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# **Nutritional, Functional Properties and Applications of Mee** (*Madhuca longifolia*) Seed Fat

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**Abstract:** Mee (*Madhuca longifolia* (Koenig) J.F. Macb. var. *longifolia*) is a salient economic tree spread throughout the subtropical region of the Indian subcontinent. Its seed constituents have distinctive chemical properties and thus extracted fat have distinctive nutritional and functional characteristics. This seed fat is edible and can Meet the fat requirements of a human diet in an economical way. Research on potential utilizations, understanding of the chemical composition, and nutritionally beneficial properties and industrial applications of Mee fat are important for efficient utilization. There are some reports available on potential applications of Mee fat in the food-processing industry. But, presently, it can only be considered as an underutilized seed fat. In India, Mee fat is used for edible purposes to a certain extent and similarly in Sri Lanka. Therefore, more scientific research should be conducted on Sri Lankan Mee varieties for edible purposes. Popularization of this valuable fat source is important. Scientific knowledge and agricultural tools have been effectively applied to make Mee fat beneficial for commercial applications. This review article summarizes recent research and studies available on botany, phytochemistry, functional properties, processing as well as food and other industrial applications of Mee fat.

Keywords: Madhuca longifolia; Mee seed; fat; phytochemistry

#### 1. Introduction

Mee (*Madhuca longifolia* (Koenig) J.F. Macb. var. *longifolia*) tree, also known as buttercup tree, is a forest tree distributed throughout the sub-tropical region of the Indian subcontinent. It belongs to genus *Madhuca* and has been represented by five species in the Indian subcontinent. They are *Madhuca longifolia* (Koenig) J.F. Macb. var. *longifolia*, *Madhuca latifolia*, *Madhuca butyracea*, *Madhuca nerifolia* and *Madhuca bourdillonii*. The most prevalent species of *Madhuca* are *M. longifolia*, *M. latifolia* and *M. butyracea* [1]. This study mainly focuses on the *M. longifolia* species. The tree *Madhuca longifolia* (Koenig) J.F. Macb. var. *longifolia* (synonymous with *Madhuca indica* Gmelin) belongs to the family Sapotaceae. People in Sri Lanka call it "Mee" in Sinhala language and "Illuppu" in Tamil language. According to Indian research studies, this tree has an important economic value in India, due to widespread uses of its seeds, fruits and flowers (Figure 1). *Madhuca longifolia* provides a solution for the three major "Fs", namely as food, forage and fuel. Furthermore, Mee contains high amounts of phytochemicals and oils which can be used to develop nutritional and pharmaceutical



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). products. Mee tree has a good potential for food and non-food applications [2]. There is a number of research studies available on the biology, phytochemical properties, and applications of Mee. According to phytochemical studies, Mee is a rich source of sugar, vitamins, protein, glycosides, alkaloids, tannins, flavonoids, steroids, terpenoids, saponins, and phenolic compounds. Thereby, it shows various pharmacological properties such as anti-inflammatory, antioxidant, analgesic, anti-hyperglycemic, hepatoprotective, anticancer, antiulcer, antitumor, neuro-pharmacological, and dermatological activities [3].



Figure 1. Mee tree (a); flowers (b); fruits (c); seeds (d).

Mee tree is a medium-to-large deciduous forest tree usually with a short trunk and a large, rounded crown. It is a large shady tree 10 m to 15 m in height. These trees are common in dry mixed deciduous forests. The tree can be grown in a wide variety of soil types and it prefers sandy soils. Mee trees can be found up to an altitude of 1200 m. It needs a mean annual temperature of 28–50 °C (maximum) and 2–12 °C (minimum), and an annual rainfall of around 550–1500 mm for proper growth [4,5]. But, Mee can also tolerate drought conditions although it is not a frost-hardy tree. This tree is a strong light demander, and its growth is suppressed under shade. The Mee tree fruits during April–May and the fruits are ovoid in shape and 2–6 cm long. They are fleshy and greenish in color, and contain 1–4 dark brown color seeds per fruit, which are rich sources of edible fat.

The seed collection was usually conducted in the months of May, June, and July. Mee seeds are comprised of quercetin, oleic, linoleic, arachidic, stearic acid, palmitic acid, aspartic acid, isoleucine, leucine, cyst cysteine alanine, proline, threonine, Mi-saponin A and B and myricetin [4]. Whole Mee seeds contain 50–61% of fat, 22% of carbohydrates, 16.9% of protein, 3.4% of ash, 3.2% of fiber, 2.5% of saponins, and 0.5% tannin [3].

Fats and oils are major constituents of a healthy diet. Hence, the consumer demand for natural and healthy edible oils is growing recently. Therefore, it is necessary to research potential edible oil seeds and their edible uses. Plant-based oils contribute to about 85% of the available oil or fat for human consumption, and there are very few plants that produce oils in commercial quantities [3]. Mee seeds produce a valuable amount of seed fat, around 50–60%, and they have the potential to be utilize in many edible and pharmaceutical products. The higher levels of linoleic acid and oleic acid content make Mee fat nutritionally

valuable. The tocopherol and sterols present in Mee fat have nutritional importance. Therefore, Mee fat can be utilized to fulfill the demands for nutraceuticals and food supplements with functional, health-promoting properties. Consumers are now focusing on nontoxic plant products which have traditional medical applications [3]. Mee fat can be used for cooking and stir-frying foods and also manufacture chocolates due to its beneficial fatty acid profiles. Other than that, the emulsifying property of Mee fat makes it an emulsifying agent in producing pharmaceutical products [6]. Scientific information on the composition, functional properties, and industrial applications of Mee tree are important to enhance its utilization.

This paper summarizes recent scientific knowledge on the Mee tree and the recent advancements in the processing and utilization of Mee fat in the food-processing industry, and reviews recent trends and patterns in processing methods and the functional properties of Mee fat. The main focus is on edible Mee fat processing and consumption. It also reviews debates, conflicts, and contradictions on the functional properties of Mee fat and focuses on gaps in the existing literature on fat-processing methods and functional properties of Mee fat to popularize its utilization for edible purposes.

#### 2. Value-Added Products from Mee Fruits and Seeds

#### 2.1. Mee Seed Fat

*M. longifolia* seeds are a novel commercial source of edible fat. Their fat is like a cocoa-like butter. Mee seeds are mechanically crushed to extract the fat and what remains is called the seed cake (Figure 2). Mee fat is light yellow in color and is retained as a semi–solid in tropical temperatures. Based on the iodine value (80 g  $I_2/100$  g Mee fat), Mee fat is considered a non-drying fat [3]. In long-term storage, its quality characteristics were deteriorated due to free fatty acid formation during the fat-extraction process. The rancidity of free fatty acids is a serious inhibiting factor for the utilization of Mee fat. The essence of toxic saponins and other lipid associates also leads Mee fat to be treated as a non-edible fat. If the extracted Mee fat was properly purified, it could use for edible purposes. Mee fat has a beneficial fatty acid profile with low saturated fatty acid content [7]. Approximately 250 mL of fat can be extracted from 1 kg of Mee seeds [4]. The seed fat contains 46% saturated fat, 37.4% monounsaturated fat, and 16.5% polyunsaturated fatty acids [3].



