
17 Calcium from Fish Bone and Other Marine Resources

*Won-Kyo Jung, Fereidoon Shahidi,
and Se-Kwon Kim*

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17.1 INTRODUCTION

Calcium is known to be an essential element required for numerous functions in our body including the strengthening of teeth and bones, nerve function, and many enzymatic reactions that require calcium as a cofactor. It is also necessary for muscle contraction and regulation of the permeability of sodium ion across cell membranes including those of nerve cells. The concentration of calcium in the blood plasma remains almost constant and varies only slightly over time for a given individual [1,2]. In marine ecosystem, there is a large amount of calcium in the major form of calcite (CaCO_3) or calcium divalent cation (Ca^{2+}). Moreover, calcium with diverse physiological roles such as fertilization of egg, formation and growth of skeleton and shell, and nutritional metabolite is widely distributed in various marine phytoorganisms, microorganisms, invertebrates, and vertebrates. The ubiquitous occurrence and plentiful mass of calcium sources in marine ecosystem

have provided a motive for study on utilization in bioavailable calcium supplements and fortifiers. However, studies on application of marine bioresources for bioavailable calcium [3–8] are scarce. This chapter focuses on current status of knowledge on the utilization of marine organisms for use as calcium supplements or fortifiers.

17.2 NEEDS FOR ALTERNATIVE CALCIUM SUPPLEMENTS OTHER THAN DAIRY PRODUCTS

Except for nutritional and physiological applications, calcium that originates from dolomite, bone meal, and oyster shell are also utilized as important ingredients in various industries such as food industry, electronic industry, and leather industry, among others. For example, calcium is used to produce acryl resin, make emulsion coagulants in the rubber industry, to produce additives in pulp and paper industry, and to make early strengthening agents (concrete strengthening agent and coating material coagulating agent, and others) in the construction industry. Especially in food and agricultural industries, calcium is utilized as a foodstuff antiseptic to prevent putrefaction of fruits and vegetables and help the process of cheese making. Although most people are aware of calcium as an important element in their bodies, it is still severely deficient in most diets. Calcium deficiency in the United States has been considered as a major cause of osteoporosis, affecting approximately 26 million people annually [9]. In 1994, the National Institute of Health (NIH) Consensus panel revised the recommendations for calcium intake [10].

As shown in Table 17.1, the optimal calcium intake has been recommended to be 800 mg/day during childhood below 5 years of age, 800–1200 mg/day for children from age 6 to 10, 1200–1500 mg/day for adolescence or young adults from age 12 to 24 and pregnant or lactating women, 1000 mg/day from age 25 to the time of estrogen deprivation or age 65, and 1500 mg/day for elderly people.

Generally, most common and trusted source of calcium (Table 17.2) is milk and other dairy products [2]. However, some people, especially Asians, do not prefer to take milk because of lactose indigestion and intolerance, which make them allergic to milk. Thus, as an alternative, these people prefer to take calcium-fortified fruit juice, calcium-rich foods, and calcium salt supplements,

TABLE 17.1
Recommended Calcium Intake for Various Population Groups (NIH Consensus Development)

Age (Years)	Calcium Needs (mg/day)
Children	800–1200
Adolescents	1200–1500
Adults	1000
Elderly	1500
<65 on hormonal replacement therapy	1000

TABLE 17.2
High-Bioavailable Calcium Sources in Foods

Food Source	Serving Size	Calcium (mg)
Milk and Yogurt	8 oz or 1 cup	300–450
Cheese	3 oz	300–450
Bones in canned sardines and salmon	3 oz	181–325
Calcium-fortified foods (i.e., orange juice, soy milk, tofu)	8 oz	200–300
Dark green, leafy vegetables	1/2 cup cooked, 1 cup raw	50–100
Nuts and seeds	1 oz	25–75

Source: <http://ag.arizona.edu/pubs/health/az1296.pdf>

such as calcium fumarate, citrate, lactate, carbonate, di- and tribasic phosphate, and gluconate. These salts are available in ingredient forms, each with their own calcium content, solubility, taste, and cost issues. Especially, the solubility and bioavailability of calcium-containing ingredients are important. Although the low pH condition in the stomach renders all calcium into its ionic form, precipitation as insoluble calcium phosphate, depending on the amount of phosphate present, can occur in the intestine, where the pH range is 6–7.

The human body cannot absorb the calcium present in precipitated calcium phosphate. To improve solubility and bioavailability of calcium, various proprietary blends of calcium salts have been developed with milk protein, food acids and sugar, polysaccharides and calcium/amino acid chelate complexes like Ca-casein phosphopeptides, as specific end-use products, depending on their final pH [1]. Casein phosphopeptides (CPPs) derived from the intestinal digestion of casein have been shown to enhance bone calcification in rats [11,12]. Calcium fortifiers like CPPs, egg yolk phosphopeptides (phosvitin), and some organic ingredients (citrate, malate, acetate, etc.) have the capacity to chelate Ca ion and to prevent precipitation of Ca-phosphate salts at neutral intestinal pH [13], thereby increasing the amount of soluble Ca available for absorption across the mucosa [14,15].

