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(54) Title: ENZYMATIC DEHUSKING OF PULSES

(57) Abstract: Disclosed is a method for dehusking pulses by pretreating them with an enzyme composition comprising a GH11 or GH8 xylanase or a GH5 endo glucanase or a polygalacturonase prior to dehusking.

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ENZYMATIC DEHUSKING OF PULSES

Reference to sequence listing

This application contains a Sequence Listing in computer readable form. The computer readable form is incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to a method of dehusking pulses. More specifically, it relates to an enzymatic method for dehusking of pulses.

BACKGROUND OF THE INVENTION

Pulses form a major source of protein component in a vegetarian diet. Economically, pulses are also one of the cheapest source of protein. Pulses are mainly consumed in the form of dehusked split pulses.

Pulse milling involves conversion of the whole unhusked pulse grain into its dehusked and split form which is commonly termed as “dal”. It involves the following processes namely, loosening of husk, dehusking and splitting of pulses. Traditionally both dry and wet milling process are employed. In traditional wet milling process soaking with water is commonly used while in traditional dry milling process, coating with oil is used. Machineries in the form of carborundum emery rollers for dehusking and burr grinders for splitting are also employed.

The traditional methods of pulse milling are labour intensive, time consuming and incurs losses. This is because dehusking is seldom complete in a single pass and requires multiple passes, each pass producing additional losses in the form of brokens and powder thus reducing the recovery of dal. It is generally assumed that the yield of split & pulses in traditional mills are only 65 to 75% due to the above losses compared to 82 to 85% of potential yield.

Indian Patent application 657/DEL/2007 discloses an enzymatic process for preparation of dehulled legume/dhals where xylanase and protease are used.

There exists a need for newer methods or processes in pulse milling which can increase yield of the pulses. In addition, there is also need for newer processes that can improve the quality of the dehusked pulse or dal obtained at the end of dehusking or milling.

SUMMARY OF THE INVENTION

The invention relates to a method of dehusking a pulse comprising

a. pretreating the pulse with an enzyme composition comprising a GH11 or GH8 xylanase;
b. dehusking the pretreated pulse.

The invention also relates to a method of dehusking a pulse comprising

a. pretreating the pulse with an enzyme composition comprising a GH5 endoglucanase;
b. dehusking the pretreated pulse.

The invention also relates to a method of dehusking a pulse comprising

a. pretreating the pulse with an enzyme composition comprising a polygalacturonase;
b. dehusking the pretreated pulse.

The invention further discloses dehusked pulse produced according to the method of the invention. The method of the invention provides for increased yield of the pulses. The pulses so obtained also have an improved quality as judged by visual appearance.

DEFINITION:

Xylanase: The term "xylanase" means a 1,4-beta-D-xylan-xylohydrolase (E.C. 3.2.1.8) that catalyses the endohydrolysis of 1,4-beta-D-xylosidic linkages in xylans. Xylanase activity can be determined with 0.2% AZCL-arabinoxylan as substrate in 0.01% TRITON® X-100 and 200 mM sodium phosphate pH 6 at 37°C. One unit of xylanase activity is defined as 1.0 µmole of azurine produced per minute at 37°C, pH 6 from 0.2% AZCL-arabinoxylan as substrate in 200 mM sodium phosphate pH 6.

GH8 Xylanase: A GH8 xylanase according to the invention is a xylanase enzyme conforming to the definition of the GH8 xylanase as set out by the Carbohydrate-active enzymes database (CAZy; http://www.cazy.org), a dedicated family classification system that correlate with the structure and molecular mechanism of CAZymes, developed by the Glycogenomics group at AFMB in Marseille, France, including any fragment derived therefrom, exhibiting xylanase activity.

GH11 Xylanase: A GH 11 xylanase according to the invention is a xylanase enzyme conforming to the definition of the GH11 xylanase as set out by the Carbohydrate-active enzymes database (CAZy; http://www.cazy.org), a dedicated family classification system that correlate with the structure and molecular mechanism of CAZymes, developed by the Glycogenomics group at AFMB in Marseille, France, including any fragment derived therefrom, exhibiting xylanase activity.

Endoglucanase: The term "endoglucanase" means an endo-1,4-(1,3; 1,4)-beta-D-glucan 4-glucanohydrolase (E.C. 3.2.1.4) that catalyzes endohydrolysis of 1,4-beta-D-glycosidic linkages in cellulose, cellulose derivatives (such as carboxymethyl cellulose and hydroxyethyl cellulose), lichenin, beta-1,4 bonds in mixed beta-1,3 glucans such as cereal beta-D-glucans or xyloglucans, and other plant material containing cellulosic components. Endoglucanase activity can be determined by measuring reduction in substrate viscosity or increase in reducing ends determined by a reducing sugar assay (Zhang et al., 2006, Biotechnology Advances 24: 452-481). Endoglucanase activity may be determined using carboxymethyl cellulose (CMC) as substrate according to the procedure of Ghose, 1987, Pure and Appl. Chem. 59: 257-268, at pH 5, 40 degrees centigrade Alternatively, the endoglucanase activity can also be determined using the procedure described in Example 8, 13 or 14 of WO13071883A1.

Cellulose is generally found, for example, in the stems, leaves, hulls, husks, and cobs of plants or leaves, branches, and wood of trees. Cellulose is a polymer of the simple sugar glucose covalently linked by beta-1,4- bonds. Many microorganisms produce enzymes that hydrolyze beta-linked glucans. These enzymes include endoglucanases, cellobiohydrolases, and beta-glucosidases. Endoglucanases digest the cellulose polymer at random locations, opening it to attack by cellobiohydrolases. Cellobiohydrolases sequentially release molecules of cellobiose from the ends of the cellulose polymer. Cellobiose is a water-soluble beta-1,4-linked dimer of glucose. Beta-glucosidases hydrolyze cellobiose to glucose.

GH5 endoglucanase: A GH5 endoglucanase according to the invention is a endoglucanase enzyme conforming to the definition of the GH5 endoglucanase as set out by the Carbohydrate-active enzymes database (CAZy; http://www.cazy.org), a dedicated family classification system that correlate with the structure and molecular mechanism of CAZymes, developed by the Glycogenomics group at AFMB in Marseille, France, including any fragment derived therefrom, exhibiting xylanase activity.

Polygalacturonase: The term “polygalacturonase” (EC 3.2.1.15) means a pectinase that catalyze random hydrolysis of (1 ,4)-alpha-D- galactosiduronic linkages in pectate and other galacturonans. They are also known as pectin depolymerase.

Pectin lyase: The term "Pectin lyase" (EC 4.2.2.10) means pectinases that catalyze eliminative cleavage of (1.4)-alpha-D- galacturonan methyl ester to give oligosaccharides with 4-deoxy-6-0-methyl-alpha-D-galact-4- enuronosyl groups at their non-reducing ends. They are alternatively known as pectolyase, polymethylgalacturonic transeliminase, pectin methyltranseliminase, pectin trans-eliminase, etc.

Pectin esterase: The term "Pectin esterase" (EC 3.1.1.11) means pectinases that hydrolyze pectin to methanol and pectate. They are alternatively known as pectin demethoxylase, pectin methoxylase, pectin methylesterase, etc. Pectin esterase catalyses the release of methanol from pectin with a resultant decrease in pH.

Pulse: Pulse is annual leguminous crops yielding from one to 12 grains or seeds of variable size, shape and colour within a pod (as defined by FAO http://www.fao.org/es/faodef/fdef04e.htm#4.01). The term pulse is limited to crops harvested solely for dry grain, thereby excluding crops harvested green for food or crops used mainly for oil extraction or leguminous crops that are used exclusively for sowing purposes.

Sequence Identity: The relatedness between two amino acid sequences is described by the parameter "sequence identity".

For purposes of the present invention, the degree of sequence identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 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 3.0.0 or later. Version 6.1.0 was used. The optional 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 labelled "longest identity" (obtained 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)

DETAILED DESCRIPTION OF THE INVENTION

Pulse forms the main source of protein in a vegetarian diet.

The United Nations Food and Agricultural organization (FAO) defines pulses as annual leguminous crops yielding from one to 12 grains or seeds of variable size, shape and colour within

a pod. They are used for both food and feed. (see http://www.fao.org/es/faodef/fdef04e.htm#4.01).

FAO also limits the term "pulse"(s) to crops harvested solely for dry grain, thereby excluding crops harvested green for food (green peas, green beans, etc.) which are classified as vegetable crops. Also excluded are those crops used mainly for oil extraction (e.g. soybean and groundnuts) and leguminous crops (e.g. seeds of clover and alfalfa) that are used exclusively for sowing purposes.

FAO recognizes 11 primary pulses.

1. Dry beans (*Phaseolus spp.* including several species now in *Vigna*)
   - Kidney bean, navy bean, pinto bean, haricot bean (*Phaseolus vulgaris*)
   - Lima bean, butter bean (*Phaseolus lunatus*)
   - Adzuki bean, azuki bean (*Vigna angularis*)
   - Mung bean, golden gram, green gram (*Vigna radiata*)
   - Black gram, urad (*Vigna mungo*)
   - Scarlet runner bean (*Phaseolus coccineus*)
   - Ricebean (*Vigna umbellata*)
   - Moth bean (*Vigna aconitifolia*)
   - Tepary bean (*Phaseolus acutifolius*)
2. Dry broad beans (*Vicia faba*)
   - Horse bean (*Vicia faba equina*)
   - Broad bean (*Vicia faba*)
   - Field bean (*Vicia faba*)
3. Dry peas (*Pisum spp.*)
   - Garden pea (*Pisum sativum* var. *sativum*)
   - Protein pea (*Pisum sativum* var. *arvense*)
4. Chickpea, garbanzo, Bengal gram (*Cicer arietinum*)
5. Dry cowpea, black-eyed pea, blackeye bean (*Vigna unguiculata*)
6. Pigeon pea, Arhar/Toor, cajan pea, Congo bean, gandules (*Cajanus cajan*)
7. Lentil (*Lens culinaris*)
8. Bambara groundnut, earth pea (*Vigna subterranea*)
9. Vetch, common vetch (*Vicia sativa*)

10. Lupins (*Lupinus spp.*)

11. Minor pulses, including:
    - Lablab, hyacinth bean (*Lablab purpureus*)
    - Jack bean (*Canavalia ensiformis*), sword bean (*Canavalia gladiata*)
    - Winged bean (*Psophocarpus tetragonolobus*)
    - Velvet bean, cowitch (*Mucuna pruriens var. utilis*)
    - Yam bean (*Pachyrhizus erosus*)

Pulses are consumed in its dehusked and split form which, traditionally in India, is termed "Dal".

Pulse milling is the process of conversion of pulse, most commonly whole unhusked pulse, to dal. It is accomplished in three major steps namely: loosening of husk, dehusking and splitting.

Commercial scale milling of pulses consists of various steps. For a detailed understanding, the description of the various steps in pulse milling is available at the Indiaagronet website (https://www.indiaagronet.com/indiaagronet/post_harvest/pulses.htm). A brief description is given below.

