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(71) Demandeur/Applicant:
NOVOZYMES A/S, DK
(72) Inventeurs/Inventors:
ELVIG, NIELS, DK;
FESTERSEN, RIKKE, (UNKNOWN)
(74) Agent: DIMOCK STRATTON LLP

(54) Titre : PROCEDE D'EMPATAGE ET COMPOSITION ENZYMATIQUE UTILE DANS LEDIT PROCEDE
(54) Title: MASHING PROCESS AND ENZYME COMPOSITION USEFUL THEREIN

(57) **Abrégé/Abstract:**

The present invention relates to a mashing and filtration step in a brewing process and to a composition comprising an endoglucanase derived from *Trichoderma reesei* and a xylanase GH10 derived from *Aspergillus*, which useful in the mashing and filtration step of a brewing process.



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(71) Applicant (for all designated States except US):
NOVOZYMES A/S [DK/DK]; Krogshøjvej 36, DK-2880
Bagsvaerd (DK).

(72) Inventor; and
(75) Inventor/Applicant (for US only): **ELVIG, Niels**
[DK/DK]; Birkebakken 19, DK-2840 Holte (DK).

(74) Common Representative: **NOVOZYMES A/S**; Patents,
Krogshøjvej 36, DK-2880 Bagsvaerd (DK).

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MASHING PROCESS AND ENZYME COMPOSITION USEFUL THEREIN

FIELD OF THE INVENTION

The present invention relates, inter alia, to a mashing and filtration step in a process for the production of an alcoholic beverage, and to a composition useful in the mashing and filtration step in such a process.

BACKGROUND OF THE INVENTION

The use of enzymes in brewing is common. Application of enzymes to the mashing step to improve mash filterability and increase extract yield is described in WO 97/42302. However, there is a need for improvement of the mashing and filtration step and for improved enzymatic compositions for use in the mashing and filtration step.

SUMMARY OF THE INVENTION

The invention provides in a first aspect a process for production of a mash having enhanced filterability and/or improved extract yield after filtration, which comprises; preparing a mash in the presence of enzyme activities and filtering the mash to obtain a wort, wherein the enzyme activities comprise endoglucanase activities derived from *Trichoderma Reesei* and a xylanase of GH family 10.

In a second aspect the invention provides a composition comprising; an endoglucanase derived from *Trichoderma reesei*; and, a xylanase GH 10 derived from *Aspergillus* sp., preferably from *Aspergillus aculeatus* (SEQ ID NO:1).

In further aspects the invention provides uses of a composition according to the second aspect in a process comprising reducing the viscosity of an aqueous solution comprising a starch hydrolysate, in a process comprising filtering of an aqueous solution comprising a starch hydrolysate, in a process wherein the aqueous solution comprising a starch hydrolysate is a mash for beer making and/or in a process wherein the aqueous solution comprising a starch hydrolysate is a feed composition.

DETAILED DESCRIPTION OF THE INVENTION

Brewing processes are well-known in the art, and generally involve the steps of malting, mashing, and fermentation. In the traditional brewing process the malting serves the purpose of converting insoluble starch to soluble starch, reducing complex proteins, generating colour and flavour compounds, generating nutrients for yeast development, and the development of enzymes. The three main steps of the malting process are steeping, germination, and kilning.

Steeping includes mixing the barley kernels with water to raise the moisture level

and activate the metabolic processes of the dormant kernel. In the next step, the wet barley is germinated by maintaining it at a suitable temperature and humidity level until adequate modification, i.e. such as degradation of starch and activation of enzymes, has been achieved. The final step is to dry the green malt in the kiln. The temperature regime in the kiln determines the colour of the barley malt and the amount of enzymes which survive for use in the mashing process. Low temperature kilning is more appropriate for malts when it is essential to preserve enzymatic activity. Malts kilned at high temperatures have very little or no enzyme activity but are very high in colouring such as caramelized sugars as well as in flavouring compounds.

Mashing is the process of converting starch from the milled barley malt and solid adjuncts into fermentable and unfermentable sugars to produce wort of the desired composition. Traditional mashing involves mixing milled barley malt and adjuncts with water at a set temperature and volume to continue the biochemical changes initiated during the malting process. The mashing process is conducted over a period of time at various temperatures in order to activate the endogenous malt enzymes responsible for the degradation of proteins and carbohydrates. By far the most important change brought about in mashing is the conversion of starch molecules into fermentable sugars. The principal enzymes responsible for starch conversion in a traditional mashing process are alpha- and beta-amylases. Alpha-amylase very rapidly reduces insoluble and soluble starch by splitting starch molecules into many shorter chains that can be attacked by beta-amylase. The disaccharide produced is maltose.