Figure 2. *Madhuca longifolia* seeds (a); seed oil (b); seed cake (c).

Mee's fat fatty acid profile consists of 21–25% of palmitic acid (16:0), 22–25% of stearic acid (18:0), 36–37% of oleic acid (18:1), 14–16% of linoleic acid (18:2) and 1.3% of arachidic acid (20:0). Sterol fraction consists 0.97% of campesterol, 7.47% of stigmasterol, 64.78% of B—sitosterol, 9.53% of  $\Delta$ 5—avenasterol, 4.08% of  $\Delta$ 7—stigmasterol and 9.67%  $\Delta$ 7—avenasterol [4]. The use of Mee fat in the food industry has been carried out on a limited scale. According to past reports, applications of this seed fat are limited mostly due to the lack of technical information regarding its properties and potential uses [7]. Semi-solid Mee fat is used in cooking, adulteration of ghee, and manufacturing chocolate. Mee fat could be used to replace hydrogenated fats, because they are free from trans fatty acids [8]. Mee seed fat could be incorporated in a number of dairy products such as cheese, ice cream, and whipping cream [8]. There is also available research data on biodegradable food packaging films which were fabricated from Mee fat-based polyurethane (PU) and chitosan (CS) incorporated with different proportions of zinc oxide nanoparticles [9].

#### 2.2. Defatted Mee Seed Cake

After extracting fat from Mee seeds, the remaining seed portion is called seed cake. It is a rich source of carbohydrates and vegetable proteins. Defatting increased the protein level in seed cake by a low percentage (16.9% to 19.68%). But, it increased the saponin level by a high percentage (2.5% to 16.7%) [3,10]. High saponin content is a limitation for food applications of seed cake. Researchers report that saponin content can be reduced by isopropanol treatment [3]. Saponin-detoxified Mee seed flour can be considered as a potential source of vegetable protein for human food and animal feed products. The defatted seed cake also showed good oil absorption and emulsification traits [3].

Researchers had analyzed proximate composition of seed cake in different agroclimatic zones in Sri Lanka. The researchers obtained powdered *M. longifolia* seed kernel from grinding the kernel and analyzed the powder samples [11]. The moisture content was determined according to AOAC method 925.13. Ash content was determined with AOAC method 923.03—the direct method. Protein and fiber contents were analyzed with AOAC official method 984.13 and AOAC method 1985. According to their study, moisture content of powdered seeds ranged from 9.5–10.86%, protein content ranged from 15.44–17.76%, ash content ranged from 1.62–2.44% and fiber content ranged from 28.03–30.59% [11]. The protein contents of widely available fat seeds are as follows, for coconut, cotton seed, ground nut, olives, palm kernel, soybean, and sunflower, values are 25.2, 40.3, 49.5, 6.3, 18.6, 47.5 and 34.1%, respectively. *M. longifolia* seed cake has a value for protein (15.44–19.68%) similar to palm kernel (18.6%) [8,10].

A research study reports that the seed cake can be used to produce low-grade fertilizer which significantly increases mushroom yield (128%) [8]. The presence of high amounts of volatiles such as proteins and starch material make the seed cake favorable in the biomethane production process. Anaerobic digestion of Mee seed cake, detoxified by simple water treatment, offers a viable way for waste to generate energy [9]. Compared to raw seed cake, detoxified *M. longifolia* seed cake has better chemical properties. The digested seed cake has an increased nutrient content and a significant reduction in cellulose (34.4%) and hemicellulose (29.7%) contents. Defatted seed cake also reduces gas production [3]. There are many other properties such as emulsification properties and foaming capacity, which give Mee seed cake potential importance in the field of the food-processing industry. This shows the suitability of Mee seed flour in the processing of certain bakery products which require flour with good absorption capacity and are also useful in the processing of products such as sausage and other meat products.

#### 3. Physical, Biological, and Chemical Properties of Mee Seed Fat

#### 3.1. Physico-Chemical Properties of Mee Seed Fat

Mee oil was extracted from dried kernel powder of Mee seeds using a Soxhlet apparatus and the extracted oil was used to determine the fatty acids as well as elements of Mee seeds [12]. The saponification value, iodine value, peroxide value, acid value, melting point, smoke point, specific gravity, and refractive index are some physicochemical parameters which were studied using AOCS (1999) and AOAC (1999) methods [12]. As coconut oil is the most cooking fat in Asian countries, it is vital to compare the physiochemical properties of Mee fat with coconut oil when popularizing Mee fat for edible uses (Table 1) [12,13].

| Type of<br>Oil/Fat | Saponification<br>Value (mg<br>KOH/g) | Iodine<br>Value<br>(gI <sub>2</sub> /100 g) | Acid Value<br>(mg<br>KOH/g) | Peroxide<br>Value<br>(meq/kg) | Melting<br>Point °C | Smoke<br>Point °C | Specific<br>Gravity<br>(at 25 °C) | Refractive<br>Index<br>(at 40 °C) |
|--------------------|---------------------------------------|---|-----------------------------|-------------------------------|---------------------|-------------------|-----------------------------------|-----------------------------------|
| Mee                | 181–184                               | 56–57                                       | 4                           | 3                             | 33–34               | 168–171           | 0.9272                            | 1.4672                            |
| Coconut            | 250–268                               | 6–11  | 0.2                         | 3                             | 24                  | 177               | 0.918                             | 1.4486                            |

Table 1. Comparison between Mee seed fat and coconut oil.

#### 3.2. Fat Content and Fatty Acid Profile of Mee Seed Fat

Most of the available research studies are on fatty acid profiles of Indian Mee varieties [7]. There is little research available on the chemical compositions of Sri Lankan Mee varieties [7,14]. There were similar results obtained for the fatty acid profile among the studies. Researchers report on 13 fatty acids which were identified in Mee seed extract [3]. Fatty acid methyl ester (FAME) analysis of fat shows oleic, stearic, palmitic, and linoleic as the major fatty acids, which together comprised more than 98.5% of the total identified FAMEs of Mee fat (Figure 3) [15–18]. Oleic acid was the main fatty acid (37.3%) followed by stearic acid (25.9%). According to this study Mee fat contains 46% saturated fatty acids, 37.4% monounsaturated FA, and 16.5% polyunsaturated FA (PUFA). Therefore, Mee contains a considerable amount of essential fatty acids (Figure 4) [3].

