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(54) **Title:** PROCESS FOR EXTRACTION OF PALM OIL USING ENZYMES

(57) **Abstract:** The present invention relates to a process for extraction of crude palm oil from palm fruitlets comprising contacting the palm fruitlets with an enzyme composition at temperature of above 65°C and extracting the crude palm oil.



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PROCESS FOR EXTRACTION OF PALM OIL USING ENZYMES**Field of the Invention**

The present invention relates to processes for extraction of palm oil from palm fruit bunches using enzymes. Particularly, the present invention relates to a process for extraction of palm oil (such as crude palm oil) from fruit bunches with an enzyme composition. More particularly, the present invention relates to a process for extraction of palm oil from fruit bunches with an enzyme composition at low temperature. The invention also relates to such compositions, for use in processes for extraction of crude palm oil.

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. Crude palm oil can be extracted from the fruit bunches after going through a sterilization, extraction and separation process. Crude palm oil extraction process can be generalized by the following major steps i.e. sterilizing the fresh palm fruit bunches, conveying the sterilized fruits in cages and subsequently discharging them into vessels commonly referred to as digesters, digesting the fruits to produce a digested mash under controlled temperature, pressing of the digested mash by screw press for subsequent recovery of oil and subjecting the pressed liquor to vibration screen and then the clarification process for production of crude palm oil (CPO).

Conventional methods for oil palm processing requires pressurized steam for heating whole fresh fruit bunch (FFB) whereby the whole FFB gradually heats up from outer palm fruitlets to inner layer and center core of the FFB. This conventional sterilization process is a wet process which requires high pressure steam. The process is carried out on a batch basis where the FFB are loaded onto a cage and pushed into the sterilizer or in a continuous process. The conventional sterilization requires large amount of water to generate steam. The steam also condenses on the oil palm fruitlets, softens them and inactivates the lipase present in the fruitlet. Crude palm oil is obtained through pressing of the mass passing to digester (MPD) by a screw press. Along with the crude palm oil, water and solids are also expressed in this pressing and must subsequently be separated from the crude oil.

The expressed crude palm oil from the screw press is pumped via vibrating screens, a decanter and sometimes a skimmer tank to a clarification tank. During this part of the process more hot water is added, typically in the exit from the screw press, to reduce viscosity for more

effective oil and water separation. The large amount of water needed in the conventional crude palm oil processing causes environmental concerns.

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. on fruit quality, and is subject to seasonal variation. On the one hand, the palm oil industry has long been in pursuit of a higher oil yield. On the other hand, mass balance calculations performed by the industry have repeatedly indicated that conventional processes for crude palm oil extraction are highly efficient and that less than 1% oil remains and is lost in various side- or waste streams from the extraction process, including the palm oil mill effluent (POME). Hence, although it would be highly desirable to improve the yield of palm oil per hectare land, it is the general opinion that little would be gained from attempting to further improve the process for extraction of crude palm oil.

Several studies have addressed the possibility of applying enzymes to assist in the extraction of crude palm oil, and promising results have been obtained in laboratory scale, for instance by adding enzymes to mashed palm fruit mesocarp or by adding enzymes in the "downstream" clarification step where pressed crude oil is separated from water and solids (sludge).

Nevertheless, enzyme assisted crude palm oil extraction has never been commercially implemented in palm oil milling. One reason may be that the conditions used in conventional palm oil milling processes have been carefully optimized to increase the OER and minimize oil loss throughout the process: the conditions include sterilization with steam at high pressure and temperature (e.g. at 38psi, more than 130°C) followed by digestion of the mesocarp at temperatures approaching 95°C or more, and subsequent screw pressing and clarification, also at high temperatures. Such conditions would not be considered optimal for enzyme technology.

The main object of the present invention is to provide a process for extraction of palm oil that would improve the yield of crude palm oil by enzymatic treatment to ensure higher recovery of oil from fresh fruit bunches. Another important object of the present invention is to improve the oil and water separation efficiency and reduce the consumption of steam and power during the oil milling process, which can results in energy savings and a higher yield for the same process.

Summary of the Invention

The present invention relates to a process for extraction of palm oil from palm fruitlets using an enzyme composition.

In one aspect, the present invention relates to a process for extraction of palm oil from palm fruitlets comprising steps of: contacting the palm fruitlet with an enzyme composition at a temperature of above 65°C and extracting the crude palm oil.

5 In one aspect, the process comprises an enzyme composition comprising one or more cellulases.

In another aspect, the process further comprises the enzyme composition comprising a hemicellulase, an amylase, a pectinase, or combination thereof.

10 In one aspect, the invention relates to a method for extraction of crude palm oil from palm fruitlets, comprising contacting the palm fruitlets with an enzyme composition comprising one or more cellulase(s).

In another aspect, the method further comprises the enzyme composition comprising a hemicellulase, an amylase, a pectinase, or combination thereof.

In one aspect, the invention relates to use of an enzyme composition comprising one or more cellulase(s) for extraction of crude palm oil from palm fruitlets.

15 In one aspect, the present invention relates to a process for extraction of palm oil from palm fruitlets comprising steps of: contacting the palm fruitlet with an amylase at a temperature of above 65°C and extracting the crude palm oil.

20 In one aspect, the present invention relates to a process for extraction of palm oil from palm fruitlets comprising steps of: contacting the palm fruitlet with a pectinase at temperature of above 65°C and extracting the crude palm oil.

In one aspect, the process comprises an enzyme composition comprising

- a. at least one cellulase or at least one hemicellulase or combination thereof;
- b. a pectinase;
- c. a combination of (a) and (b).

25 In another aspect, the process further comprises the enzyme composition comprising an amylase.

In one aspect, the invention relates to a method for extraction of crude palm oil from palm fruitlets, comprising contacting the palm fruitlets with an enzyme composition comprising

- a. at least one cellulase or at least one hemicellulase or combination thereof;
- 30 b. a pectinase;
- c. combination of (a) and (b).

In another aspect, the method further comprises the enzyme composition comprising an amylase.

In one aspect, the invention relates to use of an enzyme composition comprising a. at least one cellulase or at least one hemicellulase or combination thereof; b. a pectinase; c. combination of (a) and (b).

In another aspect, the use further comprises the enzyme composition comprising an amylase.

Finally, the invention provides a crude palm oil obtained by a process or method according to the invention.

Definitions

Acetylxyylan esterase: The term “acetylxyylan 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. Acetylxyylan 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 acetylxyylan 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 μmole 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 µmole 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.

5 **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, celooligosaccharides, 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.

15 **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).

25 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” refers to (also called a non-degummed oil) a pressed or extracted oil or a mixture thereof from. In the present context it is to be understood that the oil is palm oil, in particular un-refined palm oil.

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 μ mole 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 methylated pectins by beta-elimination), pectin 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 acetylerase (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 μ mole 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 extract rate as ratio of oil recovered and Fresh fruit branch (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: Mass balance data of control run in a commercial palm oil mill

Figure 2: Schematic representation of a production line in a commercial palm oil mill from thresher to screw press. The production line includes: Thresher (A), Conveyor(s) (B), (C) and (D), Digester (E) and Screw press (F). Enzyme application is shown at point (G); including water dosing (H) and enzyme dosing (I). Steam supply to the digester is shown as (J) and dilute crude oil (DCO) exit from screw press is shown as (K).

Detailed Description of the Invention

The present invention relates to a process for extraction of palm oil from palm fruitlets using an enzyme composition.

Generally, palm oil is extracted from fresh fruit bunches (FFB) by a thermo- mechanical process. A typical mill has many operations which include sterilization, stripping, digestion and pressing, clarification, purification, drying and storage. The present inventors have surprisingly found that despite the steep temperature gradients in industrial palm oil milling processes, and the high temperatures reached, the temperatures are in fact approaching the ideal conditions for many hydrolytic enzymes (approximately 70°C; e.g. 65-80°C) for the majority of the period during which palm fruit is passed from the thresher, to the digester and through the digester to reach the screw press.

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, stripping, digestion and pressing may be performed using equipment in a configuration as outlined in Figure 1. Stripping or threshing is 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 (A). 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 (B), (C) and (D). In the digester (E), 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 (F), 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.

Typically in the conventional process, the fruitlets coming out of the sterilization step have a temperature of up to 95°C; in some instances 95°C or above. However, during the stripping or threshing step the palm fruitlets start to cool and the temperature of the fruitlets decreases to about 45-65°C; e.g. 50-65°C or 45-55°C, as the threshed mass of fruitlets or mass passing to digester (MPD) passes towards the digester by means of one or more conveyors. The digester is supplied with continuous steam where the fruitlets are again reheated to temperatures of above 90°C for extraction of oil. The temperature in the upper one third of digester is about 45-65°C; e.g. 50-65°C or 45-55°C; in the middle one third of digester it is about 75°C and in the lower one third of the digester it is above 90°C.

The present inventors have observed that when carefully determining the mass balance of palm oil extraction, for instance by performing double Soxtherm analyses to determine the amount of oil lost in various side- or waste streams, it appears that the oil loss in a conventional palm oil extraction process is substantially greater than previously believed. Hence, contrary to what is currently accepted in the field of palm oil extraction there is indeed a potential for increasing the oil extraction rate by further improving the process of extracting oil from the palm fruitlet mesocarp.

