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MICROWAVE-ASSISTED PRETREATMENT AND ENZYMATIC HYDROLYSIS OF SARDINE PROCESSING WASTE

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Abstract

Fish waste can be used to produce many value-added products such as proteins, oil, omega-3 fatty acids, and amino acids for various applications in the food, neutraceutical and pharmaceutical industries. Fish processing wastes consist of fish heads, bones, frames and tails are produced during the processing of fish in food producing industries. The aim of this study is to evaluate the efficiency of microwave as a pretreatment prior to enzymatic hydrolysis for the extraction of protein. In this study, sardine waste was mixed with water at a ratio of 10% w/v and pre-treated under microwave heating at different power (80, 440 and 800 W) and time (5, 10, 15 minutes). Subsequently, enzymatic hydrolysis was carried out using 2.5% (w/w) Alcalase enzyme. The protein in the hydrolysate obtained was characterised using Lowry method. Results indicate that using microwave as a pretreatment gave higher protein yield as compared to untreated sample (20.2 mg/g). Generally low microwave power and shorter time give better yield. Lowest power setting for 10 mins pretreatment resulted in highest protein yield at 59.5 mg/g, followed by the medium power (57.9 mg/g). The pretreatment of fish waste using microwave prior to enzymatic hydrolysis is proven to increase the yield of protein possibly via lipid removal during the microwave pretreatment.

Keywords: Enzymatic hydrolysis, Fish waste, Microwave-assisted, Protein hydrolysate.

1. Introduction

Fish protein hydrolysate (FPH) is current being widely developed, as they are known to be a considerable solution to the utilization of fish processing waste. The fisheries industries commonly used the meat part only and discarded up to 50%-60% of total fish weight, which constituted of fish waste [1]. These fish processing waste contain approximately 20% - 30% of protein [2] and hence can be processed into fish protein hydrolysate.

Fish protein hydrolysate as defined by Pigott and Tucker [3], is a liquid product processed from fish with the help of proteolytic enzyme which enhances the hydrolysis process prior to its addition and in controlled conditions and resulted to a mixture of protein components. Chalamaiah et al. [4] stated that fish protein hydrolysates were breakdown products of enzymatic conversion of fish proteins into smaller peptides, which normally contain 2-20 amino acids. One of the benefits of fish protein hydrolysate is that they can be used as food supplement. With this being known, FPH could be utilized in order to help in reducing protein deficiency engulfing certain parts of the world's population whom suffer from malnutrition.

Protein extraction can proceed via chemical route or mechanical route. The objective is to break down the membrane of the cell. The fluid containing the lysed cells is call lysate. In terms of protein extraction, it is call protein hydrolysate. Common chemical lysis process uses alkali, enzyme and detergents which can be classed by their amphiphilic structure (both hydrophobic and hydrophilic). Mechanical route disrupt the cells membrane using sonication, homogenizer or bead beater. In commercial settings, this method is preferable as it is more economical. However, heat produced when using this method needs to be controlled carefully. The mechanism proceeds via agitation or liquid shear method. Sonication creates vibrations and cause mechanical shearing of the cell wall and normally adjusted at the highest allowable power setting. At this setting higher heat can be produced. In homogenization method, normally blender or Dounce are used to disrupt soft animal tissues. This procedure also normally produce heat thus the container needed to be pre-chilled at 4^{o}C.

In comparison to the chemical hydrolysis, enzymatic hydrolysis is preferable due to several advantages, such as mild reaction conditions, low undesirable products, and high product quality and yield. Controlled enzymatic hydrolysis of protein-rich fish wastes is believed to be a better way to transform these wastes into products. Fish hydrolysates produced are found to have functional and biological properties, which are applicable for different applications, compared to those of native proteins or common food protein ingredients [5]. An optimization of enzymatic hydrolysis of visceral waste protein of catla (*Catla catla*) for preparing protein hydrolysate using a commercial protease (Alcalase) studied by Bhaskar and Mahendrakar [6] resulted in controlled conditions of ratio of enzyme to substrate of 1.5% (v/w), pH 8.5, temperature of 50^{o}C with the hydrolysis time of 135 min as the optimum condition to obtain a higher degree of hydrolysis up to 50%. In another study by Jumardi et al. [7], protein hydrolysate produced from tilapia (*Oreochromis niloticus*) by-product showed a content of 62.71% of protein and degree of hydrolysis at 25.16% when hydrolysed at pH7.5, 50^{o}C, 2.5% (w/v) substrate concentration and 4.0% (w/w) enzyme concentration.

