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(54) Title: LAUNDRY BARS WITH IMPROVED STORAGE STABILITY

(57) Abstract: The present invention relates to a laundry bar comprising a detergent matrix, an enzyme and a phenyl boronic acid derivative, and to a process for incorporating enzymes into laundry bars. The use of said laundry bars for hand washing laundry is also claimed.



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TITLE: LAUNDRY BARS WITH IMPROVED STORAGE STABILITY**FIELD OF THE INVENTION**

The present invention relates to laundry bars with improved enzyme stability and to a process for preparing said laundry bars and to uses of the laundry bars.

5 BACKGROUND OF THE INVENTION

It is well known to add enzymes to laundry bars. When adding enzymes to laundry bars stability of the added enzymes during production and storage becomes an issue of significant importance. It is of course desirable to retain as much activity as possible.

Activity of the added enzymes can be affected by e.g. the water content of the laundry bar. A
10 high water content generally results in a lower activity during storage of the added enzyme, especially for proteases.

GB 2186883 describes laundry bars with a water content of 10-33% and containing proteases in which the proteolytic enzyme is stabilized by a mixture of a boron compound, a polyol, an organic acid or its alkali metal salt, and an alkali metal salt of an inorganic acid which is not a
15 boron compound.

WO 98/54285 describes high-moisture protease containing laundry bars with improved protease stability. The improved stability is obtained by adding a stabilizing agent made of a borate compound in conjunction with a polyol, a carboxylate salt, a carboxylic acid, or mixtures thereof.

20 In WO 97/36985 enzyme stability of a cellulase is improved by providing a laundry bar comprising from about 0.5% to about 60% synthetic detergent surfactant, about 4% or less moisture in the finished bar composition, and from about 0.1% to about 10% non-liquid, thixotropic binding agents, as well as the cellulase enzyme.

The above cited prior art all relates to enzyme stability during storage and use, however, loss
25 of enzyme activity during manufacturing the bars is also a significant problem when incorporating enzymes in laundry detergent bar compositions. This loss of enzyme stability is believed to result from denaturation of enzymes due to exposure of the enzymes to high temperature and high shear action. High temperatures are often necessary in order to process ingredients of laundry bars comprising synthetic surfactants and having relatively high moisture levels, resulting in bars having acceptable physical properties. In case enzymes are added in the form of
30 granules or prills high shear action worked on the bar composition in a mill and/or plodder destroys the integrity of the enzyme granules and enzyme prills and exposes the enzymes to attack by hostile compounds e.g. anionic surfactants.

WO 98/18897 describes a process for incorporating enzymes into laundry detergent bar compositions that minimizes the loss of enzyme stability during the manufacturing process of the
35

bar. This is achieved by a process in which the enzyme prills are added after milling and cooling and then plodding the mixture.

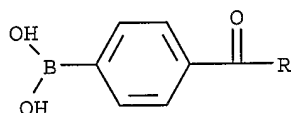
SUMMARY OF THE INVENTION

One object of the present invention is to provide enzyme containing laundry bars with improved storage stability. A further object of the present invention is to provide a process for manufacturing of a dry formulation such as laundry bars wherein the enzyme can be added in the initial phase of the laundry bar manufacturing process and still maintain a significant enzyme activity after manufacturing. We have surprisingly found that by adding phenyl boronic acid derivatives substituted in the para-position with a carbonyl group adjacent to the phenyl boronic acid to enzyme containing laundry bars the enzyme stability of the bars is significantly improved. We have further surprisingly found that by use of enzymes stabilized with phenyl boronic acid derivatives substituted in the para-position with a carbonyl group adjacent to the phenyl boronic acid we can add the enzyme during the mixing step in the preparation of laundry bars and still get the improved enzyme stability during storage.

Thus a first aspect of the present invention is a laundry bar comprising enzymes and phenyl boronic acid derivatives substituted in the para-position with a carbonyl group adjacent to the phenyl boronic acid.

A second aspect of the present invention is a process for incorporating enzymes into laundry bars comprising the steps of:

- a) preparing a mixture of a detergent matrix and enzymes and a phenyl boronic acid derivative of the following formula:



in a mixer;

- b) plodding the mixture from step (a);

The present invention is further related to the use of the laundry bars of the invention.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Laundry bar:

The term laundry bar includes soap bars, combo bars, syndet bars and detergent bars. A laundry bar is for hand washing laundry.

Introduction

The present invention relates to laundry bars which contain enzymes incorporated therein. The present invention is particularly concerned with the storage stability of laundry bars.

Despite several attempts to improve the enzyme stability during production and storage of laundry bars it is still a significant problem that the enzymes loose there activity during produc-
tion and when stored in laundry bars. We have surprisingly found that we can get significantly improved enzyme stability during production of laundry bars and upon storage of laundry bars if a phenyl boronic acid derivative substituted in the para-position with a carbonyl group adjacent to the phenyl boronic acid e.g. 4-formylphenylboronic acid, 4-FPBA, is incorporated into the laundry bar to stabilize the enzyme.

The present invention further relates to an improved process for incorporating enzymes into laundry bars by which process good enzyme stability and good mixing and thus a more homogeneous incorporation is obtained at the same time.

