Enzyme Aided Processing of Oil

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ABSTRACT

Enzyme aided process (EAP) has been identified as a viable alternative for oil extraction. This paper reviews the recent trends in EAP of soybeans during degumming, transesterification, oil extraction, and demulsification. The "common protease effect" and the degree of demulsification of oil was also examined. EAP has improved overall oil yield to about 90% and high oil quality. EAP oils have high tocopherol content, decreased turbidity, higher color index, better oxidative stability, and drastic reduction in residual phosphorus content of oils thus contributing to enhanced oil quality. We conceptualize "Common enzyme effect" as the reason for degree of demulsification (°Dem) occurring during enzyme aided extraction. The equation for rate of change of °Dem have also been proposed. Few studies on simultaneous EAP have shown the efficacy of multifunctional enzyme to enhance oil recovery and quality. The use of immobilized enzyme has also received less attention, though, could potentially reduce EAP cost.

1. Introduction

The conventional techniques used to separate oil from meal is by solvent extraction because it is economical, cheap, and easily practiced. In this process, solvent is used to wash the oilseed, thereafter, separated from the oil by evaporation and distillation. The most widely used solvent is hexane because of its unique characteristics which are easy oil recovery, narrow boiling point (63–69°C), and solubilizing ability. However, hexane is highly flammable and has exposure risk to personnel [1]. When inhaled by human, it dissolves in neural lipids and affects the neural system thereby raising safety concerns raising safety concerns. Furthermore, hexane is emitted during oil extraction process into the atmosphere, which react with pollutant to form ozone and photo chemicals. Environmental safety and Halal concerns have led to development of alternative techniques with considerations to yield of the oil to meet the growing need of food security [2].

Enzyme aided process (EAP) has been identified as a viable alternative for oil extraction. In this technique, enzymes are used to breakdown the cell structure of plant and pseudo-membrane surrounding the oil bodies. This reduces barriers to oil thus increases oil recovery. However, the high enzyme cost is prohibitive to EAP industrial feasibility. Therefore, we reviewed research that makes EAP of oil to provide a concise information and potential research gaps for future studies.

2. Oil processing

2.1 Oil Extraction

Oils are majorly utilized as edible food, nutrient supplements, or cosmetics. Common methods of oil extraction process are the mechanical process, chemical process or sometimes a combination of both [3]. Mechanical processes are less effective and often result into a reduced oil yield [4]. Although, chemical process such as solvent extraction is a highly efficient method in terms of oil yield, it is however expensive and produces poor-quality protein [5]. Moreover, the commercial solvent used in the extraction process (hexane) is of major concern. It has been labeled as a volatile organic compound by the United States Environmental Protection Agency [6]. Volatile organic gases can react with other atmospheric pollutants to form ozone which is hazardous to plants and human health. Its utilization must therefore be strictly supervised and checked. The enumerated factors have made the conventional processes of oil extraction quiet challenging, making it expedient to seek better alternatives.

Another emerging technology in the oil industry is the aqueous extraction process (AEP). AEP is a technique in which water is used as a means of extraction and separation of oil based on its insolubility in water medium [7]. Following the dissolution of soluble cellular materials, free oils are expelled into the liquid and then centrifuged and demulsified to recover free oil [8].

AEP has lots of advantages over the conventional methods of oil extraction. Apart from being environmentally friendly as compared to hexane extraction which is prone to explosion and release of volatile organic gases [7], oils and proteins are also simultaneously recovered with less protein denaturization [9]. However, over the years aqueous extraction process have become less effective in achieving enhanced oil yields [9]. Problems attached to this method are the necessity to demulsify oils formed as emulsion, treatment cost of process effluents [9] and majorly the low oil yield due to unavailability or concealment of unextracted oils in residues and protein isolates of oilseeds resulting into a reduced oil extraction efficiency [3].

Research findings have shown that plant vacuole houses a larger percentage of oil. However, diffused oil in the cytoplasm is often hard to access during extraction process and thus, oil is eventually lost to wastes as residues [10]. To forestall losses of oils to wastes and retrieve oil enveloped in the cell, the cell wall must be broken down. Breaking up of cell walls is known to aid the discharge of oil from oilseeds [7]. To break up the cell walls of plants, scientist have explored other methods of oil extraction. Based on the knowledge of location of oils in oil seeds and the mechanism of extraction, selected enzymes may be put into effective use [11].