Cleaning and Grading

Pulses received at the mill need to be cleaned and size graded for yielding good quality dal with higher recovery. Even during dehusking operation, pulses are subjected to sieving to separate out husk, brokens, splits, gota (dehusked whole pulse) and whole unhusked pulses. Usually two types of cleaners are used: reciprocating air-screen cleaners and reel screen cleaners.

In reciprocating air screen cleaners, air is blown through two screens (sieves) which separate out lighter material such as dust, stalk, dried leaves, husk etc. The upper screen has bigger perforations while the second screen has smaller perforations. The reel screen cleaners consist of 2-4 cylindrical compartments. The frame of the machine is made of wooden or mild steel sheet. In these compartments, different size perforation screens are fitted on a 5-7.5 mm diameter shaft. The machine is fitted at an inclination of 2-3 degrees. The cylindrical screen drum rotates at 5-35 rpm.

Drying of Pulses

Drying of pulses is necessary to ensure safe storage before milling as pulses received at mill have generally higher moisture content. After steeping of pulses for loosening of husk, it is also necessary to dry pulses. During splitting operation too, it is very much essential to dry the pulses to separate cotyledons. Sun drying of pulses is an economical option for drying of

pulses. The sun drying is done for a few hours to a few days depending on the season and type of pulses used. The pulses are spread over floor/roof in 5 to 7.5 cm thick layer which are intermittently stirred manually with the help of rakes or turning by foot. At night, the drying pulses are collected in heaps and covered with canvass sheet to preserve the heat. Mechanically heated air dryers, either batch type or continuous flow type are also used by the millers. The temperature of heated air for drying varies from 60 degree Centigrade to 120 degree Centigrade (C).

Loosening of Husk

This is a very important step in pulses milling as it decides the total recovery and quality of milled dal. Loosening of husk is accomplished in two different ways: wet method and dry method.

Wet Method

Cleaned and graded pulses are soaked in water for 4-12 hours for steeping, mixed with red earth for 12-16 hours and then sun dried to keep the moisture content about 10-12 %. During steeping, the husk becomes loose and thus facilitates easy dehusking and splitting. Yield is also increased due to lesser breakage. But cooking time increases when the dal is obtained by this method. Red earth is used as it imparts a good yellow colour to the end product and also helps to remove small patches of adhering husk due to its mild abrasive quality.

Dry method

In this method, husk is loosened by sequence of operations such as: oil smearing, water application, tempering and sun-drying. Cleaned and graded pulses are passed through roller dehusker in which scratches, cracks and dents are created on hard seed coat of pulses. This is known as “pitting” of pulses. The pitted pulse grains are then passed through the sieve cleaner to separate out the splits, husk and powder and later smeared with oil (100-500 gram per quintal of pulses) either manually or with auger mixer and stored for 1-5 days. During this tempering period, oil diffuses in between the husk and cotyledons and weakens the bond and thus facilitates loosening of the adhering husk. At the end of storage period, water is applied to the grains (1-5 kg/q) and stored for further 12-14 h (overnight) and at last sun-dried for 1-3 days before subjecting to milling.

Dehusking

Roller dehuskers coated with carborandum are used to dehusk the pulses. Two types of rollers viz. cylindrical and tapered are available for dehusking. Tapered rollers are placed horizontally and the diameter of roller increases from feeding side to discharge side. The difference

in diameter helps to gradually increase the pressure on pulse grains and thus helps in gradual dehusking. The cylindrical rollers are installed at an angle of 10-15 degrees which enables forward movement of pulse grains inside the machine. Annular gap between rollers varies depending upon the type of pulses being dehusked. Inlet and outlet of the roller machine can be adjusted for regulation of grain flow and retention time respectively. Small dal mills use under run disc shellers or burr mills for dehusking operation in place of Roller mills.

Conditioned pulse grains subjected to mild abrasion inside the roller machine, removes 10-25% of husk in one pass. Shelled husk, cotyledon powder, brokens and splits are separated out by Air-screen cleaners after passing the grain lot once or twice through the roller machine. Depending upon adherence of husk to grain, the pulse grains are passed through mill for two to eight times.

For hard-to-dehusk pulses (for example arhar (red gram), moong (green gram), urad (black gram)), the recovery is between 70-75% while for easy-to-dehusk pulses (bengal gram, lentil, and peas), it varies in between 78-85%.

Splitting

Splitting operation involves loosening the bond between the cotyledons and splitting. For cotyledons loosening, water at the rate of 1-5 kg/quintal is applied to dehusked pulse grain (gota) and is stored for 2-12 hours and later sun-dried for 4-8 hours. For splitting, machines like under-run-disc sheller (URD), impact machine (Phatphatia), roller mill, and hitting the gota against the metal sheet at discharge side of bucket elevator are used. In this operation, the embryo attached to two cotyledons breaks away, thereby, causing a loss in dal recovery by 1.5 to 2%.

Polishing

In this operation, dal is imparted with a glazing appearance to improve its consumers' acceptance and market value. Depending upon the need, different materials like water, oil, soapstone powder are applied to dal surface. Sometimes removal of sticking powder from dal surface is considered sufficient to improve its surface glaze.

The traditional methods of pulse milling are labour intensive, time consuming and incurs losses because dehusking is seldom complete in a single pass and requires multiple passes, each pass producing additional losses in the form of brokens and powder thus reducing the recovery of dal. It is generally assumed that the yield of split & pulses in traditional mills are only 65 to 75% due to the above losses compared to 82 to 85% of potential yield.

The inventors of the present invention have found that pretreating the pulse with an enzyme composition comprising a GH11 or GH8 xylanase prior to the dehusking process actually improves the yield of the pulse obtained after dehusking.

The inventors of the present invention have also found that pretreating the pulse with an enzyme composition comprising a GH5 endoglucanase prior to the dehusking process actually improves the yield of the pulse obtained after dehusking.

The inventors of the present invention have further found that pretreating the pulse with an enzyme composition comprising a polygalacturonase prior to the dehusking process improves the yield of the pulse obtained after dehusking.

Thus in one aspect, the invention relates to a method of dehusking a pulse comprising:

a) Pretreating the pulse with an enzyme composition comprising a GH11 or a GH8 xylanase; and

b) dehusking the pretreated pulse.

Thus in another aspect, the invention relates to a method of dehusking a pulse comprising:

a) Pretreating the pulse with an enzyme composition comprising a GH5 endoglucanase; and

b) dehusking the pretreated pulse.

Thus in another aspect, the invention relates to a method of dehusking a pulse comprising:

a) Pretreating the pulse with an enzyme composition comprising a polygalacturonase; and

b) dehusking the pretreated pulse.

Xylans are hemicelluloses found in all land plants (Popper and Tuohy, Plant Physiology, 2010, 153:373-383). They are especially abundant in secondary cell walls and xylem cells.

Plant xylans have a β-1,4-linked xylopyranose backbone that can be substituted at the O2 or O3 position with arabinose, glucuronic acid and acetic acid in a species and tissue specific manner.

The known enzymes responsible for the hydrolysis of the xylan backbone are classified into enzyme families based on sequence similarity (www.cazy.org). The enzymes with mainly *endo*-xylanase activity have previously been described in Glycoside hydrolase family (GH) 5, 8, 10, 11, 30 and 98. The enzymes within a family share some characteristics such as 3D fold and they usually share the same reaction mechanism. Some GH families have narrow or mono-specific substrate specificities while other families have broad substrate specificities. For

a detailed overview of the various families in xylanases, one can refer to the CAZY website (www.cazy.org) and the associated Cazypedia website (www.cazypedia.org).

Of the various families of xylanases, family 8, and 11 are characterized below.

GH 8 xylanases: Family 8 glycoside hydrolases or GH8 xylanases hydrolyse the beta-1,4 xylan chain. They cleave β-1,4 linkages of β-1,4 glucans, xylans (or xylooligosaccharides), chitosans, and lichenans (1,3-1,4-β-D-glucan). The majority of the enzymes are endo-acting enzymes, but one member has an exo-activity that releases β-D-xylose residues from the reducing end of xylooligosaccharides. They belong to the GH-M clan, have an $(alpha/alpha)_6$ 3D structure and are found to catalyse hydrolysis with inversion of the anomeric configuration and generally are believed to be inactive on aryl-β-glycosides of xylose, xylobiose and xylotriose.

Suitable GH8 xylanases include those of plant, archae, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful GH8 xylanases include xylanases from Achormobacter sp, Acidihalobacter sp, Acidithiobacillus sp, Acidiphilium sp., Acidobacterium sp, Actinoalloteichus sp, Actinoalloteichus sp, Aeromonas sp, Agrobacterium sp; Aliivibrio sp; Alteromonas sp, Amphibacillus sp, Amycolatopsis sp; Anaerolinea sp; Anaeromyxobacter sp; Aquifex sp, Asaia sp; Aureimonas sp; Azospirillum sp, Azotobacter sp; Bacillus sp; Bacteroides sp; Bifidobacterium sp; Bordetella sp; Brevibacillus sp; Burkholderia sp; Butyrivibrio sp; Calothrix sp; Candidatus sp; Castellaniella sp; Catenulispora sp; Caulobacter sp; Cedecea sp; Cellulomonas sp; Cellulosilyticum sp; Serratia sp; Chitiniphilus sp; Chlorobium sp; Pelodictyon luteolum sp; Chromobacterium sp; Citrobacter sp; Clostridium sp; Cronobacter sp; Enterobacter sp; Cupriavidus sp; Ralstonia sp; Curtobacterium sp; Cytophaga sp; Defluviimonas sp; Deinococcus sp; Desulfobacca sp; Desulfocapsa sp; Desulfosporosinus sp; Devosia sp; Dickeya sp; Draconibacterium sp; Dyella sp; Edwardsiella sp; Ensifer sp; Enterobacteriacea bacterium; Erwinia sp; Escherichia sp; Ethanoligenens sp; Eubacterium sp; Fibrella sp; Fibrobacter sp; Flammeovirga sp; Flavobacterium sp; Frateuria sp; Friedmanniella sp; Frondihabitans sp; Fulvimarina sp; Gallionella sp; Geodermatophilus sp; Glaciecola sp; Gloeocapsa sp; Gluconobacter sp; Granulicella sp; Hafnia sp; Hahella sp; Halomonas sp; Halotalea sp; Halothiobacillus sp; Herbaspirillum sp; Herbinix sp; Herpetosiphon sp; Janthinobacterium sp; Jeongeupia sp; Kitasatospora sp; Klebsiella sp; Kluyvera sp; Komagataeibacter sp; Gluconacetobacter sp; Kosakonia sp; Kozakia sp; Clostridium sp; Lacimicrobium sp; Lactobacillus sp; Lactococcus sp; Leclercia sp; Legionella sp; Lelliottia sp; Lentzea sp; Leptolyngbya sp; Leptospirillum sp; Leptothrix sp; Leuconostoc sp; Lysobacter sp; Mahella sp; Marinomonas sp; Marmoricola sp; Methylobacterium sp; Methylovorus sp; Microbacteriaceae bacterium; Microcoleus sp; Microvirga sp; Moorea sp; Mucilaginibacter sp; Mycobacterium sp; Rhizobium sp; Nitrospira sp; Novosphingobium sp; Obesumbacterium sp;

