RECOVERY AND UTILIZATION OF CALCIUM FROM FISH BONES BYPRODUCTS AS A RICH CALCIUM SOURCE

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ABSTRACT

Catfish bone, Salmon bone and Snapper bone were treated by alkaline treatment. The treated fish bones were milled for easy handling. The product of fish bone powder is small particle size, white colour and without fish odour. The Ca : P ratio of fish bone extract powder was close to 2:1 and its calcium content of three kinds of fish bone occurs between from 21g and 24 g per 100g of fish bone extract powder. These fish bones powder was used to fortify white bread and resulted in a calcium content ranging from 431.2 mg to 448.8 mg calcium per serving. Calcium bioavailability of fish bone extract powder fortified white bread was measured and compared with the other calcium sources. Calcium from fish bone was found to be more absorbable than calcium from calcium citrate. The calcium dialyzability of white bread fortified with fish bone extract powder ranged from 34.5% to 35.7%. The results of the sensory evaluation showed no significant difference (p > 0.05) among the three fish bone fortified white breads, control white bread, and calcium citrate fortified white bread. Fish bone extract powder could be a good alternative calcium fortificant and provides the possibility of improving calcium intake among human beings in general and in particular amongst the Vietnamese population.

Keywords: Fish bones extract powder, calcium, bioavailability, fortificant.

1. INTRODUCTION

In Vietnam, the annual output of fish catching and aquaculture is estimated to be over 2 million tonnes, with two thirds being used as raw material for seafood processors all over the country [20]. During this process of fish utilization, seafood processors annually create a great amount of byproducts and wastes. At present, these byproducts (viscera, liver, and filleting residuals) are used or sold cheaply to livestock owners and used for stock feeding. The availability of fish bones byproduct in the fisheries processors, which have fish fillets as their major product, highlights the opportunity for the fishing industry to utilize a greater part of fish bones as a higher value product. Fish bone is considered as potential high source of calcium. However, there are minimal publications addressing the bioavailability of bone calcium and its potential usability [14]. Furthermore, there has been limited studies addressing the beneficial effects of fish bone consumption and there has been no attempt to test the utilization of organic components or minerals in fish bone for human health [15]. Therefore, developing new method to use fish bone byproduct from fish processing will bring more benefit in human health and opportunities for fishery. In particular, fish bone byproduct can be used as calcium supplementation, which is necessary for the daily diet to ensure an adequate intake of calcium.

In human body, calcium (Ca) is an essential constituent of all forms of life and is critically important for good health and human nutrition [8]. It is the most important mineral in a variety

of structural elements and cell membranes. The most common source of calcium is milk and dairy products [1], however, the consumption of milk and milk product has reduced steadily over the past few decades because of their reported association with high fat levels and their association with weight increase and obesity [24]. Moreover, in some countries the majority of the population have limited or a non-existent milk intake due to lactose indigestion and intolerance [31, 25, 12]. Perman (1992) showed that the prevalence of lactose malabsorption in the Vietnamese (in the USA) group is 100 percent. In addition, calcium from plants is poorly absorbed compared to the calcium from animal sources [13]. Furthermore, in some developing countries such as Vietnam, milk is expensive in comparison to income and the calcium content in most of the available food is low generally, a diet of severe calcium deficiency is a feature of traditional Asian communities and countries [28]. Therefore, calcium-fortified products can assist in increasing the levels of calcium consumed [14]. It is well documented that consumption of whole small fish is nutritionally beneficial in providing a rich dietary calcium source and it has been proven that this calcium can be absorbed by the body as tested in vivo [15]. Furthermore, fish bone is a natural resource with a significant amount of calcium and phosphorus [9]. This source of calcium might be effectively absorbed and be an important dietary contribution, especially within population groups with low intakes of milk and dairy products.

The objective of this study was to test for fish bone discarded from industrial processing as a calcium-fortified supplement to human diet. It is also to evaluate the bioavailability of calcium from fish bone in a powder form and its application in food such as bread.

