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Review

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

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**Abstract:** Mee (*Madhuca longifolia*) is an important economic tree growing throughout the subtropical region of the Indo-Pak subcontinent. Mee seed constituents have distinctive chemical properties and thus provide nutritional and functional characteristics to the mee fat. The mee seed fat is an edible fat which can provide fat requirement of human diet in an economical way. Research on potential utilizations, understanding on the chemical composition, nutritional value and industrial applications of mee fat is important for efficient use mee seed fat. There were some reports available on potential applications of mee fat in food processing industry. But presently mee fat can be considered as an underutilized seed fat. In India mee fat used for edible purposes for a certain extent with compare to Sri Lanka. Therefore, more scientific researches should conduct on Sri Lankan mee varieties on edible purposes and popularized edible uses of Mee fat in Sri Lanka. Scientific knowledge and agricultural tools should effectively apply to make mee fat beneficial for its commercial applications. This chapter summarizes recent research work and studies available on botany, phytochemistry, functional properties, processing as well as food and non-food industrial applications of mee fat and other valuable parts of mee tree.

**Keywords:** *Madhuca longifolia*; mee seed fat; phytochemistry; underutilized

## 1. Introduction

Mee tree also known as buttercup tree is a forest tree distributed throughout the sub-tropical region of the Indo – Pakistan subcontinent. It belongs to genus *Madhuca* and represented by five species in the Indian sub-continent. They are *Madhuca longifolia*, *Madhuca latifolia*, *Madhuca butyracea*, *Madhuca nerifolia* and *Madhuca bourdillonii*. The most prevalent species of *Madhuca* are *Madhuca longifolia*, *Madhuca latifolia* and *Madhuca butyracea* [1]. In this study mainly focus on *Madhuca longifolia* species. The tree *Madhuca longifolia* (Koenig) (Synonyms, *Madhuca indica*) Gmelin is belongs to the family Sapotaceae. People in Sri Lanka call it as “Mee” in Sinhala and “Illuppu” in Tamil. This tree has an important economic value due to wide spread uses of its seeds, fruits and flowers. *Madhuca longifolia* provides an answer for the three major “F”s name as food, fodder and fuel. Furthermore, mee contains higher number of phytochemicals and oils which can use to develop nutritional and pharmaceutical products. There is a good potential for food and non-food applications for valuable parts of mee tree [2]. There was the number of research studies conducted on the biology, physiochemical properties, and applications of Mee. According to phytochemical studies, mee is a

rich source of sugar, vitamin, protein, glycosides, alkaloids, tannins, flavonoids, steroids, terpenoids, saponins, and phenolic compounds. Thereby mee shows different pharmacological properties such as anti-inflammatory, antioxidant, analgesic, anti-hyperglycemic, hepatoprotective, anticancer, antiulcer, antitumor, neuro-pharmacological, and dermatological activities [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 are producing a valuable amount of seed fat around Ca 50-60% and it has the potential to utilize in many edible and pharmaceutical products. The higher levels of linoleic acid and oleic acid content make mee fat nutritionally valuable. 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 having 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 [4]. Scientific information on the composition, functional properties, and industrial applications are important to popularize the Mee tree. 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 Mee oil processing methods and the functional properties of Mee oil. The main focus is on edible mee fat processing and consumption. It also reviews debates, conflicts, and contradictions on the functional properties of Mee oil and focuses on gaps in the existing literature on Mee oil processing methods and functional properties of Mee fat to popularize its utilization for edible purposes.

## 2. General description of mee tree

Mee (*Madhuca longifolia* L.) tree is a medium size to large deciduous forest tree usually with a short trunk and a large, rounded crown. It is a large shady tree with 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 soils. But it prefers sandy soil. Mee trees can be found up to an altitude of 1200 meters. 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 [5,6]. But Mee also can 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. 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 (Figure 1).



**Figure 1.** Mee tree, flowers, fruits, and seeds.

The seed collection was generally performed in the months of May, June, and July. Quercetin,  $\alpha$ -amyrin acetates,  $\beta$ -amyrin acetates,  $\beta$ -sitosterol and its  $3\beta$ -D-glucoside, n-hexacosanol, and dihydroquercetin are the major chemical constituents present in mee fruits. Other than that the mee seeds are comprised of quercetin, oleic, linoleic, arachidic, stearic acid, palmitic acids, aspartic acid, isoleucine, leucine, cyst cysteine alanine, proline, threonine, Mi-saponin A and B and myricetin [5]. 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]. Almost every part of the Mee tree such as bark, leaves, flowers, fruits, and seeds comprise active ingredients in small or large proportions. Some of them have medicinal properties, some have economic importance, and some compounds are toxic [7]. Mee tree has many potential uses in the field of the pharmaceutical industry, food processing industry, and biofuel processing industry.

