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(54) **DETERGENT COMPOSITION BASED ON LIPASE AND BETA-CYCLODEXTRIN**

(57) The invention relates to a composition intended for use in a detergent consisting of lipase and  $\beta$ -cyclodextrin, wherein the mass ratio of lipase and  $\beta$ -cyclodextrin is (0.0025-0.25):(0.1-1), respectively.

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## Description

## FIELD OF INVENTION

**[0001]** The invention relates to a composition and its use in a detergent, wherein the composition consists of lipase and  $\beta$ -cyclodextrin, wherein the mass ratio of lipase and  $\beta$ -cyclodextrin is (0.0025-0.25):(0.1-1), respectively. The invention provides for effective control of kinetics of enzymatic cleavage of lipids with simultaneous effective neutralization of unpleasant odours on different types of surfaces, including especially metal, polymeric, enameled, glass and wooden ones, with maintaining of continuous cleanliness and a pleasant odour.

## PRIOR ART

**[0002]** Development of household chemicals with improved consumer properties remains a promising direction in the category of household products. According to experts, in Great Britain alone by the end of 2020 the category of cleaning products will grow by 9% CAGR and will make up £485 million comparing to 2019 [Dishwashing products: Impact of COVID-19, UK, July 2020, Mintel], while in China the market volume increases by 4% CAGR annually and will make up RMB17,195 million by 2024 [Dishwashing Products - China - February 2020, Mintel].

**[0003]** Every day people use quite a number ( $>3$ ) of household chemicals for cleaning different surfaces at home: wooden, ceramic, enameled, metal, polymeric, porcelain and other ones. More often consumers pay attention to functional characteristics of products of their choice, particularly, effectiveness of removing domestic soils (types of soils, difficulty of their removal, rate of removal), additional effects, for example, odour control at home, safety for cleaned surfaces and hand skin, as well as eco-friendliness and possibility of use in residences with autonomous sewage systems and septic tanks. Based on the Lightspeed/Mintel survey in March 2019, over 38% of consumers in Great Britain tend to choose multi-functional household chemicals enabling reducing the time required for full home cleanup. According to the data of the analytical survey Dishwashing products: Impact of COVID-19, UK, July 2020 Mintel, by 2020-2025 the trend for sustainable products, with a high content of natural components, a long "free from" list, as well as presence of biodegradable components will grow. It should be emphasized that consumption of household chemicals in EAEU region does not depend on the income level of population as they are essential goods. However, according to the results of the Lightspeed/Mintel survey in March 2020, 10-16% of consumers aged 18 years and older started to use household chemicals less often because of low effectiveness (10% of the surveyed people), a high content of synthetic chemical substances and low biodegradability of products (11% of the surveyed people), high water consumption for removal of soils (36% of the surveyed people). In addition, attractiveness and possibility of purchase of household chemicals in EMEA region were assessed. The main stimulating factors are reduction of physical efforts for manual removal of soils (more than 33% of responses), reduction of water consumption for washing away soils (more than 28% of responses) and high proven effectiveness of the product (more than 27% of responses). Thus, there was revealed a need in development of innovation household chemicals that enable effective removal of domestic soils, reduce required efforts for surface cleaning and are biodegradable in the environment.

**[0004]** However, despite the demand for effective products the consumer still pays attention to dermatological comfort for hand skin after use of cleaning and washing household products. Every day people touch a large number of surfaces in public places and at home and use household chemicals. To protect oneself against development of dermatological diseases, it is necessary to carefully select household chemicals as it is dermatological comfort of hands that is a guarantee of health and, as a result, high quality of people's life. Due to frequent contact of hands with synthetic components in products, development of products with a high content of natural ingredients remains the most reliable way of maintaining skin health and, as a result, its healthy appearance. According to specialists' estimates, skin health is one of the basics of general human health. For example, 2% of sodium lauryl sulfate, known as SLS, in the composition of household products can cause loss of transepidermal moisture by 68.9 g/m<sup>2</sup>/h in relation to the normal level in 12 hours after contacting the product [Loffler, H., & Happle, R. (2003). Profile of irritant patch testing with detergents: sodium lauryl sulfate, sodium laureth sulfate and alkyl polyglucoside. Contact Dermatitis, 48(1), 26-32]. As the content of sodium lauryl sulfate in household chemicals can reach 29% in connection with a high washing capacity, this can negatively affect the hand skin condition. C10-16 alkyl sulfates cause keratin denaturation by destruction of sulfide bonds and formation of sulfhydryl groups -SH on human cells and epidermal barrier washout [Prottey C, Ferguson T. Factors which determine the skin irritation potential of soaps and detergents. J Soc Cosmet Chem. 1975;26: 29-46.], and alkyl sulfates with C12-C14 chain length, including SLS, have the maximum activity. It was revealed that aggressive anionic surfactants sodium decyl sulfate (SDS), sodium myristyl sulfate (SMS), sodium tridecyl sulfate (STS) cause washout of water-soluble epidermis proteins by 166.1%, 163.9% and 198.5%, respectively [Loffler, H., & Happle, R. (2003). Profile of irritant patch testing with detergents: sodium lauryl sulfate, sodium laureth sulfate and alkyl polyglucoside. Contact Dermatitis, 48(1), 26-32]. Thus, development of formulations of washing and cleaning products with reduced content of synthetic and semi-synthetic anionic surfactants is a top-priority direction of care of hand skin epidermis.

**[0005]** Search for effective components and their combinations as means for removal of household soils of different origin, in particular, lipid soils, is one of top-priority tasks of companies producing cleaning and washing products. The main components of household chemicals are surfactants, fillers, components providing for stability of formulations, and functional additives performing specific functions. Specifically, special functional additives include biolipase and beta-cyclodextrins.

**[0006]** Lipase, or triacylglycerol-acyl-hydrolase (enzyme classification code E.C.3.1.1.3, CAS 9001-62-1, EINECS 232-619-9) is a water-soluble enzyme of the class of hydrolases that catalyses hydrolysis of ester bonds in triglycerides of fatty acids being water-insoluble esters of glycerol and higher carboxylic acids of different structures [European Commission Cosmetic Ingredients & Substances Database: <http://ec.europa.eu/growth/tools-databases/cosing/>]. Lipase is registered as the food additive E1104 and can be used for digestion, dissolving and fractioning of fats.

**[0007]** Lipase is a catalyst of the reaction of cleavage of fats and is not a product/initial substance in the reaction of hydrolysis, which enables improving kinetics of the enzymatic reaction. One enzyme molecule is known to be capable of catalysing up to 10000 reactions per second depending on domain organization, functional activity and crude materials. A small enzyme quantity is enough for effective action as activity of commercially available lipases is above 40 U/g or U/ml. For example, an effective concentration of lipase is 0.4-0.8% (w/w) in complex additives to be included in household chemicals [<https://www.enzymeinnovation.com/lipase-detergent-everything-you-need-know/>].

**[0008]** Lipases are believed to be one of the most effective enzymes for removal of fat stains and lipid soils based on vegetable oils (sunflower, olive, rapeseed, corn, flax-seed oil and others) having different fatty-acid formulations, animal fats (butter, lard, beef tallow, mutton tallow, etc.), having a solid aggregate state in normal conditions, lubricating materials based on ester components and greasy phases of perfumes and cosmetics containing oils, waxes, emulsifiers with ester bonds such as lecithin, perfume components and essential oils. As these stains are insoluble in aqueous phase and can be removed only with micellar solutions of surfactants with limited solubility, lipase contributes to hydrolysis of ester bonds and destruction of substrates, because of which surfactants of certain origin remove surface soils better, penetrate deep into fabric and more slowly reach saturation of micelles while the washing effectiveness remains.

**[0009]** Lipases have a high activity in relation to lipid soils on different surfaces, including metal, polymeric, wooden, ceramic ones, and do not destroy the structure of these surfaces, which speaks of gentle action in formulations of washing products. The most desirable pH-optimum makes up from 5.0 to 11.0, while the activity can remain at different temperatures from 0°C to 60°C, specifically the temperature optimum makes up from 20°C to 40°C. Lipases can be stable in presence of proteases, chelating agents, peroxide compounds (hydrogen peroxide, sodium percarbonate, etc.) and surfactants, specifically, anionic ones. Lipase stabilizers, specifically, glycerol, propylene glycols, sorbitol, sugars, carboxylic acids, alkylamines, inorganic salts, non-ionic and ionic surfactants can be included in the systems, and water content in the formulation can be reduced, which enables producing a concentrated detergent with low water consumption in production of detergents. Use of lipase makes it possible to produce energy-efficient products saving electric power and water and thus to use resources in a responsible manner.

**[0010]** Lipase concentration in household chemicals depends on activity and can make up from 0.0025 to 1% (by weight, in pure form). It was established that addition of lipase with activity 100 KLU3/g to surfactants in the formulation of a laundry detergent demonstrated high effectiveness in removal of fat stains during standard washing at 40°C. The effective lipase concentration (in complex additives) with this activity is 0.2-0.6% [H. Uhlig, E. M. Linsmaier-Bednar. Industrial enzymes and their applications. Engineering, April 1998, 472 pages. ISBN: 978-0-471-19660-0. DOI:10.5860/choice.36-0333]. In detergents lipase with activity of 50 U/mL in the amount of 10 mL together with 0.5% system of anionic and non-ionic surfactants was stable in the formulation and did not influence thermal stability of detergents. Also, lipase was not destroyed in presence of 2% hydrogen peroxide solution, i.e. it retained its activity by 92% in 2 hours after addition of a bleaching agent to the solution, however the activity significantly decreased in presence of sodium hypochlorite and sodium perborate with increase of the concentration from 1 to 2%.

**[0011]** It was revealed that lipase is a necessary component in detergents for effective removal of lipid stains and fat soils. It is enzymatic activity per unit volume that determines the working concentration of the component in formulations of detergents of different applications. With time-based activity of 50 U/mL and in the amount of 10 mL, the total enzyme activity will make up 500 U. With average enzyme activity of 100-1000 U/g, the effective lipase concentration (in the formulation of a complex additive) will make up 0.05-5.00% in detergent formulation [Mamta Chauhan, Rajinder Singh Chauhan, and Vijay Kumar Garlapati. Evaluation of a New Lipase from *Staphylococcus* sp. for Detergent Additive Capability. BioMed Research International Volume 2013 |Article ID 374967 | 6 pages | <https://doi.org/10.1155/2013/374967>. <https://www.hindawi.com/journal/s/bmri/2013/3/74967/>].

**[0012]** Thus, lipase is a necessary component in detergents for effective removal of lipid stains and fat soils due to targeted action on molecules of triglycerides of fatty acids and increase of effectiveness of detergents based on surfactants, specifically, non-ionic surfactants. With the average activity of the component >100 U/g, the effective concentration of lipase in pure form is 0.0025-0.1% in cleaning and washing products for different surfaces.

**[0013]** At the same time, lipase has a specific odour. Lipase cleaves fats into glycerol and fatty acids. If, for example, these fatty acids are not removed from textile in the process of washing, consumers notice a rancid odour, which become

more intense depending on the quantity of lipase. (V.A. Averyanova. Evolution of detergents: pursuit of a sustainable ideal / Syrye i Upakovka (Raw Materials and Package). No. 02 (151), 2014). Thus, lipase can cause irritation of mucous membranes of the upper respiratory tract and lungs as well as an allergic reaction. The authors of the invention successfully overcame this restriction by creation of an effective combination.

