

Evaluation of the effect of fortification with sepia, sardine and salmon as anti-osteoporotic on the general properties of bread rolls

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Abstract

The fortification of main foods is generally accepted as an effective way for providing the daily requirements for a range of vitamins and minerals. The present study was carried out to investigate the acceptability of bread rolls fortified with fish for the prevention of osteoporosis. Four samples of bread rolls were made using fish bone powder at 5% of the total components as follows: the first sample, which is the control sample, the second sample, which contains salmon fish bones powder at 5%, the third sample, which contains sardine fish bones by 5%, and the fourth sample Which contains sepia fish bone powder at a percentage of 5%. Then a sensory evaluation was made for these samples in terms of appearance, internal color, external color, Cells uniformity, taste, smell and general acceptance. The results showed that bread rolls supplemented with sepia fish bone powder are closer to the control sample in properties except for taste, smell and general acceptance in conclusion, three types of fish. The impact of Sibia, Sardine and Salmon fish has been studied on blood stages of calcium, and phosphorus. Measurement of bone mineral content (BMC) and bone mineral density (BMD). Results found that 3 kinds of

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fish particularly sibia fish brought about. In addition, a full-size growth in calcium and phosphorus, bone mineral density and bone mineral content. In conclusion, the 3 kinds of fish specifically sibia fish defend rats against osteoporosis through their nutrition D, calcium and phosphorus content. So, it could be useful to human beings.

Keywords: Sibia, Sardine, Salmon, Prednisone Acetateas, osteoporotic

Introduction

Fish, which constitutes a major food group in diets throughout the world, contains high levels of n-3PUFAs, high-quality protein, vitamins and essential minerals, and thus may be beneficial to bone health. Some studies have reported that fish consumption can in fact reduce the risk of bone fracture (***Xia et al., 2012***). It is shown that BMD was significantly higher among participants living in a fishing village than among those living in a mountain village with less access to fish (***Calderon-Garcia et al., 2013***). Furthermore, the effect of fish consumption on bone health may vary among fish species (***Fan et al., 2013***).

Calcium-fortified products would be helpful in the enhancement of the levels of calcium intake (***Kim and Mendis, 2006***), particular for population groups with insufficient intakes of milk and dairy products due to 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 (***Nemati et al., 2017***) whereas utilization of natural calcium

sources such as fish bone can be more acceptable by consumers and be more effective in terms of the existence of calcium phosphate compound in which is similar to the human bone components (*Phiraphinyoet et al., 2006; Chuamani, 2010 and Hemung, 2013*).

Fish bones have a high Ca content, and huge quantities of this raw material are available as a by-product of the fish industry. Previously, the emphasis has been on producing high quality products from fish by-products by use of bacterial proteases. However, documentation of the nutritional value of the enzymatically rinsed Ca-rich bone fraction remains unexplored (*Malde et al., 2010a*).

In many countries, large quantities of fish waste and fish by-products are discarded into the environment and converted into an important source of pollution (*Stevanato et al., 2008*). In recent years, dried fish bone was used as a 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 represents as a significant source of minerals once included in feed (*Toppe et al., 2007*). Anyway, minimal publications are addressed regarding the bioavailability of calcium recovered from fish bone and its potential usability (*Kim and Mendis, 2006*).

It is concluded that bones from Atlantic salmon and Atlantic cod are suitable as natural Ca sources in e.g. functional foods or as supplements (*Malde et al., 2010b*). In recent years, emphasis has been put on producing high quality nutritional products from fish by-products by use of enzymes (*Liaset et al., 2003 and Sandnes et al., 2003*). The bone fraction, which comprises approximately 10-15% of the total body weight of fish (skin not included) is still regarded as waste. The Norwegian fisheries produce more than 600,000 tons of by-products annually, which is more than 20% of the gross weight of fish caught and farmed in Norway. Most of the byproducts are used

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as low-quality raw materials for feed production, and about 180,000 tons are dumped into the sea (*Malde et al.,2010b*). Thus, scientific documentation of content and use of various components from marine by-products for human consumption is warranted.

Fish bone has high calcium (Ca) content, and Ca and phosphorus (P) comprise about 2% (20 g/kg dry weight) of the whole fish. The chemical composition of fish bones varies, and in general, oily fish (e.g. salmon) have higher lipid levels, and lower protein and ash levels compared to lean species (e.g. cod) (*Toppe et al.,2007*). A recent study in growing pigs has shown that Ca from salmon, cod and saithe bones is absorbed as efficiently as CaCO₃(*Malde et al.,2010b*).The study also indicated that fish species and rinsing method may be of importance both for nutritional content and absorption. Ca-fortified foods are likely to play an important role in helping consumers to meet the calcium requirements needed to reduce the risk of osteoporosis. Thus, there is a need for evaluating Ca absorption from different types of fish bone against other commercially available products. (*Malde et al.,2010b*).

