



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<p>(21) International Application Number: PCT/DK96/00469 (22) International Filing Date: 15 November 1996 (15.11.96) (30) Priority Data: 1278/95 15 November 1995 (15.11.95) DK (71) Applicant (for all designated States except US): NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK). (72) Inventor; and (75) Inventor/Applicant (for US only): LUND, Henrik [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). (74) Common Representative: NOVO NORDISK A/S; Corporate Patents, Novo Allé, DK-2880 Bagsværd (DK).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b> <i>With international search report.</i></p>
<p>(54) Title: A PROCESS FOR COMBINED DESIZING AND "STONE-WASHING" OF DYED DENIM</p>		
<p>(57) Abstract</p> <p>A one-step process for combined desizing and "stone-washing" of dyed denim, wherein the denim is treated with an amylolytic enzyme in combination with a first abrading monocomponent endoglucanase and a second streak-reducing monocomponent endoglucanase.</p>		

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A PROCESS FOR COMBINED DESIZING AND "STONE-WASHING" OF DYED DENIM

The present invention relates to a desizing and "stone-washing" one-step process whereby dyed denim having localized variation in colour density of improved uniformity is achieved by treating dyed denim, especially dyed denim garment such as denim jeans, with an amylolytic enzyme and two different endoglucanases in the very same process step.

BACKGROUND OF THE INVENTION

During the weaving of textiles, the threads are exposed to considerable mechanical strain. Prior to weaving on mechanical looms, warp yarns are often coated with size starch or starch derivatives in order to increase their tensile strength and to prevent breaking. The most common sizing agent is starch in native or modified form, yet other polymeric compounds such as polyvinylalcohol (PVA), polyvinylpyrrolidone (PVP), polyacrylic acid (PAA) or derivatives of cellulose (e.g. carboxymethylcellulose (CMC), hydroxyethylcellulose, hydroxypropylcellulose or methylcellulose), may also be abundant in the size.

In general, after the textiles have been woven, the fabric proceeds to a desizing stage, followed by one or more additional fabric processing steps. Desizing is the act of removing size from textiles. After weaving, the size coating must be removed before further processing the fabric in order to ensure a homogeneous and wash-proof result. The preferred method of desizing is enzymatic hydrolysis of the size by the action of amylolytic enzymes.

For the manufacture of denim clothes, the fabric is cut and sown into garments, that is afterwards finished. In particular, for the manufacture of denim garment, different enzymatic finishing methods have been developed. The finishing of denim garment normally is initiated with an enzymatic desizing step, during which garments are subjected to the action of amylolytic

enzymes in order to provide softness to the fabric and make the cotton more accessible to the subsequent enzymatic finishing steps.

5 Cotton wax and other lubricants can be applied to yarns in order to increase the speed of cotton weaving. Also waxes of higher melting points are being introduced. Wax lubricants are predominantly triglyceride ester based lubricants. After desizing, the wax either remains or redeposits on the fabric and as a result, the fabric gets darker in shade, gets glossy spots,  
10 and becomes more stiff.

International Patent Application No. WO 93/13256 (Novo Nordisk A/S) describes a process for the removal of hydrophobic esters from fabric, in which process the fabric is impregnated during the desizing step with an aqueous solution of lipase.  
15 This process has been developed for use in the fabric mills only, and is carried out using existing fabric mill equipment, i.e. a pad roll, a jigger, or a J box.

JP-A 2-80673 discloses a method whereby desizing and softening are achieved by treating cellulose fibres with an  
20 aqueous solution containing both amylase and cellulase.

For many years denim jeans manufacturers have washed their garments in a finishing laundry with pumice stones to achieve a soft-hand as well as a desired fashionable "stone-washed" look. This abrasion effect is obtained by locally removing the surface  
25 bound dyestuff. Recently cellulytic enzymes have been introduced into the finishing process, turning the stone-washing process into a "bio-stoning process".

The goal of a bio-stoning process is to obtain a distinct, but homogeneous abrasion of the garments (stone-washing  
30 appearance). However, uneven stone-washing ("streaks" and "creases") are very frequently occurring. In consequence repair work ("after-painting") is needed on a major part (up to about 80%) of the stone-washed jeans that have been processed in the laundries.

35 Thus, it is an object of the present invention to provide a process which reduces the problem of streaks and creases on the finished denim garments.

## SUMMARY OF THE INVENTION

Accordingly, the present invention provides a process for the treatment of fabrics, which process improves the color distribution/uniformity, stone-wash quality, etc., and which reduces the need for after-painting of the finished clothes.

The invention provides a one-step process for enzymatically desizing and stone-washing dyed denim, which process comprises treating the denim with an amylolytic enzyme, such as an  $\alpha$ -amylase, in combination with a first abrading monocomponent endoglucanase and a second streak-reducing monocomponent endoglucanase.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a process for enzymatic treatment of fabrics, by which process it is possible to provide desized and enzymatically stone-washed dyed denim of improved visual quality.

As described above, enzymatic treatment of fabrics conventionally includes the steps of desizing the fabric by use of amylolytic enzymes, softening the garment (including the steps of bio-polishing, bio-stoning and/or garment wash) by use of cellulytic enzymes, optionally followed by dyeing the garment, washing the garment, and/or softening the garment with a chemical softening agent, typically a cationic, sometimes silicone-based, surface active compound. The process of the present invention may conveniently take place during the desizing and/or softening step of the conventional garment manufacturing steps.

Accordingly, in a preferred embodiment, the process of present invention relates to a one-step process for combined desizing and "stone-washing" of dyed denim, wherein the denim is treated with an amylolytic enzyme, such as an  $\alpha$ -amylase, in combination with a first abrading monocomponent endoglucanase and a second streak-reducing monocomponent endoglucanase.

In the present context, the term "abrading endoglucanase (or cellulase)" is intended to mean an endoglucanase which is capable of providing the surface of dyed denim fabric (usually sown into garment, especially jeans) localized variations in colour density. Examples of abrading cellulase are those mentioned in the International Patent Application PCT/US89/03274 published as WO 90/02790 which is hereby incorporated by reference.