**[0014]** Another functional biodegradable component is cyclodextrins. The direction of odour and atmosphere control at home is relevant and is in active development now. Odours play an important role in people's life as they enable recognition of different compounds and orientation in space as well as protection against toxic and allergizing substances. It is not always possible to neutralize an unpleasant odour indoors, which results in a feeling of discomfort and does not create the feeling of safety for people. Not all detergents have the ability to neutralize unpleasant odours after a thorough cleanup at home, therefore there is a need in odour control for creation of comfortable conditions.

**[0015]** Cyclodextrins are substances of natural origin, cyclic oligomers of glucose obtained enzymatically from starch [Crini G. A history of cyclodextrins //Chemical reviews. - 2014. - V. 114. - No. 21. - P. 10940-10975]. Cyclodextrins comprise at least six residues of D-(+) glucopyranose bound with  $\alpha$ -(1,4)-glucoside bonds. Glucopyranose residues form the energetically favorable "chair" conformation, due to which molecules of cyclodextrins have the shape of cones. The external surface of the latter is hydrophilic and makes it possible for them to be water-soluble, while the central cavity has hydrophobic properties because of a certain arrangement of hydroxyl groups and enables incorporating hydrophobic or amphiphilic substances. Natural cyclodextrins can be of three types:  $\alpha$ ,  $\beta$  and  $\gamma$ , which corresponds to 6, 7 and 8 glucopyranose residues [B. Bonthagarala, CH BABU RAO, N. Sreekanth. The cyclodextrins: A review// IJPRBS - 2013. - V. 2. - P. 291-304]. Each of the three types of cyclodextrins has its physical and chemical properties, specifically, molecular weight, central cavity diameter, approximate cavity volume and solubility in water, number of water molecules contained in the cavity of cyclodextrins and pKa of the substance in an aqueous medium. Molecules of  $\beta$ -cyclodextrins have the largest internal cavity diameter, which enables them to form inclusion complexes (host-guest complexes) with a wide range of solid, liquid and gaseous compounds by means of molecular complex formation. In these complexes the guest molecule is retained within the cavity of the cyclodextrin host molecule due to hydrophobic and van der Waals interactions. The hydrophobic cavity of cyclodextrin molecules provides for microenvironment where non-polar fragments of a suitable size can be included with formation of an inclusion complex [Loftsson T., Brewster M. E. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization //Journal of pharmaceutical sciences. - 1996. - V. 85. - No. 10. - P. 1017-1025]. At that, there is no formation or destruction of covalent bonds that lead to destruction of molecules. The main driving force of complex formation is release of water molecules from the cavity of cyclodextrins rich in enthalpy  $\Delta H$ . Water molecules are displaced by a more hydrophobic guest under the impact of non-covalent forces of intermolecular interaction, which results in reduction of  $\Delta G$  free energy of the cyclodextrin ring surface, and the molecule of cyclodextrins becomes stable in low-energy state [Szejtli J. Introduction and general overview of cyclodextrin chemistry //Chemical reviews. - 1998. - V. 98. - No. 5. - P. 1743-1754]. Binding of guest molecules is characterized with dynamic balance. The binding force depends on how strong non-covalent host-guest interactions are. The complexes can form either in a solution or in the crystalline state, wherein water is a preferred solvent [Hădărugă N. G. et al. A review on thermal analyses of cyclodextrins and cyclodextrin complexes //Environmental Chemistry Letters. - 2019. - V. 17. - No. 1. - P. 349-373].

**[0016]** Complex formation enables changing some properties of guest molecules: increasing solubility of hydrophobic molecules, improving stability of labile molecules, reducing volatility of aromatic compounds [B. Bonthagarala, CH BABU RAO, N. Sreekanth. The cyclodextrins: A review// IJPRBS - 2013. - V. 2. - P. 291-304]. The list of potential guests for molecular encapsulation in cyclodextrins is rather diverse and it includes such compounds as aliphatic compounds with a straight or branch chain, aldehydes, ketones, alcohols, organic acids, fatty acids, aromatic compounds, gases and polar compounds such as halogens, oxy acids and amines [Del Valle E. M. M. Cyclodextrins and their uses: a review //Process biochemistry. - 2004. - V. 39. - No. 9. - P. 1033-1046].

**[0017]** For many encapsulated substances the size of the cavity of  $\alpha$ -cyclodextrins is too small, while use of  $\gamma$ -cyclodextrins is often limited with a high cost of obtaining them. Therefore  $\beta$ -cyclodextrins are used more widely in different industries comparing to other natural cyclodextrins: They have a satisfactory cavity size, while it is economically more feasible to obtain and purify them [Lovatti Alves Q. et al. Drugs- $\beta$ -Cyclodextrin inclusion complex: Would be a new strategy to improve Antihypertensive Therapy //Clin. Res. Trials. - 2019. - V. 5. - P. 1-3].

**[0018]**  $\beta$ -cyclodextrins have various effects, specifically, they control simultaneous adsorption and prolonged release of molecules of odours, including unpleasant ones, kinetics of dyeing and release of dyes in the process of washing, stabilization of aromatic compounds (flavourings, fragrances, essential oils) in formulations and ensure thermal stability of detergents and perfumery and cosmetics.

**[0019]** Residual surfactants on the surface of fibers influence the adsorption ability of the textile surface and dyeing ability of textiles and also reduce the quality of hydrophobic finishing. Cyclodextrins are capable of forming complexes with surfactants in an aqueous solution, which enables removing a large part of adsorbed surfactants. Adding a cyclodextrin (3 g/l) to rinsing water removes residual traces of detergents in the solution and thus reduces the residual quantity

of the detergent in the fabric (from 209 to 134 parts per million) and water consumption [Ammayappan L., Moses J. J. An overview on application of cyclodextrins in textile product enhancement //J Text Assoc. - 2009. - V. 70. - No. 1. - P. 9-18].

**[0020]** There were detailed descriptions of promising directions in use of cyclodextrins in the process of fabric dyeing [Bezerra F. M. et al. The Role of  $\beta$ -Cyclodextrin in the Textile Industry //Molecules. - 2020. - V. 25. - No. 16. - P. 3624].

Cyclodextrins, when used as auxiliary substances in dyeing, influence both properties of dyes and kinetics of dyeing, making it possible to increase the degree of extraction of excessive dye and thus improve the quality of wastewater and ecological burden.

**[0021]** Molecules or functional groups causing an unpleasant taste or odour can be hidden from sense receptors by confining them to the cavity of cyclodextrins. Such complexes significantly reduce the concentration of free molecules causing an unpleasant taste or odour [Tiwari G., Tiwari R., Rai A. K. Cyclodextrins in delivery systems: Applications //Journal of Pharmacy and Bioallied Sciences. - 2010. - V. 2. - No. 2. - P. 72], specifically, dihydroxyacetone, free fatty acids and esters, derivatives of amines, glutathione, mercapto compounds, caproaldehyde as a marker of oxidation of vegetable oils and fats. Cyclodextrins, due to complex formation with aromatic compounds, can be used for concealing strong odours or reducing intensity of odours.

**[0022]** A serious problem in home or industrial kitchens is removal and separation of volatile compounds that are perceived as strong odors, especially those emitted during frying food on vegetable oils and fats. Cone-shaped molecules of  $\beta$ -cyclodextrins enable adsorbing strong and acrid odours of caproaldehyde being a marker of oxidation of oils and fats during cooking of food [Ghorani B. et al. Assembly of odour adsorbent nanofilters by incorporating cyclodextrin molecules into electrospun cellulose acetate webs //Reactive and Functional Polymers. - 2019. - V. 134. - P. 121-132], in the process of washing of different kitchen and tableware surfaces after frying of food. As fats and oils contain esters of fatty acids and glycerol, the complex based on specific lipase enzyme and  $\beta$ -cyclodextrins improves the washing process by targeted cleavage of molecules of fats and adsorption of released fatty acids. This synergetic action improves the washing capacity of detergents for different surfaces by control of kinetics of cleavage of fats and adsorption of unpleasant odours caused by fatty acids and products of their oxidation, in distinction to other detergents.

**[0023]** The documents EP0427806B1 [NOVO NORDISK A/S] published on 20.06.1994 and WO1990010687A1 published on 20.09.1990 describe the process of hydrolysis of fats in presence of lipase in water with the ratio of 1:3 to 3:1 (by weight) characterized by presence of  $\alpha$ -,  $\beta$ - or gamma-cyclodextrins, which can be optionally substituted. Cyclodextrins improve the degree of hydrolysis of fats or oils, i.e. any material with a high content of triglycerides, by means of lipase in an aqueous medium. The combination can be used with a constant dose of lipase and constant reaction time, with the use of increased pressure for solution homogenization.

**[0024]** The above-described composition from the patent application WO1990010687A1 has a number of disadvantages, specifically, impossibility of use at high temperatures above 60°C because of denaturation of enzyme quaternary structure, which causes its inactivation and reduction of effectiveness of lipid hydrolysis process as well as use of increased pressure above 0.101325 MPa, which requires special process equipment and confirmation of lipase activity under this pressure. The activity of lipase at low temperatures, a wide temperature range (temperature lability) would enable extending the field of use of the given combination. A higher fat:water and water:lipase ratio would enable achieving process scaling and increasing the yield of hydrolysis products as it would require a lower quantity of fat and lipase for this process. Also, there is a description of use of lipase of animal origin, which demonstrates an insufficiently conscious attitude to the environment and will not make it possible to obtain Vegan/Vegetarian voluntary certification for household chemicals with this component. The lipase quantity in the process can be reduced with addition of  $\beta$ -cyclodextrins acting as colipase and an activator of kinetics of cleavage of triglycerides of fatty acids.

**[0025]** The patent application WO01/18163A1 [THE PROCTER & GAMBLE COMPANY] published on 21.08.2000 discloses use of the formulation for making a laundry detergent comprising a granulated cyclodextrin. The formulation ensures removal of unpleasant odours from things connected with presence of particles of sweat and sebum, keratinized skin cells in the process of auto-washing upon presence of a cyclodextrin in the laundry detergent formulation. The active form of a stable, quickly soluble, free-flowing cyclodextrin in granulated form has a deodorizing effect and is capable of smoothening laundry after washing, primarily, cotton and also can be used for washing other surfaces such as upholstery, curtains, carpets, blankets, etc. The component can be used together with enzymes (protease, cellulase, lipase, amylase and/or a mixture of these enzymes) and fillers.

**[0026]** The above-described composition from the patent application WO01/18163A1 comprises only granulated cyclodextrins as components for odour adsorption, however it is not capable of cleaving triglycerides of fatty acids in a targeted way and increasing effectiveness of detergents in this direction as lipase is not the main active component of the composition. Thanks to the mean size of particle, it is possible to use granulated cyclodextrins only in formulation of fillers, powder mixtures or powder laundry detergents, which limits the field of application of the component as its introduction in liquid detergents for achieving this effect is complicated owing to low solubility. Introduction of the specific enzyme lipase would make it possible to increase effectiveness of removal of fat soils and odour because of presence of specific ethers responsible for an unpleasant odour, while non-granulated cyclodextrins more easily dissolve in water during mixing and are stable in liquid detergents, which extends the field of application of the components. In addition,

non-granulated cyclodextrins would adsorb products of reaction of hydrolysis of lipid substrates, would make it possible to control kinetics of cleavage of fats, would adsorb unpleasant odour molecules owing to the hydrophobic cavity and would reduce water and energy consumption per 1 wash.