2.2 Degumming

After the extraction of crude vegetable oil from seeds, oil refining is next. The process of oil refining consists of processes such as degumming, acid neutralization, bleaching and deodorization [12]. Degumming is the first refining process done to crude vegetable oil. It is defined as a process of oil refining aimed at removing impurities such as phospholipids, lecithin, mucilaginous gums and trace metals that tend to negatively impart oil quality in terms of odor, shelve life, color, and flavor [13]. Degumming is a highly essential stage in oil processing since it helps oil attain the standard of quality and stability which are the basic requirements in acceptance and marketing of oil products [13]. It makes oil highly competitive in the world market and also fit for other domestic uses such as salad dressing, frying, dairy foods and baking [14]. For degumming to be considered successful, the refining process must bring down phosphorus to <10 mg/kg [12].

Over the years, various methods of degumming have evolved. These methods are water degumming, total degumming, ultrafiltration process, super degumming, and acid degumming processes [15]. Of these methods, water degumming [16], acid degumming [17] and ultrafiltration process [18] are classified under the traditional methods of degumming. The major setback in water degumming is that nonhydratable phosphatides (NHP)) cannot be removed from vegetable oils [19, 20].

In the case of acid degumming, the residual phosphorous content is not within the range of <10 mg/kg due to the further addition of phosphoric or citric acid thus falling short of the requirements of large-scale refining [21]. Ultrafiltration process is relatively uncommon in the process of degumming and it is often prone to membrane fouling, a major issue that requires high fixing cost [18]. Total degumming and super degumming methods, often referred to as modified acid degumming processes have proven to be unreliable over the years and so rarely used in the oil industry [16, 22].

2.3 Demulsification

Demulsification simply means the separation of emulsion such as oil from a water medium. It is the extraction and recovery of oil from various sources especially oilseeds with the use of catalysts (enzymes) under varying conditions for oil yield enhancement [23]. Therefore, demulsification is the extraction of oil using demulsifiers which in most cases are surface-assimilative ingredients projected to move at the oil-water bonds to knock off the forces of emulsifying agents present. Demulsification is of various types. They include Enzymatic demulsification, chemical demulsification, mechanical demulsification, thermal demulsification, microwave demulsification, electrical demulsification, ultrasonic demulsification, membrane demulsification, hexane extraction and electrical and thermal demulsification [24]. Chemical demulsification which involves the addition of surfactants and demulsifiers bring about a minified phase separation ratio and very high demand for chemical reagents thus increasing cost [25].

2.4 Transesterification

Biodiesels are esters of fatty acid derived from animal fats or vegetable oils through a process called transesterification. Transesterification is the catalytic break up of triglycerides into fatty acid alkyl esters and glycerol in the presence of alcohol [26]. It is the most common method used for biodiesel production. Methanol and ethanol are commonly used alcohols for transesterification due to their cheapness, quick dissolution, and reaction with triglycerides [27]. Moreover, basic catalysts such as KOH and NaOH are examples of common catalysts used in transesterification of vegetable oils. Transesterification reaction occurs consecutively in three reversible steps in the presence of alcohol to form intermediate compounds such as diglycerides and monoglycerides [28]. Monoglycerides are finally converted to glycerin with each step producing one fatty acid alkyl ester molecule [29, 30]. For every one mole of triacylglycerol (TAG) that is completely converted, three moles of biodiesel and one mole of glycerol are produced [28]. Biodiesel has been reported to have various advantages over conventional fossil fuel. Such advantages include less emission of carbon monoxide when combusted by an engine, high flash point and high lubrication value [31]. They are also biodegradable and non-toxic [32].

3. Enzyme Aided Process of oil

There are 3 basic steps involved in enzyme aided process of oil for food applications (Figure 1). They include extraction, demulsification and degumming. Aqueous extracted oil is commonly subjected to enzyme aided demulsification to give enzyme demulsified oil. Enzyme aided degumming resulted into degummed oil. Several enzymes have been reportedly used to perform these 3 processes. Some researchers have equally used combination of enzymes. The following sections will provide recent reviews of enzyme aided process of oil including transesterification.