### 3. Value-added products from mee (*Madhuca longifolia*) fruits and seeds

#### 3.1. Mee seed fat

*Madhuca longifolia* seeds are a novel commercial source of edible fat. It is a cocoa-like butter. Mee fat is pale yellow in color and remains semi-solid in tropical temperatures. Based on the iodine value (ca. 80), mee fat has considered a non-drying fat [3]. It has a poor keeping quality due to free fatty acid formed during boiling of the kernel during extracting fat. 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 treat as a non-edible fat. If the extracted Mee fat was properly purified it could use for edible purposes (Figure 2). Mee fat is characterized by various nutritional and functional properties. Mee fat has a beneficial fatty acid profile with less saturated fatty acid content [8]. According to [5] approximately 250 ml of oil can be extracted from 1 kg of mee seeds. The seed fat contains 46% of saturated fat, 37.4% of monounsaturated fats, and 16.5 % of polyunsaturated fatty acids [3].





**Figure 2.** Madhuca longifolia seed oil and seed cake.

Mee 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 [5]. Mee seeds are a type of an underutilized seed type for the production of fat. The use of Mee fat in the food industry has been carried out on a limited scale. According to past reports, the utility of this seed fat is mostly due to the lack of technical information regarding its properties and potential uses [8]. Semi-solid Mee fat is used in cooking, adulteration of ghee, and manufacturing chocolate. They could be used in place of hydrogenated fats as they are free from trans fatty acids [9]. Mee seed butter may also involve in many dairy products such as cheese, ice cream, and whipping cream [9]. 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 [10].

### 3.2. Defatted Mee seed cake

After extracting fat from Mee seeds, the remaining portion is called as seed cake. There are edible seed cakes with higher nutritional value and non-edible seed cakes also. Defatting Mee seeds increased the protein level. The content of protein of widely available fat seeds is as follows For Coconut, Cotton seed, Ground nut, Olive, Palm kernel, Soybean, and Sunflower, values are 25.2, 40.3, 49.5, 6.3, 18.6, 47.5 and 34.1 %. Madhuca longifolia seed cake has a value for a protein similar to Palm kernel (18.6%). Therefore, it can be utilized as a source of protein in food applications [8]. Detoxified Mee seed flour appears to be a good source of vegetable protein for human food and animal feed products. The cake is a rich source of sugar nitrogen and protein. It contains carbohydrates (42.8%), protein (30%), saponins (9.8%), ash (6%), fiber (8.6%), lipids (1%), and tannins (1%). Saponins and tannin levels in seed cake also increase through the defatting of Mee seeds. The saponin content can be lowered by isopropanol treatment. The de-fatted seed cake showed good oil absorption and emulsification traits [3].

There powdered Madhuca longifolia seed kernel was gained from grinding the kernel. Moisture content was determined according to AOAC method 925.13. Ash content was determined with AOAC method 923.03 – direct method. Protein and Fiber contents were analyzed with AOAC official method 984.13 and AOAC method 1985. The moisture content 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 ranges from 28.03-30.59% [8]. The proximate chemical composition of whole mee seed and defatted mee seed flour is given in below (Figure 3). The composition of seed meals varies depending on their variety,

growth conditions, and fat extraction method. Phytochemical analysis done by [3] reported that defatted seed cake contains 0.65% of total ash, 23.0% of alcohol-soluble extractive, 5.95% of water-soluble ash, 0.5% of acid-insoluble ash, 17.7% of water-soluble extractive and 12.3% of chloroform extract.

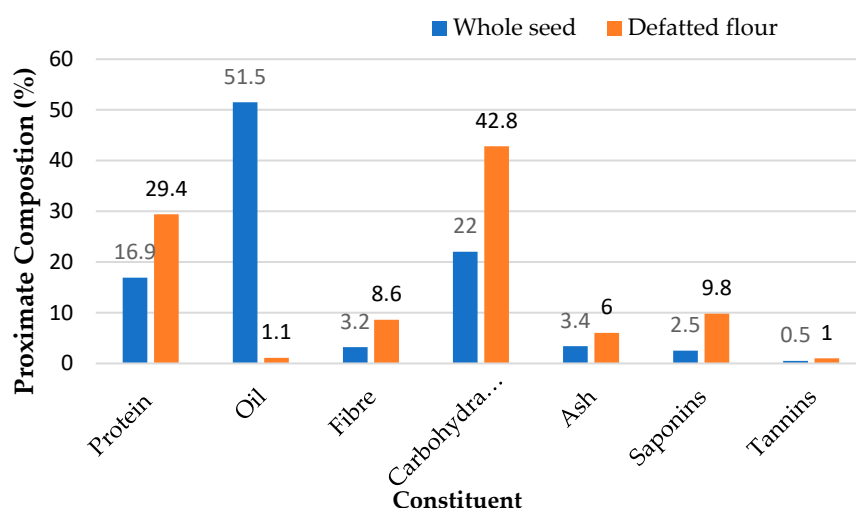


Figure 3. Proximate composition (%) of mee seed and defatted flour [11].