Furthermore, the inventors have found that, despite the steep temperature changes and the high temperatures reached in palm oil milling the temperatures during conveyance from the thresher to the digester and through the digester to the screw press do in fact approach the ideal conditions for most enzyme products that would be useful in palm oil extraction. In addition, palm oil is highly viscous and the requirement for high temperatures in the digester zone is partly in order to reduce the viscosity of the oil during pressing and subsequent separation of oil from water. However, as the use of enzymes (cellulase, amylase and pectinase, optionally in combination with other enzyme activities) leads to a viscosity reduction in the UDCO or DCO, it is also possible to slightly lower the temperatures in the digester zone, and to achieve a sufficient retention time of the palm fruit mash at a highly attractive temperature range above 65°C.

All in all, the inventors have observed that when using enzymes, in particular cellulolytic enzymes to improve conventional thermo-mechanic oil extraction, the amount of oil that can be recovered during clarification of the DCO, e.g. by centrifugation, is far greater than if enzymes are not used in the thermo-mechanical digestion process. In particular, oil recovery can be substantially improved if palm fruitlets or mass passing to digester (MPD) is/are contacted with enzymes at a temperature of above 65°C. Hence, in one aspect, the present invention relates to a process for extraction of palm oil from palm fruitlets comprising steps of: contacting the palm fruitlet with an enzyme composition at a temperature of above 65°C and extracting the crude palm oil.

In one aspect, the enzyme composition comprises one or more cellulase(s).

In one aspect, the enzyme composition further comprises a hemicellulase, an amylase, a pectinase, or combination thereof.

In another aspect, the present invention relates to a process for extraction of crude palm oil from palm fruitlets comprising the steps: contacting the palm fruitlets with an amylase at
5 temperature of above 65°C; and extracting the crude palm oil.

In further aspect, the present invention relates to a process for extraction of crude palm oil from palm fruitlets comprising the steps: contacting the palm fruitlets with a pectinase at temperature of above 65°C; extracting the crude palm oil.

In one aspect, the oil palm is from the genus *Elaeis*.

10 In particular, the inventors have found that extraction of oil with an enzyme composition is achieved at a temperature of about 65°C to about 85°C, such as a temperature within the range of 65°C to 85°C at the lower one third of the digester, and that this is very effective in providing a high oil yield compared to the conventional process of extraction of oil.

In the process according to the present invention, the palm fruitlets coming out of
15 sterilization step have a temperature of up to 95°C (in some instances 95°C or above). During the stripping or threshing step the palm fruitlets start to cool and the temperature of the fruitlets cools down to about 45-65°C; such as in the range of 45-55°C; e.g. in the range of 50-60°C or typically 55°C, as the threshed mass of fruitlets passes into the digester by means of a conveying system. The digester is supplied with continuous steam where the fruitlets are again
20 reheated for extraction of oil. The temperature in the upper one third of digester is about 45-55°C; such as in the range of 45-55°C, e.g. in the range of 55-65°C, or in the range of 50-60°C; in the middle one third of the digester it is about 65°C, such as in the range of 55-65°C, in the range of 60-70°C or in the range of 65-70°C; and in the lower one third of the digester it is about 85°C, such as about 70-85°C, e.g. in the range of 70-85°C, typically about 80°C. As the skilled
25 person will understand, the temperatures ranges in each zone within the digester may be controlled as needed, by injecting more or less steam.

In embodiments of the present invention, the process of contacting the palm fruitlet with an enzyme composition is done at a temperature of above 65°C, which would be the temperatures reached in the middle and lower third of the digester.

30 In a preferred embodiment, contacting of palm fruitlet with an enzyme composition can be at a temperature of 66-90°C, such as at a temperature of 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, and 70-85°C

In one aspect, contacting of palm fruitlet with an enzyme composition is done in presence of water.

5 The water used during the contact of palm fruitlet with an enzyme composition may comprise distilled water, sterilized water and/ or liquid condensate. In preferred embodiments, however, the water used in the process is tap water. In the process according to the invention, the water is preferably pre-heated to a temperature within the range of 65-80°C.

10 The enzyme composition used in the process according to the invention is preferably an aqueous formulation.

In preferred embodiments according to the invention, the enzyme composition is heated to 50-70°C, such as to 55-70°C, to 50-65°C, or such as to 55-65°C, prior to contacting it with the palm fruitlets or MPD.

15 It will be well within the capacity of the skilled person to optimize the dosing of the enzyme composition in view of other process parameters. In particular embodiments of the invention, the enzyme composition is dosed in amounts corresponding to 20-500 mg enzyme protein/kg palm fruitlet, such as 20-450 mg enzyme protein/kg palm fruitlet, 20-400 mg enzyme protein/kg palm fruitlet, 20-350 mg enzyme protein/kg palm fruitlet, 20-300 mg enzyme protein/kg palm fruitlet, 20-250 mg enzyme protein/kg palm fruitlet, 20-200 mg enzyme protein/kg palm fruitlet, 20-150 mg enzyme protein/kg palm fruitlet, 20-100 mg enzyme protein/kg palm fruitlet, 20-75 mg enzyme protein/kg palm fruitlet, 20-50 mg enzyme protein/kg palm fruitlet, 30-500 mg enzyme protein/kg palm fruitlet, 40-500 mg enzyme protein/kg palm fruitlet, 50-500 mg enzyme protein/kg palm fruitlet, 75-500 mg enzyme protein/kg palm fruitlet, 100-500 mg enzyme protein/kg palm fruitlet, 150-500 mg enzyme protein/kg palm fruitlet, 200-25 500 mg enzyme protein/kg palm fruitlet, 250-500 mg enzyme protein/kg palm fruitlet, 300-500 mg enzyme protein/kg palm fruitlet, 350-500 mg enzyme protein/kg palm fruitlet, 400-500 mg enzyme protein/kg palm fruitlet, 30-400 mg enzyme protein/kg palm fruitlet, 30-300 mg enzyme protein/kg palm fruitlet, 30-200 mg enzyme protein/kg palm fruitlet, 30-150 mg enzyme protein/kg palm fruitlet, 30-100 mg enzyme protein/kg palm fruitlet, 30-75 mg enzyme protein/kg palm fruitlet, or such as 30-50 mg enzyme protein/kg palm fruitlet.

30 According to some embodiments of the invention, the enzyme(s) are dosed at amounts corresponding to 100-500 ppm, such as 200-500 ppm or 250-400 ppm.

In one aspect, contacting of palm fruitlet with an enzyme composition is done for a period of 5 minutes or above.

35 In one aspect, contacting is done for a period of less than 3 hours.

In a preferred embodiment, contacting of palm fruitlet with an enzyme composition is done 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, 25-30 minutes, 25-35 minutes, 15-25 minutes, 20-25 minutes, 20-28 minutes, 15-20 minutes, 10-15 minutes or 5-10 minutes. The contacting of mash palm fruitlet with an enzyme composition can be performed before or during the loading of palm fruitlet or MPD into the digester. Some of the palm fruitlets may be bruised during threshing and conveyance, but any further disintegration, such as disintegration during maceration/pre-cooking step is preferably avoided, such that approximately 80%, preferably in the range of 30-90% of the palm fruitlets are substantially intact when arriving at the digester. Contacting with enzyme composition onto substantially intact palm fruitlets (i.e. "coating" the palm fruitlets with enzyme) reduces the phosphorous/phospholipid content in the crude oil as compared to mixing the enzyme composition with macerated fruitlets. When enzymes are mixed with substantially intact palm fruitlets; i.e. palm fruitlets which have not been subjected to maceration, less phospholipids are liberated together with the oil fraction as compared to treatment of palm fruitlets which have been subject to maceration before being contacted with enzyme and arriving at the digester.

In one aspect, the contacting of palm fruitlet or MPD with an enzyme composition is done when the fruitlet or MPD is conveyed towards the digester. In the process according to the invention, the enzyme may alternatively be dosed directly into the digester, such that it is first contacted with the palm fruitlets in the upper one third of the digester.

In one aspect, the process comprises steps of: sterilizing and threshing fresh palm fruit bunches to provide palm fruitlets; and conveying the palm fruitlets into a digester.

In one aspect, the palm fruitlets are threshed and conveyed from threshing to a digester without being subject to disintegration other than the disintegration, which occurs during threshing and conveyance, such as without being subject to maceration/pre-cooking/mashing.

In one aspect, contacting, e.g. contacting of palm fruitlets or MPD with enzyme, is done before or in the digester.

In one aspect, the palm fruitlets are contacted with the enzyme during conveyance from threshing to the digester, such as during transport of the fruitlets on a conveyer belt, in a screw conveyor or auger conveyor.

In one aspect, the palm fruitlets are contacted with the enzyme by distributing the enzyme onto the surface of the palm fruitlets, such as by sprinkling or spraying the enzyme onto the fruitlets, during conveyance.

In the present invention, enzymes are sprinkled or sprayed onto the palm fruitlets during conveyance to the digester, which leads to improved exposure of the palm fruitlets to enzyme and a more homogenous mixture as compared to mixing within the digester. The skilled person would by default add the enzymes in the digester, without having realized that more even distribution of enzyme on the fruitlet surface could be obtained by sprinkling or spraying enzyme onto the fruitlet prior to entry into the digester. A further advantage of applying the enzyme composition during conveyance of the fruitlets or MPD toward the digester is early penetration of the enzyme into the mesocarp through scratches or bruises on the exocarp. This helps in "positioning" the enzyme in the mesocarp and further reduces the reaction time needed when appropriate temperatures are reached in the digester.