The term "monocomponent endoglucanase" denotes an endoglucanase which is essentially free from other proteins, in particular other endoglucanases. Monocomponent endoglucanases are typically produced by recombinant techniques, i.e. by cloning and expression of the relevant gene in a homologous or a heterologous host.

In the present context, the term "streak-reducing endoglucanase (or cellulase)" or "levelling" endoglucanase is intended to mean an endoglucanase which is capable of reducing formation of streaks usually present on the surface of dyed denim fabric (usually sown into garment, especially jeans) which has been subjected to a "stone-washing" process, either an enzymatic stone-washing process or process using pumice for providing localized variations in colour density on the denim surface. Examples of streak-reducing or levelling cellulases are those mentioned in the International Patent Application PCT/DK95/00108 published as WO 95/24471 which is hereby incorporated by reference.

The first endoglucanase is preferably a fungal EG V type cellulase. Another useful endoglucanase is a fungal EG III type cellulase obtainable from a strain of the genus *Trichoderma*. Examples of useful fungal EG III type cellulases are those disclosed in WO 92/06184, WO 93/20208 and WO 93/20209, and WO 94/21801 which are hereby incorporated by reference.

Preferably, the EG V type endoglucanase is derived from or producible by a strain of *Scytalidium* (f. *Humicola*), *Fusarium*, *Myceliophthora*, more preferably derived from or producible by *Scytalidium thermophilum* (f. *Humicola insolens*), *Fusarium oxysporum* or *Myceliophthora themophila*, most preferably from

*Humicola insolens*, DSM 1800, *Fusarium oxysporum*, DSM 2672, or *Myceliophthora themophila*, CBS 117.65.

In one embodiment of the invention, the first endoglucanase is an endoglucanase comprising the amino acid sequence of the *Humicola insolens* endoglucanase shown in SEQ ID No. 1 or is an analogue of said endoglucanase which is at least 60% homologous with the sequence shown in SEQ ID No. 1, reacts with an antibody raised against said endoglucanase, and/or is encoded by a DNA sequence which hybridizes with the DNA sequence encoding said endoglucanase.

In another embodiment of the invention, the first endoglucanase is an endoglucanase comprising the amino acid sequence of the *Fusarium oxysporum* endoglucanase shown in SEQ ID No. 2 or is an analogue of said endoglucanase which is at least 60% homologous with the sequence shown in SEQ ID No. 2, reacts with an antibody raised against said endoglucanase, and/or is encoded by a DNA sequence which hybridizes with the DNA sequence encoding said endoglucanase.

In the present context the homology may be determined as the degree of identity between two or more amino acid sequences by means of computer programs known in the art such as GAP provided in the GCG program package (Needleman and Wunsch, 1970, *Journal of Molecular Biology* 48:443-453). For purposes of determining the degree of identity between two amino acid sequences for the present invention, GAP is used with the following settings: GAP creation penalty of 3.0 and GAP extension penalty of 0.1.

In the present context the antibody reactivity may be determined as follows:

Antibodies to be used in determining immunological cross-reactivity may be prepared by use of the relevant purified enzyme. More specifically, antiserum against the enzyme may be raised by immunizing rabbits (or other rodents) according to the procedure described by N. Axelsen et al. in: *A Manual of Quantitative Immunoelectrophoresis*, Blackwell Scientific Publications, 1973, Chapter 23, or A. Johnstone and R. Thorpe, *Immunochemistry in Practice*, Blackwell Scientific Publications,

1982 (more specifically p. 27-31). Purified immunoglobulins may be obtained from the antisera, for example by salt precipitation ((NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub>), followed by dialysis and ion exchange chromatography, e.g. on DEAE-Sephadex. Immunochemical  
5 characterization of proteins may be done either by Ouchterlony double-diffusion analysis (O. Ouchterlony in: Handbook of Experimental Immunology (D.M. Weir, Ed.), Blackwell Scientific Publications, 1967, pp. 655-706), by crossed  
10 immunoelectrophoresis (N. Axelsen et al., supra, Chapters 3 and 4), or by rocket immunoelectrophoresis (N. Axelsen et al., Chapter 2).

The hybridization may be determined by allowing the DNA (or corresponding RNA) sequences to hybridize under the following conditions:

15        Presoaking of a filter containing the DNA fragments or RNA to hybridize in 5 x SSC (Sodium chloride/Sodium citrate, Sambrook et al. 1989) for 10 min, and prehybridization of the filter in a solution of 5 x SSC, 5 x Denhardt's solution (Sambrook et al. 1989), 0.5 % SDS and 100 µg/ml of denatured  
20 sonicated salmon sperm DNA (Sambrook et al. 1989), followed by hybridization in the same solution containing a random-primed (Feinberg, A. P. and Vogelstein, B. (1983) *Anal. Biochem.* 132:6-13), <sup>32</sup>P-dCTP-labeled (specific activity > 1 x 10<sup>9</sup> cpm/µg ) probe for 12 hours at ca. 45°C. The filter is then washed twice for 30  
25 minutes in 2 x SSC, 0.5 % SDS at at least 55°C, more preferably at least 60°C, even more preferably at least 65°C, and still more preferably at least 70°C (high stringency), even more preferably at least 75°C. Molecules to which the oligonucleotide probe hybridizes under these conditions are detected using a x-  
30 ray film.

In a preferred embodiment of the process of the invention, the second endoglucanase has a catalytic activity on cellotriose at pH 8.5 corresponding to  $k_{cat}$  of at least 0.01 s<sup>-1</sup>, preferably of at least 0.1 s<sup>-1</sup>, more preferably of at least 1 s<sup>-1</sup>.

35        Preferably, the second endoglucanase is obtainable by or derived from a strain of *Humicola*, *Trichoderma*, *Myceliophthora*,



*Penicillium*, *Irpex*, *Aspergillus*, *Scytalidium* or *Fusarium*, more preferably from a strain of *Humicola insolens*, *Fusarium oxysporum* or *Trichoderma reesei*. Preferred second endoglucanases are of the EG I type.

