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Aqueous enzyme assisted oil extraction from oilseeds and emulsion de-emulsifying methods: a review

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Abstract

Regulatory, safety, and environmental issues have prompted the development of aqueous enzymatic extraction (AEE) for extracting components from oil-bearing materials. The emulsion resulting from AEE requires de-emulsification to separate the oil; when enzymes are used for this purpose, the method is known as aqueous enzymatic emulsion de-emulsification (AEED). In general, enzyme assisted oil extraction is known to yield oil having highly favourable characteristics. This review covers technological aspects of enzyme assisted oil extraction, and explores the quality characteristics of the oils obtained,

focusing particularly on recent efforts undertaken to improve process economics by recovering and reusing enzymes.

Keywords

aqueous oil extraction, enzyme treatment, oil yield, oil characteristics, emulsion separation

1. Introduction

Aqueous enzymatic extraction (AEE) is a promising method for the simultaneous extraction of oil and protein from oilseeds. The products are of superior quality and highly suited to human consumption. In the extraction process, water containing selected enzymes forms the extraction medium used for incubating the oilseeds. When enzymes are not employed, the process is termed as aqueous extraction which invariably results in lower oil yield. The use of enzymes allows separation of targeted extracted components with unchanged properties which can potentially influence, favourably, the final product in terms of taste and smell. Interest in this technological approach has also increased recently due to safety and environmental regulatory concerns. In comparison with solvent extraction, the use of an aqueous medium is much safer, environmental-friendly and economical. In addition, it contributes to a much safer and flexible operation, lower energy consumption and operational costs, and lower capital investment. A variety of temporal crops can be processed, and the extracted oil does not need further refining. Non-toxic meal and value-added fibre and protein are also produced as co-products, due to the milder operating conditions employed. In addition, the aqueous medium allows simultaneous

separation of phospholipids from the oil. Therefore, degumming step (in case of oilseeds) is not necessary and the overall cost of processing can be reduced (Latif & Anwar, 2011; Latif *et al.*, 2011; Yang Li *et al.*, 2011; Chabrand & Glatz, 2009; Jung & Mahfuz, 2009; Wu *et al.*, 2009; Soto *et al.*, 2007; Santos & Ferrari, 2005; Gros *et al.*, 2003; Hanmoungjai *et al.*, 2001; Rosenthal *et al.*, 2001; Sineiro *et al.*, 1998; Ksenija *et al.*, 1997; Rosenthal *et al.*, 1996)

Despite the advantages, the application of AEE is still limited due to long processing time and the high cost spent for the drying process after the enzyme treatment (Shah *et al.*, 2005; Dominguez *et al.*, 1996). The high cost may also be attributed to the enzymes themselves, because a significant amount is required (normally >1% of the weight of the oilseed taken). Further, the non-availability of enzymes on a commercial scale has limited the development of such processes (Rui *et al.*, 2009; Shah *et al.*, 2005). An added problem with AEE is that it is impossible to avoid emulsification of the extracted oil, which requires post extraction de-emulsification to recover and enhance oil yield (Latif & Anwar, 2011; Long *et al.*, 2011; Wu *et al.*, 2009; Chabrand *et al.*, 2008; Santos & Ferrari, 2005; Rosenthal *et al.*, 1998; Sineiro *et al.*, 1998a). Addition of suitable enzymes to the cream emulsion may be able to separate the oil, and in this paper, this particular sequence of process is termed as aqueous enzymatic emulsion de-emulsification (AEED).

In an earlier review by Rosenthal *et al.* (1996), the principles and mechanisms of: mechanical, solvent, aqueous, and aqueous enzymatic extraction methods have been addressed, besides reviewing the effects of enzymes on plant cell composition and methods employed earlier for de-emulsification. The main purpose of this review is to critically assess the information available to date, in order to conclude whether the enzymatic route

is a viable industrial option for any given oilseed. In addition, the other objectives of this review are: to discuss the effect of incubating conditions in AEE on the oil extraction efficiency; to compare AEE with other extraction methods in terms of yields and characteristics of the oils from various oil-bearing materials; to explore methods available to de-emulsify the oil- aqueous phase emulsions that are inevitably formed during extraction; and finally, to explore the possibility of re-using in the enzyme after recovery in order to make the process more cost effective.

2. Aqueous enzymatic extraction (AEE) method Table 1 lists the enzymes used in earlier research. In terms of the dispersion structure, Sineiro *et al.* (1998a) reported that aqueous extraction resulted in oil droplets with spherical shapes in the case of sunflower oil. However, with the use of enzymes, the oil aggregates possessed different shapes with less structured and irregular cell wall surface. Different oils exhibit different properties, and it is reasonable to assume that AEE of different oil-bearing materials result in oil droplets with different characteristics. The enhancement in oil yield with the use of enzymes, i.e. AEE as compared to aqueous extraction without enzymes from various oil-bearing materials are summarized in Table 2. The table also summarizes the differences observed in oil yields between AEE and solvent extraction methods. It is clearly shown that the use of enzymes increases the oil yield, yet it is still lower than the yield when solvent extraction is used. Therefore, numerous studies have been conducted to establish the most suitable enzymes that can be used, either individually or in combination, on various types of oil-bearing materials in order to increase the oil yields.

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90 2.1. Studies comparing extraction efficiencies using different enzymes

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92 Figure 1(a) and 1(b) illustrate the flow sheets of AEE for soybean and olive oil,
93 respectively. The types of enzymes added depend on the cellular composition and structure
94 of the oil-bearing material (Passos *et al.*, 2009). According to Rosenthal *et al.* (2001), the
95 use of Alcalase 2.4L (protease) increased the oil yield from heat-treated soybean flour as
96 compared to cellulase, hemicellulase, and pectinase. Similarly, Santos and Ferrari (2005)
97 reported that both Alcalase and Celluclast (cellulase) were able to increase the oil yield
98 from soybeans, with Alcalase giving higher yields. A higher yield in the case of protease
99 (96.0%) as compared to phospholipase (73.4%) was also reported by Jung *et al.* (2009) in
100 the case of extruded soybean flakes. In addition, Lamsal *et al.* (2006) reported that the use
101 of individual cellulase and a mixture of cellulase and protease did not significantly increase
102 the soybean oil yield from extruded soybean flakes (68%); yet the yield increased when
103 individual protease was added (88%). These findings illustrate the specificity of enzymes
104 and enzymatic mixtures for any given oil-bearing material. The presence of protein as a
105 major component in the cell wall of soybean seeds suggests that the oil is released more
106 easily from the cellular matrix by degrading the proteins, which is achieved by the action of
107 protease. In the case of rapeseed, pectin is reported to be the major component of its cell
108 wall (Zhang *et al.* 2007), hence the highest oil yields, up to 85.9% in emulsified form, has
109 been reported when pectinase is used which is significantly greater than the values obtained
110 with other carbohydrases. Zhang *et al.* (2007) also employed a combination of pectinase

with cellulase and β-glucanase in a ratio of 4:1:1 to result in the highest yield (91.6% emulsified oil), this marginal enhancement in yield may be attributed to the elimination of other barriers to the release of oil. Similarly, Szydłowska-Czerniak *et al.* (2010) reported that the application of pectolytic enzyme (ROHAPECT PTE) under optimum conditions prior to pressing produced higher rapeseed free oil yield (16.5%) as compared to cellulolytic enzyme (15.5%).

Different from oilseeds, addition of enzymes is done on the olive paste in the case of olive fruits, followed by its kneading process as shown in Fig. 1(b). Most studies on extraction of olive oil involved addition of an enzyme mixture consisting mainly pectinase, cellulase, hemicellulase, and other minor enzymes. The studies also reported the inadequacies of these enzymes to extract olive oil if added individually (Aliakbarian et al., 2008; De Faveri et al., 2008; Chiacchierini et al., 2007).

In general, a better oil extraction yield can be expected when a judiciously chosen mixture of enzymes is used because of possible synergy (Passos *et al.*, 2009). However, according to Rovaris *et al.* (2012), there was no significant difference in soybean oil yields when a mixture of Alcalase 2.4 L and Viscozyme was used as compared to a mixture of Alcalase 2.4 L and Celluclast 1.5 L (29.48% as against 26.82% at pH 4.5; 20.63% as against 20.23% in the case of uncontrolled pH), even though Viscozyme itself is a mixture of enzymes. There was also no significant difference in garlic oil yields upon addition of Viscozyme as compared to addition of individual pectinase, protease, and cellulase as reported by Sowbhagya *et al.* (2009). A similar outcome was reported by Tabtabaei and Diosady (2013) in yellow mustard flour oil extraction when Celluclast 1.5L and Pectinex Ultra SP-L were used, as against Viscozyme L. In addition, the use of Alcalase 2.4L and

Protex 7L resulted in highest sesame (Latif & Anwar, 2011) and *Moringa oleifera* (Latif *et al.*, 2011) seed oils, respectively, in comparison with Viscozyme L, Protex 7L, Natuzyme, Kemzyme, and Multifect CX 13L which are essentially mixtures of enzymes (Latif &Anwar, 2011; Latif *et al.*, 2011). Viscozyme, being a mixture of enzymes, was reported to have performed better in the case of sunflower oil extraction, which had been proved by Latif and Anwar (2009). A higher oil yield from bush mango kernel flour was also observed upon addition of Viscozyme (68.0%) as compared to Alcalase (35.0%) and Pectinex (42.2%) (Womeni *et al.*, 2008). The different effects of the Viscozyme on oil yields may be due to the nature of different oil-bearing materials and incubating conditions employed.

In a different study conducted by Jiang *et al.* (2010), five different proteases were tested to improve peanut oil yield, and the highest oil yield was obtained when Alcalase was used (73.45%), followed by As1398 (66.36%), Nutrase (60.08%), Protizyme (55.02%), and Protamex (48.89%). A combination of Alcalase with any of these enzymes did not increase the oil yield. Therefore, Jiang *et al.* (2010) only used Alcalase which reduced the extraction cost, and increased oil yield up to 79.32% under optimum incubating conditions. Similarly, the use of Neutrase 0.8L resulted in marginally lower *Moringa oleifera* oil yield than when its combination with other three enzymes were employed (Abdulkarim *et al.*, 2006). In the case of flaxseed oil extraction conducted by Long *et al.* (2011), the addition of cellulase, pectinase, and hemicellulase, individually, gave higher yields than β-glucosidase and proteinase. Therefore, these authors used a mixture of cellulase, pectinase, and hemicellulase (1:1:1) which resulted in a higher oil

yield of 61.7-66.1% as compared to the oil yield of each individual enzyme. With reference to Table 2, , Zhang *et al.* (2007) reported highest yield of 92.7% in the case of rapeseed oil, however, the oil remained very stably emulsified in the cream. Therefore, an alkaline extraction was conducted by using Alcalase which resulted in protein degradation along with an increase in total oil yield.

Based on the above studies, it is not possible to establish conclusively whether it is better to use enzymes individually or in combination, although there are numerous instances where there is a possibility that a mixture can work synergistically. The choice of enzyme depends on the location of the oil within the cellular architecture and the biochemical nature of the components surrounding it. It is therefore necessary, not only to look at the dominant biochemical component holding the cellular matrix together, but also investigate the cellular architecture and examine the specific components which act as a barrier against the release of oil. It is only when both these factors are considered simultaneously, the right enzyme mixture can be identified for a given oil-bearing material.