2. MATERIALS AND METHODS

2.1. Preparation of calcium concentrates from fish bone byproduct

Calcium was extracted from fish bones in order to use it as a food fortificant by using an alkaline treatment method. Fish bone materials used were from Catfish, Salmon, and Red snapper.

Catfish bones (Shutchi Catfish) (100 g), Salmon bones (100 g) and Red snapper bones (100 g) were collected from fishery processors then the flesh was separated manually, secondly these skeletal frames were dried in a hot air oven at $60^{o}C$ for 12 hours and broken up into approximately 2 – 4 cm pieces. The fish bones were boiled in 3% NaOH solution for 30 minutes with the ratio of dried bones: 3% NaOH = 1 : 4 w/v. This treatment was used to get rid of all the organic materials as well as any microbes before using as a calcium fortificant. After treatment with NaOH, the treated bone was separated with a filter cloth then washed with 1% HCl and Milli Q water until neutral bone (pH approximately 7.0). The neutralized bones were dried in a hot air oven at $100^{o}C$ for 2 hours. Finally, the good treated bones were ground in a hammer mill (Model 3100, Perten Laboratory Mill, Sweden) until passing a sieve: 0.5 mm, then the fish bone powder was used for the next stage of the experiment.

2.2. Chemical analysis

Moisture content: The samples were mixed with acid washed sand before being dried in hot oven at $105^{0}C$ until a constant weight (AOAC, 2000).

Ash content: Ash content was determined by using dry ashing technique at $550^{o}C$ (AOAC, 2000).

Calcium content: The ash of fish bone powder was dissolved in a 4N Nitric acid, and then used for the determination of calcium content. Calcium was determined by using atomic absorption spectrometer (Model SpectrAA 220, Varian Associated, Australia) at wavelengths of 422.7 nm and calcium atomic absorption standard solution (1,000 µg/ml Ca in 1% HNO_3, Catalogue 305901. Sigma-Aldrich Chemical Co.,)

Phosphorus content: The phosphorus content was determined by colorimetry (AOAC, 2000).

2.3. Preparation of breads fortified with fish bone powder and commercial calcium fortificants

White bread was chosen as the fish bone powder fortified product for the experiment. Fish bone extract powders from Catfish, Salmon, and Snapper bone were used for calcium fortificants.

Commercial calcium fortificants used in this experiment were β-Tricalcium phosphate ($Ca_3(PO_4)_2$; Catalogue No. 21218. Sigma-Aldrich Chemical Co), calcium citrate ($Ca_3(C_6H_5O_7)_2$; Catalogue No. 21120. Sigma-Aldrich Chemical Co).

The loaves of white bread were prepared following the recipe of the commercial bakery house in Gosford, NSW, Australia. The white bread without calcium fortificant was used as the control recipe in this study. The ingredients of the white bread (the control recipe) included baker flour, salt, sugar, dry milk, butter, bread improver, shortening and warm water (table 1). The ingredients of white bread fortified with fish bone or commercial calcium fortificants included the ingredient of the control recipe and fish bone extract powder or commercial calcium (table 1).

The ingredients were mixed together by an electric mixer (Model N-50, Hobart, USA). After mixing, the dough was placed into an oiled bowl and set in steam room at $60^{o}C - 70^{o}C$. The dough was allowed to rise until it doubled in size (about 40 – 45 minutes) and was then baked in a $230^{o}C$ oven for about 14 - 18 minutes.

Table 1. Ingredients of white bread and calcium fortified white bread

Ingredients	Weight of ingredients (g)	Weight of ingredient per serving (g/100 g)
Baker flour	300	59.56
Bread improver	3	0.60
Butter	3.3	0.66
Dry milk	3	0.60
Shortening	3.2	0.66
Sugar	3	0.60
Salt	4.5	0.89
Water	180	35.74
Yeast	3.7	0.73
total	503.7	100

Calcium fortified white bread		
Catfish bone extract powder	8.06	1.60
Snapper bone extract powder	7.81	1.55
Salmon bone extract powder	7.81	1.55
Tricalcium Phosphat powder	5.44	1.08
Calcium Citrate powder	23.17	4.60