A study was carried out [8] on variations of seed morphology, fat content, and fatty acid profiles of *Madhuca longifolia* grown in different agro-climatic zones in Sri Lanka. According to their study Moisture content ranged from 9.5 – 10.86%, protein from 15.44 – 17.76%, ash from 1.62 – 2.44%, and fiber from 28.03 – 30.59%. The study done by [9] shows that the seed cake can use to produce low-grade fertilizer which significantly increases the mushroom yield (128%). 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 one of the viable tools for waste to generate energy [9]. Compared to raw seed cake, detoxified *Madhuca longifolia* seed cake has better chemical properties. There is an increase in the nutrients of the digested slurry 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.

#### 4. Physical, biological, and chemical properties of mee seed fat

##### 4.1. 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. Saponification value, iodine value, peroxide value, acid value, melting point, smoke point, specific gravity, and refractive index are some physicochemical parameters studied by researchers. AOCS (1999) and AOAC (1999) methods were used for analysis. 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 (Table 1) when popularizing mee fat for edible uses.

Table 1. Comparison between mee seed fat and coconut oil [12,13].

Type of oil	Saponification value(mg KOH/g)	Iodine value (gI <sub>2</sub> /100g)	Acid value (mg KOH/g)	Peroxide value (meq/kg)	Melting point °C	Smoke point °C	Specific gravity (at 25°C)	Refractive index (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

4.2. Fat Content and Fatty Acid Profile of Mee seed fat

Many researchers have done studies on fatty acid profiles of Indian Mee varieties while [8], [14] were done their studies on Sri Lankan Mee varieties. There were similar results obtained for the fatty acid profile among the studies. Researchers [3] report on 13 fatty acids which were identified in mee seed extract. Fatty acid methyl esters (FAMES) analysis of fat shows oleic, stearic, palmitic, and linoleic (Figure 4) as the major fatty acids, which together comprised more than 98.5% of the total identified FAME of Mee fat. Oleic acid was the main fatty acid (37.3%) followed by stearic acid (25.9%). According to his study mee fat contains 46% of saturated fatty acids, 37.4% monounsaturated FA, and 16.5% polyunsaturated FA. Therefore, mee contains a considerable amount of essential fatty acids (Figure 5).

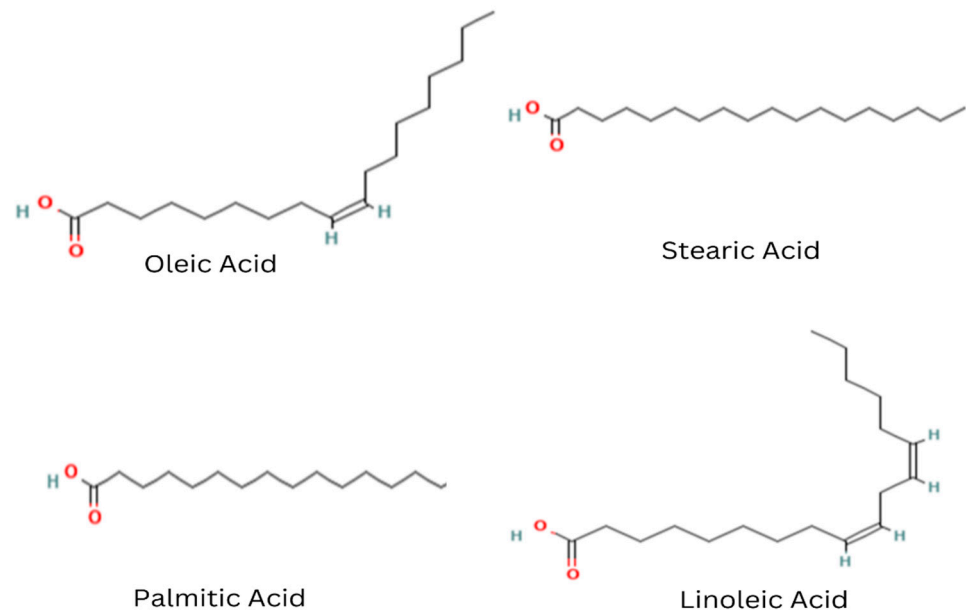
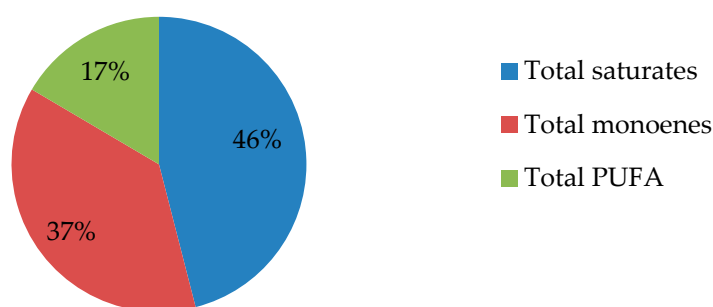
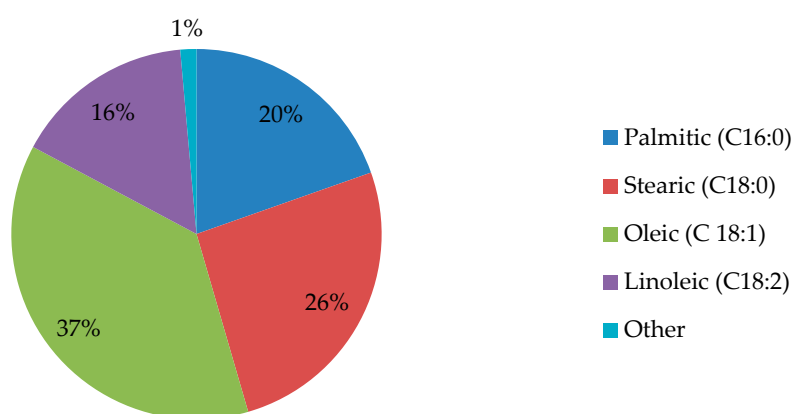


