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RESEARCH ARTICLE

Extraction of local fish waste by subcritical water

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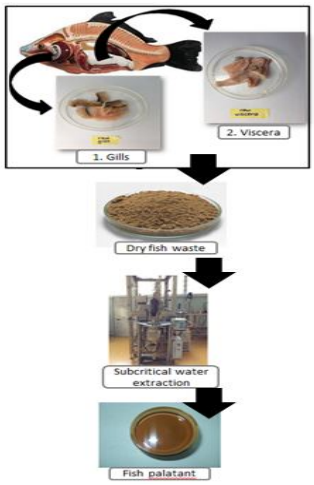
Graphical abstract



Abstract

Production from fish processing activities leads to enormous amounts of waste. This waste is substantially composed of non-edible parts, for instance viscera, gills and others. This fish waste has incredible potential as raw materials or as one of the ingredients needed for the preparation of protein foods for pets. Fish industries should recover the leftovers from fish processing into another valuable product. This can be as a good reduction strategy for industries to minimize their wastes. Hence, it helps to reduce harmful impact to the environment. Therefore, this fish waste has big potential to be commercialized. Additionally, the fish waste available in the markets actually has its own excellent probability. It can be utilized as an ingredient in animal food production such as fish palatant. So, in this study, fish waste originated from ocean fish was collected from local market in Selayang. Its nutritional compositions were firstly analyzed. Fish viscera was selected to undergo extraction as it showed up with high protein content of 84.68% rather than fish gills with protein content of only 44.62%. In order to produce safe food for cats, subcritical water extraction (SWE) method was applied in the extraction process instead of using conventional extraction method. This extraction method is considered as a green process because of usage of water as a solvent since water can replace aqueous solvent during extraction process. SWE was then heated to the desired temperature of 140°C-220°C with pressure in the reactor was fixed at 3.0 MPa. The extraction time for each sample was 5 minutes. At a temperature of 180°C, it has the highest protein content with 1.705 g/L BSA.

Keywords: Fish waste, viscera, gills, protein content, subcritical water extraction

INTRODUCTION

In landfills, one of the largest portions of the wastes component is originated from the food waste. This eventually leads our environment to be polluted with various of pollutions. Malaysia Ninth Plan Report 2005 (Leow *et al.,* 2010) stated that, the population in Malaysia produced a large amount of solid waste. It was calculated to become approximately 9.57 million of tonne metrics. Meanwhile, 25% which developed from the quantity of waste generated is equal to 2.39 million of tonne metrics which actually made up from food waste. In addition, the fish waste contributes a huge number of wastes which discarded from the fish processing industry, wet market and fish loading. They actually acts as the main contributors in fish waste production. Besides, most of the fish wastes are dumped as garbage. By cause of that, the fish leftovers are thrown away without making any intention of recovering the waste into valuable materials. Hence, without proper utilization, these wastes may promote environmental impact (Nurdiyana *et al.,* 2008).

Over the last 45 years, the population in worldwide has generated fish's consumable that reached almost double. Consequently, this leads fish processing to produce enormous quantities of waste (Gopi *et al.,* 2013). Whereas the fish canning manufacturers also induce large amount of solid waste. This is due to the estimation on the amounts of raw products that are converted into waste products that bring up about 50% by weight. Commonly, the fish waste is composed of heads, viscera, bones and scales. Opportunely, good nutritive quality has been found in the fish waste. There are plentiful in lipids and proteins and that is why it can be very useful and high possibility in animal feeding (Ivo *et al.,* 2010; Lia *et al.,* 2007). Therefore, it is strongly recommended to get an effective process or any method to convert the fish waste into valuable materials (Hiroyuki *et al.,* 1999). On top of that, these wastes come up with abundant of proteins and bio-active matters (Zhu *et al.,* 2008). In addition, fish protein hydroysate is one of the targeted molecules in the recovery of fish leftovers (Ravidran and Jaiswal, 2016).

In order to recover fish waste, fish palatant production is considered. Fish palatant is introduced since it can be utilized as a good possibility to be as protein source for pet food. This approach is an eco-friendly production. Also at the same time, it generates animal feed using economical raw materials which originated from fish waste (Nurdiyana *et al.,* 2015). Actually, palatant works as one of ingredient that is specially invented in production of pet foods. This ingredient improves taste of treats and supplements. Apart from that, it ensures that crucial nutrients are consumed by pets which are needed in their daily life (Beth, 2011). The usage of fish waste in the production of animal feed, for instance cat food, hopefully can reduce the processing

costs, the energy required as well as time consuming. Fish waste is used as one of ingredients in animal feed and becomes high interest to be an alternative way to introduce as a pet food product. This intention can benefit the environment and the community, and together help the animal food production to be more economical (Esteben *et al.,* 2007).