2.4. In vitro calcium bioavailability study

The bioavailability of calcium in all samples was determined by the in vitro equilibrium dialysis method of Miller [18]. The method simulated conditions of the stomach with pepsin and a mixture of pancreatin and bile during the small instestine stage. The proportion of compounds diffusing across a semipermeable membrane during the intestinal stage is used as prediction of the calcium's availability. The dialyzability of calcium in each product was determined in three independent replicates. The calcium content in the diluted dialyzates and in the original samples was analyzed by Atomic Absorption Spectrometer (Model SpectrAA 220, Varian Associated, Australia).

Reagents and materials

Glassware and Dialysis tubing closures: All glassware and Dialysis tubing closures were washed in the laboratory dishwasher, rinsed in distill water, soaked overnight in 1M HCl, and rinsed again with distilled water and Milli-Q water.

Water: Milli-Q water or water that is distilled, deionized, calcium free water will be used throughout all experiments.

Pepsin: Sixteen grams pepsin powder (from porcine stomach mucosa, Catalogue No. P7000, Sigma-Aldrich Chemical Co.,) was suspended in the 0.1 M HCl and brought to 100 ml with 0.1 M HCl.

Dialysis tubing: Cellulose dialysis membranes (flat width, 25 mm; internal diameter, 16 mm; molecular weight cutoff approximately 12,000 DA, Catalogue No. D-9777. Sigma-Aldrich Chemical Co.,) were soaked and stirred in Milli-Q water 3 hours. Washed in hot Milli-Q water (60°C) for 2 minutes, followed by acidification with 0.2% (v/v) solution of sulfuric acid, then rinsed with hot Milli-Q water and Milli-Q water several times to remove acid before use.

Pancreatin-bile extract mixture: 4 grams pancreatin (from porcine pancreas Catalogue No P 7545, Sigma-Aldrich Chemical Co.,) and 25 g bile extract (porcine, Catalogue No B8631 , Sigma-Aldrich Chemical Co.) was dispersed in 0.1 M $NaHCO_3$ and the mixture was brought to 1 L with 0.1 M $NaHCO_3$

Preparation Bread samples: Six bakery products: white Bread, white bread fortified with catfish bone extract powder, white bread fortified with Salmon bone extract powder, white bread fortified with Snapper bone extract powder, white bread fortified with tricalcium phosphate and white bread fortified with calcium citrate. The bread recipes are shown in Table   .

The breads will be dried at 60°C for 24 h and milled on a 0.5 mm sieve.

In vitro method with equilibrium dialysis.

The method includes three parts: peptic digestion, pH adjustment, and pancreatic digestion with equilibrium dialysis.

Peptic-HCl digestion: Dry bread sample (25 g) was suspended in 200 ml Milli-Q water in a beaker. After adjusting the pH to 2.1 with HCl, 7.5 ml pepsin suspension was added. The pH was adjusted to 2.00 ± 0.03, the weight of the sample was brought to 250 g with Milli Q water and the sample will be incubated in a shaking water-bath at $37^{o}C$ for 2 h. The pH was adjusted to 2.00 every 30 min. The unused digest was frozen for later use.

pH-adjustment for pancreatic digestion: Titratable acidity was defined on 20 g aliquot of the peptic-HCL digestion to which 5.0 ml of the pancreatin-bile extract mixture was added. Titratable acidity was determined as the number of equivalents of KOH required to titrate the combined pepsin digest pancreatin-bile extract mixture to pH 7.5 (0.5 M KOH was used in the titration).

The suspension after peptic digestion was divided into five portions of 20 g each which were transferred into beakers.

Segments of dialysis tubing containing an amount of NaHCO, (60 g/l) equivalent to the titratable acidity filled up to 25 ml with Milli Q water were placed in each beaker. The beakers were sealed with parafilm and incubated in a shaking water-bath for 30 min at $37^{o}C$ or until the pH reach about 5.