In one aspect, the palm fruitlets are contacted with the enzyme for 1-15 minutes, such as for 2-10 minutes during conveyance to the digester, such as for 3-7 minutes or such as for 4-6 minutes during conveyance to the digester.

In one aspect, the palm fruitlets are contacted with the enzyme in the digester for 10 minutes or above.

In one aspect, the palm fruitlets are contacted with the enzyme in the digester for less than 40 minutes.

As the skilled person will realize, the palm fruitlets must be retained within the digester for sufficient time to allow the enzymes to act e.g. on the cellulosic matter of the palm fruitlets. The exact retention time needed in the digester will depend on the exact conditions, and whether the enzyme composition is dosed directly in the digester or onto the palm fruitlets or MPD while being conveyed to the digester. Dosing the enzymes upstream of the digester onto the palm fruitlets or MPD while they are transported to the digester will generally lower the minimum retention time needed in the digester i.e. the average time from the fruitlets or MPD is filled into the digester until the digested fruit is discharged into the press. In a preferred embodiment, the palm fruitlets are contacted with the enzyme in the digester for 15-60 minutes, such as for 20-60 minutes, 25-60 minutes, 30-60 minutes, 40-60 minutes, 50-60 minutes, 15-50 minutes, 20-50 minutes, 25-50 minutes, 30-50 minutes, 40-50 minutes, 15-40 minutes, 20-40 minutes, 25-40 minutes, 30-40 minutes, 15-30 minutes, 20-30 minutes, 25-30 minutes, 15-25 minutes or 15-20 minutes.

In addition to slightly lowering the temperature in the digester as compared to the temperature used in conventional palm oil milling processes, it is also within the scope of the present invention to slightly increase the retention time in the digester. This may be advantageous even when applying the enzyme composition onto the palm fruitlets or MPD while being conveyed to the digester.

According to such embodiments, the temperature in the upper one third of digester may be in the range of 45-55°C, e.g. in the range of 55-65°C; in the middle one third of the digester it may be about 65°C, such as in the range of 55-65°C, in the range of 60-70°C or in the range of 65-70°C; and in the lower one third of the digester it may be about 85°C, such as in the range of 70-85°C, typically about 80°C. According to these and other embodiments contacting or incubation of the palm fruitlet or MPD with an enzyme composition is done 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, 25-30 minutes, 25-35 minutes, 15-25 minutes, 20-25 minutes, 15-20 minutes, 10-15 minutes or 5-10 minutes; the time period being calculated as the time from which the enzyme composition is applied onto the palm fruitlets or MPD and until the digested fruit is discharged into the press.

In currently preferred embodiments, the contacting or incubation of the palm fruitlet or MPD with an enzyme composition is done for a period of 25-35 minutes, more preferably a period of 25-30 minutes, most preferably a period of 25-28 minutes.

It is further to be understood that the enzymes used in the process according to the Invention, may be inactivated, or at least substantially inactivated, when the digested fruit is pressed, due to the high temperatures reached in the screw press.

In a currently preferred embodiment of the present invention, the incubation time or retention time of the palm fruit mash at temperatures above 65°C and up to 85°C is from 10-30 minutes, such as from 10-28 minutes, 15-28 minutes, 12-30 minutes, 12-28 minutes or 12-25 minutes.

Retention time at temperatures above 65°C and up to 85°C may be controlled according to need; e.g. by increasing the digester volume, or by slowing down the screw press. Also, throughout the milling process retention time at temperatures close to 65°C may be increased by the use of a predigester, and/or by use of a slow conveyor method.

In one aspect, contacting with an enzyme composition is done at one or more contact points.

In one aspect, contacting is done at least at one or more points, such as two or more points, which are then spaced at least 0.1-4.0 feet apart from each other, such as 0.1-3.0 feet, 0.1-2.5 feet, 0.1-2.0 feet, 0.1-1.5 feet, 0.1-1 feet, 0.2-4.0 feet, 0.5-4.0 feet, 1.0-4.0 feet, 1.5-4.0 feet, 2.0-4.0 feet, 2.5-4.0 feet, 3.0-4.0 feet, 0.5-2 feet, 0.5-1 foot or 1.0-2.0 feet apart from each other, during conveyance of MPD from thresher to digester.

In the present invention, the mass of palm fruitlet are conveyed into the digester by means such as but not limited to screw-conveyer or auger conveyor or belt conveyor or roller conveyor or skate-wheel conveyor or chain conveyor or bucket elevator.

5 In one aspect, the palm fruitlets are subject to temperatures during passage through the digester, which increase from 45-85°C, such as from 45-90°C, from 50-85°C or such as from 50-90°C.

10 In particular embodiments of the invention, the palm fruitlets or MPD is/are contacted with said enzyme composition under conditions which allow the enzyme(s) to weaken the mesocarp cell wall structure and thereby to reduce the number of cells, which are left intact and containing oil droplets when having been subject to screw pressing or hydraulic pressing. The reduced number of intact cells is clearly visible on photomicrographs of samples taken from POME, such as the ones disclosed in the examples herein.

15 In further embodiments of the invention, the palm fruitlets or MPD is/are contacted with said enzyme composition under conditions which allow the enzyme(s) to weaken the mesocarp cell wall structure and thereby to reduce the number of cells, which are left intact and containing oil droplets when having been subject to screw pressing or hydraulic pressing, while the average fibre length is reduced by no more than 50%, such as by no more than 45%, no more than 40%, no more than 35%, no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10%, no more than 5%, or such as no more than 2%,

20 In further embodiments of the invention, the palm fruitlets or MPD is/are contacted with said enzyme composition at a dosage and under conditions which allow the enzyme composition to reduce the number of cells, which are left intact and containing oil droplets after having been subject to screw pressing or hydraulic pressing, thereby reducing the number of unbroken mesocarp cells in the palm oil mill effluent (POME) by at least 5%, such as by at least 7%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 60%, at least 70%, or such as at least 80%.

30 In the present invention, effective extraction is also observed with enzymes, which have a temperature optimum close to, but below the processing temperature in the digester, such as the processing temperature in the lower third of the digester. The palm fruitlets are substantially disintegrated as they break when approaching the bottom of the digester due to top load of fruit in the digester; hence the interior mesocarp is mainly exposed to enzyme when the temperature is approaching 70°C at the bottom of the digester and close to screw pressing.

35 In one aspect, the palm fruitlets are subject to increasing temperatures during passage through the digester, reaching temperatures with the range of 70-85°C in the lower one third of the digester.

In one aspect, the process is a batch process, continuous and/or semi-continuous.

In one aspect, the enzyme has a temperature optimum within the range of 65-85°C.

In particular, the one or more cellulases, one or more hemicellulases, one or more pectinases, one or more amylases, one or more pectin methyl esterases, one or more polygalacturonases may each have a temperature optimum in the range of 65-85°C, such as in the range of 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.

Preferably, the one or more cellulases, one or more hemicellulases, one or more pectinases, 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.

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, hemicellulose, 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.

In currently preferred embodiments (with reference to Figure 2), the process of the invention comprises the steps of:

- i) Stripping palm fruitlets from fruit their bunches in a thresher (A), and discharging the stripped fruitlets or MPD to a conveyor; e.g. a screw conveyor (B);

ii) Applying an enzyme composition; e.g. an enzyme composition as defined above, onto the stripped fruitlets or MPD, while the stripped fruitlets or MPD is conveyed to a digester (E) on one or more conveyors; e.g conveyors (B), (C) and/or (D);

5 iii) Loading the stripped fruitlets or MPD into the digester (E), and controlling the speed of screw press (F) and/or controlling steam supply (J), so as to produce fruit mash by 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 minutes, 15-28 minutes, 12-30 minutes, 12-28 minutes or 12-25 minutes;
10 and

iv) Extracting crude oil by pressing the mashed palm fruitlets or MPD.

In one aspect, the extracted crude palm oil is subsequently refined.

In one aspect, the yield of the crude palm oil is improved by at least 0.4% compared to a process done in the absence of the added enzymes.

15 In one aspect, the ratio of fruitlets to water is in the range of 1:0.001 to 1:1.1 during digestion and pressing, preferably in the range of 1:0.007 to 1:1; more preferably in the range of 1:0.08 to 1:0.80.

In one aspect, the ratio of pressed mass to water is in the range of 1:0.6 to 1: 1.6, preferably in the range of 1:0.45 to 1:1.45, more preferably in the range of 1:0.4-1:1.04.

20 Water is added to facilitate the extraction during the contacting step where the water aids in dissolution of enzyme(s) which act on the fruit mash.

In one aspect, the pressing using screw press or hydraulic press is done at temperature of above 65°C.

25 In one aspect, the diluted pressed mass is clarified by heating to at least 85°C for a minimum of 30 minutes.

In one aspect, the enzyme inactivation is due to the heat exposure of diluted pressed mass in clarification.

In one aspect, the enzyme is in form of a liquid or a granulate.

30 In one aspect, the percent of free fatty acid (FFA) in extracted oil in the present invention is equal to the percent of FFA in oil extracted by a conventional method.

In one aspect, the Deterioration of Bleachability Index (DOBI) of the extracted oil in the present invention is equal to the percent of DOBI in oil extracted by a conventional method.

