International Food Research Journal 24(6): 2419-2426 (December 2017)
Journal homepage: *http://www.ifrj.upm.edu.my*

Development of calcium supplement from fish bone wastes of yellowfin tuna *(Thunnus albacares)* and characterization of nutritional quality

Mahnaz Nemati, Huda, N. and *Ariffin, F.

Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 Penang, Malaysia

Article history

Received: 3 October 2016
Received in revised form: 3 November 2016
Accepted: 4 November 2016

Keywords

Tuna frame
Tuna bone powder
Chemical composition

Abstract

Yellowfin tuna *(Thunnus albacares)* wastes were used to recover of tuna frame (TF) and to extract of tuna bone powder (TBP) by alkaline treatment. The amount of the calcium with 24.56% and 38.16% was the most abundant element in TF and TBP, respectively. Nine essential amino acids for human body; lysine, valine, leucine, isoleucine, methionine, threonine, histidine, phenylalanine and tryptophan are detected in TF and TBP. The amount of the collagen associated amino acids such as glycine, proline and hydroxyproline were high and the amount of glutamic acid, arginine, alanine, aspartic acid and serine were relatively more. The amount of the oleic acid, palmitic acid and gondoic acid were higher and the amount of hexadecatrienoic acid, γ-linolenic acid and dihomo-γ-linolenic acid were lower. The amount of the myristic acid, stearic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were also relatively more. Results demonstrated that TBP as inexpensive and environmentally friendly alternative source of calcium can be converted to the healthy value added products to increase of the amount of the calcium intake among people especially population groups with lactose intolerance.

Introduction

Osteoporosis is a major public disease in elderly people as a result of significantly reduction in bone density (Pongchaiyakul *et al.*, 2008; Chaimongkol, 2012; Soltan, 2013). The prevalence of osteoporosis in women is three times than in men, partially due to the changes in the feminine hormones that happen at the menopause. Statistics shows that 10% in women between 55-64 years old and 22.5% in women aged over 65 years old are involved with osteoporosis (Soltan, 2013). Conventional pharmacotherapies for osteoporosis in postmenopausal women have emphasized agents which reduce of the bone resorption including estrogen, calcitonin and bisphosphonates (Rudman *et al*., 1981; Bennet *et al*., 1984; Centrella and Canalis, 1985; Canalis *et al*., 1988; Arjmahdi *et al*., 1996). These expensive hormones with potential side effects are consumed for treatment of osteoporosis. Therefore, presentation of the natural compounds as supplement which is rich in calcium, are necessary to use as an alternative to medicine to improve bone and skeletal health (Yoon *et al*., 2005).

Osteoporosis is affected by diet, sufficient nutrition and particularly adequate intake of calcium which plays an undeniable role on prevention of osteoporosis (Love, 2003; Rizzoli *et al*., 2008). Adequate intake of calcium is affected on peak bone mass obtained in early adulthood which influences onto the skeletal system by prevention of bone loss and osteoporotic fractures later in life (Zhu and Prince, 2012). Consumption of foods rich in calcium throughout life is effective to prevent or postpone of osteoporosis in both pre- and post-menopausal women and elderly men (Spencer and Kramer, 1986; Albanese *et al*., 1986; Poneros and Erdman, 1988; Chaimongkol, 2012).

Fortification of main foods is generally accepted as an effective way for providing the daily requirements for a range of vitamins and minerals (Richardson, 1997; Fairweather and Teucher, 2002; Babarykin *et al*., 2004). Calcium-fortified products would be helpful in enhancement of the levels of calcium intake (Kim and Mendis, 2006), in particular for population groups with insufficient intakes of milk and dairy products due to the lactose intolerance (Jung *et al.*, 2006; Luu and Nguyen, 2009). Common food products successfully fortified with calcium in developing countries are including milk and milk products, wheat flour, corn flour, salt, sugar, fats and oils (Singh *et al.*, 2007). These products are fortified by using commercial calcium salts such as calcium carbonate, calcium citrate and tricalcium phosphate

*Corresponding author.
Email: *fazilah@usm.my*

(Sheik *et al*., 1987; Goldscher and Edelstein, 1996; Tateo *et al*., 1997; Singh *et al.*, 2007) whereas utilization of natural calcium sources such as fish bone can be more acceptable by consumers and be more effective in terms of existanec of calcium phosphate compound in which is similar to the human bone components (Phiraphinyo *et al.*, 2006; Chuamani, 2010; Hemung, 2013).

The amount of fish bone fraction, which is still regarded as a waste product, is approximately 10-15% of the whole body weight (Malde *et al*., 2010). In many countries, the large quantities of fish waste and fish by-product is discarded into the environment and converted to the important source of pollution (Stevanato *et al.*, 2008). In the recent years, dried fish bone was used as feed ingredient in diets for fish and other animals, with a positive effect on feed efficiency and growth compared to traditional diets (Toppe *et al.*, 2006). Fish meal obtained from whole fish or fish by products contains approximately 10% minerals, particularly high in calcium and phosphorus, and also represent as a significant source of minerals once included in feed (Toppe *et al*., 2007).