**[0027]** The patent application EP1075509A1 (WO9957254A1) [THE PROCTER & GAMBLE COMPANY] published on 30.04.1999 discloses use of a formulation for making laundry detergents comprising modified transferase. The modified enzyme comprises a catalytically active domain of transferase bound with the amino acid sequence of the domain binding cellulose for effective removal of stains and improvement of the washing process. The component can be used in combination with a system of surfactants, enzymes (protease, cellulase, lipase, amylase and/or a mixture of these enzymes), bleaching agents, fillers, releasing substances, optical whitening agents, fabric softeners, dispersing agents, inhibitors of dye wash-out from fabrics, abrasives, bactericides, fragrances. The composition based on modified transferase is directed at removal of body sebum and soils based on vegetable fibers, sugars, amylose, which are hard to remove in the washing process, especially at low temperatures. Thus, a need in a composition for soil removal, laundry smoothing and softening, control of static voltage, color retention and increasing of fabric wear resistance is described.

**[0028]** The above composition from the patent application EP1075509A1 (WO9957254A1) is intended for specific cleavage of polysaccharide substrates, specifically, glycoside dimers, oligomers and/or polymers from starch, xyloglycans, cyclodextrins, sucrose and maltose. Cyclodextrins do not increase effectiveness of the composition as they are substrates of the enzyme that cleaves them. The lipase enzyme is described as a possible additional enzyme in this composition on equal terms with protease, cellulase, amylase. In the given examples the content of lipase varies from 0.002 to 0.01%, which does not enable complete cleavage of lipid soils with short wash programs (up to 30 minutes). The composition does not have the ability to adsorb unpleasant odours fixed by sebum and lipid soils in a targeted way, but it removes them in the process of laundry washing and drying only partially. Use of the lipase enzyme with a high activity and increase of its content would enable increasing effectiveness of removal of fat soils and odours due to presence of specific ethers responsible for unpleasant odours, while non-granulated cyclodextrins would adsorb products of the reaction of hydrolysis of lipid substrates, would enable control of kinetics of cleavage of fats, would adsorb unpleasant odour molecules owing to the hydrophobic cavity and would reduce water and energy consumption per 1 wash.

**[0029]** In distinction to patent applications WO1990010687A1, WO01/18163A1 and EP1075509A1 (WO9957254A1), the authors of the present invention studied effectiveness of the biodegradable composition comprising lipase obtained by biotechnology methods and  $\beta$ -cyclodextrins and revealed the synergetic effects for control of enzyme kinetics and fast cleavage of lipid soils and depositions from different surfaces (wooden, metal, enameled, polymeric, ceramic, earthenware and other ones), supplemental emulsifying of fats and fatty acids, adsorption of unpleasant odours from different surfaces, ensuring stability of liquid detergents with aromatic components in the process of storage. The combination is safe for hand skin and ensures dermatological comfort of skin, which enables reducing the content of anionic surfactants causing increasing of hand skin sensitivity, and does not have irritating action. Thanks to the synergetic effect of lipase and  $\beta$ -cyclodextrins it becomes possible to reduce the content of anionic surfactants or a combination with them by 30% and more. Addition of  $\beta$ -cyclodextrins enables reducing the reaction temperature and reducing peroxidation of triglycerides of unsaturated fatty acids that are contained in soybean, corn, flax seed, olive and other food oils. During peroxidation oxides and peroxides of acids are formed that have a specific rancidness odour, create yellowish color and cause cloudiness of tableware made of polymeric materials.

**[0030]** The technical result of the innovation complex consists in effective removal of fat soils by control of kinetics of cleavage of lipid soils, removal of unpleasant odours and preservation of thermal stability of samples. The complex is active within a wide pH range, specifically, 5.0-10.0 units, and a wide temperature range, specifically, from +5 to +60°C, which extends the field of use in washing and cleaning products.

**[0031]** It was revealed that the combination of lipase and  $\beta$ -cyclodextrins enables increasing effectiveness of removal of complex protein and lipid soils on different surfaces, specifically, metal, polymeric, glass and wooden surfaces as well as increasing the washing effectiveness of detergents by more than 35% on different surfaces with preservation of the content of surfactants based on components of natural origin. At that, lipase and  $\beta$ -cyclodextrins mean raw materials comprising active components as well as technical-grade impurities that could form in the process of production of target raw materials.

**[0032]** Combining the components enables achieving synergetic action and maintaining effectiveness with a lower percentage of introduction of certain components. Lipase obtained by modern biotechnology methods without use of GMO is an active enzyme cleaving insoluble lipid substrates with ester bonds of triglycerides on "water-oil" or "oil-water" phase boundary.  $\beta$ -cyclodextrins act as coactivators (colipases) by binding with the terminal domain of lipase and formation of a clathrate membrane from water molecules, contributing to change of conformation of the active center of lipase and reduction of process activation energy ( $\Delta E_a$ ), which simplifies the flow of the enzymatic reaction. The lipase enzyme reduces energy of activation by increasing the number of activated molecules of triglycerides of fatty acids and glycerol that become reactive on a lower energy level, which reduces the energy barrier for further flow of the enzymatic reaction. In addition, cyclodextrins, thanks to a hydrophilic surface owing to free hydroxyl groups -OH, specifically orient lipase substrates on "water-oil" or "oil-water" phase boundary, increasing availability of an ester bond for

enzymatic hydrolysis. As the enzymatic reaction is an equilibrium reaction, displacing equilibrium towards formation of hydrolysis products requires binding free fatty acids and removing them from the system. Specifically,  $\beta$ -cyclodextrins bind reaction products thanks to emulsifying properties, displacing balance of reaction towards cleavage of lipid substrates in the system.

**[0033]** An additional property is neutralization of odorous substances represented by different classes of organic substances: aldehydes and ketones, terpenes, amines, indole, sulfur-containing components (mercaptans), organic acids and their esters, phenols and cresols. As people are more sensitive to unpleasant odours, neutralization of odorous substances becomes a main tool for emotional state control. In such cases cleanup using common household detergents does not provide for effective removal of unpleasant odours, therefore there is a need in a special combination for control and removal of unpleasant odours in addition to control of kinetics of hydrolysis of lipid soils.

**[0034]** An innovation complex including lipase and  $\beta$ -cyclodextrins is directed at effective cleaning and removal of fat soils thanks to controlling kinetics of the reaction of cleavage of lipid substrates and increasing washing effectiveness of household chemicals as well as neutralization of unpleasant odours. The complex based on natural and biodegradable components is effective within the pH range of 5.0-11.0 units in presence of different synthetic and natural components, which enables its application in a wide range of household chemicals for cleaning of different surfaces such as laundry and dishwashing detergents, floor and glass cleaners, all-purpose detergents. The components act on lipid and complex soils on different surfaces in a targeted way and also bind released products of exchange with an odour unpleasant for people. After use of the composition the cleanliness of surfaces and a pleasant scent last longer. Thus, combined use of the components in the claimed composition in one product results in increasing of kinetics of the enzymatic reaction of cleavage of complex household lipid soils by stabilization, orientation of substrates and increasing of activity of lipase thanks to  $\beta$ -cyclodextrins, which makes it possible to do cleaning quickly even using cold water. A distinctive feature is the fact that the components in claimed concentrations act only towards complex soils and do not impair the look of most surfaces, specifically, metal, wooden, polymeric and enameled ones. The composition does not include aggressive inorganic substances and organic solvents, occlusive filming agents and alcohols, therefore washing and cleaning products with this composition can be used regularly with no harm to hand skin. Combined use of these components has synergetic action ensuring complex care of different surfaces in the formulation of a single detergent for daily use.

**[0035]** Thus, the invention generally relates to a composition and its use enabling achieving such technical results as effective control of kinetics of enzymatic cleavage of lipids with simultaneous effective neutralization of unpleasant odours on different types of surfaces, including especially metal, polymeric, enameled, glass and wooden ones, with maintaining of continuous cleanliness and a pleasant odour that are not achieved or are insufficiently achieved with modern commercially available means in the given art.

## SUMMARY

**[0036]** In one aspect the invention relates to a composition intended for use in the formulation of a detergent consisting of lipase and  $\beta$ -cyclodextrin, wherein the mass ratio of lipase and  $\beta$ -cyclodextrin is (0.0025-0.25):(0.1-1), respectively.

**[0037]** The composition can differ in that the quantity of lipase in the specified mass ratio of lipase and  $\beta$ -cyclodextrin (0.0025-0.25):(0.1-1) is 0.0025, 0.005, 0.0075, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.15, 0.2 or 0.25.

**[0038]** The composition can differ in that the quantity of  $\beta$ -cyclodextrin in the given mass ratio of lipase and  $\beta$ -cyclodextrin (0.0025-0.25):(0.1-1) is 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95 or 1.

**[0039]** The composition can differ in that the specified lipase is in a hydroglyceric solution.

**[0040]** The composition can differ in that the specified hydroglyceric solution of lipase is the commercially available product Lipex® Evity® 200 L. Lipex® Evity® 200 L is available, specifically, from Novozymes and can be identified, for example, by <<https://biosolutions.novozymes.com/en/laundry/products/lipexr-evityr-200-l>>.

**[0041]** The composition can differ in that the specified hydroglyceric solution of lipase is the commercially available product Lipex® Evity® 200 L modified with an additional quantity of glycerol.

**[0042]** The composition can differ in that the composition comprises Lipex® Evity® 200 L modified with an additional quantity of glycerol and  $\beta$ -cyclodextrin, wherein the content of Lipex® Evity® 200 L is 0.4%wt. per weight of the composition, the content of the additional quantity of glycerol introduced for modification is 1%wt. per weight of the composition.

**[0043]** The composition can differ in that in the composition comprising Lipex® Evity® 200 L modified with an additional quantity of glycerol the mass ratio of Lipex® Evity® 200 L and the additional quantity of glycerol introduced for modification is (0.1-1):(0.5-1.5), respectively.

**[0044]** The composition can differ in that the quantity of Lipex® Evity® 200 L in the given mass ratio of Lipex® Evity® 200 L and the additional quantity of glycerol introduced for modification is 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95 or 1.

**[0045]** The composition can differ in that the quantity of additional glycerol introduced for modification in the given mass ratio of Lipex® Evity® 200 L and the additional quantity of glycerol introduced for modification is 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1, 1.1, 1.2, 1.3, 1.4 or 1.5.

**[0046]** The composition can be different in that it additionally comprises decylglucoside.

**[0047]** The composition can be different in that the specified decylglucoside is the commercially available product NaturalAPG HG0814CM. NaturalAPG HG0814CM is available, specifically, from Hugo and can be identified, for example, by <<https://www.hugochem.net/alkyl-polyglucoside/apg-0814/decyl-glucoside-apg-2000up.html>>.

**[0048]** The composition can be different in that the mass ratio of Lipex® Evity® 200 L and NaturalAPG HG0814CM is (0.2-0.6):(10-14).

**[0049]** The composition can be different in that the quantity of Lipex® Evity® 200 L in the specified mass ratio of Lipex® Evity® 200 L and NaturalAPG HG0814CM is 0.2, 0.3, 0.4, 0.5 or 0.6.

**[0050]** The composition can be different in that the quantity of NaturalAPG HG0814CM in the specified mass ratio of Lipex® Evity® 200 L and NaturalAPG HG0814CM is 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5 or 14.

