Identification and characterization of ketapang seeds oil 
(*Terminalia catappa L.*) using the gas chromatography-mass spectrometry (GC-MS)

M Napitupulu*1, Muhammad Firdaus1 and D K Walanda1
1Tadulako University, Jl. Soekarno Hatta Km.9, Palu 94118, Indonesia
*Email: merytn@gmail.com

Abstract. Ketapang plants (*Terminalia catappa L.*) grow on the beach and are known to contain oil. Research on oil content, lathering number, % FFA, and identification of fatty acids of ketapang fruit seed oil conducted using the Gas Chromatography-Mass Spectrometry (GC-MS) method. Results showed that ketapang seed oil content was 24.23%, saponin number was 33.3%, and % FFA was 3.65%. There are eight components identified in Ketapang fruit seeds. They are components of fatty acids: Hexadecanoic acid, octadecanoic acid, 9,12 hexadecanoic acid, 9,12 octadecanoic acid, 10-octadecanoic acid, 9-octadecanoic acid, docosanoic acid, and tetracosanoic acid identified.

1. Introduction
Ketapang plant is a plant that has the potential to produce vegetable oil. In pharmaceutical, Vegetable oil used as a solvent for injection. Because it can solubilize highly lipophilic drugs, it can be administered intramuscularly to muscle mass. Produces a depot to delay/prolong drug release. Vegetable oil is extracted from various parts of plants, usually from seeds, such as sesame seeds, cotton seeds, soybean seeds, and so on. Many plant seeds can be consumed directly or processed into snacks. Vegetable oils that are mostly used in Indonesia are those from coconut and palm oil. In contrast, apart from coconut, ketapang seeds can also be used where the sources can be extracted to produce vegetable oil.

At temperatures of 25°C, both types ketapang seed oil is 0.898 g / mL, similar to palm oil. While the refractive index identical to sesame oil is equal to 1.4648 with number acid (4.7 mg KOH/g) lower than olive oil (6.6 mg KOH/gram), so the quality is better than olive oil [3]. The lathering number (68.83 mg KOH/gram) is lower than other fats, which means that the total fatty acid content is low. The iodine number is 75.21, which corresponds to the iodine number of olive oil (75-94) [3] so that ketapang seed oil has the opportunity to be an alternative substitute for palm oil and olive oil, based on the similarity of chemical-physical properties and fatty acid content. Vegetable oil can be used in the cosmetic industry [4], soap [5], shampoo [6].

Plants and the environment in which they grow are an inseparable unit because to develop correctly, plants need an optimum environmental condition to express their genetic program fully. The existence of differences in environmental conditions where it grows can cause differences in the content and stability of ketapang seed oil. Therefore, research on oil stability from ketapang seeds grown in Central Sulawesi still needs to be done to increase scientific data on ketapang seeds (*Terminalia catappa L.*). This study focused on identifying and tracing the characteristics of oil produced from ketapang seeds.
using the GC-MS method. Ketapang seed samples were taken in Talise Village, Palu City, Central Sulawesi-Indonesia.

2. Research method
2.1 Sample preparation
Ketapang seeds that have separated from the fruit skin. It is dried by drying the ketapang seeds in the sun for four days then heated in an oven at a temperature of 40°C until they reach a constant weight. The moisture content of the ketapang seeds was determined by drying the entire sample. Ten grams of the model were dried in stages using an oven at 105°C until constant.

2.2 Oil extraction using the soxhletation method
Ketapang seed samples were mashed and then weighed as much as 100 grams, then wrapped in filter paper and extracted using Soxhletation extraction. The solvent used was n-hexane at 70°C for 6 hours. The extraction process stopped when the solvent pulled all ketapang seeds. And to obtain solvent-free ketapang seed oil, an evaporation process followed it. The resulting crude weighed, and the yield is determined. In this research, the extraction process was carried out using n-hexane as a solvent. N-hexane is a straight-chain alkane hydrocarbon having six carbon atoms with the molecular formula C6H14. The isomer of hexane is unreactive and is widely used as an inert solvent in organic reactions because it is highly non-polar. N-hexane is made from refined crude oil wherein the industrial product is the fraction that boils at a temperature of 65-70°C. Hexane is used in laboratories to extract oils and fats.

2.3 Determination of FFA
Dilute 5 grams of oil with hot alcohol with a water bath, add five drops of pp indicator and titrate with NaOH solution.