Anyway, minimal publications are addressed regarding to the bioavailability of calcium recovered from fish bone and its potential usability (Kim and Mendis, 2006). Limited studies are addressed about useful effects of fish bone utilization and no attempts are reported to test the consumption of fish bones on the human health (Larsen *et al.*, 2000; Luu and Nguyen, 2009). In this study, the first objective was to present a new calcium supplement extracted from yellowfin tuna frame by alkaline treatment as natural calcium source to human diet, and the second objective was to obtain more information about the chemical composition and nutritional quality of tuna frame and tuna bone powder.

Materials and Methods

Preparation of tuna frame (TF)

The yellowfin tuna (Thunnus albacores) wastes utilized in this study was kindly provided from tuna canning factories. The body weight and the length of yellowfin tuna was from 18-23.5 kg and 105-135 cm, respectively. The tuna frames along with residual meat obtained from factories had been frozen without any pre-cooking process in factories. They were stored at -18°C in laboratory before further processing.

For this study, around 3kg of tuna wastes were used. At first, the flesh remained on the backbone of the tuna should be removed by using knife after defrosting the frozen frame at 15°C overnight. The frames without flesh were drenched in boiling water for approximately 2-3min. Then, the residual flesh on the frame was absolutely cleared by rinsing it with cold water. Finally, the clean tuna frames were frozen before preparation of the tuna bone powder (Toppe *et al*., 2007).

Preparation of tuna bone powder (TBP)

Alkaline treatment method by Kettawan *et al.* (2002) with some modifications was used to extract of tuna bone powder as calcium source from TF (Kettawan *et al*., 2002). In preliminary study, the condition of the alkaline treatment in order to remove of the protein and fat and to obtain of the ash as major criterion from TF was optimized according to the method by Kettawan *et al*. (2002) and Kim *et al.* (2003) with some modifications. To optimize the appropriate condition for extraction of TBP, various concentrations of NaOH (1, 1.5, 2, 2.5 and 3%) and boiling times (10, 20, 30, 40 and 50min) was tested. The amount of the ash percentage in TBP was measured as criterion to select of the proper condition (Kettawan et al., 2002; Kim et al., 2003).

In this study, initially, 500 g of TF were boiled in 2% sodium hydroxide solution (NaOH) for 30 min at a ratio of 1 part TF to 3 parts NaOH solution (w/v). The soaked bones were filtered with a filter cloth and then, the filtered bones were washed with 1% hydrogen chloride (HCl) and deionized water to neutralize completely. The washed bones were then dried in a hot air oven for 2h at 100°C and were ground into the fine powder until passing a sieve of 100 mesh size.

Chemical analysis

Proximate composition analyses of TF and TBP were done in triplicate for moisture, lipid, protein and ash contents. Moisture content was conducted gravimetrically after oven drying at 105°C until reach to the constant weight. The crude protein was measured by the Kjeldahl procedure. Total ash content was measured by combusting the samples in a muffle furnace at 550°C. Crude fat was analyzed based on Soxhlet extraction method using petroleum ether as a solvent (AOAC, 2000). The results were expressed in dry-basis.

The samples were digested with nitric acid, hydrogen peroxide and hydrogen chloride until achieving the clear solution by using digester microwave (Multiwave 3000-Anton Parr). The digested samples were filtered and diluted with deionized water and made ready to measure by inductively coupled plasma optical emission spectrometry (ICP-OES). Phosphorus was measured by UV-VIS spectrophotometry using the ammonium

phosphomolybdate method (Silva, 1981).

Samples (TF and TBP) for analyses of the total amino acids were hydrolyzed in 6 M hydrogen chloride for 24 h at 110°C and analyzed by HPLC using a fluorescence technique for detection (Cohen and Michaud, 1993).

Fatty acid methyl esters (FAME) were conducted by methylation of the total lipids, as demonstrated by Joseph and Ackman (1992). In brief, the oil sample - extracted freshly from TF and TBP by using Soxhlet machine by using petroleum ether as a solvent - was transfered into a clean glass bottle. Then, boron trifluoride was added and bottles were tightly closed and put in water-bath 90-100°C for 30min. On cooling, hexane and distilled water were added to the mixture in bottles. The mixtures were vortexed and centrifuged to separate of the two phases. Finally, the upper layer was taken and was carefully transferred into the GC vial. Fatty acid esters were separated in a gas chromatograph equipped with a fused silica capilla¬ry column and flame ionization detector. Individual fatty acids were recognized and quantified in comparison with peak areas and retention times of FAMEs standards (Joseph and Ackman, 1992).

Statistical analysis

Statistical evaluations of the chemical composition, mineral contents, amino acid profiles and fatty acid contents between TF and TBP were made by using 't test' in SPSS 18 (IBM, United States).