The conventional extraction, such as liquid-liquid extraction, Soxhlet extraction, accelerated solvent extraction and solid phase extraction are extensively used in variation of applications and one of them is for waste treatment. However, organic phase solvents which are volatile organic compounds (VOCs) including halogenated hydrocarbons, aliphatic and aromatic hydrocarbons, some esters, alcohols, ethers, aldehydes and ketones are primarily used in these extraction techniques. It is notable that many of these VOCs are toxic, highly flammable and very hazardous to the environment (Plotka *et al.,* 2011).

Therefore, the subcritical water extraction (SWE) is a useful method to imply in the recovery of organic wastes. Thus, the researchers in global have been attracted towards this extraction method. This is because, SWE is sustainable, renewable, effective and safe to our nature. SWE has gained wide attention due to this method of using environmentally friendly solvent and consisting of interesting reaction medium for a variety of applications. Moreover, it is inexpensive, non-poisonous, non-flammable and non-explosive. These outstanding advantages can consider SWE as a "green technique" in the extraction process (Guanyong *et al.,* 2011).

SWE applies water instead of organic solvent as extraction solvent under subcritical conditions in which the water can be maintained in their liquid state. This is because the temperature higher than boiling point because high pressure applied throughout in the extraction process. The water is considered as subcritical at the critical temperature, Tc in the range of 100°C to 374°C whereas critical pressure, Pc is in the range of 0.10MPa to 22MPa. Therefore, this will allow the recovery of value added products in short extraction times with low impact to the environment (Espindola *et al.,* 2017). Water has gained the adoration as an extraction solvent due to its physical properties to manipulate the dielectric constant to alter over a wide range by simply changing the temperature and pressure. As a result, this can allow the dissolution of moderate or low polarity compounds. Increasing in temperature also improves the extraction efficiency due to an addition in the mass transfer rates (Vardanega *et al.,* 2017).

In this study, experiments were performed to extract fish palatant from local fish waste using subcritical water extraction.

EXPERIMENTAL

Materials

The fish waste which composed of the gills and viscera were collected from a local wet market in Selayang, Selangor. The fish waste was composed of the type of ocean fishes.

Methodology

Sample preparation

The fish waste was washed with water for several times. The reason was to detach adhering blood and any existence of undesirable fraction. Then, the fish waste samples were dried in an oven with the temperature set up to 65°C for about 15 hours. After that, the dried samples were ground and sieved through a 0.06 mm mesh filter. These samples were then kept inside a desiccator prior to usage.

Proximate composition of raw samples

Dry basis moisture content, ash content, crude protein, crude fat and crude fiber were analyzed for both dried samples of fish viscera and fish gills. The methods were as follow:

Moisture content

The sample was weighed in the crucible and dried in an oven at fixed temperature of 105°C until constant weight was achieved. The calculation of moisture content was shown in equation below (Sohaimi *et al.,* 2015):

$$Moisture\ Content(\%) = \frac{Weight\ loss\ after\ drying(g)}{Initial\ weight\ of\ sample(g)} \times 100 \quad (1)$$

Ash content

The sample was weighed in the crucible and continued with the incineration sample at 600°C for 2 hours. The ash content was calculated by using following equation:

$$Ash\ content\ (\%) = \frac{Weight\ of\ ash\ (g)}{Oven\ dry\ weight\ (g)} \times 100 \quad (2)$$

Total protein content

Determination of total protein content was analyzed according to AOAC (1995) method by Kjeldahl. Total protein content was calculated using the following equation (Romadhoni *et al.,* 1973):

$$Total\ protein\ content\ (\%) = \frac{V \times N \times 0.014 \times 6.25 \times P}{Sample\ (g)} \times 100 \quad (3)$$

In which,
V is sample titration volume
N is the solution normality of H_2SO_4 and
P is a dilution factor

Crude fat

In the determination of crude fat content, acid digestion was considered first and then continued with extraction method using petroleum ether in Soxhlet extraction process. The calculation of fat content was done by applying the equation below (Romadhoni *et al.,* 1973):

$$Fat\ content\ (\%) = \frac{Fat\ weight\ (g)}{Material\ weight\ (g)} \times 100 \quad (4)$$

