(54) Title: ENZYMATIC TREATMENT FOR THE LEATHER PROCESS

(57) Abstract: The present invention relates to a method for the processing of hides or skins into leather, comprising enzymatic treatment of the hide or skin with carbohydrate in soaking step. The present invention can achieve optimal fiber opening result in a relatively short period of time and in the same time does not cause loose grain, and meanwhile the pollution or impact on environment is reduced in a maximum way.
ENZYMATIC TREATMENT FOR THE LEATHER PROCESS

REFERENCE TO A 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 for the processing of hides and skins into leather. More specifically, the present invention relates to a method for the processing of hides or skins into leather, comprising carbohydrazte treatment of the hide or skin in soaking step.

BACKGROUND OF THE INVENTION
The hides and skins are usually received in the tanneries in the form of salted or dried raw hides or skins. The processing of hides or skins into leather comprises several different process steps including the steps of soaking, unhairing, liming, deliming, batting, pickling and tanning. These steps constitute the wet processing and are performed in the beamhouse. The traditional use of enzymes in a process for manufacturing leather is in the steps of soaking, unhairing, batting and tanning. In these processes, proteases are widely used to achieve a partial degradation of the proteins, thus making the pelt soft, supple and ready for subsequent tannage (WO 96/1 1285). WO2004/040021 describes the leather processing step: after soaking, protease is used for unhairing, and other enzymes such as amylase are added onto dehaired goatskin. WO2008/093353 relates to the use of proteolytic enzyme and carbohydrolytic enzyme in combination for unhairing.

The soaking of hides and/or skins is a critical operational step at the beginning of beamhouse operation in leather and wool-on/fur production. The conventional soaking serves to clean adhering dirt from the raw hide and skins, to remove curing salt and other preserving agents from the hides and skins, to dissolve out water soluble protein components at least partially, and to return the hides and/or skins to the degree of swelling which they had in their original condition but was lost in the course of the curing process.

Due to its importance, soaking has been studied broadly for a long time and various methods have been developed to achieve a better result. Soaking of skins and hides is currently accomplished by use of surfactants, amines and organic solvents. Recently the use of enzymes, such as protease and/or lipase in soaking has been suggested, thereby reducing or even avoiding the use of surfactants or substituting organic solvents (US5089414).
When compared to traditional methods, enzymatic soaking processes generally improve the quality of the final leather, reduce the use of chemicals and replace chemicals which have an adverse effect on the environment.

5 SUMMARY OF THE INVENTION

It is an object of the present invention to provide an enzymatic process for the preparation of leather, which process comprises carbohydrase treatment of the hide or skin in soaking step. The present invention further provides the process comprising carbohydrase in combination with lipase in soaking step.

Optionally, such enzymatic treatment can also be used in a presoaking step. Optionally, the soaking step of the present invention is followed by a conventional unhairing step, or unhairing step with the use of protease.

The process of the present invention can achieve optimal result of the removal of interfibrillar material in a relatively short period of time and in the same time does not cause loose grain. As the invention is a pure enzymatic process, the pollution or impact on environment is reduced in a maximum way.

So far, the removal of interfibrillar materials is mainly done in a soaking step with protease or in a liming step. The use of protease in soaking is somehow risky as other proteins might be damaged thus causing grain damage or loose grain, especially when the raw hide/skin is not well preserved. Soaking is the first important step in leather making, which is unavoidable. If removal of interfibrillar materials can be done in soaking, the leather making will be less dependent on liming process or even skip the liming process which generates most pollutants. Liming is the most time consuming step and moreover this process is difficult to control.

In the present invention, the use of carbohydrase such as alpha-amylase has a positive effect on the removal of interfibrillar material and the associated opening up of the fibre structure and general cleaning of the substrate. Once the enzyme assists the opening up of the fibre structure, this would be expected to enhance the unhairing by allowing better penetration of the unhairing chemicals or protease into the skin or hide. In other words, the present invention provides a better new way for leather making.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method for the processing of hides or skins into leather, comprising enzymatic treatment of the hide or skin with carbohydrase, and preferably carbohydrase in combination with lipase in soaking step.