Traditionally lager beer has often been brewed using a method referred to as "step-infusion". This mashing procedure involves a series of rests at various temperatures, each favouring one of the necessary endogenous enzyme activities. To day the double-mash infusion system is the most widely used system for industrial production of beer, especially lager type beer. This system prepares two separate mashes. It utilizes a cereal cooker for boiling adjuncts and a mash tun for well-modified, highly enzymatically active malts.

When brewing from grists low in enzymes such as high adjunct grists, mashing may be performed in the presence of added enzyme compositions comprising the enzymes necessary for the hydrolysis of the grist starch. These enzymes may comprise alpha-amylases, pullulanases, beta-amylases and glucoamylases.

After mashing, it is necessary to separate the liquid extract (the wort) from the solids (spent grains i.e. the insoluble grain and husk material forming part of grist). Wort separation is important because the solids contain large amounts of non-starch polysaccharides, protein, poorly modified starch, fatty material, silicates, and polyphenols (tannins). Important non-starch polysaccharides present in cereal grains are beta-glucan and arabinoxylan. The endosperm cell wall of barley comprises 75% beta-glucan, 20% arabinoxylan, and 5% remaining protein with small amount of cellulose, glucomannan and phenolic acids. Long chains of barley arabinoxylans, and to a lesser degree beta-glucan, which have not been

modified due to enzymatic hydrolysis may cause formation of gels when solubilised in water, these gels will strongly increase wort viscosity and reduce filterability. Likewise is it very important for the quality of the wort that the beta-glucan has been reduced to smaller oligomers, as unmodified beta-glucans later on will give rise to haze stability problems in the final beer. Therefore, enzymatic compositions comprising endoglucanases and xylanases, such as Ultraflo® or Viscozyme®, are often used in the mashing step to improve wort separation. The objectives of wort separation, *inter alia*, include the following:

- to obtain good extract recovery,
- to obtain good filterability, and
- to produce clear wort.

Extraction recovery and filterability are important for the economy in the brewing process, while the wort clarity is a must in order to produce a beer which does not develop haze. Extraction recovery, filterability and wort clarity is greatly affected by the standard of the grist, e.g. the barley malt and the types of adjunct, as well as the applied mashing procedure.

Following the separation of the wort from the spent grains the wort may be fermented with brewers yeast to produce a beer.

Definitions

Throughout this disclosure, various terms that are generally understood by those of ordinary skill in the arts are used. Several terms are used with specific meaning, however, and are meant as defined by the following.

As used herein the term “**grist**” is understood as the starch or sugar containing material that's the basis for beer production, e.g. the barley malt and the adjunct.

The term “**malt**” is understood as any malted cereal grain, in particular barley.

The term “**adjunct**” is understood as the part of the grist which is not barley malt. The adjunct may be any carbohydrate rich material.

The term “**mash**” is understood as a aqueous starch slurry, e.g. comprising crushed barley malt, crushed barley, and/or other adjunct or a combination hereof, steeped in water to make wort.

The term “**wort**” is understood as the unfermented liquor run-off following extracting the grist during mashing.

The term “**spent grains**” is understood as the drained solids remaining when the grist has been extracted and the wort separated from the mash.

The term “**beer**” is here understood as fermented wort, e.g. an alcoholic beverage brewed from barley malt, optionally adjunct and hops.

The term “**extract recovery**” in the wort is defined as the sum of soluble substances extracted from the grist (malt and adjuncts) expressed in percentage based on dry matter.

The term “**a thermostable enzyme**” is understood as an enzyme that under the

temperature regime and the incubation period applied in the processes of the present invention in the amounts added is capable of sufficient degradation of the substrate in question.

5 The term "**homology**" when used about polypeptide or DNA sequences and referred to in this disclosure is understood as the degree of homology between two sequences indicating a derivation of the first sequence from the second. The homology may suitably be determined by means of computer programs known in the art such as GAP provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, 10 USA 53711) (Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-453. The following settings for polypeptide sequence comparison are used: GAP creation penalty of 3.0 and GAP extension penalty of 0.1.

The term "**DP**" is the degree of polymerisation, herein used for average number of glucose units in polymers in a polysaccharide hydrolysate.

