1.125	12.54	-4%
7	200	2.250	12.39	-6%
8	500	5.625	12.20	-7%

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EXAMPLE 2

Improving freeness of recovered pulp with GH61 polypeptides

Pulp

5 Recovered pulp, obtained from a recycled linerboard mill, was used 'as received' in the subsequent trials. Pulp characteristics include 3% consistency and pH 6.3.

Reagents & Enzymes

Stock solutions of GH61 cofactors were prepared according to Table 1. Enzymes used in 10 the trial are described in Table 8.

Table 7. Stock solutions

Reagent	Concentration	Preparation
Pyrogallol, 279KEM00218	3.17 M	Dissolve 4.0 g of pyrogallol in 10 ml MQ H_2O
$CuSO_4$, 279KEM00098	10 mM	Dissolve 0.5 g of $CuSO_4 \cdot 5\ H_2O$ in 200 ml of MQ H_2O

Table 8. Enzymes used in the trial

Enzyme	Description
Endoglucanase	Endoglucanase shown as SEQ ID NO: 36, which is a Q120H variant of the mature endoglucanase shown as SEQ ID NO: 9 of WO 96/29397. Also available as Renozyme™ from Novozymes A/S, Denmark.
GH61	1:1:1 mixture of three mature GH61s from *Acremonium alcalophilum* shown as SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3.

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Procedure

Aliquots of the recycled fiber, equivalent to 20 oven dry grams of solids, were placed in each of eight 1 L stainless steel Lab-O-Mat beakers according to Table 9. Enzymes and 20 cofactors were added to each beaker according to the dosing schedule presented in Table 10. The beakers were sealed and placed in the Lab-O-Mat and incubation conducted according to the conditions described in Table 9. After incubation, the contents of each beaker were diluted to 2 L with deionized water, disintegrated for 10,000 revolutions and diluted to 5 L (0.4% consistency). Duplicate 'freeness' values were determined from each diluted pulp sample and 25 three 2.6 gram handsheets (equivalent to 130 g/m^2 basis weight), were prepared from each for drying and testing according to TAPPI standard procedure.

Table 9. Constant trial parameters

Parameter	Value
Substrate	Recovered fiber ('OCC')
Oven Dry weight per trial	20 g
Consistency	3% w/v
Enzyme Dose	See Table 10
Temperature	50°C
pH	6.3
Retention time	120 min
Incubation vessel	Lab-O-Mat (20 rpm, 30 s left, 30 s right), no O_2 pressurization

Table 1. Enzyme and cofactor dosing schedule

#	Total fiber (odg)	GH61 (g EP/DT)	Endoglucanase (g EP/DT)	Pyrogallol (mM)	$CuSO_4$ (mM)
1	20	0	0	2.00	0.01
2	20	15	0	2.00	0.01
3	20	30	0	2.00	0.01
4	20	300	0	2.00	0.01
5	20	0	11.25	2.00	0.01
6	20	15	11.25	2.00	0.01
7	20	30	11.25	2.00	0.01
8	20	300	11.25	2.00	0.01

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Results

Table 11 presents the results clearly demonstrating that freeness may be significantly improved by up to 21% by the pre-treatment of the pulp with GH61 alone. Furthermore, the addition of GH61 in concert with endoglucanase improved the freeness relative to the
10 endoglucanase alone. However, increasing the amount of GH61 protein within the GH61/endoglucanase blend beyond 15 grams per oven dry ton, did not significantly enhance freeness relative to lower doses of GH61.

Table 11. Canadian standard freeness (CSF) values obtained from pulp slurries (0.4%)
15 prepared from fiber pre-treated with blends of endoglucanase and GH61. Endoglucanase and GH61 doses are given as grams of enzyme protein (EP) per DT of fiber.

#	GH61 (g EP/DT)	Endoglucanase (g EP/DT)	CSF	Delta
1	0	0	173	0%
2	15	0	175	1%
3	30	0	192	10%
4	300	0	210	21%
5	0	11.25	287	65%
6	15	11.25	324	87%
7	30	11.25	331	91%
8	300	11.25	325	87%

CLAIMS:

1. A method for increasing the freeness of a pulp, increasing the short span compression strength of a paper or packaging material made from the pulp, or both, the method comprising treating the pulp with a glycoside hydrolase Family 61, wherein the glycoside hydrolase Family 61 comprises an amino acid sequence having at least 50% sequence identity to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

2. The method of claim 1, wherein a paper or packaging material is subsequently made from the treated pulp.

3. The method of claim 1 or 2, wherein the short span compression strength is measured according to TAPPI test method T 826, and the freeness is measured according to TAPPI test method T 227.