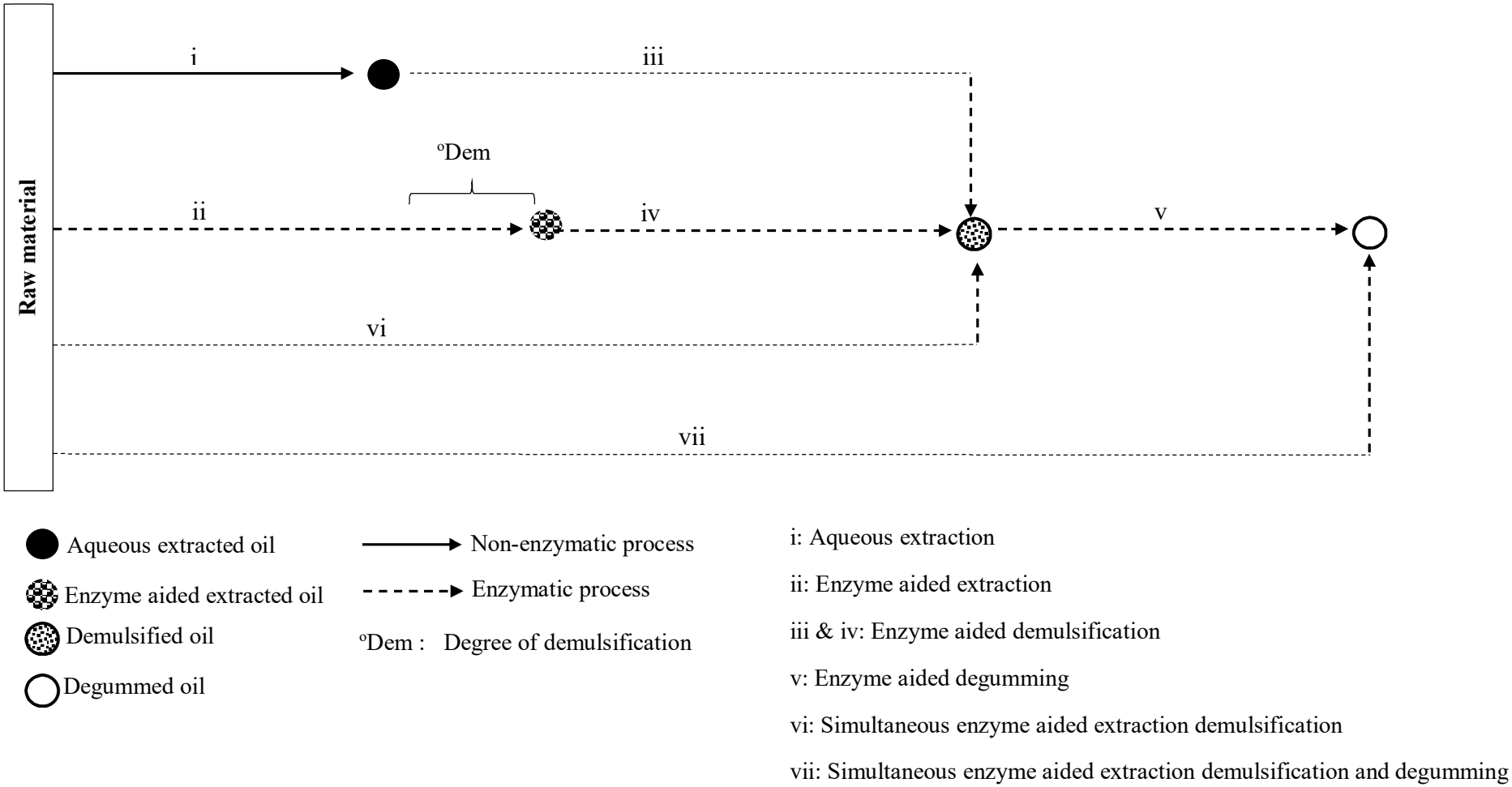

Fig.1 Various routes for enzyme aided process of oil

3.1 Enzyme Aided Oil Extraction

Enzyme-assisted extraction process (EAEP) is the process of increasing oil extraction yield where enzymes are used to collapse pseudo membranes and proteins enveloping oil bodies eradicating extraction roadblocks. EAEP is gradually gaining popularity over AEP as a better alternative due to its cheapness, better oil yield, environmental-friendliness, and safe nature [33]. According to Najafian, Ghodsvali [3], enzymatic extraction methods are quiet useful in oil manufacturing industries due to a considerably high enzyme specificity and fairly low operating temperatures. These factors in turn helps to alleviate the aqueous extraction process [34]. Research has shown that selection of enzymes for aqueous enzymatic extraction is based on differences in the composition of oilseeds [9]. Studies of various seeds majorly concentrate on oil quality and properties of the extracted oil. Application of enzymes in aqueous extraction process to step up oil yield has been variously reported [11, 34-36].

A study demonstrated the effectiveness of enzyme-assisted aqueous extraction process on aqueous enzymatic extraction of sesame oil and protein [37]. The study involved the use of 5 types of enzymes- Protex 7L, Alcalase 2.4L, Viscozyme L, Natuzyme, and Kemzyme. This is explained in Figure 1 where soy oil was subjected to simultaneous extraction and demulsification which only becomes efficient by ensuring 100% demulsification, resulting to enzyme extracted demulsified oil.

At optimum reaction conditions, results showed that Alcalase 2.4L attained the highest oil yield with a value of 57.4% of the total oil in the seed. Extracted oil characteristics such as oil density, color, refractive index (R.I), free fatty acid contents and iodine value showed no significant difference when compared with oils extract using AEP. The enzyme extracted oil had more tocopherols (a measure of natural antioxidant activity of oils as compared to AEP [3]. It also exhibited a more stable oxidative state and higher antioxidant activity.