The Processing of Hides or Skins into Leather
The hides and skins are usually received in the tanneries in the form of salted or dried raw hides or skins. The processing of hides or skins into leather comprises several different process steps including the steps of soaking, unhairing, liming, deliming, bating, pickling and tanning. These steps constitute the wet processing and are performed in the beamhouse.

Processes for the manufacture of leather are well known to the person skilled in the art and have been described in e.g. WO 94/06942, WO 90/12118, US 3840433, EP-A1-505920, GB-A 2233665 and US 3986926.

In the present invention, the enzymatic treatment with carbohydrase takes place during soaking, and even in the presoaking step. Preferably, enzymatic treatment with carbohydrase and lipase will enhance the presoaking and/or soaking effect.

The process of the present invention may be applied to any skin or hide conventionally used for leather manufacturing. In particular, the process of the invention may be applied to ovine skins, to porcine skins, to bovine hides, or to caprine skins in dry or wet conditions.

The object of the soaking step is to restore lost moisture to the salted and dried skins. In tanning industry, normally the hides or skins are washed to remove dirt, blood, salt, etc. on the surface before the soaking process, which is known as presoaking step. The purpose of presoaking is to remove the dirt, blood, salt on the surface. Furthermore, to wash out as much salt as possible from the hide/skin to get a better soaking effect in the main soaking step. Between presoaking and main soaking, fleshing can be done in order to get the optimal result of the main soak.

The soaking is generally carried out in paddle, drum or mixer as mechanical agitation accelerate the soaking process. As a guideline, hides are soaked in drum with float length of 250% and sheep skins especially for wool-on are soaked in paddle with float length of 2000%. In general, the soak float is discarded on conclusion of the soak. Subsequent to the soak, the hides or skins can be worked up further in a known way, for example conveyed to the unhairing operation.

A soaking process of the present invention may be performed at conventional soaking conditions, i.e. the pH of soak float in the range pH 4-12, preferably the range pH 6-10, most preferably the range pH 7-8; a temperature in the range 5-65°C, preferably the range 15-45°C, and a reaction time in the range 1-48 hours, preferably the range 2-24 hours, and together with known tensides and preservatives, if needed. The presoaking is simpler and shorter, normally it is just performed with water at the same temperature, alternatively a small amount of tenside is added to the water in the same vessel for 0.5 to 5 hours.

**Carbohydrases**
Carbohydrases used in the process of the invention may be any hydrolase belonging to the enzyme sub-class EC 3.2.1, i.e. enzymes hydrolysing O- and S-glycosyl compounds. The carbohydrate is preferably alpha-1,4-carbohydrase which can hydrolyze alpha 1-4 carbohydrate linkages, e.g. alpha-amylase, amyloglucosidase, maltase and so forth; and beta-1,4-carbohydrase which can hydrolyze beta 1-4 carbohydrate linkages, e.g. xylanase, mannanase, mannosidase, beta-glucomannases, cellulases and so forth. In the present invention, carbohydrase can be used individually or in combination in soaking step, preferably, carbohydrase can be one or more enzymes selected from the group consisting of xylanase, mannanase, cellulase, and alpha-amylase.

In the context of the present invention, the term "EC" (Enzyme Class) refers to the internationally recognized enzyme classification system, Recommendations (1992) of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology, Academic Press, Inc., 1992.

Mannanases
Mannanases are hemicellulases classified as EC 3.2.1.78, and called endo-1,4-beta-mannosidase. Mannanase can for example be beta-mannanase, endo-1,4-mannanase, and galacto-mannanase. Mannanase is preferably capable of catalyzing the hydrolysis of 1,4-beta-D-mannosidic linkages in mannans, including glucomannans, galactomannans and galactoglucomannans. Mannans are polysaccharides primarily or entirely composed of D-mannose units.