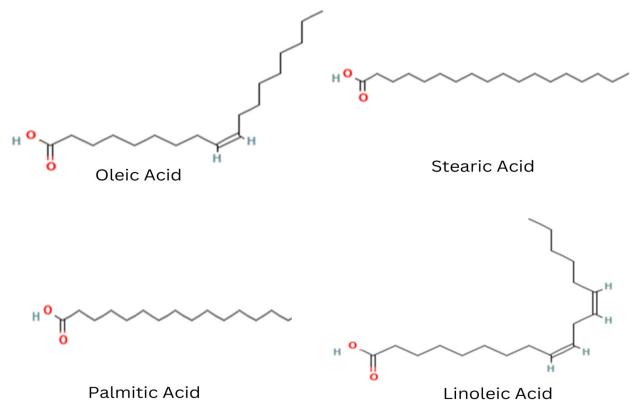


Figure 3. Major fatty acids present in Mee fat.

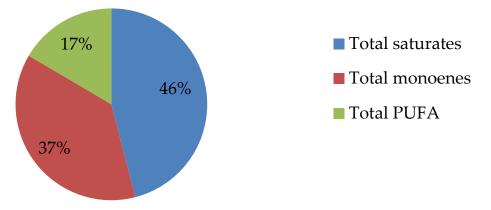


Figure 4. Total fatty acid profile (%) of Mee fat.

The fat content and the fatty acid profile of Mee seed fat from different geographical locations in Sri Lanka were studied by researchers [7]. They studied Mee fat samples processed from four different agro-climatic zones in Sri Lanka. The selected four agro-climatic zones were low-country dry zone, low-country wet zone, low-country intermediate zone, and mid-country intermediate zone. According to them, the mean fat content in seed kernels obtained from different agro-climatic zones ranged from 50.07 to 53.85%. The highest fat yield was reported in the low-country dry zone Mee seeds and the least reported in the mid-country intermediate zone. The fatty acid profiles of Mee fat samples were significantly different in four agro-climatic zones (Figure 5) [19]. A study reports how provenances significantly affect the differences in fruits and seed morphological characters [20]. According to them, there is a significant association between rainfall and the physicochemical properties of Mee seed fat. The FAMEs were prepared to determine the fatty acid profile in Mee using the method described by the Industrial Technology Institute (ITI), Sri Lanka (Figure 6) [3,7]. Around 15 types of fatty acids were identified in their study. They were oleic (C 18:1), linoleic (C 18:2), stearic (C 18:0), palmitic (C 16:0), myristic (C 14:0), palmitoleic (C 16:1), margaric (C 17:0), nonadecylic (C 19:0), cisgondoic (C 20:1), behenic (C 22:0), lignoceric (C 24:0), cemtic (C 26;0), arachidic, (C 20:0) and caprylic (C 8:0).

Between them, oleic, stearic, palmitic, and linoleic are the major fatty acids (FAs) in Mee fat [6,7]. The HPLC analysis of Mee fat shows a triacylglycerol (TAG) sequence similar to that of palm fat. Triacylglycerol formed from palmitic, oleic, and steric acids is responsible for the semi-solid nature of Mee fat. The synergistic effect of stearic and palmitic acid might elevate the melting point of Mee fat. Rich fatty acid composition and the higher levels of monounsaturated fatty acids make Mee fat a potential ingredient for nutritional applications [3]. Higher saturated fatty acid levels in vegetable oils are desirable in the food-processing industry mainly to avoid transesterification and hydrogenation processes in the production of solid fat products such as shortening and margarine, as well as to avoid the production of trans fatty acids.

Another research reports similar information on the fatty acid profile of Mee seed fat [14]. According to them, the major unsaturated FA in Mee fat is oleic acid and the major saturated FA is palmitic acid. The stearic acid content ranged from 13.70–24.50% and the highest value (24.50%) was recorded in the low-country intermediate zone [7]. The lowest level (13.70%) was reported in the mid-country intermediate zone. The oleic acid content ranged from 40.44–50.32%. The highest amount (50.32%) was reported in the low-country dry zone and the least was reported in the mid-country intermediate zone (40.44%). There is a considerable variation influenced by the environmental factors among the individual fatty acid contents.

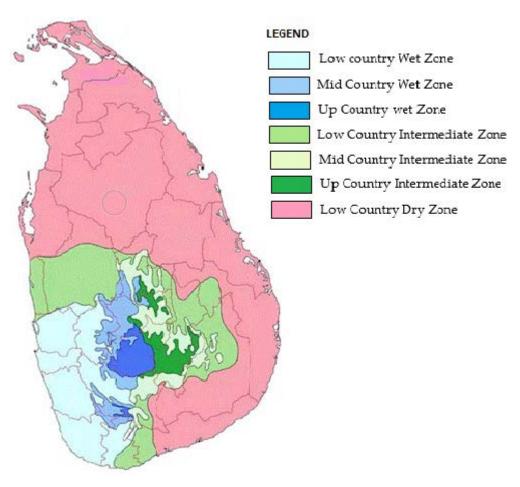
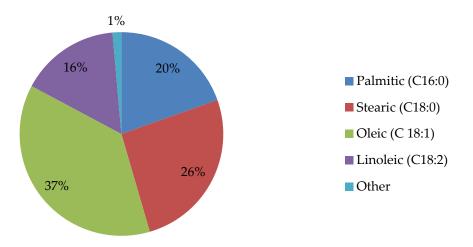
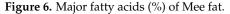


Figure 5. Agro-climatic zones of Sri Lanka.





The research was conducted to study the fatty acid profile of Mee fat using gas chromatography–mass spectroscopy (GCMS) [21]. Fatty acid methyl esters (FAMEs) were prepared from oil for the analysis. Four principal fatty acids as oleic acid, stearic acid, palmitic acid, and linoleic acid have been identified through GCMS. The Mee seed kernel quality can be determined from the oleic and linoleic acid ratio of the extracted oil.

#### 3.3. Unsaponifiable Matters Composition of Mee Seed Fat

The high unsaponifiable matter (UM) content (ca. 8 g/kg fat) in Mee is one of its distinct features. It is a limitation for Mee fat use in the manufacturing industry. Mee

fat contains a higher amount of phytosterols (3.94 g/kg TL). Phytosterol (ST) levels in vegetable fats are used to identify fats, and fat derivatives, and also to determine fat quality. Nine compounds were claimed as phytosterols (Figure 7) [3]. The 5-avenasterols consist of ca. 30.2% of the total ST content. An amount of 46% of the total ST consists of  $\beta$ -sitosterol and 5,24-stigmastadinol. Sitostanol, campesterol, stigmasterol, lanosterol, 7-avenasterol, and 7-stigmastenol were present in low quantities in Mee fat [3].

