Improvement in Nutritional Quality of Shrimp Meal with Autoclave and Chemical Treatments: an in vitro Study

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The present study was conducted to improve the nutritional quality of shrimp meal (SM) comprising of heads with hulls of black tiger shrimp (Penaeus monodon) waste by autoclaving and chemical treatments. The sun-dried SM was divided into 5 treatment groups, such as 1) control (untreated), 2) autoclaved (autoclaved at 121°C for 10 min), 3) NaOH (treated with 3% NaOH), 4) HCl (treated with 3% HCl) and 5) formic acid (treated with 3% formic acid) groups. After treatment, they were ground to pass through 1.0mm mesh screen and then used for analyses of chemical composition and in vitro dry matter (DM) and CP digestibilities. Data were subjected to one-way ANOVA and differences among treatment means (P<0.05) were distinguished with Tukey’s test. There were no significant difference in chemical composition and in vitro DM and CP digestibilities between control and autoclaved groups, except ether extract level (P<0.05), suggesting that autoclaving affected the nutritional quality of SM little. NaOH group exhibited significantly decreased CP level and in vitro DM digestibility, increased crude ash (CA) level and unchanged in vitro CP digestibility, comparing with control group. These results suggest that NaOH treatment affected the nutritional quality of SM adversely. HCl and formic acids groups showed significantly increased CP level and in vitro digestibilities of DM and CP; and decreased CA level, showing that acid treatment can improve nutritional quality of SM: formic acid treatment may be more effective because of the greater values in CP level and digestibilities and decreased crude fibre level which was not observed in HCl group (P<0.05). The results obtained here suggest acid, especially formic acid, treatment is promising to improve the nutritional quality of SM but autoclaving and NaOH treatments.

Key words: autoclaved, chemical composition, chemical treatment, nutritional quality, shrimp meal

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Introduction

The nutritional quality of shrimp meal (SM) has been investigated in our previous study to use this for an alternative protein source for broilers, and clarified that its nutritional quality was not good enough to be included more than 10% in a diet (Rahman and Koh, 2016), although SM quality was somewhat different depending on the portion and species (Rahman and Koh, 2014). Similar results have been reported in earlier studies (Fanimo et al., 1996; Gernat, 2001; Oduguwa et al., 2004; Khempaka et al., 2006a) where performances were decreased when chicken received diets containing more SM.

Several studies have been conducted to improve the nutritional quality of crustacean meals by means of physical and chemical treatments. For instance, autoclaving was applied to squilla (a stomatopod species) meal for broilers and failed to improve the nutritional quality, but assumed autoclaving temperature in their study is lower than usual one (Reddy et al., 1997). Alkali treatment, such as NaOH treatment, modified chemical composition of SM for broilers, so that CP and crude ash (CA) levels increased and crude fibre (CF) level decreased (Septinova et al., 2010). Acid treatment, such as HCl and formic acid treatments, has been reported to improve the nutritional quality of SM, but these were conducted to develop a feed ingredient not for chicken but for rats (Oduguwa et al., 1998) or shrimps (Fox et al., 1994).

In the present study, we evaluated the chemical composition and in vitro digestibilities of SM after receiving autoclaving or chemical treatments, and determined the most suitable treatment to improve the nutritional quality of SM as a CP source of chicken diets.
Materials and Methods

Preparation of Treated SM

The sun-dried SM made of heads with hulls of black tiger shrimp (Penaeus monodon) was obtained from a processing industry in Bangladesh. They were divided into five treatment groups: 1) control (untreated), 2) autoclaved, 3) NaOH treated, 4) HCl treated and 5) formic acid treated SM. The autoclave treatment was conducted according to Reddy et al. (1997) with slight modifications. Briefly, SM was autoclaved at 121°C with 2.09 kg/cm² for 10 minutes and then sun-dried until achieving the moisture content of approximately 10%. The chemical (NaOH, HCl and formic acid) treatments were performed according to Septinova et al. (2010), Oduguwa et al. (1998) and Fox et al. (1994) with slight modifications. Briefly, about 100g of sun-dried SM waste was suspended in 300mL of 3% acids (HCl or formic acid) or alkali (NaOH) solutions at room temperature for 20 minutes. After that, they were filtered by using cheese cloth and washed with distilled water to adjust pH 7. After filtration, the solid portions were sun-dried and ground to pass through 1.0 mm mesh screen and then used as chemical treated SM. Each sample was divided into 4 aliquots for quadruplicate measurements.