Oceanimonas sp; Opitutaceae sp; Opitutus sp; Paenibacillus sp; Paludibacter sp; Pandoraea sp; Pantoea sp; Paraburkholderia sp; Burkholderia sp; Paraglaciecola sp; Pectobacterium sp; Pelagibacterium sp; Photobacterium sp; Plautia sp; Plesiomonas sp; Pluralibacter sp; Polynucleobacter sp; Pontibacter sp; Prevotella sp; Proteus sp; Pseudoalteromonas sp; Pseudomonas sp; Pseudoxanthomonas sp; Rahnella sp; Ralstonia sp; Ramlibacter sp; Raoultella sp; Rhodobacter sp; Rhodovulum sp; Rivularia sp; Roseburia sp; Rufibacter sp; Ruminiclostridium sp, Ruminococcus sp; Salmonella sp; Serratia sp; Shewanella sp; Shigella sp; Sinorhizobium sp; Sodalis sp; Sorangium sp; Sphingobium sp; Sphingomonas sp; Spirosoma sp; Spongiibacter sp; Starkeya sp; Stenotrophomonas sp; Streptococcus sp; Streptomyces sp; Streptosporangium sp; Sulfuricaulis sp; Sulfurihydrogenibium sp; Teredinibacter sp; Terriglobus sp; Thermobacillus; Tistrella sp; Spirochaeta sp; Variibacter sp; Variovorax sp; Verrucomicrobia bacterium; Vibrio sp; Xanthomonas sp; Yangia sp; Yersinia sp; Zymomonas sp;

One preferred GH8 xylanase for use according to the invention is the GH8 xylanase having the sequence of SEQ ID NO: 1.

In one embodiment, the GH8 xylanase is selected among xylanases having an amino acid sequence identity of at least 80% identity, preferably at least 85% identity, preferably at least 90% identity, preferably at least 95% identity, preferably at least 96% identity, preferably at least 97% identity, preferably at least 98% identity, preferably at least 99% identity or 100% identity to the amino acid sequence of SEQ ID NO: 1.

GH11 Xylanases: Family 11 glycoside hydrolases or GH11 xylanases are endo-β-1,4-xylanases. They have the retaining mechanism and they belong to the GH-C clan have a beta-jelly roll 3D structure and generally are known to hydrolyze aryl β-glycosides of xylobiose and xylotriose at the aglyconic bond, but appear to be inactive on aryl cellobiosides.

Suitable GH11 xylanases include those of plant, archae, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful GH11 xylanases include xylanases from Halopiger sp; Halorhabdus so; Actinomadura sp; Actinoplanes sp; Actinosynnema sp; Aeromonas sp; Alteromonadaceae sp; Alteromonas sp; Amphibacillus sp; Amycolatopsis sp; Bacillus sp; Brevibacillus sp; Butyrivibrio sp; Anaerocellum sp; Caldicellulosiruptor sp; Catenulispora sp; Cellulomonas sp; Cellvibrio sp; Clostridium sp; Cohnella sp; Cytophaga sp; Dictyoglomus sp; Fibrobacter sp; Flammeovirga sp; Geobacillus sp; Herbinix sp; Jonesia sp; Kitasatospora sp; Lactococcus sp; Lentzea sp; Lutibacter sp; Microbulbifer sp; Micromonospora sp; Mucilaginibacter sp; Nesterenkonia sp; Niastella sp; Nocardiopsis sp; Opitutus sp; Paenibacillus sp; Paenibacillus sp; Pseudobutyrivibrio sp; Pseudomonas sp; Pedobacter sp; Pseudoxanthomonas sp; Ruminiclostridium sp; Ruminococcus sp; Saccharopha-

gus sp; Sorangium sp; Streptomyces sp; Teredinibacter sp; Thermoanaerobacterium sp; Thermobacillus sp; Thermobifida sp; Thermobispora sp; Thermopolyspora sp; Verrucosispora sp; Xylanimicrobium sp; Xylanimonas sp; Achaetomium sp; Achlya sp; Acremonium sp; Acrophialophora sp; Agaricus sp; Alternaria sp; Annulohypoxylon sp; Arthrobotrys sp; Ascochyta sp, Aspergillus sp; Aureobasidium sp; Bipolaris sp; Bispora sp; Botryotinia sp; Botrytis sp; Chaetomium sp; Chrysosporium sp; Cladosporium sp; Clathrus sp; Claviceps sp; Clonostachys sp; Colletotrichum sp; Coprinopsis sp; Coptotermes sp; Cryptococcus sp; Curvularia sp; Epicoccum sp; Epidinium sp; Fusarium sp; Gaeumannomyces sp; Gloeoporus sp; Gymnopilus sp; Hericium sp; Holomastigotoides sp; Humicola sp; Hypocrea sp; Lactarius sp; Lasiodiplodia sp; Lentinula sp; Lepista sp; Leptosphaeria sp; Leptosphaerulina sp; Leucoagaricus sp; Magnaporthe sp; Melanopsichium sp; Microdochium sp; Morchella sp; Myceliophthora sp; Neocallimastix sp; Neurospora sp; Ophiostoma sp; Orpinomyces sp; Paecilomyces sp; Parastagonospora sp; Penicillium sp; Phaedon sp; Phanerochaete sp; Phialophora sp; Phoma sp; Piromyces sp; Plectosphaerella sp; Pleurotus sp; Podospora sp; Polyplastron sp; Pseudallescheria sp; Pyrenophora sp; Rasamsonia sp; Reticulitermes sp; Rhizopus sp; Russula sp; Saitozyma sp; Scheffersomyces sp; Schizophyllum sp; Sclerotinia sp; Scytalidium sp; Piriformospora sp; Setosphaeria sp; Sporisorium sp; Stereum sp; Talaromyces sp; Thermomyces sp; Thielavia sp; Thraustotheca sp; Trichoderma sp; Ulocladium sp; Ustilago sp; Valsa sp; Verticillium sp.

One preferred GH11 xylanase for use according to the invention is the GH11 xylanase having the sequence of SEQ ID NO: 2.

In one embodiment, the GH11 xylanase is selected among xylanases having an amino acid sequence identity of at least 80% identity, preferably at least 85% identity, preferably at least 90% identity, preferably at least 95% identity, preferably at least 96% identity, preferably at least 97% identity, preferably at least 98% identity, preferably at least 99% identity or 100% sequence identity to the amino acid of SEQ ID NO: 2.

Endoglucanase: The term "endoglucanase" means an endo-1,4-(1,3; 1,4)-beta-D-glucan 4-glucanohydrolase (E.C. 3.2.1.4) that catalyzes endohydrolysis of 1,4-beta-D-glycosidic linkages in cellulose, cellulose derivatives (such as carboxymethyl cellulose and hydroxyethyl cellulose), lichenin, beta-1,4 bonds in mixed beta-1,3 glucans such as cereal beta-D-glucans or xyloglucans, and other plant material containing cellulosic components. Endoglucanase activity can be determined by measuring reduction in substrate viscosity or increase in reducing ends determined by a reducing sugar assay (Zhang et al., 2006, Biotechnology Advances 24: 452-481). Endoglucanase activity may be determined using carboxymethyl cellulose (CMC) as substrate according to the procedure of Ghose, 1987, Pure and Appl. Chem. 59: 257-268,

at pH 5, 40 degrees centigrade Alternatively, the endoglucanase activity can also be determined using the procedure described in Example 8, 13 or 14 of WO13071883A1.

Cellulose is generally found, for example, in the stems, leaves, hulls, husks, and cobs of plants or leaves, branches, and wood of trees. Cellulose is a polymer of the simple sugar glucose covalently linked by beta-1,4- bonds. Many microorganisms produce enzymes that hydrolyze beta-linked glucans. These enzymes include endoglucanases, cellobiohydrolases, and beta-glucosidases. Endoglucanases digest the cellulose polymer at random locations, opening it to attack by cellobiohydrolases. Cellobiohydrolases sequentially release molecules of cellobiose from the ends of the cellulose polymer. Cellobiose is a water-soluble beta-1,4-linked dimer of glucose. Beta-glucosidases hydrolyze cellobiose to glucose.

GH 5 endoglucanases: Family 5 glycoside hydrolases or GH5 endoglucanases cause endohydrolysis of (1→4)-β-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans. They will also hydrolyse 1,4-linkages in β-D-glucans also containing 1,3-linkages They belong to the GH-A clan, have a $(beta/alpha)_8$ 3D structure and are found to catalyse hydrolysis with retaining mechanism.

Suitable GH5 endoglucanases include those of plant, archae, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful GH5 endoglucanases include endoglucanases from Acidilobus sp., Caldivirga sp., Thaumarchaeota sp., Desulfurococcaceae sp., Halorhabdus sp., Halostagnicola sp., Haloterrigena sp., Ignisphaera sp., Picrophilus sp., Pyrococcus., Salinarchaeum sp., Staphylothermus sp., Sulfolobus sp., Thermococcus sp., Thermofilum sp., Thermoplasma sp., Thermoproteus sp., Vulcanisaeta sp., Acetivibrio sp., Acetobacterium sp., Acholeplasma sp., Acidimicrobium sp., Propionibacterium sp., Acidipropionibacterium sp., Acidobacteria sp., Acidothermus sp., Acidovorax sp., Acinetobacter sp., Actinobacteria sp., Actinomyces sp., Actinoalloteichus sp., Actinoplanes sp., Actinopolymorpha sp., Actinosynnema sp., Adlercreutzia sp., Aeromonas sp., Agrobacterium sp., Agromyces sp., Algibacter sp., Alicyclobacillus sp., Alistipes sp., Allokutzneria sp., Alteromonadaceae sp., Alteromonas sp., Aminobacter sp., Amphibacillus sp., Amycolatopsis sp., Anabaena sp., Anaerolinea sp., Anaeromyxobacter sp., Anoxybacillus sp., Arachidicoccus sp., Arcanobacterium sp., Archangium sp., Arthrobacter sp., Asticcacaulis sp., Atopobium sp., Aureimonas sp., Azoarcus sp., Azorhizobium sp., Azospirillum sp., Azotobacter sp., Bacillus sp., Bacteroides sp., Bacteroidetes sp., Bdellovibrio sp., Belliella sp., Belliella sp., Beutenbergia sp., Bifidobacterium sp., Blastomonas sp., Blautia sp., Bosea sp., Brachybacterium sp., Bradyrhizobium sp., Brevundimonas sp., Burkholderia sp., Butyrivibrio sp., Thermoanaerobacter sp., Caldanaerobius sp., Caldibacillus sp., Anaerocellum sp., Caldicellulosiruptor sp.,

