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(54) **Title**: AN ENZYME COMPOSITION AND PROCESS FOR EXTRACTING OIL FROM OIL PALM FRUITS

(57) **Abstract**: The present invention provides an enzyme composition and process for extracting oil from oil palm fruits particularly from mesocarp portion or mesocarp containing oil palm fruits having portion with atleast with improved efficiency and/or increased yield of atleast 90%. The process comprises steps of: incubating the mixture containing mashed mesocarp portion or mesocarp containing mashed oil palm fruit mass, water and enzyme composition comprising enzymes having exocellulolytic, pectinolytic, mannanolytic and glucanolytic activity at about 25-65 degree Celsius for atleast 60 - 120 minutes and separating the palm oil from the enzyme treated mass and water.

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TITLE : AN ENZYME COMPOSITION AND PROCESS FOR EXTRACTING OIL FROM OIL PALM FRUITS

Field of the Invention

The present invention relates to an enzyme composition and enzymatic process for extracting oil from oil bearing fruits. More particularly, the present invention relates to an enzyme composition and process for extracting oil from oil palm fruits.

Background of the Invention

Crude Palm oil is edible plant oil extracted from the pulp that is fleshy mesocarp of the fruit of the oil palm *Elaeis guineensis*. Mescocarp accounts fro 60% of the total composition of the oil palm fruit. The oil derived from the mesocarp has been long recognized as cooking ingredient and is widely used as cooking oil in West African countries and Southeast Asia. Its use has increased in the commercial food industry owing to its high oxidative stability when used for frying. It has also been used in cosmetics.

Crude Palm oil is composed of fatty acids. It is high in 16-carbon saturated fatty acid palmitic acid; another major component of crude palm oil is monounsaturated oleic acid and it is low in myristic acid with virtually no lauric acid. Such composition makes crude palm oil healthier cooking oil compared to palm kernel oil which is derived from the same plant but from seeds. Both the oils are very different in chemical composition as well as physical properties. Palm kernel oil chiefly comprises of lauric acid and myristic acid and less of palmitic acid and stearic acid. Studies have shown that both stearic acid and palmitic acid, which comprise virtually all the saturated fats in palm oil, have neutral to favorable impact on serum lipid profiles compared to lauric and myristic acid. Besides cooking, even for cosmetics, crude palm oil is a better ingredient than palm kernel oil due to their composition.

Crude or Red palm oil not only comprises healthy composition of fatty acids, but also contains a blend of vitamins, antioxidants, and other phytonutrients important for good health. Red palm oil is the richest dietary source of provitamin A carotenes (beta-carotene and alpha-carotene). In addition to beta-carotene, alpha-carotene, it contains lycopene and at least 10 other carotenes, along with tocopherols and tocotrienols (members of the vitamin E family), CoQ10, phytosterols, and glycolipids. Owing to such components now a day many processed foods contain palm oil as an ingredient, it is blended into mayonnaise and salad oil, fortified into foods for specific health use and anti-aging cosmetics.

In addition to being used in cooking and personal care items, this oil can also be distilled into biofuel, and because of its low expense, many biofuel manufacturers have turned to palm oil.

All these have increased the demand of palm oil and once regarded as cheap oil it is becoming expensive and going out of the reach of larger section of society. Besides, to meet such demand clearing of rainforests for oil palm plantations has obvious detrimental environmental effects.

In view of the above it is rather desirable to improve productivity of oil by increasing the efficiency of oil extraction.

Commercially the palm oil is extracted from the mesocarp of the palm fruits by employing very complex and harsh mechanical process. The commercial palm oil extraction consists of pulping and pressing of fruits to get oil and juice from solid material. Fruits are subjected to pressurized steam for sterilization, they are then cooked by using hot water and high heat, after which they are digested in digester commonly consisting of steam-heated cylindrical vessel fitted with a central rotating shaft carrying a number of beater (stirring) arms, to rupture and break down oil bearing

cells, and finally oil is extracted by wet or dry methods. In the 'dry' method the oil is squeezed out of a mixture of oil, moisture, fibre and nuts by applying mechanical pressure on the digested mash. The fluid coming out of the press is a mixture of palm oil, water, cell debris, fibrous material and 'non-oily solids'. Because of the non-oily solids the mixture is very thick (viscous). In wet method, hot water is added to the press output mixture to thin it. The diluted mixture is passed through a screen to remove coarse fibre. The screened mixture is boiled from one or two hours and then allowed to settle by gravity in the large tank so that the palm oil, being lighter than water can separate and rise to the top. To prevent increasing free fatty acids through autocatalytic hydrolysis of the oil, the moisture content of the oil has to be reduced to 0.15 to 0.25 percent.

