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Effect of extraction methods on the nutritional characteristics of milkfish (Chanos chanos Forskal) bone powder

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Abstract. Milkfish (Chanos chanos Forskal) bone is one of underutilized part of milkfish and considered as a waste from ’satay bandeng’ home industry in Banten. The aim of this research was to determine the effect of different extraction methods on the nutritional characteristics of milkfish bone powder. Milkfish bone powder was prepared using two different extraction methods (alkaline and acid). Milkfish filleting waste was boiled with water for 30 min, removed the remaining flesh from the frames, the frames were boiled with water for 2 hours, autoclaved, boiled in water (MBPW), sodium hydroxide solution 1 N (MBPAI) and hydrochloric acid solution 1 N (MBPAc) for 30 min at 80-90 ºC, rinsed with water, dried at 50 oC in oven drying for 24 hours, and ground. The observed parameters were % yield, moisture-, protein-, fat-, carbohydrate-, and ash-content. Milkfish bone powder was contained 27.88% protein, 7.85% lipid, 51.42% ash, and carbohydrate 7.36%. The milkfish bone power extracted by acidic and alkaline extraction methods (MBAc and MBAl, respectively) showed statistical significant difference in yield, protein-, fat-, carbohydrate-, and ash-content. The alkaline and acid-extraction methods showed higher ash content than water extraction method so these method could be used to extract the mineral content from milkfish bone.

1. Introduction
Milkfish (Chanos chanos Forskal) is one of popular raw material of Banten culininary snack, such as milkfish satay, milk fish floss, milkfish bontot, milkfish crackers and chips. The most utilized part of milkfish are flesh and skin. Whereas, milkfish bone is one of underutilized part of milkfish and considered as a waste from these industry, especially from satay bandeng home industry. Based on data of Industrial and Trade Department Office in Serang City, there are 17 Small Medium Entreprises (SME) of milkfish satay in Serang City. Milkfish bone has been produced and discarded everyday as by product of the milkfish processing line which can cause environmental pollution. This by products as a result of processing represent more than 30% of the fish being processed. The amount of fish bone fraction is approximately 10 - 15% of the fish whole body weight [1].

Fish bone powder are known as one of sources of minerals such as calcium and phosphor [2]. Fish bone powder usually extracted by boiling the fish frame with water, alkaline, acid or combination of these treatments. The alkaline extraction method of three kind fish bone powders from Grouper (Epinephelus diacanthus), Emperor (Letrinus fraenatus) and White snapper (Pristipomoides filamentosus) showed higher ash content than extraction in neutral condition so this method could be used to extract the mineral content from fish filleting waste [3]. The acidic treatment is also used to extract calcium fish bone powder in Nila fish (Oreochromis niloticus) [4]. Previous study found that the alkaline and acidic treatments could affect the nutritional characteristic of the fishbone powder [3,4].

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There are no studies addressed about the effect of extraction methods using alkaline, acidic, and neutral condition on the nutritional characteristic (macronutrients composition such as protein, fat, ash, carbohydrate) of milkfish bone powder. This study will help to effectively utilize the milkfish processing waste and reduce environmental pollution. The objectives of this research were to produce milkfish bone powder from milkfish filleting waste and to determine the effect of different extraction methods on the nutritional characteristics of milkfish bone powder.

2. Material and Methods

2.1 Sample & Chemicals
Milkfish (*Chanos chanos* forskal) filleting waste was obtained from Satay Bandeng home industry in Serang City, Banten Province. The sample was transported to the laboratory immediately and stored at -20°C until being processed. All chemicals (selenium mixture, HCl, H₂SO₄, NaOH, n-hexane, etc) were obtained from Merck.