Chemical Analysis

Proximate composition was analysed according to standard methods (AOAC, 1990). The chitin extraction process was done according to Ghanem et al. (2003), which was summarised as follows: about 1.0g of dried SM mixed with 12.5 mL of 2.5 N NaOH solution, and placed in an oven at 75°C for 6 hrs; filtrate crude chitin residue was dried at 105°C in an oven for 1 hour. About 1.0g of dried crude chitin was mixed with 10 mL of 1.7 N HCl and placed on a stir plate at room temperature for 6 hours. After filtering, the residue was washed with 95% ethanol (20 mL/g of crude chitin) followed by a final washing with distilled water and then dried. The dried content was weighed as chitin.

Digestibility Measurements

The in vitro dry matter (DM) and CP digestibilities of SM were determined according to Saunders et al. (1973) with slight modifications: briefly stated, about 250 mg of dried ground SM sample was suspended in 15 mL of 0.1 N HCl containing 1.5 mg pepsin (10,000 U/mg protein) (Nacalai Tesque Inc., Kyoto, Japan), and gently shaken at 37°C for 3 hours in multi shaker (EYELA MMS-3010). After neutralisation with 0.5 N NaOH, the digesta was mixed with 7.5 mL of phosphate buffer at pH 8.0 containing pancreatin (amylase activity 3,220 U/g, protease activity 38,500 U/g and lipase activity 1,600 U/g) (Nacalai Tesque Inc., Kyoto, Japan) and shaken at 37°C for 24 hours. The solution was then centrifuged at 240 × g for 10 min, washed with distilled water, filtered and dried.

The DM and CP digestibilities of SM were determined as follows:

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\text{DM digestibility (％) =} \frac{\text{Dried sample weight} - \text{Dried residue weight}}{\text{Dried sample weight}} \times 100
\]

\[
\text{CP digestibility (％) =} \frac{\text{Total N in sample} - \text{Total N in residue}}{\text{Total N in sample}} \times 100
\]

Statistical Analysis

The data were analysed by one-way ANOVA. Contrasts between treatment groups means were evaluated by Tukey’s test at a significance level of 5%.

Results

Chemical Composition of Treated SM (Table 1)

In control group, CP, CF, CA, ether extract (EE) and chitin accounted for 46%, 14%, 29%, 4% and 17% of dry weight of SM, respectively. These values did not change significantly by autoclave treatment, except the lower EE level (P < 0.05). When SM was treated by NaOH, comparing with the corresponding values in control group, CP and EE levels decreased whereas CF and CA levels increased significantly but chitin level was not affected. When SM was treated by acids, such as HCl and formic acid, CP and EE levels increased significantly and chitin levels were not affected. A prominent effect was found in CA level which decreased to 40% by HCl and to 50% by formic acid treatment. Interestingly, CF and chitin levels were increased by HCl treatment and decreased by formic acid treatment, comparing with the corresponding values in other treatment groups.

Table 1. Chemical composition of control (untreated) and treated shrimp meal³,2

| Treatments         | Crude protein, % | Crude fibre, % | Crude ash, % | Ether extract, % | Chitin, % |
|--------------------|------------------|----------------|--------------|-----------------|-----------|
| Control            | 45.5 ± 0.1ab     | 14.4 ± 0.4b    | 28.5 ± 0.4b  | 3.6 ± 0.2b      | 17.3 ± 0.2ab |
| Autoclaved SM      | 46.2 ± 0.3ab     | 14.8 ± 0.3ab   | 29.6 ± 0.4a  | 2.4 ± 0.1b      | 16.6 ± 0.2ab |
| NaOH treated SM    | 37.8 ± 0.2b      | 16.2 ± 0.5b    | 36.2 ± 0.2b  | 1.9 ± 0.2b      | 17.8 ± 0.2b  |
| HCl treated SM     | 54.3 ± 0.1c      | 18.3 ± 0.6c    | 16.9 ± 0.1c  | 4.2 ± 0.1c      | 19.2 ± 0.2c  |
| Formic acid treated SM | 58.1 ± 0.4d   | 12.7 ± 0.2d    | 13.6 ± 0.2d  | 5.4 ± 0.1d      | 15.3 ± 0.4d  |

¹ Values are expressed on air-dry matter basis.
² Values for each parameter represent mean ± SE values with 4 observations.
³,4 Means within the same column with different superscripts are significantly different (P < 0.05).
Digestibility of Treated SM (Table 2)

DM and CP digestibilities in control group were about 44% and 74%, respectively. Autoclave treatment did not improve these values and NaOH treatment rather deteriorated DM digestibility. In contrast, acid treatments improved DM and CP digestibilities and the magnitudes of improvements were greater in formic acid treatment.

