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Optimization of Aqueous Enzymatic Oil Extraction from Kernel of Oil Palm (*Elaeis Guineensis*) Using three Phase Partitioning and Microwave Irradiation

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Abstract

The use of microwave irradiation as a pretreatment before aqueous enzymatic oil extraction from oil palm kernel was found to be useful. The microwave irradiation for 10 min -assisted extraction was found to be a simpler and more effective alternative to the solvent extraction methods for the productions of palm kernel oil. Further enhancement was achieved when the microwave irradiated slurries were treated with a commercial enzyme preparation of proteases, followed by three phase partitioning. This resulted in 93% (w/w) oil yields form the palm kernel. The efficiency of the present technique is comparable to solvent extraction with an added advantage of being less time consuming and using *t*-butanol which is a safer solvent as compared to *n*-hexane used in conventional oil extraction process. The technique also tries to reduce the amount of enzyme used and hence reduces the overall cost.

Key words: M*icrowave irradiation; palm kernel; aqueous enzymatic extraction; three phase partitioning.*

Introduction

Industrial processes for the extraction of edible oil from oilseeds generally involve a solvent extraction step which may or may not be preceded by pressing. Extraction with *n*-hexane is a widely used approach for obtaining edible oils [1]. For such processes, it is possible to achieve oil yields in excess of 95%.

The new tendency to avoid the use of toxic organic solvents in large installations has renewed interest in alternative extraction processes (involving the use of water, alcohol aqueous solutions and supercritical fluids) and has led during the last three decades to continuous research on biorenewable solvents [2,3]. Three phase partitioning has been extensively used for both upstream and downstream steps in bioseparation of proteins/enzymes [4,5].

Although water is not a specific oil solvent, aqueous processes represent an innovation in extraction technology for any processed oilseed [3, 6-10]. Aqueous oil extraction is undoubtedly an emerging technology in the fats and oil industry since it presents no risks of fires and explosions, the solvent is not toxic, the mild processing ensures high quality in the products of the process, and does not produce volatile organic compounds (VOC) as pollutants. The operation is more flexible since start-up and shut-down are safer in the absence of flammable solvents. The main disadvantages, as compared to conventional technologies, are the lower efficiency of oil extraction, the reduction of the product stability, which contains more residual oil and the ease of microbial contamination. Permeability of the cells wall to oil passage can be increased either by mechanical or thermal conditioning or by enzymatic digestion of the cell walls. Aqueous

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enzymatic oil extraction (AEOE) has emerged as a promising technique for extraction of oil from plant materials [11,12]. Its main advantages are that it is environment friendly and does not produce volatile organic compounds as atmospheric pollutants [13]. The use of hydrolytic enzymes in aqueous enzymatic oil extraction (AEOE) has enhanced the oil yield of the aqueous extraction process. Cellulose, hemicellulose and pectic substances constitute 80-90% of the cell wall polysaccharide [14-16] and hence the use of these hydrolytic enzymes will facilitate the release of oil from oil bodies enmeshed in protein and cellulosic/hemicellulosic network [13]. One disadvantage associated with AEOE is the long process time which are necessary for enzymes to liberate oil bodies. Another factor (sometime neglected) is the use of enzymes which are not commercially available [17].

Palm oil is reddish-orange oil extracted from the mesocarp of the oil palm (*Elaeis guineensis*) fruit. A unique feature the oil palm has over other oil crops is that it can deliver two quite distinct types of oil - palm oil from the flesh of the fruit (a mixture of oleic, C18:1 and palmitic oils, C16:0 and about 50% saturated), and palm kernel oil from the seed or kernel (mainly lauric acid, C12:0, and more than 80% saturated). For every 10 tonnes of palm oil, about 1 tonne of palm kemel oil is also obtained [18-20]. The fruits of the oil palm contain between 55 and 70 percent of oil. The seeds of the fruit, the seed kernels also contain oil [21]. There are various methods used to extract the palm oil from the pulp and the seed. The method used most extensively is pressing the pulp to remove the oil. In palm oil milling, the fruits are steam-sterilized and digested for about 40 minutes, the mesocarp is separated from the kernel before pressing it to extracted the oil. The aim of this pre-treatment step is basically to break up the oil-bearing cells (also known as lipid bodies or oleosomes) in order to facilitate the release the oil [10]. The kernel oil is extracted by crushing and pressing or can be done with the help of solvents. Palm kernel may contain up to 50% oil per total weight of the kernels. Palm kernel cake (PKC) is the solid residue left from the extraction of oil from palm kernel. The oil is extracted from the kernel by expeller, i.e. continuous screw press.