In one aspect, the phosphorous/phosphatide content is reduced by at least 0.1% compared to a process done in the absence of the added enzymes. The phosphorous content in the crude palm oil may in particular be reduced by at least 0.1%, such as by at least 2%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25% or at least 30%, such as from 1-50%, from 1-40%, from 1-30%, from 1-20%, from 1-10%, from 2-50%, from 2-40%, from 2-30%, from 2-20%, from 2-10%, from 2-5%, from 5-50%, from 5-40%, from 5-30%, from 5-20%, from 5-10%, from 10-50%, from 10-40%, from 10-30%, from 10-20%, from 20-50%, from 20-40% or such as from 20-30%, compared to a process wherein palm fruitlets are processed in the digester, e.g. for 30 minutes, at a temperature reaching 90°C, in the absence of the added enzymes.

According to this and other aspects of the invention, the process comprises i) contacting the palm fruitlets or MPD with an enzyme composition; e.g an enzyme composition as defined above, at a temperature of above 65°C, and ii) extracting the crude palm oil; wherein the palm fruitlets or MPD is/are contacted with said enzyme composition under such conditions that the phosphorous content in the crude palm oil is reduced by at least 20% compared the phosphorous content of crude palm oil obtained by a process wherein palm fruitlets are processed in the digester at a temperature reaching 90°C, in the absence of the added enzymes.

In the context of the present invention, the phosphorous/phosphatide content of a palm oil sample may be determined using the official method of the American Oil Chemists' Society (AOCS), Ca 12-55, according to which the phosphorous content is determined by charring and ashing the oil sample with zinc oxide (ZnO), followed by the calorimetric measurement of phosphorus as blue phosphomolybdic acid.

In some embodiments of the invention, the process improves the oil extraction so as to add at least an additional 0.4% to the oil extraction rate (OER), such as an additional 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9% or at least an additional 2% to the OER, compared to a process, wherein palm fruitlets are processed in the digester for 30 minutes at a temperature reaching 90°C, in the absence of added enzymes.

In one aspect, the clarification time is reduced by at least 0.5% compared to a process done in the absence of the added enzymes. In particular, the crude palm oil or dilute crude oil (DCO) clarification time may be reduced by at least 0.5%, such as by at least 1%, 5%, 10%, 20%, 30%, 40% or at least 50% compared to a process, wherein palm fruitlets are processed in the digester for 30 minutes at a temperature reaching 90°C in the absence of added enzymes.

In the present invention, the process may comprise extracting the crude palm oil and subjecting it to clarification at a temperature between 85°C and 95°C. The process may also comprise clarification of extracted oil at approximately 90°C for about 3 hours to inactivate residual enzyme.

5 In one aspect, the quantity of steam passed into the digester is reduced by at least 1.5% compared to a process done in the absence of the added enzymes. In particular, the quantity of steam passed into the digester is reduced by at least 1.5%, such as by at least 2%, 3%, 4%, 5% or by at least 10%, compared to a process wherein palm fruitlets are processed in the digester for 30 minutes at a temperature reaching 90°C in the absence of the added
10 enzymes.

In one aspect, the total energy consumption to process one ton of FFB is reduced by at least 0.2%, such as by at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40% or such as by at least 50%, compared to a process wherein palm fruitlets are processed in the digester for 30 minutes at a temperature reaching 90°C in the absence of the added enzymes.

15 In one aspect, the total retention time for treatment of Palm oil mill effluent (POME) is reduced by at least 0.5% compared to the total retention time for treatment of POME generated using a process without addition of added enzymes. In particular, the total retention time for treatment of POME; e.g. the time required for anaerobic fermentation of POME, may be reduced by at least 10%, such as by 20%, 30%, 40%, 50% or by 60%, compared to the total
20 retention time for treatment of POME or time required for anaerobic fermentation of POME required when wherein palm fruitlets are processed in the digester for 30 minutes at a temperature reaching 90°C without addition of enzymes.

In one aspect, the process comprises contacting the palm fruitlets with an enzyme composition comprising one or more cellulases.

25 In another aspect, the enzyme composition further comprises a hemicellulase, an amylase, a pectinase, or combination thereof.

In one aspect, the process comprises contacting the palm fruitlet with a hemicellulase.

In one aspect, the process comprises contacting the palm fruitlet with an amylase.

In one aspect, the process comprises contacting the palm fruitlet with a pectinase.

30 In one aspect, the pectinase has pectin methyl esterase and polygalacturonase activity.

In one aspect, the enzyme composition comprises one or more (e.g., several) cellulases.

In another aspect, the enzyme composition comprises or further comprises one or more (e.g., several) proteins selected from the group consisting of a cellulase, a hemicellulase, a pectinase, an amylase, or combination thereof.

In one aspect, the one or more (e.g., several) cellulolytic enzymes comprise 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),
5 CELLUCLAST™ (Novozymes A/S), NOVOZYME™ 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
10 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
15 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. Commercially used cellulases include Renozyme®, Celluzyme®,
20 Celluclean®, Endolase® and Carezyme®. (Novozymes A/S), Clazinase®, and Puradax HA®. (Genencor Int. Inc.), and KAC-500(B).™. (Kao Corporation).

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

30 In one aspect, pectinase of the invention may comprise a single activity or at least two different activities.

Pectinases of the invention may be obtained by fermentation of organisms. Fermentation of organisms to produce enzymes is known in the art. There are different kinds of fermentation including but not limited to submerged fermentation (SmF) and surface
35 fermentation (SSF). Submerged fermentation (SmF) is known in the art and includes a process of growing a microorganism in a liquid medium. Submerged Fermentation is also alternatively

known as Submerged Liquid Fermentation or submerge fermentation Surface fermentation also called solid-state fermentation (SSF) is known in the art and is a process whereby an insoluble substrate or solid matrix is fermented with sufficient moisture but without being submerged in water. i.e., it involves growth of microorganisms on moist solid particles, in situations in which the spaces between the particles contain a continuous gas phase and a minimum of visible water. It is also known as Solid Substrate Fermentation. Most of the SSF processes are aerobic and so the term fermentation in the context of SSF is meant to mean the "controlled cultivation of organisms". Processes and apparatus for solid state fermentation are known in the art. For example, a useful reference is Mitchell D.A. *et al.*, 2006, Solid-State Fermentation Bioreactors, published by Springer Berlin Heidelberg.

In one aspect, the pectinase is obtained from a non-genetically modified organism. In another aspect, the pectinase is obtained from a genetically modified organism. Pectinase producing organisms are known in the art. They include microorganisms and higher plants. The microorganisms include bacteria, yeast and fungi. For example, *Aspergillus*, *Rhizopus*, *Bacillus*, *Pseudomonas*, *Fusarium*, *Penicillium*, *Saccharomyces*, *Erwinia* etc., are all known to produce pectinase enzymes. The procedures for carrying out the submerged and solid state fermentations for many of these organisms are well known in the art.

Even though not specifically mentioned in context of a process of the invention, it is to be understood that the enzymes (as well as other compounds) are used in an "effective amount".

The effective amount of cellulase, hemicellulase, pectinase, or amylase used in embodiments of the invention will vary with the type of enzymes used in the process, the ultrastructure and composition of the cell wall (which varies by plant type), the pretreatment or pre-processing step, and well as the as the desired yield. Commercial enzymes may be used according to their manufacturer's instructions.

In one aspect, the method for extraction of crude palm oil from palm fruitlets comprises contacting the palm fruitlets with an enzyme composition comprising one or more cellulase(s).

In another aspect, the method for extraction of crude palm oil comprises contacting the palm fruitlets with an enzyme composition, which further comprises a hemicellulase, an amylase, a pectinase or combination thereof.

In one aspect, the method comprises the steps as described above.

In one aspect, the invention provides use of an enzyme composition comprising one or more cellulase(s) for extraction of crude palm oil from palm fruitlets.

In another aspect, the invention comprises the use of an enzyme composition further comprises a hemicellulase, an amylase, a pectinase or combination thereof.

In a final aspect, the invention provides a crude palm oil obtained by the process according to the invention.

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

5

Examples

TREATMENT WITH ENZYMES

Enzyme compositions suitable for use in the present invention include composition comprising cellulase, hemicellulase, pectinase, and/or amylase or combinations thereof. The cellulase were obtained from commercial products such Ceremix™, Viscoflow™, Viscoferm™, CELLUCLAST™ (Novozymes A/S, Denmark) and from an experimental product, and are hereinafter referred to as “cellulase complex”. Enzymes were used alone or in combination.

Nut factoring methodology

Palm fruitlet mainly consists of thin outer layer (exocarp), oil rich mesocarp and inner nut which have shell and kernel. The palm fruitlets are highly non-homogenous. Due to natural variation in the nut:fruit ratio, there is difference in the actual palm mesocarp (containing CPO) in various treatment even from the same batch of fruit. Therefore, a nut factoring system was used wherein, the amount of mesocarp is normalized for each treatment in order to understand the effect of enzymes on CPO extraction from small sample size of 1 kg segregated palm fruitlets irrespective of variation in nut:fruit ratio. The amount of mesocarp of palm fruitlet is estimated by negating the inherent weight of the nuts from the total amount of 1 kg segregated palm fruitlet. The inherent weight of nut in fruit is back-calculated by adding its inherent moisture to the 60°C / 48 hours dried nut. Further, the mesocarp content across all the treatments is normalized to check the oil extracted. Then the CPO from the normalized mesocarp is then compared for % increase in CPO yield due to enzyme treatment. Inherent moisture of the nut generally present have been analyzed in lab and fixed. Inherent moisture in Indian fruitlet was 10%; Indonesian fruitlet was 5%. Amount of mesocarp generally present have been analyzed in lab. Amount of mesocarp in Indian and Indonesian palm fruitlet fruit was 770g and 650g respectively.