2.2. Studies on the use of enzyme as a pre-treatment step prior to extraction

Recently, the application of enzyme pre-treatment prior to oil extraction has been shown to increase yields (Li *et al.*, 2012). The addition of enzymes as a pre-treatment weakens the cells and facilitate the following oil extraction methods such as mechanical pressing and solvent treatment. Furthermore, the advantage of employing this approach lies in the possibility of avoiding the formation of an oil-in-water emulsion that is very difficult

178 to separate after the extraction processes. The reported enhancement in oil yields with the
179 use of enzyme pre-treatment is summarized in Table 3. In addition to the higher yield,
180 Dominguez *et al.* (1996) also reported that it was easier to extract the sunflower oil
181 remaining in a mass of pre-treated mechanically pressed cake. In the case of Chilean
182 hazelnuts, enzyme pre-treatment resulted in significantly lower residual oil in the meal as
183 reported by Zuniga *et al.* (2003). Overall, these studies indicate that enzyme pre-treatment
184 is applicable to various oil-bearing materials and can be employed prior to both mechanical
185 and solvent extraction methods. The oil yield enhancement is due to the hydrolytic action
186 of the enzymes on the cell wall and membrane components which facilitate subsequent oil
187 release.
188
189 2.3. Studies on pre-treatment step prior to enzymatic extraction
190
191 Some studies have highlighted potential pre-treatment methods, which are not
192 necessarily enzyme-based that could be followed up by AEE as summarized in Table 4. In
193 the case of high pressure processing as reported by Jung and Mahfuz (2009), the use of
194 high pressure induced protein aggregation yet it was further hydrolyzed by protease, thus
195 facilitated oil removal. On the other hand, Shan Liu *et al.* (2011) reported that ultrasound
196 generated cavitations which accelerated the leaching out of cellular components including
197 oil. The use of extrusion prior to AEE has been extensively studied by Jung and Mahfuz
198 (2009), Jung *et al.* (2009), and Wu *et al.* (2009). According to these authors, protein
199 aggregates are formed during extrusion but these entrap or interact with the oil. The

interactions could then be disrupted by the use of protease, which result in increasing the oil and protein yields. These studies have shown the potential of AEE assisted by other pre-treatment methods to increase oil yields.

2.4. Factors affecting the efficiency of enzymatic extraction

Table 5 summarizes the maximum oil yields resulting from various oil-bearing materials as influenced by the selected and optimized incubating conditions. The key factors affecting the efficiency of AEE will be discussed separately, below.

2.4.1.Particle size of the oil-bearing materials

Most of the early studies did not consider the particle size of the oil-bearing material as a key factor influencing extraction efficiency (Passos *et al.*, 2009; Rosenthal *et al.*, 2001). Theoretically, the lower the particle size, the higher the oil yield for a given set of extraction conditions, which is attributable to higher cell wall disruption during size reduction as well as the lower diffusion path length for both enzymes and cellular components. However, according to Passos *et al.* (2009), materials with high oil content but exhibiting a weak structure, may collapse and lose their microporosity when treated with solvents, which can result in non-uniform percolation and be detrimental to extraction efficiency. In addition, grinding of materials with high oil content into very low particle sizes may cause the particles to adhere, as reported by Nyam *et al.* (2009a) in the case of Kalahari melon seeds. Therefore, in industry, starting materials with very low particle size

are not recommended and there appears to be an optimum size. This illustrates the importance of selecting the right particle size prior to extraction as had been done by some authors. Sineiro *et al.* (1998a) used ground soybean and sunflower seeds having mean particle size <0.2 mm. The grape seeds used by Passos *et al.* (2009) were grouped into different particle size ranges (in mm): <0.50, 0.50-0.60, 0.60-0.71, 0.71-1.0, 1.0-1.4, 1.4-2.0, and >2.0, and increment in oil yield was observed at lower particle sizes. In the case of linseed oil, Gros *et al.* (2003) reported no oil recovery from whole linseed kernels, because the substrate was not accessible to the enzymes added. Instead, the hull broke down and the kernels expanded due to hydration. On the other hand, when the kernels were crushed to form different particle sizes including fine powders, the yields improved, particularly after applying hydraulic pressures (Gros *et al.*, 2003). Similarly, in the case of soybean, the use of flour resulted in 24% higher yield than the flakes (Jung *et al.*, 2009), while 31% yield enhancement was reported by Rosenthal *et al.* (1998) when the particle size was reduced from 400 µm to 100 µm.

2.4.2. Enzyme/substrate ratio

Higher enzyme concentration leads to greater interaction between the enzyme and substrate, thus promoting cell wall degradation and rupturing more peptide bonds (Teixeira *et al.*, 2013; Jiang *et al.*, 2010; Dominguez *et al.*, 1996). However, too high enzyme concentration may result in bitterness and off flavours, as reported by Jiang *et al.* (2010), possibly due to the extraction of undesirable components. Most authors have reported similar trends where the oil yield increased up to certain enzyme concentration only,

followed by steady or decreased rate which may be due to saturation of the substrates (Jiang *et al.*, 2010), or caramelization of soluble sugars that limit oil release (Zuniga *et al.*, 2003). In general, the actual concentration used will depend on process economics especially the cost of enzymes (Long *et al.*, 2011; Zhang *et al.*, 2007), and the quality of the oil extracted.

2.4.3. Ratio of water to oil-bearing material

The water used in AEE not only serves as an extraction medium but also enters the oil-bearing material and modifies its water activity. The resulting moisture content of the oil-bearing material can assist hydrolytic reaction, diffusion, and mobility of the enzymes and products (Yang Li *et al.*, 2011; Zhang *et al.* 2007; Sineiro *et al.*, 1998a; Dominguez *et al.*, 1996). On the other hand, very low moisture content results in the formation of thick suspensions which can prevent the enzymes from effectively penetrating into the substrate (Zhang *et al.*, 2007). Sineiro *et al.* (1998a) reported that only certain 'areas' in sunflower kernels were degraded by enzymes at low moisture content. Although, materials with higher water activity demonstrate higher extraction efficiency (Soto *et al.*, 2007), the presence of excessive moisture content in the oil-bearing material can decrease the concentration of enzymes and substrates, and have an adverse effect on extraction (Yang Li *et al.*, 2011; Zhang *et al.*, 2007; Dominguez *et al.*, 1996). Therefore, selection of appropriate moisture content is critical for the success of AEE.

2.4.4. pH of extraction medium

The pH at which enzymes attain maximum activity varies with the enzyme. In most earlier studies, the pH value of the solution, be it for soaking pre-treatment or extraction itself, was set at a value corresponding to maximum enzyme activity (Latif & Anwar, 2011; Jung & Mahfuz, 2009; Wu *et al.*, 2009; Abdulkarim *et al.*, 2005; Rosenthal *et al.*, 2001; Sineiro *et al.*, 1998). However, the optimum pH of a number of enzymes is in the range of the isoelectric pH of proteins which depends on the nature of the oilseeds; since proteins are highly insoluble in this range of pH, oil release may get inhibited. Therefore, the pH value employed must not only be conducive for the action of enzymes but it should also be remote from protein isoelectric point (Tabtabaei & Diosady, 2013; Wu *et al.*, 2009; Sineiro *et al.*, 1998; Rosenthal *et al.*, 1996). This is yet another reason why many authors considered using a mixture of enzymes which demonstrates high activity at pH values remote from the isoelectric point and remain effective for oil extraction. The enzymes are able to solubilize and hydrolyze the proteins besides disrupting other polysaccharide constituents which facilitate oil release (Rovaris *et al.*, 2012; Latif & Anwar, 2011; Passos *et al.*, 2009). Long *et al.* (2011) had used a mixture of cellulase, pectinase, and hemicellulase (1:1:1) at pH 4.5-5.0 which resulted in highest flaxseed oil yield (73.9%) as compared to oil yield of each individual enzyme. In the case of soybean oil, at pH 4.5, Rovaris *et al.* (2012) used a mixture of Alcalase 2.4L and Celluclast 1.5L which resulted in 26.82% oil (20.63% in the case of uncontrolled pH), and a mixture of Alcalase 2.4 L and Viscozyme which resulted in 29.48% oil (20.23% in the case of uncontrolled pH). A number of studies have also used Protizyme™ for the AEE (Jiang *et al.*, 2010; Gaur *et al.*, 2010; Sharma *et al.*, 2002). Protizyme™, being a mixture of proteases, possess different

optimum pH which allowed selection of any incubating pH sensitive to the isoelectric point of the major protein fraction of the seeds. Overall, proper pH selection critically influences yields of oil and other components in AEE .

2.4.5. Incubation temperature

Besides being active over a narrow range of pH, enzymes also active over a narrow temperature interval. According to Rui *et al.* (2009), the optimum temperature range for enzymatic hydrolysis is between 40-55 °C, thus many authors employ AEE temperatures which fall within this range. In practice, one often prefers to use the lowest possible temperature yielding adequate activity (Passos *et al.*, 2009). In the case of olive fruits, a lower temperature of 30 °C was found to be favourable especially to preserve the oil quality (Aliakbarian *et al.*, 2008; De Faveri *et al.*, 2008; Ranalli *et al.*, 2003; Garcia *et al.*, 2001; Ranalli *et al.*, 1999). Gros *et al.* (2003) also used a temperature of 34 °C for similar reason in linseed oil extraction. A significant effect of temperature on oil yield was reported by Sharma *et al.* (2002), where highest peanut oil yield was observed at 40 °C, but it decreased significantly when the temperature was reduced to 37 °C. According to Zúniga *et al.* (2003), at temperatures greater than 45 °C, enzymatic hydrolysis begins to decrease due to enzyme inactivation which leads to lower oil yield. The oil release from the cells may also be limited due to presence of soluble sugars in the composition which can undergo caramelization during the drying stage. Therefore, similar trends were reported from most of the conducted studies, where the oil yield increased up to certain temperature only, followed by steady or decreased rate afterwards. Thus, besides the oil yield, the oil

quality characteristics must also be taken into consideration when selecting AEE temperature.

2.4.6. Incubation time

According to Jiang *et al.* (2010), Abdulkarim *et al.* (2006), Santos and Ferrari (2005), and Dominguez *et al.* (1996), degradation of cell wall components can be enhanced by prolonging the incubation time. Passos *et al.* (2009) also reported that the use of an enzyme mixture of cellulase, protease, xylanase, and pectinase for 120 hr resulted in 3.8% higher yield as compared to 24 hr of incubation time. However, this time duration (i.e. 120 hr) is far too long to be acceptable in practice (Passos *et al.*, 2009), lower oil quality may result (Jiang *et al.*, 2010), leading to high energy usage and production of undesirable products (Abdulkarim *et al.*, 2006). In addition, Rui *et al.* (2009) highlighted that longer incubation time of AEE in relation to other solvent extraction methods is one of the disadvantages of AEE. In some cases, the oil yield decreased after a certain incubation period because the whole substrates have reacted with the enzymes; leaving negligible substrates left for further enzymatic reaction to take place (Zhang *et al.*, 2007). On the whole, these studies have shown that although oil yield may increases with time, the rate of increase may be far too slow to warrant extended operations, and the oil quality may also get compromised.

2.4.7. Agitation rate

According to Rosenthal *et al.* (1998) and Sineiro *et al.* (1998a), agitation assists in mixing and additional rupture of the cell wall, and agitation rate is one of the factors affecting the disruption of cell wall. Abdulkarim *et al.* (2006) reported that the agitation rates of 50 and 80 rpm were not adequate to separate the *Moringa oleifera* oil from other seed components, thus resulted in lower oil yield than at 120 rpm. At this agitation rate of 120 rpm, bigger oil droplets were observed to accumulate at the surface which enabled easier separation. A similar observation was reported at 80 rpm in extraction of peanut oil (Sharma *et al.*, 2002) and at 100 rpm in the extraction of Kalahari melon seed oil (Nyam *et al.*, 2009a). On the other hand, the use of higher speeds leads to higher energy consumption and cost (Rosenthal *et al.*, 1998), besides resulting in the formation of a more stable oil-aqueous phase emulsion that is difficult to separate (Nyam *et al.*, 2009a; Abdulkarim *et al.*, 2006; Sharma *et al.*, 2002, Hanmoungjai *et al.*, 2000). These studies highlight the importance of selecting appropriate agitation rate that will result in the highest oil yield possible, considering both the oil recovered and emulsion stability at the end of the AEE process.