In the preparation of laundry bars it is known practise to use dry enzymes in the form of enzyme granules as enzyme granules provide a protective layer around the enzymes which prevents hostile compounds of attacking the enzyme, whereas the liquid enzymes are completely exposed to hostile compounds in the detergent matrix when added, therefore the laundry bar industry has been reluctant to use liquid enzymes in the production of laundry bars due to the significant loss in enzyme stability. The drawback of using granules is that if they are added during mixing or plodding/milling step a part of the granules are torn apart and thereby exposing the enzymes to hostile compounds also resulting in great loss in enzyme activity, which has resulted in adding a significant excess of enzyme granules.

To prevent the granules to be torn apart during the mixing and milling step the granules can be added after said process steps, as in WO 98/18897 resulting in uneven distribution of enzymes in the laundry bars and still facing the plodding step where they still can get torn apart.

We have however surprisingly found that we by stabilizing the enzyme with a phenyl boronic acid derivative substituted in the para-position with a carbonyl group adjacent to the phenyl boronic acid are able of improving the enzyme stability of the laundry bars significantly compared to the enzyme stability of laundry bars where enzymes have been incorporated by use of enzyme containing granules stabilized with conventional enzyme stabilizers. If using liquid formulations comprising enzymes instead of granules we have surprisingly been able to obtain laundry bars with improved enzyme stability in comparison with laundry bars conventionally prepared with enzyme granules and we have furthermore been able to obtain a more homogeneous distribution of the enzymes in the laundry bars. Thereby enabling us to use both enzyme granules and liquid formulations.

The process of the invention allows the enzyme to be either on a granulate form or as a liquid formulation and to be added before or during the mixing step as opposed to the method described in WO 9854285.

It is contemplated that use of liquid enzyme formulations most likely reduces the mechanical stress on the enzyme product compared to when enzyme granulates is used like in the conventional enzyme laundry bars. This might in turn reduce the amount of frictional heat developed during mixing. The liquid enzyme formulation is also more quickly distributed in the soap mass resulting in a more homogeneous product and in a shorter time, thereby decreasing production time of the laundry bar.

Laundry bars are mainly being sold in the third world and the cost of the laundry bars is therefore a very important factor. By our process we do not need to add a significant excess of enzyme to the laundry bar which will reduce the end cost of the laundry bar, and furthermore the enzyme stabilizer added in our process is cheaper than known enzyme stabilizer systems for laundry bars which further reduces the cost of the laundry bar.

The laundry bar

The laundry bar of the present invention comprises a detergent matrix, enzymes and a phenyl boronic acid derivative substituted in the para-position with a carbonyl group adjacent to the phenyl boronic acid.

Moisture content

Moisture enhances the mixture of the bar ingredients. Moisture e.g. water can be added to the mixture by being included with an ingredient and/or as free water added to the bar mixture.

Appearance

The appearance of the laundry bars prepared with enzymes of the invention is significantly improved compared to known bars comprising enzyme stabilizers. When comparing bars stabilized with a phenyl boronic acid derivative substituted in the para-position with a carbonyl group adjacent to the phenyl boronic acid with bars stabilized with known stabilizer systems such as Borax together with MGP the bars stabilized with Borax and MGP feel slippery and gritty, whereas the bars stabilized with a phenyl boronic acid derivative substituted in the para-position with a carbonyl group adjacent to the phenyl boronic acid feel hard, smooth and non-slippery as bars without enzyme stabilizers added. Furthermore the bars prepared with liquid enzyme have a more homogeneous look, compared to bars prepared with enzyme granules where granules can be visually spotted.

Laundry bar ingredients

The laundry bar of the invention comprises enzymes, a phenyl boronic acid derivative and a detergent matrix.

Enzymes

5 The enzymes that can be stabilized by the process according to the invention are any enzyme which exerts their effects during the hand-washing process, e.g. having a cleaning, fabric care, anti-redeposition and stain removing effect in a hand-wash application and which enzymes are added for such a purpose.

The enzyme in the context of the present invention may be any enzyme or combination of dif-
10 ferent enzymes. Accordingly, when reference is made to "an enzyme" this will in general be understood to include one enzyme or a combination of enzymes.

It is to be understood that enzyme variants (produced, for example, by recombinant techniques) are included within the meaning of the term "enzyme". Examples of such enzyme variants are disclosed, e.g. in EP 251,446 (Genencor), WO 91/00345 (Novo Nordisk), EP
15 525,610 (Solvay) and WO 94/02618 (Gist-Brocades NV).

Enzymes can be classified on the basis of the handbook Enzyme Nomenclature from NC-IUBMB, 1992), see also the ENZYME site at the internet: <http://www.expasy.ch/enzyme/>. ENZYME is a repository of information relative to the nomenclature of enzymes. It is primarily based on the recommendations of the Nomenclature Committee of the International Union of
20 Biochemistry and Molecular Biology (IUB-MB), Academic Press, Inc., 1992, and it describes each type of characterized enzyme for which an EC (Enzyme Commission) number has been provided (Bairoch A. The ENZYME database, 2000, Nucleic Acids Res 28:304-305). This IUB-MB Enzyme nomenclature is based on their substrate specificity and occasionally on their molecular mechanism; such a classification does not reflect the structural features of these en-
25 zymes.