Results and discussion

Yellowfin tuna wastes from tuna canning factories were taken as raw materials and were processed to recover of TF and were then exposed to the alkaline treatment to extract of TBP as calcium source. TBP as calcium source was extracted from TF to make it available as a food fortificant by using alkaline treatment. The results of preliminary study presented in Figure 1 indicated that the amount of the ash in TBP increased and the amount of the protein decreased during boiling times. Moreover, high concentrations of NaOH have shown the positive and negative effects on the amount of the ash and protein in TBP, respectively. The results showed that the amount of the ash as major criterion in TBP extracted from TF increased after 20 min at above 1.5% NaOH concentration. Significant differences were not observed among 2, 2.5 and 3% NaOH and above 30, 40 and 50min, as well. Therefore, the amount of 2% NaOH at 30min is a suitable condition for extraction of TBP from TF (Figure 1).

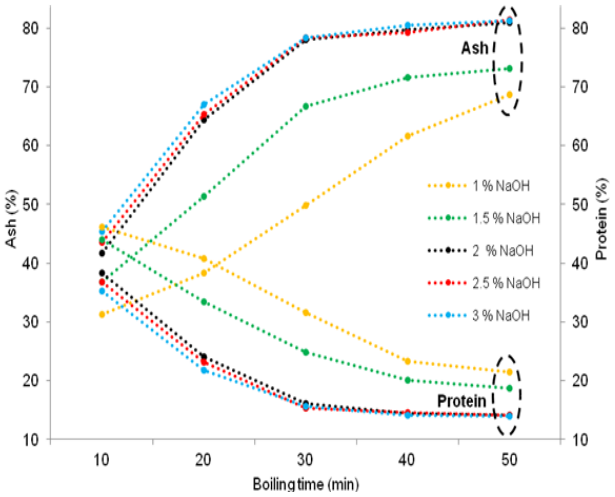

Figure 1. The percentage of ash and protein of TBP extracted by various NaOH concentrations and boiling times

The amount of TF recovered from 'tuna frame along with residual meat' was 53.75% and the amount of TBP obtained from TF was 59.79%. It means that, more than 50 percent of the waste products from tuna canning factories that recovered as TF is a valuable part and will be able to convert to TBP as new nutraceutical products to use as calcium supplement. The general appearance of TBP was in a form of fine particle size powder, white color and without any undesirable fishy odor, which makes it into the appropriate source of calcium for the purposes of development of calcium supplement or enrichment of food products. Extraction of TBP with proper characteristics indicated that alkaline treatment would be a suitable practical method to extract of calcium source from fish frames (Sittikulwitit *et al*., 2004).

The composition of the TF and TBP was unknown and was not cited in the literature. The results of the chemical composition of TF and TBP showed that the amount of the protein, fat and ash is significantly different between TF and TBP (Table 1). The amount of the protein and fat decreased in TBP, due to the effects of NaOH to remove of the fat and protein from TF (Kettawan *et al.*, 2002). Utilization of NaOH during TBP processing, dissolved and removed the organic materials from TF. That's why the amount of protein and fat in TBP decreased and the amount of ash increased.

In order to the fortification of food products by using TBP at a level of about 240 mg of calcium per serving size equal with the one cup of milk, 0.63g of TBP is needed and the amount of the protein and fat in fortified products will be 0.10% and 0.02%, respectively, which is negligible. So, the presence of protein and fat in TBP and fortified products is not a

Table 1. Proximate composition and mineral contents of TF* and TBP** (given as g/100g or mg/100g)

		TF	TBP	$CaCO_3$ (1) (Malde *et al.*, 2010)	$CaCO_3$ (2) (Malde *et al.*, 2010)
Moisture	g/100g	0.25 ± 0.02 ^{ns}	0.27 ± 0.03 ^{ns}	-	-
Protein	g/100g	33.40 ± 0.11 **	16.10 ± 0.09 **	0.56	0.53
Fat	g/100g	11.02 ± 0.09 **	3.86 ± 0.08 **	0	< 0.2
Ash	g/100g	53.43 ± 0.08 **	77.97 ± 0.17 **	78.9	98.6
K	mg/100g	0.47 ± 0.04**	0.68 ± 0.04**	80	30
Si	mg/100g	2.25 ± 0.07**	3.37 ± 0.08**	-	-
Al	mg/100g	2.40 ± 0.08**	3.50 ± 0.07**	-	-
Ba	mg/100g	0.19 ± 0.05**	0.29 ± 0.03**	-	-
Na	g/100g	0.48 ± 0.06**	0.67 ± 0.05**	0.027	0.008
As	mg/100g	0.09 ± 0.03**	0.19 ± 0.04**	0.11	0.028
Ca	g/100g	24.56 ± 0.12**	38.16 ± 0.14**	32.4	37.3
Cr	mg/100g	0.19 ± 0.04**	0.28 ± 0.04**	-	-
Cu	mg/100g	0.05 ± 0.01^{ns}	0.08 ± 0.02 ^{ns}	0.06	0.04
Fe	mg/100g	4.25 ± 0.07**	6.20 ± 0.17**	15	243.7
Mg	g/100g	0.29 ± 0.03**	0.47 ± 0.07**	0.18	0.61
Zn	mg/100g	0.04 ± 0.01**	0.08 ± 0.02**	0.19	0.26
P	g/100g	14.58 ± 0.07**	23.31 ± 0.09**	0.08	< 0.004
Cd	mg/100g	NF	NF	0.008	0.003
Pb	mg/100g	NF	NF	0.009	0.158

*TF: tuna frame

**TBP: tuna bone powder

Values are presented as the mean ± SD (n=3).