5 An example of a useful second endoglucanase is an endoglucanase comprising the amino acid sequence of the *Humicola insolens* endoglucanase shown in SEQ ID No. 3 or is an analogue of said endoglucanase which is at least 60% homologous with the sequence shown in SEQ ID No. 3, reacts with an antibody raised  
10 against said endoglucanase, and/or is encoded by a DNA sequence which hybridizes with the DNA sequence encoding said endoglucanase.

In the process of the invention, the first and second endoglucanase, respectively, can be used in an amount of corresponding to a cellulase activity between 5 and 8,000 ECU per  
15 litre of desizing/"stone-washing" liquor, preferably between 10 and 5000 ECU per litre of liquor, and more preferably between 50 and 500 ECU per litre of liquor. The first and second endoglucanase, respectively, is preferably dosed in an amount  
20 corresponding to 0.01-40 mg endoglucanase/l, more preferably 0.1-2.5 mg/l, especially 0.1-1.25 mg/l.

The substrate of the process of the invention is dyed denim. The denim may be dyed with a natural or a synthetic dye. Examples of synthetic dyes are direct dyes, fiber-reactive dyes  
25 or indirect dyes. In a preferred embodiment, the denim is dyed with indigo. Typically, the denim is cut and sown into garment before subjected to the process of the present invention. Examples of garment are jeans, jackets and skirts. An especially preferred example is indigo-dyed denim jeans.

30 In the process of the invention, conventional desizing enzymes, in particular amylolytic enzymes, can be used in order to remove starch-containing size.

Therefore, an amylolytic enzyme, preferably an  $\alpha$ -amylase, may be added during the process of the invention. Con-  
35 ventionally, bacterial  $\alpha$ -amylases are used for the desizing, e.g. an  $\alpha$ -amylases derived from a strain of *Bacillus*, particularly a

strain of *Bacillus licheniformis*, a strain of *Bacillus amyloliquefaciens*, or a strain of *Bacillus stearothermophilus*; or mutants thereof. Amino acid sequences of such amylases are apparent from, e.g., WO 95/21247. Examples of suitable commercial  $\alpha$ -amylase products are Termamyl™, Aquazym™ Ultra and Aquazym™ (available from Novo Nordisk A/S, Denmark). However, also fungal  $\alpha$ -amylases can be used. Examples of fungal  $\alpha$ -amylases are those derived from a strain of *Aspergillus*. Other useful  $\alpha$ -amylases are the oxidation-stable  $\alpha$ -amylase mutants disclosed in WO 95/21247. For instance, an  $\alpha$ -amylase mutant prepared from a parent  $\alpha$ -amylase by replacing one or more of the methionine amino acid residues with a Leu, Thr, Ala, Gly, Ser, Ile, Asn, or Asp amino acid residue, preferably a Leu, Thr, Ala, or Gly amino acid residue. Of particular interest is an  $\alpha$ -amylase mutant prepared from the *B. licheniformis*  $\alpha$ -amylase in which the methionine at position 197 has been replaced with any other amino acid residue, in particular with Leu, Thr, Ala, Gly, Ser, Ile, Asn, or Asp amino acid residue, preferably a Leu, Thr, Ala, or Gly amino acid residue.

The amylolytic enzyme may be added in amounts conventionally used in desizing processes, e.g. corresponding to an  $\alpha$ -amylase activity of from about 10 to about 10,000 KNU/l such as from 100 to about 10,000 KNU/l or from 10 to about 5,000 KNU/l. Also, in the process according to the present invention, 1-10 mM of Ca<sup>++</sup> may be added as a stabilizing agent.

The process of the present invention may be accomplished at process conditions conventionally prevailing in desizing/"stone-washing" processes, as carried out by the person skilled in the art. The process of the invention may, e.g., be carried out batch-wise in a washer extractor.

It is at present contemplated that a suitable liquor/textile ratio may be in the range of from about 20:1 to about 1:1, preferably in the range of from about 15:1 to about 5:1.

In conventional desizing and "stone-washing" processes, the

reaction time is usually in the range of from about 1 hour to about 24 hours. However, in the process of the present invention the reaction time may well be less than 1 hour, i.e. from about 5 minutes to about 55 minutes. Preferably the reaction time is within the range of from about 5 or 10 to about 120 minutes.

The pH of the reaction medium greatly depends on the enzyme in question. Preferably the process of the invention is carried out at a pH in the range of from about pH 3 to about pH 11, preferably in the range of from about pH 6 to about pH 9, or within the range of from about pH 5 to about pH 8.

A buffer may be added to the reaction medium to maintain a suitable pH for the enzymes used. The buffer may suitably be a phosphate, borate, citrate, acetate, adipate, triethanolamine, monoethanolamine, diethanolamine, carbonate (especially alkali metal or alkaline earth metal, in particular sodium or potassium carbonate, or ammonium and HCl salts), diamine, especially diaminoethane, imidazole, or amino acid buffer.

The process of the invention may be carried out in the presence of conventional textile finishing agents, including wetting agents, polymeric agents, dispersing agents, etc.

A conventional wetting agent may be used to improve the contact between the substrate and the enzymes used in the process. The wetting agent may be a nonionic surfactant, e.g. an ethoxylated fatty alcohol, an ethoxylated oxo alcohol, an ethoxylated alkyl phenol or an alkoxyated fatty alcohol.

Examples of suitable polymers include proteins (e.g. bovine serum albumin, whey, casein or legume proteins), protein hydrolysates (e.g. whey, casein or soy protein hydrolysate), polypeptides, lignosulfonates, polysaccharides and derivatives thereof, polyethylene glycol, polypropylene glycol, polyvinyl pyrrolidone, ethylene diamine condensed with ethylene or propylene oxide, ethoxylated polyamines, or ethoxylated amine polymers.

The dispersing agent may suitably be selected from nonionic, anionic, cationic, ampholytic or zwitterionic surfactants. More specifically, the dispersing agent may be selected from carboxymethylcellulose, hydroxypropylcellulose,

alkyl aryl sulphonates, long-chain alcohol sulphates (primary and secondary alkyl sulphates), sulphonated olefins, sulphated monoglycerides, sulphated ethers, sulphosuccinates, sulphated methyl ethers, alkane sulphonates, phosphate esters, alkyl  
5 isothionates, acylsarcosides, alkyltaurides, fluorosurfactants, fatty alcohol and alkylphenol condensates, fatty acid condensates, condensates of ethylene oxide with an amine, condensates of ethylene oxide with an amide, sucrose esters, sorbitan esters, alkyloamides, fatty amine oxides, ethoxylated  
10 monoamines, ethoxylated diamines, alcohol ethoxylate and mixtures thereof.

