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Original Article

Preparation of shrimp waste as aqua feedstuff: a study of physicochemical properties and *in vitro* digestibility

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Abstract

The effects of different preparation methods (boiling, microwave irradiation, oven-drying, soaking, and sun-drying) for improving the protein quality of shrimp waste (SW) when used as aqua feedstuff were observed. The findings from this study indicate that microwave irradiation maintained both the chemical composition and total carotenoid concentration of the SW. This method controlled the appropriate physicochemical characteristics thus promoting proteolytic digestion, as indicated by measurement of changes in pH, water solubility, microstructures, and thermal transition. The protein digestibility based on the use of digestive enzymes from Nile tilapia (*Oreochromis niloticus*) also increased after preparation with microwave irradiation. Based on this study, improvement in the protein quality in SW may be achieved by microwave irradiation and this method may be used as an alternative method for preparing aqua feedstuff.

Keywords: chemical composition, digestibility, feedstuff, Nile tilapia, microwave irradiation

1. Introduction

A global increase in shrimp farming and processing has led to the mass production of shrimp waste (SW). SW comprises of discarded heads, appendages and exoskeletons of prawns, which is partially converted to shrimp meal for use as animal feedstuff. Generally, sun-drying or cooking followed by coarse milling are the most common methods of preparing the shrimp meal (Fagbenro and Bello-Olusoji, 1997). Fermentation by lactic acid bacteria (Evers and Carroll, 1996; Fagbenro and Bello-Olusoji, 1997), autolysis by gradual temperature (Cao *et al*., 2009) and enzymatic pretreatment (Holanda and Netto, 2006) have also been used. However, these methods are frequently carried out either under long term heating or unhygienic conditions which can often lead to both nutritional loss and contamination by unfavorable microorganisms.

SW is a rich source of protein, chitin, carotenoids, enzymes and other nutritive components (Yanar *et al*., 2004; Holanda and Netto, 2006; Samar *et al*., 2013). These constituents vary with the species of shrimp and whether the waste comprises the head alone, the shell or the total waste. Therefore, the use of SW as food or feed has attracted much interest from researchers in recent years.

Recent studies have indicated that the method of preparation can have the effect of significantly improving the nutritional quality of the protein in many feedstuffs (Khatoon and Prakash, 2006; Sadeghi and Shawrang, 2007; Ebrahimi *et al*., 2009). These are due to the appropriate physicochemical properties of native raw materials, such as water solubility and protein subunit degradation (Sadeghi and Shawrang, 2007) that can be changed by pretreatment.

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The properties affected include the microstructure (Kristensen *et al*., 2008; Thongprajukaew, 2011) and the thermal characteristics (Tang *et al*., 2003) and these properties are important aspects of enhancing the hydrolytic properties of protein.

The goal of this study was therefore to improve the nutritional quality of SW when used as aqua feedstuff, based on the use of different methods of preparation. Three measures of improvement were employed in this study, the chemical composition of the SW, changes in its physicochemical properties, and *in vitro* protein digestibility based on the use of digestive enzymes from economic fish. The findings from this study might be applied in aquaculture production.

2. Materials and Methods

2.1 Preparation of shrimp waste

Five kilograms of fresh SW (heads and shells) was collected within 3 hrs of processing, from local markets in Hat Yai, Songkhla, Thailand. They were packed in five polyethylene bags (one kilogram per pack), stored in ice and then transported to the Department of Applied Science, Faculty of Science, Prince of Songkla University. The SW was minced roughly to reduce the variation of samples, mixed with distilled water (1: 2.5 *w/v*) and then prepared variously by 1) boiling at 100°C for 5 min, 2) cooking in a 600 ml beaker for 5 min using a 800W microwave oven (MW 71B, Samsung, Malaysia), and 3) soaking in water at 30°C for 12 hrs under 200 rpm agitation. In addition, the minced fresh SW was prepared by 4) drying at 60°C for 12 hrs using a hot air oven, and 5) sun-drying for 12 hrs at a temperature range of 33-36°C. After different treatments; the prepared SW was freeze-dried for 48 hrs (Delta 2-24 LSC, Germany) to control moisture content. The prepared samples were then ground, sieved, packed in polyethylene bags and kept in desiccators for later experiments.