NF: not found

Means with (ns) superscript are not significantly different ($p>0.05$). Means with (**) superscript are significantly different ($p<0.05$). [T-test]

Table 2. The amount of ash and calcium in previous studies in fish frame and fish bone powder (given as g/100g)

	Fish frame	Fish bone powder	Ash	Calcium
Present study	Tuna		55.43	24.56
Toppe et al., 2007	Cod		57.70	19.0
Toppe et al., 2007	Saithe		57.60	19.9
Toppe et al., 2007	Blue whiting		50.30	17.0
Toppe et al., 2007	Salmon		42.40	13.5
Toppe et al., 2007	Trout		44.10	14.7
Toppe et al., 2007	Herring		47.50	16.1
Toppe et al., 2007	Mackerel		43.80	14.3
Petenuci et al., 2010	Tilapia		21.33	-
Stevanato et al., 2008	Tilapia		20.62	-
Jung et al., 2005	Hoki		69.50	-
Kim et al., 2002	Hoki		39.78	-
Present study		Tuna	77.97	38.16
Hemung, 2013		Tilapia	75.83	-
Logesh et al., 2012		Oil sardine	91	32.73
Logesh et al., 2012		Ribbon fish	95	27.81
Luu & Nguyen, 2009		Catfish	61.8	21.00
Luu & Nguyen, 2009		Snapper	71.2	24.40
Luu & Nguyen, 2009		Salmon	65.8	22.30
Kim et al., 2002		Hoki	77.03	-
Changhu et al., 1995		pollack	-	38.27

big deal.

The major part of the proximate composition in TF and TBP was ash content, which was 53.43% and 77.97%, respectively. The amount of the ash in TBP was considerable in comparison to the results reported from other fish bone in the literature (Table 2). These results suggested that the recovery of TBP by alkaline treatment was effective way to get rid of organic materials such as fat and protein and to get the high purity of TBP (Hemung, 2013).

The results shown in Table 1 represented that calcium (Ca) with 24.56% and 38.16% was the most abundant element in TF and TBP, respectively. The second significant element in TF and TBP was phosphorus (P) with 14.58% and 23.31%, respectively. The measurement of the ratio of calcium to phosphorus (Ca:P) is important in TBP as calcium source because the ratio of Ca:P should be near to 2:1 which is similar to the human bones. In this study, the amount of the ratio was 1.64, which was near to the proper ratio for human bones, and was in agreement with the results reported for oil sardine and ribbon fish (Logesh *et al.*, 2012).

In fact, alkaline treatment method provided rather high calcium concentration (38.16 g/100g) from TF because approximately all of the protein and fat in TF was digested. Utilization of NaOH was to make sure that TBP was treated appropriately to get rid of

Table 3. Amino acid profiles of TF* and TBP** (given as g/100g or % in protein)

	TF	TBP
Hydroxyprolin	7.90 ± 0.06**	8.28 ± 0.09**
Aspartic acid	5.13 ± 0.11**	4.83 ± 0.15**
Serine	4.84 ± 0.06ns	4.77 ± 0.08ns
Glutamic a.	9.88 ± 0.15**	10.12 ± 0.07**
Glycine	18.2 ± 0.05ns	18.35 ± 0.15ns
Histidine	2.28 ± 0.08**	1.56 ± 0.08**
Arginine	7.92 ± 0.05**	8.32 ± 0.08**
Threonine	3.74 ± 0.09ns	3.76 ± 0.08ns
Alanine	7.90 ± 0.15**	8.69 ± 0.07**
Proline	8.88 ± 0.14**	9.72 ± 0.18**
Ethylglycine	3.34 ± 0.03**	3.76 ± 0.06**
Cystine/cicteine	0.13 ± 0.01ns	0.12 ± 0.01ns
Tyrosine	2.83 ± 0.04**	1.87 ± 0.05**
Valine	2.97 ± 0.04**	2.75 ± 0.05**
Methionine	2.52 ± 0.06ns	2.21 ± 0.05ns
Lysine	3.04 ± 0.05**	2.87 ± 0.04**
Isoleucine	1.82 ± 0.04**	1.65 ± 0.09**
Leucine	3.73 ± 0.06**	3.54 ± 0.04**
Phenylalanine	2.58 ± 0.05ns	2.52 ± 0.07ns
Tryptophan	0.37 ± 0.04ns	0.31 ± 0.05ns
Total amino acid	100	100

*TF: tuna frame

**TBP: tuna bone powder

Values are presented as the mean ± SD (n=3).