Caldithrix sp., Calothrix sp., Candidatus sp., Solibacter sp., Capnocytophaga sp., Catenulispora sp., Caulobacter sp., Cedecea sp., Cellulomonas sp., Cellvibrio sp., Cellulophaga., Cellulosilyticum sp., Cellulosimicrobium., Cellvibrio sp., Chelatococcus sp., Chitinophaga sp., Chloroflexus sp., Chondromyces sp., Chroococcidiopsis sp., Chryseobacterium sp., Chthonomonas sp., Citrobacter sp., Clavibacter sp., Clostridiaceae bacterium, Clostridioides sp., Peptoclostridium sp., Clostridium sp., Collimonas sp., Colwellia sp., Conexibacter sp., Coprococcus sp., Corallococcus sp., Coriobacterium sp., Corynebacterium sp., Cronobacter sp., Cryobacterium sp., Curtobacterium sp., Propionibacterium sp., Cyanobacterium sp., Cyanothece sp., Cyclobacterium sp., Cytophaga sp., Defluviitoga sp., Deinococcus sp., Dermabacter sp., Desulfitobacterium sp., Desulfobulbus sp., Desulfurivibrio sp., Dickey asp., Dictyoglomus sp., Dokdonia sp., Krokinobacter sp., Draconibacterium sp., Dyadobacter sp., Dyella sp., Echinicola sp., Elizabethkingia sp., Ensifer sp., Enterobacter sp., Enterobacteriaceae bacterium., Erwinia sp., Escherichia sp., Eubacterium sp., Exiguobacterium sp., Faecalibaculum sp., Fermentimonas sp., Fervidobacterium sp., Fibrella sp., Fibrobacter sp., Filimonas sp., Fimbriimonas sp., Fischerella sp., Flammeovirga sp., Flammeovirgaceae bacterium., Flavisolibacter sp., Flavobacteriaceae bacterium., Flavobacterium sp., Fluviicola sp., Formosa sp., Frankia sp., Friedmanniella sp., Frondihabitans sp., gamma proteobacterium., Geminocystis sp., Gemmatimonadetes bacterium., Geobacillus sp., Gillisia sp., Geobacter sp., Glaciecola sp., Gloeocapsa sp., Gluconacetobacter., Gramella sp., Granulicella sp., Acidobacterium sp., Gynuella sp., Hafnia sp., Hahella sp., Haliangium sp., Haliscomenobacter sp., Halothiobacillus sp., Herbinix sp., Herpetosiphon sp., Hirschia sp., Hymenobacter sp., Hyphomicrobium sp., Hyphomonadaceae bacterium., Ignavibacterium sp., Isoptericola sp., Isosphaera sp., Janthinobacterium sp., Jiangella sp., Jonesia sp., Kibdelosporangium sp., Kineococcus sp., Kitasatospora sp., Klebsiella sp., Gluconacetobacter sp., Komagataeibacter sp., Kosmotoga sp., Kozakia sp., Kribbella sp., Kutzneria sp., Labilithrix sp., Labrenzia sp., Lacinutrix sp., Lactobacillus sp., Lactococcus sp., Leadbetterella sp., Legionella sp., Leifsonia sp., Lelliottia sp., Lentzea sp., Leptolyngbya sp., Leptospira sp., Leptothrix sp., Leuconostoc sp., Limnochorda sp., Luteipulveratus sp., Lutibacter sp., Lysinibacillus sp., Lysobacter sp., Magnetospirillum sp., Mahella sp., Maribacter sp., Flavobacteriales bacterium., Marmoricola sp., Martelella sp., Meliioribacter sp., Melissococcus sp., Mesorhizobium sp., Methylobacillus sp., Methylobacterium sp., Methyloceanibacter sp., Methylomonas sp., Methylotenera sp., Microbacterium sp., Microbulbifer sp., Microcoleus sp., Microlunatus sp., Micromonospora sp., Microterricola sp., Mucilaginibacter sp., Mitsuaria sp., Mycobacterium sp., Myxococcus sp., Rhizobium sp., Niabella sp., Niastella sp., Nitrosospira sp., Nocardia sp., Nocardioides sp., Nocardiopsis sp., Nonlabens sp., Nostoc sp., Novosphingobium sp., Olsenella sp., Opitutaceae bacterium., Opitutus sp., Oscillatoria sp., Paenibacillaceae bacterium., Paenibacillus., Paludibacter sp., Pannonibacter sp., Pantoea sp., Burkholderia sp., Paraburkholderia sp., Paraoerskovia sp., Parascardovia

sp., Parvibaculum sp., Pectobacterium sp., Pedobacter sp., Pelosinus sp., Petrimonas sp., Photobacterium sp., Phycisphaera sp., Pirellula sp., Planctomyces sp., Pleurocapsa sp., Polaribacter sp., Polaromonas sp., Polyangium sp., Polymorphum sp., Pontibacter sp., Prevotella sp., Propionibacterium sp., Arthrobacter sp., Pseudoalteromonas sp., Pseudomonas sp., Pseudonocardia sp., Thermotoga sp., Pseudoxanthomonas sp., Psychroflexus sp., Psychromonas sp., psychrophilic marine bacterium DY3., Rahnella sp., Ralstonia sp., Ramlibacter sp., Raoultella sp., Rathayibacter sp., Rhizobium sp., Rhodococcus sp., Rhodoferax sp., Rhodopirellula sp., Rhodopseudomonas sp., Rhodothermus sp., Rivularia sp., Roseateles sp., Roseburia sp., Roseiflexus sp., Planctomyces sp., Rufibacter sp., Ruminiclostridium sp., Clostridium sp., Ruminococcus sp., Runella sp., Saccharophagus sp., Saccharopolyspora sp., Saccharothrix sp., Salinispora sp., Sandaracinus sp., Sanguibacter sp., Scardovia sp., Sediminicola sp., Serratia sp., Shewanella sp., Shinella sp., Siansivirga sp., Simiduia sp., Singulisphaera sp., Sinorhizobium sp., Solitalea sp., Sorangium sp., Sphingobacterium sp., Sphingobium sp., Sphingomonas sp., Sphingopyxis sp., Spirochaeta sp., Spirosoma sp., Stackebrandtia sp., Stanieria sp., Staphylococcus sp., Stappia sp., Starkeya sp., Stenotrophomonas sp., Stigmatella sp., Streptococcus sp., Streptomyces sp., Streptosporangium sp., Sulfuricaulis sp., Synechococcus sp., Syntrophobotulus sp., Syntrophomonas sp., Syntrophothermus sp., Tannerella sp., Tatlockia sp., Tenacibaculum sp., Teredinibacter sp., Terriglobus sp., Thauera sp., Thermacetogenium sp., Thermaerobacter sp., Thermanaerovibrio sp., Thermoactinospora sp., Thermoanaerobacter sp., Thermobaculum sp., Thermobifida sp., Thermobispora sp., Thermomonospora sp., Thermotoga sp., Thermus sp., Thiomonas sp., Treponema sp., Truepera sp., Tsukamurella sp., Turneriella sp., Variovorax sp., Verminephrobacter sp., Verrucomicrobia sp., Verrucosispora sp., Vibrio sp., Weissella sp., Wenyingzhuangia sp., Wolbachia endosymbiont., Xanthomonas sp., Xylanimonas sp., Xylella sp., Yersinia sp., Zhongshania sp., Zobellia sp., Zunongwangia sp., Achaetomium sp., Achlya sp., Acremonium sp., Actinidia sp., Adineta sp., Agaricus sp., Albugo sp., Alternaria sp., Amanita sp., Anoplophora sp., Aphelenchoides sp., Aphelenchus sp., Aplysia sp., Apriona sp., Arabidopsis sp., Armillariella sp., Arthrobotrys sp., Aspergillus sp., Aureobasidium sp., Auxenochlorella sp., Bathycoccus sp., Biomphalaria sp., Bipolaris sp., Bispora sp., Blumeria sp., Botrytis sp., Botryotinia sp., Callosobruchus sp., Candida sp., Chaetomium sp., Cherax sp., Chlorella sp., Ciboria sp., Cladosporium sp., Clonostachys sp., Coccidioides sp., Coffea sp., Colletotrichum sp., Coprinopsis sp., Coptotermes sp., Cryptococcus sp., Cryptopygus sp., Cucumis sp., Cyanea sp., Cyberlindnera sp., Cylindrocarpon sp., Cystoderma sp., Datura sp., Daucus sp., Debaryomyces sp., Ditylenchus sp., Ectocarpus sp., Epichloe sp., Epicoccum sp., Epidinium sp., Eremothecium sp., Ashbya sp., Eucalyptus sp., Eutrema sp., Flammulina sp., Fusarium sp., Ganoderma sp., Gastrophysa sp., Glaciozyma sp., Globodera sp., Gloeophyllum sp., Gloeoporus sp., Glycine sp., Gonapodya sp., Grifola sp., Gymnopilus sp., Haliotis sp., Hericium sp., Heterobasidion sp., Heterodera sp.,

Hirschmanniella sp., Hirsutella sp., Hordeum sp., Humicola sp., Hydra sp., Hypholoma sp., Hypocrea sp., Hypocrella sp., Hypothenemus sp., Inonotus sp., Irpex sp., Isaria sp., Kazachstania sp., Kluyveromyces sp., Koerneria sp., Komagataella sp., Pichia so., Lacazia sp., Laccaria sp., Lachancea sp., Lactuca sp., Lagenidium sp., Lecanicillium sp., Lentinula sp., Lepidium sp., Leptosphaeria sp., Leucoagaricus sp., Lichtheimia sp., Limacella sp., Limnoria sp., Lotus sp., Macrophomina sp., Magnaporthe sp., Malassezia sp., Malus sp., Marasmius sp., Medicago sp., Melanopsichium sp., Meloidogyne sp., Mesosa sp., Metarhizium sp., Metopus sp., Meyerozyma sp., Microdochium sp., Micromonas sp., Millerozyma sp., Morus sp., Musa sp., Mytilus sp., Naumovozyma sp., Nectria sp., Neocallimastix sp., Neotyphodium sp., Nephotettix sp., Neurospora sp., Oncideres sp., Orpinomyces sp., Oryza sp., Ostreococcus sp., Paecilomyces sp., Paracoccidioides sp., Parastagonospora sp., Penicillium sp., Pestalotiopsis sp., Phakopsora sp., Phanerochaete sp., Phaseolus sp., Phialophora sp., Phoma sp., Phytophthora sp., Picea sp., Pichia sp., Pinus sp., Piromyces sp., Plasmopara sp., Pluteus sp., Podospora sp., Polyplastron sp., Polyporus sp., Populus sp., Postia sp., Pratylenchus sp., Pristionchus sp., Prunus sp., Psacothea sp., Puccinia sp., Purpureocillium sp., Pyrenophora sp., Pythium sp., Radopholus sp., Rasamsonia sp., Rhizoctonia sp., Rhizomucor sp., Rhizopus sp., Rhodosporidium sp., Robillarda sp., Rotylenchulus sp., Saccharomyces sp., Saccharomycetaceae sp., Saitozyma sp., Schistosoma sp., Schizophyllum sp., Schizosaccharomyces sp., Schwanniomyces sp., Sclerotinia sp., Piriformospora sp., Serpula sp., Sisymbrium sp., Solanum sp., Spirotrichonympha sp., Sporisorium sp., Stereum sp., Sugiyamaella sp., Taiwanofungus sp., Talaromyces sp., Taraxacum sp., Tetrapisispora sp., Thalassiosira sp., Thermoascus sp., Myceliophthora sp., Thermothelomyces sp., Thielavia sp., Thraustotheca sp., Torulaspora sp., Trametes sp., Trichoderma sp., Trifolium sp., Triticum sp., Ulocladium sp., Ustilago sp., Vaccinium sp., Vaucheria sp., Verticillium sp., Vigna sp., Vitis sp., Volvariella sp., Wickerhamomyces sp., Williopsis sp., Xanthophyllomyces sp., Yarrowia sp., Zea sp., Zygosaccharomyces sp.,

One preferred GH5 endoglucanase for use according to the invention is the GH5 endoglucanase having the sequence of SEQ ID NO: 4.