2.2 Chemical composition and total carotenoid concentration

The chemical composition of the SW including the protein, lipid and ash content were analyzed according to standard methods of the AOAC (1980). The total concentration of carotenoid in the SW was determined according to the method described by Thongprajukaew *et al*. (2014). Briefly, a 3 mg dried sample was extracted with 1 ml acetone for 3 days in the dark at 4°C. After centrifuging at 5,000*g* for 10 min, the total carotenoid in the supernatant was determined by measuring the absorbance at 474 nm, and calculated using extinction coefficient of $E_{(1\%,\ 1\ cm)}$ = 1900 (Foss *et al*., 1984). All chemical compositions were reported on a dry matter basis.

2.3 Physicochemical properties

2.3.1 Determination of pH

One gram of prepared SW was suspended in 25 ml of water at 25°C and agitated for 10 min (Sokhey and Chinnaswamy, 1993). The measurement of pH was conducted using a pH meter (Cyber Scan 510, Eutech Instrument, Singapore).

2.3.2 Water solubility

The water solubility was determined according to the method of Chung *et al*. (2010). Briefly, 1 g of sample was mixed with 10 ml of water, gently stirred for 1 hr at room temperature and centrifuged at 1,500*g* for 10 min. The water solubility was calculated as the ratio of the dissolved solid weight in supernatant to the dried solid weight in the original sample.

2.3.3 Microstructure

Microscopic pictures of the SW were studied using a scanning electron microscope (Quanta 400, FEI, Czech Republic). The samples were mounted by double-sticky tape on an aluminum stub and coated with gold. The photographs were taken at 200, 2,000 and 10,000× magnifications under 20 kV energy potential.

2.3.4 Thermal properties

The thermal properties of the SW were measured using a differential scanning calorimeter (DSC7, Perkin Elmer, U.S.A.). Approximately 3 mg of sample was placed in an aluminum pan, sealed, allowed to equilibrate at room temperature for 1 hr, and heated from 40 to 200°C at a rate of 5°C/min. The thermal parameters including onset (T_o), peak (T_p), and conclusion (T_c) temperatures, and transition enthalpy (ΔH), were recorded automatically.

2.4 Determination of *in vitro* protein digestibility

2.4.1 Fish samples and digestive enzyme extraction

Adult Nile tilapia (*n* = 3, 800-900 g body weight, 33-36 cm total length) were randomly collected from a private farm in Hat Yai, Songkhla, Thailand. The fish were killed by chilling in ice and the intestines were carefully collected and kept in ice. The samples were homogenized in 50 mM Tris-HCl buffer (pH 8.2) containing 200 mM NaCl (1:3 *w/v*) using a micro-homogenizer (THP-220; Omni International, Kennesaw GA, U.S.A.). Centrifugation of the homogenate was carried out at 15,000*g* for 30 min at 4°C. The supernatant was collected and then kept at -20°C until used.

2.4.2 *In vitro* digestibility

The crude enzyme extracts were dialyzed overnight using an extraction buffer. An *in vitro* reaction was performed according to the method described in Thongprajukaew *et al*. (2011). The reaction mixtures contained 5 mg of SW, 10 ml of 50 mM phosphate buffer pH 8, 50 µl of 0.5% chloramphenicol and 125 µl of dialyzed digestive enzymes. The protein digestibility was determined by the quantitative analysis of liberated *DL*-alanine after incubation for 24 hrs. The digestibility values were calculated, standardized with equal trypsin activity, and was expressed as µmol *DL*-alanine equivalent/g.

2.5 Statistical analysis

Data were reported as mean ± SE from triplicate observations. The significant differences between means were analyzed by Duncan's multiple range test at 95% confidence levels. All statistical analyses were performed using SPSS Version 14 (SPSS Inc., Chicago, U.S.A.).

3. Results and Discussion

3.1 Chemical composition

The preparation methods had significant effects on the chemical composition of the SW. Highest protein contents were found in the SW prepared by microwave irradiation and also in that dried in sunlight, with the next highest content being found in the oven-dried and boiled SW, respectively. A dramatic decrease was however noted in the SW prepared by water soaking (Table 1). Previous studies have reported unchanged protein quantity between native and microwave-irradiated raw materials whereas the quality has tended to increase (Khatoon and Prakash, 2006; Ebrahimi *et al*., 2009; Thongprajukaew *et al*., 2011). Fagbenro and Bello-Olusoji (1997) reported similar protein contents between minced shrimp head (50.6% of dry matter) and solar-dried shrimp head (51.3% of dry matter). These findings suggest that both microwave irradiation and sun-drying are able to maintain the protein quantity during preparation. This is due to the both methods probably changes the molecular properties of protein by covalent cross-linkages formed or converted to higher molecular weight aggregates, resulting in a stabilization of protein structure. This presumption is well correlated with the data observed in microwave-irradiated cottonseed meal (Sadeghi and Shawrang, 2007) as well as the changes in thermal transition properties of SWs. However, the protein content found in this study was higher than those previously reported, 41.9% (Evers and Carroll, 1996), 39.4% (Fanimo *et al*., 2000; Holanda and Netto, 2006) and 32.8% (Samar *et al*., 2013) on dry matter basis. On the other hand, in a study using only shrimp head a protein content of 61.6% of dry matter has been found (Cao *et al*., 2009). This difference might be due to the variation of the raw materials used, which is the shrimp species and whether the waste comprises the head alone, the shell including appendages and exoskeletons, or the total waste recovered from peeling.