Figure 4. Major fatty acids present in mee fat [15–18].



**Figure 5.** Total fatty acid profile (%) of Mee fat [3].

The fat content and the fatty acid profile of Mee seed fat from different geographical locations in Sri Lanka were studied by researchers [8]. 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 50.07-53.85%. The highest fat yield was reported in low-country dry zone mee seeds and the least reported in the mid-country intermediate zone. The fat content in fact the y acid profile was significantly different in four agro-climatic zones. A study reports how provenances significantly affect the differences in fruits and seed morphological characters [19]. 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 (Figure 6) using the method described by ITI, Sri Lanka [8]. Around 15 types of fatty acids were identified in their study. They were Oleic (C18:1), Linoleic (C18:2), Stearic (C18:0), Palmitic (C16:0), Myristic (C14:0), Palmitoleic (C16:1), Margaric (C17:0), Nonadecylic (C19:0), cis-Gondoic (C20:1), Behenic (C22:0), Lignoceric (C24:0), Cemic (C26:0), Arachidic (C20:0) and Caprylic (C8:0).



**Figure 6.** Major fatty acids (%) of Mee fat (Source: [3]).

Between them, Oleic, Stearic, Palmitic, and Linoleic are the major fatty acids (FA) in Mee fat [6,8]. 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



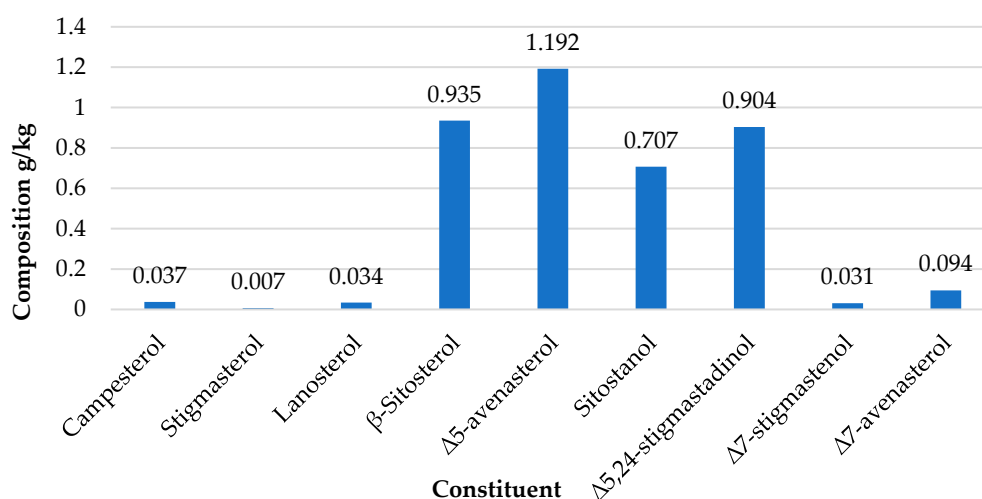
potential ingredient for nutritional applications [3]. Higher saturated fatty acid levels in vegetable oils are desirable in the food processing industry mainly to avoid transesterification and hydrogenation processes in the production of solid fat products such as shortening and margarine as well as 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 county intermediate zone [8]. The lowest level (13.70%) was reported in the mid-country intermediate zone. The Oleic acid content ranged from 40.44-50.32%. There 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.

The research was conducted to study the fatty acid profile of mee fat using Gas Chromatography-Mass Spectroscopy (GCMS) [20]. Fatty acid methyl esters (FAME) were prepared from oil for the analysis. Four major 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.