The oil palm mesocarp contains high percentage of gums in addition to oil and water which remains attached to the fibers and makes it difficult to separate gums from fibers and liberate oil. In commercially followed process despite very high temperature and harsh mechanical treatment as much as 30% or even more oil remains entrapped into fibrous, very viscous and sticky mass reducing oil extraction efficiency considerably. Also, as a result of such harsh treatments, many of the oil constituents get degraded resulting into inferior oil, further owing to large quantity of water used during the extraction, which if not removed sufficiently can increase the free fatty acid content, further deteriorating oil quality.

There have been attempts made to improve on such conventional method. Such improvement is disclosed in United States Patent No. 5039455. This process is made continuous by the steps of continuously introducing fresh fruit bunches in a receiving bin; while the fresh fruit bunches are in the receiving bin, treating the fruit bunches with steam to deactivate any enzyme responsible for the formation of free fatty acid in the fruit bunches; continuously removing fresh fruit bunches treated in the receiving bin, separating the loose fruit lets and continuously subjecting same to a combined sterilizing and stripping operation to detach fruit lets from the fruit bunches;

continuously feeding the fruitlets, each consisting of a nut surrounded by a pericarp, to a pressurized digester; continuously blowing the digested fruit to a blow tank; and continuously extracting oil by pressing the digested fruit mass.

WO/2007/038963 discloses a process and device for producing palm oil or vegetable edible oil from fresh fruits. It contain the steps of extracting crude oil from the fruits, clarifying the crude oil in a two-phase decanter and divide it into sludge and oil, and mechanically separating sludge water from the sludge of the two-phase clarification.

However, such attempts still do not get rid of harsh treatments, reduce time or increase oil extraction efficiency. Moreover, these methods require huge quantity of water during the processing leading to enormous amount of effluent at the end creating problem of its disposal and on disposal, environmental and ecological concerns. Also on account of such harsh methods being energy intensive and requiring large quantities of water make them costly.

Hence, there is need for a simpler approach for palm oil extraction, which improves efficiency, minimizes or does not employ large quantities of water and very harsh treatments, suitable at an industrial level and is viable.

Summary of the Invention

Accordingly, in one aspect the present invention provides an enzyme composition comprising enzymes having exocellulolytic, pectinolytic, mannanolytic and glucanolytic activity for extracting oil from oil palm fruits with improved efficiency.

In one specific aspect the present invention provides an enzyme composition comprising atleast one of exocellulolases, atleast one of pectinases, atleast one of mannanases and atleast one of glucanases for extracting oil from oil palm fruits, particularly from mesocarp portion or mesocarp containing oil palm fruits with atleast 90% oil yield.

In another aspect the present invention provides an enzyme composition comprising exocellulase having atleast 5000 u/gm activity, pectinase having atleast 500 u/gm activity, mannanase having atleast 10000 u/gm activity and glucanase having atleast 500 u/gm activity for extracting oil from oil palm fruits, particularly from mesocarp portion or mesocarp containing oil palm fruits with atleast 95% oil yield.

In anther aspect the present invention provides use of an enzyme composition of the present invention for extracting oil from oil palm fruits, particularly from mesocarp portion or mesocarp containing oil palm fruits with improved yield.

In another aspect the present invention provides an enzymatic process for extracting oil from oil palm fruits with improved efficiency and/or increased yield comprising steps of: incubating the mixture containing mashed mesocarp portion or mesocarp containing mashed oil palm fruit mass, water and enzyme composition of the present invention at about 25-65 degree Celsius for atleast 60 – 120 minutes and separating the palm oil from the enzyme treated mass and water.