The lipid contents varied significantly among the preparation method ($p<0.05$). Boiling maintained the highest lipid value, followed by microwave irradiation, soaking, sun-drying, and oven-drying, respectively. Holanda and Netto (2006) and Cao *et al*. (2009) reported dramatic and significant reductions of lipid content after preparing shrimp head and SW by enzymatic hydrolysis (52.8% on average) and autolysis (99.1%). This indicates that the preparation methods used in this study maintained a higher lipid content than biological pretreatments. However, the lipid content was observed to vary with time and the temperature during cooking (Stewart *et al*., 2003; Malheiro *et al*., 2009). This is because heat during preparation step can cause hydrolysis of free fatty acids, inducing the formation of hydroperoxides and secondary oxidation products, which decrease the levels of unsaturated fatty acids. Clearly, optimization of the conditions under which the SW is prepared results in the maintenance of the lipid contents.

High ash content is a major problem in feedstuff from animal sources. Previous reports indicated that the ash content found in SW was approximately 25–32% on a dry matter basis (Fagbenro and Bello-Olusoji, 1997; Holanda and Netto, 2006; Samar *et al*., 2013). In this study, the ash content was higher in the SWs prepared by both soaking and boiling than in the SWs prepared using other methods. The lower ash content compared with native SW found in previous

Table 1. Chemical composition (% on dry matter) of SW prepared by different methods.

Chemical composition	Preparation method				
	Boiling	Microwave irradiation	Oven-drying	Soaking	Sun-drying
Protein	47.48 ± 0.11^{c}	53.76 ± 0.32^{a}	50.47 ± 0.08^{b}	41.63 ± 0.01^{d}	53.72 ± 0.03^{a}
Lipid	8.91 ± 0.03^{a}	8.18 ± 0.02^{b}	5.91 ± 0.04^{e}	8.02 ± 0.06^{c}	6.81 ± 0.00^{d}
Ash	21.18 ± 0.36^{ab}	19.46 ± 0.27^{b}	19.28 ± 0.24^{b}	23.80 ± 2.84^{a}	17.66 ± 0.17^{b}

Data (Mean ± SE) were calculated from triplicate determination ($n = 3$). Significant values in each row indicate by different superscripts ($p<0.05$).

studies might have been due to a reduction of minerals during preparation. This hypothesis is in agreement with other studies which used boiling, autoclaving, and microwave irradiation (Alajaji and El-Adawy, 2006) and water soaking for 72 hrs (Akingbade *et al*., 2009). Therefore, judged on the chemical compositions noted in this study, microwave irradiation is able to both maintain and also have less effect on the main nutritional constituents of SW when compared with the other methods.

3.2 Total carotenoid concentration

One of the main carotenoids in shrimp is astaxanthin (Yanar *et al*., 2004) which provides the red-orange pigmentation in fish tissue and it is desirable that the preparation method should maintain the carotenoid content. In this study, the highest carotenoid concentrations were found in SW prepared by water soaking followed by similar levels for sun-drying, oven-drying, and microwave irradiation whereas boiling gave the lowest carotenoid concentration (Figure 1). This result might be due to the preparation methods having different abilities to destroy the carotenoid structure. However, the carotenoid concentrations found in this study were somewhat higher than those reported in previous studies, i.e. 14.1 µg/g and 16.9 µg/g in *Panaeus semisulcatus* and *Metapenaeus monoceros*, respectively (Yanar *et al*., 2004). This is because there are a number of factors which can vary and affect the deposition of carotenoid in shrimp, the method used in the analysis as well as the target organ for extraction. Based on both chemical composition and total carotenoid concentration, the results of this study indicate that microwave irradiation is able to maintain the nutritional composition of SW.