In one embodiment, the GH5 endoglucanase is selected among endoglucanases having an amino acid sequence identity of at least 80% identity, preferably at least 85% identity, preferably at least 90% identity, preferably at least 95% identity, preferably at least 96% identity, preferably at least 97% identity, preferably at least 98% identity, preferably at least 99% identity or 100% sequence identity to SEQ ID NO: 4

Pectin is a family of complex polysaccharides that contain 1,4-linked a-D- galactosyluronic acid residues. Three pectic polysaccharides have been isolated from plant primary cell walls and structurally characterized. These are:

- Homogalacturonans
- Substituted galacturonans
- Rhamnogalacturonans

Homogalacturonans are linear chains of a-(1-4)-linked D-galacturonic acid. Substituted galacturonans are characterized by the presence of saccharide appendant residues (such as D-xylose or D-apiose in the respective cases of xylogalacturonan and apiogalacturonan) branching from a backbone of D-galacturonic acid residues. Rhamnogalacturonan I pectins (RG-I) contain a backbone of the repeating disaccharide: 4)-a-D-galacturonic acid-(1,2)-a-L- rhamnose-(1. From many of the rhamnose residues, side chains of various neutral sugars branch off. The neutral sugars are mainly D-galactose, L-arabinose and D-xylose, the types and proportions of neutral sugars varying with the origin of pectin.

Another structural type of pectin is rhamnogalacturonan II (RG-I I), which is a less frequent complex, highly branched polysaccharide. Rhamnogalacturonan II is classified by some authors within the group of substituted galacturonans since the rhamnogalacturonan II backbone is made exclusively of D-galacturonic acid units.

Polygalacturonase (EC 3.2.1.15) Polygalacturonases are pectinases that catalyze random hydrolysis of (1 ,4)-alpha-D- galactosiduronic linkages in pectate and other galacturonans. They are also known as pectin depolymerase. Polygalacturonase hydrolyses the alpha-1 ,4-glycosidic bonds in polygalacturonic acid with the resultant release of galacturonic acid. This reducing sugar reacted then with 3,5- dinitrosalicylic acid (DNS).

Suitable poygalacturonases include those of plant, archae, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful polygalacturonases include polygalacturonases from Acetobacter sp., Achaetomium sp., Achlya sp., Acholeplasma sp., Acidobacterium sp., Acorus sp., Actinidia sp., Actinoplanes sp., Acutalibacter sp., Adineta sp., Agrobacterium sp., Albugo sp., Algibacter sp., Alicyclobacillus sp., Alisma sp., Alistipes sp., Alkaliphilus sp., Alkalitalea sp., Alternaria sp., Alteromonas sp., Amborella sp., Amycolatopsis sp., Apolygus sp., Apriona sp., Aquilegia sp., Arabidopsis sp., Arachidicoccus sp., Arenibacter sp., Aretaon sp., Aristolochia sp., Artemisia sp., Arthrobacter sp., Arthrobotrys sp., Asparagus sp., Aspergillus sp., Asticcacaulis sp., Athelia sp., Aulosira sp., Ba-

cillus sp., Bacteroides sp., Basilea sp., Bathycoccus sp., Belliella sp., Bifidobacterium sp., Bipolaris sp., Bispora sp., Blastomonas sp., blood sp., Botryotinia sp., Botrytis sp., Brachybacterium sp., Bradyrhizobium sp., Brassica sp., Brenneria sp., Brevundimonas sp., Burkholderia sp., Butyrivibrio sp., Buxus sp., Cabomba sp., Caldicellulosiruptor sp., Caldivirga sp., Callosobruchus sp., Candidatus sp., Canna sp., Capnocytophaga sp., Capsella sp., Capsicum sp., Carica sp., Catenulispora sp., Caulobacter sp., Cedecea sp., Celeribacter sp., Cellulophaga sp., Cellulosilyticum sp., Cellvibrio sp., Ceratophyllum sp., Chamaecyparis sp., Chania sp., Chimonanthus sp., Chitinophaga sp., Chloranthus sp., Chondrostereum sp., Chryseobacterium sp., Chrysomela sp., Chrysoporthe sp., Chthonomonas sp., Ciboria sp., Cinnamomum sp., Citrobacter sp., Citrus sp., Clavibacter sp., Claviceps sp., Clostridium sp., Coffea sp., Colletotrichum sp., Collimonas sp., Collinsella sp., Coprotinia sp., Coraliomargarita sp., Cordyceps sp., Coriobacterium sp., Cryphonectria sp., Cryptococcus sp., Cryptomeria sp., Cucumis sp., Cucurbita sp., Curtobacterium sp., Cynodon sp., Cytophagales sp., Daucus sp., Defluviimonas sp., Dendroctonus sp., Diabrotica sp., Diaporthe sp., Dickeya sp., Dictyoglomus sp., Dioscorea sp., Diospyros sp., Draconibacterium sp., Dumontinia sp., Dyadobacter sp., Echinicola sp., Elaeis sp., Emticicia sp., Ensifer sp., Enterobacter sp., Enterobacteriaceae sp., Enterococcus sp., Eremothecium sp., Erwinia sp., Erythranthe sp., Erythrobacter sp., Eucalyptus sp., Extatosoma sp., Faecalibacterium sp., Fibrella sp., Ficus sp., Filimonas sp., Fimbriimonas sp., Flammeovirga sp., Flammeovirgaceae sp., Flavisolibacter sp., Flavobacteriaceae sp., Flavobacterium sp., Formosa sp., Fragaria sp., Frateuria sp., Fremyella sp., Fusarium sp., Galactomyces sp., Gastrophysa sp., Gemmatirosa sp., Geotrichum sp., Gibbsiella sp., Gilliamella sp., Ginkgo sp., Gluconobacter sp., Glycine sp., Gossypium sp., Gramella sp., Granulicella sp., Gynuella sp., Hafnia sp., Hahella sp., Haliscomenobacter sp., Haloferax sp., Halopiger sp., Halostagnicola sp., Haloterrigena sp., Helianthus sp., Herbaspirillum sp., Hesperocyparis sp., Heterobasidion sp., Hirschia sp., Hordeum sp., Houttuynia sp., Hymenobacter sp., Hypericum sp., Hypocrella sp., Illicium sp., Ipomoea sp., Iris sp., Irpex sp., Isoptericola sp., Janthinobacterium sp., Jatropha sp., Juniperus sp., Kali sp., Kibdelosporangium sp., Kitasatospora sp., Klebsiella sp., Kluyvera sp., Kluyveromyces sp., Komagataeibacter sp., Kosakonia sp., Kribbella sp., Kutzneria sp., Lachnoclostridium sp., Lacimicrobium sp., Lactobacillus sp., Lactococcus sp., Lactuca sp., Lacunisphaera sp., Leadbetterella sp., Legionella sp., Leifsonia sp., Lelliottia sp., Leptinotarsa sp., Leptosphaeria sp., Leucoagaricus sp., Lichtheimia sp., Lilium sp., Litchi sp., Lonsdalea sp., Lotus sp., Lupinus sp., Lygus sp., Lysinibacillus sp., Magnaporthe sp., Magnolia sp., Malus sp., Mangifera sp., Manihot sp., Manilkara sp., Maribacter sp., Marinifilaceae sp., Martelella sp., Massilia sp., Medauroidea sp., Medicago sp., Melanopsichium sp., Melioribacter sp., Melissococcus sp., Meloidogyne sp., Metasequoia sp., Methylobacterium sp., Microbulbifer sp., Micromonospora sp., Microterricola

sp., Monilinia sp., Mucilaginibacter sp., Muribaculum sp., Musa sp., Myriosclerotinia sp., Neorhizobium sp., Neurospora sp., Niabella sp., Niastella sp., Nicotiana sp., Nonlabens sp., Nonomuraea sp., Novibacillus sp., Oceanobacillus sp., Oenothera sp., Olea sp., Oncidium sp., Ophiostoma sp., Opitutaceae sp., Opitutus sp., Opuntia sp., Orobanche sp., Oryza sp., Paenibacillus sp., Pandanus sp., Panicum sp., Pannonibacter sp., Pantoea sp., Parabacteroides sp., Paraburkholderia sp., Paspalum sp., Pectobacterium sp., Pediococcus sp., Pedobacter sp., Pelagibacterium sp., Pelosinus sp., Penicillium sp., Persea sp., Peruphasma sp., Petrimonas sp., Phaedon sp., Phaeodactylum sp., Phaseolus sp., Phenylobacterium sp., Phleum sp., Phomopsis sp., Phycisphaerae sp., Phytophthora sp., Picea sp., Pinellia sp., Pinus sp., Pisum sp., Plantactinospora sp., Platanus sp., Pluralibacter sp., Polaribacter sp., Pontibacter sp., Populus sp., Prevotella sp., Proteiniphilum sp., Prunus sp., Pseudarthrobacter sp., Pseudoalteromonas sp., Pseudodesulfovibrio sp., Pseudomonas sp., Pseudopedobacter sp., Pseudothermotoga sp., Pseudovibrio sp., Psidium sp., Pyrus sp., Ralstonia sp., Ramulus sp., Raoultella sp., Rathayibacter sp., Rhizobium sp., Rhizoctonia sp., Rhizopus sp., Rhodothermus sp., Rhodotorula sp., Rhynchophorus sp., Ricinus sp., Roseburia sp., Roseomonas sp., Rubus sp., Rufibacter sp., Ruminiclostridium sp., Ruminococcus sp., Runella sp., Saccharomyces sp., Saccharomycetaceae sp., Saccharophagus sp., Saccharum sp., Sagittula sp., Salix sp., Salmonella sp., Sarcandra sp., Schrenkiella sp., Sclerotinia sp., Sclerotium sp., Scytonema sp., Sebaldella sp., Secale sp., Selenomonas sp., Serendipita sp., Serratia sp., Sesamum sp., Setaria sp., Silene sp., Simiduia sp., Sinorhizobium sp., Sipyloidea sp., Sitophilus sp., Sodalis sp., Solanum sp., Solibacillus sp., Sorangium sp., Sorghum sp., Sphaerochaeta sp., Sphenophorus sp., Sphingobacteriaceae sp., Sphingobacterium sp., Sphingobium sp., Sphingomonas sp., Spirochaeta sp., Spirosoma sp., Sporisorium sp., Streptomyces sp., Sugiyamaella sp., Talaromyces sp., Tannerella sp., Tatlockia sp., Tatumella sp., Taxodium sp., Teredinibacter sp., Terriglobus sp., Thanatephorus sp., Theobroma sp., Thermoanaerobacter sp., Thermoanaerobacterium sp., Thermogutta sp., Thermothelomyces sp., Thermotoga sp., Thielavia sp., Thiobacimonas sp., Thraustotheca sp., Tolypothrix sp., Treponema sp., Trichoderma sp., Trichosanthes sp., Triticum sp., Turnera sp., uncultured sp., unidentified sp., Ustilago sp., Venturia sp., Verrucomicrobia sp., Verrucomicrobiaceae sp., Verticillium sp., Vigna sp., Vitis sp., Wenyingzhuangia sp., Xanthomonas sp., Xanthophyllomyces sp., Xylanimonas sp., Xylella sp., Yangia sp., Yersinia sp., Yucca sp., Zea sp., Zinnia sp., Zobellia sp., Zunongwangia sp., Zymoseptoria sp.,