**[0051]** The composition can be different in that the composition comprises the commercially available product Lipex® Evity® 200 L modified with an additional quantity of glycerol,  $\beta$ -cyclodextrin and the commercially available product NaturalAPG HG0814CM, wherein the content of the commercially available product Lipex® Evity® 200 L is 0.4%wt. per weight of the composition, the content of the additional quantity of glycerol introduced for modification is 1%wt. per weight of the composition, the content of the commercially available product NaturalAPG HG0814CM is 12%wt. per weight of the composition.

**[0052]** The composition can be different in that the activity of the specified lipase is at least 40 LU/g or 40 LU/ml at pH 7.0.

**[0053]** The composition can be different in that the specified household chemical is selected from a dishwashing detergent, floor cleaner and/or toilet bowl and/or wash bowl and/or bathtub and/or glass and/or pipe cleaner, laundry detergent, including delicate laundry or baby laundry detergent, fabric conditioner, stain remover for preliminary fabric treatment and washing, gel laundry detergent and conditioner.

**[0054]** In another aspect the invention relates to a dishwashing detergent comprising 0.0525-1%wt. of the composition of the present invention.

**[0055]** In another aspect the invention relates to a floor and/or toilet bowl and/or wash bowl and/or bathtub and/or glass cleaner comprising 0.0525-1%wt. of the composition of the present invention.

**[0056]** In another aspect the invention relates to an all-purpose surface cleaner comprising 0.0525-1%wt. of the composition of the present invention.

**[0057]** In another aspect the invention relates to a pipe cleaner comprising 0.0525-2%wt. of the composition of the present invention.

**[0058]** The pipe cleaner can be different in that the specified detergent comprises 0.0525-1.5%wt. of the specified composition, preferably 0.0525-1%wt. of the specified composition.

**[0059]** In another aspect the invention relates to a laundry detergent comprising 0.0525-1%wt. of the composition of the present invention.

**[0060]** The laundry detergent can be different in that the specified detergent is selected from a delicate laundry detergent and a baby laundry detergent.

**[0061]** The laundry detergent can be different in that the specified detergent is a powder laundry detergent.

**[0062]** In another aspect the invention relates to use of the composition of the present invention for control of kinetics of enzymatic cleavage of lipids and neutralization of unpleasant odours on different surfaces, with maintaining of continuous cleanliness and a pleasant odour.

**[0063]** The use can be different in that the specified surface is selected from a metal, polymeric, enameled, glass or wooden surface.

**[0064]** In another aspect the invention relates to a method of preparing the composition of the present invention including preliminary introduction of the lipase solution to  $\beta$ -cyclodextrin dispersion, wherein after addition of the last portion of lipase the dispersion is mixed during 10-15 min.

**[0065]** The invention will be disclosed in detail in its specific embodiments and will be illustrated with examples of embodiment.

## DETAILED DESCRIPTION OF THE INVENTION

**[0066]** In the detergent of the present invention the auxiliary acceptable substances can be selected from the following categories of components.

**[0067]** Anionic surfactants:

Salts of higher carboxylic acids with the general formula:  $R_1-CO_2X_1$ , where  $R_1$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms, and  $X_1$  is an alkaline and/or alkaline-earth metal, ammonium, alkylammonium, alkanolammonium, glucoammonium, basic amino acid cation;

Alkyl polyethylene glycol sulfate with the general formula:  $R_2-O(-CH_2-CH_2-O)_nSO_3X_2$ , where  $n_1$  takes a value from 1 to 10 and denotes the number of polyethylene glycol groups,  $R_2$  is an alkyl and/or alkenyl group with



hydrocarbon chain length from 6 to 22 carbon atoms and X2 is an alkaline and/or alkaline earth metal, ammonium, alkylammonium, alkanolammonium, glucoammonium cation;

Alkyl sulfate with the general formula  $R_3-OSO_3X_3$ , where  $R_3$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 6 to 22 carbon atoms and  $X_3$  is an alkaline and/or alkaline earth metal, ammonium, alkylammonium, alkanolammonium, glucoammonium cation;

A salt of a higher fatty acid amide and methylglycine with the general formula  $R_4-C(O)-N(-CH_3)-CH_2-CO_2X_4$ , where  $R_4$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms and  $X_4$  is an alkaline and/or alkaline earth metal, ammonium, alkylammonium, alkanolammonium, glucoammonium cation;

Alkyl polyethylene glycol carboxylate with the general formula:  $R_5-O(-CH_2-CH_2-O-)_{n2}CH_2-CO_2X_5$ , where  $n_2$  takes a value from 1 to 15 and denotes the number of polyethylene glycol groups,  $R_5$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 6 to 22 carbon atoms and  $X_5$  is an alkaline and/or alkaline earth metal, ammonium, alkylammonium, alkanolammonium, glucoammonium cation;

A disubstituted salt of 2-sulfocarboxylic acid with the general formula:  $R_6-CH(-SO_3X_6)-CO_2X_6$ , where  $R_6$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 4 to 20 carbon atoms and  $X_6$  is an alkaline and/or alkaline earth metal, ammonium, alkylammonium, alkanolammonium, glucoammonium cation;

A mono- or disubstituted salt of a higher carboxylic acid amide and glutamic acid with the general formula:  $R_7-C(O)-NH-CH(-CH_2-CH_2-CO_2X_7)-CO_2X_7$ , where  $R_7$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms and  $X_7$  is an alkaline and/or alkaline earth metal, ammonium, alkylammonium, alkanolammonium, glucoammonium or hydrogen cation;

A salt of a higher fatty acid amide and glycine with the general formula:  $R_8-C(O)-NH-CH_2-CO_2X_8$ , where  $R_8$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms and  $X_8$  is an alkaline and/or alkaline earth metal, ammonium, alkylammonium, alkanolammonium, glucoammonium cation;

A salt of a higher fatty acid amide and alanine with the general formula:  $R_9-C(O)-NH-CH(-CH_3)-CO_2X_9$ , where  $R_9$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms and  $X_9$  is an alkaline and/or alkaline earth metal, ammonium, alkylammonium, alkanolammonium, glucoammonium cation;

A salt of a higher fatty acid amide and 2-aminomethylethanesulfonic acid with the general formula:  $R_{10}-C(O)-N(-CH_3)-CH_2-CH_2-SO_3X_{10}$ , where  $R_{10}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms, and  $X_{10}$  is an alkaline and/or alkaline earth metal, ammonium, alkylammonium, alkanolammonium, glucoammonium cation;

Alkylpolyglucoside hydroxypropylsulfonate with the general formula:  $R_{11}-O-[G]_{p1}-O-CH_2-CH(-OH)-CH_2-SO_3X_{11}$ , where  $R_{11}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 6 to 22 carbon atoms,  $G$  is a saccharide fragment comprising 5 or 6 carbon atoms,  $p_1$  takes a value from 1 to 4 and  $X_{11}$  is an alkaline and/or alkaline earth metal, ammonium, alkylammonium, alkanolammonium, glucoammonium cation;

Alkylpolyglucoside carboxylate with the general formula:  $R_{12}-O-[G]_{p2}-O-CH_2-CO_2X_{12}$ , where  $R_{12}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 6 to 22 carbon atoms,  $G$  is a saccharide fragment comprising 5 or 6 carbon atoms,  $p_2$  takes a value from 1 to 4 and  $X_{12}$  is an alkaline and/or alkaline earth metal, ammonium, alkylammonium, alkanolammonium, glucoammonium cation;

A salt of a higher fatty acid amide and threonine with the general formula:  $R_{13}-C(O)-NH-CH(-CH(-OH)-CH_3)-CO_2X_{13}$ , where  $R_{13}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms and  $X_{13}$  is an alkaline and/or alkaline earth metal, ammonium, alkylammonium, alkanolammonium, glucoammonium cation;

A salt of a higher fatty acid amide and an aminoacid obtained by hydrolysis of proteins from plant raw materials, with the general formula:  $R_{14}-C(O)-AAX_{14}$ , where  $R_{14}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms,  $AA$  is an aminoacid or peptide obtained during hydrolysis of plant protein (possible protein sources: apple, soybeans, wheat, cotton, etc.) and  $X_{14}$  is an alkaline and/or alkaline earth metal, ammonium, alkylammonium, alkanolammonium, glucoammonium cation.

Amphoteric surfactants:

[0068]

A disubstituted salt of acylamphodiacetate with the general formula:  $R_{15}-C(O)-NH-CH_2-CH_2-N(-CH_2-CO_2X_{15})-CH_2-CH_2-O-CH_2-CO_2X_{15}$ , where  $R_{15}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms and  $X_{15}$  is an alkaline and/or alkaline earth metal, ammonium, alkylammonium, alkanolammonium, glucoammonium cation;

An acylamphoacetate salt with the general formula:  $R_{16}-C(O)-NH-CH_2-CH_2-N(-CH_2-CO_2X_{16})-CH_2-CH_2-OH$ , where  $R_{16}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms and  $X_{16}$  is an alkaline and/or alkaline earth metal, ammonium, alkylammonium, alkanolammonium, glucoammonium cation;

An alkylamphoacetate salt with the general formula:  $R_{17}-C(=N-CH_2-CH_2-N((-CH_2-CH_2-OH)-CH_2-CO_2X_{17}))$ -, where  $R_{17}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms and  $X_{17}$  is an alkaline and/or alkaline earth metal, ammonium, alkylammonium, alkanolammonium, glucoammonium cation;

Acylamidoalkylbetaine with the general formula:  $R_{18}-C(O)-NH-R_{19}-N(-CH_3)_2-CH_2-CO_2$ , where  $R_{18}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms,  $R_{19}$  is an alkyl group with hydrocarbon chain length from 1 to 4 carbon atoms;

Acylamidoalkylhydroxysultaine with the general formula:  $R_{20}-C(O)-NH-R_{21}-N(-CH_3)_2-CH_2-CH(-OH)-CH_2-SO_3$ , where  $R_{20}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms,  $R_{21}$  is an alkyl group with hydrocarbon chain length from 1 to 4 carbon atoms;

Acylamidoalkylamine oxide with the general formula:  $R_{22}-C(O)-NH-R_{23}-N(-CH_3)_2-O$ , where  $R_{22}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms,  $R_{23}$  is an alkyl group with hydrocarbon chain length from 1 to 4 carbon atoms;

Alkylbetaine with the general formula:  $R_{24}-N(-CH_3)_2-CH_2-CO_2$ , where  $R_{24}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms;

Alkylhydroxysultaine with the general formula:  $R_{25}-N(-CH_3)_2-CH_2-CH(-OH)-CH_2-SO_3$ , where  $R_{25}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 6 to 22 carbon atoms;

Alkylsultaine with the general formula:  $R_{26}-N(-CH_3)_2-CH_2-CH_2-CH_2-SO_3$ , where  $R_{26}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 6 to 22 carbon atoms;

Alkylamine oxide with the general formula:  $R_{27}-N(-CH_3)_2-O$ , where  $R_{27}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 6 to 22 carbon atoms;

Non-ionic surfactants:

#### [0069]

Alkylglucoside with the general formula:  $R_{28}-O-[G]_{p3}$ , where  $R_{28}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 4 to 22 carbon atoms,  $G$  is a saccharide fragment of 5 or 6 carbon atoms,  $p3$  can take a value from 1 to 4;

Alkylpolyethyleneglycol with the general formula:  $R_{29}-O(-CH_2-CH_2-O)_{n3}H$ , where  $n3$  can take a value from 2 to 20 and denotes the number of polyethyleneglycol groups,  $R_{29}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 6 to 22 carbon atoms;

Alkylpolyethylene/propyleneglycol with the general formula:  $R_{30}-O(-CH_2-CH_2-O)_{n4}(-CH(-CH_3)-CH_2-O)_{n5}H$ , where  $n4$  takes a value from 2 to 20 and denotes the number of polyethyleneglycol groups,  $n5$  takes a value from 2 to 20 and denotes the number of polypropyleneglycol groups,  $R_{30}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 6 to 22 carbon atoms;

Dialkylpolyethyleneglycol with the general formula:  $R_{31}-O(-CH_2-CH_2-O)_{n6}R_{32}$ , where  $n6$  takes a value from 2 to 20 and denotes the number of polyethyleneglycol groups,  $R_{31}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 6 to 22 carbon atoms,  $R_{32}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 1 to 12 carbon atoms;

Dialkylpolyethylene/propyleneglycol with the general formula:  $R_{33}-O(-CH_2-CH_2-O)_{n7}(-CH(-CH_3)-CH_2-O)_{n8}R_{34}$ , where  $n7$  can take a value from 2 to 20 and denotes the number of polyethyleneglycol groups,  $n8$  can take a value from 2 to 20 and denotes the number of polypropyleneglycol groups,  $R_{33}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 6 to 22 carbon atoms,  $R_{34}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 1 to 12 carbon atoms.