Selected enzymes have been tried on different types of oilseeds, resulting in extraction yields much higher than the original aqueous process (in some cases of over 90%). These enzymes mainly hydrolyze the structural polysaccharides which form the cell wall of oilseeds or the proteins which form the cell and lipid body membrane. This concept has already been commercialized for the production of olive oil and has also been investigated for other oil-bearing materials [10, 11, 22-24]

The present work describes an alternative approach for extraction of palm kernel oil using aqueous extraction together with protein degrading enzyme, microwave irradiation and three phase partitioning (TPP) for further optimization of oil extraction process.

Materials and Methods

Materials

From the total fruits approximately 5% of kernels would be produced. These kernels would then have to undergo another level of oil extraction. The Palm kernel (dehulled) contains approximately 50% oil and around 80-85% of the oil can be removed by extraction [20]. The oil palm fruits were a gift from MPOB Bangi, Selangor. The kernels were ground using a hammer mill running at 478 RPM. A stainless steel screen with a mesh size of 2 mm was used to obtain a uniform particle size. The ground kernels were wrapped in airtight plastic bags and stored at 4^{o}C until used.

Enzyme

The enzyme used in this study was a protease (Alcalase), which operates optimally under basic conditions. It was selected based on previous work by Rosenthal, et. al. [11] that found that extraction of oil from soybean using protease resulted in significantly higher yields of oil. The protease used in the experiments, Alcalase 2.4L (Novo Nordisk), had a declared activity of 2.4 AU/g (AU = Anson Unit), which is equivalent to 2,736 I.U. (international standard unit) when soya isolate at pH 8.0 and 50°C was used as substrate.

Methods

Preparation of palm kernel

After removing the fruits from the bunch, about 100-150g of the fruits were put in a beaker and water was added so as to submerge the fruits. The beaker was put in a microwave oven equipped with magnetic stirer and non-contact infrared continuous feedback temperature system (model RM 800, Plazmatronika, Wroclaw, Poland; operating frequency 2.5 GHz). The temperature was set at 100^{o}C (for 10 min). The next step was to pound the fruits while they are still hot and soft to separate the mesocarp from the nut. The nuts were cracked, the shell carefully removed and the kernel thus obtained were used for oil extraction.

Grinding

The palm kernels were ground using a hammer mill (Standard Model No.3, Arthur Thomas Co., Philadelphia, PA, U.S.A.) running at 478 RPM. A stainless steel screen with a mesh size of 4 mm, 2 mm, 1.0 mm and 0.75 mm was used to obtain a uniform particle size of the above range. The ground kernels were kept in air tight plastic bags until used. Some studies have shown that particle size can play an important role in oil extraction yield [11, 25].

Aqueous phase oil extraction and effect of particle size

Preliminary study was conducted to see the effect of particle size on the yield of oil. This will be used as basis for selection of best particle size for other experiments and also as a basis for calculation of oil recovery for the other method. The ground kernel (25g) of the various particle sizes as mentioned above, were dispersed in 100 ml distilled water and stirred to make a suspension. The pH of the suspension was adjusted to 8.0, and was heated to 50^{o}C overnight with constant shaking at 100 rpm (same condition as used later for enzymatic extraction). The upper oil phase was collected after centrifugation at 10,000 x g for 20 min and weighed. The amounts of oil recovered were calculated as percentages of total oil present in palm seed kernels, which was determined by soxhlet extraction using hexane as a solvent as per the standard AOAC procedure [26]. The particle size that gave the highest oil yield was then used in all subsequent experiments. The solvent extraction of oil using the AOAC method was taken as 100% recovery of oil while calculating the oil recovery by the other subsequent methods.