2.5. Multi factorial studies on AEE

A number of authors have employed statistical methods to indicate the relative importance of the AEE parameters listed above. According to Rosenthal *et al.* (2001), soybean oil yield was significantly influenced by the type of enzyme used, the particle size of the ground seeds, the ratio of water to oil-bearing material, and the interaction between

353 the two latter parameters. However, according to Hanmoungjai *et al.* (2001), only the
354 enzyme concentration had the most significant effect on the extraction of rice bran oil,
355 while both the incubation time and temperature did not significantly affect the oil yield.
356 Different AEE parameters used for other samples such as bayberry kernels (Zhang et al.,
357 2012), kalahari melon seeds (Nyam et al., 2009a), palm fruit (Teixeira et al., 2013), peanuts
358 (Jiang et al., 2010), and pine kernels (Yang Li et al., 2011) also had different degree of
359 significant effect on oil yield. These studies show that it is almost impossible to generalize
360 which factor is important and which is not, for a given material. It is necessary to undertake
361 an experimental investigation before designing and scaling up an AEE process.
362
363 **3. De-emulsification methods for aqueous enzymatic process (AEED)**
364 When oil is extracted into an aqueous enzymatic phase, it inevitably forms an emulsion,
365 which is often difficult to separate because of the added stability imparted by the
366 interfacially active cellular components which are also extracted in the same process. It is
367 therefore necessary to carefully consider the techniques employed to separate the oil,
368 because the final yield and oil quality, and the economic viability of the process, will
369 depend critically on de-emulsification steps. When AEE is followed by a centrifugation
370 step, besides oil, other fractions recovered include a skim and a cream emulsion (Figure
371 1(a)). The cream emulsion is very stable due to its protein content which acts as an
372 excellent emulsifier. Addition of suitable enzymes to the cream emulsion may be able to
373 separate the oil, and in this paper as had been mentioned earlier, this particular sequence of
374 process is termed as aqueous enzymatic emulsion de-emulsification (AEED). The enzymes

used in the AEED processes were also listed in Table 1. In this method, the enzymes added to the cream emulsion hydrolyze the interfacial proteins, thus reducing their molecular size and decreasing the rigidity of the oil droplet interface. The enzymes also remove the high molecular weight polypeptides which may occupy the emulsion interface and further reduce the interfacial membrane thickness. These enzymatic reactions lead to greater oil droplet coalescence and assist in free oil release (Tabtabaei & Diosady, 2013; Raghavendra & Raghavarao, 2010; Chabrand & Glatz, 2009; Jung & Mahfuz, 2009; Marina *et al.*, 2009; Wu *et al.*, 2009; Chabrand *et al.*, 2008). The original enzymes used in the AEE may also be carried out into the cream emulsion and assist hydrolytic reactions if suitable incubating conditions were employed (Chabrand & Glatz, 2009; Jung *et al.*, 2009). The free oil yield is commonly expressed as a percentage based on the initial weight of the cream emulsion.

In the case of oil-bearing coconut milk, the emulsion needs to be destabilized in order to obtain virgin coconut oil as shown in Figure 1(c). According to Jena and Das (2006), Garcia *et al.* (2005), Tangsuphoom and Coupland (2005), and Balasundaresan *et al.* (2002), coconut milk emulsion is low in stability due to its high fat content and the presence of coconut proteins (~65% is globulin known as cocosin) with low emulsifying properties. Therefore, these authors noted that the separation was not too challenging and concluded that the oil droplets were prone to undergo aggregation and tended to separate. In contrast, Marina *et al.* (2009), Tangsuphoom and Coupland (2008), Peamprasart and Chiewchan (2006), and McGlone *et al.* (1986) reported that a coconut cream emulsion was highly stable due to presence of natural phospholipids and coconut proteins (mainly globulins and albumins) which requires extra energy to be destabilized. It is not uncommon

to find such conflicting reports in literature, in this area, which is principally because, most papers do not take a holistic view on the whole process. Whether the downstream de-emulsification is challenging or not depends on the process conditions employed during AEE. If the conditions employed are such that the emulsion formed is very stable, then the de-emulsification will naturally become challenging. On the other hand, careful process design upstream, and use of conditions that do not favour the formation of a stable emulsion whilst releasing significant yields of oil, will simplify de-emulsification and enhance free oil yields and oil quality.

3.1. Studies comparing different enzymes for de-emulsification of cream emulsion

Table 5 summarizes the types of enzymes and the incubating conditions used in AEED methods for maximum free oil yields. In the case of yellow mustard flour, Tabtabaei and Diosady (2013) reported that Protex 6L possessed greater efficiency in the de-emulsification process, as compared to other proteases and carbohydrases tested. Lipomode (Phospholipase A2), being one of the carbohydrases, resulted in the production of lysophospholipids which is an emulsifier, thus increased the emulsion stability and decreased the free oil yield. Lysophospholipids also present in small amount in G-ZYME G999, resulted in an insignificant increase in the free oil yield. In the case of soybean oil, Lamsal and Johnson (2007) concluded that the use of Phospholipase C resulted in higher free oil yield (73±5%) as compared to the mixture of LysoMax^{TM} and G-ZYME G-999 at 1:1 ratio (68±9%) under the optimum pH and temperature of the enzymes. Wu *et al.* (2009)

have also reported that the use of enzymes shown in Table 5 at their optimum pH and temperature resulted in total de-emulsification of the cream emulsions, either the enzymes had been used individually or in combination, or sequentially. These studies indicated that the free oil yield depends on the stability of the cream emulsion which is mainly affected by the AEE, besides the incubating conditions of the AEED which are discussed below.

3.2. Factors affecting the efficiency of enzymatic de-emulsification

3.2.1. Enzyme concentration

Generally, the use of higher enzyme concentration resulted in higher free oil yield. According to Jung *et al.* (2009), at 25 °C, the use of Protex 6L resulted in higher free soybean oil yield of 96% at 2.5% (w/w) concentration when compared to a 85-89% yield while employing enzyme at 1.25% (w/w). Similarly, Wu *et al.* (2009) reported that free soybean oil yield increased with increasing enzyme concentration starting from 0.2% (w/w). In this study, when the LysoMax^{TM} enzyme was used at a concentration lower than 0.2% (w/w), the enzyme modified soybean phospholipids and caused the production of an emulsifier known as lysolecithin. This emulsifier enhanced the stability of the cream emulsion and therefore resulted in lower free oil yield. In addition, according to Wu *et al.* (2009), increasing the LysoMax^{TM} enzyme concentration did not increase the oil droplets size. These authors also reported that in the concentration range of 0.2-2.0% (w/w), the use of Protex 51FP resulted in higher free oil yield as compared to the LysoMax^{TM} which indicated the dominant role of soybean protein in stabilizing the cream emulsion.

441

442 *3.2.2. pH value*

443 As had been discussed earlier (section 2.4.4), different enzymes possess different
444 optimum pH where maximum activity is observed. Therefore, most studies employed the
445 optimum pH of the enzyme used in order to obtain the highest free oil yield (Table 5). In
446 the case of soybean oil, according to Wu *et al.* (2009), the oil droplet size and free oil yield
447 increased when the pH was lowered to 4.5, but not lower than 4.0. At the pH of 4.5, which
448 is the isoelectric point of soy protein, electrostatic repulsion between oil droplets decrease,
449 thus further enhancing oil droplets coalescence, formation of larger oil droplets, and higher
450 free oil yield (Wu *et al.*, 2009). In a study conducted by Chabrand and Glatz (2009), the
451 authors reported as high as 83% free soybean oil yield when the pH of the cream emulsion
452 was reduced to pH 4.5, and addition of enzyme (G-ZYME G999) at this similar pH
453 increased the free oil yield up to 100%. Similarly, Wu *et al.* (2009) reported that the use of
454 G-ZYME G999 and Protex 50FP separately at pH 4.5 resulted in 100% free oil yield.
455 These authors suggested that the combination of enzymatic reaction and pH reduction leads
456 to coalescence of the oil droplets and formation of much bigger droplets than when
457 enzymes are not used. Chabrand and Glatz (2009) had also reported the use of high pH on
458 the free soybean oil yield. At pH 9, only 2% of free oil yield was recovered. With the use
459 of enzymes (i.e. AEED) at pH 8 which was the original pH of the cream emulsion, no free
460 oil yield was obtained. Similarly, Wu et al. (2009) reported that the free soybean oil yield
461 decreased when the pH was increased beyond pH 4.5 up to pH 8. Therefore, the
462 significance of enzymes addition at suitable pH values for higher free oil yield is clear.

3.2.3. Incubation time and temperature

Similar to the pH value, different enzymes possess different optimum temperature where maximum activity is observed. Therefore, most earlier studies employed the optimum temperature reported for the enzyme used in order to obtain highest free oil yield (Table 5). Jung *et al.* (2009)reported the effect of different de-emulsification temperatures and times on the free soybean oil yield when Protex 6L was used. Prolonged incubation time from 2 min to 90 min enhanced the free oil yield from 86% to 100% at 65 °C. However, the incubation time did not affect the free oil yield at lower temperatures of 25 °C and 50 °C. Increment of temperature from 50 °C to 65 °C also increased the free oil yield from 90% to 100% after incubation for 90 min. In the case of coconut milk de-emulsification, Raghavendra and Raghavarao (2010) reported a higher free oil yield when the use of enzyme was followed by chilling and thawing. In this case, a higher free oil yield of 94.5% was reported at a higher temperature of 37 °C as compared to 91.0% yield at 25 °C, because according to these authors, most enzymes possess an optimum temperature of 37 °C. In addition, chilling resulted in packed oil bodies which are easier to separate (Raghavendra & Raghavarao, 2010).

It is also possible to demulsify without the use of enzymes as reported by Jung *et al.* (2009). In this study, the increase in temperature from 50 °C to 65 °C increased the free oil yield from 75% to 94%. According to the authors, the significant increase in free oil yield may be due to the action of remaining protease in the cream emulsion which was carried out from the AEE. In the case of yellow mustard flour, Tabtabaei and Diosady (2013)

subjected the emulsion recovered after AEED process to an alkaline treatment which resulted in higher oil yield than AEED alone.

Other processing parameters such as shaking, de-canting, and stirring may also influence de-emulsification efficiency (Jung *et al.*, 2009).

4. Oil characteristics

Most authors have reported the effects of extraction methods on the oil characteristics which are summarized in Table 6. With reference to the table, the oil yields from most of the enzyme treatments were lower in oxidative deterioration and rancidity, indicated by the lower free fatty acids and peroxide values as compared to the yields from solvent treatments. It was assumed that the high temperature used during the solvent extraction resulted in lower oxidative quality of the oils (Latif *et al.*, 2011; Latif & Anwar, 2011; Latif & Anwar, 2009; Latif *et al.*, 2008). The peroxide value of rice bran oil extracted by solvent was also higher than that extracted enzymatically, but the difference was too small to the limit industrial application (Hanmoungjai *et al.*, 2001). In contrast, Kalahari melon seed oil from AEE process gave higher free fatty acid and peroxide value than solvent extracted oil. This may be due to the lipase activity in the seeds during the initial heating in the case of AEE process (Nyam *et al.*, 2009).

