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Separation of *squalene* rich fraction from palm oil fatty acid distillate (*PFAD*): A review

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Abstract. Palm Oil Fatty Acid Distillate (*PFAD*) is a by-product of the palm oil industry which has many potential bioactive compounds such as vitamin E, *phytosterols* and *squalene*. To obtain multi-component bioactive compounds, saponification and extraction processes are required. The purpose of this study was to identify a review of the comparison of several methods that are more optimal in separating the *Squalene*-Rich Fraction from Palm Oil Fatty Acid Distillate (*PFAD*). The study uses the systematic literature review method, where the review will study and compare several journal descriptions regarding comparisons in managing the optimal separation of the *squalene* fraction from the three types of methods offered, namely the method using solvents, the method using high pressure supercritical fluid extraction and the isolation of squalene method using *Saccharomyces cerevisiae* strains. This review presents a descriptive analysis of the advantages and disadvantages of the three methods. The study compared three methods for separating the *squalene*-rich fraction. The review suggests that the safest method to use is separation with low temperature solvents or the so-called low temperature solvent crystallization. Reviews show that this method will not destroy bioactive compounds which are easily oxidized, be easy to apply, require low production cost and capable of producing high purity *squalene*-rich fractions.

1. Introduction

Squalene ($C_{30}H_{50}$) is an aliphatic triterpenoid hydrocarbon which was first discovered in Japan in 1906 by Mitsumaru Tsujimoto [1]. *Squalene* is involved in unsaturated hydrocarbons with six double bonds, clear oil that is not saponified, but does not contain fatty acids or COOH groups, is odorless and tasteless [2]. The molecular weight of *squalene* is 410.7 ppm [3] and has the chemical name 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-teracosahexaene [4]. It is generally known that *squalene* is abundant in liver oil from black shark (*Zameus* spp) and whale liver oil (*Squallus spp.*) With a content of up to 2,000-8,000 ppm [5], but *squalene* does not always have to be obtained from these species, they are protected by IUCN so it is necessary to find alternative sources of *squalene* [6].

Based on the alternative needs for the potential of natural ingredients from Squalene , several sources were identified to contain *squalene*, including olive oil of 564 mg/100 g [7], *Amaranthus sp* with a content of 5-8% squalene [8] and Palm fatty acid distillate (*PFAD*) which is a byproduct of refining crude palm oil (CPO) where previous studies reported that *PFAD* contains high *squalene* up to 1.03% [9].

Based on the source, research focused on the potential of palm fatty acid distillate (*PFAD*). Previous research states that *PFAD* contains several bioactive compounds such as vitamin E (*tocopherols* and *tocotrienols*), *phytosterols*, and *squalene* [10]. The background for the interest in the potential of *PFAD* is because *PFAD* is a byproduct of oil palm processing which has not been widely used as a potential natural material to extract *squalene*. So far, *PFAD* is still considered as waste and is only used for the production of biodiesel, industrial soap and animal feed [11]. However, along with the great benefits, there are several problems with the use of *squalene*. It is especially in the utilization of *squalene* obtained from *PFAD*, where a way is needed to obtain the pure fraction of *squalene* optimally. It should be underlined that the main problem is how to effectively fractionate and separate the squalene from *PFAD*. In fact, previous research has revealed several methods for performing squalene separation and determining the best separation method.

Several previous studies have stated that there are at least three types of methods to produce the optimum *squalene* fraction, including the method using solvents [13], the method using high pressure supercritical fluid extraction [11] and the isolation of squalene method using *Saccharomyces cerevisiae* strains [14]. Of the three methods offered, a review will be carried out on the advantages of the three methods in separating the *squalene* fraction from *PFAD*. Due to the difficulty level and the variety of methods available to separate *squalene*, the purpose of this research is to identify and compare several related methods to obtain the optimum high purity *squalene*-rich fraction.

2. Materials and Methods

In this study, the material will focus on oil palm waste management which will be identified in a review of the use of three different methods. The research will focus on separating fractions from *squalene* to produce a rich fraction of the materials and methods used. Separation is a technique of separating the components of a mixture so that it becomes individual fractions.