Means with (ns) superscript are not significantly different ($p>0.05$). Means with (**) superscript are significantly different ($p<0.05$). [T-test]

all the organic materials before using as a calcium fortificant (Sittikulwitit *et al*., 2004). Moreover this method was simple and low-cost method, as well (Kettawan *et al*., 2002).

According to the Table 2, in previous studies, the amount of Ca in fish frame and fish bone powder was in the range of 13-19% and 21-38%, respectively, which is agree with the results of present study. Except Ca and P, the concentration of the essential or non-essential elements in TBP was low and quite similar to the composition of the calcium carbonate as calcium supplement (Malde *et al*., 2010).

Details from two calcium carbonate supplement (first from limestone, 500 mg tablets, Weifa A/S, Oslo, Norway and second from limestone, 500mg tablets, Nordkalk Ltd., Parainen, Finland) were used as calcium source for the control in this study (Table 1) (Malde *et al*., 2010). Except calcium, the concentration of the other essential and non-essential elements in the TBP was low and somewhat similar to the calcium carbonate supplement used as control calcium source. In fact, TBP will be suitable as a potential source of calcium in food industry such as calcium carbonate and this does not mean that TBP will be a pure source of calcium (Malde *et al*., 2010).

Some differences in amount of Ca in TF and TBP with different fish bones (mentioned in Table 2) is related to the fish species, age, feed nutrition, the amount of the marrow in the different bones, fat and tendons on the surface of the bones and cartilage joined to the bones (Phiraphinyo *et al*., 2006; Luu and Nguyen, 2009). The level of magnesium and alkali metals such as sodium and potassium in TF and TBP was in good correspondence with levels reported by Toppe *et al.* (2007). This result was similar to those reported by Phiraphinyo *et al.* (2006) for fish bone powder extracted from hoki and giant seaperch.

Twenty different amino acids were found in TF and TBP. Nine essential amino acids for human body (lysine, valine, leucine, isoleucine, methionine, threonine, histidine, phenylalanine and tryptophan) are detected in TF and TBP (Table 3). In terms of amino acid profiles, differences between TF and TBP were small, however, these little differences could be commented on, which had been made as a result of alkaline treatment in TBP processing. As mentioned above, the amount of the protein in TF from 33.40 g/100g decreased to 16.10 g/100g in TBP, therefore, changes in amino acids was inevitable. TF and TBP have shown that the amount of the collagen associated amino acids such as glycine, proline and hydroxyproline were high and also the amount of other amino acids including glutamic acid, arginine, alanine and finally aspartic acid and serine were relatively more. Toppe *et al.* (2007) found that the amount of the collagen associated amino acids in horse mackerel was higher rather than other amino acids which are in agreement with the results of present study (Toppe *et al*., 2007).

Results of fatty acid profiles in TF and TBP are presented in Table 4. A total of 22 fatty acids were identified in the lipids of TF and TBP. The results showed that the amount of oleic acid, palmitic acid and gondoic acid were higher and the amount of hexadecatrienoic acid, γ-linolenic acid and dihomo-γ-linolenic acid were lower. As shown in Table

Table 4. Fatty acid profiles of TF* and TBP** (given as g/100g or % in extracted oil)

		TF	TBP
Myristic acid	14:0	7.43 ± 0.06**	9.71 ± 0.12**
Myristoleic acid	14:1n-5	0.67 ± 0.04**	0.50 ± 0.08**
Palmitic acid	16:0	14.99 ± 0.12**	18.58 ± 0.17**
Palmitoleic acid	16:1n-7	4.54 ± 0.06**	3.51 ± 0.04**
Hexadecadienoic acid	16:2n-4	0.56 ± 0.03**	0.33 ± 0.06**
Hexadecatrienoic acid	16:3n-4	0.24 ± 0.02**	0.14 ± 0.03**
Margaric acid	17:0	1.90 ± 0.07**	3.34 ± 0.09**
Stearic acid	18:0	3.89 ± 0.16**	6.70 ± 0.14**
Oleic acid	18:1n-9	23.07 ± 0.19**	20.74 ± 0.16**
α linolenic acid	18:3n-3	0.72 ± 0.06ns	0.58 ± 0.08ns
γ linolenic acid	18:3n-6	0.12 ± 0.03ns	0.08 ± 0.06ns
Gondoic acid	20:1n-9	15.99 ± 0.19**	13.38 ± 0.13**
Eicosatrienoic acid	20:3n-3	1.31 ± 0.05**	1.06 ± 0.07**
Dihomo-γ-linolenic acid	20:3n-6	0.12 ± 0.03**	0.04 ± 0.01**
Arachidonic acid	20:4n-6	0.62 ± 0.07ns	0.49 ± 0.07ns
Eicosapentaenoic acid (EPA)	20:5n-3	8.17 ± 0.14**	6.59 ± 0.12**
Heneicosapentaenoic acid	21:5n-3	0.48 ± 0.05**	0.27 ± 0.03**
Behenic acid	22:0	0.72 ± 0.07**	1.75 ± 0.05**
Docosatetraenoic acid	22:4n-6	0.60 ± 0.02ns	0.50 ± 0.08ns
Docosapentaenoic acid	22:5n-3	1.36 ± 0.07**	0.97 ± 0.04**
Docosahexaenoic acid (DHA)	22:6n-3	9.33 ± 0.11**	8.10 ± 0.09**
Nervonic acid	24:1n-9	3.17 ± 0.08**	2.63 ± 0.06**
SFA		28.93	40.07
MUFA		47.44	40.77
PUFA (n-6)		1.46	1.11
PUFA (n-3)		21.36	17.58
PUFA		23.63	19.16
n-6/n-3		0.07	0.06
PUFA/SFA		0.82	0.48
Total fatty acids		100	100

