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(54) Title: ENZYME ASSISTED PALM OIL EXTRACTION WITH CONTINUOUS STERILIZER

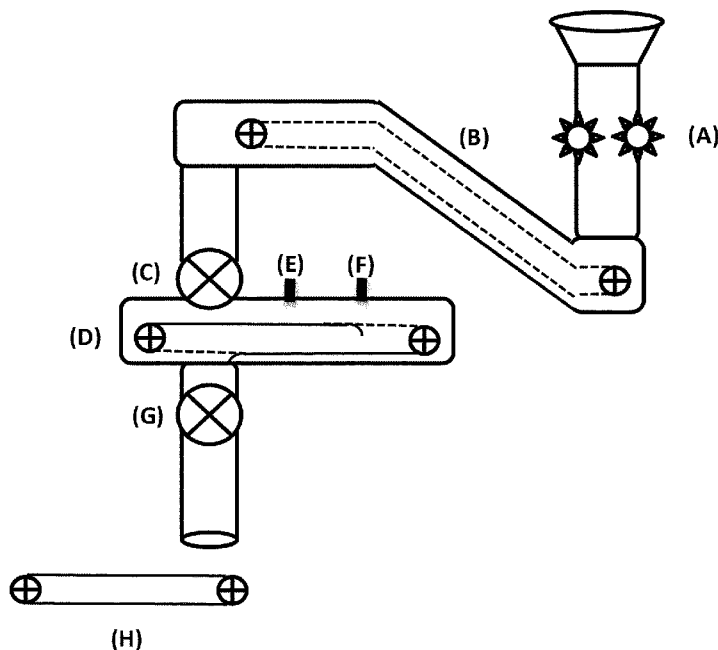


Figure 1

(57) Abstract: The present invention relates to a process for extraction of crude palm oil, comprising the use of a continuous sterilizer and an enzyme composition. The invention also relates to a palm oil obtainable by the process and to a milling line comprising a continuous sterilizer and means for dosing an enzyme composition.



ENZYME ASSISTED PALM OIL EXTRACTION WITH CONTINUOUS STERILIZER**Field of the Invention**

The present invention relates to a process for extraction of crude palm oil.

5 More particularly, the present invention relates to a process for extraction of crude palm oil, comprising the use of a continuous sterilizer and an enzyme composition.

The invention also relates to a palm oil obtainable by the process and to a milling line comprising a continuous sterilizer and means for dosing an enzyme composition.

10 Description of the Related Art

Palm oil production has huge economic implications in many developing countries in Asia, Africa and Latin America as large land banks are being devoted for growing the crop in recent years. Palm fruits or fruitlets grow in large bunches. Crude palm oil can be extracted from the palm fruitlets after the fresh fruit bunches (FFB) have gone through a sterilization and separation
15 process. In general, the crude palm oil extraction process is characterised by the following major steps:

- sterilization of the fresh palm fruit bunches process; e.g. to arrest oil quality deterioration due to enzymatic activity, and facilitate the stripping of fruits from bunch stalks and the extraction of oil;
- 20 • threshing or stripping to remove fruitlets from the bunch stalk;
- discharging the fruitlets into vessels commonly referred to as digesters and digesting the fruitlets to produce a digested mash under controlled temperature;
- pressing of the digested mash; e.g. using a screw press, for subsequent recovery of oil;
- 25 • subjecting the pressed liquor, also referred to as crude oil, to screening, e.g. to remove coarse fibre, and then to a clarification process to separate oil from water, cell debris, and any remaining fibrous material.

Palm fruit mesocarp contains large amounts of oil present as oil droplets within the mesocarp cells. Generally, the oil extraction rate (OER), which is a measure of the amount of extracted oil relative to the weight of the palm fruits is within the range of 20-24%, depending e.g.
30 on fruit quality, and is subject to seasonal variation. In general, the palm oil milling process has been carefully optimized at each mill in order to minimize oil losses to the extent possible.

At most palm oil mills, sterilization of the FFB is carried out in a batch process, in which the fruit bunches are loaded into cages or carriages, which run on railway tracks and are pushed into and out of the sterilizers, either manually or by use of winches. In the batch process, the

sterilizers are large pressure chambers in which the fruit bunches are cooked in batches using steam at approximately 40 psi. After batch sterilization, the fruit bunches are conveyed to the thresher or stripper.

The traditional batch sterilization process is problematic for several reasons: in particular the batch sterilizers, the necessary railway tracks and the equipment for loading and unloading of the carts or carriages are space consuming and may occupy as much as 70% of the entire floorage of a palm oil mill. The process is also labor-consuming and has several safety hazard due to the use of pressurized steam. Furthermore, the equipment requires frequent shut-downs for maintenance and safety inspections.

In recent years there has been a growing interest in replacing the traditional batch sterilization process with a continuous sterilization process. Continuous sterilizers have been developed, which operate under steam of atmospheric pressure: Conditioned FFB are fed into the continuous sterilizer and are moved through the whole length of the continuous sterilizer by a motor-driven conveyor, and then onto the thresher or stripper. As the FFB moves along the continuous sterilizer, it is cooked by the steam, and because the process uses low or atmospheric pressure it is easily automated.

However, to date continuous sterilizers have only been installed at a limited number of palm oil mills. Batch sterilization may still be preferred because of concerns with respect to the oil extraction rate (OER) obtained at mills operating with continuous sterilizers, and because continuous sterilization subsequently requires more extensive digestion of the palm fruitlets. This is mainly because the continuous sterilization process causes less softening of the palm fruit mesocarp.

For several reasons, the use of continuous sterilizers also leads to a more wet milling process: first of all, the use of lower temperature and ambient pressure increases the water content of the sterilized fruits as compared to batch sterilization. Furthermore, continuous sterilizers do not increase the "stripability" of the palm fruit bunches to the same extent as batch sterilizers, so the number of fruitlets remaining attached to the fruit bunch stalk after threshing or stripping is relatively high. As a consequence, one must subject the empty fruit bunches coming out of the thresher or stripper to pressing, to recover oil that would otherwise be lost with the empty fruit bunches. Pressing of the empty fruit bunches, produces a liqueur containing oil and large amounts of water, which then needs to be brought back into the milling process, if the oil is to be recovered.

There have been several reports indicating that enzymes, in particular cellulolytic enzymes, may be used successfully in palm oil milling to increase the oil yield (e.g. WO 2012/011130). In particular, enzymes have been shown to facilitate the separation of oil from

water, fibre and sludge in the crude pressed oil, and to minimize the amounts of oil lost in the heavy, water phase or sludge. However, it has also been reported that the positive effects of the enzymes on oil separation and oil lost to the sludge are compromised by dilution of the crude oil. Therefore, when applying enzymes in the milling process, the total water content of the crude oil or dilute crude oil should be kept as low as possible and should in any event not exceed 40% (AU2015101377, WO 2016/097266).

With this requirement for very low amounts of water, the enzyme- assisted milling process would not be considered to be compatible with the use of continuous sterilizers

10 Summary of the Invention

The present invention relates to palm oil milling using enzymes and milling equipment comprising a continuous sterilizer.

In a first aspect the present invention provides a process for extraction of crude palm oil CPO, comprising sterilizing fresh fruit bunches (FFB) in a continuous sterilizer and contacting mass passing to digester (MPD), fruit mash and/or crude oil with an enzyme composition.

In a further aspect the invention provides a palm oil obtainable by the process according to the invention.

Finally, the invention provides a palm oil milling line comprising a continuous sterilizer and means for dosing an enzyme composition.

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Definitions

Acetylxylan esterase: The term “acetylxylan esterase” means a carboxylesterase (EC 3.1.1.72) that catalyzes the hydrolysis of acetyl groups from polymeric xylan, acetylated xylose, acetylated glucose, alpha-naphthyl acetate, and *p*-nitrophenyl acetate. Acetylxylan esterase activity can be determined using 0.5 mM *p*-nitrophenylacetate as substrate in 50 mM sodium acetate pH 5.0 containing 0.01% TWEEN™ 20 (polyoxyethylene sorbitan monolaurate). One unit of acetylxylan esterase is defined as the amount of enzyme capable of releasing 1 μmole of *p*-nitrophenolate anion per minute at pH 5, 25°C.

Alpha-glucuronidase: The term “alpha-glucuronidase” means an alpha-D-glucosiduronate glucuronohydrolase (EC 3.2.1.139) that catalyzes the hydrolysis of an alpha-D-glucuronoside to D-glucuronate and an alcohol. Alpha-glucuronidase activity can be determined according to de Vries, 1998, *J. Bacteriol.* 180: 243-249. One unit of alpha-glucuronidase equals the amount of enzyme capable of releasing 1 μmole of glucuronic or 4-O-methylglucuronic acid per minute at pH 5, 40°C.

Beta-glucosidase: The term “beta-glucosidase” means a beta-D-glucoside glucohydrolase (E.C. 3.2.1.21) that catalyzes the hydrolysis of terminal non-reducing beta-D-glucose residues with the release of beta-D-glucose. Beta-glucosidase activity can be determined using *p*-nitrophenyl-beta-D-glucopyranoside as substrate according to the procedure of Venturi *et al.*, 2002, *J. Basic Microbiol.* 42: 55-66. One unit of beta-glucosidase is defined as 1.0 μ mole of *p*-nitrophenolate anion produced per minute at 25°C, pH 4.8 from 1 mM *p*-nitrophenyl-beta-D-glucopyranoside as substrate in 50 mM sodium citrate containing 0.01% TWEEN® 20.

Beta-xylosidase: The term “beta-xylosidase” means a beta-D-xyloside xylohydrolase (E.C. 3.2.1.37) that catalyzes the exo-hydrolysis of short beta (1→4)-xylooligosaccharides to remove successive D-xylose residues from non-reducing termini. Beta-xylosidase activity can be determined using 1 mM *p*-nitrophenyl-beta-D-xyloside as substrate in 100 mM sodium citrate containing 0.01% TWEEN® 20 at pH 5, 40°C. One unit of beta-xylosidase is defined as 1.0 μ mole of *p*-nitrophenolate anion produced per minute at 40°C, pH 5 from 1 mM *p*-nitrophenyl-beta-D-xyloside in 100 mM sodium citrate containing 0.01% TWEEN® 20.

Cellobiohydrolase: The term “cellobiohydrolase” means a 1,4-beta-D-glucan cellobiohydrolase (E.C. 3.2.1.91 and E.C. 3.2.1.176) that catalyzes the hydrolysis of 1,4-beta-D-glucosidic linkages in cellulose, cellobiosaccharides, or any beta-1,4-linked glucose containing polymer, releasing cellobiose from the reducing end (cellobiohydrolase I) or non-reducing end (cellobiohydrolase II) of the chain (Teeri, 1997, *Trends in Biotechnology* 15: 160-167; Teeri *et al.*, 1998, *Biochem. Soc. Trans.* 26: 173-178). Cellobiohydrolase activity can be determined according to the procedures described by Lever *et al.*, 1972, *Anal. Biochem.* 47: 273-279; van Tilbeurgh *et al.*, 1982, *FEBS Letters* 149: 152-156; van Tilbeurgh and Claeysens, 1985, *FEBS Letters* 187: 283-288; and Tomme *et al.*, 1988, *Eur. J. Biochem.* 170: 575-581.

Cellulolytic enzyme or cellulase: The term “cellulolytic enzyme” or “cellulase” means one or more (e.g., several) enzymes that hydrolyze a cellulosic material. Such enzymes include endoglucanase(s), cellobiohydrolase(s), beta-glucosidase(s), or combinations thereof. The two basic approaches for measuring cellulolytic enzyme activity include: (1) measuring the total cellulolytic enzyme activity, and (2) measuring the individual cellulolytic enzyme activities (endoglucanases, cellobiohydrolases, and beta-glucosidases) as reviewed in Zhang *et al.*, 2006, *Biotechnology Advances* 24: 452-481. Total cellulolytic enzyme activity can be measured using insoluble substrates, including Whatman №1 filter paper, microcrystalline cellulose, bacterial cellulose, algal cellulose, cotton, pretreated lignocellulose, etc. The most common total cellulolytic activity assay is the filter paper assay using Whatman №1 filter paper as the substrate. The assay was established by the International Union of Pure and Applied Chemistry (IUPAC) (Ghose, 1987, *Pure Appl. Chem.* 59: 257-68).

Cellulolytic enzyme activity can be determined by measuring the increase in production/release of sugars during hydrolysis of a cellulosic material by cellulolytic enzyme(s) under the following conditions: 1-50 mg of cellulolytic enzyme protein/g of cellulose in pretreated corn stover (PCS) (or other pretreated cellulosic material) for 3-7 days at a suitable temperature such as 40°C-80°C, e.g., 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 70°C, 75°C, or 80°C, and a suitable pH, such as 4-9, e.g., 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, or 9.0, compared to a control hydrolysis without addition of cellulolytic enzyme protein. Typical conditions are 1 ml reactions, washed or unwashed PCS, 5% insoluble solids (dry weight), 50 mM sodium acetate pH 5, 1 mM MnSO₄, 50°C, 55°C, or 60°C, 72 hours, sugar analysis by AMINEX® HPX-87H column chromatography (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Cellulosic material: The term “cellulosic material” means any material containing cellulose. The predominant polysaccharide in the primary cell wall of biomass is cellulose, the second most abundant is hemicellulose, and the third is pectin. The secondary cell wall, produced after the cell has stopped growing, also contains polysaccharides and is strengthened by polymeric lignin covalently cross-linked to hemicellulose. Cellulose is a homopolymer of anhydrocellobiose and thus a linear beta-(1-4)-D-glucan, while hemicelluloses include a variety of compounds, such as xylans, xyloglucans, arabinoxylans, and mannans in complex branched structures with a spectrum of substituents. Although generally polymorphous, cellulose is found in plant tissue primarily as an insoluble crystalline matrix of parallel glucan chains. Hemicelluloses usually hydrogen bond to cellulose, as well as to other hemicelluloses, which help stabilize the cell wall matrix.