The study uses the systematic literature review method, where the review will look at and compare several journals descriptions regarding comparisons in managing the optimal separation of the *squalene* fraction from the three types of methods offered, namely the method using solvents [13], the method using high pressure supercritical fluid extraction [11] and the isolation of squalene method using *Saccharomyces cerevisiae* strains [14]. The literature for this review was obtained from a variety of databases, including Google scholar, Springer, Research gate, ERIC and Science Direct. Certain keywords used to search for articles are ("*squalene*") and ("*Palm Fatty Acid Distillate*"). Then the author will do an elimination based on duplicate data from the articles obtained with the following scheme (Figure1).

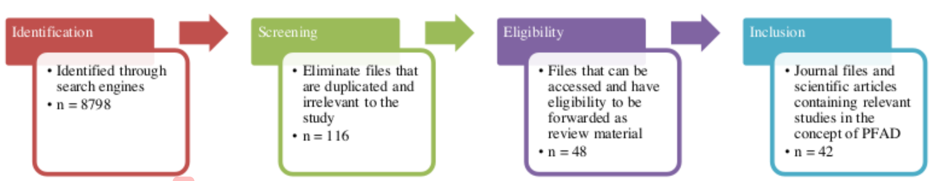

Figure 1. The process of selecting journals and scientific articles.

From 48 journals and articles that have entered the criteria, there are 42 articles and scientific journals that are following the concept of this research, namely the identities are arranged as follows (Table 1).

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Table 1. Data extraction of journals and scientific articles.

No	Search Source	Journal Eligibility	Selected / Relevant Journals
1	Google Scholar	22	20
2	Springer	7	6
3	Research Gate	5	4
4	ERIC	3	2
5	Science Direct	11	10
	TOTAL	48	42

3. Results and Discussion

3.1 Identification of journal and scientific article identity

Referring to Table 1, the use of journals is dominated by the use of Google Scholar search (48%) and Science Direct (24%). The period of publication of literature from reviewed research journals is generally dominated over 2010. All journals will lead to a division based on the analysis of the three methods, which are presented in the following Table 2. Based on Table 2, the division of a review of the method of separating *squalene* based on the three methods offered will be studied in more depth. The next discussion will direct how to analyze the advantages and disadvantages of each method of separating *squalene* from *PFAD*.

Table 2. Methods for separation of squalene.

Research focus	Relevant Journal Review	f	%
The method using solvents	3, 4, 5, 6, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21	16	38,09
The method using high pressure supercritical fluid extraction	7,8, 15, 22, 23, 26, 33, 35, 42, 45, 46	11	26.2
The isolation of squalene method using *Saccharomyces cerevisiae* Strains	24, 25, 27, 28, 30, 31,32, 34, 37, 38, 39, 40, 41, 43, 44	15	35,71
TOTAL		42	100%

3.2 Comparative analysis of the squalene separation method in PFAD

3.2.1 Solvent extraction

Solvent extraction is a standard method that is most often used. The extraction process uses organic solvents such as hexane, dichloromethane, chloroform and methanol. The method of extracting using a solvent is usually preceded by a saponification process or followed by a process followed by a degumming and deacidification process [6].

Extraction using solvents has the advantage of being carried out at low temperatures, low atmospheric pressure, does not require expensive equipment and a high level of efficiency. Conducting a *squalene* separation of soybean oil fatty acid distillate using soxhlet extraction using hexane, followed by silica gel column chromatography can produce *squalene* with a purity level of 95.90% [8]. Isolation of *squalene* from *PFAD* using a multistage liquid-liquid extraction, extraction using dichloromethane solvent was carried out in several stages.

3.2.2 Extraction with high pressure supercritical fluid extraction (SFE)

Supercritical Fluid Extraction (SFE) can be used to extract polar compounds. Supercritical fluid has the ability to diffuse like gas so that it can penetrate solid material and a high level of density. This fluid is compressible or easily changes properties with slight changes in pressure [6].