#### 4.3. Unsaponifiable matters composition of Mee seed fat

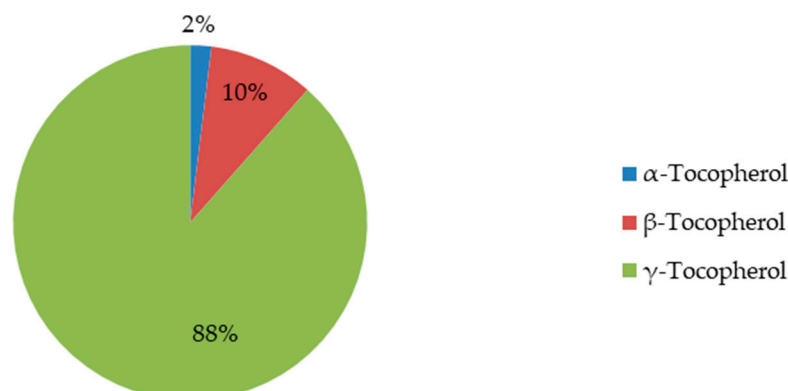
The high unsaponifiable matter (UM) content (ca. 8 g/kg butter) in Mee is a distinct feature. It is a limitation for mee fat use in the manufacturing industry. Mee fat contains a higher amount of phytosterols (3.94 g/kg TL). Phytosterols (ST) level in vegetable fats are used to identify fats, and fat derivatives and also determines the fat quality. Nine compounds (Figure 7) were claimed as phytosterols. 5 avenasterols consist of ca. 30.2% of the total ST content. 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 [3].

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). 88.8% of total tocopherols present in Mee fat are  $\gamma$ -tocopherols. 9.6% of total tocopherols are  $\beta$ -tocopherols and the rest 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 a great value to mee fat because of its nutritional value and strong stability toward oxidation [9].



**Figure 8.** Tocopherol composition of Mee fat [3].

#### 4.4. Antioxidant potential of mee seed fat

Phytonutrients present in Mee fat have antioxidant properties and thereby contribute to oxidative stability and improved shelf life of the fat. The natural antioxidants extracted from mee fat can be used as a replacer for hazardous synthetic antioxidants used in the food processing industry [21]. Tocopherols and carotene present in Mee seed fat are having significant antioxidant potential. The antiradical properties of Mee fat were compared with extra virgin olive oil [3]. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals were 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 able to reduce 9.40%. Compositional differences between fatty acids and lipid-soluble bioactive compounds such as phenolic acids, tocopherols, tocotrienols, and carotenoids present in mee fat effects the antioxidant potential. The total phenolic content and antioxidant capacity of the methanolic extract of mee seed oil were evaluated by measuring the absorbance through a spectrophotometer (Uviline 9400, SECOMAM) [21].

The antioxidant potential of mee fat extracts was evaluated 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 dose-dependent activity towards DPPH and ABTS radicals [22]. The  $IC_{50}$  value is the way of expressing the DPPH radical scavenging activity [22]. 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  $IC_{50}$  value of less than 1 mg/ml. A comparison was done between the antiradical properties of Madhuca fat and extra virgin olive oil using DPPH free radicals [1]. Study shows that Madhuca 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 on ABTS radical scavenging activity of Mee fat extract, it is 46.0% at 2.0% (w/v) concentration. This value is lower with compare 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 gives evidence that it contains a considerable amount of pigments. Ex: -  $\beta$ -carotene. Naturally, it presents as a mixture of various isomers (Cis & Trans) of the  $\beta$ -carotene molecules and they have a potent antioxidant capacity [23]. A comparison was done on the antioxidant potentials of Mee fat and coconut fat. There DPPH assay method described by Brand-William et al, (1995) with few modifications was used to assess the antioxidant potential. There BHT (Butylated hydroxytoluene) was used as a positive control [12]. The results show that the radical-scavenging activity of Mee seed fat was slightly lower compared to coconut fat. A comparison of the antioxidant activity of sesame (*Sesamum indicum*), soybean

(Glycine max), and mee (*Madhuca longifolia*) against photooxidation and autoxidation was reported [24]. 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 highest both 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.

#### *4.5. Mee seed oil content and quality upon storage*

Mee seed oil content and its quality depend on the storage conditions of the extracted oil [25]. Changes in Mee seed oil content and quality upon storage at different duration and conditions were studied by the researchers [26]. In their study, they investigated the effect of storage medium and condition of storage on the oil content and other oil parameters of mee seeds. Researchers are also revealed that the oil content and its composition can be affected by storage conditions, duration, exposure to light, and other environmental conditions. According to them, the fatty acid oxidation of seed oils 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 oil. Especially *Penicillium* and *Aspergillus* fungi genera induce seed deterioration and degradation by producing toxic substances during poor storage conditions [27].