In the following parts, we introduce dairy foodlike calcium supplements and CPP-like fortifiers derived from marine organisms, and survey current researches for bioavailable calcium.

17.3 CALCIUM FROM MARINE ORGANISMS

17.3.1 UTILIZATION OF FISH SKELETAL FRAME IN BIOACTIVE CALCIUM SUBSTANCES

Marine capture fisheries contribute over 50% of total world fish production, and more than 70% of this production has been utilized for processing [16]. As a result, every year a considerable amount of total catch is discarded as processing

leftovers and these include trimmings, fins, frames, heads, skin, and viscera. In addition to fish processing, a large quantity of processing by-products are accumulated as shells of crustaceans and shellfish from marine bioprocessing plants. Recent estimates revealed that current discards from the world's fisheries exceed 20 million tons equivalent to 25% of the total production of marine capture fisheries [16]. Therefore, there is a great potential in marine bioprocess industry to convert and utilize more of these by-products as valuable products. Majority of fisheries by-products are presently employed to produce fish oil, fishmeal, fertilizer, pet food, and fish silage [17,18]. However, most of these recycled products possess low economic value. Recently, bioactive compounds from remaining fish muscle proteins, collagen and gelatin, fish oil, fish bone, internal organs, and shellfish and crustacean shells were reviewed by Kim and Mendis [6]. Among fish by-products, fish bone or skeleton is considered as a potential source to obtain calcium, which is an essential element for the human health. However, only few studies have been carried out to identify bioavailability of fish bone calcium and its potential applications.

Generally, calcium is obtained from the diet and it is severely deficient in most of the regular diets. Therefore, to improve calcium intake, several calcium-fortified products are in the market and demand for these products is growing continuously. It is well documented that consumption of whole small fish is nutritionally beneficial providing with a rich source of calcium. Calcium in fish could be absorbed to the body as tested *in vivo* [3]. However, very little information is available on the beneficial effects of larger fish bone and few attempts have been made to test their usage for benefits of human health. Fish bone material derived from processing of large fish is a useful calcium source where the quantity of calcium is concerned. To incorporate fish bone into calcium-fortified food, it should be converted into an edible form by softening its structure. This can be achieved utilizing different methods including hot water treatment and hot acetic acid solutions. In addition, Ishikawa et al. [19] used superheated steam to reduce the loss of soluble components from fish tissue and that enabled better recovery of bone within a shorter period. Jung et al. [20] performed enzymatic degradation in acetic acid solution (pH 2.0) by pepsin to easily dissolve both mineral and organic parts of fish bone. Pepsinolytic degradation of Alaska pollack bone in acidic condition could lead to the highest degree of hydrolysis in comparison with those of other enzymes. Moreover, Jung et al. [7,8] isolated high calcium-binding oligophosphopeptide from hoki fish skeletons by an enzymatic degradation method with carnivorous intestinal crude enzymes in an ultra-filtration membrane bioreactor system. In this study, it has been observed that calcium-binding activity of the fish bone peptides (FBP) II was similar to that of CPP. Further, the pH of the reaction system was maintained at 7.8, because low pH could increase the solubility of the insoluble calcium salt. As reported by Jiang and Mine [21], the solubility of 36.3 mg/L of Ca could be obtained at 200 mg/L of the oligophosphopeptide from egg yolk phosvitin with 35% phosphate retention, and the solubility was higher than that of commercial

CPP II (Meiji Seika Co., Ltd., Tokyo, Japan). Furthermore, *in vivo* studies with osteoporosis modeling rat have elucidated that the FBP II fractionated in the molecular weight range of 5.0–1.0 kDa increased Ca solubility and bioavailability. Menopause is a time when estrogen deficiency leads to accelerated bone resorption and negative bone balance. Another study has been undertaken to evaluate the beneficial effects of FBP as a Ca fortifier in osteoporosis induced by ovariectomy and a concurrent low-Ca diet. During the experimental period corresponding to the menopause with osteoporosis disease, the loss of bone mineral (Ca) was decreased by FBP II supplementation in the ovariectomized rats. After the low-Ca diet, the FBP II diet, including both normal levels of Ca and vitamin D, significantly decreased Ca loss in feces and increased Ca retention as compared with the control. The levels of femoral total Ca, bone mineral density, and breaking strength were also significantly increased by FBP II diet to a level similar to those of the CPP diet group (no difference; $P < 0.05$). Based on these data, it was suggested that increased Ca retention by FBP II intake may lead to the prevention of mineral loss in the osteoporosis-modeling rats. As reported by Larsen et al. [3,4], the intake of small fish with bones can increase Ca bioavailability, and the small fish may be an important source of Ca, especially in population groups with low intake of milk and dairy products. Thus, these results prove the beneficial effects of fish meal in preventing Ca deficiency owing to increased Ca bioavailability by FBP intake. Furthermore, there is a potential to these fish peptides to provide a novel nutraceutical with a high bioavailability for Ca to Oriental people with lactose indigestion and intolerance and Ca-fortified supplements, such as fruit juice or Ca-rich foods, as alternatives to dairy products.