Example 1: Crude palm oil Yield from Indian Palm Fruit

1-kg of segregated (devoid of trash like leaves, thorns, stones, straws etc) Indian palm fruit was taken, to which 100 g of water was added and sterilized in a pressure cooker vessel. The sterilized fruits were transferred to a mashing bath having blades with rotational speed of

190-200 rpm. The enzyme composition was diluted with to 100ml with water. The enzyme dilution was added to the fruitlet and mashing was carried out at 70°C for 30 minutes. The resultant palm fruitlet mash was then hand pressed with 1500 g of boiling water. The hand pressed mass extract (i.e DCO – Dilute crude oil) was then passed through sieve of mesh number # 8 (2000 micron) in order to separate large size fruitlet particles. Of the 1500g of boiling water used for hand pressing, 600g of boiling water was used for initial wash of the fiber and mashing bath vessel; the resultant fiber was further washed with 300g boiling water. Palm nuts were also washed with 300g of boiling water. Finally, the bucket, cylinders (used for collecting DCO) and sieve were all washed with 300g of boiling water to ensure consistency of mass balance. The separated, water washed, pressed fibers and palm nuts are subjected to drying at 60°C for 48 hours and then their respective weights were measured. The DCO was kept for clarification in a hot air oven for 30 minutes at 90°C. During down-streaming, the DCO was further centrifuged at 5000 rpm (1558g) for 10 minutes using a Beckman Coulter centrifuge (model - Avanti JE). After the centrifugation step, three layers were formed - oil floating at the top, POME separated at the middle, and solid sludge accumulated at the bottom. The liquid stream (oil and POME) was decanted in a glass separating funnel. All the centrifuge tubes were rinsed without disturbing the solid sludge pellet with 600g of boiling water. This water used for rinsing, was added to the separating funnel. The oil (top) and POME (bottom) phases separate after keeping the whole mass stationary for at least 2 minutes. The POME fraction was drained from the bottom of the separating funnel and total weight of the POME was measured. The top oil layer was collected for determining the yield of crude palm oil from 1 kg palm fruitlets. Keeping all parameters same as above described process, the control crude palm oil extraction (without added enzymes) was performed at condition of 90°C for 15 minutes.

The results from Table 1 indicate that the percent of crude palm oil yield by enzymatic treatment increase at lower temperature compared to control.

Table 1: Crude palm oil Yield from Indian Palm Fruit

Dosage (EP (mg) per kg of palm fruitlet)	Ratio of EP (mg) per kg of palm fruitlet	Treatments	Incubation Condition	CPO (g)	% Increase of CPO Yield over Control
-	-	Control	90°C / 15 min	250	-
193.00	-	Ceremix 6XMG	70°C / 30 min	284	13.5%
46.97	-	Ceremix Plus MG	70°C / 30 min	271	8.4%
45.95	-	Ceremix 2XL	70°C / 30 min	268	7.2%
81.28	-	Viscoflow MG	70°C / 30 min	277	10.9%
81.28 + 50.29	1 : 0.62	Viscoflow MG + Viscoferm	70°C / 30 min	292	16.9%

Example 2: Crude Palm Oil Yield from Indian Palm Fruit at Different Extraction Temperatures

1-kilogram of segregated (devoid of trash like leaves, thorns, stones, straws etc) Indian palm fruit was taken, to which 100 g of water was added and sterilized in a pressure cooker vessel. The sterilized fruits were transferred to a mashing bath having blades with rotational speed of 190-200 rpm. The enzyme composition of cellulase complex having 58.42 mg EP/kg fruit was taken and then diluted with 100ml with water. The enzyme dilution was added to the fruitlet and mashing was carried out at 65-90°C for 30 minutes. The resultant palm fruitlet mash was then hand pressed with 1500 g of boiling water. The hand pressed mass extract (i.e DCO – Dilute crude oil) was then passed through sieve of mesh number # 8 (2000 micron) in order to separate large size fruitlet particles. Of the 1500g of boiling water used for hand pressing, 600g of boiling water was used for initial wash of the fiber and mashing bath vessel; the resultant fiber was further washed with 300g boiling water. Palm nuts were also washed with 300g of boiling water. Finally, the bucket, cylinders (used for collecting DCO) and sieve were all washed with 300g of boiling water to ensure consistency of mass balance. The separated, water washed, pressed fibers and palm nuts are subjected to drying at 60°C for 48 hours and then their respective weights were measured. The DCO was kept for clarification in a hot air oven for 30 minutes at 90°C. During down-streaming, the DCO was further centrifuged at 5000 rpm (1558g) for 10 minutes using a Beckman Coulter centrifuge (model - Avanti JE). After the centrifugation step, three layers were formed - oil floating at the top, POME separated at the middle, and solid sludge accumulated at the bottom. The liquid stream (oil and POME) was decanted in a glass separating funnel. All the centrifuge tubes were rinsed without disturbing the solid sludge pellet with 600g of boiling water. This water used for rinsing, was added to the separating funnel. The oil (top) and POME (bottom) phases separate after keeping the whole mass stationary for at least 2 minutes. The POME fraction was drained from the bottom of the separating funnel and total weight of the POME was measured. The top oil layer was collected for determining the yield of crude palm oil from 1 kg palm fruitlets. Keeping all parameters same as above described process, the control crude palm oil extraction (without added enzymes) was performed at condition of 90°C for 15 minutes.

The results from Table 2 indicate that there is an increase percent of crude palm oil yield by enzymatic treatment at 65°C or above temperature for 30 minutes compared to control at 90°C for 15 minutes.

Table 2: Crude Palm Oil Yield from Indian Palm Fruit at Different Extraction Temperatures

	CPO (g)	CPO (g)	% Increase in CPO Yield Over Industrial Control (90°C/15 min)	% increase in CPO Yield Over Control (Respective control)
Treatments	Control	Enzyme	Enzyme	Enzyme
90°C / 15 min	300.6	-	-	-
65°C / 30 min	294.5	312.7	4.0%	6.2%
66°C / 30 min	303.7	318.9	6.1%	5.0%
70°C / 30 min	301.6	331.1	10.1%	9.8%
75°C / 30 min	300.0	323.3	7.6%	7.8%
80°C / 30 min	300.9	317.5	5.6%	5.5%
90°C / 30 min	303.8	312.8	4.1%	3.0%

Example 3: Quality analysis of Crude Palm Oil Yield from Indian Palm Fruit at Different Extraction Temperatures

- 5 Quality analysis was done for all the extracted CPO of Table 2 using standard assays of measurement of Phosphorus content (AOCS CA 12-55), % Free Fatty Acids (%FFA) (AOCS CA 5a-40) and DOBI (Deterioration of Bleachability Index) (ES ISO-17932). The method used The results from Table 3 indicates that there is increase percent of phosphorus reduction in crude palm oil yield by enzymatic treatment at 65°C or above temperature for 30 minutes compared to control at 90°C for 15 minutes while there is no detrimental effect of %FFA & DOBI values using enzymes.
- 10

Table 3: Quality Analysis of Crude Palm Oil Yield from Indian Palm Fruit at Different Extraction Temperatures

Treatments	Phosphorus in CPO (ppm)	Phosphorus in CPO (ppm)	% Reduction in Total Phosphorus (w.r.t Industrial control)	Avg. % Reduction in Total Phosphorus (w.r.t respective temperature control)	% FFA of CPO	% FFA of CPO	DOBI of CPO	DOBI of CPO
	Control	Enzyme	Enzyme	Enzyme	Control	Enzyme	Control	Enzyme
90°C /	14.5 ±	-	-	-	12.07 ±	-	3.57 ±	-

15 min	0.0				0.08		0.02	
65°C / 30 min	11.2 ± 0.5	8.0 ± 0.7	44.4% ± 4.9%	28.0% ± 4.9%	12.98 ± 0.02	12.56 ± 0.03	3.80 ± 0.03	3.77 ± 0.02
66°C / 30 min	11.0 ± 0.1	7.7 ± 0.5	46.7% ± 3.8%	29.8% ± 4.9%	12.78 ± 0.03	12.31 ± 0.02	3.85 ± 0.02	3.85 ± 0.04
70°C / 30 min	11.7 ± 0.1	7.4 ± 0.3	49.0% ± 5.5%	37.1% ± 7.1%	12.33 ± 0.09	12.29 ± 0.07	3.74 ± 0.06	3.78 ± 0.02
75°C / 30 min	11.3 ± 0.2	7.2 ± 0.2	50.4% ± 1.4%	36.7% ± 1.8%	12.67 ± 0.01	12.34 ± 0.01	3.65 ± 0.05	3.74 ± 0.03
80°C / 30 min	11.8 ± 0.2	8.2 ± 0.4	43.6% ± 2.4%	31.0% ± 3.0%	14.73 ± 0.06	13.28 ± 0.09	3.78 ± 0.08	3.94 ± 0.06
90°C / 30 min	11.9 ± 0.1	11.3 ± 0.4	22.1% ± 2.4%	4.9% ± 2.9%	12.48 ± 0.08	10.45 ± 0.05	3.58 ± 0.03	3.88 ± 0.02

Example 4: Measurement of Oil losses in waste stream - Pressed fibre, solid sludge and POME and effect of enzyme in oil loss reduction from waste streams

The waste stream samples of Control and enzyme (70°C / 30 min) treatment of Table 3 was collected and analyzed for total oil content analysis using Gerhardt Soxtherm (Model: 840650-SOX416). The waste streams are dried at 60°C overnight. The solvent used was petroleum benzene. There are 5 standard steps in each cycle run of oil extraction in Soxtherm using solvent, namely Hot extraction, evaporation A, Rinsing tome, Evaporation B and Evaporation C. Each sample for given 3 cycle run in order to extract 100% of the inherent oil. The result in Table 4 indicates that at least 2 cycle run of oil extraction in soxtherm is required in order to extract the total inherent oil in the dried sample of palm waste streams. It was also noted that there is no significant reduction of oil in pressed fibre and solid sludge from enzyme treatment as compared to Control. However, there is significant decrease of oil content in POME from enzyme run as compared to control run. This indicates that maximum oil loss is from POME waste fraction as compared to other waste fraction.