Pancreatic digestion: Pancreatin-bile extract mixture (5.0 ml) was added to each beaker and the samples were continued to incubate in a shaking water-bath at $37^{o}C$ or 2 h. Depending on the buffering capacity of the food samples, the resulting pH after dialysis against NaHCO, and addition of the pancreatin-bile extract mixture varied between 6.7 and 7.0. At the end of the pancreatic digestion the pH was measured and the dialysis tubes were removed, and then rinsed with Milli-Q water. The solutions in dialysis tubes (dialysate) were transferred to clean beakers and rinsed in side tubes with Milli-Q water until 100 ml. The calcium contents of the dialysate factions were determined by flame atomic absorption spectrometry (Varian Spectraa 220).

The calcium bioavailabilities of the samples were calculated from amount of the calcium that had passed the dialysis membrane proportional to the total calcium content of the sample. The following equations were used

$$\text{Bioavailability (\%)} = 100 \times D/T$$

D: is the Ca content in the dialysate; T: is the Ca content in the sample.

The dialyzed calcium in each sample was expressed as mean $\pm$ SD

Preparation Bread samples: Six bakery products: white bread (control), white bread fortified with catfish bone extract powder, white bread fortified with salmon bone extract powder, white bread fortified with snapper bone extract powder, white bread fortified with tricalcium phosphate and white bread fortified with calcium citrate. The bread recipes are shown in table 1. The bakery products were dried at $60^{o}C$ for 24 h and milled on a 0.5 mm sieve. These bread samples were then used for in vitro calcium bioavailability study.

2.5. Sensory evaluation

Sensory acceptability of calcium fortified white bread was evaluated by 30 panelists. A loaf of white bread (20 g each) was served on the plastic plate to each panelist. The samples included a control white bread sample, commercial calcium (Calcium citrate) fortified white bread sample

and fish bone extract powder (catfish bone) fortified white bread sample, these samples were coded with three-digit random numbers.

Five categories of ''just-about-right'' scale (much too light/ fine=1; just about right=3; much too dark/rough=5) was used to evaluate sensory characteristics of color and general appearance before tasting. A nine-point hedonic scale was used to evaluate sensory characteristics including odor, texture, taste and overall acceptability for after tasting.

2.6. Statistical analysis

The results are expressed as the mean ± standard error. Differences between two groups with one variable were evaluated with ANOVA: Single factor (Excel). Value were considered to be significantly different at a probability level of $P < 0.05$

3. RESULT AND DISCUSSION

3.1. Qualities of fish bone extract powder

The general appearance of the fish bone powder was of fine white particle. Moreover, there was no fishy odour in fish bone powder. Therefore, fish bone powder may be suitable for incorporating to diverse products.

The amounts of moisture in fish bone extract powder were very low about 0.62 - 0.69 g / 100 g (table 2) of fish bone extract powder. Since the moisture is low it is suitable for long time storage.

Ca was the most abundant element in three fish bone species, ranging from 21.0 g to 24.4 g / 100g (table 2). The phosphorus content occurred within the range from 10.5 g to 12.8 g / 100 g of fish bone. The variability might depend on fish species, or might be related to the amount of marrow in the bone, cartilage attached to bone, or lean, fat and tendons on the surface of the bone when the alkaline treatment was not completed.

Table 2. Chemical composition of fish bone extracts powder per 100 g

Nutrient	Catfish bone	Snapper bone	Salmon bone
Moisture[a] (g) (Mean ± SD)	0.69 ± 0.02	0.62 ± 0.03	0.65 ± 0.03
Ash[b] (g) (Mean ± SD)	61.8 ± 1.58	71.2 ± 8.99	65.8 ± 1.21
Calcium[c] (g) (Mean ± SD)	21.0 ± 2.10	24.4 ± 2.26	22.3 ± 1.41
Phosphorus[c] (g) (Mean ± SD)	10.5 ± 1.07	12.8 ± 0.79	11.0 ± 1.13

[a] Mean of samples, n = 6; [b] Mean of samples, n = 10 (contents of Ash including Calcium, Phosphorus and others elements); [c] Mean of sample , n = 10.