In a preferred embodiment the mannanase is alkali tolerant, when the optimal pH condition for reaction is 7-9. The mannanase may be of any origin such as a bacterium or a fungal organism, or chemically or genetically modified mutants (variants).

In a specific embodiment, the mannanase is derived from a strain of the filamentous fungus genus Aspergillus, preferably Aspergillus niger or Aspergillus aculeatus (WO 94/25576). WO 93/24622 discloses a mannanase isolated from Trichoderma reseei useful for bleaching lignocellulosic pulps.

AM-001. A purified mannanase from *Bacillus amyloliquefaciens* is disclosed in WO 97/11164. WO 91/18974 describes a hemicellulase such as a glucanase, xylanase or mannanase active. Examples of commercially available mannanases include Mannaway™ available from Novozymes A/S Denmark and Mannastar™ available from Genencor International Inc USA.

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**Xylanases**

The xylanase used in the process of the invention may be any member of the glycoside hydrolyase families (EC 3.2.1.8) which have endo-1,4-β-xylanase activity. In a preferred embodiment the beta-xylanase is alkali tolerant, when the optimal pH condition for reaction is 7-9. Suitable beta-xylanases include those of bacterial or fungal origin, or chemically or genetically modified mutants (variants).

Fungal xylanases may be derived from strains of genera including *Aspergillus, Disporotrichum, Penicillium, Neurospora, Fusarium* and *Trichoderma*.


Examples of suitable bacterial xylanases include xylanases derived from a strain of *Bacillus*, such as *Bacillus subtilis*, such as the one disclosed in US patent no. 5,306,633 or *Bacillus agaradhaerens*, including *Bacillus agaradhaerens* AC13 disclosed in WO 94/01532, a strain of *Bacillus pumilus*, such as the one disclosed in WO 95/182109, a strain derived from of *Bacillus stearothermophilus*, such as the one disclosed in WO 95/182109.

Contemplated commercially available xylanases include SHEARZYME™, BIOFEED WHEAT™, PULPZYME™ HC (from Novozymes A/S), BioBrite™ EB (from logen, Canada) and SPEZYME™ CP (from Genencor International Inc USA).

**Alpha-amylase**

The amylase used in the process of the invention may be any alpha-amylase (EC. 3.2.1.1), which catalyzes the hydrolysis of starch and other linear and branched 1,4-glucosidic oligo- and polysaccharides. In a preferred embodiment the alpha-amylase is an alkali alpha-amylase, when the optimal pH condition for reaction is 7-9. Suitable alpha-amylases include those of bacterial or fungal origin. Chemically or genetically modified mutants (variants) are included.

In a preferred embodiment the alpha-amylase include a carbohydrate-binding module (CBM) as defined in WO 2005/00331 1, preferably a family 20 CBM as defined in WO 2005/00331 1.

In an embodiment the fungal alpha-amylase is of yeast or filamentous fungus origin. Preferred alpha-amylases include, for example, alpha-amylases obtainable from *Aspergillus* species, in
particular from *Aspergillus niger*, *A. oryzae*, and *A. awamori*, *A. kawachii*, such as the acid alpha-amylase disclosed as SWISSPROT P56271, or described in more detail in WO 89/01969 (Example 3).

In an embodiment the alpha-amylase is of bacterial origin. The bacterial alpha-amylase is preferably derived from a strain of *Bacillus*, such as *Bacillus licheniformis*, *Bacillus amyloliquifaciens*, *Bacillus stearothermophilus*, *Bacillus subtilis*, or other *Bacillus* sp., such as *Bacillus* sp. NCIB 12289, NCIB 12512 (WO 95/26397), NCIB 12513 (WO 95/26397), DSM 9375 (WO 95/26397), DSMZ 12648 (WO 00/60060), DSMZ 12649 (WO 00/60060), KSM AP1378 (WO 97/00324), KSM K36 or KSM K38 (EP 1,022,334). Preferred are the *Bacillus* sp. alpha-amylases disclosed in WO 95/26397 as SEQ ID Nos. 1 and 2, respectively, the AA560 alpha-amylase disclosed as SEQ ID NO: 2 in WO 00/60060. Preferably, *Bacillus licheniformis* alpha amylase is SEQ ID NO: 2 as disclosed in WO 96/23874.