Moreover, in the study of aqueous extraction of virgin olive oil using industrial enzymes, Najafian, Ghodsvali [3] analyzed the effects of enzymes (Pectinex Ultra SP-L and Pectinase 1.06021) employed in the treatment of three varieties of virgin olive oil. The research was carried out to investigate the probability of utilizing enzymes for increment in oil yield and quality. At optimum conditions, results obtained for enzyme-treated virgin oil established that enzymes concentration had a marked effect on the oil yield which increased from 0.9% to 2.4% wet basis. Total polyphenol content also increased from 18% to 76%. Other physicochemical properties such as turbidity decreased while color had a higher index. However, properties such as peroxide value and iodine value showed no significant difference when compared to AEP. Overall results of the research established that enzyme aided extraction, apart from being especially useful in increasing oil extraction also substantially enhanced the physicochemical properties of oil with Pectinex Ultra SP-L being the better of the two enzymes.

Latif and Anwar [37] also investigated the effect of enzymes on aqueous extraction of canola (*Brassica napus* L.) seed oil and protein using four enzymes, Protex 7L, Multifect Pectinase FE, Multifect CX 13L, and Natuzyme. At optimum conditions (Solid to Liquid ratio (S/L) of 1:6, time of 2h, agitation speed of 120 rpm and temperature of 45ºC), oil yield of the enzyme-treated canola seeds (22.2–26.0%) was found to be substantially higher than the one extracted without enzyme (16.48%). The physicochemical properties such as R.I, Iodine value, density, unsaponifiable matter, peroxide and saponification values of extracted oil were analyzed using the American Oil Chemist Society (AOCS) standards (AOCS 1997). Results showed that physicochemical properties were indifferent to the extraction methods. However, enzyme extracted oils displayed a higher oxidative stability ranging from 2.40 – 2.53 as compared to ordinary aqueous extraction method with a lower value of 2.51. Similarly, tocopherol levels also increased considerably for EAEP in comparison with non-enzyme treated aqueous extraction method. It was concluded that the canola oil enzymatic extraction further boosted the quality of oil in terms of both physical and chemical properties.