15 The numbering of **Glycoside Hydrolase Families** (GH) and **Carbohydrate Binding Modules** (CBM) applied in this disclosure follows the concept of Coutinho, P.M. & Henrissat, B. (1999) *CAZy - Carbohydrate-Active Enzymes server* at URL: <http://afmb.cnrs-mrs.fr/~cazy/CAZY/index.html> or alternatively Coutinho, P.M. & Henrissat, B. 1999; The modular structure of cellulases and other carbohydrate-active enzymes: an integrated 20 database approach. In "*Genetics, Biochemistry and Ecology of Cellulose Degradation*", K. Ohmiya, K. Hayashi, K. Sakka, Y. Kobayashi, S. Karita and T. Kimura eds., Uni Publishers Co., Tokyo, pp. 15-23, and in Bourne, Y. & Henrissat, B. 2001; Glycoside hydrolases and glycosyltransferases: families and functional modules, *Current Opinion in Structural Biology* 11:593-600. This classification system groups glucoside hydrolases based on similarities in 25 primary structure. The members of a family furthermore show the same catalytic mechanism and have similarities in the overall three-dimensional structure, although a family may contain members with substantial variation in substrate specificity.

Further information on conventional brewing processes may be found in "Technology Brewing and Malting" by Wolfgang Kunze of the Research and Teaching 30 Institute of Brewing, Berlin (VLB), 2nd revised Edition 1999, ISBN 3-921690-39-0.

Embodiments of the invention

The composition according to the proceeding aspect may be used in a process comprising reducing the viscosity of an aqueous solution comprising a starch hydrolysate.

35 The composition may even be used in a process comprising filtering of an aqueous solution comprising a starch hydrolysate. In a preferred embodiment the aqueous solution comprising a starch hydrolysate is a mash for beer making, and in another preferred embodiment the aqueous solution comprising a starch hydrolysate is a feed composition.

The process of the invention may be applied in the mashing of any grist. According

to the invention the grist may comprise any starch and/or sugar containing plant material derivable from any plant and plant part, including tubers, roots, stems, leaves and seeds. Preferably the grist comprises grain, such as grain from barley, wheat, rye, oat, corn, rice, milo, millet and sorghum, and more preferably, at least 10%, or more preferably at least 15%,
5 even more preferably at least 25%, or most preferably at least 35%, such as at least 50%, at least 75%, at least 90% or even 100% (w/w) of the grist of the wort is derived from grain. Most preferably the grist comprises malted grain, such as barley malt. Preferably, at least 10%, or more preferably at least 15%, even more preferably at least 25%, or most preferably at least 35%, such as at least 50%, at least 75%, at least 90% or even 100% (w/w) of the
10 grist of the wort is derived from malted grain.

For mashing of low malt grists the mashing enzymes may be exogenously supplied. The enzymes mostly used as starch degrading enzymes include pullulanases, alpha-amylases and amyloglucosidases. The use of starch degrading enzymes in mashing is well-known to the skilled person.

15 Adjunct comprising readily fermentable carbohydrates such as sugars or syrups may be added to the malt mash before, during or after the mashing process of the invention but is preferably added after the mashing process. A part of the adjunct may be treated with a protease and/or an endoglucanase, and/or heat treated before being added to the mash of the invention.

20 During the mashing process, starch extracted from the grist is gradually hydrolyzed into fermentable sugars and smaller dextrins. Preferably the mash is starch negative to iodine testing, before wort separation.

The application of the appropriate xylanase and endoglucanase activities in the process of the present invention results in efficient reduction of beta-glucan and arabino-
25 xylan level facilitating wort separation, thus ensuring reduced cycle time, high extract recovery and clear wort.

The wort produced by the process of the first aspect of the invention may be fermented to produce a beer. Fermentation of the wort may include pitching the wort with a yeast slurry comprising fresh yeast, i.e. yeast not previously used for the invention or the
30 yeast may be recycled yeast. The yeast applied may be any yeast suitable for beer brewing, especially yeasts selected from *Saccharomyces* spp. such as *S. cerevisiae* and *S. uvarum*, including natural or artificially produced variants of these organisms. The methods for fermentation of wort for production of beer are well known to the person skilled in the arts.

The process of the invention may include adding silica hydrogel to the fermented
35 wort to increase the colloidal stability of the beer. The processes may further include adding kieselguhr to the fermented wort and filtering to render the beer bright. The beer produced by fermenting the wort of the invention may be any type of beer, e.g. ale, strong ale, stout, porter, lager, pilsner, bitter, export beer, malt liquor, happoushu, lambic, barley wine, high-alcohol beer, low-alcohol beer, low-calorie beer or light beer.