In an embodiment of the invention, the bacterial alpha-amylase is the SP722 alpha-amylase disclosed as SEQ ID NO: 2 in WO 95/26397 or the AA560 alpha-amylase.

The alpha-amylase used in the present invention includes the polypeptide of SEQ ID NO:1 from *Bacillus licheniformis*, and the polypeptide of SEQ ID NO:4 from *Bacillus* sp., and polypeptides having at least 80% identity, preferably 90% identity, more preferably 95% identity with SEQ ID NO:1 or SEQ ID NO:4. In some embodiments, the alpha-amylase is differing from the parent protein of SEQ ID NO:1 or SEQ ID NO:4, by insertion, deletion and/or substitution of one or several amino acid residues, preferably 1, 2, 3, 4, 5, 10, 15, 20, or 30 amino acid residues.

Commercially available alpha-amylase products or products comprising alpha-amylases include product sold under the following tradenames: Termamyl™, Aquazym™ Ultra, Aquazym™, NATALASE™, STAINZYME™ (Novozymes A/S, Denmark), Bioamylase - D(G), BIOAMYLASE™ L (Biocon India Ltd.), KEMZYM™ AT 9000 (Biozym Ges. m.b.H, Austria), PURASTAR™ ST, PURASTAR™ HPAmL, PURAFECT™ OxAm, RAPIDASE™ TEX (Genencor Int. Inc, USA), KAM (KAO, Japan).

The alpha-amylase may be added in an amount of from 0.05 to 2000 KNU per kg of hide or skin, preferably from 0.1 to 1000 KNU per kg of hide or skin, more preferably from 0.5 to 500 KNU per kg of hide or skin, most preferably from 10 to 300 KNU per kg of hide or skin.

**Optional Enzymes**

**Lipases**

Suitable lipases (also termed carboxylic ester hydrolases) include those of bacterial or fungal origin, including triacylglycerol lipases (3.1.1.3) and Phospholipase A2.(3.1.1.4.). Lipases for use in the present invention include, without limitation, lipases from *Humicola* (synonym *Thermomycetes*), such as from *H. lanuginosa* (T. lanuginosus) as described in EP 258 068 and

The lipase used in the present invention includes the polypeptide of SEQ ID NO:2 from \textit{Humicola lanuginose}, and polypeptides having at least 80% identity, preferably 90% identity, more preferably 95% identity with SEQ ID NO:2. In some embodiments, the lipase is differing from the parent protein of SEQ ID NO:2, by insertion, deletion and/or substitution of one or several amino acid residues, preferably 1, 2, 3, 4, 5, 10, 15, 20, or 30 amino acid residues. In the context, amino acid changes by insertion, deletion and/or substitution are preferably of a minor nature, that is conservative amino acid substitutions that do not significantly affect the folding or activity of the protein, small deletions, typically of one to about 30 amino acids; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification, such as a poly-histidine tract, an antigenic epitope or a binding domain. See in general Ford et al., Protein Expression and Purification 2: 95-107, 1991. In the context, the degree of 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 in Genetics 16: 276-277), preferably version 3.0.0 or later. 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.

Preferred commercially available lipase enzymes include Lipolase\textregistered TM and Lipolase Ultra\textregistered TM, Lipozyme\textregistered TM, Palatase\textregistered TM, Novozym\textregistered TM 435, and Lecitase\textregistered TM (all available from Novozymes A/S, Denmark), and LIPASE P "Amano" (Amano Pharmaceutical Co. Ltd.), and L1 LIPASET\textregistered TM, LUMA FAST\textregistered TM and LIPOMAX\textregistered TM (Genencor International Inc, USA). The activity of the lipase can be determined as described in "Methods of Enzymatic Analysis", Third Edition, 1984, Verlag Chemie, Weinheim, vol. 4.
In an embodiment of the process of the invention a lipase enzyme may be added in an amount from 0.05 to 1200 KLU per kg of hide or skin, preferably 0.1 to 600 KLU per kg of hide or skin, more preferably 0.5 to 300 KLU per kg of hide or skin.