In the case of marine calcium in medicinal application, attempts have been taken to isolate fish bone-derived hydroxyapatite and use them as an alternate for synthetic hydroxyapatite [22,23]. Recently, hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ has been introduced as a bone graft material in a range of medical and dental applications because of their similar chemical composition. Generally, bone substitution materials such as autografts, allografts, and xenografts are used to solve problems related to bone fractures and damages. However, none of these materials provides a perfect bone healing owing to mechanical instability and incompatibility. Currently, calcium phosphate bioceramics such as tetracalcium phosphate, amorphous calcium phosphate, tricalcium phosphate, and hydroxyapatite are identified as most suitable bone substitution materials to address the demand. Fish bone material may serve as an important source for biomedical applications owing to the presence of hydroxyapatite as the major inorganic constituent. Unlike other calcium phosphates, hydroxyapatite does not break under physiological conditions. In fact, it is thermodynamically stable at physiological pH and actively takes part in bone bonding. This property has been exploited for rapid bone repair after major trauma or surgery. Hydroxyapatite is derived from natural materials such as coral and fish bone [24]. Generally, very high heat treatment is used for isolation of hydroxyapatite from fish bone and this temperature gives a higher strength to hydroxyapatite structure [25] and results an excellent biocompatible inorganic substance [26–28].

17.3.2 HIGH ABSORBABLE CORAL CALCIUM

Ayurveda, an ancient system of Indian medicine has mentioned several calcium preparations in the correction of bone metabolic disorders such as osteoporosis. *Praval bhasma* (PB, *Coral calx*) a natural source of rich calcium in marine ecosystem, predominantly consists of CaCO_3 and is widely used in ayurveda as calcium supplement from time immemorial. Moreover, because of appropriate ayurvedic processing of PB, it has the advantage of easy absorption from the intestine [29].

Reddy et al. [30] evaluated the efficacy of PB on the progress of bone loss in calcium-deficient diet fed ovariectomized (OVX) rats. According to the results, calcium-deficient OVX rats developed bone changes similar to those seen in osteoporotic women as indicated by a decrease in femur weight, density, and bone mineral content. Treatment with PB significantly prevented the reduction in bone density and bone mineral content, especially in calcium and phosphorus levels despite ovariectomy and calcium deficiency. The unchanged levels of calcium and phosphorus in plasma of sham and Ca-deficient/OVX (CD-OVX) group indicates that homeostatic mechanisms were able to maintain plasma levels of these minerals despite ovariectomy. Treatment with PB significantly increased and maintained the serum calcium level, thus indicating good absorption from the intestine. Fasting urinary calcium excretion is also a useful variable for estimating net bone resorption. PB-treated animals showed decreased urinary calcium excretion despite elevated serum calcium levels, suggesting that more calcium might be deposited in bones. The assumption could be true as it is evident from the increased ash weight, percent ash, and mineral content in femurs of PB-treated group compared to CD-OVX group. In addition, high-resolution radiography such as CT-scanning technique employing magnification to assess cortical bone loss has indicated a decreased cortical area and increased medullary width and cross-sectional area suggesting increased bone loss in CD-OVX animals. In conclusion, the authors have discussed that treatment with PB in the adult rat model of osteoporosis exerted desired beneficial effects on the inhibition of bone resorption, thereby justifying its continued use. In addition, a group fed with normal calcium diet as a positive control have strengthened the claim that the efficacy of PB in rats is through improvement of calcium absorption. Nevertheless, the results of this study clearly indicate the beneficial effects of PB in preventing bone loss as indicated by various parameters in the PB-treated group compared to the CD-OVX group. Furthermore, it is widely believed that calcium supplements of natural origin such as PB probably could have trace amounts of lead or other toxic substances that may cause concern for safety.

17.3.3 CALCIUM BIOAVAILABILITY OF MARINE ALGAL POWDER

Marine algae have been a valued food in Asia for thousands of years because of its highly nutritious qualities. They provide the body with a full array of nutrients including complete protein, complex carbohydrates, essential fatty acids, fiber, vitamins, minerals, enzymes, and trace elements. Marine algae are thought to

exert medicinal properties to prevent a number of disease complications in the human body.