Furthermore, in a classified research on advances in aqueous extraction processing of soybeans by Campbell, Glatz [6], oil extraction yield of up to 97–99% was achieved with extruded soybean flakes using a countercurrent 2-stage extraction strategy with operating conditions such as enzyme (Protex

6L) concentration of 0.5%, Solid to Liquid ratio (S/L) of 0.20, temperature of 50°C, pH 8 (1 h) and pH 9 (15 min) [38]. However, for extractions involving no enzyme under similar condition of S/L (0.10), temperature of 50°C, pH 7 (1 h), pH 8 (15 min), only 68% oil extraction yield was achieved [39]. The effects of enzymes on physicochemical properties of oil were however not reported.

3.2 Enzyme Aided Demulsification

Enzyme aided demulsification involves the use of enzymes to improve oil recovery from aqueous extracted emulsion [23]. Enzymatic demulsification is not only an environmental friendly invention [38] but also a low energy cost alternative [40]. Protease based enzymatic demulsification has been found to be more efficacious in demulsification than chemical methods (salts such as $CaCl_2$ and NaCl) and physical methods (heating, freezing or thawing) [41].

A study on characterization and demulsification of the oil-rich emulsion from the aqueous extraction process of almond flour revealed that enzymatic demulsification improved the physical and chemical properties of the resulting cream protein [42]. Enzymes are selected based on their powers to reduce significantly amount of protein-oil matrix [43]. Protease enhances the collapse of cell wall proteins enveloping the oil bodies thus eradicating major roadblocks to oil extraction [7]. The use of high active alkaline enzymes contributed to destabilization of the aqueous extraction processes oils [42]. It must however be noted that for optimal efficiency of demulsification enzymes, other operating parameters such as temperature, pH and incubation time must be taken into consideration [44].

Many researches have been conducted on aqueous enzymatic extraction and demulsification of seed oils. Fang, Fei [23] studied the effects of individual and combined enzymes on free oil yield from Camellia seed (*Camellia oleifera Abel.*). Enzymes used in the experiment include protease, pectinase, cellulose, hemicellulose, and amylase. Treatment with protease and cellulase resulted in an oil yield of 63.87% and 61.25% respectively. However, when camellia seed oil was subjected to treatment with hemicellulose, pectinase and amylase, oil yields drastically reduced to 32.68%, 40.56% and 31.96% respectively. This suggests that both protein and cellulose contribute to oil emulsion formation. With protease and cellulase producing the highest oil yields, they decided to experiment with the two combinations while keeping other parameters such as pH, temperature, and time in check. The combination produced the highest yield of 77.09%. This combination was subsequently used in demulsification of camellia seed oil. This discovery corroborates the use of multifunctional enzyme mixture to improve oil extraction yield.

In another study involving multifunctional enzymes for simultaneous demulsification and degumming, the efficacies of some proteases and phospholipase on oil-rich emulsion from enzyme assisted aqueous extraction of extruded soybean flakes was examined [7], The effect of enzyme concentration on demulsification using a phospholipase A2 and a protease (Protex 51FP) was determined. Each enzyme significantly destabilized the cream when applied to a 2% concentration of w/w enzyme and (cream + free oil) combination.

In a similar findings on destabilization of the emulsion formed during the enzyme-assisted aqueous extraction of oil from soybean flour by [44], substantial amount of oil was recovered with the use of enzymes proteases and phospholipase to destabilize the soybean cream. Treatment with 300 mg of Protex 6L /10 g of cream with an agitation speed of 500 rpm yielded up to 72% free oil. However, with the use of 100 mg of Protex 6L in a 2-phase demulsification process, about 95% of the emulsified oil was changed to free oil.

The extraction and demulsification of oil from wheat germ, barley germ, and rice bran using an aqueous enzymatic method by [45] featured the use of enzymes such as Protex 6L, Protex 7L, Alcalase, Fermgen, Lysomax and G-zyme 999. According to them, Protex 6L yielded a free oil of 63.8% for commercial wheat germ and 59.5 % with laboratory milled wheat germ.

So far, the yield of free oils has been improved tremendously with the use of enzymatic treatment to reduce cream emulsion stability of different types of seed oils as shown in Table 1.