5 EXAMPLES
The invention is further illustrated with reference to the following examples, which are not intended to be in any way limiting to the scope of the invention as claimed.

Materials and Reagents:
Salted English Shearling Lambskin: Great Britan origin
Salted Sheep Skin: China origin.
Salted cattle hide: USA origin.
alpha-amylase A: *Bacillus licheniformis* wild type amylase with SEQ ID NO: 1 obtained according to WO 96/23874.
lipase A: A variant lipase with two substitutions T231 R+ N233R on the *Humicola lanuginose* wild type lipase of SEQ ID NO: 2 obtained according to WO 00/60063.
alpha-amylase B: Amylase variant with substitutions D183+G184*+R1 18K +N195F+R320K+R458K on *Bacillus sp.* wild type amylase of SEQ ID NO: 3 obtained according to WO 00/60060.
mannanase: *Bacillus sp.* wild type mannanase with SEQ ID NO: 4 obtained according to US 6,566,114.
Surfactant: Eusapon OD (commercially available from BASF)
Wetting agent: Cismollan HB-C (commercially available from Lanxess)
Biocide: Myacide AS (commercially available from BASF)

Equipment:
Soaking vessel: Paddle (Yu Tong Drums Ltd.)
Soaking vessel: Drum (Wexi leather machinery ltd.)

Alpha-amylase activity (KNU)
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 color is formed, but during the break-down of the starch the blue color gets weaker and gradually turns into a reddish-brown, which is compared to a colored glass standard.
One Kilo Novo alpha amylase Unit (KNU) is defined as the amount of enzyme, which under standard conditions (i.e. at 37°C +/- 0.05; 0.0003 M Ca²⁺; and pH 5.6) dextrinizes 5260 mg starch dry substance Merck Amylum solubile.

5 Lipase Activity (LU)
The lipase activity may be determined using tributyrine as substrate. This method is based on the hydrolysis of tributyrine 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 micro mol titratable butyric acid per minute.

Example 1: Removal of Dermatan Sulphate in Sheep Skin by Alpha-Amylase
In this example, Salted English shearling lambskins were used. Alpha-amylase B was trialed at two pH conditions 7.0 and 8.0 and offered at three concentrations 0, 12 KNU per kg of skin and 120 KNU per kg of skin.

Pieces of salted sheep skins were put into soaking vessel (Drum) for washing and soaking. Percentage based on raw weight (Salted English shearling lambskin)

20 Washing: 500% Water at 25°C
   Agitate for 30 minutes,
   Discard float

Soaking: 500% Water at 25°C
25 Agitate for 10 minutes
   The pH was adjusted with 1% NaHCO₃ to pH 7 and 8 respectively.
   Add alpha-amylase B at three concentrations 0, 12KNU per kg of skin, and 120KNU per kg of skin respectively.
   Add 0.1% Myacide AS
30 Agitate for 6 hours
   Discard float

After soaking, the samples were washed, unloaded and drained. The sample was half frozen for chemical analysis of dermatan sulphate removal and histochemical staining to determine the removal of glycosaminoglycans (GAGs).
Chemical analysis of dermatan sulphate removal

Sample Preparation:

1) Cut each sample into very small pieces.
2) Weigh out accurately 0.5-1.0g of the dry sample into small necked soveril tubes.
3) Add papain digest buffer to each of the tubes followed by of papain enzyme (commercially available). Mix well with a rotary mixer.
4) Leave the samples to digest in an incubator overnight.
5) Mix the samples well on the rotary mixer, add papain enzyme to each tube and leave in the incubator until digestion is complete.
6) When completely digested add approx 1ml of diethyl ether to each tube with a pipette, mix well on a rotary mixer and centrifuge for 30 minutes.
7) Pipette off the top fat/ether layer into a beaker. Note: The ether layer can be kept for an estimation of the fat content of the samples.
8) Pipette off the top layer containing the digested skin into a clean sample tube and leave in the fridge for 12 hours to allow any remaining ether to evaporate off.