*TF: tuna frame

**TBP: tuna bone powder

Values are presented as the mean ± SD (n=3).

Means with (ns) superscript are not significantly different (p>0.05).

Means with (**) superscript are significantly different (p<0.05). [T-test]

4, the amount of the myristic acid, stearic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were also relatively more. In general, there was a higher content of monounsaturated fatty acids (MUFA) and a lower content of polyunsaturated fatty acids (PUFA). The amount of EPA and DHA in TF and TBP was 8.17 and 9.33, and 6.59 and 8.10g/100g, respectively. In fact, fish bone lipids contains the lower level of PUFA especially EPA and DHA compared to fish fillet. The amount of the EPA and DHA in the TF and TBP was in agreement with the results reported by Moreira *et al*. (2003) and Stevanato *et al.* (2008).

Among the fatty acids of most nutritional importance, the n-3 group stands out (Stevanato *et al*., 2008). α-linolenic acid as precursor in the n-3 group of fatty acids, was reported in levels of 0.72 and 0.58g/100g in TF and TBP, respectively. Petenuci *et al.* (2008) and Stevanato *et al.* (2008) studied on the fatty acid concentration in fish bone flour of tilapia and found that the amount of the palmitic acid, oleic acid and linoleic acid was the major ones. They were also the main fatty acids found in the tilapia head flour as reported by Visentainer *et al*. (2003). As revealed in Table 4, TBP was high in MUFA and saturated fatty acid (SFA) and low in PUFA, which was in good correspondence with Petenuci *et al.* (2008).

The amount of the PUFA and SFA obtained in TBP were 19.16 and 40.07g/100g, respectively. Based on the British Department of Health and Social Security, the amount of the PUFA/SFA ratio must be lower than 0.45 (DHSS, 1986), otherwise, the product would be categorized as unhealthy products, in particular for those who suffer from cardiovascular diseases (Stevanato *et al*., 2008). According to this indicator, the amount of 0.48 found for TBP as PUFA/SFA ratio proved that TBP will be a healthy product as calcium source.

Mendez *et al*. (1993) and Phleger and Wambeket (1994) reported that the most common lipid present in fish skeletal is triacylglycerol and the minor amounts of the lipid is belonging to the cholesterol and phospholipid. Lee *et al*. (1975) and Toppe *et al.* (2007) reported that triacylglycerol was the most lipid class present in fish bone oil consisting of the fatty acids 16:0, 16:1 and 18:1 in addition to the PUFA 20:5 and 22:6. These results were in good accordance with the results presented in this study (Table 4). In general, there were significantly differences between TF and TBP in terms of the percentages of the fatty acid profiles, due to the changes in the amount of lipid contents in TF and TBP caused by alkaline treatment.

The findings of the present study indicated that TBP is rich in calcium and can be considered to use as potential calcium source in fortification of food products for human consumption. The results obtained from sensory analysis conducted in parallel study showed that the bakery products such as bread and cookies fortified with TBP and tricalcium phosphate were similar in scores related to the taste, odor, color, texture, general appearance and overall

acceptability. TBP (0.63 g) and tricalcium phosphate (0.68g) were added as a calcium source to the bakery products to make available 240 mg of calcium per serving size in bread (50 g) and cookies (30 g). These results indicated that the bakery products fortified with TBP can be accepted by consumers without any undesirable effects on the sensory properties (Nemati *et al*., 2016).

Conclusion

Osteoporosis is becoming prevalent disease in all around the world because of the calcium malnourishment, and it caused that the consumption of calcium fortified food products become unavoidable. The results of the present study recommended that TBP is rich in calcium and it would be suitable to use as excellent calcium source for human utilization either directly as supplement for production of the calcium tablets or indirectly as fortificant for enrichment of the food products. Moreover, TBP can be considered as inexpensive and environmentally friendly alternative source of calcium.

Acknowledgments

The authors would like to acknowledge the support given by Universiti Sains Malaysia (USM) for this research and Research University grant (RU grant No; 1001-PTEKIND- 815072) from Universiti Sains Malaysia. M. Nemati appreciates the USM Fellowship for the financial support during the candidature.

References

Albanese, A. A., Lorenze, E. J., Edelson, A. H., Tarlow, A., Wein, E. H. and Carroll, L. 1986. Effects of dietary calcium:phosphorus ratios on utilization of dietary calcium for bone synthesis in women 20-75 years of age. Nutrition Reports International (USA) 33: 879.