The advantages of SFE extraction method is carried out at low temperatures, fluid as a solvent is separated from the extract without leaving a trace in order to obtain extracts with a high purity level and faster extraction times [11]. During the refining process of palm oil at 180-260°C and high pressure 2-8 mmHg, vitamin E is distilled in this deodorizing process and accumulates in fatty acid distillate (*PFAD*) [15]. The method is mostly used in major industries because of the high costs and complicated operating system because it uses a high pressure [20]. High temperature extraction solvents are not safe to use because they can destroy bioactive compounds and it is difficult to obtain high purity and also it requires high costs and a complex operating system.

3.2.3 Isolation of squalene using Saccharomyces cerevisiae strains

This method is the latest method to isolate *squalene*. In particular, *Saccharomyces cerevisiae* is an active microorganism to produce isoprenoid, such as *squalene* and status as Generally Recognized As Safe (GRAS) [14]. *S. cerevisiae* synthesizes squalene through the mevalonate pathway to produce sterols, such as ergosterol. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and *squalene* synthase are the main control enzymes for the production of *squalene* in the yeast metabolic pathway [16]. Excessive expression of cytosolic HMG-CoA reductase (encoded by tHMG1) causes *squalene* accumulation in *S. cerevisiae* [14].

Biotechnology has developed techniques for the industrial production of *squalene* and however, the yields obtained from *Saccharomyces cerevisiae, Botrycoccus braunii, Aurantiochytrium sp*., and *E. coli* are lower than those from plant sources (5–15 mg/g dry matter, 4.1–340.5 mg/L) and only reach less than 10% of the world production [13]. Isolation of squalene with the *Saccharomyces cerevisiae* strain method has not been able to produce a high level of *squalene* purity.

3.3 Selection of method that produces rich squalene from PFAD

In this study, the first method *(Solvent extraction)* resulted in a safer level of optimization of the rich fractionation of squalene. In this study, it was found that the *PFAD* was 1.03% (w / w) which is much more than the second and third methods. The first method with saponification was chosen because it also has a hypolipidemic effect that can affect serum and liver profiles, increase the excretion of cholesterol and bile salts, and inhibit the activity of 3-hydroxy-3-methylglutaril coenzyme A (HMG-CoA) reductase [17]. This method is also supported by the results showing that the principle of separating *tocotrienols* by crystallization is the difference in melting points between the components present in the non-soap fractions [18]. However, all of these methods have the disadvantage of requiring more complex and expensive equipment, apart from causing a higher level of bioactive compound damage and operating costs. Therefore, the use of low temperatures becomes a solution to keep bioactive compounds easily oxidized so they don't get damaged. This is in accordance with a study that fixed vitamin E from the non-therapeutic fraction of *PFAD* using low-temperature solvent crystallization [19].

This method was chosen because it provides a benefit in the crystallization process in the nucleation stage (nucleation) and crystal growth, where it focuses on when the crystal nucleus is formed it will be followed by crystal growth [22]. This method shows that the factors that can influence the crystallization conditions include the ratio of solvent to non-soap fraction, saturation level, crystallization temperature and crystallization time [23]. The solvent ratio relates to the viscosity of the solution, which can affect heat transfer and mass transfer. Conversely, if the viscosity is too high at 5: 1, the solution becomes thicker so that molecular space is limited and results in the mass transfer and heat transfer processes being inhibited [24]. The difference in the saturation level of the compounds to be separated is related to the different melting points of the compounds. The transfer of mass in the crystal nucleus causes crystal growth wherein a compound with low solubility, which is below the melting point, migrates to the crystal nucleus and crystallizes [25].

3.4 Separation squalene analysis for PFAD

PFAD is a byproduct of physical CPO refining. Crude palm oil (CPO) is a fatty extract from the fresh fruit mesocarp of the oil palm tree (*Elaeis guineensis*). The main constituent of palm oil is triacylglycerols and contains about 1% minor components such as carotenoids, tocopherols and

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tocotrienols, *phytosterols*, phospholipids, glycolipids, terpenes, and aliphatic hydrocarbons [26] as listed in Table 3.