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 contents, saponification value, acid value, and iodine value of edible oils [28]. The reports that the harvested mee seeds should be stored in appropriate storage containers with suitable relative humidity ranges, and prior to seed storage the seeds should be treated with fungicides. The change in oil content and other quality parameters by means of three types of container bags (polythene, plastic, and jute), Two storage environments (light and dark), and subjected to two air exposure (closed and open conditions) were monitored by some researchers at a monthly interval for 180 days [26]. The studies show that the oil 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 of the stored seeds were increased. The best storage method for retaining higher oil content is a closed plastic container exposed to light. Other than that closed polythene bag kept in dark was the best storage method for retaining higher saponification value.

### **5. Processing of Mee (*Madhuca longifolia*) seed fat**

Feedstock preparation before the extraction step is an important pre-requisite. First seeds were separated from Mee fruits. Then the separated seeds or kernels are 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 [29]. 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 by the researchers. 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 [30].

“Sekku” and “Peha” are traditional fat extraction methods (Figure 9) that can be tried at home to process Mee fat. Dried matured seeds were put into a “Sekku” and ground well to extract the fat. The traditional method called “Peha” also uses to extract Mee fat [14]. In this method, the first seed pith was taken out. Then the Mee seed pith was boiled and squeezed to extract the fat. There are limitations in traditional extraction methods because they that does 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 scientific data on the physical and chemical properties of Mee seeds to decide and develop processing operations, equipment development, and further industrial processing of seeds [31].



**Figure 9.** Traditional oil extraction methods: Sekku and Peha.

### 5.1. Mechanical press oil 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 done through a popular screw-type fat expeller (Figure 10) [14]. They analyzed the fat-expelling efficiency of the machine. The seeds can be subjected to a different number of extractions through the expeller. These expellers produce fat and seed cake mix continuously during the process. This affected the quality of the extracted fat. The screw-type expeller was modified to improve the fat extraction efficiency. The optimum fat yield and quality of the fat were determined by measuring the refractive index, saponification value, iodine value, unsaponifiable matter, free fatty acid, and specific gravity to assess the quality of 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. The crude fat yield, the highest yield has given at a screw speed of 90 rpm. The maximum fat yield of 35% was also achieved under this condition. There can observe 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 [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 10.** The screw type expeller.

### 5.2. Solvent oil extraction (chemical extraction)

There the fat is extracted from the solid through a leaching process using a liquid solvent. The chemical extraction by the n-hexane method gives the highest fat yield and therefore it considers the frequently used solvent [32]. The chemical extraction method was considered an effective method because it extracts higher fat yield and for its reliable performance. Chemical extraction of mee fat has been done using different solvents and different time periods according to the AOAC method [12]. The extractions were carried out for 6hrs, 5hrs, and 4hrs. The solvents n-Hexane (bp.65-70 °C) and Petroleum ether (bp.40-60 °C) were tested in the study. 4 hours of extraction time with n-Hexane can use as the method provides better Mee fat extract efficiently 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. Wconsideringider the n-hexane solvent, has a wastewater generation, higher specific energy consumption, and higher emissions of volatile organic compounds [32].

### 5.3. Ultrasonic-assisted bio-oil extraction

The effect of ultrasonic-assisted extraction for mee seeds through optimizing conventional solvent extraction procedures was studied [29]. 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 was used as solvent combinations in suitable proportions. Investigations were done to optimize various parameters like temperature, extraction time, type of solvent, solvent ratio, potassium chloride, and hydrochloric acid concentrations of 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, 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 large-scale processing of Mee fat. The oil yield and recovery of different mee oil extraction methods were compared by some researchers [21]. 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 °C) and a binary mixture of acetone and isopropanol (1:1 v/v) were utilized to get 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 doesn't alter fatty acid and triacylglycerol profiles.

## 6. Potential industrial applications of mee seed fat

### 6.1. 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 tested as sensory attributes. The test results show that in the stir-frying method, there was no significant difference ( $P>0.05$ ) between the two samples for all the tested sensory attributes. According to this research, mee fat has a good potential as cooking fat. During the frying process  $\Delta 5$ -avenasterol present in mee fat helps to protect fat from oxidative polymerization [3].