One preferred polygalacturonase for use according to the invention is the polygalacturonase having the sequence of SEQ ID NO: 5.

In one embodiment, the polygalacturonase is selected among polygalacturonases having an amino acid sequence identity of at least 80% identity, preferably at least 85% identity, preferably at least 90% identity, preferably at least 95% identity, preferably at least 96% identity, preferably at least 97% identity, preferably at least 98% identity, preferably at least 99% identity or 100% sequence identity to SEQ ID NO: 5.

In one embodiment, the pulse is a whole unhusked pulse. In the context of the present invention, an unhusked pulse is a pulse which has not been dehusked.

In another embodiment, the pulse is a split unhusked pulse.

A whole unhusked pulse is a pulse that is an unsplit grain covered with a husk.

A dehusked whole pulse is a pulse that is an unsplit grain and not covered with the husk.

A split unhusked pulse is a pulse that is split grain and covered with a husk.

The pulse for use in the invention can be of any quality - for example, it can be uncleaned or it can be variously cleaned and/or sorted and/or graded.

The pretreatment with the enzyme can be done any time prior to the dehusking step.

The pretreatment involves contacting the enzyme composition with the whole unhusked pulse or the split unhusked pulse.

In one embodiment, the pretreatment with the enzyme is combined with the soaking step during the wet milling of the pulse.

In another embodiment, the pretreatment is done separately prior to the soaking step.

In another embodiment, the pretreatment is done immediately after the cleaning step.

In one embodiment, the pretreatment involves mixing the enzyme with the water used for soaking of the whole unhusked pulse.

In yet another embodiment, the pretreatment with the enzyme is done after the pitting step.

In one embodiment, the pretreatment with the enzyme happens during the steeping step.

In another embodiment, the pretreatment with the enzyme happens after the steeping step.

In another embodiment, the pretreatment involves appropriate moisture adjustment required for enzymatic hydrolysis of pulses followed by addition of enzymes.

In one embodiment, the pulse is Cicer arietinum variously called chickpea, garbanzo or bengal gram.

The chickpea can be of any type or variety namely Desi or Kabuli or others.

In one embodiment, the pulse, before the method of invention, is a pulse chosen from pulse(s) that are traditionally dehusked using the wet method.

In one embodiment, the pulse is Pisum sativum, variously called pea or peas or garden peas or green peas or protein peas.

In one embodiment, the pulse is Lens culinaris, variously called lens esculenta or lentils, adas mercimek, messer, masser or massur and heramame.

The reaction conditions such as concentration of enzymes to be used, pH, temperature and reaction time may in principle be determined using techniques known in the field for optimizing enzymatic reactions and is completely within the skills of the average practitioner.

The concentration of enzymes for the pretreatment is typically in the range of 1 to 10,000 milligram (mg), more preferred in the range of 10 to 1000 mg and most preferred around 40 to 200 mg per kg of pulses for xylanases.

The concentration of enzymes for the pretreatment is typically in the range of 1 to10,000 mg, more preferred in the range of 10 to1000 and most preferred around 40 to 500 mg per kg of pulses for endoglucanases.

The concentration of enzymes for the pretreatment is typically in the range of 1 to 10000 milligram (mg), more preferred in the range of 10 to1000mg and most preferred around 40 to 500 mg per kg of pulses for polygalacturonases.

The activity of enzyme used for pretreatment is typically in the range of 100 to 500 FXU-W/KG pulses or 9 to 47 NXU/Kg pulses for xylanases.

The activity of enzyme used is typically 200- 600 FBG /KG pulses for endoglucanases.

The activity of enzyme used for pretreatment is typically in the range of 50 to 3000 PGNU/KG pulses.

The pH in the reaction mixture should be selected in accordance with the pH preferences of the selected enzyme and is typically in the range of 3.0 to 9.0, preferably in the range of 4.0 to 8.0, more preferred in the range of 5.0 to 7.5, measured at 25°C.

In one embodiment, the pH is regulated before the reaction is started whereas in other embodiments the pH is not regulated meaning that the pH in the reaction mixture is determined by the pH of the pulse substrate or water used.

The pretreatment temperature should be selected according to the temperature preferences and temperature stability of the selected enzyme. In general, a higher temperature is preferred to increase the reaction rate however, a higher temperature also provides for a higher inactivation rate for the enzyme, so the skilled person should select the temperature with due consideration of these factors.

The pretreatment temperature is typically in the range of 10°C to 80°C, preferably in the range of 20°C to 60°C, more preferred in the range of 25°C to 50°C.

The pretreatment should continue for a sufficient time to achieve desired degree of pretreatment in order to maximize yield and or other characteristics. The reaction time is typically below 24 hours such as in the range of 5 minutes to 24 hours, preferably in the range of 2 hours to 16 hours, preferably in the range of 4 hours to 8 hours.

The GH11 or GH8 enzyme(s) may be added as pure enzyme, as an aqueous solution thereof or as an enzyme composition that comprises the one or more enzyme(s) that work in enzymatic dehusking. It is preferred to use enzyme compositions.

The GH5 endoglucanase enzyme(s) may be added as pure enzyme, as an aqueous solution thereof or as an enzyme composition that comprises the one or more enzyme(s) that work in enzymatic dehusking. It is preferred to use enzyme compositions.

The polygalacturonase enzyme(s) may be added as pure enzyme, as an aqueous solution thereof or as an enzyme composition that comprises the one or more enzyme(s) that work in enzymatic dehusking. It is preferred to use enzyme compositions.

The enzyme(s) may be added as pure enzyme, as an aqueous solution thereof or as an enzyme composition that comprises the one or more enzyme(s) that work in enzymatic dehusking. It is preferred to use enzyme compositions.

An enzyme composition is the typical product wherein commercial enzymes are supplied and may in addition to the active enzyme comprise further enzymes, solvents, diluents, stabilizers, fillers, coloring agents etc.

The compositions may further comprise multiple enzymatic activities, such as one or more (e.g., several) enzymes selected from the group consisting of pectinases, phytase, xylanase,

galactanase, alpha-galactosidase, protease, phospholipase A1, phospholipase A2, lysophospholipase, phospholipase C, phospholipase D, amylase, lysozyme, arabinofuranosidase, beta-xylosidase, acetyl xylan esterase, feruloyl esterase, cellulase, cellobiohydrolases, beta-glucosidase, pullulanase, and beta-glucanase or any combination thereof.

In one embodiment, the enzyme composition further contains a pectinase.

In another embodiment, the enzyme composition further contains a glucanase.

In another embodiment, the enzyme composition further contains a xylanase.

In another embodiment, the enzyme composition further contains a polygalacturonase.

In another embodiment, the enzyme composition further contains a GH8 or GH11 xylanase.

In another embodiment, the enzyme composition further contains a GH5 endoglucanase.

The invention is now further described in examples which are provided for illustrative purposes and should not be considered limiting in any ways.

PREFERRED EMBODIMENTS

The invention can also be described as the following preferred embodiments:

1 A method for dehusking a pulse comprising:

a. pretreating the pulse with an enzyme composition comprising a GH11 or GH8 xylanase; and
b. dehusking the pretreated pulse.

2 A method for dehusking a pulse comprising:

a. pretreating the pulse with an enzyme composition comprising a GH5 endoglucanase; and
b. dehusking the pretreated pulse.

3 A method for dehusking a pulse comprising:

a. pretreating the pulse with an enzyme composition comprising a polygalacturonase; and
b. dehusking the pretreated pulse.

4 The method of items 1 to 3, wherein the pulse is chick pea.

5 The method of items 1 to 3, wherein the pulse is whole unhusked pulse.

6 The method of item 1, wherein the xylanase is selected among xylanases having an amino acid sequence identity of at least 80% identity, preferably at least 85% identity, preferably at least 90% identity, preferably at least 95% identity, preferably at least 96% identity, preferably at least 97% identity, preferably at least 98% identity, preferably at least 99% identity or 100% identity to SEQ ID NO: 1

7 The method of item 1, wherein the xylanase is selected among xylanases having an amino acid sequence identity of at least 80% identity, preferably at least 85% identity, preferably at least 90% identity, preferably at least 95% identity, preferably at least 96% identity, preferably at least 97% identity, preferably at least 98% identity, preferably at least 99% identity or 100% identity to SEQ ID NO: 2

8 The method of item 2, wherein the endoglucanase is selected among endoglucanases having an amino acid sequence identity of at least 80% identity, preferably at least 85% identity, preferably at least 90% identity, preferably at least 95% identity, preferably at least 96% identity, preferably at least 97% identity, preferably at least 98% identity, preferably at least 99% identity or 100% sequence identity to SEQ ID NO: 4.

9 The method of item 3, wherein the polygalacturonase is selected among polygalacturonase having an amino acid sequence identity of at least 80% identity, preferably at least 85% identity, preferably at least 90% identity, preferably at least 95% identity, preferably at least 96% identity, preferably at least 97% identity, preferably at least 98% identity, preferably at least 99% identity or 100% sequence identity to SEQ ID NO: 5.

10 The method of any of the items 1 to 9, wherein the pretreatment is carried out for a period between 5 minutes and 24 hours, preferably in the range of 2 hours to 16 hours, preferably in the range of 4 hours to 8 hours.

11 The method of any of the items 1 to 10, wherein the pretreatment is done at a temperature in the range of 10°C-80°C, preferably in the range of 20°C-60°C most preferred in the range of 25°C-50°C.