Dermatan Sulphate Analysis:

1) Make up a standard solution of dermatan sulphate and then using a 1microliter syringe put 6 straight lines of standard dermatan sulphate onto the matt side of a sheet of cellulose acetate paper and leave to dry. Do no touch the paper with your fingers as it causes stains always wear gloves.
2) Keep the remainder of the digest in capped labelled sample tubes. They are needed for the Hydroxyproline analysis.
3) Filter the stain through filter paper using suction filtration. Stain the sheets of paper as follows:
   1st stain  Lie sheet face down on surface for 2 minutes, push under surface for 2 minutes
   2nd stain  Push under surface for 11 minutes and agitate at regular intervals
   Destain   Lie face down on the surface for 2 minutes. Agitate in several 5 min changes of destain until a white background is obtained
   Note. Again do not touch the sheets with your fingers
4) Hang the sheets to dry under tension to prevent curling.
5) Once dry cut our 3 lines of each of the samples and 3 lines of the dermatan sulphate standard using a scalpel, cut each line into 3 pieces and dissolve each line separately. Also dissolve 4 background pieces to act as a control. Care is needed at this stage as the cellulose acetate paper can stick to the side of the tube. Mix on a rotary mixer before reading.
Table 1:

<table>
<thead>
<tr>
<th>Salted Skin treated by</th>
<th>Weight% dermatan sulphate on skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7</td>
<td>Enzyme dosage</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12 KNU/ kg skin</td>
</tr>
<tr>
<td></td>
<td>120 KNU/ kg skin</td>
</tr>
<tr>
<td>pH 8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12 KNU/ kg skin</td>
</tr>
<tr>
<td></td>
<td>120 KNU/ kg skin</td>
</tr>
</tbody>
</table>

The normal levels of dermatan sulphate in unprocessed hide are around 0.4%. There appeared to be a reduction in dermatan sulphate in the skin with increasing offer of enzyme (alpha-amylase B) at pH 7 and 8.

Dermatan sulphate is one of the GAGs which bond the fibers. So the reduction of dermatan sulphate reflects the removal of GAGs. The removal of GAGs will open up or loosen the fibers.

II: Histochemical analysis of GAGs removal

Method: Skin samples were stained with a dye which preferentially stains glycosaminoglycans. The detailed staining method was indicated in textbook "Theory and practice of histological techniques" Second edition 1982, Edited by John D. Bancroft and Alan Stevens, Published by Churchill Livingstone, Pages 195 to 196 about Alcian Blue technique for acid mucins using varying pH of solution.

The results of histochemical analysis were indicated in Table 2.

Table 2:

<table>
<thead>
<tr>
<th>Salted Skin treated by</th>
<th>Staining Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Enzyme dosage</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12 KNU/ kg skin</td>
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<tr>
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<td>120 KNU/ kg skin</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>12 KNU/ kg skin</td>
</tr>
<tr>
<td></td>
<td>120 KNU/ kg skin</td>
</tr>
</tbody>
</table>
There did appear to be a positive effect on the removal of GAGs with concentrations of 120 KNU/ kg skin at pH 7 and pH 8. These samples were stained lighter in color, which is to indicate some removal of the GAGs.

These results in Table 2 were consistent with the chemical analysis of dermatan sulphate at pH 7.0 and 8.0 in Table 1 where there appeared to be a correlation in the removal of dermatan sulphate with the concentration of enzymes offered.

Example 2: Sheep Skin Soaked with Carbohydrase and Lipase

In this example, salted sheep skin was treated in three different groups. In group 1, the skin was treated in soaking only with surfactants. In group 2, the skin was treated in soaking with alpha-amylase A, while in group 3, it was treated with alpha-amylase A and lipase. After the skin had been subjected to washing, soaking and unhairing processes, its cleanliness, softness and grain draw (i.e. the degree of wrinkles) were evaluated.

