



# Journal of Applied Sciences

ISSN 1812-5654

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## Ultrasonic Extraction of Oil from *Monopterus albus*: Effects of Different Ultrasonic Power, Solvent Volume and Sonication Time

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**Abstract:** Attempt was made in this research to extract the oil from *Monopterus albus* or commonly known as eel by using assisted ultrasonic extraction method. The effects of different ultrasonic power, solvents volume and sonication time on extraction yields were investigated. The ultrasonic power used was 100, 200, 300 and 450 Watts. The amount of ethanol or solvent used was 50, 100, 200 and 500 mL. In terms of sonication time it was varied at 20, 30, 50, and 60 min. Analysis was done using 785 DMP Titrino and Gas Chromatography Mass Spectrometer (GC-MS). Results obtained show that the maximum ultrasonic power of 200 Watts, ethanol volume of 500 mL and 60 min of sonication time produce the higher yields of oil extracts. The yields were 7.20% with FFA content of 0.22 (g/100 g). The FFA detected from chromatographic analysis using GC-MS was palmitic acid and stearic acid.

**Key words:** Ultrasonic extraction, FFA, *monopterus albus*, eel

### INTRODUCTION

Malays used material that comes from plant, animal and natural resources as a traditional medicine. The eel, scientifically known as *Monopterus Albus*, is included in the fish genus. The body is long and its head is rounded, with the presence of gills. According to traditional medicine practitioners, regular consuming of eels helps to boost the body's immune system, stabilizes the blood pressure, smoothens the skin texture, prevents hepatitis and enhances the memory power. However, many people are quite reluctant to eat the eel.

According to Rout *et al.* (2007) extraction is one of the key processing steps in recovering and purifying active ingredients contained in bio materials. Several types of extraction methods such as hydro distillation, soxhlet and supercritical can be used to extract the oil (Reverchon and Marco, 2006; Quitain *et al.*, 2006; Li *et al.*, 2000; Suslick and Price, 1999; Raghuram *et al.*, 1992). Trusheva *et al.* (2007), reported that ultrasonic extraction is the most efficient method based on yield, extraction time and selectivity. Other than that, the extraction process can be done at room temperature. Ultrasonic extraction process also reported as a fast, inexpensive and efficient alternative

compared to other extraction process (Kimbaris *et al.*, 2006). The sonication of liquids will generate sound waves that propagate into the liquid media resulting in alternating high-pressure and low pressure cycles.

The high-pressure cycles of the ultrasonic waves support the diffusion of solvents, such as hexane into the cell structure. As ultrasound breaks the cell wall mechanically by the cavitations shear forces, it facilitates the transfer of lipids from the cell into the solvent (Adeniyi and Bawa, 2002). Therefore, attempt was made in this research to extract oil from the eel. This research focussed on the preliminary study to identify the best operating condition in extracting oil from the eel using ultrasonic extraction method. The extracted oil has a great market value especially in pharmaceutical industries.

### MATERIALS AND METHODS

**Materials:** Fresh eels (*Monopterus albus*) purchased from Kuantan wet market were used in this research. Ethanol with 99% purity was used as solvent for extraction process. Hexane, potassium hydroxide and methanol also with 99% purity were used during samples analyses using Gases Chromatography Mass Spectrometer (GC-MS).

**Sample preparation:** Fresh eels purchased from the wet market were washed using fresh water. The internal organs were removed. Then, the Eels were cut into fillets and dried at temperature of 60°C using an oven. After that, the dried fillet was grinded into powder form by using a dry blender. Finally it was stored in a sealed plastic container and placed in a refrigerator until used.

**Extraction method:** The extraction process was done using ultrasonic extraction unit. This apparatus consists of 500 mL extraction beaker, ultrasonic bath and ultrasonic generator. The ultrasonic bath has a frequency of 25 kHz while the power can be varied up to 500W. The extraction beaker was immersed in the ultrasonic bath. Ethanol was used as solvent during the extraction process. Extraction was initially done in the absence of ultrasonic waves. For example, 10 g of dried eel was mixed with 300 mL of ethanol and placed in a 500 mL beaker. The beaker was left for 20 min at ambient conditions without sonication. After that, the sample was filtered to remove the powder and evaporated by using a rotary evaporator to get oil. The amount of extracted oil was recorded. The extracted oil was analyzed using 785 DMP Titrino and Gas Chromatography Mass Spectrometer (GCMS). Then, the same procedure was repeated using different ultrasonic powers of 100, 200, 300 and 450 Watts to determine the most suitable operating power. Next, the same procedure was repeated using different solvent volumes of 50, 100, 200 and 500 mL to determine the best solvent volume. Finally, the experiment was run at 20, 30, 50, and 60 min to determine the best sonication time.