Cellulose is generally found, for example, in the stems, leaves, hulls, husks, and cobs of plants or leaves, branches, and wood of trees. The cellulosic material can be, but is not limited to, agricultural residue, herbaceous material (including energy crops), municipal solid waste, pulp and paper mill residue, waste paper, and wood (including forestry residue) (see, for example, Wiseloge *et al.*, 1995, in *Handbook on Bioethanol* (Charles E. Wyman, editor), pp. 105-118, Taylor & Francis, Washington D.C.; Wyman, 1994, *Bioresource Technology* 50: 3-16; Lynd, 1990, *Applied Biochemistry and Biotechnology* 24/25: 695-719; Mosier *et al.*, 1999, Recent Progress in Bioconversion of Lignocellulosics, in *Advances in Biochemical Engineering/Biotechnology*, T. Scheper, managing editor, Volume 65, pp. 23-40, Springer-Verlag, New York). It is understood herein that the cellulose may be in the form of lignocellulose, a plant cell wall material containing lignin, cellulose, and hemicellulose in a mixed matrix. In one aspect, the cellulosic material is any biomass material. In another aspect, the cellulosic material is lignocellulose, which comprises cellulose, hemicelluloses, and lignin.

Crude oil: The term “crude oil” (also called a non-degummed oil) refers to a pressed or extracted oil or a mixture thereof. In the present context it is to be understood that the oil is palm oil, in

particular un-refined palm oil. In particular, the term “crude oil” refers to the effluent from the screw press of a palm oil mill; i.e. to the mixture of oil and water pressed out of the palm fruit mash, before it has been subject to clarification and separation of oil from water.

Endoglucanase: The term “endoglucanase” means a 4-(1,3;1,4)-beta-D-glucan 4-glucanohydrolase (E.C. 3.2.1.4) that catalyzes endohydrolysis of 1,4-beta-D-glycosidic linkages in cellulose, cellulose derivatives (such as carboxymethyl cellulose and hydroxyethyl cellulose), lichenin, beta-1,4 bonds in mixed beta-1,3-1,4 glucans such as cereal beta-D-glucans or xyloglucans, and other plant material containing cellulosic components. Endoglucanase activity can be determined by measuring reduction in substrate viscosity or increase in reducing ends determined by a reducing sugar assay (Zhang *et al.*, 2006, *Biotechnology Advances* 24: 452-481). Endoglucanase activity can also be determined using carboxymethyl cellulose (CMC) as substrate according to the procedure of Ghose, 1987, *Pure and Appl. Chem.* 59: 257-268, at pH 5, 40°C.

Feruloyl esterase: The term “feruloyl esterase” means a 4-hydroxy-3-methoxycinnamoyl-sugar hydrolase (EC 3.1.1.73) that catalyzes the hydrolysis of 4-hydroxy-3-methoxycinnamoyl (feruloyl) groups from esterified sugar, which is usually arabinose in natural biomass substrates, to produce ferulate (4-hydroxy-3-methoxycinnamate). Feruloyl esterase (FAE) is also known as ferulic acid esterase, hydroxycinnamoyl esterase, FAE-III, cinnamoyl ester hydrolase, FAEA, cinnAE, FAE-I, or FAE-II. Feruloyl esterase activity can be determined using 0.5 mM *p*-nitrophenylferulate as substrate in 50 mM sodium acetate pH 5.0. One unit of feruloyl esterase equals the amount of enzyme capable of releasing 1 µmole of *p*-nitrophenolate anion per minute at pH 5, 25°C.

Hemicellulolytic enzyme or hemicellulase: The term “hemicellulolytic enzyme” or “hemicellulase” means one or more (*e.g.*, several) enzymes that hydrolyze a hemicellulosic material. See, for example, Shallom and Shoham, 2003, *Current Opinion In Microbiology* 6(3): 219-228). Hemicellulases are key components in the degradation of plant biomass. Examples of hemicellulases include, but are not limited to, an acetylmannan esterase, an acetylxylan esterase, an arabinanase, an arabinofuranosidase, a coumaric acid esterase, a feruloyl esterase, a galactosidase, a glucuronidase, a glucuronoyl esterase, a mannanase, a mannosidase, a xylanase, and a xylosidase. The substrates for these enzymes, hemicelluloses, are a heterogeneous group of branched and linear polysaccharides that are bound via hydrogen bonds to the cellulose microfibrils in the plant cell wall, crosslinking them into a robust network. Hemicelluloses are also covalently attached to lignin, forming together with cellulose a highly complex structure. The variable structure and organization of hemicelluloses require the concerted action of many enzymes for its complete degradation. The catalytic modules of hemicellulases are either glycoside hydrolases (GHs) that hydrolyze glycosidic bonds, or carbohydrate esterases (CEs), which hydrolyze ester linkages of acetate or ferulic acid side

groups. These catalytic modules, based on homology of their primary sequence, can be assigned into GH and CE families. Some families, with an overall similar fold, can be further grouped into clans, marked alphabetically (e.g., GH-A). A most informative and updated classification of these and other carbohydrate active enzymes is available in the Carbohydrate-Active Enzymes (CAZy) database. Hemicellulolytic enzyme activities can be measured according to Ghose and Bisaria, 1987, *Pure & Appl. Chem.* 59: 1739-1752, at a suitable temperature such as 40°C-80°C, e.g., 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 70°C, 75°C, or 80°C, and a suitable pH such as 4-9, e.g., 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, or 9.0.

Hemicellulosic material: The term "hemicellulosic material" means any material comprising hemicelluloses. Hemicelluloses include xylan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan. These polysaccharides contain many different sugar monomers. Sugar monomers in hemicellulose can include xylose, mannose, galactose, rhamnose, and arabinose. Hemicelluloses contain most of the D-pentose sugars. Xylose is in most cases the sugar monomer present in the largest amount, although in softwoods mannose can be the most abundant sugar. Xylan contains a backbone of beta-(1-4)-linked xylose residues. Xylans of terrestrial plants are heteropolymers possessing a beta-(1-4)-D-xylopyranose backbone, which is branched by short carbohydrate chains. They comprise D-glucuronic acid or its 4-O-methyl ether, L-arabinose, and/or various oligosaccharides, composed of D-xylose, L-arabinose, D- or L-galactose, and D-glucose. Xylan-type polysaccharides can be divided into homoxylans and heteroxylans, which include glucuronoxylans, (arabino)glucuronoxylans, (glucurono)arabinoxylans, arabinoxylans, and complex heteroxylans. See, for example, Ebringerova *et al.*, 2005, *Adv. Polym. Sci.* 186: 1-67. Hemicellulosic material is also known herein as "xylan-containing material".

Sources for hemicellulosic material are essentially the same as those for cellulosic material described herein.

Pectinase: The term "pectinase" is defined as any enzyme that degrades pectic substances. Pectic substances include homogalacturonans, xylogalacturonans, and rhamnogalacturonans as well as derivatives thereof. Pectinase treatment may be achieved by one or more pectinases, such as two or more pectinases of the same type (e.g., two different pectin methylesterases) or of different types (e.g., a pectin methylesterase and an arabinanase). The pectinase may, for example, be selected from the group consisting of arabinanase (catalyses the degradation of arabinan sidechains of pectic substances), arabinofuranosidase (removes arabinosyl substituents from arabinans and arabinogalactans), galactanase (catalyses the degradation of arabinogalactan and galactan sidechains of pectic substances), pectate lyase (cleaves glycosidic bonds in polygalacturonic acid by beta-elimination), pectin acetylerase (catalyses the removal of acetyl groups from acetylated pectin), pectin lyase (cleaves the glycosidic bonds of highly

methylesterase (catalyses the removal of methanol from pectin, resulting in the formation of pectic acid, polygalacturonic acid), polygalacturonase (hydrolyses the glycosidic linkages in the polygalacturonic acid chain), rhamnogalacturonan acetyltransferase (catalyses the removal of acetyl groups from acetylated rhamnogalacturonans), and rhamnogalacturonase and rhamnogalacturonan lyase (degrade rhamnogalacturonans).

Polygalacturonases: The term “polygalacturonases” (EC 3.2.1.15) are pectinases that catalyze random hydrolysis of (1,4)-alpha-D-galactosiduronic linkages in pectate and other galacturonans. They are also known as pectin depolymerase. Polygalacturonase hydrolyses the alpha-1,4-glycosidic bonds in polygalacturonic acid with the resultant release of galacturonic acid. This reducing sugar reacted then with 3,5-dinitrosalicylic acid (DNS). The colour change produced due to the reduction of DNS is proportional to the amount of galacturonic acid released, which in turn is proportional to the activity of polygalacturonase in the sample. One polygalacturonase unit (PGNU) is defined as the amount of enzyme which will produce 1 mg of galacturonic acid sodium salt under standard conditions (acetate buffer, pH 4.5, 40°C, 10 min reaction time, 540 nm).

Xylan-containing material: The term “xylan-containing material” means any material comprising a plant cell wall polysaccharide containing a backbone of beta-(1-4)-linked xylose residues. Xylans of terrestrial plants are heteropolymers possessing a beta-(1-4)-D-xylopyranose backbone, which is branched by short carbohydrate chains. They comprise D-glucuronic acid or its 4-O-methyl ether, L-arabinose, and/or various oligosaccharides, composed of D-xylose, L-arabinose, D- or L-galactose, and D-glucose. Xylan-type polysaccharides can be divided into homoxylans and heteroxylans, which include glucuronoxylans, (arabino)glucuronoxylans, (glucurono)arabinoxylans, arabinoxylans, and complex heteroxylans. See, for example, Ebringerova *et al.*, 2005, *Adv. Polym. Sci.* 186: 1-67.

Amylase: For the purpose of the present invention “amylase” refers to an enzyme that catalyses the hydrolysis of starch into sugars. Amylase activity may be determined as described by Joseph D. Teller, Measurement of amylase activity, *J. Biol. Chem.* 1950, 185:701-704.

Xylan degrading activity or xylanolytic activity: The term “xylan degrading activity” or “xylanolytic activity” means a biological activity that hydrolyzes xylan-containing material. The two basic approaches for measuring xylanolytic activity include: (1) measuring the total xylanolytic activity, and (2) measuring the individual xylanolytic activities (e.g., endoxylanases, beta-xylosidases). Recent progress in assays of xylanolytic enzymes was summarized in several publications including Biely and Puchard, 2006, *Journal of the Science of Food and Agriculture* 86(11): 1636-1647; Spanikova and Biely, 2006, *FEBS Letters* 580(19): 4597-4601; Herrmann *et al.*, 1997, *Biochemical Journal* 321: 375-381.

Total xylan degrading activity can be measured by determining the reducing sugars formed from various types of xylan, including, for example, oat spelt, beechwood, and larchwood xylans, or by photometric determination of dyed xylan fragments released from various covalently dyed xylans. A common total xylanolytic activity assay is based on production of reducing sugars from polymeric 4-O-methyl glucuronoxylan as described in Bailey *et al.*, 1992, Interlaboratory testing of methods for assay of xylanase activity, *Journal of Biotechnology* 23(3): 257-270. Xylanase activity can also be determined with 0.2% AZCL-arabinoxylan as substrate in 0.01% TRITON® X-100 and 200 mM sodium phosphate pH 6 at 37°C. One unit of xylanase activity is defined as 1.0 µmole of azurine produced per minute at 37°C, pH 6 from 0.2% AZCL-arabinoxylan as substrate in 200 mM sodium phosphate pH 6.

Xylan degrading activity can be determined by measuring the increase in hydrolysis of birchwood xylan (Sigma Chemical Co., Inc., St. Louis, MO, USA) by xylan-degrading enzyme(s) under the following typical conditions: 1 ml reactions, 5 mg/ml substrate (total solids), 5 mg of xylanolytic protein/g of substrate, 50 mM sodium acetate pH 5, 50°C, 24 hours, sugar analysis using *p*-hydroxybenzoic acid hydrazide (PHBAH) assay as described by Lever, 1972, *Anal. Biochem.* 47: 273-279.

Xylanase: The term “xylanase” means a 1,4-beta-D-xylan-xylohydrolase (E.C. 3.2.1.8) that catalyzes the endohydrolysis of 1,4-beta-D-xylosidic linkages in xylans. Xylanase activity can be determined with 0.2% AZCL-arabinoxylan as substrate in 0.01% TRITON® X-100 and 200 mM sodium phosphate pH 6 at 37°C. One unit of xylanase activity is defined as 1.0 µmole of azurine produced per minute at 37°C, pH 6 from 0.2% AZCL-arabinoxylan as substrate in 200 mM sodium phosphate pH 6.

Pectin methylesterase (PME), EC 3.1.1.11, is an enzyme that acts mainly in the hydrolysis of methyl ester groups in pectin chains to form carboxylate groups, releasing methanol and H₃O⁺ (Jayani, R.S.; Saxena, S.; Gupta, R. Microbial pectinolytic enzymes: a review. *Process Biochemistry*, London, v.40, p.2931-2944, 2005). Pectin methyl esterase activity may be determined e.g. as described by Lemke Gonzalez et al., Pectin methylesterase activity determined by different methods and thermal inactivation of exogenous pme in mango juice. *Ciênc. agrotec.* vol.35 no.5 Lavras Sept./Oct. 2011

Palm oil mill effluent (POME): Palm oil mill effluent (POME) is the waste water discharged e.g. from the sterilization process, crude oil clarification process.

Oil extraction rate (OER): For the purpose of the present invention, “Oil extraction rate (OER)” may be defined as by Chang *et al.*, oil palm Industry economic journal, volume 3, 2003[9]. Chang *et al.* defines the Oil extraction rate as ratio of oil recovered to Fresh fruit bunch (FFB) times 100.