However, marine algal-derived valuable proteins, minerals, and isoflavons on bone metabolism has not yet been clarified. Recently, the effect of various algae on bone calcification in the femoral-metaphyseal tissues of rats was investigated by Yamaguchi et al. [31]. The study was undertaken to determine the effect of various marine algae on bone calcification in the femoral metaphyseal tissue of rats *in vivo* and *in vitro*. Marine algae (*Undaria pinnatifida*, *Sargassum horneri*, *Eisenia bicyclis*, *Cryptonemia scmitziana*, *Gelidium amansii*, and *Ulva pertusa* Kjellman), which are utilized in food, have been used in this study. Water suspensions of marine algae powder were orally administered to rats and it was observed that bone calcium content was significantly increased by the administration of *U. pinnatifida*, *S. horneri*, *E. bicyclis*, or *C. scmitziana*. Bone alkaline phosphatase activity, which is an enzyme for calcification, was significantly enhanced by the administration of *S. horneri* or *G. amansii*. Moreover, bone calcium content was significantly elevated in the presence of *S. horneri* extract (25 and 50 $\mu\text{g/mL}$). It was elucidated that *S. horneri* extract had an anabolic effect on bone calcification *in vivo* and *in vitro*. In this study, it was suggested that prolonged intake of *S. horneri* extract may play a role in the prevention of bone loss with increasing age. These data suggest that much more remains to be elucidated in animal models of osteoporosis. Thus, marine algae (*S. horneri*) extract has an anabolic effect on bone calcification in animal models of osteoporosis and hence may play a role in the prevention of osteoporosis.

17.3.4 LOW MOLECULAR PHOSPHORYLATED CHITOOLOGOSACCHARIDES DERIVED FROM CRAB SHELL AS A CALCIUM FORTIFIER

Chitosan is a deacetylated polymer of *N*-acetylglucosamine, which is obtained after alkaline deacetylation of the chitin derived from the exoskeletons of crustaceans and arthropods. Recently, considerable attention has been given for its commercial applications in biomedical, food, and chemical industries. In addition, chitosan has been widely used in vastly diverse fields such as pharmaceuticals, medicine, and biotechnology. However, increasing attention has recently been paid to convert chitosan into its oligosaccharides because of their biological activities, such as antitumor activity [32,33], immunostimulating effects [34,35], enhancing protective effects against infection with some pathogens in mice [36], antifungal activity [37], antimicrobial activity [38–40], angiotensin I converting enzyme (ACE)-inhibitory activity [41], and radical scavenging activity [42,43]. Kim and Mendis [6], reported that phosphorylated chitooligosaccharides (P-COSs) exhibited inhibitory activity against the formation of insoluble calcium phosphate at neutral pH. Furthermore, P-COS with low molecular weight exhibited the highest inhibitory activity of calcium phosphate precipitation. Its inhibitory activity, especially at concentrations of more than 4 mg/mL, was similar to that of CPP, which is widely used as a calcium-fortifying agent that improves calcium absorbability. Therefore, this study illustrated that phosphorylated chitooligosaccharides can be

considered as potential inhibitors of calcium phosphate precipitation. Furthermore, *in vivo* effect of COSs was also elucidated by Jung et al. [8]. In this study, low-molecular-weight COSs obtained using an ultrafiltration (UF) membrane reactor system, inhibited the formation of insoluble calcium salts in the neutral pH. *In vivo* effects of COSs on Ca bioavailability were further studied in the osteoporosis rats model induced by ovariectomy and concurrent low calcium intake. During the experimental period corresponding to the menopause with the osteoporosis disease, calcium retention was increased and bone turnover was decreased by COS IV (molecular mass of <5.0 kDa) supplementation in the OVX rats. After a low-Ca diet, COS IV diet including both normal level of calcium and vitamin D significantly decreased calcium loss in feces and increased calcium retention compared to the control diet. The levels of femoral total calcium, bone mineral density (BMD), and femoral strength were also significantly increased by the COS IV diet in a similar level to those of CPP diet group. In the study, the results proved the beneficial effects of low-molecular-weight COS IV in preventing negative mineral balance.

In addition, Kim et al. [44] previously reported that COSs do not exert any toxic effects in experimental diet groups of Sprague–Dawley rats fed with 500, 1000, and 2000 mg/kg/day. This suggestion was made observing weight change, general symptoms, food consumption, urinalysis, hematology, blood biochemistry, and relative organ weights of COS-treated rats. Further, this study states that further studies are needed to confirm the bioavailability of the chitosan derivative, P-COS using *in vivo* studies and it may serve as potential inhibitors of calcium phosphate precipitation.

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