### 6.2. An alternative ingredient for halal fats

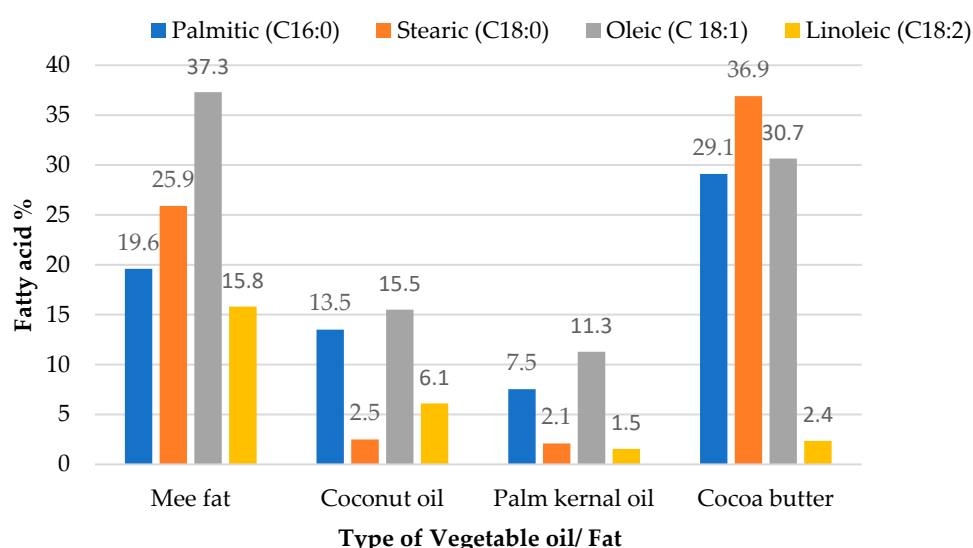
The potential of mee fat as an alternative ingredient for halal fats was tested by some researchers [33]. Pork fat is a common halal fat used in food processing that has an animal origin. Mee fat is plant-based non-halal fat with good processing potential. Studies show that pork fat has more unsaturated 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 very little amount of linoleic acid (7.85%) with compare to pork fat. Mee fat has higher saturated fatty acid content (42.93%) with compare 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) is analyzed and compared with each other. The results show that mee fat has a higher SMP value with compare to pork fat. With compare to native samples, stearin fractions had a higher SMP. There 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 with compare 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 with compare 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. In the temperature range of 0 – 25 °C, both pork fat and mee fat display similar saturated fatty acid profiles. 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 [34]. There *Madhuca longifolia* seed fat is composed of palm oil to form a substitute for lard (pork fat). There 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 the most important physical property 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 consider 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 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.

### 6.3. A cocoa butter substitute

Mee fat has a similar fatty acid profile to cocoa butter (Figure 11). The high levels of oleic and stearic acids in Mee fat give it the suitability to be a cocoa butter substitute [35,36]. Therefore Mee fat has the potential to produce chocolates, confectionery products, and shortenings.



**Figure 11.** Comparison of average levels of major fatty acids (%) in Mee fat with selected natural semi-solid fats and oils [3,37–39].

### 6.4. 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 materials 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 overcome by combining them with other polymeric materials which enhance the desired properties 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 used for food packaging applications. According to available reports, polyurethanes (PU) are one of the most widely studied film-forming polymers with a wide range of applications in food packaging. Polyurethanes are generally prepared by polymerization reaction between polyols and diisocyanates. Focus on polyols derived from vegetable fats such as castor fat, soybean fat, jatropha fat, and rapeseed fat have 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 properties. Therefore they were in cooperated with inorganic agents or blended with other synthetic or natural polymers.

A study was done 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 fabrication of biodegradable nanocomposite films from mee fat polyol-based polyurethane, chitosan (CS), and ZnO nanoparticles. Thermal, mechanical, antibacterial, biodegradation, transparency, and barrier properties of the plain CS, plain PU, PU-CS blends, and PU/CS/ZnO films were compared in their study. According to them, ZnO can improve the hydrophobicity of the film by about 63% compared to the PU/CS blend film. Chitosan affects the oxygen permeability and PU has positively modified the water vapor transmission rate and surface wettability of the films. The synergistic interactions of all three components have led to the composite film being a prospective packaging material. These researchers concluded that ZnO-reinforced PU/CS film can be proposed as a potential biodegradable food packaging material.

#### 6.5. *Pharmaceutical product manufacturing*

Natural-based 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 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 [40]. The cream was formulated using erythromycin stearate, stearic acid, potassium hydroxide, glycerine, Mee fat, and methylparaben. Different cream formulas were developed and evaluated the physical and chemical properties of each cream formulation.

#### 6.6. *Formulation of coating binders*

The development of polyetherimide-based corrosion protective polyurethane coating from Mee fat was carried out [41]. 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 it suitable as an anticorrosive coatings material.

#### 6.7. *Biodiesel production*

Mee fat also has good potential in biodiesel production and can partially replace petroleum diesel. Due to less environmental pollution and low-cost biomass get attention in biodiesel manufacture. Vegetable fats such as soybean, rapeseed, sunflower, and safflower are generally used to prepare biodiesel [42]. Biomass is one of the technically and economically feasible options against fossil fuels. Biofuel also can be used in any mixture with fossil fuels because of their similar characteristics. 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) [43]. Fatty acid methyl esters (FAMES) of seed fat are used as a substitute fuel in a diesel engine.