A dispersion medium for a polysaccharide/solvent:

#### [0070]

An organic alcohol with the general formula:  $R_{35}(-OH)_{s1}$ , where  $R_{35}$  is an alkyl group with hydrocarbon chain length from 3 to 12 carbon atoms,  $s1$  can take a value from 1 to 12 and denotes the number of hydroxyl groups arranged in the hydrocarbon radical in any order with respect to each other;

Alkylpolypropyleneglycol with the general formula:  $H(-CH(-CH_3)-CH_2-O)_{n9}R_{36}$ , where  $n9$  can take a value from 2 to 10 and denotes the number of polypropyleneglycol groups,  $R_{36}$  is an alkyl group with hydrocarbon chain length from 1 to 10 carbon atoms.

PH adjusters:

**[0071]**

Organic acids with the general formula:  $R_{37}(-OH)_{s2}(-COOH)_{m1}$ , where  $R_{37}$  is an alkyl group with hydrocarbon chain length from 1 to 12 carbon atoms,  $S_2$  can take a value from 1 to 12 and denotes the number of hydroxyl groups arranged in the hydrocarbon radical in any order with respect to each other,  $M_1$  can take a value from 1 to 4 and denotes the number of carboxyl groups arranged in the hydrocarbon radical in any order with respect to each other;

Solutions of hydroxides of alkaline and/or alkaline earth metals, ammonia, primary or tertiary alkylamines, primary or tertiary alkanolamines, primary or tertiary glucamines, a basic amino acid, a citric acid disodium salt, a citric acid trisodium salt.

A chelating agent:

**[0072]**

A trisodium salt of methylglycinediacetic acid, a tetrasodium salt of gluteminediacetic acid, a trisodium salt of ethylenediamine-(N,N)-disuccinate;

Organic acids as well as salts of alkaline metals, ammonium, alkylammonium, alkanolammonium, glucoammonium corresponding to the following acids: citric acid, malic acid, tartaric acid, glutaric acid, adipic acid, glucuronic acid, galacturonic acid, galactaric acid, gluconic acid, phytic acid, polytaconic acid, polyacrylic acid, polymethacrylic acid, a copolymer of acrylic and maleic acids, as well as organic acids with the general formula  $R_{38}(-OH)_{s3}(-COOH)_{m2}$ , where  $R_{38}$  is an alkyl group with hydrocarbon chain length from 1 to 12 carbon atoms,  $S_3$  can take a value from 1 to 12 and denotes the number of hydroxyl groups arranged in the hydrocarbon radical in any order with respect to each other,  $M_2$  can take a value from 1 to 4 and denotes the number of carboxyl groups arranged in the hydrocarbon radical in any order with respect to each other.

Soil redeposition inhibitors:

**[0073]**

Derivatives of polysaccharides: carboxymethylpolysaccharide sodium salt, hydroxyalkylpolysaccharide, alkyl-polysaccharide;

Polyvinylpyrrolidone;

Water-soluble salts of polyacrylic acid, polymethacrylic acid, a copolymer of acrylic/methacrylic and maleic acid.

Anti-foaming agents:

**[0074]**

Higher carboxylic acids with the general formula:  $R_{39}-CO_2H$ , where  $R_{39}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms;

Higher carboxylic alcohols with the general formula:  $R_{40}-COH$ , where  $R_{40}$  is an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms;

Ethers of higher carboxylic alcohols with the general formula:  $R_{41}-O-R_{42}$ , where  $R_{41}$ ,  $R_{42}$  are an alkyl and/or alkenyl group with hydrocarbon chain length from 4 to 22 carbon atoms;

Bisamides of alkyldiamines and higher carboxylic acids with the general formula:  $R_{43}-C(O)-NH-R_{44}-NH-C(O)-R_{45}$ , where  $R_{43}$ ,  $R_{45}$  are an alkyl and/or alkenyl group with hydrocarbon chain length from 5 to 21 carbon atoms and  $R_{44}$  is an alkyl radical with hydrocarbon chain length from 1 to 12 carbon atoms;

Preservatives:

**[0075]**

Organic acids and salts of alkaline and alkaline-earth metals, ammonium, alkylammonium, alkanolammonium, glucoammonium corresponding to the following acids: benzoic acid, sorbic acid, 4-methoxybenzoic acid, salicylic acid, undecylenic acid;

Organic alcohols and phenols: phenoxyethanol, benzyl alcohol, caprylyl glycol, ethylhexylglycerol, phenethyl alcohol, 3-methyl-4-isopropylphenol, 2,4-dichlorobenzyl alcohol;  
Biocides of a wide range of action: benzisothiazolinone, dodecylidipropylene triamine;

- 5 **[0076]** Fungicides: sodium pyrithione, climbazole.
- [0077]** Enzymes: protease, amylase, pectate lyase, mannanase, cellulase, aminooxidase, nuclease, feruloyl esterase, gluconase, tannase and other commercially available enzymes used in laundry and dishwashing detergents, floor, glass cleaners, all-purpose detergents.
- 10 **[0078]** Bleaching agents based on oxygen compounds: hydrogen peroxide, calcium peroxide, carbamide peroxide,  $\epsilon$ -phthalimidoperoxyacaproic acid and other commercially available components.
- [0079]** Generally, the present invention can be characterized with the following peculiarities.
- [0080]** In one aspect the invention relates to a composition intended for use in the formulation of a detergent consisting of lipase and  $\beta$ -cyclodextrin, wherein the mass ratio of lipase and  $\beta$ -cyclodextrin is (0.0025-0.25):(0.1-1), respectively.
- 15 **[0081]** The composition can differ in that the quantity of lipase in the specified mass ratio of lipase and  $\beta$ -cyclodextrin (0.0025-0.25):(0.1-1) is 0.0025, 0.005, 0.0075, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.15, 0.2 or 0.25. The composition can differ in that the quantity of  $\beta$ -cyclodextrin in the given mass ratio of lipase and  $\beta$ -cyclodextrin (0.0025-0.25):(0.1-1) is 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95 or 1.
- [0082]** The composition can differ in that the specified lipase is in a hydroglyceric solution.
- 20 **[0083]** The composition can differ in that the specified hydroglyceric solution of lipase is the commercially available product Lipex® Evity® 200 L. The composition can differ in that the specified hydroglyceric solution of lipase is the commercially available product Lipex® Evity® 200 L modified with an additional quantity of glycerol. The composition can differ in that the composition comprises Lipex® Evity® 200 L modified with an additional quantity of glycerol and  $\beta$ -cyclodextrin, wherein the content of Lipex® Evity® 200 L is 0.4%wt. per weight of the composition, the content of the additional quantity of glycerol introduced for modification is 1%wt. per weight of the composition. The composition can differ in that in the composition comprising Lipex® Evity® 200 L modified with an additional quantity of glycerol the mass ratio of Lipex® Evity® 200 L and the additional quantity of glycerol introduced for modification is (0.1-1):(0.5-1.5), respectively.
- 25 **[0084]** The composition can differ in that the quantity of Lipex® Evity® 200 L in the given mass ratio of Lipex® Evity® 200 L and the additional quantity of glycerol introduced for modification is 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95 or 1. The composition can differ in that the quantity of additional glycerol introduced for modification in the given mass ratio of Lipex® Evity® 200 L and the additional quantity of glycerol introduced for modification is 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1, 1.1, 1.2, 1.3, 1.4 or 1.5.
- 30 **[0085]** The composition can be different in that it additionally comprises decylglucoside. The composition can be different in that the specified decylglucoside is the commercially available product NaturalAPG HG0814CM. The composition can be different in that the mass ratio of Lipex® Evity® 200 L and NaturalAPG HG0814CM is (0.2-0.6):(10-14). The composition can be different in that the quantity of Lipex® Evity® 200 L in the specified mass ratio of Lipex® Evity® 200 L and NaturalAPG HG0814CM is 0.2, 0.3, 0.4, 0.5 or 0.6. The composition can be different in that the quantity of NaturalAPG HG0814CM in the specified mass ratio of Lipex® Evity® 200 L and NaturalAPG HG0814CM is 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5 or 14. The composition can be different in that the composition comprises the commercially available product Lipex® Evity® 200 L modified with an additional quantity of glycerol,  $\beta$ -cyclodextrin and the commercially available product NaturalAPG HG0814CM, wherein the content of the commercially available product Lipex® Evity® 200 L is 0.4%wt. per weight of the composition, the content of the additional quantity of glycerol introduced for modification is 1%wt. per weight of the composition, the content of the commercially available product NaturalAPG HG0814CM is 12%wt. per weight of the composition.
- 40 **[0086]** The composition can be different in that the activity of the specified lipase is at least 40 LU/g or 40 LU/ml at pH 7.0.
- [0087]** The composition can be different in that the specified household chemical is selected from a dishwashing detergent, floor cleaner and/or toilet bowl and/or wash bowl and/or bathtub and/or glass and/or pipe cleaner, laundry detergent, including delicate laundry or baby laundry detergent, fabric conditioner, stain remover for preliminary fabric treatment and washing, gel laundry detergent and conditioner.
- 45 **[0088]** In another aspect the invention relates to a dishwashing detergent comprising 0.0525-1%wt. of the composition of the present invention.
- [0089]** In another aspect the invention relates to a floor and/or toilet bowl and/or wash bowl and/or bathtub and/or glass cleaner comprising 0.0525-1%wt. of the composition of the present invention.
- 50 **[0090]** In another aspect the invention relates to an all-purpose surface cleaner comprising 0.0525-1%wt. of the composition of the present invention.
- 55 **[0091]** In another aspect the invention relates to a pipe cleaner comprising 0.0525-2%wt. of the composition of the present invention. The pipe cleaner can be different in that the specified detergent comprises 0.0525-1.5%wt. of the specified composition, preferably 0.0525-1%wt. of the specified composition.

**[0092]** In another aspect the invention relates to a laundry detergent comprising 0.0525-1%wt. of the composition of the present invention. The laundry detergent can be different in that the specified detergent is selected from a delicate laundry detergent and a baby laundry detergent. The laundry detergent can be different in that the specified detergent is a powder laundry detergent.