The beer produced by the process of the invention may be distilled to recover ethanol, e.g. for whisky production. Contemplated are any kind of whisky (spelled "whiskey" in US and Ireland) include bourbon, Canadian whisky, Irish whiskey, rye, and scotch.

5

Xylanase

For the present purposes a xylanase is an enzyme classified as EC 3.2.1.8. The official name is endo-1,4-beta-xylanase. The systematic name is 1,4-beta-D-xylan xylanohydrolase. Other names may be used, such as endo-(1-4)-beta-xylanase; (1-4)-beta-xylan 4-xylanohydrolase; endo-1,4-xylanase; xylanase; beta-1,4-xylanase; endo-1,4-xylanase; endo-beta-1,4-xylanase; endo-1,4-beta-D-xylanase; 1,4-beta-xylan xylanohydrolase; beta-xylanase; beta-1,4-xylan xylanohydrolase; endo-1,4-beta-xylanase; beta-D-xylanase. The reaction catalysed is the endohydrolysis of 1,4-beta-D-xylosidic linkages in xylans.

While the xylanase to be used for the present invention may be of any origin including mammalian, plant or animal origin it is presently preferred that the xylanase is of microbial origin. In particular the xylanase may be one derivable from a filamentous fungus or a yeast.

Xylanases have been found in a number of fungal species, in particular species of *Aspergillus*, such as *A. niger*, *A. awamori*, *A. aculeatus* and *A. oryzae*, *Trichoderma*, such as *T. reesei* or *T. harzianum*, *Penicillium*, such as *P. camembertii*, *Fusarium*, such as *F. oxysporum*, *Humicola*, such as *H. insolens*, and *Thermomyces lanuginosa*. Xylanases have also been found in bacterial species, e.g. within the genus *Bacillus*, such as *B. pumilus*.

Preferably, according to the process of the invention the xylanase is derived from a filamentous fungus such as from *Aspergillus* sp., *Bacillus* sp., *Humicola* sp., *Myceliophthora* sp., *Poitrasia* sp. or *Rhizomucor* sp.

Preferably the xylanase to be used in the present invention is a Glycoside Hydrolase Family 10 (GH10) xylanase. As the GH10 enzymes are able to go closer to the branched xylose units, they form smaller oligosaccharides than the GH11 xylanases.

Most preferably the xylanase to be used according to the process of the invention the xylanase from *Aspergillus aculeatus* shown as SEQ ID NO:1 (XYL II) in the present disclosure and/or described in WO9421785A1 and comprising the partial amino acid sequence shown as SEQ ID NO:5 therein. Also preferred are any xylanase having and or comprising a sequence having at least 50%, at least 60%, at least 70%, at least 80%, or even at least 90% homology to any of the aforementioned sequences.

Endoglucanases

For the present purposes an endoglucanase is an enzyme classified as EC 3.2.1.4. The endoglucanase activity to be used for the present invention may be any composition

derived from *Trichoderma reesei*, preferably the composition is obtained by submerged fermentation, such as the composition Celluclast® available from Novozymes A/S. Celluclast has a pronounced viscosity-reducing effect on soluble cellulosic substrates. Also preferred are the cellulase composition Laminex BG®, a commercial cellulase preparation produced by *Trichoderma reesei* and available from Genencor International Inc.

MATERIALS AND METHODS

Xylanolytic Activity

The xylanolytic activity can be expressed in FXU(S)-units, determined at pH 6.0 with Azo-Wheat arabinoxylan (arabinoxylan dyed with remazol brilliant blue, Megazyme) as substrate.

A xylanase sample is incubated with the remazol-xylan substrate. The background of non-degraded dyed substrate is precipitated by ethanol. The remaining blue colour in the supernatant (as determined spectrophotometrically at 585 nm) is proportional to the xylanase activity, and the xylanase units are then determined relatively to an enzyme standard at standard reaction conditions, i.e. Substrate concentration 0.45% w/v, Enzyme concentration 0.04 – 0.14 FXU(S)/mL at 50.0 °C, pH 6.0, and in 30 minutes reaction time. Xylanase activity in FXU(S) is measured relative to a Novozymes FXU(S) enzyme standard comprising the monocomponent xylanase preparation Shearzyme from *Aspergillus aculeatus*.

A folder EB-SM-0397.02 describing this analytical method in more detail is available upon request to Novozymes A/S, Denmark, which folder is hereby included by reference.