In another preferred embodiment of the invention, the process may be performed using a lipolytic enzyme that is capable of carrying out lipolysis at elevated temperatures. In order to  
15 efficiently hydrolyse hydrophobic esters of high melting points, lipolytic enzymes that possess sufficient thermostability and lipolytic activity at temperatures of about 60°C or above, are preferred. Adequate hydrolysis can be obtained even above or below the optimum temperature of the lipolytic enzyme by  
20 increasing the enzyme dosage.

The lipolytic enzyme may be of animal, plant or microbial origin. Examples of microorganisms producing such thermostable lipolytic enzymes are strains of *Humicola*, preferably a strain of *Humicola brevispora*, a strain of *Humicola lanuginosa*, a  
25 strain of *Humicola brevis var. thermoidea*, a strain of *Humicola insolens*, a strain of *Fusarium*, preferably a strain of *Fusarium oxysporum*, a strain of *Rhizomucor*, preferably a strain of *Rhizomucor miehei*, a strain of *Chromobacterium*, preferably a strain of *Chromobacterium viscosum*, and a strain of *Aspergillus*, preferably a strain of *Aspergillus niger*. Preferred thermostable  
30 lipolytic enzymes are derived from strains of *Candida* or *Pseudomonas*, particularly a strain of *Candida antarctica*, a strain of *Candida tsukubaensis*, a strain of *Candida auriculariae*, a strain of *Candida humicola*, a strain of *Candida foliarum*, a strain of *Candida cylindracea* (also called *Candida rugosa*), a strain of *Pseudomonas cepacia*, a strain of  
35 *Pseudomonas fluorescens*, a strain of *Pseudomonas fragi*, a strain

of *Pseudomonas stutzeri*, or a strain of *Thermomyces lanuginosus*.

Lipolytic enzymes from strains of *Candida antarctica* and *Pseudomonas cepacia* are preferred, in particular lipase A from *Candida antarctica*. Such lipolytic enzymes, and methods for their production, are known from e.g. WO 88/02775, US 4,876,024, and WO 89/01032, which publications are hereby included by reference.

The enzyme dosage is dependent upon several factors, including the enzyme in question, the desired reaction time, the temperature, the liquid/textile ratio, etc. It is at present contemplated that the lipolytic enzyme may be dosed in an amount corresponding to of from about 0.01 to about 10,000 KLU/l, preferably of from about 0.1 to about 1000 KLU/l.

Conventional finishing agents that may be present in a process of the invention include, but are not limited to pumice stones and perlite. Perlite is a naturally occurring volcanic rock. Preferably, heat expanded perlite may be used. The heat expanded perlite may e.g. be present in an amount of 20-95 w/w% based on the total weight of the composition.

### **Cellulytic Activity**

The cellulytic activity may be measured in endo-cellulase units (ECU), determined at pH 7.5, with carboxymethyl cellulose (CMC) as substrate.

The ECU assay quantifies the amount of catalytic activity present in the sample by measuring the ability of the sample to reduce the viscosity of a solution of carboxy-methylcellulose (CMC). The assay is carried out at 40°C; pH 7.5; 0.1M phosphate buffer; time 30 min; using a relative enzyme standard for reducing the viscosity of the CMC Hercules 7 LFD substrate; enzyme concentration approx. 0.15 ECU/ml. The arch standard is defined to 8200 ECU/g.

### **Amylolytic Activity**

The amylolytic activity may be determined using potato starch as substrate. This method is based on the break-down of modified potato starch by the enzyme, and the reaction is

followed by mixing samples of the starch/enzyme solution with an iodine solution. Initially, a blackish-blue colour is formed, but during the break-down of the starch the blue colour gets weaker and gradually turns into a reddish-brown, which is compared to a coloured glass standard.

One Kilo Novo alfa Amylase Unit (KNU) is defined as the amount of enzyme which, under standard conditions (i.e. at 37°C +/- 0.05; 0.0003 M Ca<sup>2+</sup>; and pH 5.6) dextrinizes 5.26 g starch dry substance Merck Amylum solubile.

A folder AF 9/6 describing this analytical method in more detail is available upon request to Novo Nordisk A/S, Denmark, which folder is hereby included by reference.

#### **Lipolytic Activity**

The lipolytic activity may be determined using tributyrine as substrate. This method is based on the hydrolysis of tributyrin by the enzyme, and the alkali consumption is registered as a function of time.

One Lipase Unit (LU) is defined as the amount of enzyme which, under standard conditions (i.e. at 30.0°C; pH 7.0; with Gum Arabic as emulsifier and tributyrine as substrate) liberates 1 µmol titrable butyric acid per minute (1 KLU = 1000 LU).

A folder AF 95/5 describing this analytical method in more detail is available upon request to Novo Nordisk A/S, Denmark, which folder is hereby included by reference.

#### **EXAMPLE 1**

The following example illustrates the effect of adding a streak-reducing or levelling endoglucanase to the combined desizing-abrasion process in order to reduce the number of streaks on denim jeans or other garment and to produce denim garment, especially jeans, with a uniformly localized color variation.

Wash trials were carried out under the following conditions:

**Textile:**

Blue denim DAKOTA, 14½ oz, 100 % cotton.

The denim was cut and sewed into "legs" of approximately 37.5x100 cm (about 375 g each).