Effect of microwave on aqueous oil extraction (AOE)

To see the effect of microwave irradiation, the ground kernels (25g) of particle size 0.75 mm (since it gave slightly higher oil yield as compared with larger size particles as determined above) were dispersed in 100 ml distilled water and stirred to make a suspension. The pH of the suspension was adjusted to 8.0 as above. The slurry was heated to using microwave irradiation (output power 700 Watt) with constant stirring using build in magnetic stirrer for 2, 4, 6, 8 and 10 min. Another sample which was not treated with microwave was used as a control. The upper oil phase was collected after centrifugation at 10,000 x g for 20 min and weighed. The amounts of oil recovered were calculated as percentages of total oil present in palm seed kernels using the standard AOAC procedure as above [26].

Microwave assisted aqueous enzymatic oil extraction process (AEOE)

The hydrolytic enzymatic treatment to enhance oil extractability was performed. As above, the ground kernels (25g) of particle size 0.75 mm were dispersed in 100 ml distilled water and stirred to make a suspension. The pH of the suspension was adjusted to 8.0 (optimum pH for enzyme activity). As mentioned above in 2.2.2 the sample was subjected to microwave irradiation (output power 700 Watt) with constant stirring using build in magnetic stirrer for 6 min. For comparison another sample was subjected to aqueous extraction using the similar method as described in 2.2.3, at 50^{o}C for overnight with constant shaking at 100 rpm (no microwave irradiation). Then 1% (v/w) of the protease enzyme Alcalase was added separately to both samples and

the mixture were incubated at 50°C for 1 hr. The upper oil phase was collected as described above and the amounts of oil recovered from both samples were calculated as previously mentioned.

Extraction of oil by three phase partitioning after enzyme treatment

Recently three phase portioning has been reported as an alternative method for oil extraction [12]. Both the samples (microwave treated and non microwave treated) after enzymatic treatment were subjected to three phase partitioning as described previously by Sharma et al., (2002). The pH was adjusted to 7. Varying amount of ammonium sulphate (20%, 30%, 40%, 50% and 60% (w/v) was added to the samples and mixed gently, followed by the addition of the 100 ml of *t*-butanol (ratio of sample slurry to *t*-butanol is 1:1; v/v). The mixture was mixed gently and allowed to stand for 1 hr at 25°C for the three phase formation. The three phases formed were separated by centrifugation at 2000*g* for 10min. The upper organic layer was collected and evaporated on a rotary evaporator to obtain oil extracted in this phase. The amounts of oil recovered were calculated as percentages of total oil present in the kernel. The amount of oil present in these sources was determined by soxhlet extraction using hexane as a solvent as per the standard procedure as mentioned above [26]. The solvent extraction of mango kernel/soybean/rice bran gave yields of 16, 25 and 16.5 g/100 g of plant sources taken (w/w), respectively.

Further study on the effect of varying pH was done. Using the optimal ammonium sulphate concentration as obtain above, the pH of the samples was varied. Before the addition of ammonium sulphate, the pH of the samples were adjusted to 4, 7 or 9 by adding 0.1N HCl or 0.1N NaOH. The sample then gentle stirred with a magnetic stirrer. Subsequently the samples were subjected to three phase partitioning as described previously.

Calculation of the oil recovery

The amount of oil released was calculated as a percentage of the total oil present in the palm kernel. The latter was determined by soxhlet extraction using hexane as a solvent as per the standard AOAC procedure [26]. The soxhlet solvent extraction of palm kernel gave a yield of 42 g oil/100g of palm kernel. For calculation of the oil recovery by aqueous extraction, aqueous enzymatic extraction methods and three phase partitioning, a value of 42 g oil /100g palm kernel was taken as 100% recovery of the oil. Each extraction was run in duplicate and the yields were found to agree in duplicates within 3%.