According to this definition, the mathematical formula is:

$$\text{OER} = (\text{weight of oil recovered} / \text{weight of FFB processed}) \times 100$$

Temperature optimum: In the context of the invention, the term “temperature optimum” refers to the temperature at which an enzyme's catalytic activity is at its greatest. Below the temperature, reacting molecules have more and more kinetic energy as the temperature rises. This increases the chances of a successful collision and so the rate increases. Above temperature optimum the enzyme structure begins to denature since at higher temperatures intra- and intermolecular bonds are broken as the enzyme molecules gain even more kinetic energy.

A temperature optimum may be determined by assessing the enzyme activity; e.g. the cellulase activity, of a purified enzyme, a crude extract of the enzyme or an enzyme in a whole broth, over a range of temperatures (e.g. 40 to 90°C) at a relevant pH (e.g. pH 5) and for an appropriate incubation period; e.g. for a period of 5-60 minutes, such as 5-30 minutes, 10-30 minutes or 20-30 minutes, or 20-25 minutes. For determination of cellulase activity, the buffers, substrate and assay principle disclosed below, in the definition of “thermostability” may be used in a determination of temperature optimum.

Thermostability: As used herein, “thermostability” refers to the stability of an enzyme when the enzyme is tested or left at a specific high temperature, such as 70°C. The thermostability may be determined by incubating the enzyme in an appropriate buffer (e.g. 0.1 M Na-OAc buffer pH 5.0) at an elevated temperature, e.g. 70 degrees Celsius for varying time periods (i.e. 0 min, 20 min, 40 min and 60 minutes) followed by transfer of the samples to ice, and then determine the residual enzyme activity. For instance, residual cellulase activity may be determined on Konelab using the following protocol:

- Substrate: Carboxymethyl cellulose (CMC), 5 g/L in Na-AOAc buffer pH 5.0
- Sample dissolution and dilution buffer: 0.1 M Na-OAc buffer pH 5.0
- Stop and detection reagent: PAHBAH, 50 g/L K-Na-tartrate, 20 g/L PAHBAH, 5.52g/L Bismuth(III)-acetate, 0.5 M NaOH
- Assay principle: Cellulose is hydrolyzed and form reducing carbohydrate. The substrate carboxymethyl cellulose (CMC) is a substituted form of cellulose. The reaction is stopped by an alkaline reagent containing PAHBAH and Bismuth that forms complexes with reducing sugar. The complex formation results in colour production which can be read at 405 nm by a spectrophotometer. The produced colour is proportional to the cellulose activity.
- The residual activity is calculated and plotted against time of heat treatment

In the present application, reference to “about” a value or parameter herein includes aspects that are directed to that value or parameter *per se*. For example, description referring to “about X” includes the aspect “X”.

As used herein and in the appended claims, the singular forms “a,” “or,” and “the” include plural referents unless the context clearly dictates otherwise. It is understood that the aspects of the invention described herein include “consisting” and/or “consisting essentially of” aspects.

Unless defined otherwise or clearly indicated by context, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

Description of the figures

Figure 1: Schematic representation of a continuous sterilizer. The continuous sterilizer comprises a crusher (A), depicted as a double roll crusher, a conveyor (B), a first steam lock (C), a sterilizing chamber (D) which has conveying means and steam injection nozzles (E) and (F), a second steam lock (G) and a discharge conveyor (H).

Figure 2: Schematic representation of a production line in a commercial palm oil mill from thresher to screw press. The production line includes: Thresher (I), Conveyor(s) (J), (K) and (L), Digester (M) and Screw press (N). Enzyme application is shown at points (O); including water dosing (S) and enzyme dosing (R). Steam supply to the digester is shown as (P) and dilute crude oil (DCO) exit from screw press is shown as (Q).

Figure 3: Palm oil milling equipment configuration comprising pre-cooker. The equipment comprises a thresher or stripper (A), a pre-cooker (R), such as a vertical pre-cooker, a digester (M) with steam supply (P), such as a vertical digester, and a screw press (N), connected in a series.

Figure 4: Palm oil milling equipment comprising a continuous sterilizer, which comprises a crusher (A), such as a double roll crusher, a conveyor (B), such as a chain-type conveyor, a first steam lock (C), a sterilizing chamber (D) with conveying means and steam injection nozzles (E) and (F), a second steam lock (G), and optionally a discharge conveyor (H), linked to a thresher or stripper (I), a digester (M) with steam supply (P), and a screw press (N),

Figure 5: Palm oil milling equipment comprising a continuous sterilizer and pre-cooker. The equipment comprises a continuous sterilizer, which comprises a crusher (A), such as a double roll crusher, a conveyor (B), such as a chain-type conveyor, a first steam lock (C), a sterilizing chamber (D) with conveying means and steam injection nozzles (E) and (F), a second steam lock (G), and optionally a discharge conveyor (H), linked to a thresher or stripper (I), a pre-cooker (T), such as a vertical pre-cooker, a digester (M) with steam supply (P), such as a vertical digester, and a screw press (N), which are serially connected; e.g. by means of conveyors (J), (K), and/or (L).

Detailed Description of the Invention

The present invention relates to a process for extraction of crude palm oil using enzymes, and in addition, the process according to the invention involves sterilizing fruit bunches in a sterilizer using steam at low or atmospheric pressure such as a continuous sterilizer.

The present inventors have surprisingly found that oil extraction efficiency is maintained with enzyme treatment even at a relatively high content of moisture in the fruit mash or press liquid (dilute crude oil (DCO)). Furthermore, the inventors found that amount of emulsion formed during clarification decreases with enzyme treatment at higher %moisture, indicating that enzymes is able to break down emulsion even at relatively high %moisture, especially from 46-64 %moisture

The inventors have also found that there is no significant increase in the amounts of free fatty acids with recycling of sterilizer condensate (SC) for dilution of undilute crude oil (UDCO) to produce dilute crude oil (DCO). In particular, the inventors have found that the % FFA in the final oil is less than 5%.

Together these findings show that enzyme-assisted extraction of crude palm oil is feasible even in an extraction process with high moisture. Since crude palm oil extraction processes using continuous sterilizers are characterized by a high moisture content the data also confirm that enzyme assisted crude palm oil extraction is possible in milling processes using continuous sterilizers.

Sterilization is the first step in the process, which is crucial to the final oil quality as well as the strippability of fruits. Steam sterilization of the FFBs facilitates fruits being stripped from bunches to give the palm fruitlet. The sterilization step has several advantages, one being that it softens the fruit mesocarp for subsequent digestion.

After sterilization, the palm fruits are subject to stripping, digestion and pressing. Stripping or threshing is generally carried out in a mechanized system having a rotating drum or fixed drum equipped with rotary beater bars detach the fruit from the bunch, leaving the spikelets on the stem. After stripping, the palm fruitlets (also referred to as "mass passing to digester" ("MPD")) are moved into a digester by one or more transportation means. In the digester (M), the fruitlets are further reheated to loosen the pericarp. The digester is typically a steam heated vessels, which has rotating shafts to which are attached stirring arms. The fruitlets are rotated about, causing the loosening of the pericarps from the nuts and degradation of the mesocarp. The digester is kept full and as the digested fruit is drawn out, freshly stripped fruits are brought in. The digested mass is passed into a screw press, from which a mixture of oil, water, press cake or fibre and nuts are discharged. The mixture of oil, water and solids (Undiluted Dilute Crude Oil,

(UDCO) or Diluted Crude Oil (DCO)) from the fruitlets is delivered from the press to a clarification tank for further processing.

In a first aspect the present invention provides a process for extraction of crude palm oil CPO, comprising sterilizing fresh fruit bunches (FFB) in a continuous sterilizer and contacting mass
5 passing to digester (MPD), fruit mash and/or crude oil with an enzyme composition.

In the process according to the invention, the FFBs are subject to conditioning prior to sterilization, so as to loosen the arrangement of spikelets in the FFB; e.g. by use of a crusher or bunch splitter, such as a double-roll crusher. Subsequent to this, the FFB is then fed into the Continuous Sterilizer, for instance via a sealed scrapper bar type feeding conveyor. This conveyor
10 may be designed to take the advantage of excess steam from the Continuous Sterilizer to pre-heat as well as to evacuate the air from the conditioned FFB. In doing so, it contains and controls the excessive steam from leaking into the atmosphere and pre-heats the conditioned FFB at the same time.

In further embodiments the said continuous sterilizer comprises one or more non-
15 pressurized chamber(s) fitted with steam injection nozzles and means for conveying the FFB through the chamber.

The said means for conveying may in particular be a chain-type conveyor.

In particular embodiments of the invention the continuous sterilizer has a configuration as shown in Figure 1, comprising a crusher/bunch splitter (A), such as a double roll crusher, one or
20 more conveyor(s) (B), such as one or more chain-type conveyor(s), a first steam lock (C), one or more sterilizing chamber(s) (D) with conveying means and steam injection nozzles (E) and (F), a second steam lock (G), and optionally a discharge conveyor (H).

The crude oil may in particular be extracted from sterilized fruit using milling equipment configured as shown in Figure 2, said equipment comprising a thresher or stripper (I), a digester (M)
25 with steam supply (P), such as a vertical digester, and a screw press (N), connected in a series.

In some embodiments, the crude oil is extracted from sterilized fruit using milling equipment configured as shown in Figure 3, said equipment comprising a thresher or stripper (A), a pre-cooker (R), such as a vertical pre-cooker, a digester (M) with steam supply (P), such as a vertical digester, and a screw press (N), connected in a series.

In other embodiments, the crude oil is extracted using milling equipment configured as shown in Figure 4, said equipment comprising a continuous sterilizer, which comprises a crusher (A), such as a double roll crusher, a conveyor (B), such as a chain-type conveyor, a first steam lock (C), a
30 sterilizing chamber (D) with conveying means and steam injection nozzles (E) and (F), a second steam lock (G), and optionally a discharge conveyor (H), linked to a thresher or stripper (I), a digester

(M) with steam supply (P), such as a vertical digester, and a screw press (N), which are serially connected; e.g. by means of conveyors (J), (K), and/or (L).

The serial connection allows fruit bunches to be conveyed via the sterilizer into the thresher or stripper, palm fruitlets or "mass passing to digester" to be conveyed from the thresher or stripper to the digester, optionally via the pre-cooker, and transport of fruit mash from the digester into the screw press. In further embodiments the exit of the screw press is fluidically connected to downstream equipment for separation of oil from water and sludge. In the process according to the invention the crude oil may be extracted using milling equipment configured as shown in Figure 5, said equipment comprising a continuous sterilizer, which comprises a crusher (A), such as a double roll crusher, a conveyor (B), such as a chain-type conveyor, a first steam lock (C), a sterilizing chamber (D) with conveying means and steam injection nozzles (E) and (F), a second steam lock (G), and optionally a discharge conveyor (H), linked to a thresher or stripper (I), a pre-cooker (T), such as a vertical pre-cooker, a digester (M) with steam supply (P), such as a vertical digester, and a screw press (N), which are serially connected; e.g. by means of conveyors (J), (K), and/or (L).

The sterilization in said continuous sterilizer may proceed from 60 to 120 minutes, such as from 80 to 120 minutes, from 80-110 minutes, from between 80 minutes to 100 minutes, or such as from 80-90 minutes.

The enzyme composition used in the process according to the invention may comprise one or more hydrolases, such as one or more cellulases. The cellulases are optionally in combination with one or more hemicellulases, one or more pectinases, one or more amylases, or a combination thereof.

In one embodiment, the enzyme composition used in the process of the invention comprises a commercial cellulolytic enzyme preparation. Examples of commercial cellulolytic enzyme preparations suitable for use in the present invention include, for example, CELLIC® CTec (Novozymes A/S), CELLIC® CTec2 (Novozymes A/S), CELLIC® CTec3 (Novozymes A/S), CELLUCLAST™ (Novozymes A/S), NOVOZYM™ 188 (Novozymes A/S), SPEZYME™ CP (Genencor Int.), ACCELERASE™ TRIO (DuPont), FILTRASE® NL (DSM); METHAPLUS® S/L 100 (DSM), ROHAMENT™ 7069 W (Röhm GmbH), or ALTERNAFUEL® CMAX3® (Dyadic International, Inc.).

Suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Suitable cellulases include cellulases from the genera *Bacillus*, *Pseudomonas*, *Humicola*, *Fusarium*, *Thielavia*, *Acremonium*, e.g. the fungal cellulases produced from *Humicola insolens*, *Myceliophthora thermophila* and *Fusarium oxysporum* disclosed in U.S. Pat. No. 5,648,263, U.S. Pat. No. 5,691,178, U.S. Pat. No. 5,776,757 and WO 89/09259. Especially suitable cellulases are the alkaline or neutral cellulases having colour care

and whiteness maintenance benefits. Examples of such cellulases are cellulases described in EP 0 531 372, WO 96/11262, WO 96/29397, WO 98/08940. Other examples are cellulase variants such as those described in WO 94/07998, EP 0 531 315, U.S. Pat. No. 5,457,046, U.S. Pat. No. 5,686,593, U.S. Pat. No. 5,763,254, WO 95/24471, WO 98/12307 and PCT/DK98/00299.

- 5 Commercially used cellulases include Renozyme®, Celluzyme®, Celluclean®, Endolase® and Carezyme®. (Novozymes A/S), Clazinase®, and Puradax HA®. (Genencor Int. Inc), and KAC-500(B)TM (Kao Corporation).

In currently preferred embodiments, the enzyme composition comprises Palmora® OER, which is commercially available from Novozymes A/S.