Endoglucanase activity

The cellulytic activity may be measured in fungal endoglucanase units (FBG), determined on a 0.5% beta-glucan substrate at 30°C, pH 5.0 and reaction time 30 min. Fungal endoglucanase reacts with beta-glucan releases glucose or reducing carbohydrate which is determined as reducing sugar according to the Somogyi-Nelson method.

1 fungal endoglucanase unit (FBG) is the amount of enzyme which according to the above outlined standard conditions, releases glucose or reducing carbohydrate with a reduction capacity equivalent to 1 micromol glucose per minute.

Cellulytic Activity

The cellulytic activity may be measured in endo-glucanase units (EGU), determined at pH 6.0 with carboxymethyl cellulose (CMC) as substrate.

A substrate solution is prepared, containing 34.0 g/l CMC (Hercules 7 LFD) in 0.1 M phosphate buffer at pH 6.0. The enzyme sample to be analyzed is dissolved in the same buffer.

5 ml substrate solution and 0.15 ml enzyme solution are mixed and transferred to a vibration viscosimeter (e.g. MIVI 3000 from Sofraser, France), thermostated at 40°C for 30 minutes.

One EGU is defined as the amount of enzyme that reduces the viscosity to one half under these conditions. The amount of enzyme sample should be adjusted to provide 0.01-0.02 EGU/ml in the reaction mixture. The arch standard is defined as 880 EGU/g.

Enzymes

A: Celluclast ® 1.5 L, a commercial preparation produced by *Trichoderma reesei* comprising cellulase activities 700 EGU/g and xylanase activities 200 FXU(S)/g.

B: composition of the invention comprising the cellulase and xylanase activities, respectively 700 EGU/g and 200 FXU(S)/g, from *Trichoderma reesei* and the GH10 xylanase from *Aspergillus aculeatus* shown in SEQ ID NO:1 250 FXU(S)/g.

C: Laminex BG®, a commercial preparation produced by *Trichoderma reesei* comprising cellulase activities 1370 EGU/g and xylanase activities.

Methods

Mash preparation

Unless otherwise stated mashing was performed as follows. Except when noted (e.g. with regard to enzyme dosage) the mash was prepared according to EBC: 4.5.1 using malt grounded according to EBC: 1.1. Mashing trials were performed in 500 ml lidded vessels incubated in water bath with stirring and each containing a mash with 44 g malt and adjusted to a total weight of 300±0.2 g with water preheated to 50°C. The wort produced was app. 12% Plato.

Mashing temperature profile

Unless otherwise stated mashing was carried out using an initial incubation temperature at 50°C for 30 minutes, followed by an 17 min increasing step with 1°C/min to 67°C remaining here for 40 min. The profile is continued with an 8 min increasing step with 1C/min to 75°C and remaining here for 20 min. The mash is cooled to 20°C during 15 min, which result in a total incubation period of 131 min.

Additional methods

Methods for analysis of raw products, wort, beer etc. can be found in *Analytica-EBC*, Analysis Committee of EBC, the European Brewing Convention (1998), Verlag Hans Carl Geranke-Fachverlag. For the present invention the methods applied for determination of the following parameters were as indicated below.

Plato: refractometer.

Beta-glucan: EBC: 8.13.2 (High Molecular weight beta-glucan content of wort: Fluorimetric Method).

Turbidity: EBC: 4.7.1

5 Filterability: Volume of filtrate (ml) determination: According to EBC: 4.5.1 (Extract of Malt: Congress Mash) subsection 8.2. Filterability: Filtration volume is read after 1 hour of filtration through fluted filter paper, 320 mm diameter. Schleicher and Schüll No.597 ½, Machery, Nagel and Co. in funnels, 200 mm diameter, fitted in 500 ml flasks.