With reference to Table 6, some of the enzymatically extracted oils gave higher iodine value (IV) than aqueous and solvent extracted oils. Hanmoungjai *et al.* (2001) and Long *et al.* (2011) reported that the higher IV indicated higher polyunsaturated fatty acid content which therefore suggested a higher antioxidant activity. In addition, highest total

tocopherols was observed in most seed oils obtained from the AEE, followed by aqueous and solvent extracted oils. It was suggested that the higher temperature employed in the solvent treatment reduced the tocopherol content in the oil (Latif *et al.*, 2011; Latif & Anwar, 2011). The total tocopherols in olive oils reported by Ranalli *et al.* (2001) and Ranalli *et al.* (2003) were also higher when AEE was employed as compared to aqueous extractions without enzymes. In contrast, Nyam *et al.* (2009) reported lower total tocopherol content in the Kalahari melon oil obtained by AEE than solvent extraction method. This may be due to the production of components during the digestion process in the AEE that can influence the amount of non-saponifiable matter, including tocopherols (Gunstone, 2000),

In terms of total phenolic content, the values varied with different oil-bearing materials, extraction methods employed, and the types of enzymes used in the AEE process. In the case of olive oil, AEE resulted in higher total phenolic content than the aqueous extractions without enzymes. This may be due to cell wall hydrolysis by the enzymes used which further assists partitioning of the phenolics into the oil. The phenolic content positively influences oxidative stability, shelf life, nutritional, sensory, and health properties of the olive oil, besides flavour which got a greater sensory score (Latif & Anwar, 2009, 2011; Aliakbarian *et al.*, 2008; Ranalli *et al.*, 2003; Ranalli *et al.*, 1999; Ranalli & De Mattia, 1997). Najafian *et al.* (2009) also reported that at higher enzyme concentration, the phenolic content increased whilst the oil turbidity decreased, which may be due to the enzymatic effect in reducing the amount of colloidal particles.

In terms of the fatty acid compositions (FAC), most authors reported similarities between the oils obtained from solvent and enzymatic extraction methods (Teixeira *et al.*, 2013; Li *et al.*, 2012; Zhang *et al.*, 2012; Latif *et al.*, 2011; Latif & Anwar, 2009, 2011; Jung *et al.*, 2009; Nyam *et al.*, 2009, 2009a; Latif *et al.*, 2008). In a study conducted by Rui *et al.* (2009), the FAC of the pitaya oil obtained from microwave-pre-treated enzyme treatment was similar to the recommended FAC by the US dietary standard. Rui *et al.* (2009) suggested that microwave irradiation enhanced volumetric swelling of the cells in the seed kernels which caused cell walls rupture, while the enzymes hydrolyzed the cell wall and the bonds between the protein or pectin. A combination of these methods led to extraction of pitaya oil with varying fatty acid types as compared to other methods. In the case of flaxseed oil, Long *et al.* (2011) reported that the oil yield from enzyme-pre-treated ultrasonication possessed higher monounsaturated and polyunsaturated fatty acids than the flaxseed oil obtained by solvent extraction. According to the authors, the use of water allowed diffusion of water-soluble components instead of the oil. Therefore, the oil possessed approximately similar FAC as the original flaxseed oil (Long *et al.*, 2011).

In addition to the characteristics listed in Table 6, the colour intensity of oil had also been reported in some studies based on red and yellow units; higher values of these units correspond to higher colour intensity. In the case of *Moringa oleifera* seeds, according to Latif *et al.* (2011) and Abdulkarim *et al.* (2006), the different enzymes used in the AEE processes act on different components of the seeds which resulted in oil yields having different colour intensity. However, the difference was more significant between the oil obtained by AEE and solvent extraction methods, which is similar to the results reported by

550 Nyam *et al.* (2009) and Latif *et al.* (2008) for Kalahari melon and canola seed oil,
551 respectively. The solvent-extracted oil had higher colour intensity which may due to the
552 pigments extracted by the solvent into the oil, such as carotenes and chlorophylls. The oil
553 obtained from AEE process may not need refining due to low colour intensity which
554 reduces the processing costs (Latif & Anwar, 2009; Nyam *et al.*, 2009; Latif *et al.*, 2008;
555 Abdulkarim *et al.*, 2006, Abdulkarim *et al.*, 2005).

556 Besides the colour of the oils, the sterols were also significantly lower in oil
557 obtained by AEE than solvent extracted oil, which suggests the ability of the solvent used
558 to extract lipid-soluble components (Nyam *et al.*, 2009). In addition to these characteristics,
559 Sowbhagya *et al.* (2009) reported that the use of enzymes as a pre-treatment prior to steam
560 distillation or hydrodistillation resulted in garlic oil with higher concentration of dithiins
561 which possess health benefits and highly desirable from a nutraceutical point of view. In
562 the case of soybean oil, with the use of enzymes, Jung *et al.* (2009) reported lower
563 phosphorus content (<200ppm) which comply with the specification of the National
564 Oilseed Processors Association trading rules for crude degummed soybean oil. In a study
565 done by Ranalli *et al.* (1999), the Cytolase 0 enzyme used in olive oil extraction was
566 harmless and water-soluble. Therefore, after the enzyme exerted all its effects on oil
567 extraction, it came out into the water (i.e. olive juice) and left no residue in the oil. Thus the
568 olive oil composition was not modified.

569 In extraction of virgin coconut oil from coconut milk emulsion, a combination of
570 AEED, chilling, and thawing for the coconut milk destabilization resulted in highest
571 creaming index as compared to other destabilization methods which indicated faster oil

droplets movement and higher droplets aggregation. As compared to commercial coconut oil sample, the coconut oil possessed higher caprylic (9.4%), capric (6.3%), and medium chain (69.7%) fatty acids. These fatty acid types are known to impart health benefits, and contribute to higher oxidative stability to the oil itself. In addition, the resulting coconut oil was also lower in acid value (0.27%) which also corresponds to lower free fatty acids, as compared to the commercial coconut oil (0.91%). The free fatty acids are responsible for undesirable flavour in the oil. Therefore overall, the coconut oil obtained from AEED followed by chilling and thawing seems to possess greater oxidative stability, and the attributes measured were within the Asian and Pacific Coconut Community standards (Raghavendra & Raghavarao, 2010).

Overall, enzyme based extraction methods result in oils with better characteristics as compared to oil obtained from solvent and aqueous extraction methods. Therefore, further studies are desirable to enable industrial application by scaling up.

5. Potentials for re-using enzymes in enzymatic extraction methods

Rosenthal *et al.* (1996) highlighted the possible alternatives for improvement of aqueous extraction, including the use of enzymes (i.e. AEE), the optimization of both extraction and de-emulsification processes, utilization of membrane technology, and the potential of water recycling (i.e. enzyme recycling in the case of AEE). Enzyme recycling may assist in reducing the cost of AEE which bears the potential to compete with conventional extraction method based on the market price commanded by the oil (Nyam *et al.*, 2009a)

According to Jung *et al.* (2009), after conducting AEE (Protex 6L) to produce soybean oil, the aqueous phase recovered contained 84.7% of the remaining Protex 6L activity. After separation, a major part of this enzyme activity was recovered in the skim fraction (Jung *et al.*, 2009). Similarly, 100% of Protex 6L activity remained in the skim fraction in a study conducted by Chabrand and Glatz (2009). These findings indicate the possibility of recovering and re-using the skim fraction as a source of water and enzyme at the upstream end of the process (Jung *et al.*, 2009). In addition, Jung *et al.* (2009) reported lower Protex 6L activity in the cream emulsion, yet adequate to increase the free oil yield with the use of suitable incubation time and temperature. Droplet coalescence was also promoted by the gentle stirring during the incubation of the cream emulsion (Jung *et al.*, 2009).

Studies concerning the enzyme recycling were conducted in order to improve process economics and lower the environmental impact of the process. Another method which has gained recent interests is the enzyme immobilization, where the enzymes are separated from the treated products before being re-used. It was reported that the separated enzymes possessed enhanced stability (Long *et al.*, 2011; Wan *et al.*, 2008; Roy *et al.*, 2004). The increasing demands on enzyme-based methods have resulted in production of more enzymes at lower production costs (Roy *et al.*, 2004; Mondal *et al.*, 2003; Sharma *et al.*, 2003; Chase, 1994).

6. Concluding remarks

This review has highlighted the main process, advantages, and disadvantages of AEE and AEED as alternative methods for conventional solvent based extraction methods. In order to enhance the oil yield, a combination of AEE with other non-enzymatic processing methods prior to, or after AEE, has been widely conducted and relevant studies have been reviewed in this paper. The process factors influencing AEE and AEED efficiencies, as well as the oil characteristics, have also been discussed. On the whole, the process factors are correlated with each other, and statistical optimization is currently the best solution for investigating the interacting effects between the contributing factors for obtaining highest oil yield with favourable quality. The high cost of enzymes and production of lower oil yield than that of solvent extraction method have been the major drawbacks of AEE process. Despite the problems, the interest in this method for oil and protein extraction has progressively increased due to the perceived environmental advantages.

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Figure caption

Fig. 1. Flow sheet for (a) production of extruded soybean oil by aqueous enzymatic extraction and free soybean oil recovery by aqueous enzymatic emulsion de-emulsification method (Adapted from Lamsal and Johnson, 2007; Jung *et al.*, 2009; Wu *et al.*, 2009; Chabrand and Glatz, 2009); (b) production of olive oil by aqueous enzymatic extraction with different post-treatments (Adapted from Ranalli *et al.*, 1999; Garcia *et al.*, 2001; Ranalli *et al.*, 2001; Ranalli *et al.*, 2003; De Faveri *et al.*, 2008; Najafian *et al.*, 2009); and (c) production of virgin coconut oil by aqueous enzymatic emulsion de-emulsification method (Adapted from Raghavendra and Raghavarao, 2010).

Table captions

Table 1. Commercial enzymes used for aqueous enzymatic extraction (AEE) and aqueous enzymatic emulsion de-emulsification (AEED) processes: descriptions and compositions.

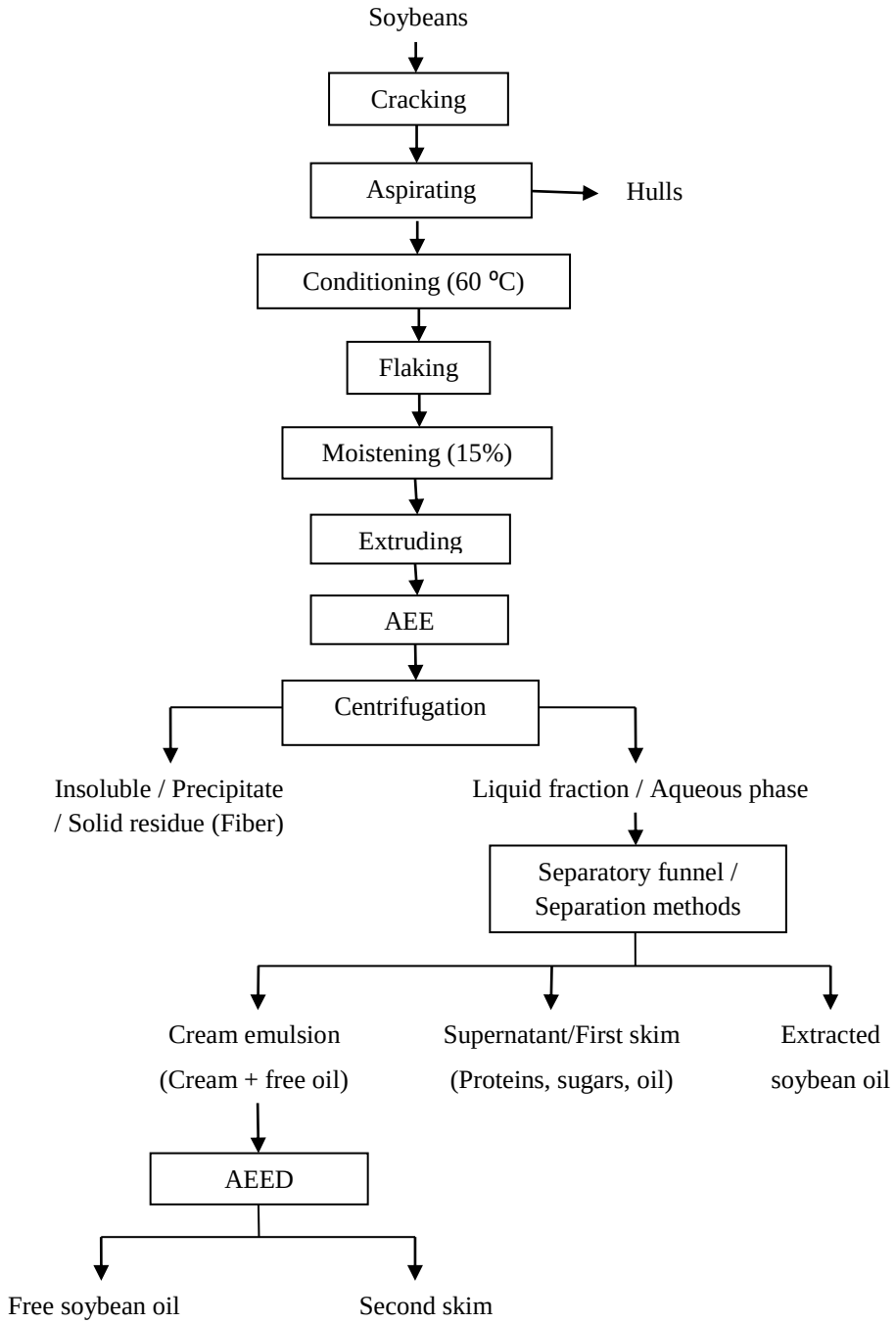
Table 2. The oil yield enhancement with the use of enzymes, and the oil yield difference between the enzyme and solvent extraction methods.