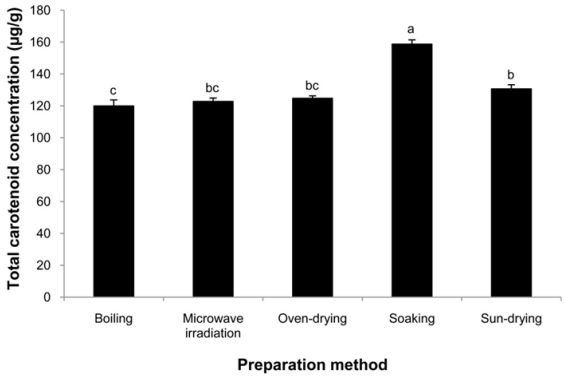

Figure 1. Total carotenoid concentration (µg/g) of SWs prepared by different methods. Data with different superscripts are significantly different ($p<0.05$).

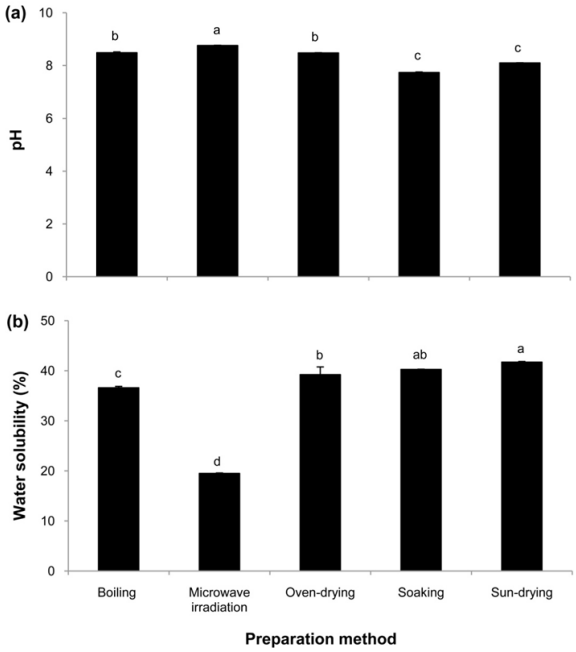

Figure 2. pH (a) and water solubility (b) of SWs prepared by different methods. Data with different superscripts are significantly different ($p<0.05$).

3.3 Physicochemical properties

3.3.1 pH value

Differences in pH were observed among preparation methods. Changes in pH can play an important role in determining the liberation of functional groups after preparation. SW prepared by microwave irradiation exhibited the highest pH (Figure 2a). This finding might be due to the removal of the NH_2-group during chitin breakdown causing an alkaline condition after irradiation. This is consistent with Roy *et al*. (2003), who reported that treatment of chitin by microwave irradiation causes more efficient enzymatic hydrolysis of macromolecules. In addition, this phenomenon might have occurred as a result of an increase in the NH_2-group after proteolytic digestion under microwave irradiation similar to that reported by Izquierdo *et al*. (2005) in milk protein, β-lactoglobulin AB. Boiling and oven-drying produced moderate and similar pH with the lowest value being found in the soaked and sun-dried SW. This reduction could be due to the heat treatment methods that induced the formation of stable free radicals (Morehouse and Desrosiers, 1993) and by this inducing the formation of carboxyl groups. For soaking, this method could also decrease the pH by the activity of autolytic enzymes. Similar changes in pH have also been found in many food and feed ingredients after pretreatment (Chung and Liu, 2009, 2010; Chumwaengwapee *et al*., 2013).

3.3.2 Water solubility

Higher water solubility was noted in the SW prepared by oven-drying, soaking and sun-drying than in that prepared by boiling (Figure 2b) with the value decreasing dramatically in the SW prepared by microwave irradiation. This finding is in agreement with the findings reported by

Sadeghi and Shawrang (2007) of microwave irradiation decreasing protein solubility in cottonseed meal with the *in vitro* protein digestibility increasing. This might be due to the cross-linking and aggregation (denaturation) of protein chains altering the surface hydrophobic (protein-protein) and hydrophilic (protein-solvent) interactions after heating. This transformation similarly tends to reduce the water binding capacity (Taha and Mohamed, 2004) as well as exposing hydrophobic amino acids (especially aromatics) on the surface, which is the active site for proteolytic digestion by pepsin and trypsin (Murray *et al*., 2003).