5 Two new legs and one old (used one time) leg were used in each trial (a total of approx. 1100 g textile).

**Enzyme:**

Trial A: Amylase: Termamyl®, dosage: 200 KNU/l

10 Endoglucanase (cellulase):

EG V (a monocomponent ~43 kD endoglucanase from *Humicola insolens*, DSM 1800, having the amino acid sequence of SEQ ID No. 1),

dosage: 10 ECU/g denim

15

Trial B: Amylase: Termamyl®, dosage: 200 KNU/l

Endoglucanase (cellulase):

EG V (as in trial A), dosage: 10 ECU/g denim

20 EG I (monocomponent endoglucanase from *Humicola insolens*, DSM 1800, having the amino acid sequence of SEQ ID No. 3), dosage: 10 ECU/g denim

**Washing** was carried out in a wascator (FOM71 LAB).

Wash-program:

25 1) Main wash at 55°C, 20 l water, 120 min, buffer and enzyme added.

Buffer: 30 g KH<sub>2</sub>PO<sub>4</sub> + 20 g Na<sub>2</sub>HPO<sub>4</sub>, pH7

2) Drain 30 sec.

30 3) Rinse at 80°C, normal action, 32 l water, 15 min.; 20 g Na<sub>2</sub>CO<sub>3</sub> added

4) Drain 30 sec.

5) Rinse at 54°C, normal action, 32 l water, 5 min.

35 6) Drain 30 sec.

7) Rinse at 14°C, normal action, 32 l water,

5 min.

8) Drain 30 sec.

9) Spinning 40 sec. at low speed and 50 sec. at high speed.

5

**Drying:** The samples were dried in a tumble-dryer.

The jeans from the two trials were abraded to almost the same level.

10 **Evaluation:**

5 persons skilled in the art of evaluating denim were asked to grade the denim legs (two legs from each trial, leg "1" and "3" from trial B, leg "2" and "4" from trial A) from 1 to 4, where 1 was the least streaked denim leg and 4 was the leg with most streaks on.

15

Grading were as shown in the table below:

	Person 1	Person 2	Person 3	Person 4	Person 5
Grade 1	1	3	3	3	3
Grade 2	3	1	1	1	1
Grade 3	4	2	2	2	2
Grade 4	2	4	4	4	4

20 As can be seen from the table, the denim legs treated in the combi-process of the invention with a combination of two monocomponent endoglucanases having abrading and strak-reducing properties, respectively, e.g. an EG V type and EG I type cellulase, are all rated to have the best appearance with respect to streaking and uniformity of the localized color variation.

25

**Figures 1 and 2:**

To illustrate the change in uniformity that can be obtained by using a streak-reducing or levelling endoglucanase



(cellulase) in the process of the invention, swatches from trial A and B were scanned (HP ScanJet II CX) into a computer and printed in black-and-white.

Figure 1 show part of a denim leg from trial B and figure  
5 2 show part of a denim leg from trial A.

## SEQUENCE LISTING

## INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 415 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## 10 (vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Humicola insolens*  
 (B) STRAIN: DSM 1800

## SEQUENCE DESCRIPTION: SEQ ID NO:1:

15

Gln Lys Pro Gly Glu Thr Lys Glu Val His Pro Gln Leu Thr Thr Phe  
 1 5 10 15

20

Arg Cys Thr Lys Arg Gly Gly Cys Lys Pro Ala Thr Asn Phe Ile Val  
 20 25 30

Leu Asp Ser Leu Ser His Pro Ile His Arg Ala Glu Gly Leu Gly Pro  
 35 40 45

25

Gly Gly Cys Gly Asp Trp Gly Asn Pro Pro Pro Lys Asp Val Cys Pro  
 50 55 60

Asp Val Glu Ser Cys Ala Lys Asn Cys Ile Met Glu Gly Ile Pro Asp  
 65 70 75 80

30

Tyr Ser Gln Tyr Gly Val Thr Thr Asn Gly Thr Ser Leu Arg Leu Gln  
 85 90 95

35

His Ile Leu Pro Asp Gly Arg Val Pro Ser Pro Arg Val Tyr Leu Leu  
 100 105 110

Asp Lys Thr Lys Arg Arg Tyr Glu Met Leu His Leu Thr Gly Phe Glu  
 115 120 125

40

Phe Thr Phe Asp Val Asp Ala Thr Lys Leu Pro Cys Gly Met Asn Ser  
 130 135 140

Ala Leu Tyr Leu Ser Glu Met His Pro Thr Gly Ala Lys Ser Lys Tyr  
 145 150 155 160

5 Asn Pro Gly Gly Ala Tyr Tyr Gly Thr Gly Tyr Cys Asp Ala Gln Cys  
 165 170 175

Phe Val Thr Pro Phe Ile Asn Gly Leu Gly Asn Ile Glu Gly Lys Gly  
 180 185 190

10 Ser Cys Cys Asn Glu Met Asp Ile Trp Glu Ala Asn Ser Arg Ala Ser  
 195 200 205

His Val Ala Pro His Thr Cys Asn Lys Lys Gly Leu Tyr Leu Cys Glu  
 210 215 220

15 Gly Glu Glu Cys Ala Phe Glu Gly Val Cys Asp Lys Asn Gly Cys Gly  
 225 230 235 240

20 Trp Asn Asn Tyr Arg Val Asn Val Thr Asp Tyr Tyr Gly Arg Gly Glu  
 245 250 255

Glu Phe Lys Val Asn Thr Leu Lys Pro Phe Thr Val Val Thr Gln Phe  
 260 265 270

25 Leu Ala Asn Arg Arg Gly Lys Leu Glu Lys Ile His Arg Phe Tyr Val  
 275 280 285

Gln Asp Gly Lys Val Ile Glu Ser Phe Tyr Thr Asn Lys Glu Gly Val  
 290 295 300

30 Pro Tyr Thr Asn Met Ile Asp Asp Glu Phe Cys Glu Ala Thr Gly Ser  
 305 310 315 320

35 Arg Lys Tyr Met Glu Leu Gly Ala Thr Gln Gly Met Gly Glu Ala Leu  
 325 330 335

Thr Arg Gly Met Val Leu Ala Met Ser Ile Trp Trp Asp Gln Gly Gly  
 340 345 350

40 Asn Met Glu Trp Leu Asp His Gly Glu Ala Gly Pro Cys Ala Lys Gly  
 355 360 365

Glu Gly Ala Pro Ser Asn Ile Val Gln Val Glu Pro Phe Pro Glu Val  
 370 375 380

Thr Tyr Thr Asn Leu Arg Trp Gly Glu Ile Gly Ser Thr Tyr Gln Glu  
 385 390 395 400