Table 3. Enhancement in oil yield due to presence of enzyme pre-treatment prior to the extraction method, as compared to the extraction method alone.

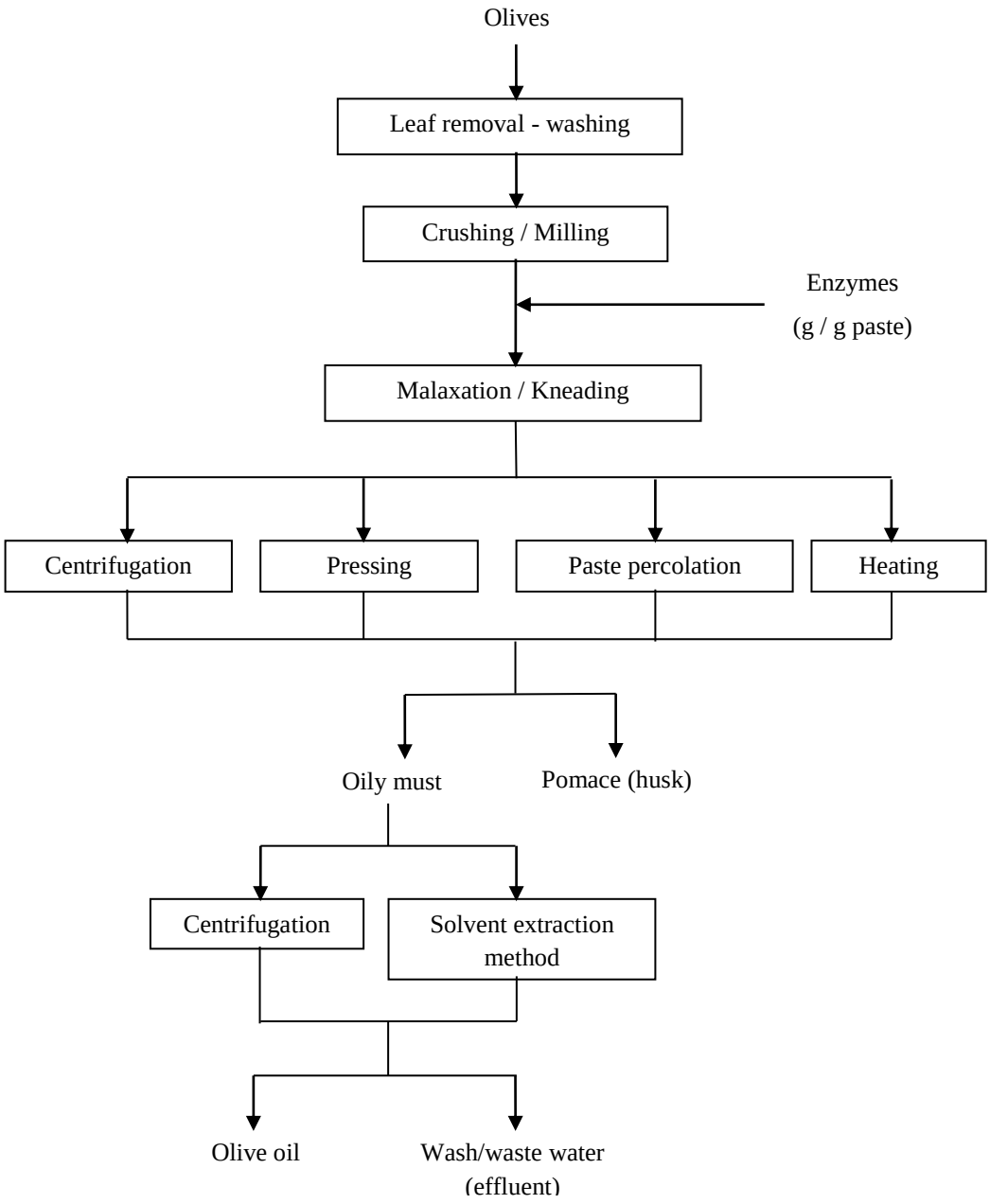
Table 4. The advantages of the use of pre-treatments (non-enzymatic) prior to the enzymatic extraction method.

Table 5. Maximum oil yields as affected by the selected and optimized incubating conditions of the aqueous enzymatic extraction and aqueous enzymatic emulsion de-emulsification methods.

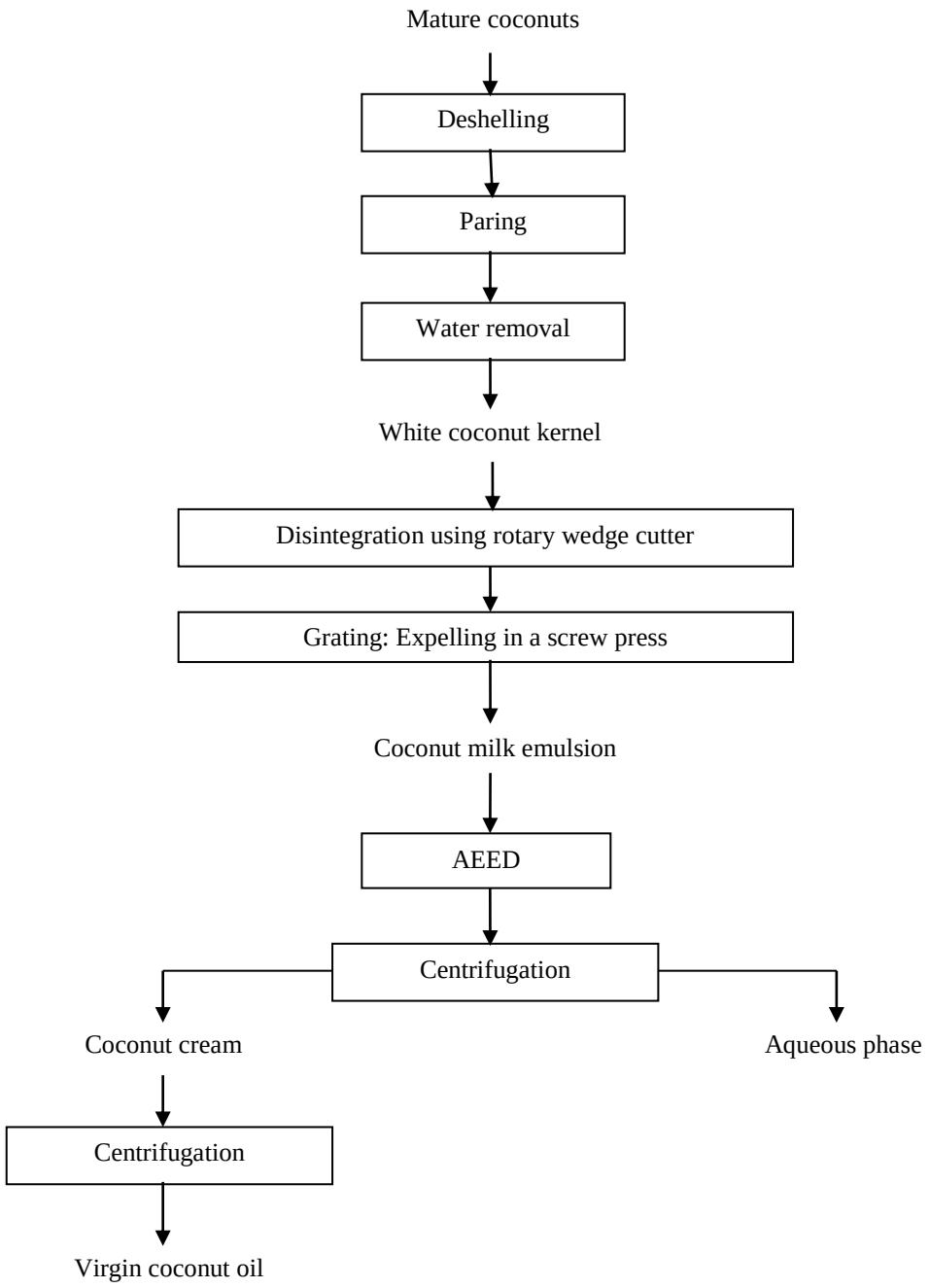
Table 6. The characteristics of oil yields from solvent, aqueous, and aqueous enzymatic extraction methods.


Soybeans
Cracking
Aspirating
Hulls
Conditioning (60 °C)
Flaking
Moistening (15%)
Extruding
AEE
Centrifugation
Insoluble / Precipitate / Solid residue (Fiber)
Liquid fraction / Aqueous phase
Separatory funnel / Separation methods
Cream emulsion (Cream + free oil)
Supernatant/First skim (Proteins, sugars, oil)
Extracted soybean oil
AEED
Free soybean oil
Second skim

(a)


Olives
Leaf removal - washing
Crushing / Milling
Enzymes
(g / g paste)
Malaxation / Kneading
Centrifugation
Pressing
Paste percolation
Heating
Oily must
Pomace (husk)
Centrifugation
Solvent extraction method
Olive oil
Wash/waste water (effluent)

(b)


Mature coconuts
Deshelling
Paring
Water removal
White coconut kernel
Disintegration using rotary wedge cutter
Grating: Expelling in a screw press
Coconut milk emulsion
AEED
Centrifugation
Coconut cream
Aqueous phase
Centrifugation
Virgin coconut oil

(c)

Table 1. Commercial enzymes used for aqueous enzymatic extraction (AEE) and aqueous enzymatic emulsion de-emulsification (AEED) processes: descriptions and compositions.