2.4 Determination of the soaping number
2 grams of oil is put in a flask and added with 25 mL of 0.5 N KOH alcohol. The flask was then connected to an upright cooler and heated using an electric bath for 1 hour. Ten drops of the pp indicator are added and then titrated using 0.5 N HCl.

2.5 Identification of fatty acids in ketapang seed oil using Gas Chromatography-Mass Spectrometry (GC-MS) Method
The 200 µL ketapang fruit seed oil sample was esterified first using BF3 methanol with a volume of 600 mL. Then the samples refluxed at 70°C for 1 hour. They were then extracted using n-hexane. Furthermore, the samples were analyzed using GC-MS

3. Result and discussion
3.1 Ketapang seed oil extraction
The separation occurs based on the different solubility capabilities of the ingredients in the mix [7]. Extraction is the separation of one or more materials from a solid or liquid with a solvent's help. Extraction is also the process of separating the components from a homogeneous mixture using a liquid solvent (solvent).

The Soxhlet extraction method is a method of extracting materials in solids with a continuous liquid solvent. The equipment used is called a Soxhlet extractor. The Soxhlet device consists of several parts, such as a flask for the extracting solvent, a place for extracted materials, chiffon, and a condenser. In the extraction process, the solvent will evaporate by heating, and then the steam will rise to the condenser through the side pipe. Because there is cold water in the condenser, the moisture will condense and drip off as a liquid. This solvent will soak the extracted material (extraction process). After the liquid soaking is as high as chiffon, the extract's solvent will circulate back to the flask.
3.2 Characterization of physical and chemical properties

Analyzing the samples' water content is done using a drying method in an oven. The technique is based on calculating the difference in mass of a piece before and after drying. The mass difference obtained is the mass of water contained in the sample. This study showed that the water content of ketapang seeds was 8.889%, while the moisture content of ketapang seed oil was 0.23%. This amount fulfills SNI 01-3555-1998, where the oil quality requirement for foodstuffs has a maximum moisture content of 0.5%.

Levels of FFA (Free Fatty Acids) describe the amount of free fatty acid in the oil. The lower the FFA value, the higher the quality of the oil. Free fatty acids are fatty acids that have been separated from the glycerol molecule. These fatty acids bind with glycerol to form triglycerides (glycerol which binds three fatty acids). Triglycerides will react with alcohol, assisted by alkaline catalysts, to produce biodiesel. However, if the fatty acids are released from the glycerol, the free fatty acids will react with an alkaline catalyst to form soap, an unnecessary byproduct. The FFA value of ketapang seed oil exceeds the maximum amount set for food oil (cooking oil), which is 0.3%. The fatty acid content of ketapang fruit seeds obtained was 3.65%; this figure indicates that ketapang fruit seed oil is not suitable for consumption. Because it has high FFA levels compared to the Indonesian national standard, the ketapang seed oil does not meet the quality standards.

The saponification number of ketapang seed oil obtained was 33.3 mg/g. If some oil or fat samples are soaked with excess KOH solution in alcohol, then KOH will react with triglycerides, namely three KOH molecules reacting with one oil or fat molecule. The remaining alkaline solution is determined by titration with HCL thus the reacted KOH can be measured. The lathering number for coconut oil is 255-265 mg KOH/g of fat. This low lathering number indicates that the oil has a large molecular mass and does not contain low fatty acids. The smaller the soaping number suggests, the less alkaline content needed to lather the oil [2]. The oil quality test was conducted to determine the physical and chemical properties of the oil produced. The result was referring to SNI 01-3555-1998. The characterization results obtained are shown in Table 1.

| No. | Treatment                                      | Result  |
|-----|-----------------------------------------------|---------|
| 1   | Water content of ketapang seeds               | 8.88%   |
| 2   | Ketapang seed oil content                      | 24.22%  |
| 3   | Water content of ketapang seed oil            | 0.23%   |
| 4   | % FFA determination                            | 3.65%   |
| 5   | Determination of the soaping number           | 33.30%  |

3.3 Analysis of the fatty acid components of Ketapang seed oil

Analysis of fatty acid components in ketapang seed samples was carried out using Gas Chromatography-Mass Spectrometry (GC-MS). The mass spectrum in GC-MS functions as a detector; each piece that comes out of the GC was immediately shot with a high-energy electron beam. This shot will turn neutral molecules into molecular ions (M+), molecular ions will turn into fragments. The most stable piece will appear at the highest peak. The value of M+ shows the amount of the molecular mass of a compound [10]. The chromatogram analysis results of the ketapang fruit seed oil sample can be seen in Figure 1.