Results and Discussion

The effect of particle size on oil extraction yield was determined. Particle size reduction from more than 1 mm to 0.75 mm gave slight improvement in the oil extraction for the smaller sizes. The increased was around 7-8 % higher for the smaller size particles. Since it was found that particle size of 0.75 mm gave slight better yield compared to other larger particle sizes, it was chosen for further experiments. The effect of particle size in aqueous extraction has been shown previously in studies on aqueous extraction of oil from sunflower kernel [23] and soybean [11].

Microwave irradiation is emerging as a powerful tool to accelerate many chemical and physical processes. Previously it has been shown that ultrasonication can increase the oil yield during aqueous oil extraction (AOE) [17, 27]. It was shown that using ultrasonication as a pretreatment allows one to cut down the process time to about 6 h without reducing the over all yield [17]. Fig. 1 shows the effect of microwave irradiation exposure time on the oil yield by aqueous oil extraction method as mentioned in section 2.2.4. Six minutes of microwave exposure gave about 52% oil yield while the sample that was not exposed to microwave irradiation (at time 0 min.) gave only about 25% oil yield. This clearly shows that microwave treatment greatly enhances the oil extraction process. Although the enhancement in rate of many chemical reactions have been reported using microwave irradiation, it is still not very clear whether the enhancement are purely due to thermal effects or other non thermal effects [28]. It has been shown that microwave irradiation causes the some protein structural changes which make them become more granular shape instead of 'splattered' structure. This

presumably allows much better accessibility of oil and other substance from the internal structure of the kernel. The result from previous studies using aqueous extraction gave results close to that of the control samples (aqueous extraction without microwave) processed under same conditions. Aqueous oil extraction reported for palm kernel was 27% [3] while for *Jatropha curcas L* was reported as 38% [29]. There would be a limit to the microwave treatment time because it is likely that longer exposure times would lead to thermally induced chemical transformations of the oil resulting in a reduction in the oil yield and quality. Protein engineering has been used to alter the catalytic properties of enzymes by site directed mutagenesis. We can also say that microwave irradiation has the ability to alter the substrate so that they become more accessible towards the enzyme and hence produce much faster reaction rates.

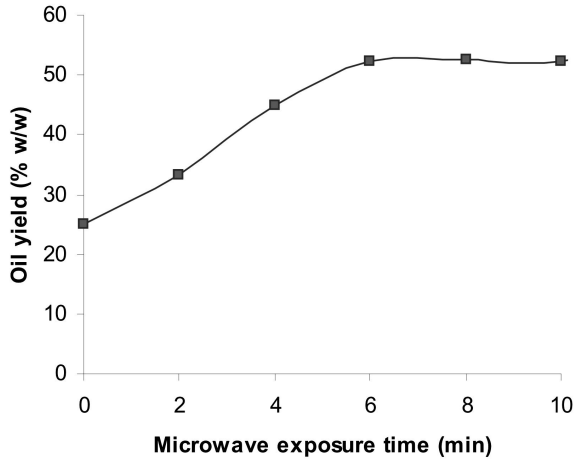

Figure 1. Effect of microwave irradiation exposure on % of oil yield by AOE method

(The ground kernels (25g) of particle size 0.75 mm, dispersed in 100 ml distilled water (pH 8) The slurry, heated using microwave (output power 700 Watt) with constant stirring for 0 (control) 2, 4, 6, 8 and 10 min. The oil was recovered as described in text).