Another classification of certain glycoside hydrolase enzymes, such as endoglucanase, xy-
lanase, galactanase, mannanase, dextranase and alpha-galactosidase, in families based on amino acid sequence similarities has been proposed a few years ago. They currently fall into 90 different families: See the CAZy(ModO) internet site (Coutinho, P.M. & Henrissat, B. (1999)
30 Carbohydrate-Active Enzymes server at URL:

<http://afmb.cnrs-mrs.fr/~cazy/CAZY/index.html> (corresponding papers: Coutinho, P.M. & Henrissat, B. (1999) Carbohydrate-active enzymes: an integrated database approach. In "Recent Advances in Carbohydrate Bioengineering", H.J. Gilbert, G. Davies, B. Henrissat and B. Svensson eds., The Royal Society of Chemistry, Cambridge, pp. 3-12; Coutinho, P.M. & Henrissat, B. (1999) The modular structure of cellulases and other carbohydrate-active enzymes:
35 an integrated database approach. In "Genetics, Biochemistry and Ecology of Cellulose Degrada-

dation"., K. Ohmiya, K. Hayashi, K. Sakka, Y. Kobayashi, S. Karita and T. Kimura eds., Uni Publishers Co., Tokyo, pp. 15-23).

The types of enzymes which may be incorporated in laundry bars of the invention include oxidoreductases (EC 1.-.-.-), transferases (EC 2.-.-.-), hydrolases (EC 3.-.-.-), lyases (EC 4.-.-.-), isomerases (EC 5.-.-.-) and ligases (EC 6.-.-.-).

Particularly suitable enzymes include lyases or hydrolases (EC 3.-.-.-), particularly proteases, amylases, lipases, pectate lyases, carbohydrases and/or cellulases.

Preferred oxidoreductases in the context of the invention are peroxidases (EC 1.11.1), laccases (EC 1.10.3.2) and glucose oxidases (EC 1.1.3.4)]. An Example of a commercially available oxidoreductase (EC 1.-.-.-) is Gluzyme™ (enzyme available from Novozymes A/S). Further oxidoreductases are available from other suppliers. Preferred transferases are transferases in any of the following sub-classes:

- a Transferases transferring one-carbon groups (EC 2.1);
- b transferases transferring aldehyde or ketone residues (EC 2.2); acyltransferases (EC 2.3);
- c glycosyltransferases (EC 2.4);
- d transferases transferring alkyl or aryl groups, other than methyl groups (EC 2.5); and
- e transferases transferring nitrogenous groups (EC 2.6).

A most preferred type of transferase in the context of the invention is a transglutaminase (protein-glutamine γ -glutamyltransferase; EC 2.3.2.13).

Further examples of suitable transglutaminases are described in WO 96/06931 (Novo Nordisk A/S).

Preferred hydrolases in the context of the invention are: carboxylic ester hydrolases (EC 3.1.1.-) such as lipases (EC 3.1.1.3); phytases (EC 3.1.3.-), e.g. 3-phytases (EC 3.1.3.8) and 6-phytases (EC 3.1.3.26); glycosidases (EC 3.2, which fall within a group denoted herein as "carbohydrases"), such as α -amylases (EC 3.2.1.1); peptidases (EC 3.4, also known as proteases); and other carbonyl hydrolases. Examples of commercially available phytases include Bio-Feed™ Phytase (Novozymes), Ronozyme™ P (DSM Nutritional Products), Natuphos™ (BASF), Finase™ (AB Enzymes), and the Phyzyme™ product series (Danisco). Other preferred phytases include those described in WO 98/28408, WO 00/43503, and WO 03/066847.

In the present context, the term "carbohydrase" is used to denote not only enzymes capable of breaking down carbohydrate chains (e.g. starches or cellulose) of especially five- and six-membered ring structures (i.e. glycosidases, EC 3.2), but also enzymes capable of isomerizing carbohydrates, e.g. six-membered ring structures such as D-glucose to five-membered ring structures such as D-fructose.

Carbohydrases of relevance include the following (EC numbers in parentheses):

α -amylases (EC 3.2.1.1), β -amylases (EC 3.2.1.2), glucan 1,4- α -glucosidases (EC 3.2.1.3), endo-1,4-beta-glucanase (cellulases, EC 3.2.1.4), endo-1,3(4)- β -glucanases (EC 3.2.1.6), endo-1,4- β -xylanases (EC 3.2.1.8), dextranases (EC 3.2.1.11), chitinases (EC 3.2.1.14), polygalacturonases (EC 3.2.1.15), lysozymes (EC 3.2.1.17), β -glucosidases (EC 3.2.1.21), α -galactosidases (EC 3.2.1.22), β -galactosidases (EC 3.2.1.23), amylo-1,6-glucosidases (EC 3.2.1.33), xylan 1,4- β -xylosidases (EC 3.2.1.37), glucan endo-1,3- β -D-glucosidases (EC 3.2.1.39), α -dextrin endo-1,6- α -glucosidases (EC 3.2.1.41), sucrose α -glucosidases (EC 3.2.1.48), glucan endo-1,3- α -glucosidases (EC 3.2.1.59), glucan 1,4- β -glucosidases (EC 3.2.1.74), glucan endo-1,6- β -glucosidases (EC 3.2.1.75), galactanases (EC 3.2.1.89), arabinan endo-1,5- α -L-arabinosidases (EC 3.2.1.99), lactases (EC 3.2.1.108), chitosanases (EC 3.2.1.132), endo-mannanase (EC 3.2.1.78) and xylose isomerases (EC 5.3.1.5).