Table 1. Enzymatic treatment of Oil Seeds for improved cream emulsion destabilization

Oil source	Enzymes	Processing condition	Oil yield (%)	Reference
Peanuts	Papain	enzymes concentration was 1.5% (w/w) and at their optimal conditions	90.7 ± 2.2[hi]	[41]
camellia seed oil	Protease	camellia seed to water ratio of 1:6, pH 4.5, enzyme conc. of 1% (v/w), and a hydrolysis temperature of 50°C for 8 h	63.87 ± 2.41[a]	[23]
Wheat germ	Protease	ratio of substrate to water of 2:10, 10 % enzyme (V/W), pH 8 and a hydrolysis temperature of 50 C and a duration of 20 h	57.1 ± 3.4[y]	[45]
Soybean	Protex 6L	Agitation speed of 500rpm, pH 9 and 50 ◦C	65 ± 4[z]	[44]

[a] An assumption that 100% oil recovered with the hexane extraction
[y] Significant differences at the 5 % level
[z] The oil yield was calculated based on the 65% (95%CI = 2%) average value of oil content from 3 batches of enzyme-assisted aqueous extraction processing (EA-AEP) cream.
[hi] Data are expressed as the mean ± SD

Enzyme aided oil extraction raises a pertinent question about the measure of degree of demulsification (°Dem) achieved due to "common protease effect". This concept explains the fact that enzyme protease used initially to degrade aqueous extracted oil during enzyme aided extraction is also likely to have helped during enzyme aided demulsification. Therefore, the emulsion in route iv is less than that of route iii in Figure 1. Hence, enzyme aided extracted oil requires less demulsification when compared to the demulsification of aqueous extracted oil. We have proposed a concept of degree of demulsification °Dem to represent the demulsification taking place during enzyme aided extraction. After further degumming, we expect that the resulting oil is characterized by improved oil yield and higher quality due to retention of tocopherol and removal of phospholipids.

The degree of demulsification can thus be calculated as follows:

$$^{o}Dem \quad = \frac{C_{(EnExt)} - C_{Aq}}{C_{Aq}} * 100 \qquad () \qquad (1)$$

Where
°Dem = Degree of demulsification
C_{EnExt} = Concentration of enzyme extracted oil
C_{Ag} = Concentration of aqueous extracted oil

The rate at which demulsification occurs can also be achieved by integrating with respect to time thus:

$$R = \int_{t1}^{t2} \Delta D_{Dem} \qquad (2)$$

Where
R = Net rate of demulsification
t1 = initial time
t2 = final time
D_{Dem} = Change in degree of demulsification

3.3 Enzyme Aided Degumming

Enzymatic degumming is another renowned, often reported approach to degumming. Chakrabarti, Rao [46] reported that it is probably the most effective method available nowadays for achieving a reduced phosphorus content of vegetable oils (<10 mg/kg). The research has shown that actions of enzymes on phospholipids vary based on their types. However, they are all known to step-up the oil yield reclaimed during the degumming process [46]. Examples of enzymes used in the enzymatic treatment of vegetable oils are phospholipase A1 (PLA1) and phospholipase A2 (PLA2) [17, 46], phospholipase B (PLB) [46] and phospholipase C (PLC) [17].

The efficacy of enzymatic degumming cannot be overemphasized. Application of PLA1 under optimum experimental conditions and enzyme dosage of 200 U/kg of oil for 3h brought about a sharp reduction in phosphorus content from 544.51 mg/kg to 3.02 mg/kg. Similarly, PLA2 treatment under the same reaction conditions reduced phosphorus content from 544.51 mg/kg to 5.81 mg/kg. The overall reaction achieved about 97% degumming. The oils obtained are therefore of good quality on account of its residual phosphorus content [12]. More so, the enzymatic treatment of crude oil with *Pseudomonas fluorescens* BIT-18 enzyme yielded a phospholipid concentration of 5 mg/kg under a reaction temperature of 40ºC and time of 5h. Furthermore, in the treatment of vegetable oil using magnetic immobilized PLA1 under a temperature of 56 ºC, reaction time of 3h, enzyme dosage of 0.10 g/kg, and added water of 2.13 ml/100g, the degumming process for soybean oil yielded a final residual phosphorus of 10.38 mg/kg [47].