3.3.3 Microstructures

The preparation method had a direct effect on the microscopic structures of the SW. The SWs prepared by microwave irradiation (Figures 3d–f), oven-drying (Figures 3g–i), and sun-drying (Figures 3m–o) were mainly found to have a rough surface with deep concave indentations, whereas only shallow concave indentations were observed in the SW treated by soaking (Figures 3j–l). Such increases in surface roughness have been reported to play an important role in improving the digestive efficiency of feed ingredients (Kristensen *et al*., 2008; Thongprajukaew, 2011; Thongprajukaew *et al*., 2013). Oria *et al*. (2000) reported that the increased surface area of endosperm protein bodies in mutant cultivar can cause a 2.5 times increase in protein digestibility based on pepsin digestion when compared with native sorghum. Surface indentation provides a large surface-to-volume ratio, promoting highly efficient proteolysis, which favors high enzyme loading (Ji *et al*., 2008). Therefore, methods of preparing SW which cause an increase in surface roughness may contribute to enzymatic hydrolysis along the alimentary tracts of animals. It was observed that the SW prepared by boiling (Figures 3a–c) had a smoother surface structure than that found in the samples prepared by other methods.

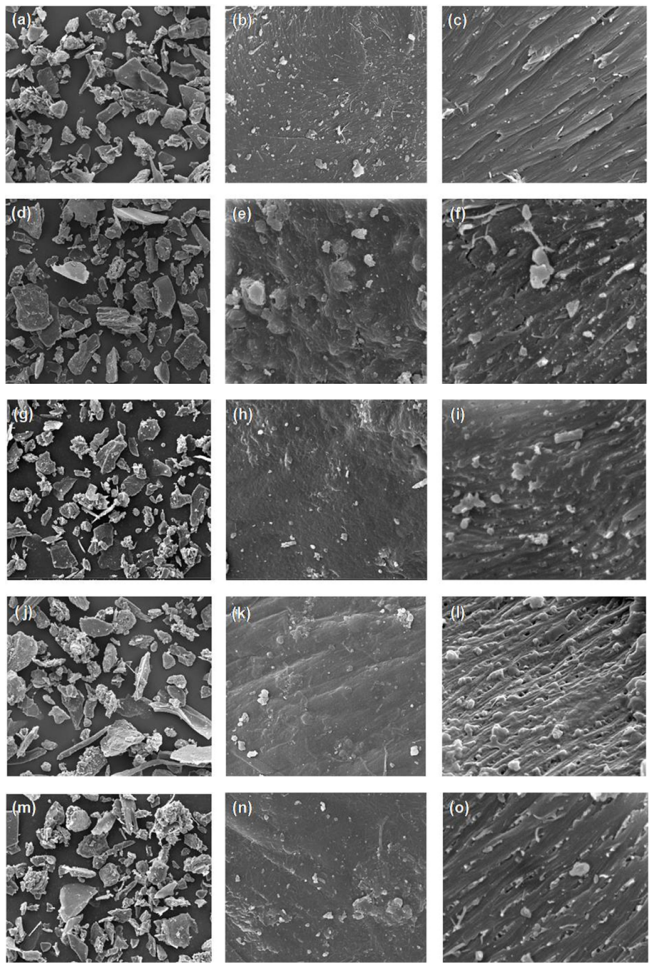

Figure 3. Microstructures of SWs prepared by boiling (a–c), microwave irradiation (d–f), oven-drying (g–i), soaking (j–l) and sun-drying (m–o). Photographs were taken at magnifications of 200× (left panel), 2,000× (middle panel) and 10,000× (right panel).

3.3.4 Thermal properties

The thermal transition properties (T_o, T_p, and T_c), melting temperature range (T_c–T_o), and transition enthalpy (ΔH) were different among the SWs prepared by different methods (Table 2). The presence of endothermic peak is usually related to the disruption of hydrogen bonds which maintain the integrity of the tertiary structure of the protein (Wang *et al*., 2008). SW prepared by different methods appeared to induce varying conformational changes in proteins. Of all the methods tested, microwave irradiation of SW provided the highest T_c which resulting in the widest T_c–T_o, when compared with the other methods. Bao and Corke (2002) suggested that the T_c–T_o value might be increased directly by the heterogeneity of crystalline polymers. Therefore, the width of the temperature range observed in the microwave-irradiated SW is probably due to the heterogeneity of the cleaved length of the polypeptide chains after preparation as well as the protein subunit segregation of the raw materials pretreated by microwave irradiation (Sadeghi and Shawrang, 2007; Ebrahimi *et al*., 2009). This is supported by the finding of Cao *et al*. (2009) who reported a large number of smaller polypeptides after preparing SW by autolysis.