**Group 1 (Control)**

Salted sheep skin was cut into 12 pieces with total weight of 28.5 kg, and was all put into soaking vessel (paddle) for washing and soaking.

Washing: 300L Water at 28°C
- Stop for 1 hour, agitate for 1 hour
- Discard float

Soaking: 300L Water at 28°C
- Add 57 Gram Eusapon OD
- Add 86 Gram Cismollan HB-C
- Add 114 Gram Soda to adjust the pH of float to 9
- Agitate for 2 hours, then agitate 15 minutes every hour. Total soaking time:15 hours.
- Discard float.

Unhairing:

1) Apply 50 gram per square foot (sqft) of sodium sulphide solution containing 180 gram sodium sulphide per liter on the flesh side of skin. 60 minutes later, remove the hair by hand with beam and blunt knife.
2) Washing: 30L water at 25°C
- Agitate for 1 hour, then add 45L more water at 25°C and agitate for one more hour.
- Discard float, take out the skin for evaluation.
Group 2 (Soaking with amylase)
Salted sheep skin was cut into 12 pieces with total weight of 27.8 kg and were all put into a soaking vessel (paddle) for washing and soaking.

Washing: 300L Water at 28°C
Stop for 1 hour, agitate for 1 hour
Discard float

Soaking: 300L Water at 28°C
Add 360 KNU alpha-amylase A / kg skin

Discard float

Soaking: 300L Water at 28°C
Add 111 Gram Soda to adjust the pH of float to 9
Agitate for 2 hours, then agitate 15 minutes every hour. Total soaking time: 15 hours.
Discard the float

Unhairing:
1) Apply 50 gram per sqft of sodium sulphide solution containing 180 gram sodium sulphide per liter on the flesh side of each skin. 60 minutes later, remove the hair by hand with beam and blunt knife.
2) Washing: 30L water at 25°C
Agitate for 1 hour, add 45L more water at 25°C and agitate for one more hour.
Discard float, take out the skin for evaluation.

Group 3 (Soaking with amylase + lipase)
Salted sheep skin was cut into 12 pieces with total weight of 29.1 kg and were all put into a soaking vessel (paddle) for washing and soaking.

Washing: 300L Water at 28°C
Stop for 1 hour, agitate for 1 hour
Discard float

Soaking: 300L Water at 28°C
Add 120 KNU alpha-amylase A / kg skin
Add 200 KLU lipase A/ kg skin
Add 116 Gram Soda to adjust the pH of float to 9
Agitate for 2 hours, then agitate 15 minutes every hour. Total soaking time: 15 hours.
Discard float.

Unhairing:
1) Apply 50 gram per sqft of sodium sulphide solution containing 180 gram sodium sulphide per liter on the flesh side of each skin. 60 minutes later, remove the hair by hand with beam and blunt knife.

2) Washing: 30L water at 25°C

Agitate for 1 hour, then add 45L more water at 25°C and agitate for one more hour. Discard float, take out the skin for evaluation.

Tests and Results

After unhairing, the appearance of the surface cleanliness, the softness and grain draw (i.e. the degree of wrinkles) of the skin were evaluated by trained panelists.

The skins treated in Group 3 showed the best soaking effect among all three groups, which were cleanest and softest than those treated in two groups and had less wrinkles. Furthermore, the skins treated in Group 2 showed the better soaking effect than control group, which was cleaner and softer than the control groups and had less wrinkles.

Therefore, the results showed that carbohydrase, especially amylase, can improve the leather quality when used in a soaking step. Carbohydrase, especially amylase, used together with lipase improved the soaking effect even more.

Example 3: Cattle Hide Soaked With Carbohvdrase and Lipase

In this example, cattle hide (USA origin) was treated in two different groups. In group 1, the skin was treated in soaking with mannanase and lipase. In group 2, the skin was treated in soaking with alpha-amylase B and lipase. After the skin had been subjected to washing, soaking and unhairing processes, its cleanliness, softness and grain draw were evaluated.