5 Val Gln Lys Pro Lys Pro Lys Pro Gly His Gly Pro Arg Ser Asp  
 405 410 415

INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 409 amino acids
- 10 (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- 15 (A) ORGANISM: *Fusarium oxysporum*
- (B) STRAIN: DSM 2672

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

20 Gln Thr Pro Asp Lys Ala Lys Glu Gln His Pro Lys Leu Glu Thr Tyr  
 1 5 10 15

Arg Cys Thr Lys Ala Ser Gly Cys Lys Lys Gln Thr Asn Tyr Ile Val  
 20 25 30

25 Ala Asp Ala Gly Ile His Gly Ile Arg Arg Ser Ala Gly Cys Gly Asp  
 35 40 45

30 Trp Gly Gln Lys Pro Asn Ala Thr Ala Cys Pro Asp Glu Ala Ser Cys  
 50 55 60

Ala Lys Asn Cys Ile Leu Ser Gly Met Asp Ser Asn Ala Tyr Lys Asn  
 65 70 75 80

35 Ala Gly Ile Thr Thr Ser Gly Asn Lys Leu Arg Leu Gln Gln Leu Ile  
 85 90 95

Asn Asn Gln Leu Val Ser Pro Arg Val Tyr Leu Leu Glu Glu Asn Lys  
 100 105 110

40

Lys Lys Tyr Glu Met Leu His Leu Thr Gly Thr Glu Phe Ser Phe Asp  
 115 120 125

Val Glu Met Glu Lys Leu Pro Cys Gly Met Asn Gly Ala Leu Tyr Leu  
 5 130 135 140

Ser Glu Met Pro Gln Asp Gly Gly Lys Ser Thr Ser Arg Asn Ser Lys  
 145 150 155 160

10 Ala Gly Ala Tyr Tyr Gly Ala Gly Tyr Cys Asp Ala Gln Cys Tyr Val  
 165 170 175

Thr Pro Phe Ile Asn Gly Val Gly Asn Ile Lys Gly Gln Gly Val Cys  
 15 180 185 190

Cys Asn Glu Leu Asp Ile Trp Glu Ala Asn Ser Arg Ala Thr His Ile  
 195 200 205

Ala Pro His Pro Cys Ser Lys Pro Gly Leu Tyr Gly Cys Thr Gly Asp  
 20 210 215 220

Glu Cys Gly Ser Ser Gly Ile Cys Asp Lys Ala Gly Cys Gly Trp Asn  
 225 230 235 240

25 His Asn Arg Ile Asn Val Thr Asp Phe Tyr Gly Arg Gly Lys Gln Tyr  
 245 250 255

Lys Val Asp Ser Thr Arg Lys Phe Thr Val Thr Ser Gln Phe Val Ala  
 260 265 270

30 Asn Lys Gln Gly Asp Leu Ile Glu Leu His Arg His Tyr Ile Gln Asp  
 275 280 285

Asn Lys Val Ile Glu Ser Ala Val Val Asn Ile Ser Gly Pro Pro Lys  
 35 290 295 300

Ile Asn Phe Ile Asn Asp Lys Tyr Cys Ala Ala Thr Gly Ala Asn Glu  
 305 310 315 320

40 Tyr Met Arg Leu Gly Gly Thr Lys Gln Met Gly Asp Ala Met Ser Arg  
 325 330 335

Gly Met Val Leu Ala Met Ser Val Trp Trp Ser Glu Gly Asp Phe Met  
 340 345 350

Ala Trp Leu Asp Gln Gly Val Ala Gly Pro Cys Asp Ala Thr Glu Gly  
 355 360 365

Asp Pro Lys Asn Ile Val Lys Val Gln Pro Asn Pro Glu Val Thr Phe  
 5 370 375 380

Ser Asn Ile Arg Ile Gly Glu Ile Gly Ser Thr Ser Ser Val Lys Ala  
 385 390 395 400

10 Pro Ala Tyr Pro Gly Pro His Arg Leu  
 405

INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - 15 (A) LENGTH: 415 (435) amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- 20 (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Humicola insolens
  - (B) STRAIN: DSM 1800

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:  
 25

SEQUENCE LISTING

(May be renumbered from -21 to 415, total 435 aa)

Met Ala Arg Gly Thr Ala Leu Leu Gly Leu Thr Ala Leu Leu Leu Gly  
 30 1 5 10 15

Leu Val Asn Gly Gln Lys Pro Gly Glu Thr Lys Glu Val His Pro Gln  
 20 25 30

35 Leu Thr Thr Phe Arg Cys Thr Lys Arg Gly Gly Cys Lys Pro Ala Thr  
 35 40 45

Asn Phe Ile Val Leu Asp Ser Leu Ser His Pro Ile His Arg Ala Glu  
 50 55 60

40 Gly Leu Gly Pro Gly Gly Cys Gly Asp Trp Gly Asn Pro Pro Pro Lys  
 65 70 75 80

Asp Val Cys Pro Asp Val Glu Ser Cys Ala Lys Asn Cys Ile Met Glu  
 85 90 95

Gly Ile Pro Asp Tyr Ser Gln Tyr Gly Val Thr Thr Asn Gly Thr Ser  
 5 100 105 110

Leu Arg Leu Gln His Ile Leu Pro Asp Gly Arg Val Pro Ser Pro Arg  
 115 120 125

10 Val Tyr Leu Leu Asp Lys Thr Lys Arg Arg Tyr Glu Met Leu His Leu  
 130 135 140

Thr Gly Phe Glu Phe Thr Phe Asp Val Asp Ala Thr Lys Leu Pro Cys  
 145 150 155 160

15 Gly Met Asn Ser Ala Leu Tyr Leu Ser Glu Met His Pro Thr Gly Ala  
 165 170 175

Lys Ser Lys Tyr Asn Ser Gly Gly Ala Tyr Tyr Gly Thr Gly Tyr Cys  
 20 180 185 190