- 10 Examples of commercial hemicellulolytic enzyme preparations suitable for use in the present invention include, for example, SHEARZYMETM (Novozymes A/S), CELLIC® HTec (Novozymes A/S), CELLIC® HTec2 (Novozymes A/S), CELLIC® HTec3 (Novozymes A/S), ULTRAFLO® (Novozymes A/S), PULPZYME® HC (Novozymes A/S), MULTIFECT® Xylanase (Genencor), ACCELLERASE® XY (Genencor), ACCELLERASE® XC (Genencor), ECOPULP®
 15 TX-200A (AB Enzymes), HSP 6000 Xylanase (DSM), DEPOLTM 333P (Biocatalysts Limit, Wales, UK), DEPOLTM 740L. (Biocatalysts Limit, Wales, UK), and DEPOLTM 762P (Biocatalysts Limit, Wales, UK).

In particular, the said enzyme composition may comprise one or more cellulases and/or one or more amylases and/or one or more pectinases.

- 20 In further embodiments, the process according to the invention comprises
- i. Sterilizing the FFB to produce sterilized FFB,
 - ii. Subjecting the sterilized FFB to stripping or threshing to provide stripped fruitlets or MPD,
 - iii. Conveying the stripped fruitlets or MPD to a digester or to a pre-cooker and then
 25 to a digester,
 - iv. Subjecting the stripped fruitlets or MPD to a digestion procedure to produce fruit mash,
 - v. Subjecting the fruit mash to pressing to produce a crude oil comprising oil and water, together with cell debris, and/or fibrous material, and
 - vi. Separating the oil in said crude oil from the water and from said cell debris,
 30 and/or fibrous material.
 - vii. and/or fibrous material.

- It is to be understood that the enzyme composition used according to the invention may be applied at any point in the crude palm oil extraction process, after the palm fruit bunches have been sterilized and until the oil is separated from water the water and from cell debris and fibrous
 35 material, which is also present in the liquid which is obtained by pressing of the mashed palm

fruitlets. In particular, the enzyme composition may be applied to a substrate selected from the group consisting of palm fruitlets, mass passing to digester (MPD), mashed or partly mashed palm fruitlets or MPD, and palm press liquid, such as DCO or UDCO.

5 In the process according to the invention the enzyme composition or the one or more enzymes; e.g. one or more enzymes as defined above may be applied in step iii), such as by application onto the stripped fruitlets or MPD, while the stripped fruitlets or MPD is/are conveyed to a digester (I) on one or more conveyors; e.g. conveyors (J), (K) and/or (L) in figure 2.

In some embodiments, the enzyme composition or the one or more enzymes; e.g. one or more enzymes as defined above are applied in step iv).

10 In other embodiments, the enzyme composition or the one or more enzymes; e.g. one or more enzymes as defined above, are applied after step v) and before the oil in said crude oil is separated from the water and from the cell debris, and/or the fibrous material.

In some embodiments step iv) comprises retaining the stripped fruitlets or MPD in the digester at temperatures above 65°C and up to 85°C from 10-30 minutes, such as from 10-28
15 minutes, 15-28 minutes, 12-30 minutes, 12-28 minutes or 12-25 minutes; e.g. by controlling the speed of the screw press (N) and/or by controlling steam supply (P).

In further embodiments step iv) comprises retaining the stripped fruitlets or MPD in the digester at temperatures between 55 and 90°C, such as between 60 and 90°C, between 60 and 85°C, between 60 and 80°C, between 65 and 85°C, or such as between 65 and 80°C from 7-30
20 minutes, from 10-30 minutes, such as from 7-28 minutes, from 10-28 minutes, 15-28 minutes, 12-30 minutes, 12-28 minutes, from 7-25 minutes, from 10-25 minutes, from 7-20 minutes, from 10-20 minutes or 12-25 minutes; e.g. by controlling the speed of the screw press (N) and/or by controlling steam supply (P).

The process according to the invention may comprise the steps of:

- 25 a. Contacting the palm fruitlets, said MPD, said fruit mash and/or said crude oil with said enzyme composition at a temperature of above 65°C;
b. Extracting the CPO.

The palm fruitlets, the MPD, the fruit mash and/or the crude oil may be contacted with said enzyme composition at a temperature within the range of 55-90°C, such as a temperature within the range
30 of 55-85°C, 55-80°C, 60-90°C, 60-85°C, 60-80°C, 66-90°C, 67-90°C, 68-90°C, 69-90°C, 70-90°C, 66-85°C, 66-80°C, 67-80°C, 66-79°C, 66-78°C, 66-77°C, 66-76°C, 66-75°C, 66-74°C, 66-73°C, 66-72°C, 66-71°C, 67-80°C, 67-79°C, 67-78°C, 67-77°C, 67-76°C, 67-75°C, 67-74°C, 67-73°C, 67-72°C, 67-71°C, 68-79°C, 68-78°C, 68-77°C, 68-76°C, 68-75°C, 68-74°C, 68-73°C, 68-72°C, 68-71°C, 69-79°C, 69-78°C, 69-77°C, 69-76°C, 69-75°C, 69-74°C, 69-73°C, 69-72°C, 69-71°C,
35 70-90°C, 70-89°C, 70-88°C, 70-87°C, 70-86°C, or 70-85°C.

The enzyme composition may be dosed in amounts corresponding to 20-1000 mg enzyme protein/kg palm fruitlet, MPD, fruit mash, or crude oil, 20-750 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 20-500 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, such as 20-450 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 20-400 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 20-350 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 20-300 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 20-250 mg enzyme protein/kg palm fruitlet, MPD or crude oil, 20-200 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 20-150 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 20-100 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 20-75 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 20-50 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 30-500 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 40-500 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 50-500 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 75-500 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 100-500 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 150-500 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 200-500 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 250-500 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 300-500 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 350-500 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 400-500 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 200-1000 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 200-750 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 30-400 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 30-300 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 30-200 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 30-150 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 30-100 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, 30-75 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil, or such as 30-50 mg enzyme protein/kg palm fruitlet, MPD, fruit mash or crude oil.

The enzyme composition may be dosed in amounts corresponding to 5-200 mg enzyme protein per metric ton of fresh fruit bunches (FFBs) entering the oil extraction process, such as 10-200 mg enzyme protein, such as 20-200 mg enzyme protein, such as 5-150 mg enzyme protein, such as 5-100 mg enzyme protein, such as 5-50 mg enzyme protein, such as 10-200 mg enzyme protein, such as 10-100 mg enzyme protein, such as 10-75 mg enzyme protein, or such as 10-50 mg enzyme protein per metric ton of fresh fruit bunches (FFBs) entering the oil extraction process.

The enzyme composition may also be dosed such that the amount of enzyme protein corresponds to 100-1000 ppm, such as 200-1000 ppm, 100-500 ppm, such as 200-500 ppm, 250-400 ppm or 350-1000 ppm relative to the amount of palm fruitlet, MPD, fruit mash or crude oil.

In further embodiments the palm fruitlet, MPD, fruit mash or crude oil is contacted with the enzyme composition for a period of 5-60 minutes, such as for a period of 20-60 minutes, 25-60 minutes, 30-60 minutes, 15-50 minutes, 20-50 minutes, 25-50 minutes, 30-50 minutes, 15-40 minutes, 20-40 minutes, 25-40 minutes, 30-40 minutes, 15-30 minutes, 20-30 minutes, 25-28 minutes, 25-30 minutes, 25-35 minutes, 15-25 minutes, 20-25 minutes, 20-28 minutes, 15-20 minutes, 10-15 minutes or 5-10 minutes.

The process may in some embodiments comprise retaining the palm fruitlets or MPD, fruit mash or crude oil at temperatures above 65°C and up to 85°C for 10-30 minutes, such as for 10-28 minutes, 15-28 minutes, 12-30 minutes, 12-28 minutes or 12-25 minutes.

In particular embodiments, the process according to the invention comprises retaining the palm fruitlets or MPD in a pre-cooker at a temperature between 40 and 85°C, such as between 50 and 85°C, such as between 65 and 85°C, such as between 60 and 85°C, or such as between 70 and 80°C. In currently preferred embodiments the palm fruitlets or MPD are retained in a pre-cooker together with enzyme at a temperature between 65 and 75°C.

The palm fruitlets or MPD may be retained in said pre-cooker for a period of 15 – 120 minutes, such as 15-60 minutes, such as 30-120 minutes, or such as for a period of 30-60 minutes.

In some embodiments according to the invention the retention time in the pre-cooker may be even shorter. Hence, the palm fruitlets or MPD may be retained in said pre-cooker for a period of 2-20 minutes, such as 3-20 minutes, such as 5-20 minutes, such as 2-15 minutes, such as 3-15 minutes, such as 3-10 minutes, such as 2-100 minutes, or such as for a period of 5-10 minutes.

The palm fruitlets or MPD may be retained in said pre-cooker for an amount of time which is sufficient to provide a total retention time of 15 minutes or more, such as 20 minutes or more, such as a retention time of 15 minutes – 1 hour, such as a retention time of 20 minutes – 1 hour, such as a retention time of 20 – 45 minutes or such as a retention time of 20 -30 minutes; the retention time being calculated as the time from application or dosing of enzyme to the time point at which the fruit mash is subject to pressing.

The enzyme or enzyme composition, such as the one or more cellulases, one or more hemicellulases, one or more pectinases, and/or one or more amylases, may have a temperature optimum in the range of 60-85°C, such as in the range of 65-85°C, 66-85°C, 67-85°C, 68-85°C, 69-85°C, 70-85°C, 65-79°C, 65-80°C, 66-80°C, 67-80°C, 66-79°C, 66-78°C, 66-77°C, 66-76°C, 66-75°C, 66-74°C, 66-73°C, 66-72°C, 66-71°C, 67-80°C, 67-79°C, 67-78°C, 67-77°C, 67-76°C,

67-75°C, 67-74°C, 67-73°C, 67-72°C, 67-71°C, 68-79°C, 68-78°C, 68-77°C, 68-76°C, 68-75°C, 68-74°C, 68-73°C, 68-72°C, 68-71°C, 69-79°C, 69-78°C, 69-77°C, 69-76°C, 69-75°C, 69-74°C, 69-73°C, 69-72°C, 69-71°C, and 70-85°C.

In the process according to the invention the one or more cellulases, one or more hemicellulases, one or more pectinases and/or one or more amylases, may be thermostable to such an extent that at least 15% of the enzyme activity (i.e. the cellulase, hemicellulase, amylase and/or pectinase activity) is retained after incubation at 70°C for 20 minutes, to such an extent that at least 20% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 25% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 30% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 35% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 40% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 50% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 60% of the enzyme activity is retained after incubation at 70°C for 20 minutes, or to such an extent that at least 70% of the enzyme activity is retained after incubation at 70°C for 20 minutes.

The thermostability may in particular be determined by incubation at 70°C for 20 minutes in a 0.1 M Na-OAc buffer pH 5.0, followed by transfer to ice and determination of residual enzyme activity (i.e. residual cellulase, amylase and/or pectinase activity) on Konelab by a method comprising: hydrolyzing substrate (e.g. carboxymethyl cellulose (CMC) form reducing carbohydrate; stopping the hydrolyzation by an alkaline reagent containing PAHBAH and Bismuth, which that forms complexes with reducing sugar; and measuring color production by complex formation at 405 nm in a spectrophotometer

A further aspect of the invention provides palm oil obtainable by the method according to the invention.

A still further aspect of the invention provides a palm oil milling line comprising a continuous sterilizer, e.g. a continuous sterilizer as defined in any of the above embodiments, and means for dosing an enzyme composition.

In the palm oil milling line according to the invention, the means for dosing an enzyme composition are for dosing the enzyme composition to MPD being conveyed to a digester, for dosing the enzyme composition into a digester and/or for dosing the enzyme composition to a crude oil, such as an undiluted crude oil (UDCO) or a diluted crude oil (DCO).

According to specific embodiments, the palm oil milling line according to the invention is configured as shown in Figure 4, said milling line comprising

a continuous sterilizer, which comprises:

- i) A crusher or bunch splitter (A), such as a double roll crusher,
- ii) Optionally a conveyor (B), such as a chain-type conveyor,
- iii) A first steam lock (C),
- iv) One or more sterilizing chamber(s) (D) with conveying means and steam injection
- 5 nozzles (E) and (F),
- v) A second steam lock (G), and
- vi) optionally a discharge conveyor (H);

linked to:

- vii) A thresher or stripper (I),
- 10 viii) A digester (M) with steam supply (P), such as a vertical digester, and
- ix) A screw press (N);

which are serially connected; e.g. by means of conveyors (J), (K), and/or (L).

In further embodiments, the palm oil milling line is configured as shown in Figure 5, said milling line comprising

15 A continuous sterilizer, which comprises:

- i) A crusher or bunch splitter (A), such as a double roll crusher,
- ii) Optionally a conveyor (B), such as a chain-type conveyor,
- iii) A first steam lock (C),
- iv) One or more sterilizing chamber(s) (D) with conveying means and steam injection
- 20 nozzles (E) and (F),
- v) A second steam lock (G), and
- vi) optionally a discharge conveyor (H);

linked to:

- vii) A thresher or stripper (I),
- 25 viii) A pre-cooker (T), such as a vertical pre-cooker,
- ix) A digester (M) with steam supply (P), such as a vertical digester, and
- x) A screw press (N);

which are serially connected; e.g. by means of conveyors (J), (K), and/or (L).

The present invention is further described by the following examples that should not be

30 construed as limiting the scope of the invention.

Examples

Example 1: Effect of dilution on Oil recovery in downstream from UDCO.

Experimental procedure:

Approximately 2 Kg of UDCO was collected from Screw Press during peak processing hours and was mixed properly.

The UDCO (28 %moisture and 65%oil) was aliquoted in 50ml graduated Falcon tubes and diluted with hot water (HW) to make final volume to 40ml such that the final sample %Moisture ranged from 18% to 82% as shown in table 1.