The ratio of Ca: P of three fish species is close to 2: 1. This finding was in agreement with some studies in animal bones and fish bones [9, 23, 33, 26].

Some studies suggested that phosphate is needed for calcium transportation [36]. Calvo, (1993) reported that high phosphorus and low calcium consumption are not conductive to

optimizing peak bone mass. Therefore, with ratio of Ca: P (2:1), fish bone may be the optimum ration for calcium transportation or optimizing peak bone mass.

3.2. Calcium in fortified product

The amounts of calcium in white bread (control) and white breads fortification are showed in table 3. The content of calcium in white bread (control) is significantly less than the content of calcium in white bread fortification. The content of calcium of fish bone fortified white bread showed that during the period of use of fish bone extract powder for white bread fortification, the content of calcium concentrate was sufficiently stable in white bread under the customary conditions of processing, storage, distribution and use. Furthermore, it does not unduly shorten shelf life; providing one advantage of fish bone extract powder for fortification.

Table 3. Calcium content in calcium fortified white bread

Product	Weight of sample (g/serving)	Calcium content (mg/serving) Mean ± SD
White Bread (control)	100	124.09 ± 11.75
White Bread fortified		
Catfish Bone (white bread fortified with catfish Bone exact powder)	100	451.71 ± 7.61
Salmon bone (white bread fortified with Salmon bone extract powder)	100	431.19 ± 28.84
Snapper bone (white bread fortified with Snapper bone extract powder)	100	448.83 ± 19.49
Tricalcium Phosphat (White bread fortified with Tricalcum phosphat)	100	500.77 ± 19.32
Calcium Citrate (White bread fortified with calcium citrate)	100	507.01 ± 8.93

3.3. Sensory evaluation of calcium fortified white bread

According to table 4 scores for colour of three formulas ranged from (moderately dark: 2) to (moderately light: 3). (2.88 – 3.20). General appearance scores ranged from "just about right" to "moderately rough" (3.05 – 3.26). The odor, texture and taste of all formulas ranged from "either like or dislike" to "like moderately" (5.01 – 5.89). The scale values, which represented overall acceptability of three formulas from "neither like nor dislike" to "like moderately" (5.08 – 5.66). None of the formula scores were significantly different in sensory characteristics (P = 0.05).

Table 4. Sensory acceptability scores of calcium fortified white breads (n = 30)

| Formula | Sensory characteristics (means ± SD)[a] | | | | | |
| | Before tasting | | After tasting | | | |
	Colour[b]	[c]General appearance	Odor[d]	Texture[d]	Taste[d]	Overall acceptability[d]
NFB	2.88 ± 0.81	3.05 ± 0.78	5.05 ± 1.53	5.01 ± 1.64	5.00 ± 1.56	5.08 ± 1.61
FFB	3.20 ± 0.74	3.09 ± 0.70	5.85 ± 1.28	5.42 ± 1.27	5.71 ± 1.47	5.56 ± 1.44
CFB	2.93 ± 0.75	3.26 ± 0.70	5.65 ± 1.61	5.41 ± 1.67	5.89 ± 1.69	5.66 ± 1.50

NFB: Non calcium fortified white bread; FFB: Fish bone powder (catfish) fortified white bread; CFB: Calcium citrate (CaCi) fortified white bread

[a] Mean ± standard deviation. The scores show no significant difference at P = 0.05

[b] Five categories just right scale ranging from "Too dark: 1"; "Just right: 3"; "Too light: 5".

[c] Five categories just right scale ranging from "Too fine: 1"; "Just right: 3"; "Too rough: 5".

[d] Nine-point hedonic scale ranging from "Dislike extremely: 1"; "Neither like nor dislike: 5;"; "Like extremely: 9".

In the sensory acceptability evaluation, no formulas of white bread were significantly different in characteristics (p > 0.05). The fortified fish bone does not affect the color, odor, texture or taste of white bread. It is confirmed that the fish bone extract powder fortified white bread was well accepted by the consumer as there was no significant difference in overall acceptability scores between fish bone powder fortified white bread and control samples. It would also be possible that fish bone powder extract can be fortified in other products such as noodles, prawn chips, cookies and other foods that are consumed by the population at risk of calcium deficiency.