Group 1 (mnnanase and lipase)

8 kg presoaked and fleshed salted cattle hide was put into soaking vessel (drum with speed of 3 rpm) for soaking.

Washing: 24L water at 28°C

Agitate for 30 min.

Discard float

Soaking: 24L water at 28°C

Add 8mg mannanase/ kg hide
Add 100 KLU Lipase A/ kg hide
Add 32 gram soda to adjust the pH of float to 9.5

Agitate for 18 hours.

Discard float.
Group 2 (alpha-amylase B and lipase)

32.6 kg presoaked and fleshed salted cattle hide cut into 4 quarters was put into a soaking vessel (drum with speed of 6 rpm) for soaking.

Washing: 82L water at 28°C
   Agitate for 30 min.
   Discard float

Soaking: 82L water at 28°C
   Add 240 KNU alpha-amylase B/ kg hide
   Add 100 KLU Lipase A/ kg hide
   130 gram soda to adjust the pH of float to 9.5
   Agitate 60 min. then agitate 5 min, rest 15 min for 18 hours.
   Discard float.

The cattle hides treated in Group 1 and 2 were clean and soft.
CLAIMS

1. A method for the processing of hides or skins into leather, comprising enzymatic treatment of the hide or skin with carbohydrate in a soaking step.

2. The method according to claim 1, in which the enzymatic treatment is the treatment of hide or skin with carbohydrate and lipase.

3. The method according to claim 1 or 2, in which the carbohydrate is selected from the group consisting of alpha-1,4-carbohydrase and beta-1,4-carbohydrase.

4. The method according to claim 3, in which the alpha-1,4-carbohydrase is alpha-amylase.

5. The method according to claim 3, in which the beta-1,4-carbohydrase is selected from the group consisting of xylanase and mannanase.

6. The method according to claim 4, in which the alpha-amylase is of bacterial origin, such as strain of Bacillus.

7. The method according to claim 6, in which the alpha-amylase is derived from Bacillus licheniformis, Bacillus amyloliquefaciens, Bacillus stearothermophilus or Bacillus subtilis.

8. The method according to claim 4, in which the alpha-amylase is of fungal origin, such as strain of Aspergillus.

9. The method according to claim 8, in which the alpha-amylase is derived from Aspergillus niger, Aspergillus oryzae, Aspergillus awamori or Aspergillus kawachii.

10. The method according to any of the preceding claims, in which the process is carried out at a temperature in the range of from 5 to 65°C, preferably 15 to 45°C.

11. The method according to any of the preceding claims, in which the process is carried out in the range of pH 4-12, preferably pH 6-10.

12. The method according to any of the preceding claims, in which the reaction time is in the range of 1-48 hours, preferably 2-24 hours.

13. The method according to any of the preceding claims, in which the alpha-amylase is added in an amount from 0.05 to 2000 KNU per kg of hide or skin, preferably from 0.1 to 1000 KNU per kg of hide or skin, more preferably from 0.5 to 500 KNU per kg of hide or skin, most preferably from 10 to 300 KNU per kg of hide or skin.

14. The method according to any of the preceding claims, in which the lipase is added in an amount from 0.05 to 1200 KLU per kg of hide or skin, preferably 0.1 to 600 KLU per kg of hide or skin, more preferably 0.5 to 300 KLU per kg of hide or skin.

15. The method according to any of the preceding claims, which method is followed by a unhairing step comprising protease.
INTERNATIONAL SEARCH REPORT  

International application No  
PCT/EP2009/063585

A. CLASSIFICATION/ SUBJECT MATTER  
INV. C14C1/04

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)  
C14C

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT  

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Further documents are listed in the continuation of Box C

See patent family annex

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21 January 2010

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Fax (+31-70) 340-3016

Authorized officer  
Neugebauer, Ute
**INTERNATIONAL SEARCH REPORT**

**International application No**
PCT/EP2009/063585

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