Table 1:

UDCO (ml)	Inherent Moisture (ml)	Added HW (ml)	HW to make up enzyme vol. (ml)	Total Moisture (ml)	% Moisture
40	10.8	0	0.75	11.55	28%
35	9.45	5	0.75	15.2	37%
30	8.1	10	0.75	18.85	46%
25	6.75	15	0.75	22.5	55%
20	5.4	20	0.75	26.15	64%
15	4.05	25	0.75	29.8	73%
10	2.7	30	0.75	33.45	82%

Enzymes were added (Comprising Cellic® CTec 2 and Cellic®Htec, both commercially available from Novzymes A/S) at 0.25ml dose for each (corresponding to 45 mg active enzyme protein for Cellic® CTec 2; and to 28.75mg active enzyme protein for Cellic®Htec). The entire mass was mixed thoroughly and incubated at 65°C ($\pm 3^\circ$) with intermittent mixing for 2h. Clarification was done at 90°C for 1h in static condition and the clarified extract was centrifuged at 4000 rpm for 5 min to separate oil from the sludge. Oil, emulsion, water and sludge quantities were estimated.

Results:

The results are presented in Table 2 below.

The results show that Oil Extraction efficiency from UDCO is maintained with enzyme treatment even at higher %Moisture >55% compared to control. Emulsion% decreases with enzyme treatment at higher %Moisture indicating that enzyme is able to break down emulsion even at higher %Moisture, especially from 46-64%Moisture.

Table 2:

Sample	UDCO (ml)	Inherent Moisture (ml)	Added HW (ml)	HW to make up enzyme vol (ml)	Total Moisture (ml)	% Moisture	Control/Enzyme Treatment	Centrifuge at 4000 rpm/5min	Total volume (ml)
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1	40	10,8	0	0,75	11,55	28%			40,75
1	35	9,45	5	0,75	15,2	37%			40,75
3	30	8,1	10	0,75	18,85	46%			40,75
4	25	6,75	15	0,75	22,5	55%			40,75
5	20	5,4	20	0,75	26,15	64%			40,75
6	15	4,05	25	0,75	29,8	73%			40,75
7	10	2,7	30	0,75	33,45	82%			40,75

Table 2, cont'd:

Sample	Control				Enzyme			
	Free Oil (ml)	Emulsion (ml)	Free Water (ml)	Wet sludge (ml)	Free Oil (ml)	Emulsion (ml)	Free Water (ml)	Wet sludge (ml)
1	25	1,5	2	12	25	1,5	2	12
1	21,5	3	7,2	9	22	2,5	12	4,2
3	18	2,5	13	7	19,5	1,5	15	4,1
4	14,1	2,4	17	6,75	15,5	1,1	20	4
5	12	1,5	21	6	12,75	0,48	23	4
6	9	0,52	26	5	9,5	0,5	27	3,5
7	6,1	0,5	28,5	5	6,5	0,4	31	3

5

Table 2, cont'd:

Sample	Total Extractable oil (ml)	Control- % Efficiency based on Free Oil extracted	Enzyme- % Efficiency based on Free Oil extracted	Enzymatic Process Efficiency delta over control (%)
1	26	96%	96%	0%
1	22,75	95%	97%	2%

3	19,5	92%	100%	8%
4	16,25	87%	95%	9%
5	13	92%	98%	6%
6	9,75	92%	97%	5%
7	6,5	94%	100%	6%

Example 2: Use of cellulolytic enzymes at a continuous sterilizer palm oil mill.

Trial design:

- 5 Dosing of cellulolytic enzymes to increase oil yield was tested at a Malaysian palm oil mill operating at 30 metric tonnes FFB per hour with a continuous sterilizer configuration essentially as depicted in Figure 6.

The enzyme (Palmora®OER, commercially available from Novozymes A/S) was dosed in amounts corresponding to 37.95 mg Enzyme protein per metric ton of FFB by spraying onto the mass passing to digester (MPD) during conveyance from thresher to pre-cooker and digester.

The total retention time from dosing of enzyme to pressing was estimated to be 22 minutes. The temperature during enzyme incubation ranged from 60-90°C; the pre-cooker being operated with temperatures ranging from 65-80, the digester being operated with temperatures ranging from 60-90°C.

- 15 Water content in MPD, UDCO and DCO was determined by oven method.

Determination of water content in UDCO and DCO samples performed on samples having an initial weight of 20 g. The samples were dried at 105°C for 12h.

Determination of water content in MPD was performed on samples having an initial weight of 10 g. The samples were prepared by separating mesocarp from the good fruits of the MPD, blending it and mixing it with bad fruits (no nuts/perthenocarp) and trash from the rest of the MPD materials to arrive at the final sample. Samples were dried at 105°C for 8h;

Oil content in underflow and heavy phase was determined by Soxhlet analysis according to the following procedure:

Different samples (30g for liquid samples) and 10g for MPD were taken for soxhlet extraction for measuring oil content.

Samples were prepared by first drying them in hot air oven for 8-12 hrs at 105°C.

Soxhlet extraction was carried out by taking dried samples into cellulose thimbles, followed by extraction using 200 ml of hexane for 4 hrs until the thimble was colourless.

The extracted oil samples were dried in hot air oven at 105°C for 2 hrs for removal of residual hexane and kept in desiccator until constant weight.

- 5 After cooling, the samples were weighed and the weight of the extracted oil was noted.

The effect of the enzyme was tested in alternate control and enzyme test runs as indicated in table 3 below, each sample/set of data being the average of samples collected every two hours on the respective day during the trial period.

Results:

- 10 Results are shown below in table 3.

Table 3:

Sample		#1	#2	#3	#4	#5	#6	#7	#8
Mode	Control: "CON" Enzyme: "ENZ"	CON	CON	CON	CON	CON	CON	CON	CON
% water content (w/w)	MPD	43,93	48,04	45,82	46,09	45,79	46,76	45,92	44,55
	UDCO								
	DCO								
Oil extraction rate (OER)		17,14	17,28	17,03	16,65	16,9	16,91	17,08	17,04
Oil loss	Underflow	10,26	10,34	9,61	9,19	8,73	8,71	8,29	8,46
	Heavy Phase	1,67	1,85	1,86	1,75	1,61	1,56	1,82	1,61

Table 3, cont'd:

#9	#10	#11	#12	#13	#14	#15	#16	#17	#18	#19
CON	ENZ	ENZ	ENZ	ENZ	ENZ	ENZ	ENZ	ENZ	ENZ	ENZ
45,11	54,28								48,86	48
		49,46	54,99	54,68	54,51	48,93	48,27	51,42	50,71	51,64
		60,66	61,09	66,71	68,22	45,34	46,12	55,05	66,84	65,69
17,14	17,5	17,49	17,44	17,52	17,55	17,45	17,42	17,46	17,46	17,43
8,25	8,49	8,00	8,28	8,49	7,9	8,24	8,51	8,28	8,8	8,01
1,59	1,45	1,52	1,3	1,34	1,41	1,18	1,35	1,47	1,61	1,46

Table 3, cont'd:

#20	#21	#22	#23	#24	#25	#26	#27	#28	#29	#30
ENZ	ENZ	CON	CON	CON	CON	CON	ENZ	ENZ	ENZ	ENZ
50,62									44,1	
51,26	51,55	50,88	50,33	49,27	56,67	52,78	60,00	47,08	55,14	40,72
65,88	66,19	65,98	62,30	60,75	68,61	70,47	68,49	70,61	63,22	69,73
17,55	17,13	17,24	17,25	17,27	17,35	17,42	17,72	17,82	17,53	18,07
9,11	8,69	8,57	8,74	9,13	8,41	9,01	9,12	8,43	8,18	8,69
1,62	1,55	1,53	1,46	1,55	1,59	1,52	1,54	1,4	1,42	1,52

Table 3, cont'd:

#31	#32	#33	#34	#35	#36	#37	#38	#39	#40	#41	#42
ENZ	ENZ	ENZ	ENZ	ENZ	ENZ	ENZ	ENZ	CON	CON	CON	CON
46,38				46,99	47,02	51,44	48,65	50,69	40,81	47,67	43,97
57,97	56,51	53,94	55,30	58,04	47,15	50,64	57,72	40,61		55,97	
70,91	68,34	66,15	64,88	60,63	55,05	62,16	69,77	59,63		67,48	
18,05	18,09	17,53	18,5	18,16	18,33	18,31	18,24	17,1	17,52	17,12	17,25
8,34	8,98	8,11	9,24	8,83	9,21	8,41	8,21	8,72			
1,5	1,4	1,33	1,56	1,31	1,37	1,33	1,33	1,41			

5 Table 3, cont'd:

Mode	Control: "CON" Enzyme: "ENZ"	Average	
		ENZ	CON
% water content (w/w)	MPD	48,6	45,8
	UDCO	52,5	50,9
	DCO	63,4	65,0
Oil extraction rate (OER)		17,7	17,1
Oil loss	Underflow	8,5	9,0
	Heavy Phase	1,4	1,6

The data show that, at a continuous sterilizer mill, the average moisture content in all streams in the oil milling process, from mass passing to digester (MPD) to undilute and dilute crude oil (UDCO and DCO), was considerably higher than 40% (w/w).

Nevertheless, the data confirm that the dosing of cellulolytic enzymes during extraction of the crude resulted in increased oil extraction as determined a higher oil extraction rate (OER). Despite the high water content, dosing of enzymes also improved the separation of oil from water and solids at the clarifier tank as shown by a lower content of oil in the Clarifier Tank Underflow, as

5 well as reduced oil content in the Heavy Phase (or Sludge Phase).

CLAIMS

1. A process for extraction of crude palm oil CPO, comprising sterilizing fresh fruit bunches (FFB) in a continuous sterilizer and contacting mass passing to digester (MPD), fruit mash and/or crude oil with an enzyme composition.
2. The process according to claim 1, wherein the FFB are subject to conditioning prior to sterilization, so as to loosen the arrangement of spikelets in the FFB; e.g. by use of a crusher or bunch splitter, such as a double-roll crusher.
3. The process according to any of the preceding claims, wherein said continuous sterilizer comprises one or more non-pressurized chamber(s) fitted with steam injection nozzles and means for conveying the FFB through the chamber.
4. The process according to any of the preceding claims, wherein said means for conveying is a chain-type conveyor.
5. The process according to any of the preceding claims, wherein the continuous sterilizer has a configuration as shown in Figure 1, comprising a crusher/bunch splitter (A), such as a double roll crusher, one or more conveyor(s) (B), such as one or more chain-type conveyor(s), a first steam lock (C), one or more sterilizing chamber(s) (D) with conveying means and steam injection nozzles (E) and (F), a second steam lock (G), and optionally a discharge conveyor (H).
6. The process according to any of the preceding claims, wherein the crude oil is extracted from sterilized fruit using milling equipment configured as shown in Figure 2, said equipment comprising a thresher or stripper (I), a digester (M) with steam supply (P), such as a vertical digester, and a screw press (N), connected in a series.
7. The process according to any of the preceding claims, wherein the crude oil is extracted from sterilized fruit using milling equipment configured as shown in Figure 3, said equipment comprising a thresher or stripper (A), a pre-cooker (R), such as a vertical pre-cooker, a digester (M) with steam supply (P), such as a vertical digester, and a screw press (N), connected in a series.
8. The process according to any of the preceding claims, wherein the crude oil is extracted using milling equipment configured as shown in Figure 4, said equipment comprising a continuous sterilizer, which comprises a crusher (A), such as a double roll crusher, a conveyor (B), such as a chain-type conveyor, a first steam lock (C), a sterilizing chamber (D) with conveying means and steam injection nozzles (E) and (F), a second steam lock (G), and optionally a discharge conveyor (H), linked to a thresher or stripper (I), a digester

(M) with steam supply (P), such as a vertical digester, and a screw press (N), which are serially connected; e.g. by means of conveyors (J), (K), and/or (L).

9. The process according to any of the preceding claims, wherein the crude oil is extracted using milling equipment configured as shown in Figure 5, said equipment comprising a continuous sterilizer, which comprises a crusher (A), such as a double roll crusher, a conveyor (B), such as a chain-type conveyor, a first steam lock (C), a sterilizing chamber (D) with conveying means and steam injection nozzles (E) and (F), a second steam lock (G), and optionally a discharge conveyor (H), linked to a thresher or stripper (I), a pre-cooker (T), such as a vertical pre-cooker, a digester (M) with steam supply (P), such as a vertical digester, and a screw press (N), which are serially connected; e.g. by means of conveyors (J), (K), and/or (L).
10. The process according to any of the preceding claims, wherein sterilization in said continuous sterilizer proceeds from 60 to 120 minutes, such as from 80 to 120 minutes, from 80-110 minutes, from between 80 minutes to 100 minutes, or such as from 80-90 minutes.
11. The process according to any of the preceding claims, comprising the use of an enzyme composition comprising one or more hydrolases, such as one or more cellulases.
12. The process according to any of the preceding claims, wherein said enzyme composition comprises one or more cellulases and/or one or more amylases and/or one or more pectinases.
13. The process according to any of the preceding steps, comprising
- i. Sterilizing the FFB to produce sterilized FFB,
 - ii. Subjecting the sterilized FFB to stripping or threshing to provide stripped fruitlets or MPD,
 - iii. Conveying the stripped fruitlets or MPD to a digester or to a pre-cooker and then to a digester,
 - iv. Subjecting the stripped fruitlets or MPD to a digestion procedure to produce fruit mash,
 - v. Subjecting the fruit mash to pressing to produce a crude oil comprising oil and water, together with cell debris, and/or fibrous material, and
 - vi. Separating the oil in said crude oil from the water and from said cell debris, and/or fibrous material.
14. The process according to claim 13, wherein one or more enzymes; e.g. one or more enzymes as defined in any of claims 11 and 12, are applied in step iii), such as by application onto the stripped fruitlets or MPD, while the stripped fruitlets or MPD is/are

conveyed to a digester (I) on one or more conveyors; e.g. conveyors (J), (K) and/or (L) in figure 2.

15. The process according to claim 13, wherein one or more enzymes; e.g. one or more enzymes as defined in any of claims 11 and 12, are applied in step iv).