Figure 1 shows 8 peaks out of 72 with a reasonably high abundance, namely peaks with a retention time of 16.395; 32.032; 32.931; 35.304; 37.453; 37.918; 45.126; and 50.395. The obtained fatty acid were; hexadecenoic acid, octadecanoic acid, 9,12-hexadecadienoic acid, 9,12-octadecadienoic acid, 10-octadecenoic acid 9-octadecenoic acid, docosanoic acid, tetracosanoic acid. These compounds do not come out from the column simultaneously because of the difference in boiling points. The compound with the lowest boiling point will exit the column earlier, whereas the compound with the largest boiling point will exit the column later. Besides the boiling point factor, the column's difference in exit is due to its affinity to the compound. Compounds with a strong affinity for the column or held firmly by the column will slowly exit from the column. The compound that leaves the slowest from the column will
have the greatest retention time. The peak height is proportional to the concentration of a compound's components in the injected sample mixture.

The GC-MS results showed that the dominant fatty acids contained in the sample with the largest area were two peaks. They are hexadecanoic acid with an area of 182062355, and octadecanoic acid with an area of 50312930. The percentage of each of these fatty acids was 23.40% hexadecanoic acid and 6.47% octadecanoic acid. The two fatty acids are the most dominant among the six identified fatty acids. However, the most prevalent fatty acid found at peak 18 was hexadecanoic acid, with a percentage of 23.40%. The results of the analysis of ketapang fruit fatty acid components shown in Table 2

3.3.1 Hexadecanoic acid. Hexadecanoic acid, better known as palmitic acid, has the chemical formula C₁₆H₃₂O₂, a saturated fatty acid found in various oils sourced from vegetable materials and located in animals, plants, and microorganisms. Palmitic acid is the main component of the oil from the coconut tree (palm oil), but it can also find in meat, cheese, butter, and dairy products. Palmitate is the term for the salt and ester of palmitic acid. Palmitic acid is naturally produced by a variety of other plants and organisms, usually at low levels. Ketapang oil contains methyl palmitate (35.63%) [11]. The high content of palmitic acid in the ester form is similar to the palmitic acid content in palm oil.

3.3.2 Octadecanoic acid. Octadecanoic acid or stearic acid is a saturated fat with various functions such as additional composition in foods, cosmetics, and products industry. Stearic acid can be extracted from various animal fats, vegetable fats, and several other oils. These compounds are also widely used to change the consistency or melting temperature, as a lubricant, or prevent oxidation. Stearic acid is very commonly used in soap production as an additive to harden the texture, give it a pearl white color, and make it easy to rinse off (give a coarse effect). In cosmetics, stearic acid is used to make a stable base
for deodorants, lotions, and creams. Stearic acid is a fatty acid with the chemical formula C\(_{18}\)H\(_{36}\)O\(_2\). Stearic acid is soluble in mixed solvents of (water + ethanol) and (ethanol + ethyl acetate) [12].

3.3.3 9,12-hexadecadienoic acid and 9,12-octadecadienoic acid. 9,12-hexadecadienoic acid or linoleic acid has a molecular formula of C\(_{18}\)H\(_{36}\)O\(_2\) belonging to the unsaturated fatty acid class and 252.39 g/mol. 9,12-Octodekadienoic acid or linoleic an unsaturated fatty acid has the molecular formula C\(_{18}\)H\(_{36}\)O\(_2\) classified as essential fatty acids. The 9,12-octadecadienoic acid was identified at the peak in GC-MS. An area of 8810112 and an area percentage of 1.13% and a molecular weight of 280 g/mol. Linoleic and linolenic acids are long-chain unsaturated fatty acids and classified as essential fatty acids. Both linoleic acid and linolenic acid are necessary for the body. Therefore, they must be obtained from food. Linoleic acid is isolated from soybean oil deodorizer distillate, known as trans-cis fatty acid [13].