Crude fibre

In order to examine the crude fiber content in the sample, hydrolysis of sulphuric acid, H_2SO_4 and sodium hydroxide, NaOH was applied. The analysis was preceded by calcination at 550°C until constant weight was achieved. The calculation for crude fibre was done by using equation below ((Esteben *et al.,* 2007):

$$Crude\ fibre\ (\%) = \frac{Loss\ in\ weight\ on\ ignition\ (g)}{Weight\ of\ sample\ (g)} \times 100 \quad (5)$$

Subcritical water extraction

The fish viscera or fish gills that containing the highest composition of nutrients was selected for subcritical water extraction (SWE). The SWE was performed using the fabricated 1Litre Subcritical Water Extractor located at Shizen Conversion and Separation *i*kohza (Shizen *i*kohza), Universiti Teknologi Malaysia, Kuala Lumpur, as shown in Fig. 1.

The ratio of 1:20 was preferred as solid solvent ratio and then charged into the reactor of SWE. So, about 20 g of powdered, dried fish waste sample was weighed and 400 ml distilled water was added. Firstly, the reactor was heated up to the desired temperature of 140°C with a fixed pressure of 3.0 MPa. Hence, 5 minutes was fixed for the extraction time. When the extraction was finished, the reactor was cooled down, and then followed by the collection of the liquid portion formed from extracted product in the reactor. Then, the product was centrifuged at 10000 rpm before proceeding for analysis (Uddin *et al.,* 2010). The same procedures were repeated for other temperatures (160, 180, 200 and 220) °C where extraction time and pressure were maintained the same with 5 minutes and 3.0 MPa, respectively.

Fig. 1 Subcritical water extractor at Shizen ikohza UTM KL.

Analysis of soluble protein

The Lowry method (1951) was considered to analyze the soluble protein content in extracted fish palatant. The protein was estimated using bovine serum albumin (BSA) as a standard protein. For a standard curve, a series of standard protein, i.e. 0 mg/L, 20 mg/L, 40 mg/L, 60 mg/L and 80 mg/L was constructed. Protein determination of sample (for different temperatures) was similar as standard protein determination. But, the dilution factor was considered in sample determination. All absorptions for standard and sample were measured at 750 nm using UV-Vis Spectrometer (Romadhoni *et al.,* 1973).

RESULTS AND DISCUSSION

Proximate analysis

In order to examine the nutrient composition, this proximate analysis was conducted on each fish waste sample (fish viscera and fish gills). Thus, the result of compositions for both samples was stated as shown in Table 1 below.

Table 1 The proximate composition of fish viscera and fish gills.

Composition	Fish viscera (%)	Fish gills (%)
Moisture	6.21	4.43
Ash	1.68	24.33
Crude protein	84.68	44.62
Crude fat	4.56	27.38
Crude fibre	Not detected	0.04

The high moisture content was found in both waste samples, which were fish viscera with 6.2% and fish gills with 4.4%, respectively. For another result of nutrient composition, 24.3% of ash content was appeared in fish gills. This data indicated that fish gills were rich in mineral content source compared to the ash content of fish viscera with only 1.7%. This result was in agreement with previous studies of the ash content of fish waste which fell within the range of 1.6%-2.0% (Peter, 2003). Fish waste provided one great element because of its high protein source which was found in the fish viscera (84.7%). This revealed that the amount of protein content presented in fish waste was higher than the protein content existed in the commercial fish meal. Usually, the amount of protein content in fish meal was within the range from 60% to 70% (Ricque-Marie *et al.,* 1998). On the other hand, lower fat content was found in fish viscera with 4.6% while fish gills came out with larger amount of fat which was about 27.4%. Hence, in order to continue a study on subcritical water extraction, fish viscera was preferable as a sample for extraction to produce fish palatant as a result of its high protein content, low in organic matter content and low fat content.

Extraction of fish viscera by subcritical water

The fish palatant extracted from fish viscera using subcritical water at different temperatures (140, 160, 180, 200 and 220) °C were shown in Fig. 2. After the reaction during SWE, a liquid phase for each extraction was produced. Some parts of the fish viscera were remained as solid residual. Also, the changing of colour in the extracted fish palatant indicated from light to dark brown as increasing in temperatures.

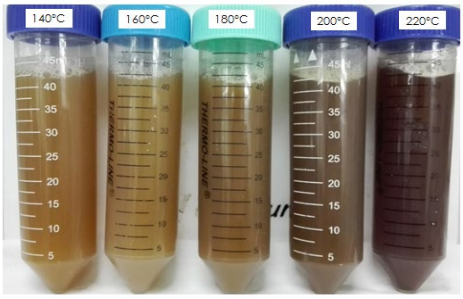


Fig. 2 Products of fish palatant extracted by SWE at different temperatures.