Detailed Description of the Invention

The present invention is directed to an enzyme composition and a process for extracting oil from oil palm that is *Elaeis guineensis* fruits with improved efficiency. The present invention is specifically directed to an enzyme composition and a process for extracting oil from mesocarp portion or mesocarp containing oil palm fruits with improved efficiency.

The mesocarp portion is the outer fleshy, fibrous part of oil palm fruit surrounding the kernel or seed. The "mesocarp containing oil palm fruits" as used in the present invention refers to the entire oil palm fruits essentially containing mesocarp portion as well as other part of the fruit that is kernel.

In accordance with the present invention an enzyme composition comprises enzymes capable of extracting oil from from mesocarp portion or mesocarp containing oil palm fruits with improved efficiency and/or increased yield of atleast 90%, more preferably with atleast 95% yield. The enzymes composition of the present invention preferably comprises enzymes that extract oil from the mesocarp portion of the oil palm fruit but which essentially do not extract oil from the kernel or the seed part of the oil palm fruit.

In one embodiment the present invention provides an enzyme composition comprising enzymes having activities selected from exocellulolytic, pectinolytic, mannanolytic, and glucanolytic for extracting oil from oil palm fruits with improved efficiency.

In another embodiment the present invention provides an enzyme composition comprising atleast one of exocellulolases, atleast one of pectinases, atleast one of mannanases and atleast one of glucanases for extracting oil from oil palm fruits with atleast 90% oil recovery.

The enzyme composition of the present invention comprises enzymes in an effective amount so as to extract oil from oil palm fruit with improved efficiency and/or increased yield.

In still another embodiment the present invention provides an enzyme composition comprising exocellulase having atleast 5000 u/gm activity, pectinase having atleast 500 u/gm activity, mannanase having atleast 10000 u/gm activity and glucanase having atleast 500 u/gm activity. It was surprisingly found that the composition of the present invention comprising these specified enzymes in specified quantities provided enhanced oil extraction efficiency with ability of extracting atleast 95% of oil, particularly from mesocarp portion of the oil palm fruit.

In certain embodiments, the enzyme composition comprises exocellulase having 5000 u/gm to 20000 u/gm activity.

In certain embodiments, the enzyme composition comprises pectinase having 500 u/gm to 5000 u/gm activity.

In certain embodiments, the enzyme composition comprises mannanase having 10000 u/gm to 35000 u/gm activity.

In certain embodiments, the enzyme composition comprises glucanase having 500 u/gm to 5000 u/gm activity.

Alternately, enzymes may be added on the basis of percentage in the enzyme composition. For example exocellulase may be included in the range of about 2.5% to 8.5%. Pectinase may be included in the range of about 0.5% to 5%. Mannanase may be included in the range of about 25% to 65%. Glucanase may be included in the range of about 0.35% to 3.5%.

The enzyme composition of the present invention may optionally further comprise one or more additional enzyme having activity selected from amylolytic, arabinolytic, glucosidolytic, amyloglucosidolytic, endocellulolytic, ferulic acid esterolytic, hemicellulolytic, xylanolytic, proteolytic and/or phytolytic.

The enzyme composition of the present invention may be formulated in the dry powder form or liquid form.

The enzyme composition in powder form may be formulated by blending enzymes with inert fillers selected from the group consisting of but not limited to lactose, maltodextrin, starch, microcrystalline cellulose, sucrose and/or dextrin.

The enzyme composition of the present invention in liquid form may be formulated by dispersing enzymes in the base medium selected from group consisting of but not limited to glycerin, sorbitol and/or water.

In anther embodiment the present invention provides use of an enzyme composition of the present invention for extracting oil from oil palm fruits, particularly from mesocarp portion or mesocarp containing oil palm fruit with improved efficiency, preferably atleast 90% recovery, more preferably with atleast 95% recovery of oil.

In one embodiment the present invention provides a process for extracting oil from oil palm fruits with an increased yield, wherein process comprises steps of: incubating the mixture containing mashed mesocarp portion or mesocarp containing mashed oil palm fruit mass, water and enzyme composition of the present invention at about 25-65 degree Celsius for atleast 60 – 120 minutes and separating the palm oil from the enzyme treated mass and water.

