PALM OIL EXTRACTION USING ENZYME MIXTURE TREATMENT

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Specially dedicated to my beloved family and friends for the continuous support, encouragement and motivation

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ABSTRACT

In this study, application of aqueous enzymatic process to enhance the recovery of palm oil was studied. Experiments were conducted to study the effect of different combinations of enzyme mixture towards the percentage of oil extracted with respect to total oil content in palm mesocarp. The optimum combination of enzymes comprising of cellulase (X1), peptinase (X2), and xylanase (X3) for Aqueous Enzymatic Oil Extraction Process were determined using Simple Lattice mixture design (Design of Experiments). Maximum oil recovery of 85.95% was achieved with ratio of enzyme at 0.67:0.17:0.17 (X1: X2: X3), at enzyme loading of 30 mg protein/10 g substrate, substrate loading of 50% w/v, pH 4.8 and 2.0 hours of incubation at 50 ^{0}C. The concentration of reducing sugars at corresponding experimental runs was measured to evaluate the degree of hydrolysis and oil extracted. Concentration of reducing sugar trend was found not to be similar of the trend of oil extraction Analysis using Design Expert software for optimum condition showed a cellulase to xylanase ratio of 0.78:0.22 for 84.79% of oil recovery. A confirmation run performed produced 83.8% palm oil recovery.

ABSTRAK

Dalam kajian ini, penggunaan enzim untuk meningkatkan pengekstrakan minyak sawit telah dikaji. Eksperimen telah dijalankan untuk mengkaji kesan gabungan campuran enzim yang berlainan ke atas peratusan minyak yang diekstrak serta jumlah kandungan minyak dalam mesokarp sawit. Gabungan optimum enzim yang terdiri daripada selulase (X1), peptinase (X2), dan xylanase (X3) untuk Proses Pengekstrakan Minyak Enzimatik Aqueous telah ditentukan menggunakan reka bentuk campuran Simple Lattice (Reka Bentuk Eksperimen). Pengekstrakan minyak maksimum sebanyak 85.95% dicapai dengan nisbah enzim pada 0.67: 0.17: 0.17 (X1: X2: X3), pada pembebanan enzim 30 mg protein / 10 g substrate, pembebanan substrat sebanyak 50% w / v, pH 4.8 dan 2.0 jam inkubasi pada 50 ^{0}C. Kandungan gula dalam mesokarp diukur untuk menilai hubungkait antara tahap hidrolisis serta minyak yang diekstrak. Kepekatan mengurangkan trend gula didapati tidak sama dengan trend pengekstrakan minyak. Analisis menggunakan perisian Design Expert untuk mengetahui keadaan optimum menunjukkan nisbah selulase kepada xylanase 0.78: 0.22 akan menghasilkan pengekstrakan minyak sebanyak 84.79%. Ujikaji pengesahan yang dilakukan menghasilkan pengekstrakan minyak sawit sebanyak 83.8%

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LIST OF ABBREVIATION

α	-	alpha
β	-	beta
^{0}C	-	degree Celcius
µ	-	micro
%	-	percentage
AEOE	-	aqueous enzymatic oil extraction
AEOEP	-	aqueous enzymatic oil extraction process
AEP	-	aqueous extraction process
BSA	-	bovine serum albumin
BOD	-	biochemical oxygen demand
C	-	carbon
CBHII	-	Cellobiohydrolase II gene
COD	-	chemical oxygen demand
CPO	-	crude palm oil
CSTR	-	continuous stirred tank reactor.
DNS	-	dinitrosalicylic acid
EBB	-	empty fruit branches
ETP	-	effluent treatment plant.
FFA	-	free fatty acid
FFB	-	fresh fruit branches
G	-	gram
Ha	-	hectare
LDL	-	low density lipoprotein
Kg	-	kilogram
M	-	meter
M	-	Molar
MDF	-	medium density fibreboard

mg/L	-	milligram per Litre
Nm	-	Nano
OD	-	optical density
pI	-	isoelectric point
POME	-	palm oil mill effluent
ppm	-	parts per million
RBDO	-	refined, bleached and deodorized oil
Rpm	-	revolution per minute
T	-	tonne
TAG	-	triacylglycerol
UASB	-	up-flow anaerobic sludge blanket
UASFF	-	up-flow anaerobic sludge fixed-film
USD	-	United States Dollar
USDA	-	United States Department of Agriculture.
v/w	-	volume by weight
w/w	-	weight by weight

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CHAPTER 1

INTRODUCTION

1.1 Background

Palm oil is one of the 17 major oils and fats produced and traded worldwide (Jaafar and Sukaimi, 2001). It is widely used as edible product and its importance is increasing as dietary component for over one billion people. Edible fats are used as vegetable oil to produce margarine, shortenings and functional food. The dietary trend today is to replace animal fats with vegetable origin fats. Although oil palm diet can lead to higher cholesterol as compared to corn, soyabean, safflower seed and sunflower oil, intake of palm oil leads to endogenous cholesterol level to drop (Edem, D.O, 2002). It is believed that this is assisted by the presence of tocotrienols and peculiar isomeric position of fatty acids. Palm oil benefits to health include reduction in risk of arterial thrombosis and atherosclerosis, aggregation of platelet, inhibition of endogenous cholesterol biosynthesis and reduction in blood pressure (Edem, D.O, 2002).