5 16. The process according to claim 13, wherein one or more enzymes; e.g. one or more enzymes as defined in any of claims 11 and 12, are applied after step v) and before the oil in said crude oil is separated from the water and from the cell debris, and/or the fibrous material.

10 17. The process according to any of claims 13-16, wherein step iv) comprises retaining the stripped fruitlets or MPD in the digester at temperatures between 55 and 90°C, such as between 60 and 90°C, between 60 and 85°C, between 60 and 80°C, between 65 and 85°C, or such as between 65 and 80°C from 7-30 minutes, from 10-30 minutes, such as from 7-28 minutes, from 10-28 minutes, 15-28 minutes, 12-30 minutes, 12-28 minutes, from 7-25 minutes, from 10-25 minutes, from 7-20 minutes, from 10-20 minutes or 12-25
15 minutes; e.g. by controlling the speed of the screw press (N) and/or by controlling steam supply (P).

18. The process according to any of the preceding claims, comprising the steps of:

- a. Contacting the palm fruitlets, said MPD, said fruit mash and/or said crude oil with said enzyme composition at a temperature of above 65°C;
- 20 b. Extracting the CPO.

19. The process according to any of the preceding claims, wherein the palm fruitlets, the MPD, the fruit mash and/or the crude oil is contacted with said enzyme composition at a temperature within the range of 55-90°C, such as a temperature within the range of 55-85°C, 55-80°C, 60-90°C, 60-85°C, 60-80°C, 66-90°C, 67-90°C, 68-90°C, 69-90°C, 70-90°C, 66-85°C, 66-80°C, 67-80°C, 66-79°C, 66-78°C, 66-77°C, 66-76°C, 66-75°C, 66-74°C, 66-73°C, 66-72°C, 66-71°C, 67-80°C, 67-79°C, 67-78°C, 67-77°C, 67-76°C, 67-75°C, 67-74°C, 67-73°C, 67-72°C, 67-71°C, 68-79°C, 68-78°C, 68-77°C, 68-76°C, 68-75°C, 68-74°C, 68-73°C, 68-72°C, 68-71°C, 69-79°C, 69-78°C, 69-77°C, 69-76°C, 69-75°C, 69-74°C, 69-73°C, 69-72°C, 69-71°C, 70-90°C, 70-89°C, 70-88°C, 70-87°C, 70-86°C, or 70-85°C.

20. The process according to any of the preceding claims, wherein the enzyme composition is dosed in amounts corresponding to 5-200 mg enzyme protein per metric ton of fresh fruit bunches (FFBs) entering the oil extraction process, such as 10-200 mg enzyme protein, such as 20-200 mg enzyme protein, such as 5-150 mg enzyme protein, such as
35 5-100 mg enzyme protein, such as 5-50 mg enzyme protein, such as 10-200 mg enzyme

protein, such as 10-100 mg enzyme protein, such as 10-75 mg enzyme protein, or such as 10-50 mg enzyme protein per metric ton of fresh fruit bunches (FFBs) entering the oil extraction process.

- 5 21. The process according to any of the preceding claims, wherein the enzyme composition is dosed such that the amount of enzyme protein corresponds to 100-1000 ppm, such as 200-1000 ppm, 100-500 ppm, such as 200-500 ppm, 250-400 ppm or 350-1000 ppm relative to the amount of palm fruitlet, MPD, fruit mash or crude oil.
- 10 22. The process according to any of the preceding claims, wherein the palm fruitlet, MPD, fruit mash or crude oil is contacted with the enzyme composition for a period of 5-60 minutes, such as for a period of 20-60 minutes, 25-60 minutes, 30-60 minutes, 15-50 minutes, 20-50 minutes, 25-50 minutes, 30-50 minutes, 15-40 minutes, 20-40 minutes, 25-40 minutes, 30-40 minutes, 15-30 minutes, 20-30 minutes, 25-28 minutes, 25-30 minutes, 25-35 minutes, 15-25 minutes, 20-25 minutes, 20-28 minutes, 15-20 minutes, 10-15 minutes or 5-10 minutes.
- 15 23. The process according to any of the preceding claims, the process comprising retaining the palm fruitlets or MPD, fruit mash or crude oil at temperatures above 65°C and up to 85°C for 10-30 minutes, such as for 10-28 minutes, 15-28 minutes, 12-30 minutes, 12-28 minutes or 12-25 minutes.
- 20 24. The process according to any of the preceding claims, comprising retaining the palm fruitlets or MPD in a pre-cooker at a temperature between 40 and 85°C, such as between 50 and 85°C, such as between 65 and 85°C, such as between 60 and 85°C, or such as between 70 and 80°C, or such as between 65 and 75°C.
- 25 25. The process according to claim 24, wherein the palm fruitlets or MPD is retained in said pre-cooker for a period of 2-20 minutes, such as 3-20 minutes, such as 5-20 minutes, such as 2-15 minutes, such as 3-15 minutes, such as 3-10 minutes, such as 2-100 minutes, or such as for a period of 5-10 minutes.
- 30 26. The process according to any of the preceding claims, wherein the enzyme, such as the one or more cellulases, one or more hemicellulases, one or more pectinases, and/or one or more amylases, has a temperature optimum in the range of 60-85°C, such as in the range of 65-85°C, 67-85°C, 68-85°C, 69-85°C, 70-85°C, 65-79°C, 65-80°C, 66-80°C, 67-80°C, 66-79°C, 66-78°C, 66-77°C, 66-76°C, 66-75°C, 66-74°C, 66-73°C, 66-72°C, 66-71°C, 67-80°C, 67-79°C, 67-78°C, 67-77°C, 67-76°C, 67-75°C, 67-74°C, 67-73°C, 67-72°C, 67-71°C, 68-79°C, 68-78°C, 68-77°C, 68-76°C, 68-75°C, 68-74°C, 68-73°C, 68-72°C, 68-71°C, 69-79°C, 69-78°C, 69-77°C, 69-76°C, 69-75°C, 69-74°C, 69-73°C, 69-72°C, 69-71°C, and 70-85°C.
- 35

27. The process according to any of preceding claims, wherein the one or more cellulases, one or more hemicellulases, one or more pectinases and/or one or more amylases, are thermostable to such an extent that at least 15% of the enzyme activity (i.e. the cellulase, hemicellulose, amylase and/or pectinase activity) is retained after incubation at 70°C for 20 minutes, to such an extent that at least 20% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 25% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 30% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 35% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 40% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 50% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 60% of the enzyme activity is retained after incubation at 70°C for 20 minutes, or to such an extent that at least 70% of the enzyme activity is retained after incubation at 70°C for 20 minutes.

28. A palm oil obtainable by the process defined in any of the preceding claims.

29. A palm oil milling line comprising a continuous sterilizer, e.g. a continuous sterilizer as defined in any of claims 2-5, and means for dosing an enzyme composition.

30. The palm oil milling line according to claim 29, wherein the means for dosing an enzyme composition are for dosing the enzyme composition to MPD being conveyed to a digester, for dosing the enzyme composition into a digester and/or for dosing the enzyme composition to a crude oil, such as an undiluted crude oil (UDCO) or a diluted crude oil (DCO).

31. The oil milling line according to claim 29 or 30, wherein said means for dosing an enzyme composition comprises an air propellant assisted liquid delivery system, such as one or more spray nozzles.

32. The palm oil milling line according to any of claims 29-31, wherein the milling line is configured as shown in Figure 4, said milling line comprising

A continuous sterilizer, which comprises:

- i) A crusher or bunch splitter (A), such as a double roll crusher,
- ii) Optionally a conveyor (B), such as a chain-type conveyor,
- iii) A first steam lock (C),
- iv) One or more sterilizing chamber(s) (D) with conveying means and steam injection nozzles (E) and (F),
- v) A second steam lock (G), and

vi) optionally a discharge conveyor (H);

linked to:

vii) A thresher or stripper (I),

viii) A digester (M) with steam supply (P), such as a vertical digester, and

5 ix) A screw press (N);

which are serially connected; e.g. by means of conveyors (J), (K), and/or (L).

33. The palm oil milling line according to any of claims 29-31, wherein the milling line is configured as shown in Figure 5, said milling line comprising

A continuous sterilizer, which comprises:

10 i) A crusher or bunch splitter (A), such as a double roll crusher,

ii) Optionally a conveyor (B), such as a chain-type conveyor,

iii) A first steam lock (C),

iv) One or more sterilizing chamber(s) (D) with conveying means and steam injection nozzles (E) and (F),

15 v) A second steam lock (G), and

vi) optionally a discharge conveyor (H);

linked to:

vii) A thresher or stripper (I),

viii) A pre-cooker (T), such as a vertical pre-cooker,

20 ix) A digester (M) with steam supply (P), such as a vertical digester, and

x) A screw press (N);

which are serially connected; e.g. by means of conveyors (J), (K), and/or (L).

AMENDED CLAIMS

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CLAIMS

1. A process for extraction of crude palm oil CPO, comprising sterilizing fresh fruit bunches (FFB) in a continuous sterilizer and contacting mass passing to digester (MPD), fruit mash and/or crude oil with an enzyme composition.
- 5 2. The process according to claim 1, wherein the FFB are subject to conditioning prior to sterilization, so as to loosen the arrangement of spikelets in the FFB; e.g. by use of a crusher or bunch splitter, such as a double-roll crusher.
3. The process according to any of the preceding claims, wherein said continuous sterilizer comprises one or more non-pressurized chamber(s) fitted with steam injection nozzles and means for
10 conveying the FFB through the chamber.
4. The process according to any of the preceding claims, wherein said means for conveying is a chain-type conveyor.
5. The process according to any of the preceding claims, wherein the continuous sterilizer has a configuration as shown in Figure 1, comprising a crusher/bunch splitter (A), such as a double roll
15 crusher, one or more conveyor(s) (B), such as one or more chain-type conveyor(s), a first steam lock (C), one or more sterilizing chamber(s) (D) with conveying means and steam injection nozzles (E) and (F), a second steam lock (G), and optionally a discharge conveyor (H).
6. The process according to any of the preceding claims, wherein the crude oil is extracted from sterilized fruit using milling equipment configured as shown in Figure 2, said equipment comprising a
20 thresher or stripper (I), a digester (M) with steam supply (P), such as a vertical digester, and a screw press (N), connected in a series.
7. The process according to any of the preceding claims, wherein the crude oil is extracted from sterilized fruit using milling equipment configured as shown in Figure 3, said equipment comprising a
25 thresher or stripper (A), a pre-cooker (R), such as a vertical pre-cooker, a digester (M) with steam supply (P), such as a vertical digester, and a screw press (N), connected in a series.
8. The process according to any of the preceding claims, wherein the crude oil is extracted using milling equipment configured as shown in Figure 4, said equipment comprising a continuous
30 sterilizer, which comprises a crusher (A), such as a double roll crusher, a conveyor (B), such as a chain-type conveyor, a first steam lock (C), a sterilizing chamber (D) with conveying means and steam injection nozzles (E) and (F), a second steam lock (G), and optionally a discharge conveyor (H), linked to a thresher or stripper (I), a digester (M) with steam supply (P), such as a vertical digester, and a screw press (N), which are serially connected; e.g. by means of conveyors (J), (K), and/or (L).
9. The process according to any of the preceding claims, wherein the crude oil is extracted using
35 milling equipment configured as shown in Figure 5, said equipment comprising a continuous

sterilizer, which comprises a crusher (A), such as a double roll crusher, a conveyor (B), such as a chain-type conveyor, a first steam lock (C), a sterilizing chamber (D) with conveying means and steam injection nozzles (E) and (F), a second steam lock (G), and optionally a discharge conveyor (H), linked to a thresher or stripper (I), a pre-cooker (T), such as a vertical pre-cooker, a digester (M) with steam supply (P), such as a vertical digester, and a screw press (N), which are serially connected; e.g. by means of conveyors (J), (K), and/or (L).

10. The process according to any of the preceding claims, wherein sterilization in said continuous sterilizer proceeds from 60 to 120 minutes, such as from 80 to 120 minutes, from 80-110 minutes, from between 80 minutes to 100 minutes, or such as from 80-90 minutes.

11. The process according to any of the preceding claims, comprising the use of an enzyme composition comprising one or more hydrolases, such as one or more cellulases.

12. The process according to any of the preceding claims, wherein said enzyme composition comprises one or more cellulases and/or one or more amylases and/or one or more pectinases.

13. The process according to any of the preceding steps, comprising

- i. Sterilizing the FFB to produce sterilized FFB,
- ii. Subjecting the sterilized FFB to stripping or threshing to provide stripped fruitlets or MPD,
- iii. Conveying the stripped fruitlets or MPD to a digester or to a pre-cooker and then to a digester,
- iv. Subjecting the stripped fruitlets or MPD to a digestion procedure to produce fruit mash,
- v. Subjecting the fruit mash to pressing to produce a crude oil comprising oil and water, together with cell debris, and/or fibrous material, and
- vi. Separating the oil in said crude oil from the water and from said cell debris, and/or fibrous material.

14. The process according to claim 13, wherein one or more enzymes; e.g. one or more enzymes as defined in any of claims 11 and 12, are applied in step iii), such as by application onto the stripped fruitlets or MPD, while the stripped fruitlets or MPD is/are conveyed to a digester (I) on one or more conveyors; e.g. conveyors (J), (K) and/or (L) in figure 2.

15. The process according to claim 13, wherein one or more enzymes; e.g. one or more enzymes as defined in any of claims 11 and 12, are applied in step iv).

16. The process according to claim 13, wherein one or more enzymes; e.g. one or more enzymes as defined in any of claims 11 and 12, are applied after step v) and before the oil in said crude oil is separated from the water and from the cell debris, and/or the fibrous material.