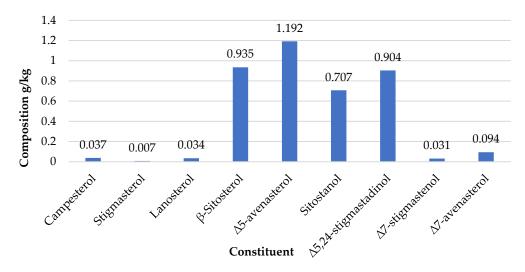


Figure 7. Sterol composition of Mee fat.

Other than phytosterols, tocopherols also play an important role as an unsaponifiable matter in Mee fat. There are four types of tocopherol isomers and three of them are present in Mee fat (Figure 8) [3]. An amount of 88.8% of total tocopherols present in Mee fat are  $\gamma$ -tocopherols. An amount of 9.6% of total tocopherols are  $\beta$ -tocopherols and the remaining 1.9% of total tocopherols are  $\alpha$ -tocopherols. Among them,  $\alpha$ -Tocopherol is the most efficient antioxidant of tocopherol isomers.  $\beta$ -tocopherol has 25–50% of the antioxidant activity of  $\alpha$ -tocopherol, and  $\gamma$ -tocopherol has 10–35% of the antioxidant activity of  $\alpha$ -tocopherol. Tocopherols add great value to Mee fat because of their nutritional value and strong stability toward oxidation [8].

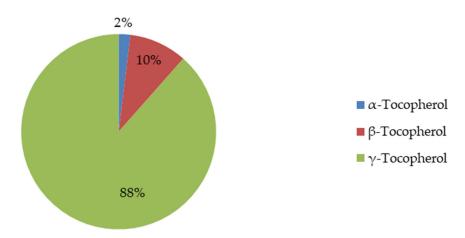


Figure 8. Tocopherol composition of Mee fat.

#### 3.4. Antioxidant Potential of Mee Seed Fat

Phytonutrients present in Mee fat have antioxidant properties and thereby contribute to the oxidative stability and improved shelf life of the fat. The natural antioxidants extracted from Mee fat can be used as a replacer for hazardous artificial antioxidants applied in the food-manufacturing industry [22,23]. Tocopherols and carotene present in Mee

seed fat have significant antioxidant potential. The antiradical properties of Mee fat were compared with extra virgin olive oil [3]. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals' quenching activity was assessed by the above researchers. After one hour of incubation, Mee fat has shown a higher antiradical potential than extra virgin olive oil. Mee fat has been reduced by approximately 25% of DPPH radicals whereas olive oil has been reduced by 9.40%. Compositional differences between fatty acids and lipid-soluble bioactive compounds such as phenolic acids, tocopherols, tocotrienols, and carotenoids present in Mee fat affect the antioxidant potential. The total phenolic content and antioxidant capacity of the methanolic extract of Mee seed oil was evaluated by measuring the absorbance through a spectrophotometer (Uviline 9400, SECOMAM) [22].

The antioxidant potential of Mee fat was measured using DPPH and 3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging assays and  $\beta$ -carotene/linoleate model system using  $\alpha$ -tocopherol as the reference antioxidant. The Mee fat extract has shown a dosedependent activity towards DPPH and ABTS radicals [24]. The IC50 value is the way of expressing the DPPH radical scavenging activity [24]. This value is 0.078 mg/mL for Mee fat extract and 0.031 mg/mL for  $\alpha$ -tocopherol. High antioxidant potential compounds have an IC50 value of less than 1 mg/mL. A comparison was performed between the antiradical properties of Madhuca fat and extra virgin olive oil using DPPH free radicals [1]. *M. longifolia* fat had higher antiradical potential than olive oil. Due to the different compositions of fatty acids and lipid-solubles in Mee fat and virgin olive oil, they have different antiradical actions. When considering the ABTS radical scavenging activity of Mee fat extract, it is 46.0% at a 2.0% (*w*/*v*) concentration. This value is lower compared to the radical scavenging activity of  $\beta$ -carotene (64.0%). But, there was a good potential for radical scavenging activity.

The bright yellow color of *Madhuca longifolia* seed fat provides evidence that it contains a considerable amount of pigments, e.g.,  $\beta$ -carotene. Naturally, it presents as a blend of cis and trans isomers of the  $\beta$ -carotene molecules and they have a potent antioxidant capacity [11]. A comparison was performed on the antioxidant potentials of Mee fat and coconut fat. In the DPPH assay method, the antiradical activity of antioxidants was evaluated using the radical form of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) [25]. This method was used to assess the antioxidant potential of Mee fat and coconut oil with few modifications to the method. BHT (butylated hydroxytoluene) was the positive control in this method [12]. The results show that the radical scavenging activity of Mee seed fat was moderately low compared to coconut fat. A comparison of the antioxidant activity of sesame (Sesamum indicum L.), soybean (Glycine max (L.) Merr.), and Mee (Madhuca longifolia (Koenig) J.F. Macb. var. longifolia) against photooxidation and autoxidation was reported [26]. The level of oxidation was determined by measuring peroxide value, thiobarbituric acid reactive substances, conjugated dienes, and conjugated trienes. According to their study, Mee fat has the least stability, and both the highest photo-oxidation and autoxidation as measured by primary oxidative products. By measuring secondary oxidative products, it demonstrates the strongest stability of Mee fat against both photo-oxidation and autoxidation.

#### 3.5. Mee Seed Fat Content and Quality upon Storage

Mee seed fat content and its quality depend on the storage conditions of the extracted fat [27]. Changes in Mee seed fat content and quality upon storage at different durations and conditions were studied by researchers [28]. In their study, researchers have investigated the effect of storage medium and storage condition on the fat content and other fat parameters of Mee seeds. Researchers also proved that the fat content and its composition can be changed by storage conditions, duration, exposure to light, and other environmental factors. According to them, the fatty acid oxidation of seed fats can be seen during long-term storage. Postharvest handling and storage of Mee fruits and seeds affect the final composition and the quality of processed fat. Especially, *Penicillium* and *Aspergillus* fungi genera induce seed deterioration and degradation by forming toxic materials on poor storage conditions [29].