3.3.4 9-octadecenoic acid and 10-octadecenoic acid. The 9-octadecanoic or oleic acid identified at peak MS has a molecular formula of C\(_{18}\)H\(_{34}\)O\(_2\) and a molecular weight of 282.5 g/mol and isoleic acid having the chemical formula C\(_{18}\)H\(_{34}\)O\(_2\). Isooleic acid is an acid with 10 C atoms and one double bond after the 9th C atom from the base (carboxyl group). This acid is composed of 18 C atoms with a double bond between the 9th and 10th C atoms. Apart from olive oil (55-80%), this fatty acid is also found in specific cultivars of sunflower oil, rapeseed oil, and grapeseed oil.

10-Octadecenoic acid or commonly called isoleic acid identified at peak MS having a molecular weight of 282.5 g/mol and isoleic acid having the chemical formula C\(_{18}\)H\(_{34}\)O\(_2\). Isoleic acid is a mixture of C 18 - unsaturated branched-chain fatty acid isomers with a methyl group on the alkyl chain [14].

3.3.5 Docosanoic acid. Docosanoic acid or behenic acid identified peak MS has the molecular formula C\(_{22}\)H\(_{44}\)O\(_2\) and a 350 g/mol weight. Behenic acid is a saturated fatty acid and consists of white crystal or cream or powder with a melting point of 80°C and a 306°C boiling point. It has a carbon chain of at least 12; insoluble behenate acid is neutral in the water. Behenic acid is found in multiple biofluids, such as stool, blood, and urine. The incorporation of behenic acid [15] was found using sal, kokum, and mango as a substrate.

3.3.6 Tetracosanoic acid. Tetracosanoic acid or acid lignoceric, identified at the MS peak with the molecular formula C\(_{24}\)H\(_{48}\)O\(_2\) and a molecular weight of 378 g/mol. The fatty acids from peanut oils contain a small amount of lignoceric acid (1.1% - 2.2%). These fatty acids are also a byproduct of lignin production.

4. Conclusion
There were eight fatty acids identified in Ketapang seeds. They are; hexadecanoic acid or palmitate acid, octadecanoic acid or stearic acid, 9,12-hexadecadienoic acid, 9,12-octadecadienoic acid or linoleic acid, 10-octadecenoic acid or isoleic acid, 9- octadecenoic or oleic acid, dosanoic acid or behenic acid, and tetracosanoic acid or lignoseric acid. The free fatty acid content reached 3.65 %. One way to use ketapang seed oil is by making ketapang seed oil as a material for biodiesel.

Acknowledgments
Authors wishing to acknowledge assistance from students of the chemistry study program, special work by technical staff and Lab assistant at Chemistry Laboratory of Tadulako University.

References
[1] Balogun A 1985 Food Chemistry 17 175–82
[2] Hariani P L, Riyanti F and Oktaviani H 2007 Results of Sokletasi 10 327–34
[3] Aug C M, Menkiti M C, Nwabanne J T and Onukwuli O D 2019 Industrial Crops and Products 140 111727
[4] Chaikul P, Lourith N and Kanlayavattanakul M 2017 *Industrial Crops and Products* **108** 56–62
[5] Melchor J J and Fortes I C P 2018 *Journal of Analytical and Applied Pyrolysis* **135** 101–10
[6] Satchell A C, Saurajen A, Bell C and Barnetson R S C 2002 *Journal of the American Academy of Dermatology* **47** 852–5
[7] Suhendra D, Gunawan E R, Nurita A D, Komalasari D and Ardianto T 2017 *J. Oleo Sci.* **66** 209–15
[8] Manzoor M, Anwar F, Iqbal T and Bhanger M I 2007 *Amer Oil Chem Soc* **84** 413–9
[9] Jenkins T L, Jin E and Sutherland J W 2020 *Forest Policy and Economics* **111** 102053
[10] Rasyid H A and Nasir R 2020 *J. Pijar MIPA* **15** 77
[11] C A 2006 *J. of Nutrition* **5** 306–7
[12] Noubigh A 2019 *Journal of Molecular Liquids* **296** 112101
[13] Gunawan S, Melwita E and Ju Y H 2010 *Food Chemistry* **121** 752–7
[14] Ngo H L, Dunn R O and Hoh E 2013 *Eur. J. Lipid Sci. Technol.* **115** 676–83
[15] Bebarta B M J, Kotasthane P and Sunkireddy Y R 2013 *Food Chemistry* **136** 889–94