For use in the process of the present invention, the mashed mass of the oil palm fruit may be obtained by crushing the oil palm fruits with seeds and mesocarp in suitable *mashing equipment for a sufficient time and preferably at a higher temperature* to get the mashed mass of oil palm fruits with seeds remaining intact. Mashing equipments to obtain the mashed mass of oil palm fruit should be such which mashes the fibrous part essentially comprising of mesocarp but keeps the seed intact, such mashing equipment may be selected from mixer, spiral mixer and or shredder or stripper or other suitable equipment. Mashing time of oil palm fruit may depend upon the mashing equipment

used, for example when spiral mixer is used mashing may be carried out for 10 minutes to 30 minutes.

Prior to mashing oil palm fruits are steamed and /or sterilized by subjecting them to suitable steaming and or sterilizing method. Preferably oil palm fruits are steamed at 130 degrees Celsius to 150 degree Celsius temperature and under high pressure for example 2.5 to 3.5kg pressure.

The steamed and mashed mesocarp portion or oil palm fruit mass is mixed with water and the enzyme composition as per the present invention by any suitable homogenizing means for example by stirring.

The mixture containing mashed mashed oil palm fruit mass and water is obtained by adding water in a specific ratio to substrate that is mesocarp portion or mesocarp containing oil palm fruit. In specific embodiments the ratio of substrate to water is from about 1: 0.5 to about 1:2.5, preferably in the ratio of 1:0.75 to 1:1.75.

The enzyme composition of the present invention may be added in a range from about 0.1 % to about 5% on w/w basis. Preferably the enzyme composition is added in an amount from about 0.5 % to about 3.5% on w/w basis.

The mixture comprising the mashed oil palm fruit mass, enzyme composition and water is incubated for sufficient time and under suitable conditions allowing enzyme composition to act on the crushed oil palm fruit mass and provide the oil with higher yield.

In certain embodiments the mixture of crushed oil palm fruit mass, enzyme and water is incubated for about 1 hour to about 24 hours, preferably from about 2 hrs to about 6

hrs. The mixture is incubated at the temperature of about 25 – 70 degree Celsius, preferably from 45 – 65 degree Celsius.

The enzyme treated mixture thus obtained has the liquid consistency so as to provide much ease in further processing as against the commercial methods. In the commercial methods the oil palm fruit mass after digestion is highly thick viscous mass having gums adhering to the fibers thereby making the extraction of oil therefrom very difficult. Further, by the conventional method of dry extraction the oil extracted from such mass is incomplete and does not give good quality oil. Whereas the conventional wet extraction method uses very high quantity of water which makes process very lengthy and difficult with respect to separation of oil and water and creates large quantity of effluent. Contrary to this surprisingly it has been found that the enzyme composition of the present invention makes the process very efficient by requiring very less water and despite such less use of water the enzyme treated mixture is of liquid consistency and having much less viscosity thereby allowing the ease in separation of oil from leftover mass of fruit as well as water adding much ease in the processing and rendering process efficient and energy efficient as compared to the conventional methods.

The enzyme treated oil palm fruit mass is subjected to suitable separation means and method for separating oil and water from the fruit residue comprising fibers and entire seeds in case when the whole fruits are used. For example fiber mass and entire seeds are separated with the help of Vibrio-separator. The oil and water mixture is subjected to suitable separation means to separate water and obtain oil.

The process as per the present invention provides crude palm oil with an increased yield of atleast 90%, preferably more than 95% or upto 98 % or even more. The oil is clear, dark red in colour has good viscosity and bleachability index and has low FFA content (free fatty acid). Thus, the enzyme composition as well as process of the

present invention provides superior quality oil without any degradation of oil or adverse effect on the quality of oil.

The left over water containing the remainder of the enzyme is reused as such or after adjusting the amount of the enzyme as required for another batch for extracting oil. The process of the present invention is made economically feasible by reusing left over water containing enzyme. This has huge economic advantage and helps in rapid reduction of costly enzymes. It also helps in recycling the water and thus making whole process effluent free. Thus, the present invention process is advantageous over the current commercially used process which leaves behind huge effluent and creates environmental hazards.