A study was conducted on the storage behavior of Mahua seed in relation to seed longevity to determine the preferable storage medium and optimum duration to preserve better oil content and other oil quality factors such as specific gravity, free fatty acid profiles, saponification value, acid value, and iodine value of edible fats [30]. The study reports that the harvested Mee seeds should be stored in preferable storage containers by providing appropriate relative humidity ranges, and prior to seed storage, the seeds should be treated with fungicides. The change in fat content and other quality parameters by means of three types of container bags (polythene, plastic, and jute), by means of two storage environments (light and dark), and subjected to two air exposure conditions (closed and open conditions) were monitored by some researchers at a monthly interval for 180 days [28]. The fat content, saponification value, and iodine value of the stored Mee seeds were reduced with time. But, the specific gravity, acid value, and free fatty acid content of the stored seeds were increased. The best storage method for retaining higher oil content is a closed plastic container uncover to light. Other than that, a closed polythene bag remaining in the dark was the best storage method for preserving higher saponification value [28].

#### 4. Processing of Mee Seed Fat

Feedstock preparation for Mee seeds before the extraction step is an important prerequisite. First, seeds were separated from Mee fruits. Then, the separated seeds or kernels were sieved, cleaned, and stored at room temperature. Before storing, seeds were dried to reduce the moisture content. The seeds were sun-dried for a week to remove moisture content and then the kernel was separated according to the researchers [31]. The kernels were further oven-dried at about 60 °C for 72 h to remove excess moisture and stored at 37 °C since they were easily susceptible to fungal and insect attacks. The stored kernels were ground into a very fine powder and used for the extraction process.

After feedstock preparation, the raw material is ready for fat extraction. There are a few types of fat-extraction methods reported in the research studies. The main three extraction methods are mechanical extraction, chemical or solvent extraction, and enzymatic extraction. In addition, accelerated solvent extraction (ASE), supercritical fluid extraction (SFE), and microwave-assisted extraction (MAE) methods were also practiced to extract the fat. Mechanical pressing and solvent extraction are the most frequently used methods [32].

Traditional fat-extraction methods are still used to extract Mee fat by people in rural areas of India and Sri Lanka. "Sekku" and "Peha" are two examples for traditional fat-extraction methods (Figure 9). "Sekku" involves a stationary motar and a pestle which is rotated around its own axis. It is made out of wood or stone. This system is powered by an animal or hand tractor to crush oil seeds [33]. Dried matured seeds were put into a "Sekku" and ground well to extract the fat. The traditional method called "Peha" is also used to extract Mee fat [14]. In this method, the first seed pith was taken out. Then, the Mee seed pith was boiled and squeezed inside the "Peha" to extract the fat. There are limitations in traditional extraction methods because they do not yield sufficient quantities of fat for commercial purposes. Therefore, it is necessary to develop an efficient Mee fat-extraction method. It is important to know the scientific data on the physical and chemical properties of Mee seeds to decide and develop processing operations, equipment development, and further the industrial processing of seeds [34].

#### 4.1. Mechanical Press Fat Extraction

Mechanical press fat extraction uses a manual ram press or an engine-driven screw press to extract the fat. Mee seed fat extraction was conducted through a traditional screwtype fat expeller [14]. Researchers have analyzed the fat-expelling efficiency of the machine. The seeds can be subjected to a different number of extractions through the expeller and thereby produce seed fat and seed cake mixture continuously during the process. The researchers had modified the screw-type expeller to improve the fat-extraction efficiency. The optimum fat yield and quality of the fat were determined by evaluating the refractive index, saponification value, iodine value, unsaponifiable matter, free fatty acid content, and specific gravity to assess the quality of the refined fat [14]. Modified machine performances were evaluated. The crude fat yield of seeds, machine capacity, and energy consumption at different screw shaft speeds were calculated in this study. For the crude fat yield, the highest yield was obtained at a screw speed of 90 rpm. The maximum fat yield of 35% was also achieved under this condition. There can be observed a correlation between the fat yield and the speed levels. The energy consumption is lowest at speeds of 90 and 120 rpm. According to previous studies, the saponification value of Mee fat was reported in the range of 181-184 mg KOH/g Mee fat [12]. The value near this range was observed at a screw speed of 90 rpm. The color of extracted oil was lighter when the modified machine speed decreases.



Figure 9. Traditional fat-extraction methods: Sekku (a); Peha (b).

#### 4.2. Solvent Oil Extraction (Chemical Extraction)

The fat is extracted from the solid through a leaching process using a liquid solvent. The chemical extraction using the n-hexane method gives the highest fat yield [35]. The chemical extraction method was considered an effective method because it extracts higher fat yield and due to its reliable performance. The chemical extraction of Mee fat has been conducted using different solvents and different time periods according to the AOAC method [12]. The extractions were carried out for 6 h, 5 h, and 4 h. The solvents n-Hexane (bp. 65–70 °C) and petroleum ether (bp. 40–60 °C) were tested in the study. Four hours of extraction time with n-hexane can be used as the method provides better Mee fat extract efficiency with good quality. The rate of solvent extraction depends on many factors such as particle size, the type of solvent used, temperature, and agitation speed. According to the reports, solvent extraction is only economical in large-scale fat extractions. There are negative environmental impacts from the solvent extraction method. When considering the n-hexane solvent, it has wastewater generation, higher specific energy consumption, and higher emissions of volatile organic compounds [35].

#### 4.3. Ultrasonic-Assisted Bio-Oil Extraction

The effect of ultrasonic-assisted extraction for Mee seeds through optimizing conventional solvent extraction procedures was studied [31]. Ground Mee seed powder was mixed with different solvents to study the extraction process. Both single solvents and combinations of solvents were tested. There, diethyl ether/ethanol, chloroform/methanol, and isopropanol/methanol were used as solvent combinations in suitable proportions. Investigations were performed to optimize various parameters like temperature, extraction time, type of solvent, solvent ratio, potassium chloride, and hydrochloric acid concentrations of the extracted fat. Further increases in fat extraction from Mee seeds were achieved through the ultrasonic-assisted extraction method. They characterized Mee fat by gas chromatography-mass spectrometry (GC-MS) and studied the fat characteristics. Through solvent extraction, a 77.9% fat yield was obtained from Mee seeds. Their mixed solvents, diethyl ether, and ethanol were taken in a 3:1 ratio at 50 °C for 20 min. as the extraction medium. This fat yield was increased while performing the ultrasonic-assisted solvent extraction. The extracted Mee fat yield was observed as 82% in ultrasonic-assisted solvent extraction. GC-MS confirmed that the Mee fat contains octadecenoic acid. Extraction kinetics and optimization parameters were analyzed in this study which can be further incorporated with the large-scale processing of Mee fat. The oil yield and recovery of different Mee oil extraction methods were compared by some researchers [22]. The results revealed that ultrasound-assisted solvent extraction (UAE) is more efficient than Soxhlet extraction and mechanical extractions. UAE produced 99.54% of oil recovery and an oil yield of 56.97% under minimum energy consumption. Milder conditions such as shorter extraction time (35 min.) and lower temperature (35  $^{\circ}$ C), and a binary mixture of acetone and isopropanol (1:1 v/v) were utilized to obtain the above-mentioned oil yield and recovery from UAE. This method also showed a higher antioxidant capacity to the extracted oil than Soxhlet extraction and mechanical extraction, and does not alter fatty acid and triacylglycerol profiles.