The standard of Bovine Serum Albumin (BSA) was applied in this protein determination in order to develop a standard curve through a series of standard. The straight line generated from the BSA standard curve indicated the measured absorbance for each series of BSA standard in order to create a linear equation. Thus, this equation was used to determine the protein concentration in respective extracted fish palatant produced from different temperatures. A graph in Fig. 3 shows that y represented for absorbance at 750 nm and x represented protein concentration that expressed in milligrams per liter (mg/L).

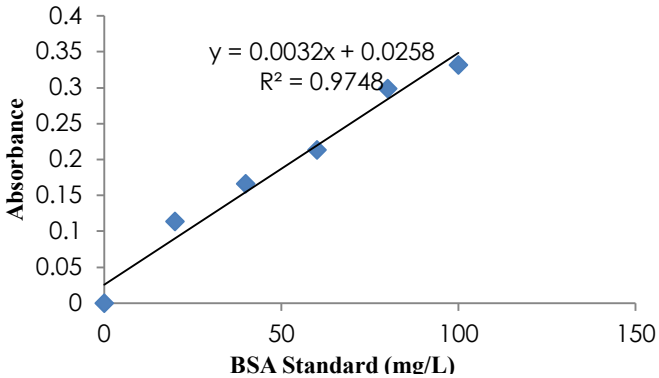


Fig. 3 A Lowry protein standard curve using BSA at (0, 20, 40, 60, and 80) mg/L. A linear regression by the line y = 0.0032x + 0.0258 with R^2 value of 0.9748.

Therefore, when SWE was performed to the fish viscera, the absorbances for all extracted products for each temperature were analyzed. The result was shown in Table 2. Among a series of temperature between (140, 160, 180, 200 and 220) °C, absorbance was the most abundant at temperature of 180°C. However, absorbance was started to decrease with the rising temperature from 200°C to 220°C.

Table 2 The absorption of extracted fish palatant from subcritical water extraction at different temperatures.

Temperature (°C)	Absorption
140	0.937
160	0.943
180	1.117
200	1.108
220	1.036

Hence, to get the amount of protein content in extracted fish palatant for each temperature, a linear regression as stated in Fig. 3 was considered. Thus, according to the Fig. 4, the protein content was larger as the temperature for SWE was increased from 140°C to 180°C. On the other hand, at a temperature of SWE was 180°C, it has the highest protein content with a 17.05 g/L BSA. But, the protein content in extracted fish palatant was started to decrease when the temperature was further increased to 200°C and 220°C.

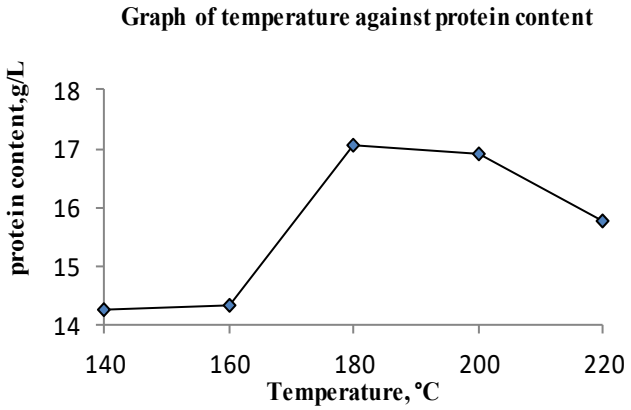


Fig. 4 Protein content of extracted fish palatant from SWE at different temperatures.

Probably this situation was happened due to protein degradation in which the temperatures were actually enhanced the rate of protein decomposition in the subcritical water. Meanwhile, at higher temperatures of 200°C and 220°C, the concentrations of hydronium (H_3O^+) and hydroxide (OH^-) ions were increased in the subcritical water. Thus, this encouraged protein to decompose into smaller particles. Similar results were found in SWE of squid viscera (Uddin *et al.,* 2010).

CONCLUSION

In conclusion, fish waste like fish viscera could be an excellent potential as a source of protein. Besides, fundamental information could be generated based on proximate composition of fish waste which could be studied further. The fish palatant extracted from subcritical water extraction (SWE) also could be one of the ingredients in pet food. Moreover, SWE has a great potential to extract valuable materials such as protein in the fish waste instead of using conventional extraction method.

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