3.4. In vitro calcium bioavailability

In vitro dialyzable calcium was placed in white bread and white bread fortified with fish bones extract powder (FEP), Tricalcium phosphate (CaP), Calcium citrate (CaCi). It can be seen from table 4. The white bread fortified with calcium fortificant that included white bread fortified with tricalcium phosphate, calcium citrate and fishbone showed a significant higher degree of calcium bioavailability than white bread (control samples) (table 5). The reason for this difference may be resulted from a high availability of this elemental form of calcium (Bosscher et al., 1998), or the presence of calcium fortificant source may reduce the effect of inhibitory food component on their dialyzability.

Nickel et al. (1996) (Nickel et al., 1996) and Ünal et al. (2005) (Ünal et al., 2005) found that calcium content does not affect the calcium bioavailability. However, Roig et al. (1999) (Roig et al., 1999) and Bosscher et al. (1998) suggested that the most determinant parameter of calcium dialysability is the content of this element in the samples. On the other hand, from the result of this study the content of white bread fortified with calcium citrate is 507.01 mg/100g (table 3) is higher than that of white bread fortified with fish bone (average of three kinds of fish bone approximately 443.91 mg /100 g (table 3)) however the dialyzable calcium of white bread fortified with calcium citrate is 31.13% in comparison to 35.21% white bread fortified with fish

bone (average of three kinds of fish bone approximately 35.21%). In this result the bioavailability seemed to be less dependent on the calcium content of samples. It is possible that the presence of good calcium resources reduce the effect of inhibitory food components on their dialyzability. Therefore, the result of this study supports that the calcium content does not affect the bioavailability.

Table 5. The percentage dialyzable Ca of breads

Product	Amount (g) of sample in used for analysis	% Dialyzable Ca (mean ± SD) (25g sample)
White Bread (control)	124.09 ± 11.75	28.19 ± 0.68a
White Bread fortified		
Catfish Bone (white bread fortified with catfish Bone exact powder)	451.71 ± 7.61	35.40 ± 1.49b
Salmon bone (white bread fortified with Salmon bone extract powder)	431.19 ± 28.84	35.73 ± 1.17b
Snapper bone (white bread fortified with Snapper bone extract powder)	448.83 ± 19.49	34.50 ± 1.65b
Tricalcium Phosphat (White bread fortified with Tricalcum phosphat)	500.77 ± 19.32	39.84 ± 1.18c
Calcium Citrate (White bread fortified with calcium citrate)	507.01 ± 8.93	31.13 ± 0.97d

Mean having same letter indicates no significant different at P < 0.05. Different letter having significant different at P < 0.05.

The calcium bioavailability of white bread, white bread fortified with fish bone and white bread fortified with commercial calcium fortificant were significantly different. This result confirms the fact that calcium bioavailability is affected by different types of calcium fortificants [21, 19, 33]. Garcia-Lopez and Miller (1991) [7] determined that there was no significant difference in Ca bioavailability from the source of tricalcium phosphate, calcium citrate, calcium carbonate and reduced particle size calcium citrate, in rats. However, Nicar and Pak (1985), Sakhaee et al. (1999) (Sakhaee et al., 1999) and Heller et al. (1999) (Heller et al., 1999) concluded that calcium citrate provides a more optimum calcium bioavailability than calcium carbonate. Furthermore, Sittikulwitit et al. (2004) reported that tricalcium phosphate was the best the calcium dialyzability in comparison to the five calcium fortificants (calcium carbonate, tricalcium phosphate, calcium lactate, calcium citrate and calcium lactogluconate) when they applied *in vitro* method. In this study, the bioavailability of tricalcium phosphate is better than that of calcium citrate. The dialysis rate of calcium in white bread fortified with Tricalcium phosphate has the highest calcium bioavailability, 39.84% ± 1.18%. However, the dialysis rate of calcium obtained from white bread fortified with calcium citrate is lowest (31.13% ± 0.97%) among the three sources of calcium fortified bread. This result is similar to those reported for Tricalcium phosphate (CaP) that is the best calcium dialyzability compared to the five calcium fortificants [33].