However, the used of enzyme is expensive, thus large-scale production is not economically viable. If the reduction of enzyme or higher protein yield can be achieved via this extraction technique, it can be more attractive. Therefore, a

pretreatment of the raw material for the enhancement of cell lysis and separation of the lipid at the same time could possible increase the protein yield [8]. Microwave-assisted pretreatment is proposed in this study as a physico-chemical pretreatment method. Since protein can be dissolved in water and water is a good absorber of microwave, it can be a good solvent in microwave-assisted pretreatment. Since protein is heat sensitive, high power and time of treatment could negative impact on the yield, the use of microwave is probably more beneficial to break the cell wall to ease the enzyme to get inside the cell for the hydrolysis step.

A microwave oven, commonly referred to as a microwave, is a kitchen appliance that heats and cooks food by exposing it to electromagnetic radiation in the microwave spectrum. This induces polar molecules in the food to rotate and produce thermal energy in a process known as dielectric heating. Few benefits from the application of microwave include low solvent volume and short heating time with the ability to process multiple extractions. Ha et al. [9] reported on microwave-assisted pretreatment of cellulose in ionic liquid for accelerated enzymatic hydrolysis and it was shown that the pretreatment increased the rate of enzymatic hydrolysis of cotton cellulose. Meanwhile a study by Mehmood et al. [10] showed a significant increment in lipid extraction with highest extracted lipid content of 0.052 g/g (92.81%) as compared to 0.016 g/g yielded from non-treated sample. Solubilisation of protein was found to be affected by the presence of fats in raw material, where higher amount of fats content of raw materials produced lowest percentage of solubilized protein [11].

Following these findings, this study is made in search of a new method for fish protein hydrolysate production incorporating microwave heating as the pretreatment process. Thus, the objectives of this paper is to evaluate the use of microwave-assisted heating of sardine processing waste as the pretreatment method prior to enzymatic hydrolysis for the production of protein hydrolysate.

2. Materials and Methods

Figure 1 below shows the flow of the experimental procedure for microwave-assisted pretreatment and enzymatic hydrolysis of sardine processing waste.

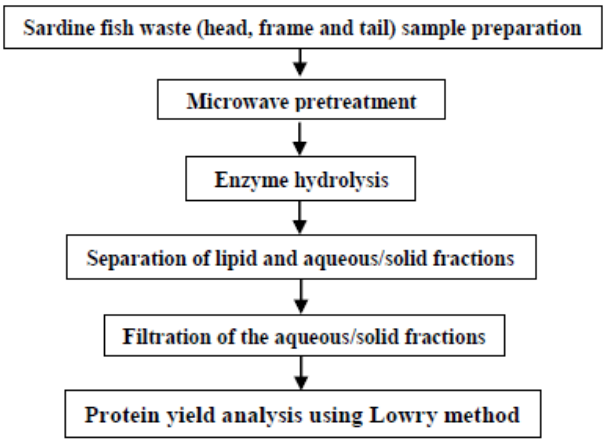

Fig. 1. Experimental procedure for microwave-assisted pre-treatment and enzymatic hydrolysis of fish wastes

2.1. Preparation of fish waste sample

Fresh sardine fish (*Sardiella*) was bought from a wet market in Seri Kembangan, Selangor. The fish was rinse with tap water, eviscerated and filleted to separate the fish muscle from the waste (head, frame (bone sections) and tail). The waste samples were then blended in kitchen blender and kept in 10 g portions at -20°C until further use.

2.2. Pretreatment experiments

Microwave-assisted pretreatment was carried out using a modified domestic microwave (Samsung, ME711K) equipped with a temperature control device which has a working temperature ranged between 20 and 240°C with a frequency of 2.45GHz and maximum output power of 800 and Graham condenser cooled using tap water. The schematic drawing of the experimental setup is illustrated in Fig. 2. A 25 mm-round hole was made at the center of the top of the microwave cavity to facilitate the connection between the reaction flask and the reflux condenser.