Asp Ala Gln Cys Phe Val Thr Pro Phe Ile Asn Gly Leu Gly Asn Ile  
 195 200 205

25 Glu Gly Lys Gly Ser Cys Cys Asn Glu Met Asp Ile Trp Glu Val Asn  
 210 215 220

Ser Arg Ala Ser His Val Val Pro His Thr Cys Asn Lys Lys Gly Leu  
 225 230 235 240

30 Tyr Leu Cys Glu Gly Glu Glu Cys Ala Phe Glu Gly Val Cys Asp Lys  
 245 250 255

Asn Gly Cys Gly Trp Asn Asn Tyr Arg Val Asn Val Thr Asp Tyr Tyr  
 35 260 265 270

Gly Arg Gly Glu Glu Phe Lys Val Asn Thr Leu Lys Pro Phe Thr Val  
 275 280 285

40 Val Thr Gln Phe Leu Ala Asn Arg Arg Gly Lys Leu Glu Lys Ile His  
 290 295 300

Arg Phe Tyr Val Gln Asp Gly Lys Val Ile Glu Ser Phe Tyr Thr Asn  
 305 310 315 320

Lys Glu Gly Val Pro Tyr Thr Asn Met Ile Asp Asp Glu Phe Cys Glu  
 325 330 335

Ala Thr Gly Ser Arg Lys Tyr Met Glu Leu Gly Ala Thr Gln Gly Met  
 5 340 345 350

Gly Glu Ala Leu Thr Arg Gly Met Val Leu Ala Met Ser Ile Trp Trp  
 355 360 365

10 Asp Gln Gly Gly Asn Met Glu Trp Leu Asp His Gly Glu Ala Gly Pro  
 370 375 380

Cys Ala Lys Gly Glu Gly Ala Pro Ser Asn Ile Val Gln Val Glu Pro  
 385 390 395 400

15 Phe Pro Glu Val Thr Tyr Thr Asn Leu Arg Trp Gly Glu Ile Gly Ser  
 405 410 415

Thr Tyr Gln Glu Val Gln Lys Pro Lys Pro Lys Pro Gly His Gly Pro  
 20 420 425 430

Arg Ser Asp  
 435



## CLAIMS

1. A one-step process for combined desizing and "stone-washing" of dyed denim, wherein the denim is treated with an amylolytic enzyme in combination with a first abrading monocomponent endoglucanase and a second streak-reducing monocomponent endoglucanase.

2. The process according to claim 1, wherein the amylolytic enzyme is an  $\alpha$ -amylase, preferably a microbial  $\alpha$ -amylase, such as a bacterial or a fungal  $\alpha$ -amylase.

3. The process according to claim 2, wherein the  $\alpha$ -amylase is producible by the bacterium *Bacillus*, or by the fungus *Aspergillus*.

4. The process according to claim 2, wherein the  $\alpha$ -amylase is producible by the *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Bacillus subtilis* or *Bacillus stearothermophilus*; or mutants thereof.

5. The process according to claim 4, wherein the  $\alpha$ -amylase are selected from the oxidation-stable  $\alpha$ -amylase mutants disclosed in International Patent Application PCT/DK94/00371 (WO 95/21247).

6. The process according to any of the claims 1-5, wherein the first endoglucanase is a fungal EG V type cellulase, or a fungal EG III type cellulase obtainable from a strain of the genus *Trichoderma*.

7. The process according to claim 6, wherein the EG V type endoglucanase is derived from or producible by a strain of *Scytalidium* (f. *Humicola*), *Fusarium*, or *Myceliophthora*.

8. The process according to claim 7, wherein the EG V is derived from or producible by *Scytalidium thermophilum* (f. *Humicola insolens*), *Fusarium oxysporum* or *Myceliophthora themophila*, preferably from *Humicola insolens*, DSM 1800, *Fusarium oxysporum*, DSM 2672, or *Myceliophthora themophila*, CBS 117.65.

9. The process according to claim 8, in which the endoglucanase comprises the amino acid sequence of the *Humicola*

*insolens* endoglucanase shown in SEQ ID No. 1 or is an analogue of said endoglucanase which

i) is at least 60% homologous with the sequence shown in SEQ ID No. 1,

5 ii) reacts with an antibody raised against said endoglucanase, and/or

iii) is encoded by a DNA sequence which hybridizes with the DNA sequence encoding said endoglucanase.

10 10. The process according to claim 8, in which the endoglucanase comprises the amino acid sequence of the *Fusarium oxysporum* endoglucanase shown in SEQ ID No. 2 or is an analogue of said endoglucanase which

i) is at least 60% homologous with the sequence shown in SEQ ID No. 2,

15 ii) reacts with an antibody raised against said endoglucanase, and/or

iii) is encoded by a DNA sequence which hybridizes with the DNA sequence encoding said endoglucanase.

20 11. The process according to any of the claims 1 - 10, wherein the second endoglucanase has a catalytic activity on celotriose at pH 8.5 corresponding to  $k_{cat}$  of at least  $0.01 \text{ s}^{-1}$ , preferably of at least  $0.1 \text{ s}^{-1}$ , more preferably of at least  $1 \text{ s}^{-1}$ .

25 12. The process according to claim 11, wherein the second endoglucanase is obtainable by or derived from a strain of *Humicola*, *Trichoderma*, *Myceliophthora*, *Penicillium*, *Irpex*, *Aspergillus*, *Scytalidium* or *Fusarium*.

30 13. The process according to claim 12, wherein the endoglucanase is derivable from a strain of *Humicola insolens*, *Fusarium oxysporum* or *Trichoderma reesei*.

14. The process according to claim 12, in which the endoglucanase comprises the amino acid sequence of the *Humicola insolens* endoglucanase shown in SEQ ID No. 3 or is an analogue of said endoglucanase which

35 i) is at least 60% homologous with the sequence shown in SEQ ID No. 3,

ii) reacts with an antibody raised against said endoglucanase, and/or

iii) is encoded by a DNA sequence which hybridizes with the DNA sequence encoding said endoglucanase.

5 15. The process according to any of the claims 1-14, in which the first and second endoglucanase, respectively, is used in an amount of corresponding to a cellulase activity between 5 and 8000 ECU per litre of desizing/"stone-washing" liquor, preferably between 50 and 500 ECU per litre of liquor.