12 The method of any of the items 1 to 11, wherein enzyme composition further comprises a pectinase.

13 The method of any of the items 1 to 12, wherein enzyme composition further comprises a glucanase.

14 The method of any of the items 1 to 13, wherein pretreatment is done in the presence of water.

15 The method of any of the items 1 to 14wherein the enzyme composition further comprises one or more additional enzymes.

16 A completely or partially dehusked pulse prepared using the method according to any of the items 1 to 15.

17 An enzyme composition comprising a GH8 or GH11 xylanase for dehusking of pulse.

18 An enzyme composition comprising a GH5 endoglucanase for dehusking of pulse.

19 An enzyme composition comprising a polygalacturonase for dehusking of pulse.

20 The enzyme composition according to item 17 wherein, the xylanase is a xylanase having at least 80% identity, preferably at least 85% identity, preferably at least 90% identity, preferably at least 95% identity, preferably at least 96% identity, preferably at least 97% identity, preferably at least 98% identity, preferably at least 99% identity or 100% identity to SEQ ID NO: 1 or 2

21 The enzyme composition according to item 18 wherein, the endoglucanase is an endoglucanase having an aminoacid sequence having at least 80% identity, preferably at least 85% identity, preferably at least 90% identity, preferably at least 95% identity, preferably at least 96% identity, preferably at least 97% identity, preferably at least 98% identity, preferably at least 99% identity or 100% identity to SEQ ID NO: 4

22 The enzyme composition according to item 19 wherein, the polygalacturonase is a polygalacturonase having an aminoacid sequence having at least 80% identity, preferably at least 85% identity, preferably at least 90% identity, preferably at least 95% identity, preferably at least 96% identity, preferably at least 97% identity, preferably at least 98% identity, preferably at least 99% identity or 100% identity to SEQ ID NO: 5

23 An enzyme composition comprising a GH8 or GH11 xylanase and a GH 5 endoglucanase for dehusking of pulse.

24 An enzyme composition comprising a GH8 or GH11 xylanase and a polygalacturonase for dehusking of pulse.

25 An enzyme composition comprising a GH5 endoglucanase and a polygalacturonase for dehusking of pulse.

26 The enzyme composition according to items 17 to 25 further comprising one or more additional enzymes.

EXAMPLES

Materials and Methods

Materials:

Chick pea available locally as a commodity and bought from local market in Karnataka India was used for all experiments.

Water used was ground water or water supplied by the municipal corporation and the pH was found to be between 6.5 to 8.0.

Enzymes used:

GH8 xylanase of SEQ ID no 1 was prepared as described in WO2011070101-A1.

GH11 xylanase of SEQ ID no 2 was prepared as described in WO2005059084-A1.

GH10 xylanase of SEQ ID no 3 was prepared as described in WO2009074650-A2.

GH5 endoglucanase of SEQ ID no 4 was prepared as described in WO2012122518-A1.

Polygalacturonase of SEQ ID no 5 was prepared using standard recombinant methods as described in for example in WO2011070101-A1.

Pectinesterase of SEQ ID no 6 was prepared as described in WO2011103812-A1.

Pectin lyase of SEQ ID no 7 was prepared as described in EP353188-A.

Methods for analysis

Assay for enzyme activity:

Konelab analyser (Thermo Fisher Scientific) was used for the enzyme assay.

Xylanase activity was assayed using wheat arabinoxylan as substrate at pH 6.0 and 50 degrees C. The reaction is stopped by an alkaline reagent containing PAHBAH and bismuth which forms complexes with reducing sugar, producing color detected at 405 nm. The produced color is proportional to the xylanase activity. One FXU-W is defined as the endoxylanase activity using Bio-feed Wheat (Novozymes A/S) as a standard. In other words, The FXU-W endoxylanase activity is determined relative to Bio-feed Wheat (Novozymes A/S) as a standard. One NXU is defined as the endoxylanase activity using Panzea (Novozymes A/S) as a standard. In other words, The NXU endoxylanase activity is determined relative to Panzea (Novozymes A/S) as a standard.

Betaglucanase activity was assayed using Betaglucan as substrate at 50 degrees C and pH 5. This reaction is stopped by an alkaline reagent including PAHBAH and Bi3+, which complexes with reducing sugar, producing color that is detected at 405 nm. The color is proportional to the betaglucanase activity. One FBG unit is the amount of enzyme that produces reducing carbohydrate equivalent to 1 µmol of glucose per minute at 50 degrees C and pH 5. The activity is determined relative to Energex (Novozymes A/S) as enzyme standard.

Polygalacturonase hydrolyses the alpha-1 ,4-glycosidic bonds in polygalacturonic acid with the resultant release of reducing carbohydrate. This reaction is stopped by an alkaline reagent including p-hydroxybenzoic acid hydrazide (PAHBAH) and Bi3+, which complexes with reducing sugar, producing colour, detected at 405 nm. The produced colour is proportional to the polygalacturonase activity. One PGNU(PL) is defined as the amount of enzyme which produces reducing carbohydrates equivalent to 1 mg of galacturonic acid sodium salt under standard conditions (50 degrees C and 4.5 pH).

Pectin Lyase activity is assayed by an enzymatic reaction that consists of splitting alpha 1-4 galacturonosidyl bond producing unsaturated delta 4,5 uronide. The double bond with carbonyl function in C6 has an absorption in UV Optical density at 235 nm assays the pectin lyase activity. One Pectin lyase unit (PELU) is the quantity of enzyme that catalyses the split of bound endo alpha 1-4 galacturonosidyl (C6 methyl ester) forming one micromole of delta 4,5 unsaturated product in one minute, at conditions of 45°C and pH 5.5.

Pectin esterase catalyses the release of methanol from pectin with a resultant decrease in pH. Sodium hydroxide is added to maintain the pH at 4.5. The amount of sodium hydroxide consumed is an indication of the enzyme activity. One unit of PE activity is that amount of enzyme

which consumes 1 micro equivalent of sodium hydroxide per minute under standard conditions (30°C, pH 4.5).

General process for enzymatic dehusking: The following process was used for enzymatic dehusking.

Chickpea grains were cleaned and pitted to about 50% using mini dal mill roller. The pitted grains were mixed with about 2-3% water to increase the moisture content of the grain to about 13.5 to 14%.

Enzyme was incorporated to the pitted grains through the required amount of water used for adjusting the moisture content of the grains. All the grains were mixed for uniformly in water distribution through all the grains. The grains were incubated at about 30 to 35 deg C for 15 hours. Post incubation the moisture content of the grains was readjusted to 10 to 11% using hot air dryer/oven.

Dehusking of the pulses were carried out using Random orbit sander from Bosch model no GEX 125-1 AE Professional. The sander was fitted with 40 no emery coated disc and 10g of pitted grains were kept on 120no emery coated paper and dehusking was done by placing the sander on the grain and running at speed 1 for 1:30 minutes. The seeds were passed through classifier to separate the whole grains (husked and dehusked), dehusked splits, husk, broken and powdered samples.

The dehusking efficiency (DE) of the pulses was calculated using the following formula.

$$DE = \frac{W1-(W3+Wh+Wb)}{W1} X100$$

Where

W1: Total input material post Cleaning

W3: Total Unhulled grains

Wh: Total husk

Wb: Total Broken and Powder

Or other way to calculate Dehusking efficiency is

$$DE = \frac{W2}{\text{----------}} X100$$

W1

Where

W1: Total input material Post cleaning

W2: Total dehulled/dehusked Split and Whole grains.

EXAMPLE 1: ENZYMATIC DEHUSKING OF PULSE USING GH 11 XYLANASES

TABLE 1.1: CONCENTRATION OF XYLANASE FAMILY GH-11

	1	2	3	4	5	6
XYLANASE GH11	0ppm (control)	20ppm	40ppm	80ppm	160ppm	200ppm

RESULTS:

TABLE 1.2: DEHUSKING EFFICIENCY OF CHICKPEA USING GH11 XYLANASE

	1	2	3	4	5	6
Dehusking Efficiency % (DE%)	47.77	55.10	58.98	61.85	57.28	55.17
Standard Deviation along with means comparison using Tukey Kramer HSD (in superscript)	$1.4^{(C)}$	$2.6^{(B)}$	$7.0^{(AB)}$	$2.4^{(A)}$	$5.1^{(AB)}$	$4.0^{(B)}$

In the Tukey Kramer HSD analysis, the levels not connected by the same letter are significantly different. From the above table and statistical analysis, it is found that Xylanase from GH-11 can significantly increase dehusking efficiency of Chickpea.

EXAMPLE 2: ENZYMATIC DEHUSKING OF PULSE USING GH 8 XYLANASES.

TABLE 2.1: CONCENTRATION OF XYLANASE FAMILY GH-8

	1	2	3	4	5	6
XYLANASE GH8	0ppm (control)	20ppm	40ppm	80ppm	160ppm	200ppm

RESULTS:

TABLE 2.2: DEHUSKING EFFICIENCY OF CHICKPEA USING XYLANASE FROM FAMILY GH 8

	1	2	3	4	5	6
Dehusking Efficiency % (DE%)	43.1	49.3	54.1	51.2	54.2	54.7
Standard Deviation along with means comparison using Tukey Kramer HSD (in superscript)	$5.2^{(B)}$	$4.2^{(A)}$	$2.9^{(A)}$	$5.3^{(A)}$	$2.2^{(A)}$	$2.2^{(A)}$

In the Tukey Kramer HSD analysis, the levels not connected by the same letter are significantly different. From the above table and statistical analysis, it is found that Xylanase from GH-8 can significantly increase dehusking efficiency of Chickpea.

EXAMPLE 3: ENZYMATIC DEHUSKING OF PULSE USING GH 10 XYLANASES.

TABLE 3.1: CONCENTRATION OF XYLANASE FAMILY GH-10

	1	2	3	4
XYLANASE GH-10	0ppm	50ppm	100ppm	500ppm

RESULTS:

TABLE 3.2: DEHUSKING EFFICIENCY OF CHICKPEA USING GH10 XYLANASE

	1	2	3	4
Dehusking Efficiency % (DE%)	55.5	54.0	49.2	58.5
Standard Deviation along with means comparison using Tukey Kramer HSD (in superscript)	$2.9^{(A)}$	$4.0^{(A)}$	$16.1^{(A)}$	$13.4^{(A)}$

In the Tukey Kramer HSD analysis, the levels connected by the same letter are not significantly different. From the above table and statistical analysis, it is found that Xylanase from GH-10 though increases dehusking efficiency of Chickpea, the difference in increase is not statistically significant.