The quality of FAMES determines by 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 produce from raw fat through different methods. Transesterification, dilution, microemulsions, and pyrolysis are some examples of such methods. Among the above methods transesterification process is the most common method to produce biodiesel from fat [44]. According to some researchers, the transesterification method affects by factors such as the amount of alcohol and catalyst, pressure, speed, time, reaction temperature, water in raw fat, and content of free fatty acids [45]. An experimental investigation on Mee methyl ester blended with diesel fuel in a compression ignition diesel engine was conducted by researchers [46]. Mee Methyl ester (MME) was formed through the transesterification process. The physical properties of developed MME-incorporated fuel formulas were compared with the requirement of standards. According to the tests, the emission characteristics of carbon monoxide, hydrocarbons, and smoke opacity were reduced for all the fuel formulas. The good quality performance and lower emissions make it a good alternative fuel to engines without any modifications [9,47]. Three methods of biodiesel manufacturing as the conventional method, ultrasonic horn method, and ultrasonic bath method were evaluated by a research group [42]. The highest biodiesel yield was gained from the ultrasonic horn method and the least biodiesel yield was gained through the conventional method.

## 7. 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 the India, 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 [48]. 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 median lethal dose LD50 of saponin extracted from Mee was found to be 1000 mg/kg in mice on oral administration. According to the European Food Safety Association, the excess dose of *Madhuca* fat may lead to anti-fertility [3]. It is important to identify and minimize risks associated with mee oil consumption.

## 8. Health-promoting potential and nutritional properties of mee (*Madhuca longifolia*) seed fat

Mee can be considered a medicinal plant with a good pharmacological profile. It provides convincing support 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, anti-ulcer activity, laxative properties, etc. of mee seed fat [7,35,49]. The anti-inflammatory action of mee on rats was also evaluated by a research group [50]. Mee fat also has demulcent properties. Therefore can be used to treat skin diseases, rheumatism, headache, laxative, and piles and also as a galactagogue. The ethanol extract and saponin mixture of Mee seeds were evaluated for anti-inflammatory activity using acute, sub-acute, and chronic models of inflammation in rats [51]. There ethanol extract and saponin mixture of Mee at a different dose level was studied. The edema induced 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 *Madhuca 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 [52]. The study found that various extracts of Mee seeds have very good to moderate anticancer activity.

The ethanolic extract of *Madhuca longifolia* significantly reduced the plasma glucose level in normal albino rats in a dose-dependent manner. This hypoglycemic effect is 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 incidences of heart attacks, and reduction of muscle damage from oxygen-free radicals produced during exercises [9]. Certain sterols such as  $\beta$ -sitosterol have the favorable effect of lowering the amount of cholesterol in the blood [3].

### 9. Future perspectives of edible mee (*Madhuca longifolia*) seed fat

Research work on *Madhuca longifolia* should step forward on the transparency of reliable information on the identity of the plant, its characteristics, postharvest techniques, and their utilization. Prerequisites 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 get a large scale of quality mee seeds and flowers it is essential to introduce high-yielding genotypes to the agroforestry [31]. There is less research literature on 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 [53]. They have reviewed on importance of genetic diversity and phytochemical assessment of *Madhuca* spp. in Sri Lanka. 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 research 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 could introduce to Agroforestry will help to increase the availability of quality mee seeds and flowers on large scale and be beneficial to farmers to get a sustainable harvest, value-addition, and marketing [2]. Similar to many other tropical fruit seeds, *Madhuca 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 edible fat in the field of food processing. Under tropical conditions, *Madhuca longifolia* fat generally exists in semi-solid nature. Therefore it has a chance to its 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 food packaging material [10]. Their further research should carry out to study the practical applications of biodegradable food packaging material 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 [43]. They are Accelerated solvent extraction (ASE), aqueous enzymatic fat extraction (AEOE) method, Microwave-assisted extraction (MAE), and Supercritical fluid extraction (SFE) methods. Their fat quality, fat yield and their physicochemical properties, and fatty acid profiles of extracted fat were studied in these methods. The extraction time and solvent consumption for ASE are lower with compare to the other solvent extraction techniques according to the study. These modern methods can be used as potential fat Extraction Methods for mee fat extraction. Although there were no research studies on fat extraction from the above modern methods, there can be conducted research trials regarding determining the mee fat extraction efficiency and the quality of extracted oil.

The characterization of *Madhuca longifolia* seeds in relation to 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 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 post-harvest types of equipment. The researchers have experimented with physical properties such as principle 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 analyzed by the researchers to advance the processing of edible mee fat.

## 10. Conclusions

Mee seeds are a good source of edible fat after a proper extraction and detoxification process. It gives a significant amount of fat. The mee fat is easily available and low cost. This fat can be considered as a good source of essential fatty acids and lipid-soluble bio-actives. High oleic and linoleic acid content make mee fat a nutritional component for a regular diet. The higher antiradical potential due to tocopherol and phytosterol content 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. Therefore it is a preferable fat to process chocolates and other confectionery products in tropical regions. It could be also used in the production of shortenings, and margarine and as a base for cosmetics and pharmaceuticals. Other than that mee fat also has the potential for alternative fuel options for diesel. Since the valuable utilization of Mee fat in the food industry, it is important to develop the fields of cultivation, propagation, postharvest, and processing technology of Mee.

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