In a similar research on the enzymatic degumming of soybean oil with magnetic immobilized PLA2 (PLA2-Fe3O4/SiOx-g-P(GMA)) as reported by [48], PLA2 was utilized for enhancement of a static degumming activity. With a reaction time of over 5h, enzyme dosage of 0.24 g/kg, temperature of 55ºC and pH of 5.0, the degumming process for soybean oil yielded a final residual phosphorus of 9.8 mg/kg and a free fatty acid contents of 0.84 g/100 g.

A pilot-scale experiment also reported the use of PLC in the optimization of the degumming process for camellia oil [49]. Under optimum experimental conditions and enzyme dosage (PLC) of 400mg/kg, the degumming process for camellia oil yielded a final residual phosphorus of 15.14 mg/kg with a 98.2% camellia oil yield. It was further discovered from the research that camellia oil treatment with another type of enzyme (PLA) may further keep down phosphorus content to about 6.84 mg/kg. Indications from these results suggests that combining PLA and PLC treatments in degumming process may be a better substitute for a host of other degumming methods [49].

So far, as demonstrated in most of the studies on enzymatic degumming, the residual phosphorus content of oils was drastically reduced thus contributing to an enhanced oil quality. Table 2 shows the effect of enzymatic degumming on residual phosphorus content of oil.

Table 2. Effect of Enzymatic degumming on residual phosphorus content of oil

Oil source	Enzymes	Processing condition	Residual Phosphorus (mg/kg)	Reference
Soybean	Phospholipase A1	56 ºC temperature, reaction time of 3h, enzyme dosage of 0.10 g/kg, and added water of 2.13 ml/100g	10.38	[47]
Soybean	Magnetic immobilized Phospholipase A2	Reaction time of over 5h, enzyme dosage of 0.24 g/kg, temperature of 55ºC and pH of 5.0.	9.8	[48]
Sunflower	Phospholipase A2	Reaction time of 3h temperature of 50 °C, pH 5 and an enzyme dosage of 200 U/kg of oil	5.81	[50]

Camellia oil	Phospholipase C	Reaction time of 2.2h, temperature of 53°C, pH of 5.4, and enzyme dosage of 400mg/kg	15.14	[49]
Sunflower	Phospholipase A1	Reaction time of 3h temperature of 50 °C, pH 5 and an enzyme dosage of 200 U/kg of oil	3.02	[50]
Crude Oil	Pseudomonas fluorescens BIT-18	Temperature of 40°C and time of 5h.	5.00	[49]

3.4 Enzyme Aided Transesterification

Enzymatic transesterification of oils using lipase has also been extensively studied. Though, commonly used in acidosis, alcoholysis and glycerol hydrolysis, the use of enzyme lipase for esterification and transesterification has also been discovered [51]. Studies show that enzyme lipase enhances better product separation. It is also highly stable to heat, and its immobilized residue can still be reused effectively [52]. It is however expensive and prone to initial loss of enzyme activity.

Candida antarctica lipase is the most utilized enzyme for transesterification [41]. More so, in a classical research on biodiesel production through transesterification of waste cooking oil, a 90% biodiesel yield was recovered [53]. It was discovered during transesterification that the use of methanol caused a reduction in lipase activity. This problem was however solved by a bit-by-bit addition of methanol to the reaction mixture. Enzymatic transesterification of oils with reference to lipase catalysis ease glycerol recovery and improves biodiesel yield.

4. Conclusion and recomendation

Researchers have opted for the enzymatic aqueous extraction process of oil seeds based on its numerous advantages over aqueous extraction process and other methods. It has not only improved overall oil yield to as much as 90% but also improved the overall quality of resulting oil in terms of increased tocopherol content [37], decreased turbidity, higher color index [3] and better oxidative stability [37]. Other properties such as refractive index, iodine value, density, unsaponifiable matter, peroxide, and saponification values however were not affected [33]. In a similar manner, the efficacy of enzymatic degumming has also been established in a study on the effect of degumming process on physicochemical properties of sunflower oil using enzymes PLA1, PLA2 and water degumming process [50]. Furthermore, enzymes of demulsification play a highly significant role in the free oil recovery from seeds [25]. The use of Enzymatic treatment to destabilize the cream emulsion in order to improve oil recovery yield has been widely used in demulsification process of Aqueous Extraction Process (EAP) [7, 54].

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