4. The method of any one of claims 1-3, wherein the amino acid sequence of the glycoside hydrolase Family 61 comprises the motif:
[ILMV]-P-x(4,5)-G-x-Y-[ILMV]-x-R-x-[EQ]-x(4)-[HNQ],
H-x(1,2)-G-P-x(3)-[YW]-[AILMV],
[EQ]-x-Y-x(2)-C-x-[EHQN]-[FILV]-x-[ILV],
or a combination thereof.

5. The method of any one of claims 1-4, wherein the glycoside hydrolase Family 61 comprises an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

6. The method of claim 5, wherein the amino acid sequence of the glycoside hydrolase Family 61 comprises an amino acid sequence that has at least 65% sequence identity to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

7. The method of claim 5, wherein the glycoside hydrolase Family 61 comprises amino acid sequence having at least 70% sequence identity the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

8. The method of claim 5, wherein the glycoside hydrolase Family 61 comprises an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

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9. The method of claim 5, wherein the glycoside hydrolase Family 61 comprises an amino acid sequence having at least 85% sequence identity to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

10. The method of claim 5, wherein the glycoside hydrolase Family 61 comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

11. The method of claim 5, wherein the glycoside hydrolase Family 61 comprises an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

12. The method of any one of claims 1-11, wherein the glycoside hydrolase Family 61 comprises an amino acid sequence having up to 10, up to 9, up to 8, up to 7, up to 6, up to 5, up to 4, up to 3, up to 2, or up to 1 substitution(s) as compared to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

13. The method of any one of claims 1-12, wherein the glycoside hydrolase Family 61 comprises the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

14. The method of any one of claims 1-4, wherein the glycoside hydrolase Family 61 consists of an amino acid sequence having at least 50% sequence identity to the amino acid sequence SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

15. The method of claim 14, wherein the glycoside hydrolase Family 61 consists of an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

16. The method of claim 13, wherein the glycoside hydrolase Family 61 consists of an amino acid sequence that has at least 65% sequence identity to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

17. The method of claim 14, wherein the glycoside hydrolase Family 61 consists of an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

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18. The method of claim 14, wherein the glycoside hydrolase Family 61 consists of an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

19. The method of claim 14, wherein the glycoside hydrolase Family 61 consists of an amino acid sequence having at least 85% sequence identity to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

20. The method of claim 14, wherein the glycoside hydrolase Family 61 consists of an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

21. The method of claim 14, wherein the glycoside hydrolase Family 61 consists of an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

22. The method of any one of claims 14-21, wherein the glycoside hydrolase Family 61 consists of an amino acid sequence having up to 10, up to 9, up to 8, up to 7, up to 6, up to 5, up to 4, up to 3, up to 2, or up to 1 substitution(s) as compared to the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

23. The method of any one of claims 14-22, wherein the glycoside hydrolase Family 61 the amino acid sequence consists of the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

24. The method of any one of claims 1-23, wherein the pulp is also treated with an endoglucanase.

25. The method of any one of claims 1-24, wherein the pulp is a recovered, recycled or secondary pulp.

26. The method of any one of claims 1-25, wherein the pulp is Kraft pulp or sulphite pulp.

27. The method of any one of claims 1-26, wherein the pulp is wood pulp.

28. The method of claim 27, wherein the wood pulp is hardwood pulp or softwood pulp.

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29. The method of claim 28, wherein the hardwood pulp is eucalyptus pulp.

30. The method of claim 28, wherein the softwood pulp is pine pulp.

31. The method of any one of claims 1-30, wherein the freeness of the pulp, the short span compression strength of the paper or packaging material, or both, are increased as compared to not treating the pulp with a glycoside hydrolase Family 61.

32. The method of any one of claims 1-31, wherein the pulp exhibits improved drainage/dewatering.

33. A paper or packaging material made by the method of any one of claims 1-32.

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