17. The process according to any of claims 13-16, wherein step iv) comprises retaining the stripped fruitlets or MPD in the digester at temperatures between 55 and 90°C, such as between 60 and 90°C, between 60 and 85°C, between 60 and 80°C, between 65 and 85°C, or such as between 65 and 80°C from 7-30 minutes, from 10-30 minutes, such as from 7-28 minutes, from 10-28 minutes,

15-28 minutes, 12-30 minutes, 12-28 minutes, from 7-25 minutes, from 10-25 minutes, from 7-20 minutes, from 10-20 minutes or 12-25 minutes; e.g. by controlling the speed of the screw press (N) and/or by controlling steam supply (P).

18. The process according to any of the preceding claims, comprising the steps of:

- 5 a. Contacting the palm fruitlets, said MPD, said fruit mash and/or said crude oil with said enzyme composition at a temperature of above 65°C;
- b. Extracting the CPO.

19. The process according to any of the preceding claims, wherein the palm fruitlets, the MPD, the fruit mash and/or the crude oil is contacted with said enzyme composition at a temperature within the
 10 range of 55-90°C, such as a temperature within the range of 55-85°C, 55-80°C, 60-90°C, 60-85°C, 60-80°C, 66-90°C, 67-90°C, 68-90°C, 69-90°C, 70-90°C, 66-85°C, 66-80°C, 67-80°C, 66-79°C, 66-78°C, 66-77°C, 66-76°C, 66-75°C, 66-74°C, 66-73°C, 66-72°C, 66-71°C, 67-80°C, 67-79°C, 67-78°C, 67-77°C, 67-76°C, 67-75°C, 67-74°C, 67-73°C, 67-72°C, 67-71°C, 68-79°C, 68-78°C, 68-77°C, 68-76°C, 68-75°C, 68-74°C, 68-73°C, 68-72°C, 68-71°C, 69-79°C, 69-78°C, 69-77°C,
 15 69-76°C, 69-75°C, 69-74°C, 69-73°C, 69-72°C, 69-71°C, 70-90°C, 70-89°C, 70-88°C, 70-87°C, 70-86°C, or 70-85°C.

20. The process according to any of the preceding claims, wherein the enzyme composition is dosed in amounts corresponding to 5-200 mg enzyme protein per metric ton of fresh fruit bunches (FFBs) entering the oil extraction process, such as 10-200 mg enzyme protein, such as 20-200 mg enzyme
 20 protein, such as 5-150 mg enzyme protein, such as 5-100 mg enzyme protein, such as 5-50 mg enzyme protein, such as 10-200 mg enzyme protein, such as 10-100 mg enzyme protein, such as 10-75 mg enzyme protein, or such as 10-50 mg enzyme protein per metric ton of fresh fruit bunches (FFBs) entering the oil extraction process.

21. The process according to any of the preceding claims, wherein the enzyme composition is dosed
 25 such that the amount of enzyme protein corresponds to 100-1000 ppm, such as 200-1000 ppm, 100-500 ppm, such as 200-500 ppm, 250-400 ppm or 350-1000 ppm relative to the amount of palm fruitlet, MPD, fruit mash or crude oil.

22. The process according to any of the preceding claims, wherein the palm fruitlet, MPD, fruit mash or crude oil is contacted with the enzyme composition for a period of 5-60 minutes, such as for a
 30 period of 20-60 minutes, 25-60 minutes, 30-60 minutes, 15-50 minutes, 20-50 minutes, 25-50 minutes, 30-50 minutes, 15-40 minutes, 20-40 minutes, 25-40 minutes, 30-40 minutes, 15-30 minutes, 20-30 minutes, 25-28 minutes, 25-30 minutes, 25-35 minutes, 15-25 minutes, 20-25 minutes, 20-28 minutes, 15-20 minutes, 10-15 minutes or 5-10 minutes.

23. The process according to any of the preceding claims, the process comprising retaining the palm
 35 fruitlets or MPD, fruit mash or crude oil at temperatures above 65°C and up to 85°C for 10-30

minutes, such as for 10-28 minutes, 15-28 minutes, 12-30 minutes, 12-28 minutes or 12-25 minutes.

24. The process according to any of the preceding claims, comprising retaining the palm fruitlets or MPD in a pre-cooker at a temperature between 40 and 85°C, such as between 50 and 85°C, such as between 65 and 85°C, such as between 60 and 85°C, or such as between 70 and 80°C, or such as between 65 and 75°C.

25. The process according to claim 24, wherein the palm fruitlets or MPD is retained in said pre-cooker for a period of 2-20 minutes, such as 3-20 minutes, such as 5-20 minutes, such as 2-15 minutes, such as 3-15 minutes, such as 3-10 minutes, such as 2-100 minutes, or such as for a period of 5-10 minutes.

26. The process according to any of the preceding claims, wherein the enzyme, such as the one or more cellulases, one or more hemicellulases, one or more pectinases, and/or one or more amylases, has a temperature optimum in the range of 60-85°C, such as in the range of 65-85°C, 67-85°C, 68-85°C, 69-85°C, 70-85°C, 65-79°C, 65-80°C, 66-80°C, 67-80°C, 66-79°C, 66-78°C, 66-77°C, 66-76°C, 66-75°C, 66-74°C, 66-73°C, 66-72°C, 66-71°C, 67-80°C, 67-79°C, 67-78°C, 67-77°C, 67-76°C, 67-75°C, 67-74°C, 67-73°C, 67-72°C, 67-71°C, 68-79°C, 68-78°C, 68-77°C, 68-76°C, 68-75°C, 68-74°C, 68-73°C, 68-72°C, 68-71°C, 69-79°C, 69-78°C, 69-77°C, 69-76°C, 69-75°C, 69-74°C, 69-73°C, 69-72°C, 69-71°C, and 70-85°C.

27. The process according to any of preceding claims, wherein the one or more cellulases, one or more hemicellulases, one or more pectinases and/or one or more amylases, are thermostable to such an extent that at least 15% of the enzyme activity (i.e. the cellulase, hemicellulose, amylase and/or pectinase activity) is retained after incubation at 70°C for 20 minutes, to such an extent that at least 20% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 25% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 30% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 35% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 40% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 50% of the enzyme activity is retained after incubation at 70°C for 20 minutes, to such an extent that at least 60% of the enzyme activity is retained after incubation at 70°C for 20 minutes, or to such an extent that at least 70% of the enzyme activity is retained after incubation at 70°C for 20 minutes.

28. A palm oil milling line comprising a continuous sterilizer, e.g. a continuous sterilizer as defined in any of claims 2-5, and means for dosing an enzyme composition.

29. The palm oil milling line according to claim 28, wherein the means for dosing an enzyme composition are for dosing the enzyme composition to MPD being conveyed to a digester, for dosing

the enzyme composition into a digester and/or for dosing the enzyme composition to a crude oil, such as an undiluted crude oil (UDCO) or a diluted crude oil (DCO).

30. The oil milling line according to claim 28 or 29, wherein said means for dosing an enzyme composition comprises and air propellant assisted liquid delivery system, such as one or more spray nozzles.

31. The palm oil milling line according to any of claims 28-30, wherein the milling line is configured as shown in Figure 4, said milling line comprising

A continuous sterilizer, which comprises:

- i) A crusher or bunch splitter (A), such as a double roll crusher,
- ii) Optionally a conveyor (B), such as a chain-type conveyor,
- iii) A first steam lock (C),
- iv) One or more sterilizing chamber(s) (D) with conveying means and steam injection nozzles (E) and (F),
- v) A second steam lock (G), and
- vi) optionally a discharge conveyor (H);

linked to:

- vii) A thresher or stripper (I),
- viii) A digester (M) with steam supply (P), such as a vertical digester, and
- ix) A screw press (N);

which are serially connected; e.g. by means of conveyors (J), (K), and/or (L).

32. The palm oil milling line according to any of claims 28-30, wherein the milling line is configured as shown in Figure 5, said milling line comprising

A continuous sterilizer, which comprises:

- i) A crusher or bunch splitter (A), such as a double roll crusher,
- ii) Optionally a conveyor (B), such as a chain-type conveyor,
- iii) A first steam lock (C),
- iv) One or more sterilizing chamber(s) (D) with conveying means and steam injection nozzles (E) and (F),
- v) A second steam lock (G), and
- vi) optionally a discharge conveyor (H);

linked to:

- vii) A thresher or stripper (I),
- viii) A pre-cooker (T), such as a vertical pre-cooker,
- ix) A digester (M) with steam supply (P), such as a vertical digester, and

x) A screw press (N);

which are serially connected; e.g. by means of conveyors (J), (K), and/or (L).

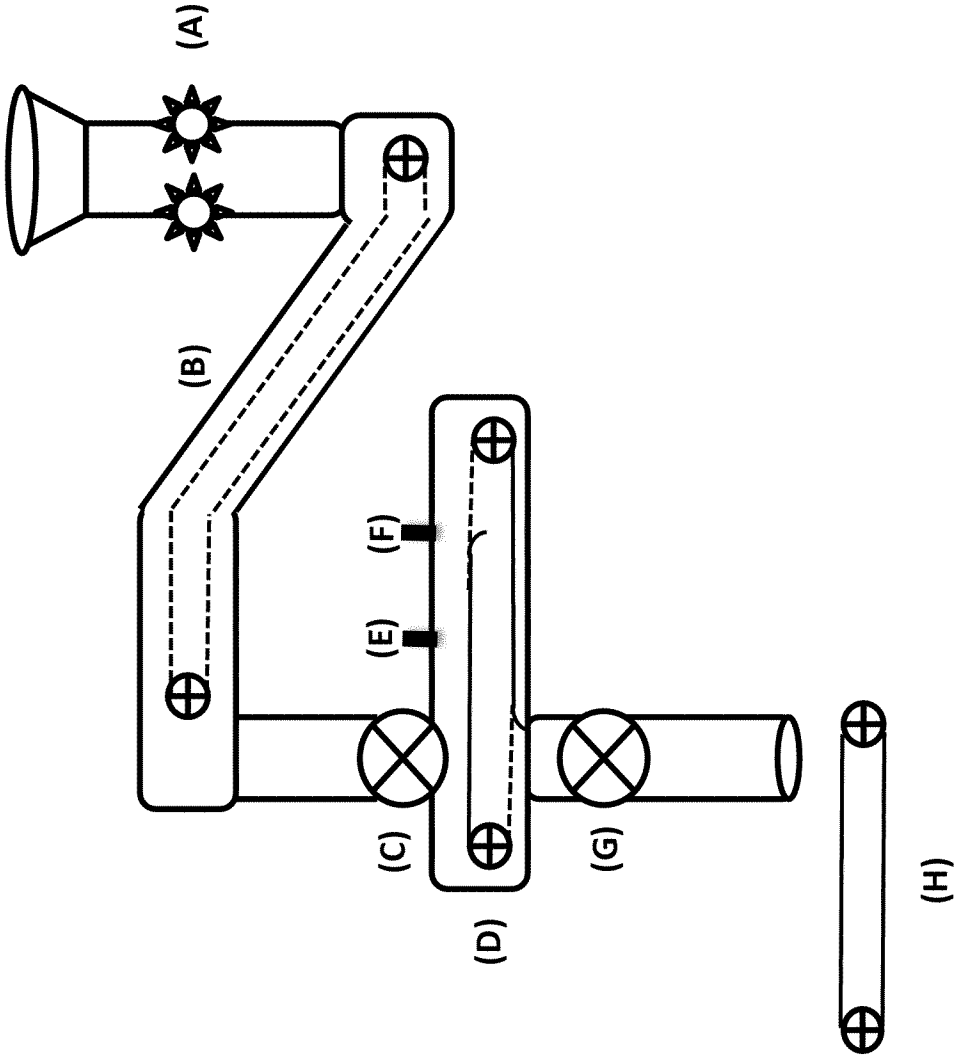
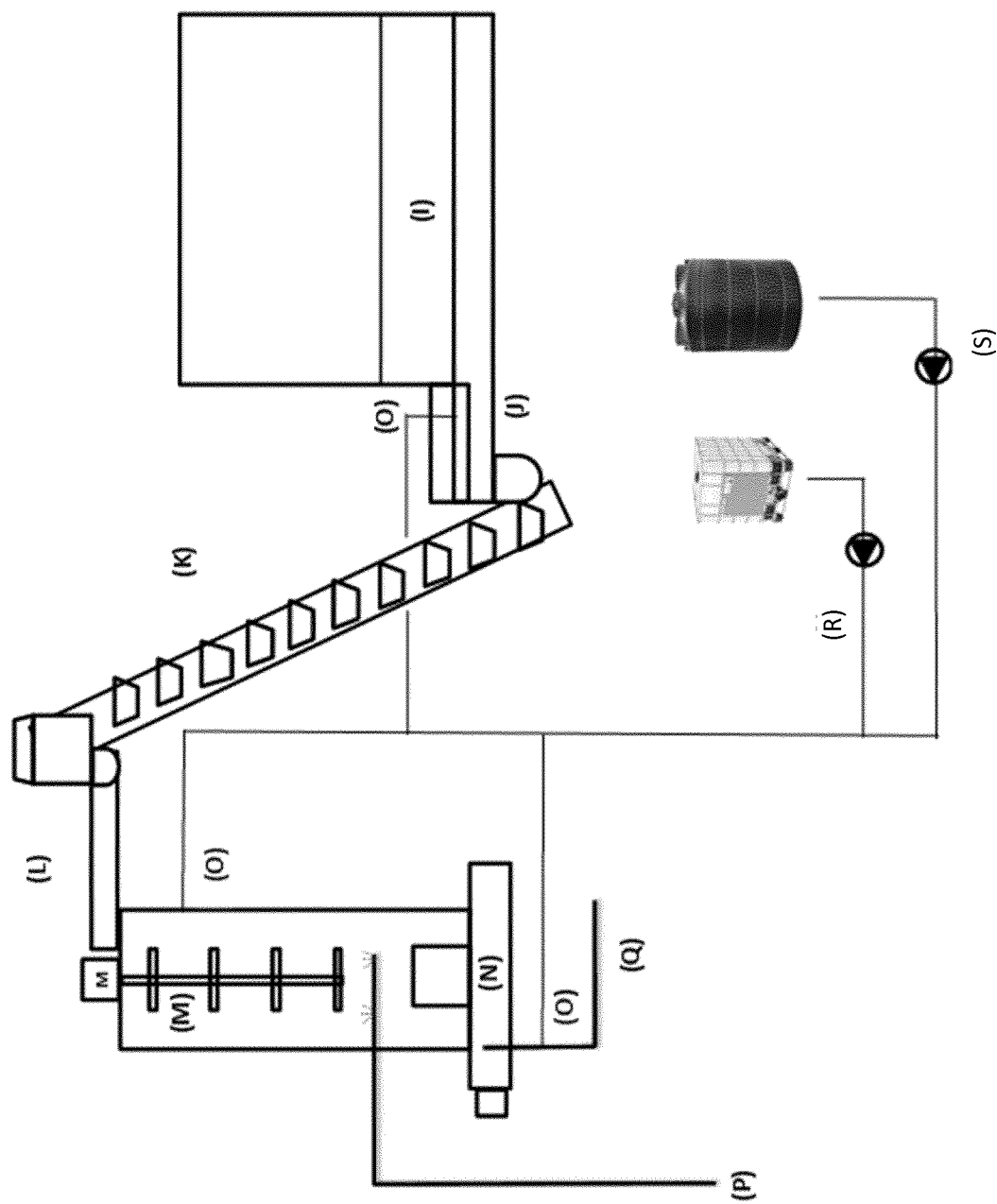