Thus the enzyme composition and the process of the present invention of extracting oil from oil palm fruits is advantageous and superior over the hitherto known methods on account of requiring less water during processing, adding much ease during processing, minimizing or avoiding harsh treatment, being less energy intensive, reducing time, not having any detrimental effect on the quality of oil and increasing yield.

The invention is further illustrated by the following, nonlimiting examples.

Example 1:

Formulation of the Enzyme Composition:

Example 1A: Comparative Enzyme Composition:

The comparative enzyme composition was formulated by blending exocellulase enzyme having activity range from 7500-15000 u/gm with percent ranging from 3.5% -6.5%, pectinase enzyme having activity 1000 – 2500 u/gm with the percent ranging from 1%-2.5% with the inert filler liklactose/maltodextrin with Q.S.

Example 1B: Enzyme Composition of the Present Invention:

The enzyme composition as per the preferred embodiment present invention was formulated by blending exocellulase having activity range from 7500-15000 u/gm with percent ranging from 3.5% -6.5%, pectinase enzyme having activity 1000 – 2500 u/gm with the percent ranging from 1%- 2.5%, mannanase enzyme having activity ranging from 15000-25000 u/gm with percent ranging from 35%-55%, glucanase enzyme having activity range from 750 -2500 u/gm with percent ranging from 0.5% -1.5%, with inert filler like lactose/maltodextrin with percent ranging from 20-30%.

Example 2:

Extraction of oil from Mesocarp Portion of Oil Palm Fruit:

Three sets were taken up for oil extraction:

2A: Using comparative enzyme composition as per Example 1A.

2B: Using enzyme composition of the present invention as per Example 1B.

2C: Control set that is without adding any enzyme.

Oil palm fruits were initially checked for their oil content by standard Soxhlet method and was found to have oil content of 65.99%. Three sets 1.4 kg each were taken for oil extraction. Palm fruits of all three sets were cleaned and steam cooked for 90 minutes at 146 degree Celsius for 3.4 kg pressure. The steamed fruits were crushed in the mixer and mashed mass was mixed with 1.4 ltr of water. To set 2A enzyme composition of Example 1A was added at the dose of 1.5%, to set 2B, enzyme composition of the present invention as per Example 1B was added at the dose of 1.5% and to set 2C no enzyme composition was added. Mixture of three sets were incubated at 55 degrees for 2 Hours. Set 2A and 2B were allowed to stand for 80 to 90 c for 15 min and then subjected to centrifugation to separate mass containing fibers with seed from oil and water layer. However for set 2C control, 30% more water was added on w/w basis to make it liquid as it being very thick and viscous mass before subjecting it to centrifugation step. The quantity of oil content was measured, FFA (free fatty acid)

content and deterioration of bleachability index of oil were measured and sugar in the water layer was measured by AOAC method.

Parameters	Set 2C	Set 2A	Set 2B
Viscosity of liquid after enzyme treatment in centipoises at 55^0	1815	401.12	3.44
Liquidity after enzyme treatment	Thick mass	Thick	Liquidity ++++++
Oil quantity in gm	30	41	65
Clarity of water layer	Not Clear at All	Less Clear	Very Clear
Gums attached to the fiber	gums and fiber completely attached	Gums attached with fiber	Gums detached from fiber
FFA Content	3.88%	3.85%	3.79 %
Deterioration of Bleachability index value by spectrophotometer	3.78	3.81	3.89
Sugar in the water layer in %	1.05%	6.87%	14.28%

The less FAA content in oil obtained by 2B indicates that the oil obtained would not be susceptible to rancidity.

Also, the deterioration of Bleach ability index(DOBI) value was compared with the standard values as below for the palm oil which is the numerical ratio of the spectrophotometer absorbance at 446nm to absorbance at 269nm. The DOBI value of the oil obtained as per the present invention is more than 3.24 and hence the palm oil is of superior grade.

BLEACHABILITY INDEX OF PALM OIL:

DOBI	GRADE
≤1.68	Sludge palm oil
1.68-2.3	Poor
2.31-2.92	Fair
2.93-3.24	Good
>3.24	excellent

From the above example it is clear that the enzyme composition of the present invention and process provides much enhanced recovery of the oil which is as good as 99% without any deterioration of the oil quality.