AOAC. 2000. Official Methods of Analysis, 17th ed. Arlington, USA: Association of Official Analytical Chemists.

Arjmahdi, B. H., Alekel, L., Mollis, W., Amin, D., Sapuntzakis, M. S., Guo, P. S. and Kukreja, C. 1996. Dietary soybean protein prevents bone loss in an ovariectomized rat model of osteoporosis. The Journal of Nutrition 126: 161-167.

Babarykin, D., Adamsone, I., Amerika, D., Spudass, A., Moisejev, V., Berzina, N., Michule, L. and Rozental, R. 2004. Calcium enriched bread for treatment of uremic hyperphosphatemia. Journal of Renal Nutrition 14: 149-156.

Chaimongkol, L. 2012. Use of Selected Natural Calcium Sources for Calcium Enrichment of Crisp Rice. KKU Science Journal 40(4): 1214-1224

Chuamani, P. 2010. Calcium Enrichment in Crispy Snacks by Using Fish Bone Powder. Suratthani: Rachaphat Surathani University. (in Thai)

Cohen, S. A. and Michaud, K. E. 1993. Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via high-performance liquid chromatography. Analytical Biochemistry 211: 279–287.

DHSS - Department of Health and Social Security. 1984. Report on health and social subjects n. 28. Diet and cardiovascular Disease. London. Meat Science. 42: 443-456.

Fairweather, T. S. J. and Teucher, B. 2002. Iron and calcium bioavailability of fortified foods and dietary supplements. Nutrition Reviews 60: 360-367.

Goldscher, R. L. and Edelstein, S. 1996. Calcium citrate: A revised look at calcium fortification. Food Technology 50: 96–98.

Hemung, B. O. 2013. Properties of tilapia bone powder and its calcium bioavailability based on transglutaminase assay. International Journal of Bioscience, Biochemistry and Bioinformatics 3(4): 306-309.

Joseph, J. D. and Ackman, R. G. 1992. Capillary column gas chromatography method for analysis of encapsulated fish oil and fish oil ethyl esters: collaborative study. Journal of AOAC International, Arlington 75: 488-506.

Jung, W. K., Karawita, R., Heo, S. J., Lee, B. J., Kim, S. K. and Jeon, Y. J. 2006. Recovery of a novel Ca-binding peptide from Alaska Pollack *(Theragra chalcogramma)* backbone by pepsinolytic hydrolysis. Process Biochemistry 41(9): 2097-2100.

Kettawan, A., Sungpuag, P., Chavasit, V. and Sirichakwal, P. P. 2002. Chicken bone calcium extraction and its application as a food fortificant. Journal of the National Research Council of Thailand 34: 163-180.

Kim, S. K., Park, P. J., Byun, H. G., Je, J. Y., Moon, S. H. and Kim, S. H. 2003. Recovery of fish bone from hoki *(Johnius belengeri)* frame using a proteolytic enzyme isolated from mackerel intestine. Journal of food biochemistry 27(3): 255-266.

Kim, S. K. and Mendis, E. 2006. Bioactive compounds from marine processing byproducts. A review. Food Research International 39: 383-393.

Larsen, T., Thilsted, S. H., Konsbak, K. and Hansen, M. 2000. Whole small fish as a rich calcium source. British Journal of Nutrition 83: 191-196.

Lee, R. F., Phleger, C. F. and Horn, M. H. 1975. Composition of oil in fish bones: possible function in neutral buoyancy. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 50(1): 13-16.

Logesh, A. R., Pravinkumar, M., Raffi, S. M. and Kalaiselvam, M. 2012. Calcium and phosphorus determination in bones of low value fishes, *Sardinella longiceps* (Valenciennes) and *Trichiurus savala* (Cuvier), from Parangipettai, Southeast Coast of India. Asian Pacific Journal of Tropical Disease 2:

S254-S256.

Love, C. 2003. Dietary needs for bone health and prevention of osteoporosis. British Journal Nutrition 12(1): 12-21.

Luu, P. H. and Nguyen, M. H. 2009. Recovery and utilization of calcium from fish bones by-products as a rich calcium source. Tap Chí Khoa Hoc Và Công Nghe 47: 91-103.

Malde, M. K., Graff, I. E., Siljander-Rasi, H., Venalainen, E., Julshamn, K., Pedersen, J. I. and Valaja, J. 2010. Fish bones – a highly available calcium source for growing pigs. Journal of Animal Physiology and Animal Nutrition 94: 66-76.

Mendez, E., Jachmanian, I. and Grompone, M. A. 1993. Lipid distribution in blackbelly rosefish (*Helicolenus dactylopterus* lahillei) in relation to its possible functions as hydrostatic agent and energy reserve. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 105(1): 193-198.

Moreira, A. B., Visentainer, J. V., de Souza, N. E. and Matsushita, M. 2001. Fatty acids profile and cholesterol contents of three Brazilian Brycon freshwater fishes. Journal of Food Composition and Analysis 14(6): 565-574.