Table 4: Oil content in various waste fraction of Crude Palm Oil processing

Fractions	% Oil content (on wet weight basis)						% Extraction of Oil in 1st run of Soxtherm	
	1 st run of Soxtherm		2 nd run of Soxtherm		3 rd run of Soxtherm		Control (Incubation: 90°C / 15 min)	Enzyme (Incubation: 70°C / 30 min)
	Control (Incubation: 90°C / 15 min)	Enzyme (Incubation: 70°C / 30 min)	Control (Incubation: 90°C / 15 min)	Enzyme (Incubation: 70°C / 30 min)	Control (Incubation: 90°C / 15 min)	Enzyme (Incubation: 70°C / 30 min)		
Pressed Fibre	15.1%	17.9%	0.4%	0.30%	0.0%	0.00%	97%	98%

Solid Sludge	15.1%	15.9%	0.2%	0.20%	0.0%	0.00%	99%	99%
POME	24.5%	10.8%	1.4%	0.90%	0.0%	0.00%	95%	92%

Example 5: Crude Palm Oil Extraction in a Commercial Palm Oil Mill using enzyme

Full-scale plant trials were conducted in a local palm mill in Karnataka with 5 tons per hour capacity in India. Fresh fruit bunches (FFB) procured from nearby palm plantations were autoclaved in vertical sterilizer (10 tons capacity) at steam pressure (2 kg/cm²), temperature (130-150 °C) and sterilization time of 40-80 minutes. In order to separate MPD (mass passing to digester) from FFB, the sterilized FFBs were passed at throughput rate of 5 tons per hour for control run through thresher (stripper) having horizontal rotating drum. The resultant empty fruit bunches (EFBs) were collected separately and weighed. Then, the MPD lifted to the single digester having 2 tons capacity (working at 80% operational capacity) through a bucket elevator. From bucket elevator, MPD were transferred to the inlet at the top of the digester via a inclined chute. In control run, the steam pressure at the digester is maintained upto 1.5 kg/cm² and the temperature is around 90-95°C. Water is also added to the digester at 1800-2000 litre per hour. The mashed MPD from the digester is passed through the screw-press and 2 fractions are obtained; i.e, total extract and pressed cake (pressed fibres and palm nuts). The total extract is passed through vibratory sieve separator and the resultant mass is DCO (dilute crude oil) which is pumped to the vertical clarifier. The temperature in the vertical clarifier is maintained at 90°C for minimum 2 hours. The clarified CPO at the top is skimmed out to the CPO tank. The remaining heavy fraction was centrifuged through sludge centrifuge separator and the CPO fraction is pumped to the CPO tank while the POME stream is passed to the POME pit. The OER is measured as percentage of total CPO extracted in the CPO tank to the total FFB processed. For the enzyme run, throughput of the FFB was decreased to 2.5 tons per hour; digester temperature maintained at 65-70°C; enzyme concentration in enzyme dilution was 1% and flowrate of enzyme dilution was 82.5 litre per hour. Rest of the procedure was similar to the control run. The results from Table 5 indicate that there is increase percent of crude palm oil yield by enzyme at 65°C or above temperature for 30 minutes compared to control at 90°C for 15 minutes.

Table 5: OER data in a Commercial Palm Oil Mill trial using enzymes

Important Parameter	Control	Enzyme
Incubation Condition	(90°C / 15 min)	(65-70°C / 30 min)
Dosage (ppm on FFB basis)	0 ppm	330 ppm
FFB (kg)	31392	38085
EFB (kg)	6475	7311

Total Fruitlet (kg)	24917	30774
Palm Nut (kg)	3313	3917
Pressed Fibers (kg)	2367	3808
Extracted CPO (kg)	5052	6763
Extractable % OER	16.1%	17.8%

Example 6: Schematic Mass balance of Commercial Palm Oil Mill

Schematic mass balance for the control run of table 5 is illustrated in Figure 1. Maximum Input OER was taken as sum of OER in DCO (Dilute crude oil) and OER loss in 3 streams, namely EFB (Empty fruit bunch), steam condensate and pressed fibre. The OER loss in POME was calculated as difference between maximum input OER and the extractable OER. The mass balance depicted in Figure 1 showed that in POME there is more than 4% OER loss.

The invention described and claimed herein is not to be limited in scope by the specific aspects herein disclosed, since these aspects are intended as illustrations of several aspects of the invention. Any equivalent aspects are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. In the case of conflict, the present disclosure including definitions will control.

Example 7: Crude Palm Oil Yield from Indonesian Palm Fruit.

Crude palm oil extraction from Indonesian palm fruits was carried out at 90°C (control) and at 70°C (enzyme: Experimental cellulase) following the procedure set forth in Example 2.

Oil in pressed fibre and POME was determined as described in example 4.

Table 6: OER data

Dosage (ppm on Fruitlet basis)	Dosage (EP (mg) per kg of palm fruitlet)	Ratio of EP (mg) per kg of palm fruitlet	Treatments	Incubation Condition	CPO Yield (g)	% Increase of CPO Yield over Control	% of Oil in pressed fibers to CPO Yield	% of Oil in POME to CPO Yield
0	0	-	Control	90°C / 15 min	284.12	0%	2.74%	26.39%
330	5.84	-	Enzyme	70°C / 30 min	329.01	15.80%	2.06%	5.77%
500	8.85	-	Enzyme	70°C / 30 min	328.45	15.60%	2.10%	8.21%

1. A process for extraction of crude palm oil from palm fruitlets or mass passing to digester (MPD) comprising the steps:

- Contacting the palm fruitlets with an enzyme composition at temperature of above 65°C;
- Extracting the crude palm oil.

2. The process according to claim 1, wherein the enzyme composition comprises one or more cellulase(s).

3. The process according to any of the preceding claims, wherein the enzyme composition further comprises a hemicellulase, an amylase, a pectinase, or combination thereof.

4. The process according to any of the preceding claims, wherein the temperature is 66-90°C, such as at a temperature of, 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, and 70-85°C.

5. The process according to any of the preceding claims, wherein said contacting is done in presence of water.

6. The process according to any of the preceding claims, wherein said enzyme composition is an aqueous formulation.

7. The process according to any of the preceding claims, wherein said enzyme composition is heated to 50-70°C, such as to 55-70°C, to 50-65°C, or such as to 55-65°C, prior to contacting it with the palm fruitlets or MPD.

8. The process according to any of the preceding claims, wherein the enzyme composition is dosed in amounts corresponding to 20-500 mg enzyme protein/kg palm fruitlet, such as 20-450 mg enzyme protein/kg palm fruitlet, 20-400 mg enzyme protein/kg palm fruitlet, 20-350 mg enzyme protein/kg palm fruitlet, 20-300 mg enzyme protein/kg palm fruitlet, 20-250 mg enzyme protein/kg palm fruitlet, 20-200 mg enzyme protein/kg palm

fruitlet, 20-150 mg enzyme protein/kg palm fruitlet, 20-100 mg enzyme protein/kg palm fruitlet, 20-75 mg enzyme protein/kg palm fruitlet, 20-50 mg enzyme protein/kg palm fruitlet, 30-500 mg enzyme protein/kg palm fruitlet, 40-500 mg enzyme protein/kg palm fruitlet, 50-500 mg enzyme protein/kg palm fruitlet, 75-500 mg enzyme protein/kg palm fruitlet, 100-500 mg enzyme protein/kg palm fruitlet, 150-500 mg enzyme protein/kg palm fruitlet, 200-500 mg enzyme protein/kg palm fruitlet, 250-500 mg enzyme protein/kg palm fruitlet, 300-500 mg enzyme protein/kg palm fruitlet, 350-500 mg enzyme protein/kg palm fruitlet, 400-500 mg enzyme protein/kg palm fruitlet, 30-400 mg enzyme protein/kg palm fruitlet, 30-300 mg enzyme protein/kg palm fruitlet, 30-200 mg enzyme protein/kg palm fruitlet, 30-150 mg enzyme protein/kg palm fruitlet, 30-100 mg enzyme protein/kg palm fruitlet, 30-75 mg enzyme protein/kg palm fruitlet, or such as 30-50 mg enzyme protein/kg palm fruitlet.

9. The process according to any of the preceding claims, wherein said contacting is done for a period of 5 minutes or above.