In a particular embodiment of the present invention the enzyme is a protease. In a particular embodiment the enzyme is a bacterial protease.

Examples of commercially available proteases (peptidases) include KannaseTM, EverlaseTM, EsperaseTM, AlcalaseTM, Alcalase UltraTM, NeutraseTM, DurazymTM, SavinaseTM, Savinase UltraTM, OvozymeTM, PyraseTM, Pancreatic Trypsin NOVO (PTN), Bio-FeedTM Pro and Clear-LensTM Pro (all available from Novozymes A/S, Bagsvaerd, Denmark). Other preferred proteases include those described in WO 01/58275 and WO 01/58276.

Other commercially available proteases include RonozymeTM Pro, MaxataseTM, MaxacalTM, MaxapemTM, OpticleanTM, PropeaseTM, PurafectTM and Purafect OxTM (available from Genencor International Inc., Gist-Brocades, BASF, or DSM Nutritional Products).

Examples of commercially available lipases include LipexTM, LipoprimeTM, LipopanTM, LipolaseTM, LipolaseTM Ultra, LipozymeTM, PalataseTM, ResinaseTM, NovozymTM 435 and LecitaseTM (all available from Novozymes A/S).

Other commercially available lipases include LumafastTM (*Pseudomonas mendocina* lipase from Genencor International Inc.); LipomaxTM (*Ps. pseudoalcaligenes* lipase from Gist-Brocades/Genencor Int. Inc.; and *Bacillus* sp. lipase from Solvay enzymes. Further lipases are available from other suppliers.

Examples of commercially available carbohydrases include Alpha-GalTM, Bio-FeedTM Alpha, Bio-FeedTM Beta, Bio-FeedTM Plus, Bio-FeedTM Wheat, Bio-FeedTM Z, NovozymeTM 188, CarezymeTM, CelluclastTM, CellusoftTM, CelluzymeTM, CeremylTM, CitrozymTM, DenimaxTM, DezymeTM, DextrozymeTM, DuramylTM, EnergexTM, FinizymTM, FungamylTM, GamanaseTM, GlucanexTM, LactozymTM, LiquezymeTM, MaltogenaseTM, NatalaseTM, PentopanTM, PectinexTM, PromozymeTM, PulpzymeTM, NovamylTM, TermamylTM, AMGTM (Amyloglucosidase Novo), MaltogenaseTM, SweetzymeTM and AquazymTM (all available from Novozymes A/S). Further

carbohydrases are available from other suppliers, such as the RoxazymeTM and RonozymeTM product series (DSM Nutritional Products), the AvizymeTM, PorzymeTM and GrindazymeTM product series (Danisco, Finnfeeds), and NatugrainTM (BASF), PurastarTM and PurastarTM OxAm (Genencor).

- 5 Other commercially available enzymes include MannawayTM, PectawayTM, StainzymeTM and RenozymeTM.

In a particular embodiment of the present invention the enzyme is a protease.

Protease: Any protease suitable for use in a laundry bar can be used. Suitable proteases include those of animal, vegetable or microbial origin. Microbial origin is preferred. Chemically or
10 genetically modified mutants are included. It may be a serine protease, preferably an alkaline microbial protease or a trypsin-like protease. Examples of alkaline proteases are subtilisins, especially those derived from Bacillus, e.g. subtilisin Novo, subtilisin Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168 (described in WO 89/06279).

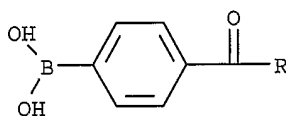
In a particular embodiment of the present invention the enzymes added to the laundry bar are a
15 mixture of proteases and one or more enzymes selected from the group consisting of cellulases, lipases, amylases, pectate lyases and carbohydrases.

The enzyme can either be incorporated in a granule or in a liquid formulation. In a particular embodiment the present invention the enzyme is added to the process of the invention as a granule. In a more particular embodiment of the present invention the enzyme is added to the
20 process as a liquid formulation.

Phenyl Boronic Acid Derivatives

We have surprisingly found that particularly useful enzyme stabilizing agents in dry formulations such as laundry bars are phenyl boronic acid derivatives substituted in the para-position with carbonyl group adjacent to the phenyl boronic acid.

- 25 The phenyl boronic acid derivative enzyme stabilizer has the following formula:



wherein R is selected from the group consisting of hydrogen, hydroxy, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₁-C₆ alkenyl and substituted C₁-C₆ alkenyl.

In a particular embodiment of the present invention the laundry bar comprise an enzyme and a
30 phenyl boronic acid derivative enzyme stabilizer of the formula disclosed above, wherein R is a C₁-C₆ alkyl, in particular wherein R is CH₃, CH₃CH₂ or CH₃CH₂CH₂, or wherein R is hydrogen.

In a further particular embodiment of the present invention the laundry bar comprise a surfactant, an enzyme and a phenyl boronic acid derivative enzyme stabilizer of the formula disclosed above.

We have surprisingly found that 4-formyl-phenyl-boronic acid (4-FPBA) is significantly better than other boron stabilizing agents known in the art such as borax at stabilizing enzymes in laundry bars. In a particular embodiment of the present invention the phenyl boronic acid derivative enzyme stabilizer is 4-FPBA.