EXAMPLE 4: ENZYMATIC DEHUSKING OF PULSE USING GH5 ENDOGLUCANASE

TABLE 4.1: CONCENTRATION OF GH5 ENDOGLUCANASE

	1	2	3	4	5
ENDOGLUCANASE	0ppm (control)	20ppm	80ppm	200ppm	500ppm

RESULTS:

TABLE 4.2: DEHUSKING EFFICIENCY OF CHICKPEA USING GH5 ENDOGLUCANASE

	1	2	3	4	5

Dehusking Efficiency % (DE%)	66.7	75.3	74.5	76.1	74.0
Standard Deviation along with means comparison using Tukey Kramer HSD (in superscript)	1.3 (C)	0.7 (AB)	1.1 (B)	1.1(A)	0.3 (B)

In the Tukey Kramer HSD analysis, the levels not connected by the same letter are significantly different. From the above table and statistical analysis, it is found that GH5 endoglucanase can significantly increase dehusking efficiency of Chickpea.

EXAMPLE 5: ENZYMATIC DEHUSKING OF CHICKPEA USING PECTIN LYASE

TABLE 5.1: CONCENTRATION OF PECTIN LYASE for CHICKPEA DEHUSKING

	1	2	3	4
PECTIN LYASE DOSAGE	0ppm	20ppm	40ppm	80ppm

RESULTS:

TABLE 5.2: DEHUSKING EFFICIENCY OF CHICKPEA USING PECTIN LYASE

	1	2	3	4
Dehusking Efficiency % (DE%)	65.8	57.1	48.2	46.3
Standard Deviation along with means comparison using Tukey Kramer HSD (in superscript)	1.48 (A)	1.39 (B)	1.89 (C)	1.55 (C)

In the Tukey Kramer HSD analysis, the levels not connected by the same letter are significantly different. From the above table and statistical analysis, it is found that Pectin Lyase does not increase dehusking efficiency of Chickpea.

EXAMPLE 6: ENZYMATIC DEHUSKING OF CHICKPEA USING POLYGALACTURONASE

TABLE 6.1: CONCENTRATION OF POLYGALACTURONASE FOR CHICKPEA DEHUSKING

	1	2	3	4
POLYGALACTURONASE DOSAGE	0ppm	20ppm	40ppm	160ppm

RESULTS:

TABLE 6.2: DEHUSKING EFFICIENCY OF CHICKPEA USING POLYGALACTURONASE

	1	2	3	4
Dehusking Efficiency % (DE%)	40.32	46.58	48.33	60.62
Standard Deviation along with means comparison using Tukey Kramer HSD (in superscript)	2.6 (C)	1.43 (BC)	0.89 (B)	1.78 (A)

In the Tukey Kramer HSD analysis, the levels not connected by the same letter are significantly different. From the above table and statistical analysis, it is found that Polygalacturonase can significantly increase dehusking efficiency of Chickpea.

EXAMPLE 7: ENZYMATIC DEHUSKING USING COMBINATION OF POLYGALACTURONASE, PECTIN METHYL ESTERASE IN CHICKPEA DEHUSKING

TABLE 7.1: CONCENTRATION OF POLYGALACTURONASE AND PECTIN METHYL ESTERASE IN CHICKPEA DEHUSKING

DOSAGES	1	2	3	4

POLYGALACTURONASE	0	4924units/kg		4924units/kg
PECTIN METHYL ESTERASE	0		1034 units/kg	1034 units/kg

RESULTS:

TABLE 7.2: DEHUSKING EFFICIENCY OF CHICKPEA USING POLYGALACTURONASE AND PECTIN METHYL ESTERASE

	1	2	3	4
Dehusking Efficiency % (DE%)	44.3	61.3	51.9	60.2
Standard Deviation along with means comparison using Tukey Kramer HSD (in superscript)	3.58 (C)	2.43 (A)	8.13 (BC)	6.22 (A)

In the Tukey Kramer HSD analysis, the levels not connected by the same letter are significantly different. From the above table and statistical analysis, it is found that Polygalacturonase with or without Pectin Methyl Esterase can significantly increase dehusking efficiency of Chickpea.

Claims

1. A method for dehusking a pulse comprising:
    a. pretreating the pulse with an enzyme composition comprising a GH11 or GH8 xylanase; and
    b. dehusking the pretreated pulse.

2. A method for dehusking a pulse comprising:
    a. pretreating the pulse with an enzyme composition comprising a GH5 endoglucanase; and
    b. dehusking the pretreated pulse.

3. A method for dehusking a pulse comprising:
    a. pretreating the pulse with an enzyme composition comprising a polygalacturonase; and
    b. dehusking the pretreated pulse.

4. The method according to any of claims 1 to 3, wherein the pulse is chick pea.

5. The method according to any of claims 1 to 4, wherein the pulse is whole unhusked pulse.

6. The method according to claim 1, wherein the xylanase is selected among xylanases having an amino acid sequence identity of at least 80% identity, preferably at least 85% identity, preferably at least 90% identity, preferably at least 95% identity, preferably at least 96% identity, preferably at least 97% identity, preferably at least 98% identity, preferably at least 99% identity or 100% sequence identity to SEQ ID NO: 1.

7. The method according to claim 1, wherein the xylanase is selected among xylanases having an amino acid sequence identity of at least 80% identity, preferably at least 85% identity, preferably at least 90% identity, preferably at least 95% identity, preferably at least 96% identity, preferably at least 97% identity, preferably at least 98% identity, preferably at least 99% identity or 100% sequence identity to SEQ ID NO: 2.

8. The method according to claim 2, wherein the endoglucanase is selected among endoglucanases having an amino acid sequence identity of at least 80% identity, preferably at least 85% identity, preferably at least 90% identity, preferably at least 95% identity, preferably at least 96% identity, preferably at least 97% identity, preferably at least 98% identity, preferably at least 99% identity or 100% sequence identity to SEQ ID NO: 4.

9. The method according to claim 3, wherein the polygalacturonase is selected among polygalacturonases having an amino acid sequence identity of at least 80% identity, preferably at least 85% identity, preferably at least 90% identity, preferably at least 95% identity, preferably at least 96% identity, preferably at least 97% identity, preferably at least 98% identity, preferably at least 99% identity or 100% sequence identity to SEQ ID NO: 5.

10. The method according to any of the preceding claims, wherein the pretreatment is carried out for a period between 5 minutes and 24 hours, preferably in the range of 2 hours to 16 hours, preferably in the range of 4 hours to 8 hours.

11. The method according to any of the preceding claims, wherein the pretreatment is done at a temperature in the range of 10°C-80°C, preferably in the range of 20°C-60°C most preferably in the range of 25°C-50°C.

12. The method according to any of the preceding claims, wherein the enzyme composition further comprises a pectinase.

13. The method according to any of the preceding claims, wherein the enzyme composition further comprises a glucanase.

14. The method according to any of the preceding claims, wherein the enzyme composition further comprises a xylanase.

15. The method according to any of the preceding claims, wherein the enzyme composition further comprises a polygalacturonase.

16. A completely or partially dehusked pulse prepared using the method according to any of the claims 1-15.

17. An enzyme composition comprising a GH8 or GH11 xylanase for dehusking of pulse.

18. An enzyme composition comprising a GH5 endoglucanase for dehusking of pulse.

19. An enzyme composition comprising a polygalacturonase for dehusking of pulse.

20. The enzyme composition according to any of claims 17 to 19 further comprising one or more additional enzymes.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/050051

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23L11/30
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)
A23L C12N

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 practicable, search terms used)
EPO-Internal, BIOSIS, WPI Data, COMPENDEX, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; November 2000 (2000-11), SINGH U ET AL: "Influence of dehulling, soaking solution and enzyme treatment on the cooking quality of improved varieties of pulses", XP002779060, Database accession no. PREV200100116357 abstract -/--	4,12,15

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 March 2018	06/06/2018

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	& SINGH U ET AL: "Influence of dehulling, soaking solution and enzyme treatment on the cooking quality of improved varieties of pulses", JOURNAL OF FOOD SCIENCE AND TECHNOLOGY, vol. 37, no. 6, November 2000 (2000-11), pages 627-630, ISSN: 0022-1155	

X	SREERAMA Y N ET AL: "Effect of enzyme pre-dehulling treatments on dehulling and cooking properties of legumes", JOURNAL OF FOOD ENGINEERING, BARKING, ESSEX, GB, vol. 92, no. 4, 1 June 2009 (2009-06-01), pages 389-395, XP026001062, ISSN: 0260-8774, DOI: 10.1016/J.JFOODENG.2008.12.008 [retrieved on 2009-03-03]	1,5,10, 11,16,17
Y	Section 2.3. "Xylanase pre-dehulling treatment"	1,4,6,7, 10-16

Y	V P Sangani ET AL: "Optimization of enzymatic hydrolysis of pigeon pea for cooking quality of dhal", International Journal of Agricultural and Biological Engineering, 1 October 2014 (2014-10-01), page 123, XP055458479, Beijing DOI: 10.3965/j.ijabe.20140705.014 Retrieved from the Internet: URL:https://ijabe.org/index.php/ijabe/article/download/922/pdf the whole document	1,4-7, 10-16

Y	R P Murumkar ET AL: "EFFECT OF ENZYME PRE-TREATMENTS ON MILLING OF PIGEONPEA", International Journal of Science, Environment and Technology, 2 December 2016 (2016-12-02), pages 4029-4051, XP055458773, Retrieved from the Internet: URL:http://www.ijset.net/journal/1415.pdf [retrieved on 2018-03-13] the whole document	1,4-7, 10-16

X	WO 2011/070101 A1 (NOVOZYMES AS [DK]; BEIER LARS [DK]; TOSCANO MIGUEL DUARTE [DK]; FRIIS) 16 June 2011 (2011-06-16) sequence 3 claims 37,40,41	17,20

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/050051

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/034449 A1 (CLARIANT PRODUKTE DEUTSCHLAND [DE]) 10 March 2016 (2016-03-10) sequence 8 claims 13,14 -----	17,20

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2018/050051

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

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

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

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

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

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1, 6, 7, 17(completely); 4, 5, 10-16, 20(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 6, 7, 17(completely); 4, 5, 10-16, 20(partially)

   Method for dehusking a pulse comprising the pulse pretreatment with GH11 or GH8 xylanase.

   ---

2. claims: 2, 8, 18(completely); 4, 5, 10-16, 20(partially)

   Method for dehusking a pulse comprising the pulse pretreatment with GH5 endoglucanase.

   ---

3. claims: 3, 9, 19(completely); 4, 5, 10-16, 20(partially)

   Method for dehusking a pulse comprising the pulse pretreatment with polygalacturonase.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2018/050051

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011070101 A1	16-06-2011	AU 2010329917 A1	14-06-2012
		CA 2783418 A1	16-06-2011
		CN 102753023 A	24-10-2012
		CN 106929494 A	07-07-2017
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		EP 2509427 A1	17-10-2012
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		WO 2011070101 A1	16-06-2011
WO 2016034449 A1	10-03-2016	AR 102410 A1	01-03-2017
		AU 2015311115 A1	23-02-2017
		CA 2959457 A1	10-03-2016
		CN 106687586 A	17-05-2017
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		US 2017327809 A1	16-11-2017
		WO 2016034449 A1	10-03-2016