Enzymes commercial names	Description/Composition	Reference
Single enzyme		
Alcalase®	Protease	Womeni *et al.* (2008)
Alcalase 2.4L	Protease	Rosenthal *et al.* (2001)
		Latif & Anwar (2009)
		Jiang *et al.* (2010)
		Latif & Anwar (2011)
		Rovaris *et al.* (2012)
		Tabtabaei & Diosady (2013)
As1398	Protease	Jiang *et al.* (2010)
Celluclast 1.5L®	Cellulase	Dominguez *et al.* (1996)
		Sineiro *et al.* (1998)
		Abdulkarim *et al.* (2006)
		Rovaris *et al.* (2012)
		Tabtabaei & Diosady (2013)
		Teixeira *et al.* (2013)
Flavourzyme® 1000 L	Protease	Nyam *et al.* (2009)
		Nyam *et al.* (2009a)
Glucanex	Glucosidases	Garcia *et al.* (2001)
G-ZYME® G999	Lysophospholipase A1	Chabrand & Glatz (2009)
		Wu *et al.* (2009)
		Tabtabaei & Diosady (2013)
Lipomod 699L	Phospholipase A2	Tabtabaei & Diosady (2013)
LysoMax™	Phospholipase A2	Wu *et al.* (2009)
Multifect Neutral®	Protease	Lamsal & Johnson (2007)
Neutrase 0.8L	Bacterial neutral protease	Abdulkarim *et al.* (2005)
		Abdulkarim *et al.* (2006)
		Nyam *et al.* (2009)
		Nyam *et al.* (2009a)
Nutrase	Xylanase	Jiang *et al.* (2010)
Papain	Protease	Jiang *et al.* (2010)
Pectinase 1.06021	Pectinase	Najafian *et al.* (2009)
Pectinase Multieffect FE®	Pectinase	Teixeira *et al.* (2013)

Pectinex®	Pectinase	Womeni *et al.* (2008)
Pectinex Ultra SP	Pectinase	Dominguez *et al.* (1996)
Pectinex Ultra SP-L	Pectinase	Abdulkarim *et al.* (2006) Tabtabaei & Diosady (2013)
Promozyme	Pullulanase	Shah *et al.* (2005)
Protamex	Protease	Jiang *et al.* (2010)
Protex 6L	Alkaline serine endopeptidase	Chabrand & Glatz (2009) Jung *et al.* (2009) Wu *et al.* (2009) Shan Liu *et al.* (2011) Xiaonan Sui *et al.* (2011) Tabtabaei & Diosady (2013)
Protex 7L	Natural metallo endopeptidase	Latif *et al.* (2008) Chabrand & Glatz (2009) Jung & Mahfuz (2009) Latif & Anwar (2009) Wu *et al.* (2009) Latif & Anwar (2011) Latif *et al.* (2011)
Protex 30L	Alkaline serine endopeptidase	Chabrand & Glatz (2009)
Protex 50FP	Acid fungal endopeptidase-exopeptidase complex	Wu *et al.* (2009)
Protex 51FP	Neutral fungal endopeptidase-exopeptidase complex	Wu *et al.* (2009) Tabtabaei & Diosady (2013)
Protex 89L	Endopeptidase	Tabtabaei & Diosady (2013)
ROHALASE® OS	Cellulase	Szydłowska-Czerniak *et al.* (2010)
ROHAPECT® PTE	Pectinase	
Termamyl 120L	α-amylase	Abdulkarim *et al.* (2006)
Enzymes mixture		
Bioliva	Cellulase, hemicellulase, pectinase, other minor enzymes	Ranalli *et al.* (2003)
Cytolase 0	Cellulase, hemicellulase, pectinase, other minor enzymes	Ranalli *et al.* (1999) Ranalli *et al.* (2003)
Kemzyme	Cellulase complex, hemi-cellulase complex, α-amylase, β-glucanase, protease, xylanase	Latif & Anwar (2009) Latif & Anwar (2011) Latif *et al.* (2011)

Maxoliva	Cellulase, hemicellulase, pectinase, other minor enzymes	Ranalli *et al.* (2003)
Multifect CX 13L	Cellulase, hemicellulase, β-glucanase, arabinoxylans	Latif *et al.* (2008) Latif *et al.* (2011)
Multifect Pectinase FE	Pectinase, cellulase, hemicellulase	Latif *et al.* (2008)
Natuzyme	Cellulase, xylanase, phytase, α-amylase, pectinase	Latif *et al.* (2008) Latif & Anwar (2009) Latif & Anwar (2011) Latif *et al.* (2011)
Olivex	Cellulase, hemicellulase, pectinase	Garcia *et al.* (2001)
Olivex-Celluclast	50%: Cellulase, hemicellulase pectinase 50%: Cellulase, hemicellulase	Soto *et al.* (2007)
Pectinex Ultra SP-L	Cellulase, pectinase, xylanase	Shah *et al.* (2005) Najafian *et al.* (2009) Tabtabaei & Diosady (2013)
Protizyme™	Three different proteases with pH optima 3-4, 5-7, 7-10	Sharma *et al.* (2002) Gaur *et al.* (2007) Jiang *et al.* (2010)
Rapidase® Liq plus	Hemicellulases, pectinases, cellulases	Gros *et al.* (2003)
Viscozyme®	(Carbohydrases): Cellulase, hemicellulase, arabinase, xylanase, amylase, β-glucanase	Sowbhagya *et al.* (2009) Womeni *et al.* (2008)
Viscozyme L	(Carbohydrases): Cellulase, hemicellulase, arabinase, xylanase, β-glucanase	Latif & Anwar (2009) Latif & Anwar (2011) Latif *et al.* (2011) Rovaris *et al.* (2012) Tabtabaei & Diosady (2013)

Table 2. Oil yield difference between the aqueous and aqueous enzymatic extraction, and between solvent and aqueous enzymatic extraction methods.

Oil-bearing material	Type of enzyme	Difference in oil yield (%)		Reference
		Aqueous extraction and aqueous enzymatic extraction	Solvent treatment and aqueous enzymatic extraction	
Crushed borage seeds (≤2.0 mm)	Olivex / Celluclast (1:1)	7.80	-	Soto *et al.* (2007)
Extruded soybean flakes	Protease	20.00	-	Lamsal *et al.* (2006)
	Multifect Neutral®	13.40	-	Lamsal & Johnson (2007)
	Protex 7L	22.10	-	Jung & Mahfuz (2009)
	Protex 51FP	16.00[a]	-	Wu *et al.* (2009)
	Protex 6L	20.00[a]	-	
	Protex 7L	17.00[a]	-	
Ground canola seeds	Multifect CX 13L	9.50	17.10	Latif *et al.* (2008)
	Protex 7L	6.90	19.70	
	Natuzyme	6.20	20.40	
Ground *Jatropha* seed kernels (inedible)	Protizyme™	26.00		Shah *et al.* (2005)
Ground Kalahari melon seeds	Neutrase 0.8L		9.58	Nyam *et al.* (2009a)
	Flavourzyme 1000L		8.67	
Ground *Moringa. oleifera* seeds	Neutrase 0.8L		8.20	Abdulkarim *et al.* (2005)
	Neutrase 0.8L	12.12	9.39	Abdulkarim *et al.* (2006)
	Termamyl 120L	10.15	11.36	
	Pectinex Ultra SP-L	6.98	14.53	
	Celluclast 1.5L	10.12	11.39	
	Neutrase 0.8L / Termamyl 120L / Pectinex Ultra SP-L / Celluclast 1.5L	12.83	8.68	

	Natuzyme	9.10	23.30	Latif *et al.* (2011)
	Kemzyme	10.30	22.10	
	Multifect CX 13L	14.00	18.40	
	Protex 7L	14.70	17.70	
	Viscozyme L	13.10	19.30	
Ground peanuts	Alcalase	42.86	-	Jiang *et al.* (2010)
	As1398	35.77	-	
	Nutrase	29.49	-	
	Protizyme	24.43	-	
	Protamex	18.30	-	
	Protizyme™	-	3.36-5.88	Sharma *et al.* (2002)
	Papain	-	10.08	
	Chymotrypsin	-	16.38	
	Trypsin	-	13.86	
Ground sesame seeds	Alcalase 2.4L	12.50	25.40	Latif & Anwar (2011)
	Natuzyme	4.50	33.40	
	Protex 7L	6.40	31.50	
	Viscozyme L	9.10	28.80	
	Kemzyme	4.20	33.70	
Ground sunflower seeds (0.75-1 mm)	Celluclast 1.5L	35.00	-	Sineiro *et al.* (1998)
Ground sunflower seeds	Alcalase 2.4L	8.30	18.90	Latif & Anwar (2009)
	Kemzyme	13.90	13.30	
	Natuzyme	17.20	10.00	
	Protex 7L	10.00	17.20	
	Viscozyme L	21.40	5.80	
Heat-treated soybean flour	Alcalase 2.4L	16.90	-	Rosenthal *et al.* (2001)

Kernel flour of bush mango	Alcalase®	7.60	-	Womeni *et al.* (2008)
	Pectinex®	14.80	-	
	Viscozyme®	40.60	-	
Minced yellow horn seed kernels	Cellulase / Hemicellulase / Pectinase (1.8 : 1.3 : 2.5)		9.00	Li *et al.* (2013)
Olive paste	Bioliva	1.20	-	Ranalli *et al.* (2003)
	Maxoliva	1.37	-	
	Cytolase 0	1.44	-	
	A (pectinase, cellulase, hemicellulase)	152.00 (30 min)	-	Aliakbarian *et al.* (2008)
	/ B (pectinase, hemicellulase) / C (pectolytic enzyme) (1:1:1)	91.40 (150 min)	-	
	Pectinex Ultra SP-L	1.96[b]	-	Najafian *et al.* (2009)
	Pectinase 1.6021	1.41[b]	-	
Palm fruit	Pectinase / cellulase	35.57	5.36	Teixeira *et al.* (2013)
	Pectinase / cellulase / tannase	35.90	5.03	
	Tannase	12.70	28.23	
Rapeseed slurry	Pectinase	38.10	-	Zhang *et al.* (2007)
	Cellulase	21.50	-	
	B-glucanase	16.20	-	
	Pectinase / Cellulase / β-glucanase (4:1:1)	43.80	-	
	Multifect Pectinae FE	5.70	-	
Shattered bayberry kernels (60-mesh sieved)	Cellulase / Neutral protease (1:2)		31.85	Zhang *et al.* (2012)
Yellow mustard flour	Celluclast 1.5L	3.74	10.59	Tabtabaei & Diosady (2013)
	Pectinex Ultra SP-L	3.03	11.30	

	Viscozyme L	3.99	10.34
	Celluclast 1.5L / Pectinex Ultra SP-L / Viscozyme L (1: 1:1)	6.70	7.63

The oil yield differences were determined based on the oil yields under the best incubating conditions of each enzyme used, or based on the fixed incubating conditions for all enzymes used, in the conducted studies.

All aqueous enzymatic extractions resulted in higher oil yields than aqueous extractions, and all solvent treatments resulted in higher oil yields than aqueous enzymatic extractions.

[a] total oil as in the skim and cream emulsion

[b] average oil yield enhancements from three olive species with the use of enzymes at high concentrations

Table 3. Enhancement in oil yield due to presence of enzyme pre-treatment prior to the extraction method, as compared to the extraction method alone.