Various modifications may be made to the various embodiments of the present invention in part or whole without departing from the spirit and scope of the appended claims.

CLAIMS :

1. An enzyme composition comprising enzymes having exocellulolytic, pectinolytic, mannanolytic and glucanolytic activity for extracting oil from oil palm fruits with atleast 90% oil yield.
2. The enzyme composition as claimed in claim 1, wherein the enzyme composition comprises atleast one of exocellulolases, atleast one of pectinases, atleast one of mannanases and atleast one of glucanases.
3. The enzyme composition as claimed in claim 2, wherein the enzyme composition comprises exocellulase having atleast 5000 u/gm activity, pectinase having atleast 500 u/gm activity, mannanase having atleast 10000 u/gm activity and glucanase having atleast 500 u/gm activity
4. The enzyme composition as claimed in claim 3, wherein the exocellulase is present in the range of having 5000 u/gm to 20000 u/gm activity.
5. The enzyme composition as claimed in claim 3, wherein the pectinase is present in the range of having 500 u/gm to 5000 u/gm activity.
6. The enzyme composition as claimed in claim 3, wherein the mannanase is present in the range of having 10000 u/gm to 35000 u/gm activity.
7. The enzyme composition as claimed in claim 3, wherein the glucanase is present in the range of having 500 u/gm to 5000 u/gm activity.
8. The enzyme composition as claimed in claim 1, wherein the composition optionally further comprises one or more additional enzyme having activity selected from amylolytic, arabinolytic, glucosidolytic, amyloglucosidolytic, endocellulolytic, ferulic acid esterolytic, hemicellulolytic, xylanolytic, proteolytic and/or phytolytic.
9. The enzyme composition as claimed in claim 1, wherein the enzyme composition is formulated in the dry powder form or liquid form.
10. The enzyme composition as claimed in claim 9, wherein the enzyme composition in powder is formulated by blending enzymes with inert fillers

selected from the group consisting of but not limited to lactose, maltodextrin, starch, microcrystalline cellulose, sucrose and/or dextrin.

11. The enzyme composition as claimed in claim 9, wherein the enzyme composition in liquid form is formulated by dispersing enzymes in the base medium selected from group consisting of but not limited to glycerin, sorbitol and/or water.

12. Use of an enzyme composition of any one of claims 1 to 11 for extracting oil from mesocarp portion or mesocarp containing oil palm fruit with atleast 90% yield of oil.

13. A process for extracting oil from oil palm fruits with improved yield comprising steps of: incubating the mixture containing mashed mesocarp portion or mesocarp containing mashed oil palm fruit mass, water and enzyme composition as claimed in any one claims 1 to 11 at about 25-65 degree Celsius for atleast 60 – 120 minutes and separating the palm oil from the enzyme treated mass and water.

14. The process for extracting oil from oil palm fruits as claimed in claim 13, wherein, the mashed mass of the oil palm fruit comprising of mesocarp and intact seed is obtained by crushing oil palm fruit with equipment selected from mixer, spiral mixer, shredder or stripper.

15. The process for extracting oil from oil palm fruits as claimed in claim 14, wherein, prior to mashing, the oil palm fruits are steamed and /or sterilized by subjecting them to steaming at 130 degrees Celsius to 150 degree Celsius temperature under 2.5 to 3.5kg pressure.

16. The process for extracting oil from oil palm fruits as claimed in claim 13, wherein the mixture containing mashed mesocarp portion or mesocarp containing mashed oil palm fruit mass and water is obtained by adding water in the ratio of substrate to water from 1: 0.5 to 1:2.5, preferably in the ratio of 1:0.75 to 1:1.75.

17. The process for extracting oil from oil palm fruits as claimed in claim 13, wherein the enzyme composition is added in the range from 0.1 % 5% on w/w basis, preferably from 0.5 % to 3.5% on w/w basis.

18. The process for extracting oil from oil palm fruits as claimed in claim 13, wherein the yield of the oil is atleast 90%, preferably more than 95% or upto 98 % or even more and the oil obtained is clear, dark red in colour has good viscosity, bleachability index and has low FFA content (free fatty acid).