10. The process according to any of the preceding claims, wherein said contacting is done for a period of less than 3 hours.

11. The process according to any of the preceding claims, wherein contacting is done for a period of 15-60 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, 25-30 minutes, 15-25 minutes, 20-25 minutes, 15-20 minutes, 10-15 minutes or 5-10 minutes.

12. The process according to any of the preceding claims, said process comprising:

- (i) Sterilizing and threshing fresh palm fruit bunches to provide palm fruitlets or MPD; and
- (ii) Conveying the palm fruitlets into a digester.

13. The process according to any of the preceding claims, wherein the palm fruitlets are substantially intact when entering the digester.

14. The process according to any of the preceding claims, wherein the palm fruitlets are threshed and conveyed from threshing to a digester without being subject to disintegration other than the disintegration, which occurs during threshing and conveyance, such as without being subject to maceration/pre-cooking.

15. The process according to any of the preceding claims, wherein said contacting is done before or in the digester.
- 5 16. The process according to any of the preceding claims, wherein the palm fruitlets or MPD are contacted with the enzyme composition during conveyance from threshing to the digester, such as during transport of the fruitlets on a conveyer (e.g. a screw conveyer, a conveyer belt, a bucket elevator).
- 10 17. The process according to any of the preceding claims, wherein the palm fruitlets or MPD are contacted with the enzyme composition by distributing the enzyme onto the surface of the palm fruitlets, such as by sprinkling or spraying the enzyme onto the fruitlets, during conveyance.
- 15 18. The process according to any of the preceding claims, wherein the said contacting is done at least at one or more points, such as two or more points, which are spaced at least 0.1-4.0 feet apart from each other during conveyance of the palm fruitlets or MPD from thresher to digester.
- 20 19. The process according to any of the preceding claims, wherein the palm fruitlets or MPD are contacted with the enzyme for 30 seconds to 15 minutes during conveyance to the digester, such as for 1-15 minutes, such as for 2-10 minutes or such as for 3-7 minutes or such as for 4-6 minutes during conveyance to the digester.
- 25 20. The process according to claim 16, wherein the palm fruitlets or MPD are contacted with the enzyme in the digester for 10 minutes or above.
21. The process according to claim 17, wherein the palm fruitlets or MPD are contacted with the enzyme in the digester for less than 40 minutes.
- 30 22. The process according to claim 16 or 17, wherein the palm fruitlets or MPD are contacted with the enzyme in the digester for 15-60 minutes, 20-60 minutes, 25-60 minutes, 30-60 minutes, 40-60 minutes, 50-60 minutes, 15-50 minutes, 20-50 minutes, 25-50 minutes, 30-50 minutes, 40-50 minutes, 15-40 minutes, 20-40 minutes, 25-40 minutes, 30-40 minutes, 15-30 minutes, 20-30 minutes, 25-30 minutes, 15-25 minutes or 15-20 minutes.
- 35

23. The process according to any of the preceding claims, wherein the palm fruitlets are subject to temperatures during passage through the digester, which increase from 45-85°C.

24. The process according to any of the preceding claims, wherein the palm fruitlets or MPD are incubated or retained at temperatures above 65°C and up to 85°C from 12-28 minutes.

25. The process according to any of the preceding claims, wherein said palm fruitlets or MPD is/are contacted with said enzyme composition under conditions which allow the enzyme(s) to weaken the mesocarp cell wall structure and thereby to reduce the number of cells, which are left intact and containing oil droplets when having been subject to screw pressing or hydraulic pressing.

26. The process according to any of the preceding claims, wherein said palm fruitlets or MPD is/are contacted with said enzyme composition under conditions which allow the enzyme(s) to weaken the mesocarp cell wall structure and thereby to reduce the number of cells, which are left intact and containing oil droplets when having been subject to screw pressing or hydraulic pressing, while the average fibre length is reduced by no more than 50%, such as by no more than 45%, no more than 40%, no more than 35%, no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10%, no more than 5%, or such as no more than 2%.

27. The process according to any of the preceding claims, wherein the said palm fruitlets or MPD is/are contacted with said enzyme composition at a dosage and under conditions which allow the enzyme composition to reduce the number of cells, which are left intact and containing oil droplets after having been subject to screw pressing or hydraulic pressing, thereby reducing the number of unbroken mesocarp cells in the palm oil mill effluent (POME) by at least 5%, such as by at least 7%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 60%, at least 70%, or such as at least 80%.

28. The process according to any of the preceding claims, wherein the palm fruitlets are subject to increasing temperatures during passage through the digester, reaching temperatures with the range of 65-85°C in the lower one third of the digester.

29. The process according to any of the preceding claims, wherein said process is a batch process, continuous and/or semi-continuous.

30. The process according to any of the preceding claims, wherein the enzyme has a temperature optimum within the range of 65-85°C.

5 31. The process according to any of the preceding claims, wherein the extracted crude palm oil is subsequently refined.

32. The process according to any of the preceding claims, wherein the pressing using screw press or hydraulic press is done at temperature of above 65°C.

10 33. The process according to any of the preceding claims, wherein the ratio of fruitlets to water is in the range of 1:0.001 to 1:1.1 during digestion and pressing, preferably in the range of 1:0.007 to 1:1; more preferably in the range of 1:0.08 to 1:0.80.

15 34. The process according to any of the preceding claims, wherein the ratio of pressed mass to water is in the range of 1:0.6 to 1: 1.6, preferably in the range of 1:0.45 to 1:1.45, more preferably in the range of 1:0.4-1:1.04.

20 35. The process according to any of the preceding claims, wherein diluted pressed mass is clarified by heating to at least 85°C for a minimum of 30 minutes.

36. The process according to any of the preceding claims, wherein the enzyme inactivation is due to the heat exposure of diluted pressed mass in clarification.

25 37. The process according to any of the preceding claims, said process improves the oil extraction so as to add at least an additional 0.4% to the oil extraction rate (OER), such as an additional 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9% or at least an additional 2% to the OER, compared to a process, wherein palm fruitlets are processed in the digester for 30 minutes at a
30 temperature reaching 90°C, in the absence of added enzymes.

38. The process according to any of the preceding claims, wherein the phosphorous content in the crude palm oil is reduced by at least 0.1%, such as by at least 2%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25% or at least 30%, such as from 1-50%,
35 from 1-40%, from 1-30%, from 1-20%, from 1-10%, from 2-50%, from 2-40%, from 2-30%, from 2-20%, from 2-10%, from 2-5%, from 5-50%, from 5-40%, from 5-30%, from 5-20%, from 5-10%, from 10-50%, from 10-40%, from 10-30%, from 10-20%, from 20-50%, from 20-40% or such as from 20-30%, compared to a process done when palm

fruitlets are processed in the digester at 90°C for 30 minutes in the absence of the added enzymes.

39. The process according to any of the preceding claims, wherein the crude palm oil or dilute crude oil (DCO) clarification time is reduced by at least 0.5%, such as by at least 1%, 5%, 10%, 20%, 30%, 40% or at least 50% compared to a process, wherein palm fruitlets are processed in the digester for 30 minutes at a temperature reaching 90°C in the absence of added enzymes.

40. The process according to any of the preceding claims, wherein the quantity of steam passed into the digester is reduced by at least 1.5%, such as by at least 2%, 3%, 4%, 5% or by at least 10%, compared to a process wherein palm fruitlets are processed in the digester for 30 minutes at a temperature reaching 90°C in the absence of the added enzymes.

41. The process according to any of the preceding claims, wherein the total energy consumption to process one ton of FFB is reduced by at least 0.2%, such as by at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40% or such as by at least 50%, compared to a process wherein palm fruitlets are processed in the digester for 30 minutes at a temperature reaching 90°C in the absence of the added enzymes.

42. The process according to any of the preceding claims, wherein the total retention time for treatment of Palm oil mill effluent (POME); e.g. the time required for anaerobic fermentation of POME, is reduced by at least 10%, such as by 20%, 30%, 40%, 50% or by 60%, compared to the total retention time for treatment of POME or time required for anaerobic fermentation of POME required when wherein palm fruitlets are processed in the digester for 30 minutes at a temperature reaching 90°C without addition of enzymes.

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

- v) Stripping palm fruitlets from fruit their bunches in a thresher (A), and discharging the stripped fruitlets or MPD to a conveyor; e.g. a screw conveyor (B);
- vi) Applying an enzyme composition; e.g. an enzyme composition as defined above, onto the stripped fruitlets or MPD, while the stripped fruitlets or MPD is conveyed to a digester (E) on one or more conveyors; e.g. conveyors (B), (C) and/or (D);

- vii) Loading the stripped fruitlets or MPD into the digester (E), and controlling the speed of screw press (F) and/or controlling steam supply (J), so as to produce fruit mash by 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 minutes, 15-28 minutes, 12-30 minutes, 12-28 minutes or 12-25 minutes; and
- viii) Extracting crude oil by pressing the mashed palm fruitlets or MPD.