**[0093]** In another aspect the invention relates to use of the composition of the present invention for control of kinetics of enzymatic cleavage of lipids and neutralization of unpleasant odours on different surfaces, with maintaining of continuous cleanliness and a pleasant odour. The use can be different in that the specified surface is selected from a metal, polymeric, enameled, glass or wooden surface.

**[0094]** In another aspect the invention relates to a method of preparing the composition of the present invention including preliminary introduction of the lipase solution to a  $\beta$ -cyclodextrin dispersion, wherein after addition of the last portion of lipase the dispersion is mixed during 10-15 min. This makes it possible to obtain a homogeneous solution.

## EXPERIMENTAL PART

**[0095]** The examples included in the present description do not limit the claimed invention and are given only with the aim of illustration and confirmation of achievement of expected technical results. These examples are among many experimental data obtained by the authors of the invention that prove effectiveness of the detergents within the scope of the invention.

**[0096]** Pre-clinical studies were conducted to assess effectiveness of the composition of the present invention.

### Example 1.

**[0097]** A laboratory analysis of washing effectiveness of active components, namely lipase and  $\beta$ -cyclodextrines, in the formulation of detergents with the same base was carried out.

**[0098]** The base for addition of complex components was a mixture consisting of purified water, anionic surfactants 5-15%, non-ionic surfactants based on glycosides <5%, glycerol, cotton extract, tetrasodium diacetate glutamate, citric acid monohydrate, sodium hydroxide, a preservative and an aqueous solution of citric acid and silver citrate (Table No.1).

**[0099]** The base of the dishwashing detergent was prepared in the following way: solubilizer glycerol and the chelating agent glutamate diacetate tetrasodium salt were mixed with purified water in a common mixer till mixture homogeneity. First, anionic surfactants were homogenized with a sufficient amount of purified water while heating at 40-60°C. Then non-ionic surfactants and an anionic surfactant solution were added. Also, cotton extract and an aqueous solution of citric acid and silver citrate were added. At the very end there was added an organic acid for pH control, a preservative for ensuring microbiological stability during the storage period. The ingredients were mixed until a homogeneous transparent solution was obtained. After mixing pH of the product was measured and adjusted by adding sodium hydroxide until the required value was obtained.

Table No. 1 Detergent base formulation

No.	Component
1	Purified water
2	Glycerol 99.5%
3	Anionic surfactants 5-15%
4	Non-ionic surfactants based on glycosides <5%
5	Cotton extract
6	Aqueous solution of citric acid and silver citrate
7	Glutamate diacetate tetrasodium salt
8	Citric acid monohydrate
9	Sodium hydroxide
10	Preservative active at the given pH

**[0100]** The test method was based on OST 6-15-1662-90 "Household detergents". The method of determination of washing effectiveness for different surfaces is that the mass of removed artificial oil and fat soil was determined during certain time with the test sample in relation to the sample by the gravimetric method. Glass plates were selected as

surfaces. The glass plates were thoroughly washed, rinsed and dried in a drying oven at the temperature of 120° during 60 minutes and wiped with ethyl alcohol. After preparation the plates were weighed to determine the baseline. Then there was prepared complex oil and fat soiling consisting of grease based on leached industrial oil, lanolin, lecithin emulsifier, egg yolk, flax seed oil, sunflower oil, oleic acid and distilled water at the ratio of 4.1:4.9:1.0:5.1:7.3:2.1:4.5:20, respectively. Charges of grease and lanolin were mixed at the room temperature, then heated in a water bath up to 70°C and the emulsifier was added, the mixture was cooled and egg yolk and other ingredients were added. The contaminant was applied on plates with a pipette and left at the room temperature for 30 minutes, then it was transferred to a drying oven and kept at 220±5°C during 8 minutes. Then it was cooled and weighed. The samples were used in pure form. The control was a standard detergent with a similar quantitative composition.

**[0101]** To conduct the test each plate was placed into ajar with a lid, capron fabric was put on top and one jar with a plate was filled with a clean sample of the test detergent, another jar with plates was filled with 40 ml of the control so that the water:lipophilic soil (fat, oil, glycerides) ratio was 9:1. The jars were placed into an agitator for liquids and agitated for 5 minutes. After agitation the plates were washed in running water, then rinsed with distilled water and dried in the drying oven at 120°C for an hour, then cooled at the room temperature and weighed.

**[0102]** The obtained data were used to calculate the mass fraction of removed soil. The mass fraction of the removed soil was calculated by the following formula:

$$X = \frac{A-B}{A-B} * 100,$$

where

A is the weight of a piece of the plate with the soil before washing with the detergent, g;

B is the weight of the piece of the plate with the soil after washing with the detergent, g.

B is the weight of the clean plate, g.

**[0103]** The parameter for general assessment of the washing effectiveness and kinetics of cleavage of lipid soils included in the detergent was calculation of % of the washing effectiveness determined by the lab technician based on the test results. The indicator of the mass fraction of the removed soil makes it possible to assess washing effectiveness with respect to complex lipid-based soils with daily use of the composition included in the dishwashing detergent. The general positive trend of use of the dishwashing detergent with the claimed composition is improvement of effectiveness of the dishwashing detergent and cleaner of different surfaces.

**[0104]** For quantitative data, the group arithmetic mean (M), standard deviation (SD) and standard error of the mean (SEM) were calculated. The obtained data were processed by means of MS Excel program. The probability of differences of the mean at different moments of time was determined with the use of the Student's t-test with normal distribution in independent and dependent samplings. The differences were considered to be significant with the level of significance p<0.05.

Results.

**[0105]** Based on the results of assessing washing effectiveness of the samples it was established that the test composition included in the dishwashing detergent has strong washing effect with respect to complex lipid and protein soils comparing to the control that did not comprise components of the claimed composition.

**[0106]** At the end of the study marked changes of the assessed parameter of washing effectiveness were observed. According to the parameter dynamics, in relation to the control sample without components of the claimed composition, effectiveness of removal of a complex lipid and protein soil increased by 53.6%, on average. The control version did not demonstrate achieving 100%, which speaks of insufficient effectiveness of the combination of surfactants for removal of lipid and protein soils (Table No.2).

Table No.2. Assessment of washing effectiveness of samples

Test sample	Components of composition	Mass of removed contaminant, %
Sample No.1	no	82.0±4.0
Sample No.2	lipase 0.036%	98.7±4.0
Sample No.3	lipase 0.036% + β-cyclodextrins 0.5%	135.6±4.0

**[0107]** Complex lipid and protein soils are hard to remove from different surfaces owing to fixation and insufficient action of surfactants.

**[0108]** The composition base of a dishwashing detergent will mildly remove soils from tableware surfaces (glass, metal, polymeric, ceramic ones) and increase access of active components from the claimed composition to hard-to-get areas, thus improving effectiveness of the composition with respect to complex lipid and protein soils, incl. old ones. The analysis of the dishwashing detergent with the claimed composition demonstrated marked synergetic effect with respect to soils that are hard to remove using common dishwashing detergents based on surfactants. The composition enables replacing synthetic surfactants with surfactants of natural origin without reduction of detergent effectiveness, which makes it possible to reduce the environmental load, maintain the approach to human health and ensure a high percentage of natural ingredients in the formulation of household chemicals for consumers.

**[0109]** As the composition includes natural lipase obtained by biotechnology methods and natural  $\beta$ -cyclodextrins, single-time use will enable a high % of removal of complex soils with low labor inputs and with time saving. Combination of the components in the composition makes it possible to control kinetics of enzymatic cleavage of lipid substrates fixing complex soils. Lipase enables targeted cleavage of lipid soils in different phase systems, while cyclodextrins are cofactors for control of activity of the enzyme and increasing of affinity for triglycerides, enabling changes of the conformation of the protein domain with an active center and correct orientation of substrates. In addition, cyclodextrins bind released products of cleavage having an unpleasant odour, which enables neutralizing odorous aromatic molecules and controlling the atmosphere indoors for people's psychological comfort.

**[0110]** Thus, combination of the components enables achieving synergetic effect with respect to complex lipid and protein soils by targeted action and control of kinetics of the hydrolysis reaction of triglycerides, additionally neutralizing unpleasant odours, which makes it possible to regularly use them in the formulations of household chemicals for different surfaces.

#### Example 2.

**[0111]** A laboratory analysis of washing effectiveness of active components, namely, lipase and  $\beta$ -cyclodextrins, was carried out. The base for addition of complex components was purified water and glycerol for solubilization of  $\beta$ -cyclodextrins.

**[0112]** The test method was based on OST 6-15-1662-90 "Household detergents". The method of determination of washing effectiveness for different surfaces is that the mass of removed artificial oil and fat soil was determined during certain time with the test sample in relation to the sample by the gravimetric method. The selected surfaces were metal and plastic plates. 8 metal and 8 plastic plates were carefully washed, rinsed and dried in a drying oven at 120°C during 60 minutes and rinsed with ethyl alcohol. After preparation the plates were weighed to determine the baseline. Then there was prepared complex oil and fat soiling consisting of grease based on leached industrial oil, lanolin, lecithin emulsifier, egg yolk, flax seed oil, sunflower oil, oleic acid and distilled water at the ratio of 4.1:4.9:1.0:5.1:7.3:2.1:4.5:20, respectively. Charges of grease and lanolin were mixed at the room temperature, then heated in a water bath up to 70°C and the emulsifier was added, the mixture was cooled and egg yolk and other ingredients were added. The contaminant was applied on plates with a pipette and left at the room temperature for 30 minutes, then it was transferred to a drying oven and kept at  $220 \pm 5^\circ\text{C}$  during 8 minutes. Then it was cooled and weighed. The samples were used in pure form. The control was distilled water free of the claimed composition.

**[0113]** To conduct the test each plate was placed into ajar with a lid, capron fabric was put on top and one jar with a plate was filled with a clean sample of the test detergent, another jar with plates was filled with 40 ml of the control so that the water:lipophilic soil (fat, oil, glycerides) ratio was 9:1. The jars were placed into an agitator for liquids and agitated for 5 minutes. After agitation the plates were washed in running water, then rinsed with distilled water and dried in the drying oven at 120°C for an hour, then cooled at the room temperature and weighed.

**[0114]** The obtained data were used to calculate the mass fraction of removed soil. The mass fraction of the removed soil was calculated by the following formula:

$$X = \frac{A-B}{A-B} * 100,$$

where

A is the weight of a piece of the plate with the soil before washing with the sample, g;

B is the weight of the piece of the plate with the soil after washing with the sample, g.

B is the weight of the clean plate, g.

**[0115]** The parameter for general assessment of the washing effectiveness and kinetics of cleavage of lipid soils included in the detergent was calculation of % of the washing effectiveness determined by the lab technician based on the test results. The indicator of the mass fraction of the removed soil makes it possible to assess washing effectiveness with respect to complex lipid-based soils (oils and fats with fixing additives) with daily use of the composition included in the dishwashing detergent. The general positive trend of use of the dishwashing detergent with the claimed composition is improvement of effectiveness of the dishwashing detergent and cleaner of different surfaces.

**[0116]** For quantitative data, the group arithmetic mean (M), standard deviation (SD) and standard error of the mean (SEM) were calculated. The obtained data were processed by means of MS Excel program. The probability of differences of the mean at different moments of time was determined with the use of the Student's t-test with normal distribution in independent and dependent samplings. The differences were considered to be significant with the level of significance  $p < 0.05$ .