There are no significant differences between the three kinds of fish bone as calcium fortificants source for white bread, (Catfish bone: 35.40%, Salmon bone: 35.73% and Snapper bone: 34.50%). The inorganic constituent of three kinds of fish bone is similar with Ca: P ration approximately 2: 1. It is perhaps possible that there is no significant difference of the crystal structure and elemental composition between the three species of fish bone, therefore the calcium bioavailability of three kinds of fishbone as calcium fortificant is not significantly different. However, other species of fish with different crystal structures and elemental compositions of bone may not have the same bioavailability.

The calcium bioavailability of white breads fortified with fish bone is a good calcium source with respect to the bioavailability of calcium. Calcium from fishbone was more absorbable than that from calcium citrate and white bread control. Sittikulwitit et al. (2004) indicated that the bioavailability of calcium citrate is higher than that of milk powder. Larsen et al. (2000) reported that calcium sources from small fish with bones is available and useful for growth in rats. The good result of fish bones as calcium fortificant also agree well with previous studies using animal bones as calcium fortificant source. There are total bone extraction of bovine reported by Miura and Nakano (1998), bone meal studied by Heaney et al. (1990), and chicken bone reported by Sittikulwitit et al. (2004). The good calcium bioavailability of fish bone might be due to the presence of good calcium Ca: P ration and crystal structure. Therefore, it is more solution and dissociated in soluble than calcium citrate.

Calcium bioavailability from dairy products are usually considered superior to non-dairy products. Milk contains lactose and is known to promote calcium absorption [5, 6, 4, 8]. However, its calcium may not be highly absorbable when ingested in a mixed diet [27, 33]. Shanil Juma et al. (1999) found that calcium-enriched bread (bread-based diet) could serve as a good source of bioavailable calcium in comparison with calcium-enriched milk (milk-based diet). Martin *et al*. (2002) (Martin et al., 2002) indicated that the absorption of the calcium salt from the bread compares favorably with that of milk and does not differ when compared to calcium lactate and calcium carbonate.

The value of bioavailability of calcium depends on many factors of food components. In bakery products, the effect of phytate and dietary fiber are seen to be the main factors. The presence of phytate and dietary fiber can act as inhibitors on calcium bioavailability [16, 10, 37, 33]. However, sour-dough fermentation of bread can lead to a significant reduction of the phytic acid [35, 37]. Sittikulwitit et al. (2004) showed that in white bread, the amount of phytate and dietary fiber are 41 mg / 100 g and 3.6 g / 100g, respectively. Wolter et al. (1993) postulated that in normal white bread, phytic acid content is about 0.1 g/kg. the amount of these phytate and dietary fiber in white bread are much lower than that of other bakery products [37, 33]. This level of phytate and dietary fiber in white bread appears to be too low to negatively affect calcium availability. Beside phytate and dietary fiber, the phosphate produced from the phytic acid during sour-dough fermentation also has a negative effect on the calcium bioavailability [37] and the higher fat content in the white bread may influence calcium absorption [32]. In general, this study supports the use of white bread as calcium fortified product to be good calcium supplement product. Nevertheless, bread formulation varies from one brand to another. Therefore, further studies concerning the composition of the bread and its calcium content need to be undertaken to optimize the absorption of calcium

4. CONCLUSION

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The finding of this study suggests that fish bone as a source of calcium in fortified bread might be a feasible alternative for those who do not consume milk and milk product. The use of a fishbone fortified white bread is therefore an option for calcium supplement. However, research analyzing the full composition of the product before commercialization is required. It is also necessary to develop a safe and cost-effective preservation method for fish byproduct and to identify methods to further utilize the remaining fish byproduct for the benefit of the fish industry in general and the Vietnamese population in particular.

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