Microwave assisted aqueous enzymatic oil extraction (AEOE) process showed further enhancement in oil yield from palm kernel. The oil yield from microwave assisted aqueous enzymatic extraction, using the protease enzyme Alcalase with one hour incubation at 50^{o}C (in which the kernel had been previously subjected to microwave heating with output power 700 W for 10 min at pH 8) was found to be in the range of 70 – 75 %. In aqueous enzymatic extraction, enzymes are used to facilitate release of oil from oil bodies enmeshed in protein and cellulosic / hemicellulosic network [13]. Fig. 2 shows that the oil yield from palm kernel using protease enzyme is around 73%. The further enhancement in the yield of oil (from 52% to around 73%) using alkaline protease enzyme indicates that oil is trapped in oil bodies which are enmeshed in protein network. It was established previously that only alkaline proteases should be used during AEOE from *J. curcas* L. seed kernels [17, 30]. It was also shown that oil extraction from soybean was improved with the use of protease but other enzymes such as hemicellulases and cellulases had no significant improvement [11]. It was postulated that enzymes that can hydrolyse the proteins that form the cell and oil bodies membrane should favor oil extraction. This is on the basis of the observation that oil bodies enmeshed in the cytoplasm, which is predominantly composed of protein, are themselves surrounded by proteinaceous membrane [11, 31].

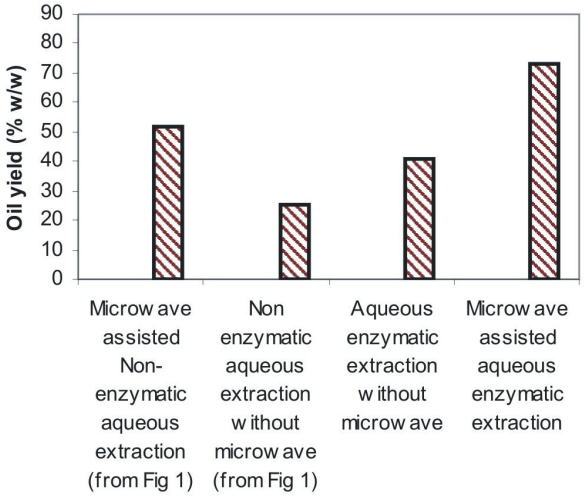

Figure 2. Effect of proteolytic enzyme on % of oil yield by EAOE method

(The ground kernels (25g) of particle size 0.75 mm, dispersed in 100 ml distilled water (pH 8.0). The slurry was heated using microwave as described in 2.2.5 (output power 700 Watt) for 6 min. For control, conventional heating at 50^{o}C for overnight (no microwave irradiation). Then 1% (v/w) of the protease enzyme Alcalase was added separately to both samples and the mixture were incubated at 50^{o}C for 1 hr. The oil was recovered as described in text).

Three phase partitioning has been extensively used for both upstream and downstream steps in bioseparation of proteins/enzymes [4, 5]. Previously, it was shown that enzyme pretreatment before carrying out three phase partitioning (TPP) led to significant improvement in oil yields in the case of soybean, rice bran and mango kernel [32]. A study was carried out to see if TPP would also enhance oil yield from palm kernel. The effect of varying ammonium sulphate on the amount of oil extracted from palm kernel in the *t*-butanol phase is shown in Fig. 3.

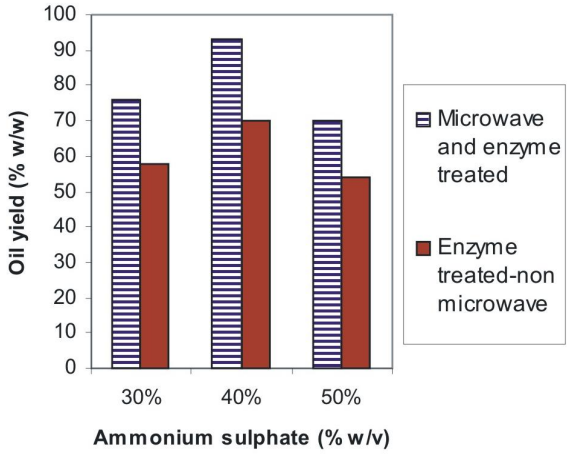

Figure 3. Effect of varying amounts of ammonium sulphate in TPP on oil extraction at pH 7