2.2 Preparation of Milkfish Bone Powder
Milkfish filleting waste was further processed to produce milkfish bone powder. Milkfish filleting waste was boiled with water for 30 min, removed the remaining flesh from the frames, the frames were boiled with water for 2 hours, autoclaved, boiled in water, sodium hydroxide solution 1 N and hydrocloric acid solution 1 N for 30 min at 80-90°C, rinsed with water, dried at 50°C in oven drying for 24 hours, and ground. Milkfish bone powder production steps was shown in figure 1. The product obtained, referred to as milkfish bone powder produced by alkaline condition, acidic condition, and neutral condition (further named as MBPAL, MBPAC, and MBPW, respectively). Water milkfish bone powder were packaged in polypropilene plastic bag, and stored at 4°C until analisys.

![Flow chart of milkfish bone powder production](image)

**Figure 1.** Flow chart of milkfish bone powder production.
2.3 Nutritional Analysis

Milkfish bone powders were analyzed for their nutritional characteristics (macronutrients) such as protein-, fat-, carbohydrate- and ash- contents using the standard methods of AOAC [5]. Crude protein content was determined by the Kjeldahl method, moisture content was determined by thermogravimetry and total fat content by soxhlet extraction method, ash content was determined by combusting the samples in a muffle furnace at 550°C and carbohydrate content was determined by difference. The results were expressed in dry-basis.

2.4 Statistical Analysis

Statistical analysis was done using Excel spreadsheet and Statistical package for social scientists (SPSS) version 20. Analysis of variance (ANOVA) and Duncan test were used to test significant differences between samples (P < 0.05).

3. Result And Discussion

Milkfish filleting wastes from satay bandeng industry were taken as raw materials and processed to recover of milkfish frame then exposed to the alkaline and acidic treatment to extract of milkfish bone powder. The moisture content and yield of milkfish bone powder extracted by water, acidic, and alkaline condition are shown in Table 1.

The moisture contents of all samples have no significantly differences. The moisture content range from 5.19 % to 5.49 %. The moisture content of fish bone powder are vary depends on the fish species. The moisture content of fish bone powder from Grouper, Emperor, White snapper, Nile Tilapia are 5.96%, 4.12%, 3.21% [3], 8.4% [6], respectively.

The yields are 67.57%, 39.67%, and 50.87%, for water, alkaline, and acidic extraction methods, respectively. The highest yield was obtained by water extraction method. Both of the alkaline and acidic treatments can cause loss in some compounds, such as protein and fat-contents as shown in Table 2. The alkaline extraction method cause more significant loss in yield than acidic extraction method, because greater loss of protein and fat in the alkaline- than the acidic- extraction method.

| Parameters          | MBPW  | MBPAI | MBPAc |
|---------------------|-------|-------|-------|
| Moisture content (%)| 5.49  | 5.27  | 5.19  |
| Yield (%)           | 67.57 | 39.67 | 50.87 |

Different lowercase superscripts within the same row indicates significant difference (p < 0.05)

MBPW = Milkfish Bone Powder extracted with water
MBPAI = Milkfish Bone Powder extracted with alkaline
MBPAc = Milkfish Bone Powder extracted with acid

| Parameters (%)      | MBPW  | MBPAI | MBPAc |
|---------------------|-------|-------|-------|
| Protein content     | 27.88 | 1.74  | 4.68  |
| Fat content         | 7.85  | 1.83  | 2.45  |
| Ash content         | 51.42 | 80.84 | 73.35 |
| Carbohydrate content (by difference) | 7.36 | 10.62 | 14.33 |

Different lowercase superscripts within the same row indicates significant difference (p < 0.05)

