

Microwave-assisted extraction of lipid from fish waste

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Abstract. Processing fish waste for extraction of value added products such as protein, lipid, gelatin, amino acids, collagen and oil has become one of the most intriguing researches due to its valuable properties. In this study the extraction of lipid from sardine fish waste was carried out using microwave-assisted extraction (MAE) and compared with Soxhlets and Hara and Radin methods. A mixture of two organic solvents isopropanol/hexane and distilled water were used for MAE and Hara and Radin methods. Meanwhile, Soxhlet method utilized only hexane as solvent. The results show that the higher yield of lipid 80.5 mg/g was achieved using distilled water in MAE method at 10 min extraction time. Soxhlet extraction method only produced 46.6 mg/g of lipid after 4 hours of extraction time. Lowest yield of lipid was found at 15.8 mg/g using Hara and Radin method. Based on aforementioned results, it can be concluded MAE method is superior compared to the Soxhlet and Hara and Radin methods which make it an attractive route to extract lipid from fish waste.

1. Introduction

Fish is one of protein sources for humans. It has high value of nutrient and rich in not only protein, but also packed with essential fatty acids, minerals and micronutrients. Fish demands in recent years have increased, due to the growing of population worldwide and the demand for fish for functional food. Malaysia is also known as fish eating nation. Therefore, fish demand in years has increased. In year 2008 the fisheries sector produced 1.75 million tonnes of fish with a value of RM 7.5 million in Malaysian Ringgit [1]. Fish also can easily be digested not only as protein, but for its essential fatty acids, mineral and vitamins to Malaysian diet.

During the fish processing, 20-80% amount of fish waste are produced, depending on the type of fish used and usually fish waste are disposed in the ocean. The commercial fish industry is more export-oriented and encompasses of processing of prawns, canning of fish, and the production of surimi and surimi-based products. The export values exceed RM1.7 billion per annum of which frozen shrimps and prawns constitute more than RM 1 billion. In Malaysia, there are 120 commercial and 5,000 small and medium fish processing plants actively operating over the country. Most of these processing plants only utilize only fish flesh and discards the skins, bones, head, scales, fins, offal and viscera. These by-products are good source of essential amino acids and rich in energy, minerals and essential fatty acids, potential to be converted to the functional foods, nutraceutical and pharmaceutical products [2].



The standard method used to extract lipid in chemical method is solvent extraction. This is the traditional method to extract lipid. Oils are highly soluble in organic solvents such as hexane, ethers, benzene, cyclohexane, acetone and chloroform. Factors to be considered in choosing an organic solvent include preferential solubility of the compound of interest, low boiling point for easy recovery, economics, toxicity, availability, safety and re-usability. Hexane is one of the few solvents with such qualities and used in large scale extraction [3]. There are various forms of lipids in tissue matrix. The simple lipids exist as part of large aggregates in storage and are readily extractable. The complex lipids usually exist as constituent of membranes in close association with protein and polysaccharides. Therefore, the interaction with these compounds is not extracted easily [4].

A Soxhlet extraction method is one of the oldest methods that were originally designed for the extraction of a lipid from solid materials [5]. In recent years, microwave-assisted extraction has been successfully applied for extraction which involves deriving natural compounds from raw plants [6]. Microwave extraction allows organic compounds to be extracted more rapidly, with similar or better yield as compared to conventional extraction methods [7] as a results for unique features of microwave heating such as high heating rate and ease of operation [8]. Lipid investigators [9] have established an alternative method for extraction using low toxicity solvent such as isopropanol/hexane. Therefore this study aims to evaluate the extraction efficiency of lipid yield from fish waste by the microwave-assisted extraction method and compared to soxhlet extraction method and Hara and Radin method.

2. Methodology

2.1. Equipment and apparatus

The microwave-assisted extraction was carried out in a domestic microwave oven (NN-ST340M, Panasonic, Kadoma, Osaka Prefecture, Japan) at a frequency of 2.45 GHz with maximum output power 800 Watt. Soxhlet apparatus was used for soxhlet extraction. A 250 ml conical flask was used for extraction in Hara and Radin method. All chemicals are of reagent grade.

2.2 Substrate preparation

The fresh sardine fish was purchased from local market and the cut to obtain the head, tail and bones. These parts were chopped to small size using household blender with the adding water to the blender with ratio 1:3 (1 of water to 3 of wastes). Then, the mixture was filtered to separate solid parts and stored in plastic bag at -18°C until further use.