**Method**

**Free fatty acid and acid value determination:** Free Fatty Acids (FFA) values were used as the quality indicator of oil. It can be determined by using a free fatty analyzer model 785 DMP Titrino. This equipment can also determine the acid value of the oil without using a conventional method which is titration. The Acid Value (AV), which is defined as the number of milligrams of KOH required to neutralize the free fatty acids in 1 g of sample, is a measure of FFA content or a measure of the amount of free acids present in a given amount of fat. Five milliliters of fish oil were diluted with 50 mL of ethanol and the free fatty acid content and acid value were detected by this equipment.

**Chemical composition determination:** Agilent 6890 GCMS equipped with a Flame Ionization Detector (FID) and automated split injection 7683 auto sampler was used to determine the fatty acid composition in the extracted oil. The inlet temperature and the detector temperature for GCMS were set at 250 and 280°C, respectively. The injection volume was set at 1 µL. Hydrogen gas was used as detector.

**Yields determination:** The extraction yield was calculated using Eq. 1:

$$\text{Extraction yields} = \frac{W_o}{W_s} \times 100 \tag{1}$$

where,  $W_o$  denotes the weight of extracted oil in grams and  $W_s$  denotes the weight of eel powder used in grams.

**RESULTS AND DISCUSSION**

**Influence of ultrasonic power on oil yield:** The oil yields at different ultrasonic powers are shown in Fig. 1. The yields for ultrasonic powers of 100, 200, 300 and 450 Watts were 2.20, 2.50, 2.40 and 2.30%, respectively. The results show that the best ultrasonic power was obtained at 200 Watts. Improved oil extracts from eel at ultrasonic powers less than 200 Watts may be explained in terms of cavitation effects caused by the application of ultrasonic waves. Cavitation normally takes place in a liquid medium once the media is subjected to rapid, alternating high pressure. Voids containing small micro bubbles are created when the differences between the amplitude pressure of ultrasonic waves and the hydrostatic pressure in the liquid is large enough to exceed the local tensile strength of the liquid medium. These bubbles expand during the negative part of the pressure cycle or rarefaction cycle, reach the maximum radius and then collapse at the onset of the positive pressure cycle or compression cycle (Ensminger, 1998). Bubble collapse may cause strong shear forces to be exerted that can cause micro fractures to be formed in biological tissues (Vinatorua *et al.*, 1996).

According to Mason (1990), an increase in ultrasonic intensity will contribute to an increase in cavitation effect. Larger ultrasonic intensity indicates greater ultrasonic energy entering the liquid system, thereby producing more cavitation micro bubbles. This consequently enhances stronger shear forces to be exerted during the bubble collapse and can cause more microfractures to be formed in biological tissues. This will ease the penetration of lipids or oil from the eel powder.

However, as the ultrasonic power increased beyond 200 Watts, the extraction yields were decreased. This may be due to the formation of a large amount of cavitation micro bubbles at intensity above 200 Watts. When a large

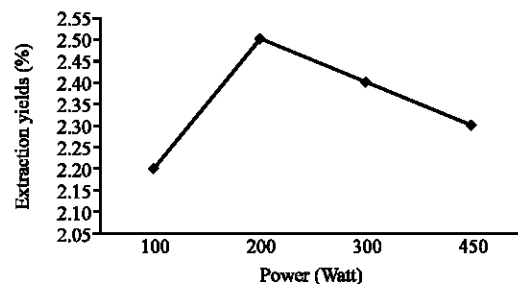


Fig. 1: Oil yields vs. ultrasonic power in 300 mL ethanol and 20 min sonication time