#### Results.

**[0117]** Based on the results of assessing washing effectiveness of the samples it was established that the test composition has strong washing effect with respect to complex lipid and protein soils comparing to the control sample that did not comprise components of the claimed composition.

**[0118]** At the end of the analysis there were marked changes of the assessed indicator of washing effectiveness on a hydrophilic metal surface of stainless steel. According to the indicator dynamics, with respect to the control sample free of components of the claimed composition, increase of the content of components led to statistically significant ( $p < 0.05$ ) growth of removal of the soil with the composition free of the base with surfactants. First there is geometric growth of effectiveness and then transition to the stationary phase, when increase of the content of components does not influence growth of effectiveness because of displacement of equilibrium in the enzymatic reaction (Table No.3).

Fast accumulation of reaction products results in the chemical reaction constant  $K_{eq}$  and displacement of equilibrium towards the reverse reaction thanks to the concentrated gradient as well as pH change in the system.

**[0119]** Change of washing effectiveness on a hydrophobic polymeric surface of plastic similarly to a metal surface, however soils are removed better even with a minimum percentage of composition introduction due to physical and chemical peculiarities of the surface. According to the indicator dynamics, with respect to the control sample free of components of the claimed composition, increase of the content of components led to statistically significant ( $p < 0.05$ ) growth of removal of the soil with the composition free of the base with surfactants. First there is geometric growth of effectiveness and then transition to the stationary phase, when increase of the content of components does not influence growth of effectiveness because of displacement of equilibrium in the enzymatic reaction (Table No.4).

**[0120]** The control version did not demonstrate effective removal of soils, which speaks of targeted action and high activity of the composition within a certain concentration range.

Table No.3. Assessment of washing effectiveness on a metal surface

Surface	Test sample	Weight of removed soil, $M \pm SEM$ %
Metal	Distilled water	$0.00 \pm 0.00$
	Lipase 0.006% +	$14.42 \pm 0.91$
	$\beta$ -cyclodextrins 0.10%	
	Lipase 0.015% + $\beta$ -cyclodextrins 0.25%	$26.51 \pm 1.14$
	Lipase 0.030% + $\beta$ -cyclodextrins 0.50%	$35.55 \pm 2.33$
	Lipase 0.060% + $\beta$ -cyclodextrins 1.00%	$31.22 \pm 2.31$



Table No. 4. Assessment of washing effectiveness on a polymeric surface

Surface	Test sample	Weight of removed soil, M $\pm$ SEM %
Polymeric	Distilled water	0.00 $\pm$ 0.00
	Lipase 0.006% + $\beta$ -cyclodextrins 0.10%	87.74 $\pm$ 2.78
	Lipase 0.015% + $\beta$ -cyclodextrins 0.25%	92.44 $\pm$ 3.03
	Lipase 0.030% + $\beta$ -cyclodextrins 0.50%	93.51 $\pm$ 1.11
	Lipase 0.060% + $\beta$ -cyclodextrins 1.00%	93.61 $\pm$ 1.15

**[0121]** Complex lipid and protein soils are hard to remove from different household surfaces thanks to fixation and insufficient action of surfactants from household chemicals, despite their solubilizing properties.

**[0122]** The composition base of a dishwashing detergent will mildly remove soils from tableware surfaces (metal, polymeric, glass and other ones) and increase access of active components from the claimed composition to hard-to-get areas, thus improving effectiveness of the composition with respect to complex lipid and protein soils, incl. old ones. Analysis of washing effectiveness of the composition demonstrated marked synergetic effect with respect to soils hard to remove using common dishwashing detergents based on surfactants of natural origin. The composition enables replacing synthetic surfactants with surfactants of natural origin or reducing their content without reduction of detergent effectiveness, which makes it possible to reduce the environmental load, maintain the approach to human health and ensure a high percentage of natural ingredients in the formulation of household chemicals for consumers.

**[0123]** As the composition includes lipase obtained by biotechnology methods and natural  $\beta$ -cyclodextrins, single-time use will enable a high % of removal of complex soils with low labor inputs and with time saving. Combination of the components in the composition makes it possible to control kinetics of enzymatic cleavage of lipid substrates fixing complex soils. Lipase enables targeted cleavage of lipid soils in different phase systems, while cyclodextrins are cofactors for control of activity of the enzyme and increasing of affinity for triglycerides, enabling changes of the conformation of the protein domain with an active center and correct orientation of substrates. In addition, cyclodextrins bind released products of cleavage having an unpleasant odour, which enables neutralizing odorous aromatic molecules and controlling the atmosphere indoors for people's psychological comfort.

**[0124]** As the kinetics of the reaction is prone to impact of reaction products, specifically, non-competitive impact of released fatty acids on allosteric sites of lipase,  $\beta$ -cyclodextrins act as activity stabilizers and bind reaction products from the system. Combined use of the components preserves high activity with different % of composition introduction depending on the type of the household chemical and its formulation.

**[0125]** Thus, combination of the components enables achieving synergetic effect with respect to complex lipid and protein soils by targeted action and control of kinetics of the hydrolysis reaction of triglycerides, additionally neutralizing unpleasant odours, which makes it possible to regularly use them in the formulations of household chemicals for different surfaces.

Example 3.

**[0126]** A laboratory analysis of deodorizing effectiveness of the active components, namely lipase and  $\beta$ -cyclodextrins, and household chemicals with these substances was carried out. The base for inclusion of the complex components was purified water and glycerol for solubilization of  $\beta$ -cyclodextrins, as well as the mixture specified in Table No. 1.

**[0127]** The test method was based on the article "Deodorizing Ability of *Houttuynia cordata* Thunb. (Dokudami) for Masking Garlic Odor - Hiromi Ikeura" and it enables assessment of the ability of the components to neutralize unpleasant odours by the organoleptic method.

**[0128]** The selected unpleasant odours were organic compounds of the group of thioethers  $R_1-S-R_2$  and esters  $R_3-CO-OR_4$  from *Allium sativum* garlic fruit obtained by the method of ultrasound extraction with 96% alcohol and filtration through a Schott funnel. The auxiliary materials used were sterile medical containers of a polymeric material. A pipette was used to apply 0.5 g of alcohol extract strictly on the bottom of each polymeric container and then they were placed into a heated drying oven (with set temperature of 60°C) for 15-30 minutes. Then 1.0 g of purified water was poured on the bottom of containers and left for 10 minutes. Then 0.8-1.0 g of the tested household chemical was applied on the bottom of glasses and mixed during 3-5 minutes by means of a vibration shaker. In 3 minutes each container was rinsed with 1 liter of warm tap water of mean water hardness for complete removal of liquid and then dried at room temperature. The deodorizing effect was determined by the organoleptic method and assessed based on the developed 5-score scale, wherein score 0 means complete absence of deodorizing effect and 5 scores means complete deodorizing effect.

**[0129]** The samples of household chemicals were used in pure form. The control was purified water free of the claimed

composition and a water-soluble zinc salt having prominent deodorizing properties due to chemical structure of  $Zn^{2+}$  zinc ions. To assess the result a group of 5 people was engaged as there could be individual variability of odour intensity. For quantitative data the average score obtained by the study results was calculated.

## Results.

**[0130]** It was established based on the study results that the tested composition in the formulation of household chemicals has prominent deodorizing effect comparing to the control free of the components of the claimed composition. Each of the components contributes to the general effect and their combination enables achieving neutralization of unpleasant odours up to 100% depending on the concentration of substances of the claims (Table No.5). The synergetic effect of lipase and  $\beta$ -cyclodextrins reveals itself in increasing of effectiveness of unpleasant odour removal by +0.5 score, which is a good change with combination of components in addition to effects described in examples 1 and 2.

Table No.5. Assessment of the deodorizing effect of components of the composition

Test sample	Active components	Effectiveness of odour removal, scores from 0 to 5	
		Absolute values	Relative values
Sample No.1	distilled water (negative control)	0.0	-
Sample No.2	Lipase 0.006%	0.5	+0.5
Sample No.3	Lipase 0.015%	1.0	+1.0
Sample No.4	Lipase 0.030%	1.0	+1.0
Sample No. 5	Lipase 0.036%	1.0	+1.0
Sample No.6	Lipase 0.060%	1.0	+1.0
Sample No.7	$\beta$ -cyclodextrins 0.10%	2.0	+2.0
Sample No.8	$\beta$ -cyclodextrins 0.25%	2.5	+2.5
Sample No.9	$\beta$ -cyclodextrins 0.50%	3.0	+3.0
Sample No.10	$\beta$ -cyclodextrins 1.00%	4.0	+4.0
Sample No.11	Lipase 0.036% + $\beta$ -cyclodextrins 0.25%	4.0	+4.0
Sample No.12	Lipase 0.036% + $\beta$ -cyclodextrins 0.50%	4.5	+4.5
Sample No.13	Lipase 0.036% + $\beta$ -cyclodextrins 1.00%	5.0	+5.0
Sample No.14	Lipase 0.036% + water-soluble zinc salt 1.0%	5.0	+5.0

**[0131]** The composition base of an all-purpose surface cleaner will mildly remove soils from surfaces (metal, polymeric, glass and other ones) and increase access of active components from the claimed composition to hard-to-get areas, thus improving effectiveness of the composition in neutralization of persistent unpleasant odours. Analysis of the deodorizing effect of the composition demonstrated marked synergetic effect with respect to unpleasant odours hard to conceal or wash away with common surface cleaners based on surfactants of natural origin. The composition enables replacing synthetic adsorbers of unpleasant odours, synthetic fragrances or aromatic composition in the detergent formulation without reduction of effectiveness, which makes it possible to preserve safe influence on human health, incl. people with allergic diseases of the respiratory tract, and ensure a high percentage of natural ingredients in the formulation of household chemicals for consumers.

**[0132]** As the formulation of the composition includes lipase obtained by biotechnology methods and natural  $\beta$ -cyclodextrins, single-time use will ensure a high % of neutralization of unpleasant odours because of sulfur-containing components (mercaptans), organic acids, their ethers and other compounds. Combination of the components in the composition enables targeted elimination of the cause of unpleasant odours, but not temporarily concealing it with aromatic substances with more expressed perception intensity.

**[0133]** Thus, combination of the components enables achieving synergetic action with respect to persistent unpleasant odours and a high affinity for organic molecules of odour, which enables their regular use in formulations of household chemicals for different surfaces and control of atmosphere indoors for people's psychological comfort.

## Example 4.

**[0134]** 9.00 g of glyceryl oleate contained in different fats and vegetable oils were mixed with 90.7 g of purified water until the mixture became homogeneous with heating up to 45°C and mixing at 200 rpm using a magnetic mixer. After that heating was stopped and the mixture was cooled to the room temperature. In a separate container there was prepared a dispersion of 0.0027 g of lipase with activity >100 LU/g and 0.27 g of  $\beta$ -cyclodextrins making up 3% of the weight of the lipophilic substrate, in 0.02 g of the dispersion medium mixture (glycerol-sorbitol) with the ratio of 1:1.4. Then a hydroglyceric solution of components was added while mixing actively to a medium with the substrate and incubated at 30°C during 4 hours. The initial pH of the solution was  $8.20 \pm 0.10$ .