Figure 1

Figure 2



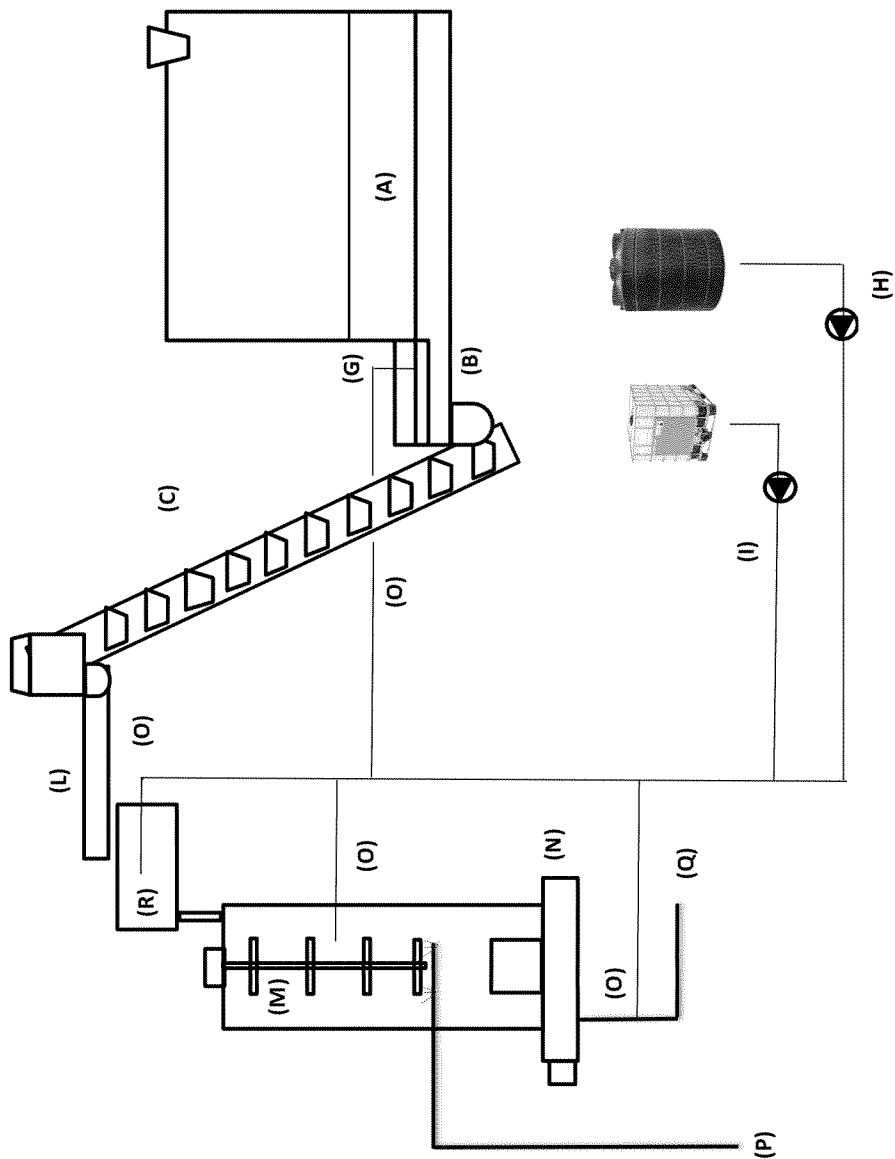


Figure 3

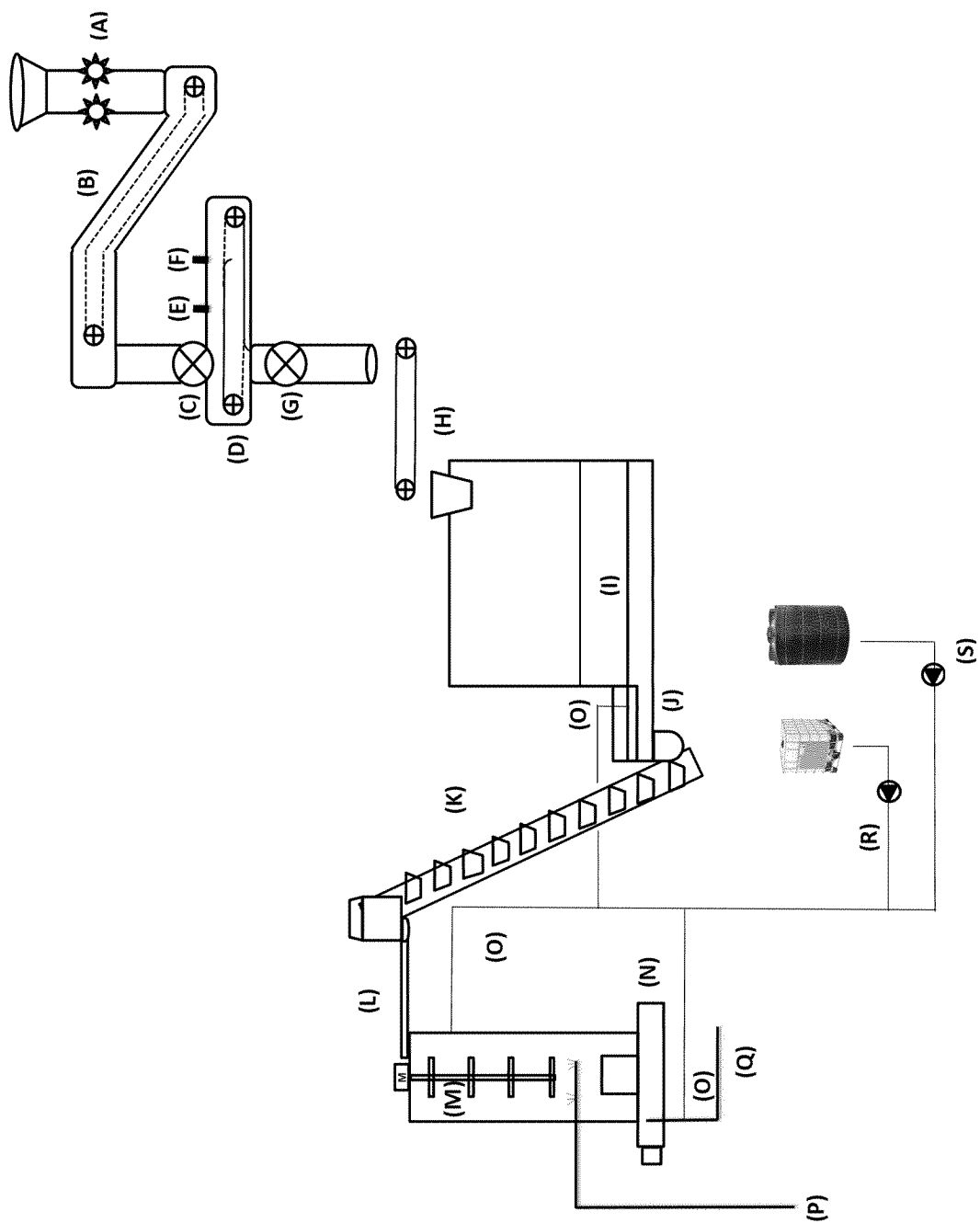


Figure 4

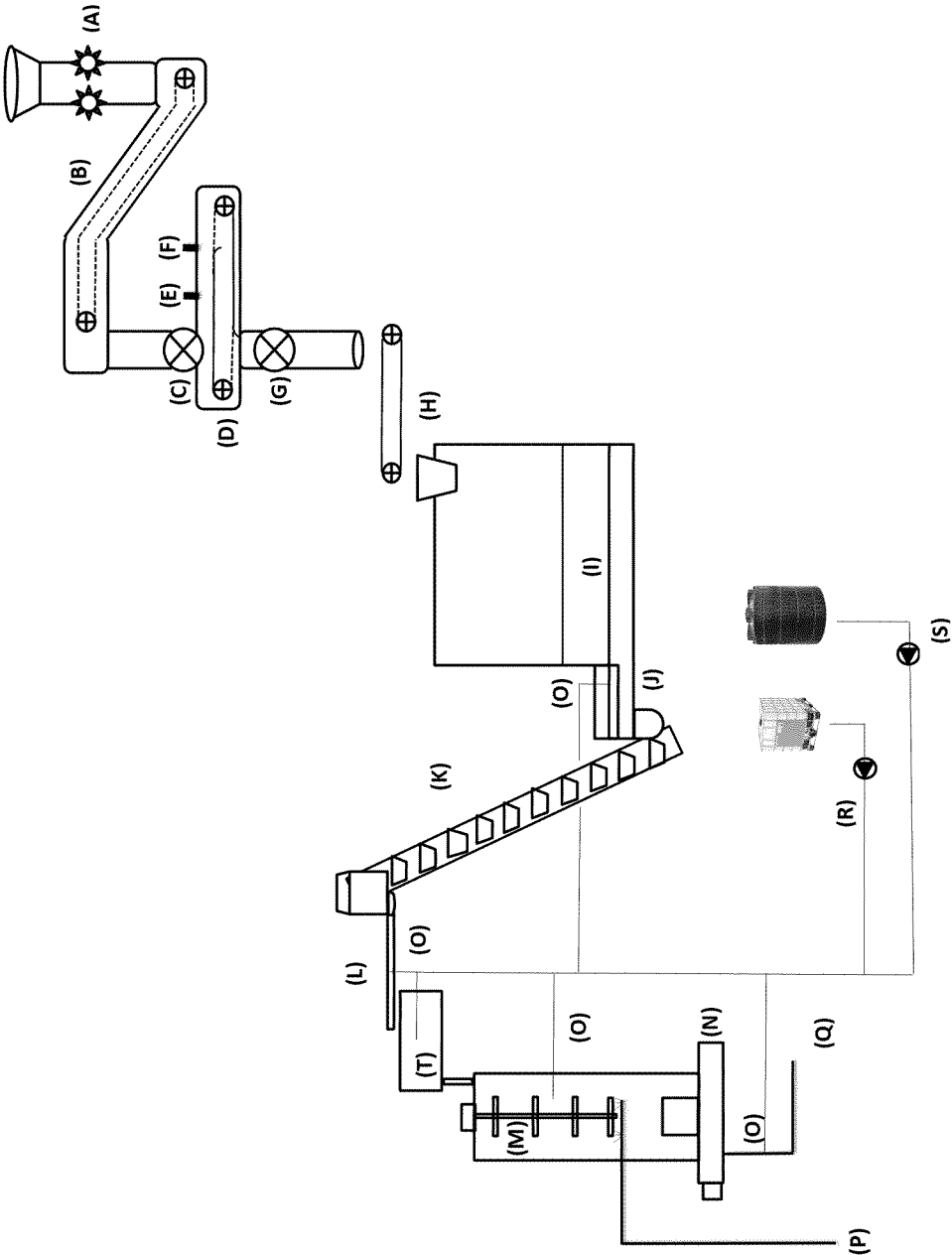


Figure 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/059576

A. CLASSIFICATION OF SUBJECT MATTER		
INV.	C11B1/02 C12N9/24	C11B1/04 C11B1/06 C11B1/10 C11B1/14
ADD.		
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) C11B C12N		
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) EPO-Internal, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2016/162507 A1 (NOVOZYMES AS [DK]) 13 October 2016 (2016-10-13) claims 1, 12, 29	1,28,29
X	----- WO 2012/011130 A2 (ADVANCED ENZYME TECHNOLOGIES LTD [IN]; RATHI CHANDRAKANT LAXMINARAYAN) 26 January 2012 (2012-01-26) cited in the application	28
A	claims 13, 15	1-27, 29-33
X	----- US 5 039 455 A (KOOI BOON-LAM [CA]) 13 August 1991 (1991-08-13)	28
A	column 12, line 1 - line 4; claim 1	1-27, 29-33
	----- -/-	
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 15 June 2017		Date of mailing of the international search report 26/06/2017
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Picout, David

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/059576

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2009/017389 A2 (MALAYSIAN PALM OIL BOARD [MY]; KANDIAH SIVASOTHY [MY]; BATUMALAI RAMAC) 5 February 2009 (2009-02-05) the whole document -----	1-33

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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WO 2016162507 A1	13-10-2016	AU 2016100384 A4 WO 2016162507 A1	19-05-2016 13-10-2016
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