The macronutrients composition of three milkfish bone powder are shown in table 2. The results of the macronutrients composition of MBPAI, MBPAc, and MBPW showed that the amount of the protein, fat, ash and carbohydrate is significantly different among all samples. The alkaline and acid treatment significantly decrease protein and fat of milkfish bone, and significantly increase ash content. Milkfish bone powder extracted with water extraction method was contained 27.88% protein, 7.85% lipid 51.42% ash, and carbohydrate 7.36%. The results of milkfish bone powder protein content obtained in the this study was comparable with previous studies [6, 7].The crude protein content of
fishbone powder without alkaline and acidic treatments in tilapia fish bone powder and tuna frame powder were 31.5% [7] and 28.66% [6]. The alkaline and acidic extraction methods showed the the protein content in the Alkaline and Acidic were 1.74 % and 9.68%, respectively, while the fat content were 1.83 % and 2.45%, respectively. Both of the MBPAI and the MBPAc have lower protein content than the MBPW. The alkaline and acidic extraction methods are boiling bones with NaOH solution and HCl solution (respectively), so that it will remove the protein from fish bone matrices. The alkaline extraction methods with NaOH 1N solution as the boiling media not only can cause protein denaturation and hydrolisis, but also fat saponification, result in lower protein- and fat- content compared to water and acidic extraction methods. Previous study concluded that alkaline solution would be effective way to leach out protein from the bone [2]. The acidic or alkaline pretreatment can hydrolyze the crosslinking bonds between polypeptides in protein [8].

Similar results related to the content of protein and ash in fish bone powder was obtained by Nemati et al [9] in production of tuna bone powder. The alkaline extraction method using of NaOH solution will increase the ash content but decrease the protein content of the tuna bone powder. Utilization of NaOH during MBP processing, dissolved and removed the organic materials from bone result in protein and fat contents in MBP decreased and ash content increased [9].

The significant difference in total fat content of fish bone powder was found in all treatments. Milkfish bone powder showed total fat content of 7.85% in MBPW, 1.83% in MBPAI, and 2.45% in MBPAc. These results are in agreement with several studies reported in tuna fish bone powder [6] and tia fish bone powder extracted by alkaline solution (NaOH) [9]. The fat content in tuna fishbone powder without pre treatments (alkaline) was 11 %, [6,9] and in tuna bone powder extracted by alkaline solution was 3.86% [9]. Having a low fat content in MBP is beneficial because the product can not easily oxidized so that it has a longer shelf life.

The major compound in the milkfish bone powder was ash content which was found to be 51.41% - 73.35%. The amount of the ash in MBPAl and MBPAc were higher than MBPW. The ash content in MBP were 51.42%, 80.84%, 73.35% for MBPW, MBPAI, and MBPAc, respectively. The amount of the ash in MBP was considerable in comparison to the results reported from other fish bone in the literature such as tuna fish bone without treatment 53.43%, with alkaline treatment 77.97% [9], tilapia bone powder 75.3%, catfish bone powder 61.8% [10]. The alkaline treatment method provided rather high ash content because all of the protein and fat in bone was removed. After some amount of fat and protein in milkfish bone were removed, the minerals became concentrated [11]. The ash content usually correlated to the high mineral content in fish bone powder [2], especially calcium content in tuna bone powder [9]. The alkaline solution (NaOH) usually used in production of biocalcium and calcined fishbone powder [11].

The ratio of ash/protein is the important criteria to indicate the bone mineralization, associated with hardness of the bone [2]. The ash/protein ratio in salmon, trout, and mackerel bone were lower than 1.00 [2]. In this study, the ratio ash/protein were 1.84, 46.46, 15.67 for MBPW, MBPAI, MBPAc respectively. The ash/protein ratio in MBPAI and MBPAc were higher than MBPW, its showed that both treatment with alkaline and acidic solution in extraction method of MBP could affect the ratio of ash/and protein. Similar result was found in production of tilapia bone powder and the value of ratio ash/protein could be used to indicate the purity of bone powder [12]. Both alkaline and acidic treatment in extraction methods could provide higher purity of milkfish bone powder.

4. Conclusion
The alkaline and acid- extraction methods showed higher ash content than water extraction method so these method could be used to extract the mineral content from milkfish bone so that both alkaline and acidic extraction methods could provide higher purity of milkfish bone powder. The results demonstrated that extracted milkfish bone powder are nutritious. Further, the recovered minerals of this milkfish bone powder may also have potential application in various pharmaceutical, and food industrial applications.
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