- 5 In a particular embodiment of the present invention the laundry bar only comprises a phenyl boronic acid derivative as enzyme stabilizing agent. In an even more particular embodiment of the present invention the laundry bar only comprises a 4-FPBA as enzyme stabilizing agent. The detergent composition may contain up to 500 mM of the stabilizer (the phenyl boronic acid derivative), preferably the detergent composition may contain 0.001-250 mM of the stabilizer, 10 more preferably the composition may contain 0.005-100 mM of the stabilizer, most preferably the composition may contain 0.01-10 mM of the stabilizer. The phenyl boronic acid derivative may be an acid or the alkali metal salt of said acid. In a particular embodiment the laundry bar comprises at least 0.001% w/w of the stabilizer.

Detergent matrix

- 15 The detergent matrix may comprise but are not limited to ingredients such as soap, surfactants e.g. anionic synthetic surfactants, builders, polymeric soil release agents, detergent chelants, glycerine, stabilizing agents, fillers, dyes, colorants, dye transfer inhibitors, alkoxylated polycarbonates, suds suppressers, structurants, binders, leaching agents, bleaching activators, clay soil removal agents, anti-redeposition agents, polymeric dispersing agents, brighteners, fabric 20 softeners, perfumes and/or other compounds known in the art.

Soap

- The soap suitable for use according to the present invention includes water soluble salts of higher fatty acids. Soap can be made by direct saponification of fats and oils or by neutralisation of free fatty acids. Suitable soaps are sodium, potassium, ammonium, and alkyloammonium salts of higher fatty acids containing from about 8 to about 24 carbon atoms, such as 25 from 12 to about 18 carbon atoms. In a particular embodiment the soap is selected from sodium and potassium salts of mixtures of fatty acid derived from coconut oil and tallow, such as sodium or potassium tallow and coconut soaps.

Surfactants

- 30 Synthetic anionic surfactants which are suitable for use herein include the water soluble salts, preferably the alkali metal, ammonium and alkyl ammonium salts of organic sulphuric reaction products having in their molecular structure an alkyl group containing from about 10 to about 20 carbon atoms and a sulfonic acid or sulphuric acid ester group. Examples of this group of synthetic surfactants are the sodium and potassium alkyl sulfates, especially those obtained by 35 sulfating the higher alcohols (C_{8-18} carbon atoms) such as those produced by reducing the glycerides of tallow or coconut oil; and the sodium and potassium alkyl benzene sulfonates in

which the alkyl group contains from about 9 to about 15 carbon atoms, in straight chain or branched chain configuration. Especially valuable are the linear straight chain alkyl benzene sulfonates (LAS) in which the average number of carbon atoms in the alkyl group is from about 11-13, abbreviated as C₁₁₋₁₃ LAS. The alkali metal salts, particularly the sodium salts of these surfactants are preferred.

Other examples of an anionic synthetic detergent suitable for use herein are the sodium alkyl glyceryl ether sulfonates (AES), especially those ethers of higher alcohols derived from tallow and coconut oil; sodium coconut oil fatty acid monoglyceride sulfonates and sulfates; and sodium or potassium salts of alkyl ethylene oxide ether sulfates containing about 1 to about 10 units ethylene oxide per molecule and wherein the alkyl group contains from about 10 to about 20 carbon atoms.

In addition, a suitable anionic synthetic detergent also includes the water soluble salts of ester of alpha- sulphonated fatty acids containing from about 6 to about 20 carbon atoms in the fatty acid group and from about 1 to about 10 carbon atoms in the ester group; water soluble salts of 2-acyloxyalkane-1-sulfonic acids containing from about 2 to about 9 carbon atoms in the acyl group and from 9 to about 23 carbon atoms in the alkane moiety; water soluble salts of olefin and paraffin sulfonates containing from about 12 to about 20 carbon atoms; and beta-alkyloxy alkane sulfonates containing from about 1 to about 3 carbon atoms in the alkyl group and from about 8 to about 20 carbon atoms in the alkane moiety.

Preferred anionic synthetic surfactant examples are C₁₀₋₁₈ alkyl sulfates (AS), C₁₀₋₁₈ linear alkyl benzene sulfonates (LAS), C₁₀₋₁₄ alkyl glyceryl ether sulfonates (AES), and mixtures thereof.

An example of ingredients comprised in a conventional laundry bar (syndet bar) is:

Linear alkyl benzene sulfonate, coco fatty alcohol sulphate, soda ash, sulphuric acid, sodium tripolyphosphate, calcium carbonate, coco fatty alcohol, TiO₂, cellulase, Diethylenetriamine penta, coco monoethanolamide, fluorcent agents, substituted methylcellulose, perfume, moisture.

An example of detergent ingredients comprised in a conventional laundry bar (combo bar) is:

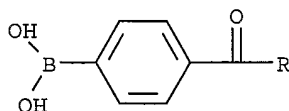
LAS, soap, cellulase, protease, boric acid, borax, sodium formate, sodium citrate, sodium carbonate, glycerol, propylene glycerol, ethylene glycol, MgSO₄, soda ash, STPP, talc, moisture.

Preparation of laundry bars

The laundry bars of the present invention may be processed in conventional laundry bar making equipment such as but not limited to: mixer, plodder, mill, e.g a two stage vacuum plodder, extruder, cutter, logo-stamper, cooling tunnel and wrapper.