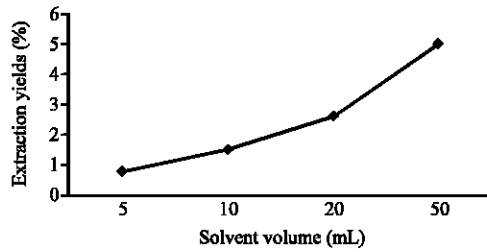


Fig. 2: Oil yields vs. solvent volume in 200 Watts ultrasonic power and 20 min sonication time

amount of cavitation bubbles are present inside the liquid medium, the tendency of the bubbles to collide becomes higher. Upon collision, bigger micro bubbles are created at ultrasonic power higher than 200 Watts. Since, the time available for the bubbles to collapse is insufficient, the bubbles will form a bubble cushion at the radiating face of the ultrasonic transducer, thereby reducing the effects of coupling sound energy to the liquid system. Such phenomenon tends to reduce the amount of ultrasonic energy being transmitted to the liquid medium and produces less cavitational effects. This ultimately results in reduced formation of micro fractures in the biological tissues. This phenomenon explains why further increase in ultrasonic power beyond 200 Watts decreases the extraction yields.

**Influence of solvent volume on oil yield:** Figure 2 shows that the oil yields started at 0.80% and increased to 1.50% and 2.60% when the amount of solvent are increased. The highest extraction yields which were 5.00% were determined at solvent volume of 500 mL. As ultrasound breaks the cell wall mechanically by the cavitation shear forces, it also facilitates the lipid transfer from the cell in to the solvent. Larger solvent volume promotes an increasing concentration gradient between solvent and solid samples. As a consequence, a larger mass transfer between solid and solvent occurs. This finding is aligned with those reported by Franco *et al.* (2007).

**Influence of sonication time on oil yield:** Figure 3 shows the percentage of oil yield as a function of sonication time. The oil yields are increases with the increased of sonication time. The amount of extraction yields without sonication is 4.20%. The highest extraction yields, which were 7.20% was obtained at 60 min sonication time. The increase of sonication time, increased the duration of cavitation process occurs in the extraction process. Therefore, increase the oil yields.

**Free fatty acid contents in oil yield:** The amount of Free Fatty Acid (FFA) contents in the oil yields are tabulated in Table 1-3.

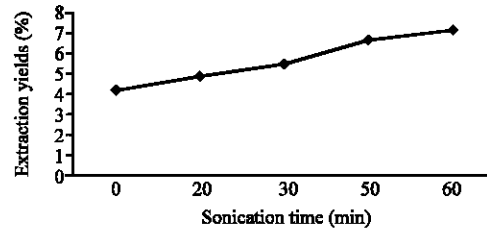


Fig. 3: Oil yields vs. sonication time in 500 mL of ethanol and 200 Watts ultrasonic power

Table 1: Amount of free fatty acid in the oil yields using 300 mL of ethanol and 20 min sonication time with different ultrasonic power

Ultrasonic power (Watts)	FFA (g/100 g)
100	0.53
200	0.28
300	0.17
450	0.13

Table 2: Amount of free fatty acid in the oil yields using 200 Watts of ultrasonic power and 20 min sonication time with different solvent volume

Solvent volume (mL)	FFA (g/100 g)
50	0.09
100	0.07
200	0.08
500	0.14

Table 3: Amount of free fatty acid in the oil yields using 200 Watts of ultrasonic power and 500 mL solvent volume with different sonication time

Sonication time (min)	FFA (g/100 g)
20	0.14
30	0.15
50	0.20
60	0.22

The results particularly show that FFA volume increased with increased sonication time as well as ultrasonic power. Solvent volume also plays an important role in increasing the yield of FFA volume. As what has been claimed by Boran *et al.* (2005) acid value is generally associated with lipase activity originating from microorganisms or biological tissue. The maximum acid value obtained through this study is 0.22 g/100 g and are within the acceptable limit as to compare to what have been reported by Bimbo (1998).

### CONCLUSION

The effects of ultrasonic power, solvent to solid ratio and sonication time on extraction yields were investigated. The best parameter to extract the oil was ultrasonic power of 200 Watts, 500 mL of ethanol and 60 min sonication time. The amount of oil extracted was 7.2% with FFA contents of 0.22 (g/100 g).

#### ACKNOWLEDGMENTS

The authors would like to express their heartiest gratitude to the members of separation pyramid in the Faculty of Chemical and Natural Resources Engineering, University Malaysia Pahang.

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