#### 5. Potential Industrial Applications of Mee Seed Fat

#### 5.1. Stir Frying Application

Sensory evaluations were conducted for Mee fat and coconut fat for frying purposes. There, Mee fat and coconut fat were compared with each other for deep-fat frying and stir-frying of french fries using the above two types of fat [12]. Taste, odor, mouth feel and organoleptic acceptability were evaluated as sensory attributes. The test results show that in the stir-frying method, there was no significant difference (p > 0.05) between Mee fat and coconut oil for the evaluated sensory attributes. According to this research, Mee fat has a good potential as cooking fat. During the frying process the  $\Delta$ 5-avenasterol present in Mee fat helps to protect fat from oxidative polymerization [3].

#### 5.2. An Alternative Ingredient for Halal Fats

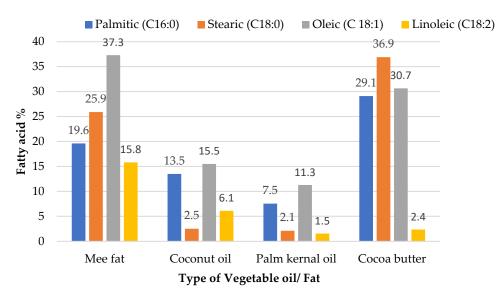
The potential of Mee fat as an alternative ingredient for halal fats (fats which are permitted under the Islamic Law and which Muslims are allow to eat without any punishment) was tested by some researchers [36,37]. Pork fat is a common halal fat used in food processing that has an animal origin. Mee fat is a plant-based non-halal fat with good processing potential. Studies show that pork fat has more unsaturated fatty acids (USFA) than saturated fatty acids (SFA). Oleic acid (38.24%) is the major fatty acid in pork fat. Other fatty acids available in pork fat are palmitic (22.68%) and linoleic (20.39%) acids. In Mee fat, major fatty acids are oleic (44.02%), stearic (22.05%), and palmitic (20.88%) acids. But, it has a very small amount of linoleic acid (7.85%) compared to pork fat. Mee fat has a higher saturated fatty acid content (42.93%) compared to pork fat (36.62%). Fractionation results in stearin fractions (solid) and olein fractions (liquid) of the fat. In the above study, the basic physico-chemical parameters of pork fat, Mee fat, and their fractions were evaluated. Their slip melting point (SMP), cloud point (CP), and iodine value (IV) were analyzed and compared with each other. The results show that Mee fat has a higher SMP value compared to pork fat. Compared to native samples, stearin fractions had a higher SMP. The SMP of pork fat stearin (45.75 °C) was lower than Mee fat stearin (46.50 °C). CP values were used to evaluate the thermal characteristics of olein fractions. Pork fat olein has a lower CP compared to Mee fat olein. IV represents the degree of unsaturation of fats. Pork fat shows an IV of 73.80 while Mee fat shows an IV of 61.10. When considering stearin fraction, pork fat has a lower IV than Mee fat stearin. However, pork fat olein has a higher IV value compared to Mee fat olein. This study reports some common thermal characteristics of pork fat and Mee fat. Both fats have thermal transitions at low and high temperatures, and these fats yield solid stearin and liquid olein. Both pork fat and Mee fat display similar

saturated fatty acid profiles between 0  $^{\circ}$ C to 25  $^{\circ}$ C. Therefore, Mee fat could be used as an alternative ingredient for halal fats.

The physicochemical properties and thermal behavior of the Mee seed fat and palm oil blends were evaluated by [38]. There, Madhuca longifolia seed fat is composed of palm oil to form a substitute for lard (pork fat). Moreover, the solidification and melting characteristics of Mee seed oil and palm oil were evaluated by the researchers. Various binary blends of Mee oil (ML) and palm oil (PO) were formulated in order to develop a similar substitute for lard. Three blends were prepared: ML: PO (97:3; w/w), ML: PO (95:5; w/w), and ML: PO (93:7; w/w). Each blend was compared with lard (LD) in terms of fatty acid composition, triacylglycerol composition, and thermal properties. Solid fat content (SFC) is one of the principal physical characteristics of fats. SFC profile can be used to evaluate the special applications of fats. Chemical properties of fat are also an important quality characteristic that should be considered during formulation mainly in terms of the nutritional value of fat-based food products. According to the Solid Fat Content profile, both lard and palm oil have similar solidification behavior within the 25-40 °C temperature range, whereas Mee oil (ML) is found to be compatible with that of LD, within a 0–25 °C temperature range. The addition of PO into ML can provide a better compatibility of solidification behavior to LD. The formulated blends were significantly different from LD with regard to fatty acid and triacylglycerol compositions. But, there were some similarities regarding thermal properties and solid fat content profiles. The blend of ML: PO (97:3; w/w) displayed closer similarity to LD with respect to melting transition at -3.59 °C and its solid fat content profile showed the least difference to that of LD throughout the measured temperature range.

#### 5.3. A cocoa Butter Substitute

Mee fat has a similar fatty acid profile to cocoa butter (Figure 10) [3,39–41]. The high levels of oleic and stearic acids in Mee fat give it the suitability to be a cocoa butter substitute [42,43]. Therefore, Mee fat has the potential to produce chocolates, confectionery products, and shortenings.



**Figure 10.** Comparison of average levels of major fatty acids (%) in Mee fat with selected natural semi-solid fats and oils.

#### 5.4. Development of Food Packaging Material

Food packaging films made out of antimicrobial compounds are important in food safety to protect food from microbial contamination and increase its shelf life [10]. Petroleum-based polymeric substances such as polyethylene, polycarbonate, polyethylene terephthalate, polyvinylchloride, polypropylene, polystyrene, and polyamides are the most common packaging materials. Due to environmental concerns, bio-based polymer films are successful alternatives for food packaging due to their easy availability, low cost, and biodegradability. But, these polymers lack tensile strength and water absorption. These can be controlled by combining them with other polymeric substances which improve the desired characteristics of the film. Pure polysaccharides can composite with nanoparticles such as AgO, TiO<sub>2</sub>, and ZnO according to research data available. Other than that, plant extracts such as neem, papain, grape, and green tea extract also have moderate to very good antibacterial activity and could be incorporated in food-packaging developments. According to available reports, polyurethanes (PU) are one of the broadly studied film-forming polymers with a wide range of applications in food packaging. Polyurethanes are generally developed by the polymerization reaction between polyols and diisocyanates. Consideration of polyols extract from vegetable fats including castor fat, soybean fat, jatropha fat, and rapeseed fat has been engaged due to environmental safety considerations. Due to their low toxicity, sustainability, and biodegradability, these polyols are reported as better alternatives to fossil fuels. But, they also have drawbacks such as low mechanical strength and degradation effects. Therefore, they were combined with inorganic compounds or mixed with other synthetic or natural polymers.