Oil-bearing material	Type of enzyme (pre-treatment)	Extraction method	Enhancement in oil yield (%)	Reference
Crushed borage seeds (≤2.0 mm)	Olivex / Celluclast (1:1)	Double pressing	5.40[a]	Soto *et al.* (2007)
Crushed garlic cloves	Cellulase	Steam distillation	0.11	Sowbhagya *et al.* (2009)
	Pectinase		0.23	
	Protease		0.22	
	Viscozyme		0.18	
	Cellulase	Hydrodistillation	0.14	
	Pectinase		0.26	
	Protease		0.24	
	Viscozyme		0.19	
Ground flaxseeds	Cellulase / Pectinase / Hemicellulase (1:1:1)	Ultrasonication	29.50	Long *et al.* (2011)
Ground rapeseeds	ROHAPECT® PTE	Pressing	5.70	Szydłowska-Czerniak *et al.* (2010)
	ROHALASE® OS		1.70	
Milled grape seeds	A mixture of cellulase, xylanase, protease, pectinase	Solvent extraction (24 hr)	106.00	Passos *et al.* (2009)
		Solvent extraction (120 hr)	163.00	
Minced yellow horn seed kernels	Cellulase / hemicellulase / pectinase (1.8 : 1.3 : 2.5)	Microwave	4.30 (oil yield enhancement as compared to AEE alone)	Li *et al.* (2013)
Pre-heated ground Chilean hazelnut seeds (inedible, ≤1.4 mm)	Ultrazyme / Celluclast (1:1)	Double pressing (hydraulic pressing at each of 39.2 MPa)	~8.00	Zuniga *et al.* (2003)
Silybum marianum seed powders	Cellulase / Xylanase / Pectinase / Protease (2:1:1:2)	Solvent extraction (1.5 hr)	10.46	Li *et al.* (2012)
		Solvent extraction (14.0 hr)	50.72	
Whole sunflower kernels	Celluclast 1.5L / Pectinex Ultra SP (2:1)	Pressing (Batch press)	13.11	Dominguez *et al.* (1996)
Mango kernel powders	Protizyme™	Three-phase partitioning method	16.00	Gaur *et al.* (2007)
Soybean flour			8.00	

Rice bran powders	14.00

[a] the oil yield enhancement was based on the difference between an enzymatic and non-enzymatic pre-treatment, followed by double pressing

Table 4. The advantages of the use of pre-treatments (non-enzymatic) prior to the enzymatic extraction method.

Oil-bearing material	Pre-treatment	Type of enzyme	Advantages	Reference
Ground *Isatis indigotica* seeds	Microwave	Cellulase / Proteinase / Pectinase (1:1:1)	- In combination with AEE, the use of optimal microwave irradiation power increased the oil yield up to 59.27%, and the oil yield had greater antioxidant properties than solvent-extracted oil.	Gai *et al.* (2013)
Ground *Jatropha* seed kernels (inedible)	Ultrasonication (5 min)	Protizyme^{TM}	The enzyme treatment time was reduced from 18 hr to 6 hr for maximum of 74% oil yield	Shah *et al.* (2005)
Ground linseeds	Electrical discharge	-	Mucilage (stabilizing agent) is removed which caused easier oil separation from the resulted residue by using enzyme treatment	Gros *et al.* (2003)
Grounds peanuts	Alkaline extraction	Alcalase	Oil yield of 5.87% higher than AEE alone	Jiang *et al.* (2010)
Ground pitaya seeds (40-mesh sieved)	Microwave	Pectinase / Cellulase / Acid protease (1:1:1)	- Oil yield of 0.84% higher than AEE alone	Rui *et al.* (2009)
Ground watermelon kernels	Ultrasound	Protex 6L	-Under the fixed parameters of the ultrasound, the yield was 20.67% higher than AEE alone -Under the selected parameters of ultrasound for maximum oil yield, the yield was 21.39% higher than AEE alone	Xiaonan Sui *et al.* (2011), Shan Liu *et al.* (2011)
Soybean flakes	High pressure processing (200 MPa)	Protex 7L	Oil yield of 3.20% higher than AEE alone	Jung & Mahfuz (2009)
	High pressure processing (500 MPa)		Oil yield of 1.30% higher than AEE alone	
	Extrusion		- Oil yield of 29.90% higher than AEE alone - Free oil yield of 17.00% higher than AEE	

			alone	
	Extrusion	Protex 6L	- Oil yield of 35.52% higher than AEE alone - After de-emulsification: Free oil from cream emulsion of 62.00% higher than AEE alone	Jung *et al.* (2009)

AEE: aqueous enzymatic extraction.

Table 5. Maximum oil yields as affected by the selected and optimized incubating conditions of the aqueous enzymatic extraction and aqueous enzymatic emulsion de-emulsification methods.

Oil-bearing material	Type of enzyme	Moisture / Material ratio (w/w; for aqueous enzymatic extraction)	Enzyme / Material ratio	pH	Tempera-ture (°C)	Time (hr)	Agitation rate (rpm)	Oil yield (%)	Reference
Selected(*) and optimized (**) incubating conditions used for maximum oil yield in aqueous enzymatic extraction									
Crushed borage seeds (≤2.0 mm)	Olivex / Celluclast (1:1)[a]	20%* (corresponded to 1:5)	0.25%*	-	45.0*	9.00*	-	85.50	Soto *et al.* (2007)
Ground *Jatropha* seed kernels (inedible)	Protizyme™ [a]	6:1	0.25 (w/w)%	9.00*	50.0*	18.00	100	64.00	Shah *et al.* (2005)
Ground *Moringa. oleifera* seeds	Celluclast 1.5L[a]	6:1	2.00% (v/w)*	4.80***	60.0*	36.00*	120*	22.01	Abdulkarim *et al.* (2006)
	Termamyl 120L[a]			5.50***				22.04	
	Pectinex Ultra SP-L [a]			3.50***	45.0*			18.87	
	Neutrase 0.8L [a]			6.80***				24.02	
	Neutrase 0.8L / Termamyl 120L / Pectinex Ultra SP-L / Celluclast 1.5L [a]			7.50***				24.72	
Ground peanuts	Alcalase [a]	5:1*	1.50% (w/w)*	8.50*	60.0*	5.00*	-	73.45	Jiang *et al.* (2010)

	Protizyme™ [a]	2:1	2.50% (w/w)*	4.00*	40.0*	18.00*	80*	36.12-38.64	Sharma *et al.* (2002)
Ground pitaya seeds (40-mesh sieved)	Pectinase / Cellulase / Acid protease (1:1:1) [a]	8:1	-	7.00	50.0*	1.00	90	6.94	Rui *et al.* (2009)
Ground rice bran (16-mesh sieved)	Alcalase 0.6L [a]	-	1.00% (w/w)*	9.00	60.0*	3.00*	1000	79.10	Hanmoungjai *et al.* (2001)
Ground sunflower seeds (0.75-1 mm)	Celluclast 1.5L [a]	5:1*	2.00% (w/w)*	4.80***	50.0***	2.00*	150	35.65	Sineiro *et al.* (1998)
Heat-treated soybean flour	Alcalase 2.4L [a]	-	3.00% (v/w)*	8.00 ***	50.0***	1.00	200	58.70	Rosenthal *et al.* (2001)
Olive paste	A (pectinase, cellulase, hemicellulase) / B (pectinase, hemicellulase) / C (pectolytic enzyme) (1:1:1) [a]	-	0.25% (v/w)*	-	30.0	2 hr 30 min*	10 (kneading)	17.50	Aliakbarian *et al.* (2008)
Rapeseed slurry	Pectinase / Cellulase / β-glucanase (4:1:1) [a]	5:1*	2.50% (v/w)*	5.00	48.0	4.00*	200	92.70	Zhang *et al.* (2007)
Ground Kalahari melon seeds	Neutrase 0.8L [a]	-	2.50% (w/w)**	7.00**	58.0**	31.00**	100	68.58	Nyam *et al.* (2009a)
	Flavourzyme® 1000 L [a]	-	2.10% (w/w)**	6.00**	50.0**	36.00**	100	71.55	
Ground *Moringa. oleifera* seeds	Neutrase 0.8L [a]	6:1 (v/w)	2.00% (v/w)	6.80 ***	45.0**	24.00**	120	22.60	Abdulkarim *et al.* (2005)

Ground pine kernels	Alcalase endo-protease [a]	5:1**	1.97%**	8.40**	51.0**	3.00**	-	89.12	Yang Li *et al.* (2011)
Ground pumpkin seeds	Cellulase [a]	-	1.70% (w/w)**	-	47.0**	2.64**	-	89.12	Hu & Zou (2013)
Ground watermelon kernels	Protex 6L [a]	4.35:1**	2.63%**	7.89**	47.1**	4.29**	-	77.25	Xiaonan Sui *et al.* (2011); Shan Liu *et al.* (2011)
Palm fruits	Pectinase / Cellulase / Tannase (1:1:1) [a]	2:1 (v/w)**	4.00**	4.00**	50.0	0.50*	200	91.52	Teixeira *et al.* (2013)
Shattered bayberry kernels (60-mesh sieved)	Cellulase / Neutral protease (1:2) [a]	4.91:1 (v/w)**	3.17%**	-	51.6**	4.00**	-	31.15	Zhang *et al.* (2012)
Selected (*) and optimized (**) incubating conditions for maximum free oil yield in aqueous enzymatic emulsion de-emulsification method									
Alkaline pre-treated ground peanuts	Alcalase 2.4L[a]	As1398[b]	1.00%	-	-	2.0 hr	-	12.66	Jiang *et al.* (2010)
Coconut milk emulsion	-	Aspartic protease (endoprotease)[b]	0.10%	-	37.0*	3.0 hr	-	83.00	Raghavendra & Raghavarao (2010)
Extruded soybean flakes	Protease Multifect Neutral®[a]	LysoMax™ / G-ZYME G999 (1:1) [b]	-	4.5***	60.0***	1 hr 30 min	-	68.00	Lamsal & Johnson (2007)
		Phospholipase	-	7.0***	37.0***	1 hr 30	-	73.00	

		C[b]				min			
	Protex 6L[a]	Protex 6L [b]	2.50%*	4.5*	50.0	1 hr 30 min	-	100.00	de Moura *et al.* (2008)
	Protex 6L[a]	Protex 6L [b]	1.25%**	-	50.0**	1 hr 30 min**	-	100.00	Jung *et al.* (2009)
	Protex 7L[a]	LysoMax™ [b]	2.00%	8.0***	40.0***	1 hr 30 min	-	100.00	Wu *et al.* (2009)
		G-ZYME® G999[b]		4.5***	50.0***				
		Protex 6L[b]		8.0***	50.0***				
		Protex 7L[b]		7.0***	50.0***				
		Protex 50FP[b]		4.5***	50.0***				
		Protex 51FP[b]		8.0***	50.0***				
Ground *Perilla frutescens* seeds	-	Protex 6L[b]	1.90%**	9.4**	62.6**	1.6 hr**	-	85.52	Zhang *et al.* (2013)
Soybean flour	Protex 7L[a]	G-ZYME G999[b]	2.00%*	4.5***	50.0	3.0 hr	700*	100.00	Chabrand & Glatz (2009)
		Protex 6L[b]	3.00%*	9.0***	50.0	3.0 hr	500*	72.00	
Yellow mustard flour	Celluclast 1.5L / Viscozyme L / Pectinex Ultra SP-L (1:1:1) [a]	Protex 6L[b]	2.50%	4.5-6.0***	50-60***	3.0 hr	-	91.30	Tabtabaei & Diosady (2013)
		Alcalase 2.4L[b]		6.5-8.5***	45-65***			42.10	
		Lipomode 699L[b]		8.0***	40.0***			1.30	

	G-ZYME G999[b]	4.5***	50-60***	41.20

Values without any notation are fixed incubating conditions.

[a] Type of enzymes used for aqueous enzymatic extraction

[b] Type of enzymes used for aqueous enzymatic emulsion de-emulsification

*selected incubating condition; the authors varied the level of each incubating condition and finalized the conditions which resulted in highest oil yield.

**optimized incubating condition; the authors varied the level of each incubating condition and optimized the conditions which resulted in highest oil yield based on an experimental design and statistical software used.

*** optimum incubating condition of the enzyme used; different types of enzymes possess different optimum pH and temperature where the enzymes attain maximum activity

Table 6. The characteristics of oil yields from solvent, aqueous, and aqueous enzymatic extraction methods.