The ΔH indicates the amount of energy required to denature the protein structure. This value was higher in the SW pretreated by boiling, followed by soaking, microwave irradiation, sun-drying, and oven-drying, respectively (Table 2). This finding suggests that a higher amount of undenatured protein was retained in the boiled and soaked SWs than in the microwave-irradiated SW. Therefore, based on equal heating time, microwave irradiation provides better ΔH characteristics when compared with boiling. In addition, Arntfield and Murray (1981) suggested that ΔH was a reflection of the extent of ordered protein structure. This finding suggests the boiled SW had a more ordered structure than

Table 2. Thermal transition property of SW prepared by different methods.

Thermal property	Preparation method				
	Boiling	Microwave irradiation	Oven-drying	Soaking	Sun-drying
T_o (ºC)	47.64 ± 0.02^a	45.12 ± 0.01^d	46.69 ± 0.02^b	46.65 ± 0.04^b	45.97 ± 0.03^c
T_p (ºC)	77.42 ± 0.03^a	70.50 ± 0.02^b	62.50 ± 0.03^e	68.75 ± 0.02^c	63.08 ± 0.04^d
T_c (ºC)	103.03 ± 0.01^b	124.79 ± 0.03^a	78.40 ± 0.01^e	88.97 ± 0.03^c	79.61 ± 0.03^d
T_c–T_o (ºC)	55.40 ± 0.02^b	79.65 ± 0.02^a	31.70 ± 0.02^e	42.34 ± 0.03^c	33.63 ± 0.01^d
ΔH (J/g)	86.96 ± 0.02^a	58.84 ± 0.02^c	23.16 ± 0.01^e	75.82 ± 0.01^b	26.80 ± 0.03^d

T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperature, T_c–T_o = melting temperature range, ΔH = enthalpy. Significant values in each row indicate by different superscripts (p<0.05).

that of the microwave irradiated SW indicating that a more easily denaturable protein content with lower thermal stability results from microwave irradiation.

3.4 *In vitro* protein digestibility

The different methods of preparation affected the *in vitro* protein digestion, based on the effect of digestive enzymes from the intestinal section of Nile tilapia (Figure 4). The liberated product was higher in microwave-irradiated SW than in oven-dried, soaked, and sun-dried SWs (p>0.05) with the lowest protein digestion being found in the boiled SW. This observation accords with the physicochemical alterations governing enzymatic hydrolysis described above. Izquierdo *et al*. (2005) reported enhanced enzymatic hydrolysis of protein resulting from microwave irradiation based on amino acid analysis (Marconi *et al*., 1995). Similar increases in protein digestibility in feedstuffs treated by microwave irradiation have been reported previously (Alajaji and El-Adawy, 2006; Khatoon and Prakash, 2006). This has been attributed to alterations of amino acid recemizations (Stenberg *et al*., 2001) and protein subunits (Sadeghi and Shawrang, 2007; Ebrahimi *et al*., 2009) in the feedstuffs. Therefore, it is apparent that microwave irradiation of SW is a viable alternative method for improving the *in vitro* protein digestion. However, *in vivo* trials on growth and efficiency of protein utilization when fed with the microwave-irradiated SW should be performed in aquatic animals.

4. Conclusions

Microwave irradiation of SW maintained its chemical composition, provided nearly all the required characteristics for promoting proteolytic hydrolysis as well as increasing the *in vitro* protein digestibility. Therefore, industrial preparation of SW by microwave irradiation may be used as an alternative method for producing better quality aquatic feedstuff.

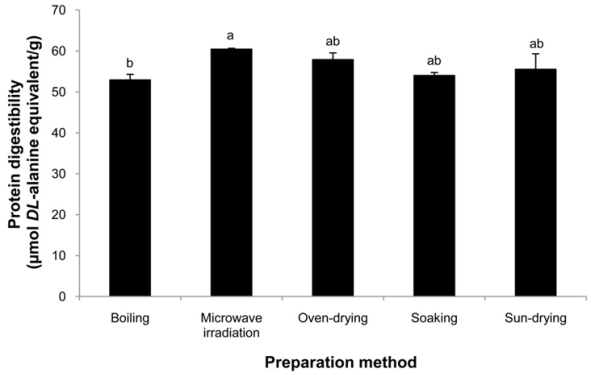

Figure 4. *In vitro* protein digestibility (µmol *DL*-alanine equivalent/g) of SWs prepared by different methods using digestive enzyme extracts from Nile tilapia (10U trypsin activity). Data with different superscripts are significantly different (p<0.05).

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