According to the invention the process is a way of incorporating enzymes into laundry bars comprising the steps of:

- a) preparing a mixture of a detergent matrix, enzymes and a phenyl boronic acid derivative of the following formula:



where R is selected from the group consisting of hydrogen, hydroxy, C₁-C₆ alkyl substituted C₁-C₆ alkyl, C₁-C₆ alkenyl and substituted C₁-C₆ alkenyl, together in a mixer;

- b) plodding the mixture from step a).

Besides the mixing step and the plodding step the process may further comprise the steps of; milling, extruding, cutting, stamping, cooling and/or wrapping.

The phenyl boronic acid derivative can be added in several ways. It can be added to the enzyme formulation before mixing, or it can be added to any of the ingredients comprised in the detergent matrix, e.g. it can be incorporated in the soap before mixing or it can be added to the mixing on its own.

In a particular embodiment of the present invention the phenyl boronic acid derivative is added to the enzyme formulation before the mixing step.

In a particular embodiment of the present invention the enzyme added is on liquid form.

In a particular embodiment of the present invention the enzyme is added at the same time as the phenyl boronic acid derivative, e.g. 4-FPBA.

The mixing may take place in a mixer e.g. in a Double Sigma Amalgator type MSA-100 mixer.

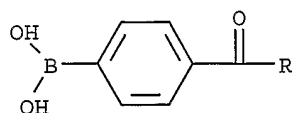
The mixing step takes preferably no more than 1 min., 5 min., 10 min., 15 min. 30 min., 45 min., no more than 1 hour.

After mixing the mixture is transported into a plodder e.g. a Duplex plodder, in which the plodding step is performed. The plodder operates preferably at high vacuum, so that entrapped air/gas is removed.

The product is extruded and the extruded bar subsequently moves to the cutter where the bar is cut to the desired bar length. The pieces are stamped into their final shape in stamping presses. High-speed presses are normally used on high-volume production lines. The form of the stamp is established by designers, taking into account legal considerations such as the minimum net weight for a laundry bar. Optionally the bars are printed with the product brand name. The bar can be cooled, e.g. in a cooling tunnel, before it following normal procedure is wrapped, cased and sent to storage.

The process of the invention further relates to a way of incorporating enzymes into laundry bars comprising the steps of:

- 5 a) preparing a mixture of a detergent matrix, an enzyme and a phenyl boronic acid derivative of the following formula:



wherein R is C₁-C₆ alkyl, such as CH₃, CH₂CH₃, CH₂CH₂CH₃.

The phenyl boronic acid derivative is in a particular embodiment 4-formylphenylboronic acid.

- 10 In a particular embodiment the enzyme is added as a liquid formulation. In another particular embodiment the enzyme is added to the process as a powder or a granulate.

In a particular embodiment the enzyme of the mixture is a protease. In a further embodiment the mixture further comprises an enzyme selected from the group consisting of cellulases, lipases, amylases, pectate lyases and carbohydrases.

- 15 In a particular embodiment the phenyl boronic acid derivative is added to the enzyme formulation before step a). In another embodiment the phenyl boronic acid derivative is added to any ingredients being part of the detergent matrix before step a). In a third embodiment the phenyl boronic acid derivative is added to step a) on its own.

- 20 The present invention further relates to a laundry bar obtainable by the process of the invention

Use of laundry bars

The laundry bar of the invention is for use in hand washing laundry.

25

The present invention is further described by the following examples which should not be construed as limiting the scope of the invention.

EXAMPLES

Example 1

- 30 The enzymes used in the study were:

A Liquid protease "Savinase Ultra 16 XL"

A protease comprising granule "Savinase 12 T, W"

The Ultra system is a liquid stabilized by 4-FPBA, whereas the T enzyme is a granulate.
 Soap Matrix: 80/20 Tallow/Coco. 78% TFM. 85% Na soap, produced by *Italsilva*.

- 5 Enzyme Dosages: 0.94% (w/w) of the Ultra liquid
 1.00% (w/w) of the T granulate.

Process: Batch size (per enzyme): 50 kg of soap mass.

- 10 The enzyme formulation was added to the soap mass in a Double Sigma Amalgator type MSA-100.

Mixing time was 5 minutes.

- Soap mass and enzyme then flowed into a Duplex plodder comprising two steps, i.e. a milling compartment type B 100 followed by a plodder & extruder type MP 150 with a screw type normally used for production of laundry bars.

The temperature in MP 150 was regulated to 36-37°C.

After MP 150, the resulting extruded soap bar moved continuously to the cutter and finally, the single shaped bars flowed continuously to the stamper in which laundry bars were produced.

- 20 The samples produced with the Ultra liquid looked fully homogeneous, whereas the T granulates could be visually identified in their laundry bars.

Example 2.

The laundry bars produced in example 1 were analysed for loss of enzyme activity during storage in up to 8 weeks. The bars were stored at 30°C and the results are given in table 1 below.

25

Enzyme	Sample Code	Week 1	Week 6	Week 8
Savinase Ultra 16 XL	A1	94	95	89
	A2	90	97	91
Savinase 12 T	B1	81	51	46
	B2	95	51	46

Table 1. The values of % residual activity were obtained through the eight weeks storage of the laundry bars at 30°C.