The 10 g thawed samples were transferred to a 1,000 ml round bottom flask that contained 100 ml water. Then, the flask was placed inside the microwave cavity. Three different powers were investigated: 80 W (Low), 440 W (Medium) and 800 W (High) at different durations (5-15 mins). Water was used as the solvent and the solid loading was maintained at 10% (w/v). After the pretreatment experiments, the samples were transferred to 250 ml Erlenmeyer flasks and incubated at 60°C for 20 minutes in a water bath.

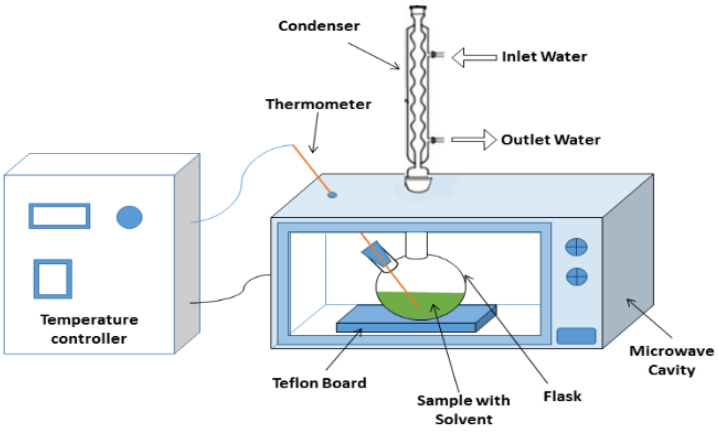

Fig. 1. Schematic drawing of the microwave-assisted system.

2.3. Enzymatic hydrolysis of pretreated samples

After the samples were incubated, 15%(w/v) buffer to maintain pH 7.5 and Alcalase enzyme® 2.4 L (Novo Industry, Denmark) were added at 2.5% (w/w). The flasks were then placed on incubator shaker at temperature 55°C and 50 rpm for 2 hours.

Then the samples was heated at 90°C for 10 minutes to deactivate the enzyme and cooled. Samples were transferred to centrifuge container and centrifuge at 10,000 rpm for 15 mins at 4°C. Lipid layer on the surface of the aqueous layer were removed using micropipette and the remaining samples were filtered using P5 filter

paper. Filtrate (Fish protein hydrolysate) samples were stored at 4^{o}C before protein analysis was done.

2.4. Analysis of protein

The protein yield in the FPH was evaluated using Lowry Method. Two reagents were prepared. Reagent A was prepared by mixing 48 ml of 2% Na_2CO_3 in 0.1 NaOH, 1 ml of 1% NaK tartrate in H_2O and 1 ml of 0.5% $CuSO_4.5H_2O$ in H_2O. Reagent B was prepared by mixing 2 N Folin-Phenol with distilled water at 1:1 ratio.

1 mg/ml Bovine Serum Albumin (BSA) was used as the standard. The standard curve was prepared using BSA mixed with the reagents. The different concentrations of prepared standards were measured using UV-Spectrophotometer (Thermo and Genesys 10UV) at 660 nm. The samples were prepared and analysed using the same method as the standard.

3. Results and Discussion

3.1. Chemical composition of fish waste

Chemical analysis was done and the result are as shown in Table 1. The weights represent the raw content of sardine processing waste. With high content of crude protein (438.09 mg/g), it shows that significant potential for high yield of protein for protein hydrolysate. The composition of fat, moisture, ash and carbohydrates are 76.8, 30.5, 280.0 and 175.0 mg/g, respectively. The fat content at 76.8 mg/g shows that the waste does contain significant percentage of fat and thus the pretreatment with microwave heating was done to enhance the protein yield in the fish protein hydrolysate.

Table 1. Chemical analysis of sardine processing waste.