**[0135]** The degree of hydrolysis was determined by measuring pH by releasing weak oleic acid having  $pK_a(\text{Acid}) = 4.8 \pm 0.1$  [<https://echa.europa.eu/registration-dossier/-/registered-dossier/12335/4/22>]. Specifically, the ratio of substrate and aqueous phase of 1:3333 enabled achieving the hydrolysis degree of 76% during 270 minutes, which demonstrates high washing effectiveness of the composition.

## Example 5.

**[0136]** An instrumental physical and chemical study was carried out to prove synergism of lipase and  $\beta$ -cyclodextrins in case of combined use in the composition. Spectroscopy methods, specifically, spectroscopy in UV-region and fluorescent spectroscopy, are the most suitable methods for description of biological properties and interactions in organic molecules, specifically, enzymes, polysaccharides and others. Aromatic aminoacids in lipase such as tryptophane, tyrosine, phenylalanine and histidine, have adsorption in the ultraviolet range of radiation (180-400 nm) and emit fluorescent quanta.  $\beta$ -cyclodextrins interact with aminoacid residues of lipase subunits forming clathrate complexes on the enzyme surface, which changes the microenvironment of chromophoric groups, thus changing the ability of adsorbing and emitting light quanta of different spectrum regions.

**[0137]** For the test lipase solutions 2 g/l with dispersion of  $\beta$ -cyclodextrins with the concentration from 0 to 4.54 g/l at pH 7.4 were prepared with account for the technology aspect of making of the composition described in Example 6.

**[0138]** For UV-spectroscopy there were determined adsorption spectra with wavelength of 180-340 nm and the optical density of solutions A was calculated that demonstrated adsorption of lipase amino acids and energetically favorable conformation of the enzyme for enzymatic cleavage of triglycerides in formulations of lipid and complex protein and lipid soils. It was established based on the study results that with adsorption maximum of 190-200 nm the lipase spectrum practically does not change, however in the region of 270-290 nm there is an additional peak with modified spectrum in case of addition of a dispersion of  $\beta$ -cyclodextrins. The optical density of the peak at 270-290 nm decreased with addition of cyclodextrins to the concentration of 2.27 g/l, however, then the optical density returns to the initial value, which demonstrates formation of a stable clathrate complex between lipase and  $\beta$ -cyclodextrins. It should be noted that the optimal lipase:cyclodextrins ratio is from 1:15 to 1:40 with no change of effectiveness of the composition.

**[0139]** For fluorescent spectroscopy there were determined fluorescence quanta emission spectra with wavelength of 280-450 nm and relative fluorescence intensity F was calculated that demonstrated energetically favorable conformation of the enzyme and the ability of aminoacid residues to adsorb and emit light quanta. It was established based on the study results that with the adsorption maximum of 350-360 nm the emission spectrum does not change with addition of a dispersion of  $\beta$ -cyclodextrins. The fluorescence intensity of the peak at 340-350 nm decreased with addition of cyclodextrins to the concentration of 2.27 g/l, however, then the optical density returned to the initial value, which demonstrates formation of a stable clathrate complex between lipase and  $\beta$ -cyclodextrins. It should be noted that the optimal lipase:cyclodextrins ratio is from 1:15 to 1:40 with no change of effectiveness of the composition.

## Example 6.

**[0140]** The method of preparing the detergent of the present invention includes the following stages.

Preparation of a dispersion of lipase and  $\beta$ -cyclodextrins.

**[0141]** The main process of detergent preparation is preceded by the stage of preliminary application of a lipase solution on a dispersion of  $\beta$ -cyclodextrins. Powdered  $\beta$ -cyclodextrins are added to a dispersion medium, the cyclodextrins:dispersion medium ratio is 1:1.20-2.50. The process takes place at room temperature and under atmospheric pressure. Cyclodextrins are added in small portions, with continuous mixing. After adding the last portion of cyclodextrins the dispersion is mixed during 10-15 minutes, the signal of transition to the next stage is absence of large inclusions of cyclodextrins in the dispersion. Then, a lipase solution is added to the obtained dispersion in small portions, the ratio of cyclodextrins (in solids) to lipase solution is 1:0.5-1.50. After addition of the last lipase portion the dispersion is mixed during 10-15 minutes.

Preparation of a detergent with the given composition.

**[0142]** After settling, the obtained dispersion of lipase and  $\beta$ -cyclodextrins is dissolved in a primary reactor for making a ready detergent, for example, a dishwashing detergent. The dispersion is pumped to preliminarily warmed water, the resulting solution is mixed until completely dissolved. If technically possible, a high-rate disperser is used for intensification of dissolution. Upon completion of dispersion dissolution the following components specified in the description of the invention, according to the technical formulation, can be loaded to the reactor to obtain the final product.

## Claims

1. A composition intended for use in the formulation of a detergent consisting of lipase and  $\beta$ -cyclodextrin, wherein the mass ratio of lipase and  $\beta$ -cyclodextrin makes up (0.0025-0.25):(0.1-1), respectively.
2. The composition of claim 1, wherein the quantity of lipase in the specified mass ratio of lipase and  $\beta$ -cyclodextrin (0.0025-0.25):(0.1-1) is 0.0025, 0.005, 0.0075, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.15, 0.2 or 0.25.
3. The composition of claim 1, wherein the quantity of  $\beta$ -cyclodextrin in the given mass ratio of lipase and  $\beta$ -cyclodextrin (0.0025-0.25):(0.1-1) is 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95 or 1.
4. The composition of claim 1, wherein the specified lipase is in a hydroglyceric solution.
5. The composition of claim 4, wherein the specified hydroglyceric solution of lipase is the commercially available product Lipex® Evity® 200 L.
6. The composition of claim 5, wherein the specified hydroglyceric solution of lipase is the commercially available product Lipex® Evity® 200 L modified with an additional quantity of glycerol.
7. The composition of claim 6, wherein the composition comprises Lipex® Evity® 200 L modified with an additional quantity of glycerol and  $\beta$ -cyclodextrin, wherein the content of Lipex® Evity® 200 L is 0.4%wt. per weight of the composition, the content of the additional quantity of glycerol introduced for modification is 1%wt. per weight of the composition.
8. The composition of claim 6, wherein the composition comprising Lipex® Evity® 200 L modified with an additional quantity of glycerol the mass ratio of Lipex® Evity® 200 L and the additional quantity of glycerol introduced for modification is (0.1-1):(0.5-1.5), respectively.
9. The composition of claim 8, wherein the quantity of Lipex® Evity® 200 L in the given mass ratio of Lipex® Evity® 200 L and the additional quantity of glycerol introduced for modification is 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95 or 1.
10. The composition of claim 8, wherein the quantity of additional glycerol introduced for modification in the given mass ratio of Lipex® Evity® 200 L and the additional quantity of glycerol introduced for modification is 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1, 1.1, 1.2, 1.3, 1.4 or 1.5.
11. The composition of claim 1, wherein it additionally comprises decylglucoside.
12. The composition of claim 11, wherein the specified decylglucoside is the commercially available product NaturalAPG HG0814CM.
13. The composition of claim 12, wherein the mass ratio of Lipex® Evity® 200 L and NaturalAPG HG0814CM is (0.2-0.6):(10-14).
14. The composition of claim 13, wherein the quantity of Lipex® Evity® 200 L in the specified mass ratio of Lipex® Evity® 200 L and NaturalAPG HG0814CM is 0.2, 0.3, 0.4, 0.5 or 0.6.
15. The composition of claim 13, wherein the quantity of NaturalAPG HG0814CM in the specified mass ratio of Lipex®

Evity® 200 L and NaturalAPG HG0814CM is 10, 10,5, 11, 11,5, 12, 12,5, 13, 13,5 or 14.

- 5 16. The composition of claim 1, wherein the composition comprises the commercially available product Lipex® Evity® 200 L modified with an additional quantity of glycerol,  $\beta$ -cyclodextrin and the commercially available product NaturalAPG HG0814CM, wherein the content of the commercially available product Lipex® Evity® 200 L is 0.4%wt. per weight of the composition, the content of the additional quantity of glycerol introduced for modification is 1%wt. per weight of the composition, the content of the commercially available product NaturalAPG HG0814CM is 12%wt. per weight of the composition.
- 10 17. The composition of claim 1, wherein the activity of the specified lipase is at least 40 LU/g or 40 LU/ml at pH 7.0.
- 15 18. The composition of claim 1, wherein the specified household chemical is selected from a dishwashing detergent, floor cleaner and/or toilet bowl and/or wash bowl and/or bathtub and/or glass and/or pipe cleaner, laundry detergent, including delicate laundry or baby laundry detergent, fabric conditioner, stain remover for preliminary fabric treatment and washing, gel laundry detergent and conditioner.
- 20 19. A dishwashing detergent comprising 0.0525-1%wt. of the composition of any of claims 1-18.
- 25 20. A floor and/or toilet bowl and/or wash bowl and/or bathtub and/or glass cleaner comprising 0.0525-1%wt. of the composition of any of claims 1-18.
- 30 21. An all-purpose surface cleaner comprising 0.0525-1%wt. of the composition of any of claims 1-18.
- 35 22. A pipe cleaner comprising 0.0525-1%wt. of the composition of any of claims 1-18.
- 40 23. The cleaner of claim 22, wherein the specified cleaner comprises 0.0525-1.5%wt. of the specified composition, preferably 0.0525-1%wt. of the specified composition.
- 45 24. A laundry detergent comprising 0.0525-1%wt. of the composition of any of claims 1-18.
- 50 25. The detergent of claim 24, wherein the specified detergent is selected from a delicate laundry detergent and a baby laundry detergent.
- 55 26. The detergent of claim 24, wherein the specified detergent is a powder laundry detergent.
27. Use of the composition of any of claims 1-18 for control of kinetics of enzymatic cleavage of lipids and neutralization of unpleasant odour on different surfaces, maintaining continuous cleanliness and a pleasant odour.
28. Use of claim 27, wherein the specified surface is selected from a metal, polymeric, enameled, glass or wooden surface.
29. The method of obtaining the composition of any of claims 1-18 comprising preliminary addition of a lipase solution to a dispersion of  $\beta$ -cyclodextrin, wherein after addition of the last portion of lipase the dispersion is mixed during 10-15 min.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/RU 2021/000271

## A. CLASSIFICATION OF SUBJECT MATTER

C11D 1/66 (2006.01) C11D 3/22 (2006.01) C11D 3/386 (2006.01) C11D 3/60 (2006.01)

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 practicable, search terms used)

EAPATIS, ESPACENET, PatSearch (RUPTO internal), USPTO, PATENTSCOPE, eLIBRARY, Science Direct, Google Patents

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	JP H01256596 A (KAO CORP) 13.10.1989, abstract, description	1-3, 18, 24-27, 29 4-17, 19-23, 28
Y	RU 2654031 C2 (BASF SE) 15.05.2018, claims 13-15, p. 4, lines 26-29, p. 8, line 47 - p. 9, line 12	19-23, 28
Y	WO 2020/074302 A1 (UNILEVER N.V. et al.) 16.04.2020, claims 1, 3, p. 25, lines 1-18, p. 26, lines 20-25	4-17
Y	WO 2017/091674 A1 (THE PROCTER & GAMBLE COMPANY) 01.06.2017, claim 1, p. 9, lines 32-33	11-16

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

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"&amp;" document member of the same patent family

Date of the actual completion of the international search

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20 January 2022 (20.01.2022)

Name and mailing address of the ISA/ RU

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## REFERENCES CITED IN THE DESCRIPTION

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