Oil characteris-tic	Oil-bearing material	Solvent extraction	Aqueous extraction	Aqueous enzymatic extraction		Reference
Free fatty acids (%)	Extruded soybean flakes	0.26	*	0.18	Protex 6L	Jung *et al.* (2009)
	Ground canola seeds	0.81	0.56	0.52	Multifect CX 13L	Latif *et al.* (2008)
				0.57	Protex 7L	
				0.55	Natuzyme	
				0.54	Multifect Pectinae FE	
	Ground Kalahari melon seeds	0.60	*	0.90	Flavourzyme® 1000 L	Nyam *et al.* (2009)
				0.90	Neutrase 0.8L	
	Ground *Moringa. oleifera* seeds	2.48	*	1.13	Neutrase 0.8L	Abdulkarim *et al.* (2005)
		2.48	1.22	1.13	Neutrase 0.8L	Abdulkarim *et al.* (2006)
				1.24	Termamyl 120L	
				1.22	Pectinex Ultra SP-L	
				1.25	Celluclast 1.5L	
				1.23	Neutrase 0.8L / Termamyl 120L / Pectinex Ultra SP-L / Celluclast 1.5L	
		1.26	0.42	0.43	Natuzyme	Latif *et al.* (2011)
				0.41	Kemzyme	
				0.39	Multifect CX 13L	
				0.38	Protex 7L	
				0.42	Viscozyme L	
	Ground rice bran (16-mesh sieved)	7.40	*	2.36	Alcalase 0.6L	Hanmoungjai *et al.* (2001)
	Ground sesame seeds	0.54c	0.48	0.47	Natuzyme	Latif & Anwar (2011)
				0.44	Kemzyme	
				0.51	Protex 7L	
				0.46	Alcalase 2.4L	
				0.44	Viscozyme L	
	Ground sunflower seeds	0.94	0.68	0.66	Alcalase 2.4L	Latif & Anwar (2009)
				0.65	Kemzyme	

				0.67	Natuzyme	
				0.69	Protex 7L	
				0.64	Viscozyme L	
Iodine value (g / 100g)	Ground canola seeds	117.00	114.00	116.00	Multifect CX 13L	Latif *et al.* (2008)
				114.00	Protex 7L	
				117.00	Natuzyme	
				116.00	Multifect Pectinae FE	
	Ground flaxseeds	140.80	*	161.20	Cellulase / Pectinase / Hemicellulase (1:1:1)	Long *et al.* (2011)
	Ground Kalahari melon seeds	125.00	*	141.00	Flavourzyme® 1000 L	Nyam *et al.* (2009)
				135.20	Neutrase 0.8L	
	Ground *Moringa. oleifera* seeds	65.40	*	66.10	Neutrase 0.8L	Abdulkarim *et al.* (2005)
		65.40	66.00	67.10	Neutrase 0.8L	Abdulkarim *et al.* (2006)
				66.50	Termamyl 120L	
				67.20	Pectinex Ultra SP-L	
				66.50	Celluclast 1.5L	
				67.00	Neutrase 0.8L / Termamyl 120L / Pectinex Ultra SP-L / Celluclast 1.5L	
		67.00	70.00	76.00	Natuzyme	Latif *et al.* (2011)
				73.00	Kemzyme	
				75.00	Multifect CX 13L	
				74.00	Protex 7L	
				76.00	Viscozyme L	
	Ground pitaya seeds (40-mesh sieved)	173.10	*	118.00	Pectinase / Cellulase / Acid protease (1:1:1)	Rui *et al.* (2009)
	Ground rice bran (16-mesh sieved)	95.40	*	97.18	Alcalase 0.6L	Hanmoungjai *et al.* (2001)
	Ground sesame seeds	107.00	106.00	104.00	Natuzyme	Latif & Anwar (2011)
				109.00	Kemzyme	
				108.00	Protex 7L	
				105.00	Alcalase 2.4L	
				103.00	Viscozyme L	
	Ground sunflower	127.00	120.00	124.00	Alcalase 2.4L	Latif &

	seeds			121.00	Kemzyme	Anwar (2009)
				123.00	Natuzyme	
				122.00	Protex 7L	
				121.00	Viscozyme L	
Peroxide value (meq O_2 / kg)	Extruded soybean flakes	6.50	*	4.05	Protex 6L	Jung *et al.* (2009)
	Ground canola seeds	1.29	0.69	0.72	Multifect CX 13L	Latif *et al.* (2008)
				0.70	Protex 7L	
				0.71	Natuzyme	
				0.64	Multifect Pectinae FE	
	Ground flaxseeds	1.20	*	1.00	Cellulase / Pectinase / Hemicellulase (1:1:1)	Long *et al.* (2011)
	Ground Kalahari melon seeds	2.30	*	6.40	Flavourzyme® 1000 L	Nyam *et al.* (2009)
				7.30	Neutrase 0.8L	
	Ground *Moringa. oleifera* seeds	2.09	1.60	1.58	Natuzyme	Latif *et al.* (2011)
				1.56	Kemzyme	
				1.61	Multifect CX 13L	
				1.63	Protex 7L	
				1.59	Viscozyme L	
	Ground pitaya seeds (40-mesh sieved)	1.93	*	1.44	Pectinase / Cellulase / Acid protease (1:1:1)	Rui *et al.* (2005)
	Ground rice bran (16-mesh sieved)	8.20	*	12.01	Alcalase 0.6L	Hanmoungjai *et al.* (2001)
	Ground sesame seeds	1.50	1.30	0.90	Natuzyme	Latif & Anwar (2011)
				1.30	Kemzyme	
				1.40	Protex 7L	
				1.10	Alcalase 2.4L	
				1.20	Viscozyme L	
	Ground sunflower seeds	1.78	1.36	1.25	Alcalase 2.4L	Latif & Anwar (2009)
				1.33	Kemzyme	
				1.32	Natuzyme	
				1.31	Protex 7L	
				1.37	Viscozyme L	
Saponifica-tion value	Ground Kalahari melon seeds	173.20	*	185.20	Flavourzyme® 1000 L	Nyam *et al.* (2009)
				184.80	Neutrase 0.8L	

(mg KOH / g oil)	Ground *Moringa. oleifera* seeds	164.00	*	163.00	Neutrase 0.8L	Abdulkarim *et al.* (2005)
		164.00	158.00	156.00	Natuzyme	Latif *et al.* (2011)
				158.00	Kemzyme	
				155.00	Multifect CX 13L	
				159.00	Protex 7L	
				156.00	Viscozyme L	
	Ground pitaya seeds (40-mesh sieved)	194.40	*	191.10	Pectinase / Cellulase / Acid protease (1:1:1)	Rui *et al.* (2005)
	Ground rice bran (16-mesh sieved)	187.60	*	188.72	Alcalase 0.6L	Hanmoungjai *et al.* (2001)
	Ground sesame seeds	169.00	159.00	158.00	Natuzyme	Latif & Anwar (2011)
				162.00	Kemzyme	
				167.00	Protex 7L	
				164.00	Alcalase 2.4L	
				156.00	Viscozyme L	
	Ground sunflower seeds	190.00	187.00	187.00	Alcalase 2.4L	Latif & Anwar (2009)
				186.00	Kemzyme	
				187.00	Natuzyme	
				187.00	Protex 7L	
				185.00	Viscozyme L	
Total tocopherols; α, δ, and γ (α, β, δ, and γ for Kalahari melon seeds and olive paste) (mg / kg oil) .	Ground canola seeds	739.00	598.00	794.00	Multifect CX 13L	Latif *et al.* (2008)
				805.00	Protex 7L	
				783.00	Natuzyme	
				819.00	Multifect Pectinae FE	
	Ground Kalahari melon seeds	174.80	*	143.20	Flavourzyme® 1000 L	Nyam *et al.* (2009)
				143.30	Neutrase 0.8L	
	Ground *Moringa oleifera* seeds	179.30	216.90	220.80	Natuzyme	Latif *et al.* (2011)
				228.50	Kemzyme	
				221.70	Multifect CX 13L	
				221.50	Protex 7L	
				228.30	Viscozyme L	
	Ground sesame seeds	584.10	603.30	628.50	Natuzyme	Latif & Anwar (2011)
				641.20	Kemzyme	
				627.30	Protex 7L	
				619.80	Alcalase 2.4L	

					612.80	Viscozyme L	
	Ground sunflower seeds		799.00	778.00	845.00	Alcalase 2.4L	Latif & Anwar (2009)
					849.00	Kemzyme	
					849.00	Natuzyme	
					842.00	Protex 7L	
					833.00	Viscozyme L	
	Olive paste	Cipressino	*	77.30	89.20	Cytolase 0	Ranalli *et al.* (2001)
		Cassanese		95.20	114.10		
		Leccino		117.00	135.40		
		Dritta	*	231.00	288.00	Cytolase 0	Ranalli *et al.* (2003)
					279.00	Maxoliva	
					266.00	Bioliva	
		Caroleo	*	218.00	273.00	Cytolase 0	
					269.00	Maxoliva	
					252.00	Bioliva	
		Coratina	*	244.00	305.00	Cytolase 0	
					300.00	Maxoliva	
					289.00	Bioliva	
	Palm fruit		*	325.27	251.11	Pectinase / Cellulase	Teixeira *et al.* (2013)
					200.54	Pectinase / Cellulase / Tannase	
					204.26	Tannase	
Total phenolic content (mg / kg oil), as in gallic acid equivalent for sesame seeds, sunflower seeds, *Moringa oleifera* seeds, and palm fruit;	Ground Kalahari melon seeds		18.00	*	18.00	Flavourzyme® 1000 L	Nyam *et al.* (2009)
					19.00	Neutrase 0.8L	
	Ground *Moringa oleifera* seeds		12.00	13.00	15.00	Natuzyme	Latif *et al.* (2011)
					14.00	Kemzyme	
					13.00	Multifect CX 13L	
					14.00	Protex 7L	
					18.00	Viscozyme L	
	Ground sesame seeds		17.00	18.00	19.00	Natuzyme	Latif & Anwar (2011)
					18.00	Kemzyme	
					22.00	Protex 7L	
					21.00	Alcalase 2.4L	
					24.00	Viscozyme L	
	Ground sunflower seeds		8.00	9.00	13.00	Alcalase 2.4L	Latif & Anwar (2009)
					14.00	Kemzyme	

caffeic acid					13.00	Natuzyme	
equivalent for					13.00	Protex 7L	
olive paste;					15.00	Viscozyme L	
and sum of	Olive	Cipressino	*	90.00	105.00	Cytolase 0	Ranalli *et al.*
phenolic	paste	Cassanese		122.00	153.00		(2001)
acids for		Leccino		112.00	131.00		
Kalahari		Dritta	*	314.00	435.00	Cytolase 0	Ranalli *et al.*
melon seeds					427.00	Maxoliva	(2003)
					388.00	Bioliva	
		Caroleo	*	222.00	329.00	Cytolase 0	
					318.00	Maxoliva	
					287.00	Bioliva	
		Coratina	*	382.00	479.00	Cytolase 0	
					462.00	Maxoliva	
					431.00	Bioliva	
		Coratina	*	691.30	751.00	A / B / C** (1:1:1)	Aliakbarian *et al.* (2008)
		Coratina	*	574.50	804.30	A / B / C** (1:1:1)	De Faveri *et al.* (2008)
		Koroneiki	*	179.00	309.00	Pectinex	Najafian *et al.* (2009)
					245.00	Pectinase	
		Iranian	*	302.33	357.67	Pectinex	
		oleaginous			359.00	Pectinase	
		Mission	*	199.67	306.67	Pectinex	
					258.33	Pectinase	
	Palm fruit		*	21.43	17.43	Pectinase / Cellulase	Teixeira *et al.*
					14.76	Pectinase / Cellulase / Tannase	(2013)
					26.43	Tannase	

The column adjacent to the olive paste refers to the different olive species used.

*data not reported

**A: pectinase, cellulase, hemicellulase; B: pectinase, hemicellulase; C: pectolytic enzyme