Content	Weight (mg/g)
Fat	76.8
Moisture	30.5
Ash	280.0
Carbohydrate	175.0
Crude Protein	438.0

3.2. Effect of microwave-assisted pretreatment parameters on protein yield

The results of protein yield for protein hydrolysate production using microwave-assisted pretreatment prior to enzymatic hydrolysis are shown in Fig. 2. The effect of microwave pretreatment of protein yield was significant where the protein yield for untreated sample prior to enzymatic hydrolysis is only 20.2 mg/g as compared to microwave-assisted pre-treated samples. According to Fig. 3, the protein yield pre-treated at low and medium power at 10 minutes is not significantly different. However, using high power for pretreatment resulted in reduced protein yield with more negative affect for prolonged pretreatment time. Generally 10 minutes at low and medium microwave power give high yield protein above 55 mg/g. Extending the pretreatment time to 15 minutes did not increase the yield possibly due to protein denaturing at higher temperature resulted from longer heating. It is important to maintain optimum time in

microwave heating as it affects the process of removing the fat to extract more protein from the sample. The yield of protein is however still low as compared to the composition of crude protein in the samples. Only 12.6% of protein were recovered in this study.

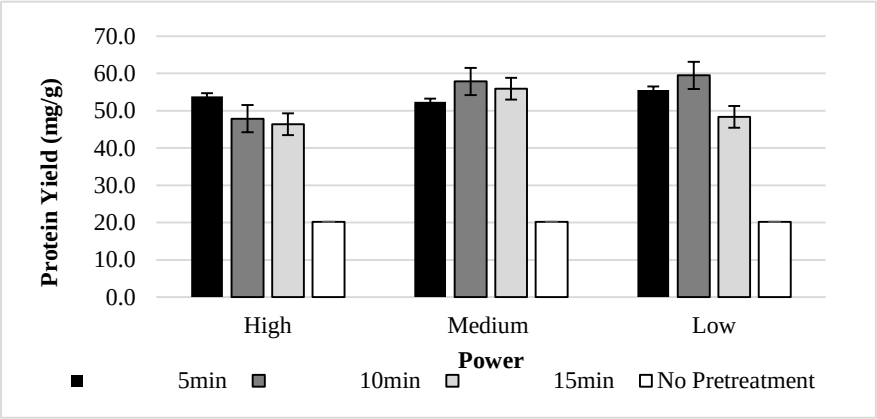

Fig. 3. Protein yield of protein hydrolysate after enzymatic hydrolysis.

SEM analysis was conducted to support the findings from the experimental work and the finding is shown in Fig. 4. It is observed that the microwave pre-treated fish waste sample, Fig. 4(b) has a rougher surface while the no pretreatment fish waste sample has a smoother surface, Fig. 4(a). Protein hydrolysate produced from pre-treated sample also showed higher protein yield. It is due to cell wall were broken during microwave heating, releasing lipids and hence the better hydrolysis of protein was able.

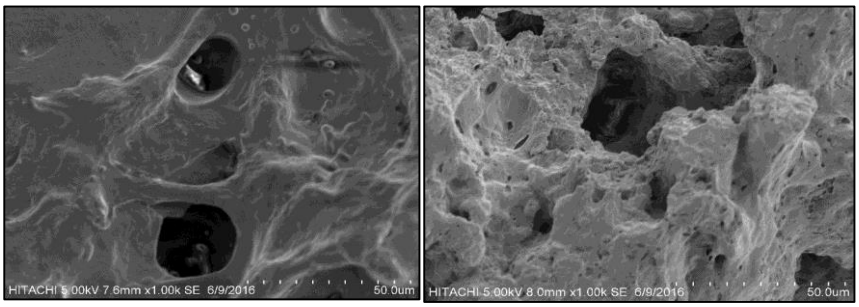

Fig. 4. Surface image of (a) untreated and (b) microwave pre-treated fish waste sample.

4. Conclusions

Microwave-assisted pretreatment prior to enzymatic hydrolysis of sardine waste shows better protein extraction for the fish waste. Sample pre-treated in microwave at low and medium power for only 10 minutes yielded higher protein content compared to high power and untreated samples. Although the findings is at the early stage, microwave as a pretreatment tool for enzyme hydrolysis offers an interesting alternative for protein production from fish wastes.

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