The oil palm was introduced from West Africa to the Bogor Botanical Gardens, Indonesia in 1848, arrived on Malaysian shores in 1871 and known an ornamental or decorative plant at that time. In less than 100 years oil palm has moved from being a relatively minor subsistence crop in West and Central Africa to one of the world's major agricultural commodities (Wicke *et al*, 2011). Malaysia and Indonesia are two largest producers accounting for approximately 85% of world's oil palm production (Sulaiman *et al* 2011).

The oil palm was commercially exploited as an oil crop only from 1911 when the first oil palm estate was established (Basiron *et al*, 2000). The fruits produce two main products – crude palm oil from the palm fruit oil (outer mesocarp) and palm kernel oil from the kernel within the fruit. In December 2015, Malaysia has produced 1.4 million tonnes of crude palm oil and 200 thousand tonnes of kernel oil (MPOB 2001). This shows that we are the major key player in palm oil industry and contributing to economic growth.

The tree grows up to 20 to 30 meters high, has an economic life span of 25 to 30 years. The female bunch bears about 2500-3000 fruits borne on 100-120 spikelet attached to a peduncle from the axil of a frond and weighs as much as 30-40 kg (MPOB 2001). Palm oil is extracted from highly perishable oil palm fruit through a series of processing which involves harvesting, sterilization, stripping, digestion, clarification, purification, vacuum drying and nut recovery (Basiron et al 2000).

There have been a significant number of researches on enzymatic oil extraction from plant seeds such as rapeseed, soybean, coconut, avacoda, sunflower and peanut. Oil extracted from this seeds has been promising, yielding about 60-90%, mainly depending on enzyme used and other contributing factors such as oilseeds size, pH, time, temperature, solid water ratio, moisture, number of extraction stages and agitation degree (Cater C M et al., 1974). Research by Rosenthal A, et al, 1996 showed that palm oil has very high oil content, of about 97.7%, followed by coconut 80-90%, soybean, 86% and avocado, 75%. This research would be highlighting on the extraction of oil from palm oil by using different combination of enzymes as this has a potential to recover 80-90% of oil content in the palm oil. To further support this, there have been researches conducted on palm oil extraction using mixture of enzymes with good output yield of about 80-90% (Silvamany, H. & Jahim, J.M. 2015). This research is intended to study the different combination of three enzymes as compared to previous research and to see how different combination enzymes affects the oil output.

1.2 Problem Statement

The ideal composition of palm fruit bunch is usually as such – kernel per fruit: 5-8%, mesocarp per fruit: 85-92%, oil per mesocarp: 20-25%, oil per bunch: 23-25%. In the palm oil industry, the complete process of extraction of edible oil from oil palm involves mechanical pressing at temperature ranging from 90 ^{0}C to 140 ^{0}C. Generally, fresh fruit bunches undergo sterilization process at 140 ^{0}C for about 75 to 90 minutes to deactivate hydrolytic enzyme responsible for the breakdown of oil to free fatty acid (FFA) and also to loosen the fruits on the bunch to facilitate stripping (Mohammad N.E. *et al.*, 2015).

Separated fruits are then heated in a digester aided with rotating paddle impeller at a temperature of 85 to 90 ^{0}C to mash the fruit which results in release of 20 to 30 % of free oil from fruit mesocarp. The crude palm oil is extracted with a screw press under high pressure and then clarified to remove dirt, fibres or gums. The crude palm oil is further processed to obtain refined, bleached and deodorized oil (RBDO). The oil that was not extracted remains in solid residue and end up as waste oil. However, aqueous enzymatic oil extraction can be employed in our palm oil industry due to its potential as an environmentally cleaner alternative technology for oil extraction and produce significant increase in oil yield. The release of oil facilitated by cell wall degrading enzyme is able to exhibit greater than 90% oil extraction efficiency (Rosenthal, A. *et al.*, 1996). Hence, enzymatic oil extraction is a promising area of study as compared to other oil extraction methods and is chosen as part of research for this study. Design expert® software with process variable of Simplex lattice is utilised in this study to analyse the outcome of the research. This class of design is chosen since a simplex lattice analyses mixture variable only provided all components have same range and no constrains on design space.

Aqueous enzymatic oil extraction from plant material is said to increase oil yield. The combination of enzymes which favours the oil extraction from mesocarp might not be at its optimum. Suitable combination is not exactly known. Since no single enzyme is adequate for the efficient maceration and extraction of oil, the best combination ratio of cellulose, xylanase and pectinase enzymes can give the highest oil extraction (Faveri D.D. *et al*., 2008). Duration of reaction and optimum temperature will be fixed in order to study the relationship between percentage of oil extraction and reducing sugar concentration. The concentration of reducing sugar may give an indication of the extent of breakdown of cell wall in the palm oil mesocarp. A higher reducing sugar concentration indicates a higher degree of breakdown of cellulose to simpler forms of sugar, thus increasing the oil extraction percentage. Hence, the correlation of trend of reducing sugar concentration and percentage of oil extraction is not known and will be studied in this research. This is vital to further understand the behaviour of enzymatic reactions.

1.3 Objective

The objectives of this research are:

1. To formulate best enzyme mixture for aqueous enzymatic oil extraction by using Design Expert® version 10.

2. To study the reducing sugar concentration formation in an aqueous oil extraction process. This may provide a degree of knowledge on enzymatic degradation of cell wall.

1.4 Scope

The scopes of study include:

1. Pre-treatment of palm oil mesocarp.
2. Enzyme protein concentration determination.
3. Formulation of best enzyme mixture, based on different combination of enzyme mixture.
4. Aqueous oil extraction by organic solvent in a Soxhlet apparatus.
5. Analysis of reducing sugar concentration by using Dinitrosalicylic acid (DNS) method.

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