A research study was performed on Mee fat-based polyurethane, chitosan, and nano zinc oxide (ZnO) composite films for biodegradable food-packaging applications. There, they first converted Mee fat into polyols through epoxidation followed by hydroxylation [10]. Their work was focused on the creation of biodegradable nanocomposite films from Mee fat polyol-based polyurethane, chitosan (CS), and ZnO nanoparticles. Thermal, mechanical, antibacterial, biodegradation, transparency, and hurdle properties of the plain CS, plain PU, PU-CS blends, and PU/CS/ZnO films were compared in their study. The research outcomes emphasize that ZnO can enhance the hydrophobicity of the film by about 63% compared to the PU/CS blend film. Chitosan influences the oxygen permeability and PU positively alters the water vapor transmission rate and surface wettability of the films. The synergistic interactions of all three constituents have led to the composite film being a potential packaging material. These researchers concluded that ZnO-reinforced PU/CS film can be proposed as a potential biodegradable food-packaging material.

#### 5.5. Pharmaceutical Product Manufacturing

Natural products are gaining a high demand in the present in many industrial fields. Medicinal product manufacturing is one of them. The major components of a medicinal cream are the active pharmaceutical ingredient (API) and the excipient. The excipients sometimes act as "non-active agents" and also have been found to enhance the activity of the API. Agar, alginates, cellulose, gelatin, guar gum, pectin, starch, and xanthan gum are some of the natural pharmaceutical excipients used in the pharmaceutical products manufacturing industry. They are composed of therapeutic supplementary properties and also have applications such as binding agents, coating material, disintegrating agents, gelling agents, stabilizers, sustaining agents, thickening agents, etc. The role of Mee fat as an emulsifier for formulating the w/o cream was studied by a group of researchers [44]. The cream was formulated using erythromycin stearate, stearic acid, potassium hydroxide, glycerine, Mee fat, and methylparaben. Separate cream formulas were developed and the physical and chemical properties of each cream formulation was evaluated.

#### 5.6. Formulation of Coating Binders

The development of polyetherimide-based corrosion-protective polyurethane coating from Mee fat was carried out [45]. The Mee fat was successfully used in the preparation of polyetherimide resin (MPEA)-derived polyurethanes. There, Mee fat was converted to fatty amide (MFA) and then converted into polyetherimide (MPEA). The synthesized materials were characterized by spectroscopic methods. It possesses good coating properties such as gloss, pencil hardness, and thermal stability. Mee fat-based polyurethane resins have excellent potential properties for use in the formulation of coating binders. The prepared

coatings applied on metal and particle board panels and the performance of the coatings were analyzed and found to be suitable as an anticorrosive coating material.

#### 5.7. Biodiesel Production

Mee fat also has good potential in biodiesel production and can partially replace petroleum diesel. Due to less environmental pollution, low-cost biomass receives attention in biodiesel manufacture. Vegetable fats such as soybean, rapeseed, sunflower, and safflower are generally used to prepare biodiesel [46]. Biomass is one of the technically and economically feasible options against fossil fuels. Biofuel can also be used in any mixture with fossil fuels because of their similar characteristics. This includes common straight fatty acids such as palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) [47]. Fatty acid methyl esters (FAMEs) of seed fat are utilized as a substitute fuel in a diesel engine.

The quality of FAMEs is decided by their saponification value (SV), iodine value (IV), and cetane number (CN). For Mee fat, the saponification value is 198.3–202.8, the iodine value is 52.0–68.6 and the cetane number is 58.0–61.6. Biodiesel can be produced from raw fat through different methods. Transesterification, dilution, microemulsions, and pyrolysis are some examples of such methods. Among the above methods, the transesterification process is the most common method to produce biodiesel from fat [48]. According to some researchers, the transesterification method is affected by factors such as the quantity of alcohol and catalyst, pressure, speed, time, reaction temperature, water in raw fat, and content of free fatty acids [49]. An experimental research on Mee methyl ester combined with diesel fuel in a compression ignition diesel engine was conducted by researchers [50]. Mee Methyl ester (MME) was formed through the transesterification process. The physical properties of the developed MME-incorporated fuel formulas were compared with the requirements of standards. According to the analysis, the emission attributes of carbon monoxide, hydrocarbons, and smoke opacity were decreased for all the fuel formulas. The quality performance and lower emissions make it a good replacement fuel to engines without any modifications [8,51]. Three methods of biodiesel manufacturing as the conventional method, ultrasonic horn method, and ultrasonic bath method were evaluated by a research group [45]. The highest biodiesel yield was gained from the ultrasonic horn method and the least biodiesel yield was gained through the conventional method.

#### 6. Limitations and Gaps in Processing and Applications of Mee Fat

The raw material supply chain of Mee flowers and seeds does not occur consistently. In many parts of India, the Mee tree is utilized to a certain extent. But in Sri Lanka, it has a very limited utilization. Most of the Mee tree populations available are in their old age. The frequency of natural rejuvenation is very poor for the Mee tree [52]. Therefore, even quality raw materials for industrial applications are limited. Inadequate knowledge of postharvest handling of Mee seed and flower affects the quality of raw materials for industrial applications of Mee. Currently, there is limited work available on the postharvest technology of Mee. The research work on Mee postharvest should improve to popularize industrial applications of Mee. There is also a compositional limitation of the utilization of Mee seeds because seeds contain a considerable amount of saponins and tannins. The mean lethal dose LD50 of saponin extracted from Mee was found to be 1000 mg/kg in mice on oral intake. According to the European Food Safety Association, an intolerable dose of Mee fat could result in anti-fertility [3]. It is important to identify and minimize risks associated with Mee oil consumption.