The sample codes refer to the following dosage combinations:

A: 468 g enzyme per 50 kg soap, i.e. 0.94 % (w/w) (nom. 0.1504 KNPU/g)

B: 500 g enzyme per 50 kg soap, i.e. 1.00 % (w/w) (nom. 0.12 KNPU/g)

- 5 It is obvious that Savinase Ultra 16 XL has a very high stability performance in the laundry bar produced according to the process described above.

Example 3.

Calculated nominal "start values" based upon the amounts of the four enzyme products added to the soap matrix in the amalgator:

10	Savinase Ultra 16 XL:	0.1504 KNPU(S)/g
	Savinase 12 T:	0.120 KNPU(S)/g.

Values measured at the start of the storage stability test described above (Week 0 values):

Savinase Ultra 16 XL:	0.092 KNPU(S)/g
Savinase 12 T:	0.067 KNPU(S)/g.

- 15 This means that there is an initial loss of activity during the entire manufacturing process described above (mixing – plodding – extrusion – cutting – stamping) as follows:

Savinase Ultra 16 XL:	38.8%
Savinase 12 T:	44.2%.

- 20 Thus, we learn from this test that the liquid protease stabilized with 4-FPBA does have the lowest loss of activity during the bar production steps.

- 25 Proteolytic Activity (KNPU): In the present specification, proteolytic activity is expressed in Kilo Novo Protease Units (KNPU). The activity is determined relative to an enzyme standard, and the determination is based on the digestion of a dimethyl-casein (DMC) solution by the proteolytic enzyme under standard conditions (50°C, pH 8.3, 9 min. reaction time, 3 min. measurement time). A brochure (AF 220/1) providing further details of the analytical method is available upon request from Novozymes A/S, Bagsvaerd, Denmark.

Example 4

Materials:

- 30 Laundry Soap Base: Soap noodles "Cremersap BV 601"
TFM = 78.5%
Moisture: 12.9%
Preservatives: Present
Glycerine: 0.43%
35 FFA = 0.6%.

Liquid Protease enzyme formulations:

Savinase 16 L EX (not stabilized)

Savinase Ultra 16 XL (stabilized with 4-FPBA)

Stabilizing agents: Borax

5 MPG (monopropylene glycol)

Systems Produced:

A1: 0.2% Savinase 16 L EX, 1 run.

A2: 0.2% Savinase 16 L EX, 2 runs.

10 B1: 0.2% Savinase 16 L EX + 2% Borax + 4% MPG, 1 run.

B2: 0.2% Savinase 16 L EX + 2% Borax + 4% MPG, 2 runs.

C1: 0.2% Savinase Ultra 16 XL, 1 run.

C2: 0.2% Savinase Ultra 16 XL, 2 runs.

15 Equipment and Process:

The soap noodles and enzyme formulations, and in system B Borax and MPG, were added into the amalgator (Werner & Pfleiderer). Materials used: 200 kg of soap noodles + 400 g of enzyme (0.2%) + (in system No. 2) 4 kg of Borax (2%) + 8 kg of MPG (4%).

Blending was performed for 15 minutes - temperature: 38-42°C.

20 Subsequently the batch moved into the plodder (Mazzoni Double Sigma, 2 steps, vacuum).

The temperature after the last plodder, i.e. before the extruder, was 55-60°C.

Finally, the extruded cylinder was cut into pieces of 100 g. Cutter: "Marsina", 70-72 mm/sec.

25 In order to simulate the normal recycling of scraps and spills from extrusion, cutting, and stamping, we sent part of the enzymatic bars through the amalgator, plodder, extruder, and cutter once again. This was done with all three systems. Thus, the resulting bars were subjected to double exposure to the mechanical and thermal stress in the equipment.

Washing performance test

30 The storage period was eight weeks at 30°C. The washing efficiency was measured of week 0 samples, and of week 6 and 8 samples.

There were measured on test swatches EMPA 117 (blood, milk and carbon black) and EMPA 164 (grass).

Remission Values at 460 nm:

35

Soap Bars with enzymes. 15°dH water hardness.

5

Enzyme	Weeks	EMPA 117	Delta R	EMPA 164	Delta R
A1+ Savinase 16 L EX	Ro	38.57	0	33.33	0.00
	0	42.05	3.48	33.34	0.01
	6	40.39	1.82	33.13	-0.20
	8	40.19	1.62	32.74	-0.59
A2+ Savinase 16 L EX	Ro	38.57	0	33.33	0.00
	0	42.43	3.86	32.96	-0.37
	6	41.02	2.45	32.80	-0.53
	8	40.92	2.35	32.30	-1.03

Enzyme	Weeks	EMPA 117	Delta R	EMPA 164	Delta R
B1+ Savinase 16 L EX + Borax + MPG	Ro	38.57	0	33.33	0
	0	52.03	13.46	34.64	1.32
	6	52.20	13.63	34.31	0.98
	8	50.36	11.79	33.68	0.35
B2+ Savinase 16 L EX + Borax + MPG	Ro	38.57	0	33.33	0
	0	48.62	10.05	36.87	3.54
	6	47.93	9.36	35.03	1.70
	8	49.57	11.00	36.27	2.94

10

Enzyme	Weeks	EMPA 117	Delta R	EMPA 164	Delta R
C1 + Savinase Ultra 16 L	Ro	38.57	0	33.33	0
	0	56.61	18.04	39.20	5.87
	6	57.23	18.66	38.90	5.57
	8	57.34	18.77	38.65	5.32
C2 + Savinase Ultra 16 L	Ro	38.57	0	33.33	0
	0	58.20	19.63	38.96	5.63
	6	58.22	19.65	38.55	5.22
	8	58.23	19.66	37.74	4.41

Conclusion

From the results obtained, the following can clearly be concluded:

15 Savinase Ultra 16 XL compared to Savinase 16 L EX with or without traditional stabilizers (Borax + MPG) maintains the highest washing efficiency of the laundry bar after both 1 and 2 runs through the production process.