2.3 Experimental procedure

The overall layout of extraction process of the three extraction methods which include microwave-assisted extraction, Soxhlet, and Hara and Radin methods is described in the flowchart as shown in Figure 1.

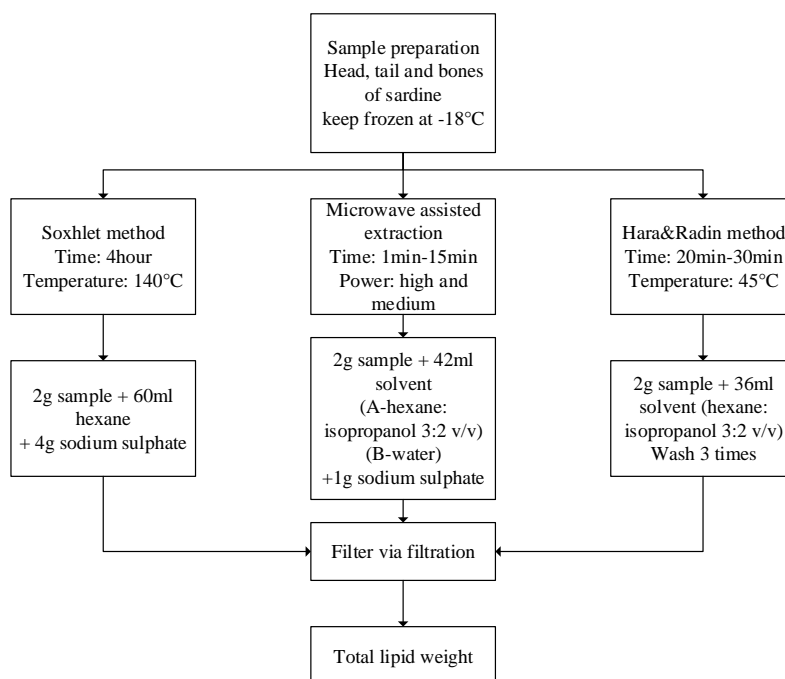


Figure 1. Experimental layout of extraction process

2.3.1 Microwave-assisted extraction method. Microwave-assisted extraction was carried out using distilled water and hexane/isopropanol mixture with ratio 3:2 (v/v) as extraction solvents. 2 g of fish waste was mixed with 1 g sodium sulphate and soaked in 42 ml of the extraction solvent. Then the mixture was subjected to microwave power at 800 Watt. At this power the sample irradiated was for 2, 3, 5, 7 and 10 min when using water as solvent and 1-4 mins when using the organic solvent. After that, mixture was filter using filter paper. 5 ml hexane was added to separate the filtrate mixture. The upper layer was taken and dried. After that, lipid weight was measured.

2.3.2 Soxhlet method. Soxhlet apparatus was used for the extraction of lipid from the fish waste. 2g of fish waste was mixed with 60 ml hexane and 4 g of sodium sulphate was added to the mixture before fixing the Soxhlet apparatus. The temperature of the Soxhlet was set at 140°C for 4 hour. By the end of the extraction time, the mixture was filtered using filter paper. Then, the filtrate was dried in rotary evaporator at 45°C and the dried part was weighed to obtain the lipid weight.

2.3.3 Hara and Radin method. In this method distilled water and hexane:isopropanol with ratio 3:2 (v/v) were employed as extraction solvents [9]. 2 g of sample was added to 36 ml solvent and homogenize for 30 seconds. After homogenize, the mixture was filtered into a round bottom flask. The residual on the filter paper was rinsed twice with additional 10 ml hexane which was also filtered into the same round bottom flask. 24 ml aqueous sodium sulfate was added to the filtrate. The aqueous sodium sulphate was prepared by mix 30 ml water and 1 g sodium sulphate. After addition of the aqueous sodium sulphate solution two layers were formed. The lipids were in the upper layer, pipetted out and dried to obtain the lipid weight. Figure 2 shows the layout of the procedure for Hara and Radin method.