#### 7. Therapeutic Potential and Nutritional Properties of Mee Seed Fat

Mee can be considered a medicinal plant with a good pharmacological profile. It provides potent aid for its future clinical uses in modern medicine [7]. Mee (*Madhuca longifolia*) seed fat has a number of medicinal and nutritional properties. Many researchers have reported on the anti-inflammatory property, anti-cancer properties, hypoglycemic

properties, anti-ulcer activity, laxative properties, etc. of Mee seed fat [42,53,54]. The antiinflammatory action of Mee on rats was also evaluated by a research group [55]. Mee fat also has demulcent properties. Therefore, it can be used to treat skin diseases, rheumatism, headache, laxative, and piles, and also as a galactagogue. The ethanol extract and saponin blend of Mee seeds was evaluated for anti-inflammatory action using acute, sub-acute, and chronic models of inflammation in rats [56]. There, ethanol extract and a saponin mixture of Mee at a different dose level was studied. The edema caused by carrageenan in the acute model of inflammation was significantly reduced by the above mixture. Both extracts had a more effective response than the reference drug in the sub-acute inflammation model. Results prove the significant anti-inflammatory activity of *M. longifolia* saponins. An in vitro study on the anticancer activity of different extracts of Mee seeds against human cancer cell lines was experimented with and evaluates the cell growth inhibition [57]. The study found that various extracts of Mee seeds have very good to moderate anticancer activity.

The ethanolic extract of *M. longifolia* significantly reduced the plasma glucose level in normal albino rats in a dose-dependent way. This hypoglycemic effect occurred due to the stimulating effect of seed extract to release insulin from the  $\beta$ -cells and/or increase the uptake of glucose from the plasma. The benefits of tocopherols to the human body are the protection of PUFA from peroxidation, reduction of prevalence of heart attacks, and reduction of muscle damage from oxygen-free radicals produced during exercise [8]. Certain sterols such as  $\beta$ -sitosterol have the favorable effect of lowering the amount of cholesterol in the blood [3].

#### 8. Future Perspectives of Edible Mee (Madhuca longifolia) Seed Fat

Research work on Madhuca longifolia is a step forward on the transparency of reliable facts and data on the identity of the plant, its characteristics, postharvest techniques, and utilization. Prerequisite information for the safety and value of medicinal plants must be addressed through scientific research. There is a need for strong legislation and conservation measures to regulate the damage that occurs to the Mee trees and their ecosystem, and protect good genotypes of Mee. To obtain a large scale of quality Mee seeds and flowers, it is essential to introduce high-yielding genotypes to the agroforestry [34]. There is less research on the food applications of Mee fat and genetic analysis of the Mee tree. Since there are many potential applications of Mee fat, there is a direct necessity in increasing the cultivation of this valuable plant. There is also a need to achieve improvements in its genetic characteristics to expand its commercial utilization [58]. The significance of genetic diversity and phytochemical evaluation of *Madhuca* spp. in Sri Lanka was reviewed. According to their studies, there are seven Madhuca species currently found in Sri Lanka and four of them are endemic. As a plant with a wide scope of potential applications, presently, there is only one study available in the published literature on the phytochemical analysis of four Madhuca species found in Sri Lanka and there are no supporting materials on genetic studies of genus *Madhuca* in Sri Lanka.

Therefore, it is necessary to conduct such phytochemical analysis of different Madhuca spp. in Sri Lanka and to study genetic data and molecular verification studies to fill the gaps in phytochemical studies of Sri Lankan Mee varieties. The need of keeping the genetic data of the genus *Madhuca* in databases for public access for future research purposes and effective viable germplasm management of the Mee tree will widen the research scope and potential application development of this precious plant. High-yielding genotypes of Mee introduced to Agroforestry will help to increase the availability of quality Mee seeds and flowers at a large scale and be beneficial to farmers to achieve a sustainable harvest, value addition, and marketing [2]. Similar to most of the tropical fruit seeds, *M. longifolia* seeds are also underutilized for edible fat production. This could possibly be due to the lack of scientific information with concern for its properties and potential uses. Presently, there are some reports already available in the literature highlighting the compositional characteristics of the Mee seed fat and specifying its potential uses as an edible fat in the field of food processing. Under tropical conditions, *M. longifolia* fat generally exists with a

semi-solid nature. Therefore, it has a chance to be separated into solid and liquid fractions. This may be a good prospect to develop its application in many food applications.

A ZnO-reinforced PU/CS film was proposed as a potential biodegradable foodpackaging material [9]. Further research should be carried out to study the practical applications of biodegradable food-packaging materials in the protection of a variety of food items. Few types of modern fat-extraction methods were studied by researchers for fat extraction from flaxseeds [47]. They are the accelerated solvent extraction (ASE), aqueous enzymatic fat extraction (AEOE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE) methods. Their fat quality, fat yield and the physicochemical properties, and fatty acid profiles of the extracted fat were studied in these methods. The extraction time and solvent consumption for ASE method is low compared to the other solvent extraction techniques according to the study. These modern methods can be used as potential fat-extraction from the above modern methods, research trials determining the Mee fat-extraction efficiency and the quality of extracted oil can be conducted.

The extensive study of *M. longifolia* seeds regarding the processing of Mee oil and designing related processing equipment is necessary to popularize edible Mee fat. The research was conducted with the aim of the characterization of Mee seed in terms of its physical, mechanical, and chemical properties [31]. These characteristics are important in deciding the processing methods, conditions, and the designing of postharvest types of equipment. The researchers have experimented with physical properties such as principal dimensions, sphericity, surface area, density, and frictional properties. The mechanical properties such as hardness, deformation at rupture, percentage deformation at rupture, and practicability were assessed during their study. Other than that, chemical properties such as moisture, protein, oil, carbohydrates, and ash content were also analyzed by the researchers to advance the processing of edible Mee fat.

#### 9. Conclusions

Mee seeds are a satisfactory source of edible fat after a proper extraction and detoxification process. They yield a significant amount of fat. Mee fat is easily available and is of low cost. This fat can be considered as a potential source of essential fatty acids and lipid-soluble bio-actives. High oleic and linoleic acid composition make Mee fat a nutritional component for a regular diet. The higher antiradical potential due to tocopherol and phytosterol contents also makes Mee fat more valuable in food processing. There, the high levels of oleic and stearic acids in the triglyceride composition are similar to that of cocoa butter. Thus, it is a preferable fat to process chocolates and other confectionery products in countries of the tropical territories. It could be also used in the development of shortenings and margarine, and as a base for cosmetics and pharmaceuticals. Other than that, Mee fat also has potential as a substitute fuel choice for diesel. Since the valuable utilization of Mee fat in the food industry, it would be beneficial to develop the fields of cultivation, propagation, postharvest, and processing technology of Mee. Sustainable development strategies for Madhuca longifolia (Mee) are needed, balancing environmental, social, and economic considerations. They will reduce the negative impacts of practices like palm oil plantations.

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