Discussion

The obtained results revealed that autoclaving failed to improve the nutritional quality of SM. Similar observation was found in squilla meal reported by Reddy et al. (1997). Our autoclaving condition (at 121°C for 10 min) seemed to be more severe than their condition (at 1.09 kg/cm² for 5 min), because according to Antoine equation (Thomson, 1946) of temperature in an autoclave increased until 102.05 °C at a pressure of 1.09 kg/cm². This may suggest that this level of autoclaving is not effective to alter the chemical composition of crustacean meals. In this connection, autoclaving can improve pepsin digestibility of feather meal significantly, even though there was no significant impact on the chemical composition (Kim and Patterson, 2000). This makes us expect an improved in vitro digestibility of autoclaved SM in the present study, but the fact was different: not only DM but also CP digestibilities did not show improved values. Consequently, autoclaving may not be suitable to improve the nutritional quality of SM.

NaOH treated SM had lower CP level and higher CA level than other treatment groups (P<0.05), which was contrary to the results of Septinova et al. (2010) who found increased CP, CA levels and decreased CF level in NaOH treated SM. Thus, it is quite difficult to discuss this inconsistency, because there was no difference in treatment condition between them. Our results exhibited decreased DM and CP digestibilities and it may assume that CP retention should be low in chickens, although Septinova et al. (2010) found increased protein retention in broilers given diet containing NaOH treated SM. In this connection, it has been reported that NaOH treated feather meal showed decreased protein and increased ash levels, although in vitro protein digestibility increased (Papadopoulos et al., 1985) and no significant difference in CP level (Steiner et al., 1983). Therefore, further investigation is necessary to confirm the effect of NaOH treatment on nutritional quality of SM.

Beneficial effect was obtained in two acid treatment experiments: significantly higher CP and lower CA levels were observed in both acid treated SM, comparing with the corresponding values of other treatment groups. This may be results of leaching the minerals, such as calcium, in exoskeleton (No et al., 1989; Fox et al., 1994; Odugwua et al., 1998), and accordingly, relative content of CP increased. In this context, increased CF and chitin levels in HCl treated SM can also be explained. However, it is very interesting that formic acid treatment decreased CF and chitin levels, suggesting that chitin, main source of CF and possible factor to decrease digestibility (Austin et al., 1981; Fanimo et al., 2006; Khempaka et al., 2006b), was leached from SM by formic acid treatment. In this regard, Win and Stevens (2001) reported that formic acid treatment results in a weakening of the crystal structure and able to dissolve the shrimp chitin.

Treating the SM with acid solution resulted in increased DM and CP digestibilities relative to other treatments (P<0.05). Similarly, it has been reported that apparent protein digestibility increased in rats (Odugwua et al., 1998) and protein retention increased in broilers (Septinova et al., 2010) when HCl treated SM was included in diet. Moreover, significantly greater digestibilities observed in formic acid treated SM, may be because of the higher CP level and lower CF, CA and chitin levels in formic acid treated SM. The positive response of formic acid treated SM was also observed by Fox et al. (1994) who reported an improvement in growth and survival rate in marine shrimps (Penaeus monodon) fed diet containing formic acid treated SM.

In conclusion, the obtained results revealed that formic acid treatment showed improved nutritional quality of SM over autoclaving, NaOH and HCl treatments, and suggest that formic acid treated SM can be used as a potential source of protein in chicken diets.

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Table 2. In vitro DM and CP digestibilities of control (untreated) and treated shrimp meal1,2

| Treatments          | DM digestibility, % | CP digestibility, % |
|---------------------|---------------------|---------------------|
| Control             | 44.3 ±0.3a          | 73.9 ±0.4*          |
| Autoclaved SM       | 43.9 ±0.3*          | 73.8 ±0.3*          |
| NaOH treated SM     | 39.6 ±0.4b          | 72.8 ±0.3*          |
| HCl treated SM      | 48.1 ±0.5c          | 76.2 ±0.5b          |
| Formic acid treated SM | 51.6 ±0.3d        | 79.1 ±0.3          |

1 Values are expressed on air-dry matter basis.
2 Values for each parameter represent mean ± SE values with 4 observations.
3-4 Means within the same column with different superscripts are significantly different (P<0.05).
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