Nemati, M., Kamilah, H., Huda, N. and Ariffin, F. 2016. In vitro calcium availability in bakery products fortified with tuna bone powder as a natural calcium source. International Journal of Food Sciences and Nutrition. *http://dx.doi.org/10.1080/09637486.2016.1179269*

Petenuci, M. E., Stevanato, F. B., Laguila Visentainer, J. E., Matsushita, M., Garcia, E. E., Souza, N. E. D. and Visentainer, J. V. 2008. Fatty acid concentration, proximate composition, and mineral composition in fishbone flour of Nile Tilapia. Archivos Latinoamericanos De Nutricion 58(1): 87-90.

Phleger, C. F. and Wambeke, S. R. 1994. Bone lipids and fatty acids of Peru fish. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 109(1): 145-152.

Phiraphinyo, P., Taepakpurenat, S., Lakkanatinaporn, P., Suntornsuk, W. and Suntornsuk, L. 2006. Physical and chemical properties of fish and chicken bones as calcium source for mineral supplements. Songklanarin Journal of Science and Technology 28(2): 327-335.

Poneros, A. G. and Erdman, J. W. 1988. Bioavailability of calcium from tofu, tortillas, nonfat dry milk and mozzarella cheese in rats: effect of supplemental ascorbic acid. Journal of Food Science 53(1): 208-210.

Pongchaiyakul, C., Songpattanasilp, T. and Taechakraichana, N. 2008. Burden of osteoporosis in Thailand. Journal of Medicine Association of Thailand 91(2): 261-267.

Rizzoli, R., Boonen, S., Brand, M. L., Burlet, N., Delmos, P. and Register, J. Y. 2008. The role of calcium and vitamin D in the management of osteoporosis. Bone 42: 246-249.

Sheikh, M. S., Santa Ana, C. A., Nicar, M. J., Schiller, L. R. and Fordtran, J. S. 1987. Gastrointestinal absorption of calcium from milk and calcium salts. New England Journal of Medicine 317: 532–536.

Silva, D. J. 1981. Analysis of food. In Silva, D. J., (Ed.) Chemical methods and biological. Viçosa: University Press.

Singh, G., Arora, S., Sharma, G. S., Sindhu, J. S., Kansal, V. K. and Sangwan, R. B. 2007. Heat stability and calcium bioavailability of calcium-fortified milk. LWT-Food Science and Technology 40(4): 625-631.

Sittikulwitit, S., Sirichakwal, P. P., Puwastien, P., Chavasit, V. and Sungpuag, P. 2004. In vitro bioavailabilities of calcium from chicken bone extract powder and its fortified products. Journal of Food Composition and Analysis 17: 321–329.

Soltan, S. S. 2013. The Effects of Skimmed Milk, Soybean Flour and Sardine Fish Powder on Osteoporotic Female Rats. Middle-East Journal of Scientific Research 15(7): 984-997.

Stevanato, F. B., Almeida, V. V., Matsushita, M., Oliveira, C. C., Souza, N. E. and Visentainer, J. V. 2008. Fatty acids and nutrients in the flour made from tilapia *(Oreochromis niloticus)* heads. Food Science and Technology (Campinas) 28(2): 440-443.

Spencer, H. and Kramer, L. 1986. Factors contributing to osteoporosis. The Journal of nutrition 116(2): 316-319.

Tateo, F., Bononi, M., Testolin, G., Ybarra, L. and Fumagalli, M. 1997. Experiments in the use of calcium and magnesium lactates in milk enrichment. Industrie Alimentari (Italy) 36(359): 614–617.

Toppe, J., Aksnes, A., Hope, B. and Albrektsen, S. 2006. Inclusion of fish bone and crab by-products in diets for Atlantic cod, Gadus morhua. Aquaculture 253: 636–645.

Toppe, J., Lbrektsen, S., Hope, B. and Aksnes, A. 2007. Chemical composition, mineral content and amino acid and lipid profiles in bones from various fish species. Comparative Biochemistry and Physiology, Part B 146: 395–401.

Visentainer, J. V., Gomes, S. T., Hayashi, C., Santos-Júnior, O. O., Da-Silva, A. B., Justi, K. C., Souza, N. E. and Matsushita, M. 2003. Effect of feed delivery time supplemented with linseed oil on the physical and chemical and acid composition. Ciencia E Tecnologia De Alimentos 23(3): 478-484.

Vignesh, R. and Srinivasan, M. 2012. Nutritional quality of processed head and bone flours of Tilapia (*Oreochromis mossambicus*, Peters 1852) from Parangipettai estuary, South East Coast of India. Asian Pacific Journal of Tropical Biomedicine 2(1): S368-S372.

Yoon, G. A., Kim, Y. M., Chi, G. Y. and Hwang, H. J. 2005. Effects of tuna bone and herbal extract on bone metabolism in ovariectomized rats. Nutrition Research 25(11): 1013-1019.

Zhu K, Prince R. 2012. Calcium and bone. Clinical Biochemistry 45(12): 936-942.