Table 3. Components in *PFAD.*

Components	%
Fatty Acids and Glycerides	96,1
Phytosterols	0,37
Tocopherols and Tocotrienols	0,48
Squalene	0,76
Hydrocarbons	0,71

Some of the minor components, especially carotenoids and tocopherols and *tocotrienols*, not only maintain the stability and quality of palm oil but also have significant biological health properties [27]. Fatty acids and glycerides are the largest components in *PFAD* up to 96.1%, besides that there are also other bioactive compounds which are listed in Table 4.

Table 4. Composition of vitamins E and Phytosterols in *PFAD.*

Components	%
α-tocopherol	23,15
α-tocotrienols	17,16
Ƴ-tocotrienols	45,5
δ-tocotrienols	14,18
Campesterol	13,24
β-sitosterol	73,42
stigmasterol	15,99

Based on research stated that the mean composition of vitamin E in *PFAD* from several samples of palm oil was α-tocopherol (23.15%), α-*tocotrienols* (17.16%), Ƴ-*tocotrienols* (45.5%) and δ-*tocotrienols* (14, 18%). While the composition of the *phytosterols* is campesterol (13.24%), β-sitosterol (73.42%) and stigmasterol (15.99%) [25].

3.5 Benefits of fractionated squalene from PFAD

Squalene is now one of the most expensive ingredients for making cosmetics and moisturizers and is often sold in pill form as a supplement that can treat a wide variety of ailments [28]. Therefore, humans need the additional intake of squalene from the outside because it can act as an antioxidant and anti-cancer and can be used to inhibit cholesterol synthesis [29]. In addition, *squalene* can also maintain moisture and elasticity and has anti-tumor and anti-inflammatory activity [30]. *PFAD* in this study is said to be one of the richest sources of vitamin E [31] because it consists of *tocotrienols* and tocopherols with a ratio of 70:30 [32]. Tocopherols and *tocotrienols* (tocochromanols) have potential as antioxidants due to their lipoperoxide radical-binding activity [33]. However, the potency of *tocotrienols* which are unsaturated vitamin E has stronger antioxidant abilities than tocopherols.

The antioxidant activity of *tocotrienols* is due to the unsaturated side chains that can be well distributed in the fat layer of the cell membrane and penetrate the tissues that have saturated fat layers such as brain and liver tissue [34]. The antioxidant activity of *tocotrienols* in liver microsomes is 40-60 times greater than that of α-tocopherol [35]. *Tocotrienols* are able to inhibit cholesterol biosynthesis and have neuroprotective and anti-cancer properties [36], an enzyme in the liver responsible for cholesterol synthesis [37]. Therefore, in the binding of *squalene*, it is of course expected that the potential of *tocotrienols* derived from PFDA can be utilized as a potential that has the ability to reduce cholesterol, anti-inflammatory and neuroprotective effects in humans [38].

Phytosterols are also bioactive components in *PFAD* [39]. The most important benefit of *phytosterols* is the effect of lowering cholesterol levels in the blood through partial inhibition of intestinal cholesterol absorption [40]. Decreasing blood cholesterol levels will have an impact on improving the health of the cardiovascular system [41]. Other benefits of *phytosterols* include anti-atherogenic effects (especially beta-sitosterol), immune stimulation and anti-inflammatory activity, inhibition of the development of various types of cancer, such as colorectal, breast and prostate cancer [42].

4. Conclusions

The review suggests that the second method, namely isolation *squalene* with solvent extraction methods, is more appropriate to be used in developing the potential of *squalene* contained in *PFAD*. Palm oil fatty acid distillate (*PFAD*) has several useful bioactive compounds such as vitamin E, *phytosterols* and *squalene* found in the saponified fraction. The best method for separating *squalene* compounds from *PFAD* must be through saponification and separation processes using low temperature crystallization solvents because they will not damage bioactive compounds which are easily oxidized, easy to operate, low cost for the operating system and capable of producing high *squalene* levels.

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