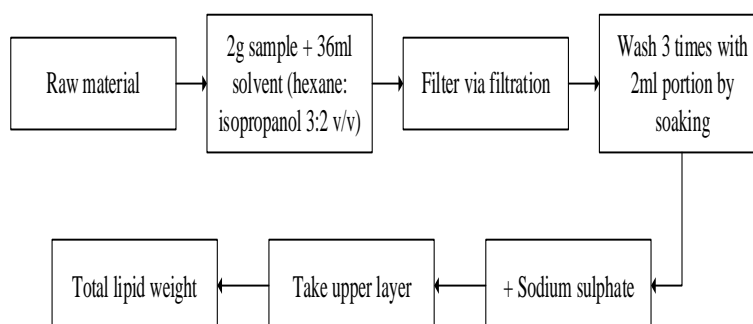


Figure 2. Hara and Radin method procedure

3. Results and Discussion

Figure 3 shows the lipid yield from fish waste when distilled water was used at high microwave power. The results revealed that lipid weight increased from 33.0 mg/g to 80.5 mg/g by increase the treatment time from 2 min to 10 min. The highest yield at 80.5 mg/g resulted from 10 min treatment time can be attributed to the microwave heating which based on the polar characteristic of the treatment solvent [10,11]. Water as solvent has high dielectric constant which can enhance the internal heat generation within the sample that helps to extract out the lipid from fish particle waste. Moreover, extending the treatment time at high power level caused an increase in the heat generation within the sample. The highest yield when using organic solvent as solvent at 4 mins is at 61 mg/ as shown in Figure 4. Increasing treatment time beyond 4 mins only resulted in evaporation of solvent. Increasing the time gave positive result to the lipid yield. In fact, at 4 mins, the yield drastically increased. This could be attributed by the heating effect of the solvent and sample, which could have approach a suitable temperature for extraction. However, the yield is 24% lower than the yield in MAE water extraction albeit at less than half the time. Comparative amount of lipid was extracted in MAE-water as early as 3 mins, possibly due to the fact that water has better dielectric characteristics (dielectric constant = 80 at 20°C) compared to hexane/isopropanol mixture (dielectric constant=20 at 20°C) which absorbs the microwave faster and converts to heat better. The higher yield when using water as the solvent is promising as it is a green solvent plus the separation of lipid and water is almost instant.

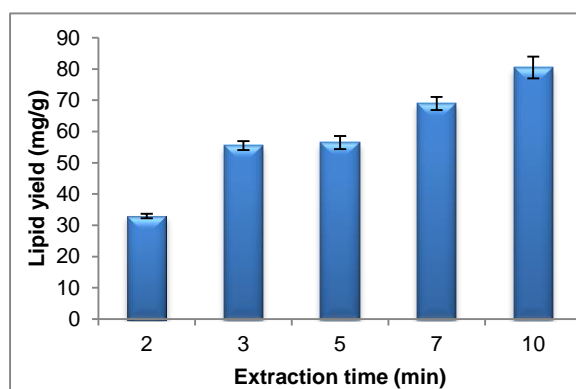


Figure 3. Lipid yield during microwave assisted extraction using distilled water.

For Soxhlet method, in this process the hexane was selected and used as extraction solvent. The results show the lipid yield was 46.6 mg/g after 4 hours extraction time and at 140°C. The yield is only 58% of yield found during microwave-assisted extraction. In Hara and Radin method, the same solvents with microwave-assisted extraction were employed to compare the microwave effect. Only 15.8 mg/g lipid was recovered when using Hara and Radin method, only 25% of the MAE-organic solvent maximum yield. This shows that higher temperature of solvent is needed to extract lipid out of the cells.

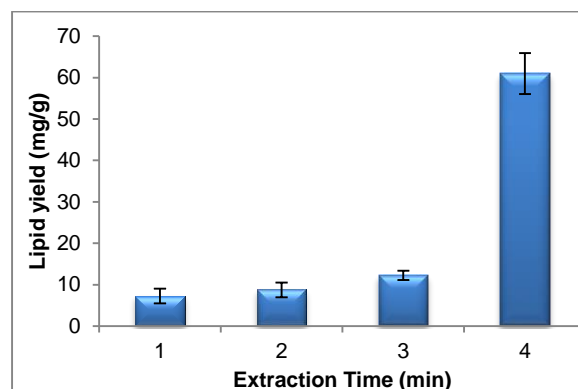


Figure 4. Lipid yield during microwave assisted extraction using hexane: isopropanol

4. Conclusions

The extraction of lipid was done using the three different methods. Among the experimented methods, the microwave- assisted extraction achieved the highest yield of lipid from fish wastes using distilled water as an extraction solvent meanwhile Soxhlet, and Hara and Radin methods yielded 58 % and 20% of the highest yield found using MAE method. These results suggest that the microwave-assisted extraction process can enhance the lipid extraction from fish waste using a green solvent such as water which makes this process an attractive route. In future study it is essential to identify the optimal extraction conditions such as microwave power and extraction time.

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