44. A crude palm oil obtained by the process according to any of the preceding claims.

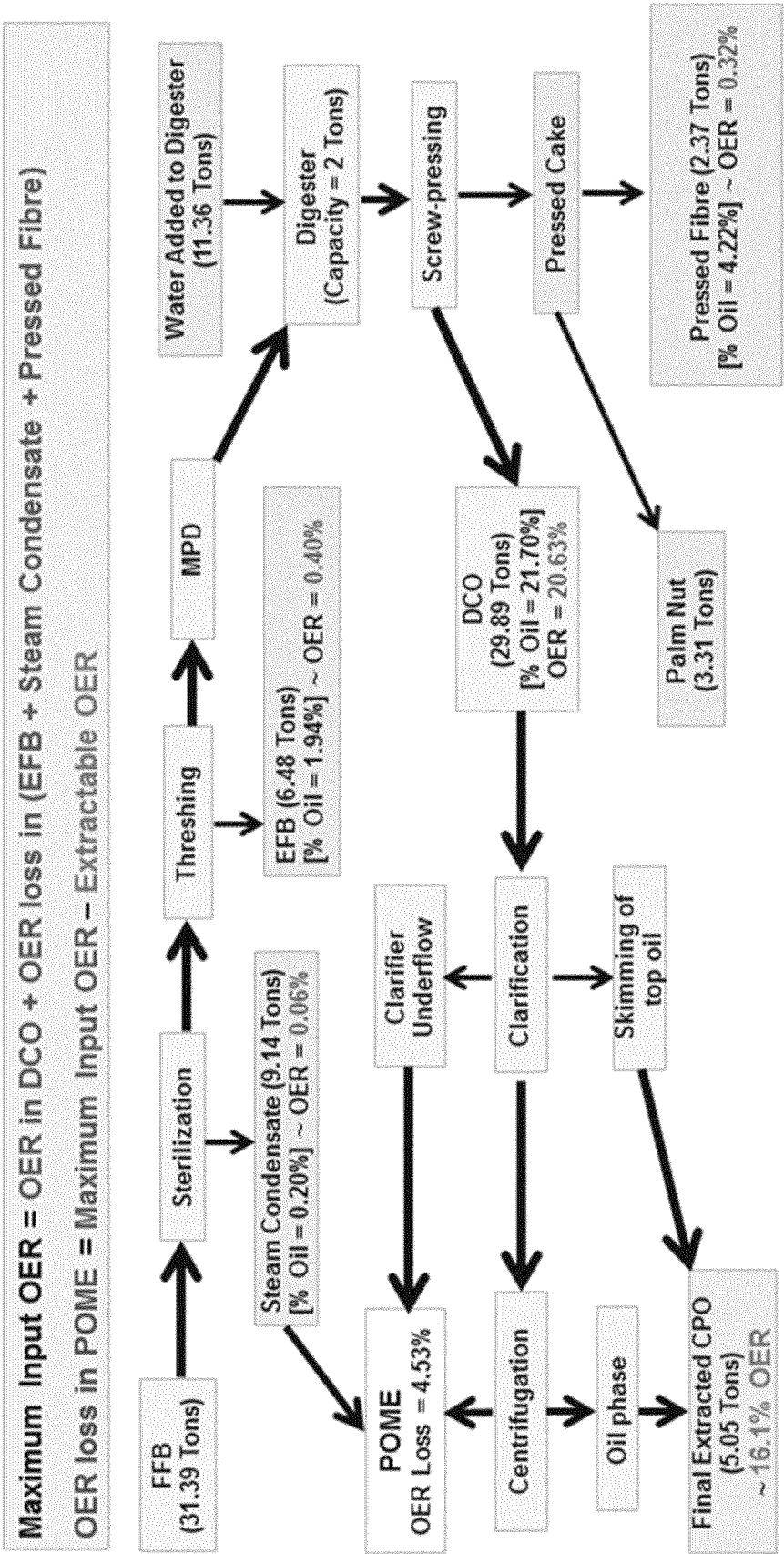


Figure 1

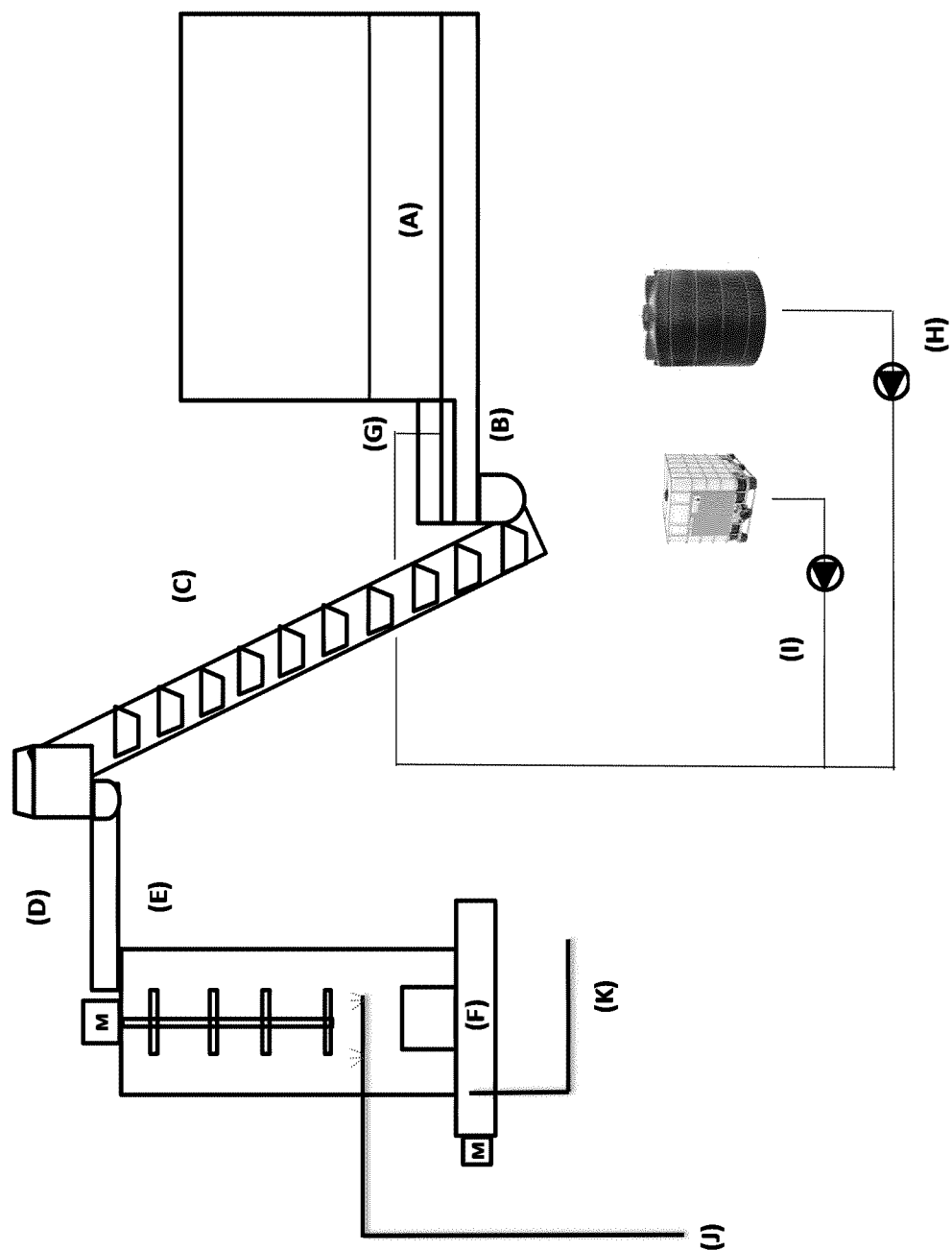


Figure 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/057802

A. CLASSIFICATION OF SUBJECT MATTER
INV. C11B1/02 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

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, BIOSIS, COMPENDEX, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	AU 2015 101 377 A4 (DUPONT NUTRITION BIOSCI APS) 5 November 2015 (2015-11-05) abstract; claims 1-6 page 33, line 13 - page 34, line 30 -----	1-44
X,P	WO 2015/150372 A1 (DUPONT NUTRITION BIOSCI APS [DK]) 8 October 2015 (2015-10-08) abstract; claims 1-5, 7, 14; figure 1 page 13, lines 11-16 -----	1-44
X	WO 2012/011130 A2 (ADVANCED ENZYME TECHNOLOGIES LTD [IN]; RATHI CHANDRAKANT LAXMINARAYAN) 26 January 2012 (2012-01-26)	44
Y	abstract; claims 13-18; example 2 page 9, line 27 - page 10, line 2 ----- -/-	1-43



Further documents are listed in the continuation of Box C.



See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"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

8 June 2016

Date of mailing of the international search report

15/06/2016

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/057802

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	HEMAVATHI SILVAMANY ET AL: "Enhancement of palm oil extraction using cell wall degrading enzyme formulation", MALAYSIAN JOURNAL OF ANALYTICAL SCIENCES, MALAYSIAN ANALYTICAL SCIENCES SOCIETY (ANALIS), MY , vol. 19, no. 1 1 January 2015 (2015-01-01), pages 77-87, XP002746751, ISSN: 1394-2506 Retrieved from the Internet: URL: http://www.ukm.my/mjas/v19_n1/pdf/Silvaman_19_1_10.pdf abstract pages 78-79 -----	44
A	abstract pages 78-79 -----	1-43
A	WO 2011/095643 A2 (NAT UNIV IRELAND [IE]; AER SUSTAINABLE ENERGY LTD [IE]; MURRAY PATRICK) 11 August 2011 (2011-08-11) page 9, paragraph 1 - page 10, last paragraph page 13, paragraph 2 -----	1-6

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2016/057802

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