10 Extract recovery: EBC: 4.5.1 (Extract of Malt: Congress Mash, Extract in dry). The term extract recovery in the wort is defined as the sum of soluble substances (glucose, sucrose, maltose, maltotriose, dextrans, protein, gums, inorganic, other substances) extracted from the grist (malt and adjuncts) expressed in percentage based on dry matter. The remaining insoluble part is defined as spent grains.

$$a) E_1 = \frac{P(M + 800)}{100 - P}$$

$$b) E_2 = \frac{E_1 \cdot 100}{100 - M}$$

where;

15 E_1 = the extract content of sample, in % (m/m)

E_2 = the extract content of dry grist, in % (m/m)

P = the extract content in wort, in % Plato

M = the moisture content of the grist, in % (m/m)

800 = the amount of distilled water added into the mash to 100 g of grist

Viscosity: Automated Microviscometer (AMVn) is based on the rolling ball principle. The sample to be measured is introduced into a glass capillary in which a steel ball rolls. The viscous properties of the test fluid can be determined by measuring the rolling time of the steel ball. The rolling time t_0 of a ball over a defined measuring distance in a capillary is measured. The dynamic viscosity η of the sample is calculated from the calibration constant $K_1(\alpha)$ of the measuring system, the rolling time t_0 and the difference of density $\Delta\rho$ between the ball and the sample. The following equation is used:

$$\eta = K_1(\alpha) \cdot t_0 \cdot (\rho_k - \rho_s), \text{ where}$$

η = Dynamic viscosity of the sample, [mPa · s]

$K(\alpha)$ = Calibration constant for the Measuring system [mPa · scm³ / g]

t_0 = Rolling time for 100mm [s]

ρ_k = Ball density [7,85g / cm³]

ρ_s = Density of the sample measured [g / cm³]

The viscosity is presented based on the extract (Plato°) as is, or converted to 8,6°Plato based upon a Congress mashing procedure.

5 Example 1.

A mash was prepared from a well modified malt (172 mg β -glucan/l). Four treatments according to table 1 were applied. The results are presented in table 2

10 The treatment B wherein the enzyme composition comprised a GH10 xylanase had a significantly better performance on viscosity reduction than blank, as well as than the treatments A and C consisting only of a *T. reesei* preparation. The differences are significant an at least the 99.9% level.

Table 1. The enzyme composition applied.	
Blank	No enzymes added
A	1250 ppm of <i>T. reesei</i> 700 EGU/g+200 FXU(S)/g
B	200 ppm of <i>T. reesei</i> 700 EGU/g + 200 FXU(S)/g and <i>A.aculeatus</i> GH10 xylanase 250 FXU(S)/g
C	750 ppm of Laminex BG 1370 EGU/g

Table 2.				
	Blank	A	B	C
Viscosity mPa*s as is	1.737 (n = 8) *	1.654	1.585	1.646 (n = 7) *
Viscosity mPa*s as 8.6°Plato	1.417 (n = 8) *	1.323	1.264	1.327 (n = 7) *
mg β -glucan/l	306 (n = 8) *	22	35	20 (n = 7) *

* n = 8 : Average of 8 mashing

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JUMBO APPLICATIONS/PATENTS

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CLAIMS

- 5 1. A process for production of a mash having enhanced filterability and/or improved extract yield after filtration, which comprises; preparing a mash in the presence of enzyme activities and filtering the mash to obtain a wort, wherein the enzyme activities comprise endoglucanase activities derived from *Trichoderma Reesei* and a xylanase of GH family 10.
- 10 2. The process of the proceeding claims wherein the xylanase of GH family 10 is derived from a filamentous fungi such as from a strain of an *Aspergillus* sp., preferably from *Aspergillus aculeatus*,
- 15 3. The process of the proceeding claims wherein the xylanase of GH family 10 is a xylanase having the amino acid sequence shown in SEQ ID NO:1.
4. The process of the proceeding claims wherein the xylanase of GH family 10 has a homology of at least 50% to the amino acid sequence shown in SEQ ID NO:1.
- 20 5. The process of the proceeding claims wherein at least one additional enzyme is present, which enzyme is selected from the list comprising; arabinofuranosidase, ferulic acid esterase and xylan acetyl esterase.
- 25 6. The process of the proceeding claims, wherein the aqueous solution comprising a starch hydrolysate is a mash for beer making or a feed composition.
7. A composition comprising;
 - a. an endoglucanase derived from *Trichoderma reesei*; and,
 - b. a xylanase GH 10 derived from *Aspergillus* sp., preferably from *Aspergillus*
30 *aculeatus* (SEQ ID NO:1).
8. Use of a composition according to claim 7 in a process comprising reducing the

viscosity of an aqueous solution comprising a starch hydrolysate.

9. Use of a composition according to the proceeding claims in a process comprising filtering of an aqueous solution comprising a starch hydrolysate.

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10. Use of a composition according to the proceeding claims in a process wherein the aqueous solution comprising a starch hydrolysate is a mash for beer making.

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11. Use of a composition according to the proceeding claims in a process wherein the aqueous solution comprising a starch hydrolysate is a feed composition.