(The ground kernels (25g) of particle size 0.75 mm, dispersed in 100 ml distilled water (pH 7.0). After microwave and enzyme treatment as in 2.2.5, varying amounts of ammonium sulphate (%, w/v) were added followed by addition of *t*-butanol (20mL). For non microwave conventional heating was done. The three phases formed after incubating the slurries at 37 °C for 1 h were then separated by centrifugation at 2000*g* for 10 min. The oil was recovered from upper *t*-butanol layer by following the procedure described in text).

With palm kernel 40% (weight/volume (w/v)) ammonium sulphate gave maximum oil yields of 93% at pH 7. Use of greater than 50% (w/v) ammonium sulphate did not produce a three phase formation. Further optimization by varying the pH was performed. The effect of varying pH of slurries before TPP on oil yield is shown in Fig. 4. The best pH for palm kernel was 7.0 at 40% (w/v) ammonium sulphate concentration, giving an oil yield of 90% (w/w). The efficiency of the present technique is comparable to solvent extraction with an added advantage of being less time consuming and using *t*-butanol which is a safer solvent as compared to hexane used in conventional oil extraction process. The strategy outlined here should make TPP a more widely used technique. Three-phase affinity with the combination of microwave irradiation and enzyme treatment should prove to be a very useful and powerful technique, particularly with its ease of scaling up. An additional feature of this process is the possibility of simultaneous recovery of protein (hydrolysates). The data shown here also indicates that using microwave irradiation as a pretreatment allows one to cut down the process time drastically without reducing the over all yield. Aqueous enzymatic extraction with microwave and TPP is a better and more efficient alternative approach to oil extraction. An important aspect in this study was to use a minimal amount of enzyme, so as to reduce the cost of the entire process.

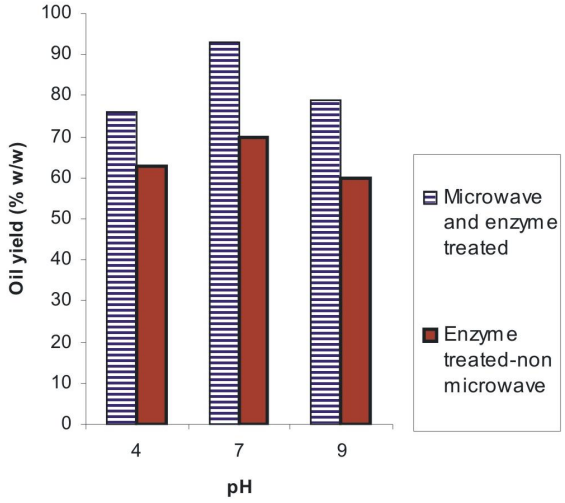

Figure 4. Effect of varying amounts of ammonium sulphate in TPP on oil extraction at pH 7

(The ground kernels (25g) of particle size 0.75 mm, dispersed in 100 ml distilled water (pH 7.0). After microwave and enzyme treatment as in 2.2.5, varying amounts of ammonium sulphate (%, w/v) were added followed by addition of *t*-butanol (20mL). For non microwave conventional heating was done. The three phases formed after incubating the slurries at 37°C for 1 h were then separated by centrifugation at 2000*g* for 10 min. The oil was recovered from upper *t*-butanol layer by following the procedure described in text.)

The cost of enzyme might be a factor in the consideration of alternative approach for oil extraction. Any strategy that can lower the cost of enzyme will definitely encourage use of alternative biotechnological processes. In fact using three

phase partitioning allows smaller amounts of enzyme to be used to achieve the optimal effect. In such cases, the overall effective cost of the enzymes would decrease substantially. A similar approach need to be tried with other plant materials, the results described here indicate that aqueous enzymatic extraction with combination of microwave irradiation and three phase partitioning may be a useful technique for extraction of oil from plant materials.

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