10 16. The process according to any of the claims 1-15, in which the treatment is performed at a temperature in the range of 30-100°C, preferably 30-60°C, and a pH in the range of 3-11, preferably 7-9.

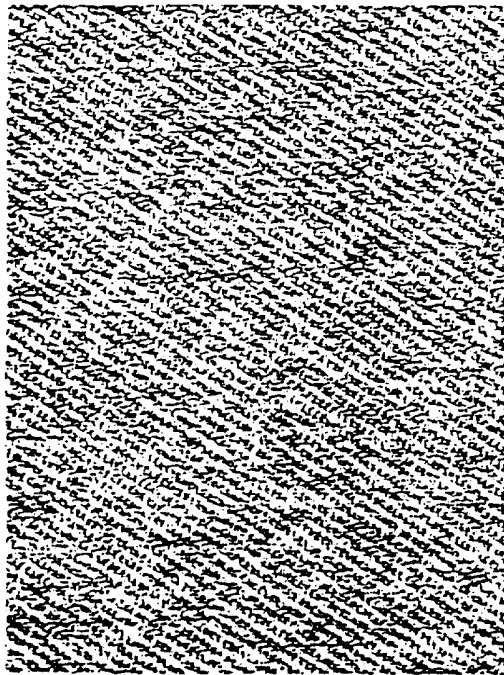
15 17. The process according to any of the claims 1-16, wherein denim is dyed with a natural dye, preferably indigo; or a synthetic dye, preferably direct dye, indirect dye and fiber-reactive dye.

20 18. The process according to any of the claims 1-17, in which the denim additionally is treated with a thermostable lipolytic enzyme; preferably a thermostable lipolytic enzyme derived from a strain of *Pseudomonas*, more preferably a strain of *Pseudomonas fragi*; a strain of *Pseudomonas stutzeri*, a strain of *Pseudomonas cepacia*, a strain of *Pseudomonas fluorescens*, or a strain of *Candida*, preferably a strain of *Candida cylindracea* (also called *Candida rugosa*), or a strain of *Candida antarctica*.

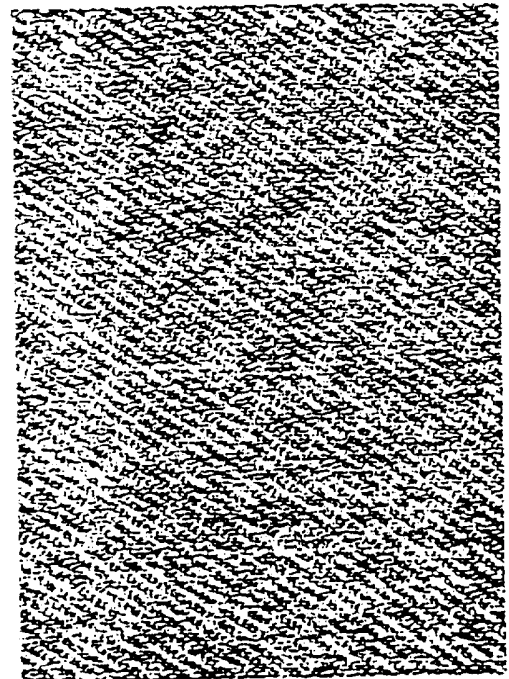
25 19. The process according to claim 18, in which the lipolytic enzyme is dosed in an amount of from about 0.01 to about 10,000 KLU/l, preferably of from about 0.1 to about 1000 KLU/l.

30 20. The process according to any of the claims 1-19, in which the  $\alpha$ -amylase is dosed in an amount of from about 100 to about 10,000 KNU/l.

1/1



**Fig. 1**



**Fig. 2**

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 96/00469

A. CLASSIFICATION OF SUBJECT MATTER		
IPC6: C11D 3/386, D06L 1/14, D06M 16/00 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)		
IPC6: C11D, D06M		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
SE,DK,FI,NO classes as above		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5460966 A (MICHAEL W. DIXON), 24 October 1995 (24.10.95), column 1, line 23 - line 35; column 5, line 1 - line 7 --	1-17
Y	WO 9117243 A1 (NOVO NORDISK A/S), 14 November 1991 (14.11.91), see example 6 --	1-4,6
Y	WO 9407983 A1 (GENENCOR INTERNATIONAL, INC.), 14 April 1994 (14.04.94), page 12, line 31 - page 13, line 16, and claims --	1,6,11-13
Y	WO 9524471 A1 (NOVO NORDISK A/S), 14 Sept 1995 (14.09.95), figures 1-3, claims 20 and 21 --	1,6-17
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> 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 document 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
17 February 1997		28.02.97
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86		Authorized officer  Carl-Olof Gustafsson Telephone No. +46 8 782 25 00

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 96/00469

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9521247 A1 (NOVO NORDISK A/S), 10 August 1995 (10.08.95)  -- -----	5

# INTERNATIONAL SEARCH REPORT

Information on patent family members

03/02/97

International application No.

PCT/DK 96/00469

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 5460966	24/10/95	BE-A- 1008269	05/03/96
		DE-A- 4407801	22/09/94
		FR-A,B- 2702779	23/09/94
		GB-A,B- 2276178	21/09/94
		GB-D- 9404712	00/00/00
		IT-D- RM940135	00/00/00
		-----	
WO-A1- 9117243	14/11/91	AT-T- 118545	15/03/95
		AU-B- 639570	29/07/93
		AU-A- 7887491	27/11/91
		CA-A- 2082279	10/11/91
		DE-D,T- 69107455	12/10/95
		EP-A,B- 0531372	17/03/93
		ES-T- 2068586	16/04/95
		JP-T- 5509223	22/12/93
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		EP-A- 0663004	19/07/95
		JP-T- 8502101	05/03/96
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WO-A1- 9524471	14/09/95	AU-A- 1890095	25/09/95
		DE-U- 29509606	08/02/96
		EP-A- 0749473	27/12/96
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WO-A1- 9521247	10/08/95	AU-A- 7807594	21/08/95
		EP-A- 0742817	20/11/96
		FI-A- 963043	01/08/96
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