Savinase Ultra 16 XL has the absolutely best storage stability (eight weeks, 30°C), and thus offers a significantly higher washing efficiency than Savinase 16 L EX with and without Borax +
20 MPG as stabilizers, respectively.

Savinase 16 L EX as such without the stabilizers suffers a very high loss of efficiency during the production process. On test swatches EMPA 117 with 1 run, ΔR is 3.5 and addition of Bo-

rax + MPG causes an increase of ΔR to 13.5 whereas Savinase Ultra 16 XL has a ΔR value of 18.0.

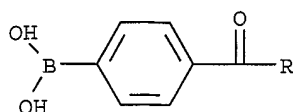
During the storage period (eight weeks at 30°C), the low ΔR values for Savinase 16 L EX are further reduced whereas addition of Borax + MPG has some stabilizing effect. Savinase Ultra
5 16 L EX suffers practically no loss of washing efficiency.

Overall Conclusion

The present example proves that a laundry bar produced with a liquid protease stabilized with 4-FPBA produced in industrial scale has a very satisfactory stability during the manufacturing
10 process and during a storage period of eight weeks at 30°C. The stability performance measured as washing efficiency of the laundry bar produced with liquid protease stabilized with 4-FPBA is significantly higher than that of the laundry bar produced with liquid protease stabilized with Borax + MPG (4%), which in turn is more stable than the laundry bar comprising a liquid protease as such (without stabilizer).

CLAIMS

1. A laundry bar comprising a detergent matrix, an enzyme and a phenyl boronic acid derivative of the following formula:



5 where R is selected from the group consisting of hydrogen, hydroxy, C₁-C₆ alkyl substituted C₁-C₆ alkyl, C₁-C₆ alkenyl and substituted C₁-C₆ alkenyl.

2. The laundry bar of claim 1, wherein R is C₁-C₆ alkyl.

3. The laundry bar of claim 1, wherein the phenyl boronic acid derivative is 4-formylphenylboronic acid.

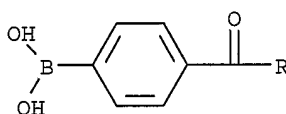
10 4. The laundry bar of claim 1, wherein the enzyme is a protease.

5. The laundry bar of claim 4, wherein the laundry bar further comprises one or more enzymes selected from the group consisting of cellulases, lipases, amylases, pectate lyases and carbohydrases.

6. A process for incorporating enzymes into laundry bars comprising the steps of:

15

a) preparing a mixture of a detergent matrix, enzymes and a phenyl boronic acid derivative of the following formula:



20

where R is selected from the group consisting of hydrogen, hydroxy, C₁-C₆ alkyl substituted C₁-C₆ alkyl, C₁-C₆ alkenyl and substituted C₁-C₆ alkenyl, together in a mixer;

b) plodding the mixture from step a).

7. The process according to claim 6, wherein R is C₁-C₆ alkyl.

8. The process according to claim 6, wherein the phenyl boronic acid derivative is 4-formylphenylboronic acid.

25

9. The process according to claim 6, wherein the enzyme is added as a liquid formulation of enzymes.
10. The process according to claim 6, wherein the enzyme is added as a granule.
11. The process according to claim 6, wherein the enzyme of the mixture is a protease.
- 5 12. The process according to claim 11, wherein the mixture further comprises an enzyme selected from the group consisting of cellulases, lipases, amylases, pectate lyases and carbohydrases.
13. The process according to claim 6, wherein the phenyl boronic acid derivative is added to the enzyme formulation before step a).
- 10 14. The process according to claim 6, wherein the phenyl boronic acid derivative is added to any ingredients being part of the detergent matrix before step a).
15. The process according to claim 6, wherein the phenyl boronic acid derivative is added to step a) on its own.
16. A laundry bar obtainable by the process of claim 6.
- 15 17. Use of a laundry bar according to claims 1 and 16 for hand washing laundry.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK2005/000674

A. CLASSIFICATION OF SUBJECT MATTER

C11D3/16

C11D9/22

C11D17/00

C11D3/386

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)

C11D

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, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98/18897 A (PROCTER & GAMBLE) 7 May 1998 (1998-05-07) cited in the application page 2, lines 1-31 claims; examples	1-17
A	WO 98/54285 A (PROCTER & GAMBLE) 3 December 1998 (1998-12-03) cited in the application page 2, lines 18-31 claims; examples 1-9	1-17
A	WO 96/21716 A (NOVO NORDISK) 18 July 1996 (1996-07-18) page 4, lines 13-17 table 1 claims 1-13	1-17
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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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- *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
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- * & * document member of the same patent family

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INTERNATIONAL SEARCH REPORT

International Application No

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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 96/41859 A (NOVO NORDISK) 27 December 1996 (1996-12-27) page 2, lines 12-26 claims 1-16 -----	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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