Cuttlebone (CB), otherwise called cuttlefish spine, is an inner shell of marine creatures known as cuttlefish (*Sepia*), having a place with the phylum mollusca, class cephalopod, arrange Sepiidae(*Zhao et al., 2011 and North et al., 2017*). Cuttlefish bone is for the most part made out of calcium carbonate. A few tests considerhaving been directed at normal cuttlefish bone as a calcium hotspot for bone substitutes (*Negm, 2018*). Cuttlefish bone will in general be utilized and masterminded by various fillers as a marine typical opposing to Osteoporosis (*Hemmatti et al., 2018*).

Materials and methods

Materials:

Sibia, Sardine and Salmon fish: Egyptian sibia, sardine and salmon fish were obtained from the local markets and its bone were collected and removed for fortification study on the baked products (bread rolls).

Wheat flour: wheat flour was obtained from the local market to make the baked product (bread rolls).

Rats: Fifty adult female albino rats of Sprague Dawley strain weighing (170 ± 10 g) were obtained from Helwan farm for experimental animals, in Cairo, Egypt.

Diet: Casein, vitamins, cellulose, minerals, methionine and choline were obtained from Morgan Company for Chemicals, Cairo, Egypt.

Chemicals: Kits for biochemical analysis were purchased from the Biodiagnostic Company for Pharmaceutical and Chemicals, Dokki, Egypt.

Prednisone Acetate as a source of glucocorticoid was obtained from Pharmacy, Cairo, Egypt.

Methods:

Chemical composition: Protein, calcium, phosphorus, magnesium, zinc and vitamin D content in sibia, sardine and salmon were determined according to the official methods (**AOAC., 2005**).

Sibia, Sardine and Salmon fish: were dried in the oven for 15 minutes at 40°C, grinded up in a grinder and crushed in a ceramic mortar.

Preparation of Sibia, Sardine and Salmon bone Powder: blocked fish bone was removed from the sibia, sardine and salmon, each one gently washed with distilled water, and dried in free air in order to lose its smell. After drying, the clean fish bone was powdered.

Preparation of fortified baked product (bread rolls): baked product (bread rolls) were prepared according to the procedures

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reported by (Gisslen.,2007) using bone powder as a fortification by 5% of the total ingredients. The products were prepared in the laboratory of nutrition and food science, faculty of Home Economics, Helwan University.

Experimental Design:

Fifty adult female albino rats were housed in well-aerated cages under hygienic conditions and were fed on a basal diet for one week for adaptation. After this week the rats were divided into five groups as follows:

The first groups (10 rats) were kept as a negative control group and were fed only on a basal diet. The other four groups (10 rats each) were fed on a basal diet containing 100 mg Prednisone Acetate as a source of glucocorticoid/ kg diet for two weeks to induce osteoporosis (*Liao et al., 2003*), four rats were selected to insure the induction of osteoporosis by DEXA scan then the osteoporotic group were divided as follow:

The second group served as a positive control group and fed on a basal diet.

The third group was fed on a basal diet supplemented with sibia powder at the level of (5%).

The fourth group was fed on a basal diet supplemented with sardine powder at the level of (5%).

The fifth group was fed on a basal diet supplemented with salmon powder at the level of (5%).

All rats were observed each day. Their feed intake was determined daily and body weights were obtained every week. Feed Efficiency Ratio and body weight gain were calculated according to the method of (*Chapman et al., 1959*). At the end of the experiment (8 weeks) the rats fasted for 12 hours, and then were sacrificed under ether anesthesia. Blood samples were collected from the medial

canthus of the eyes of rats by means of fine capillary glass tubes in a centrifuge tube without any anticoagulant and were centrifuged for 20 minutes at 3000 r.p.m. to obtain the serum which was stored at -20°C until used for subsequent analysis.

Four samples of bread rolls were made using fish bone powder at 5% of the total components as follows: the first sample, which is the control sample, the second sample, which contains salmon fish bones powder at 5%, the third sample, which contains sardine fish bones by 5%, and the fourth sample Which contains sepia fish bone powder at a percentage of 5%.

Biochemical analysis:

Serum calcium and phosphorus were determined according to (*Gindler and King., 1972*) and(*El-Merzabani et al., 1977*), respectively. Measurement of bone mineral density (BMD) and bone mineral content (BMC): The BMD and BMC of femur bone were estimated in anesthetized rats using dual energy X-ray absorptiometry (DEXA) according to (*Gao et al., 2013*).

Organoleptic (Sensory) evaluation:

Sensory evaluation was carried out by (10) panelists from the Department of Nutrition and food science at the Faculty of Home Economics, Helwan university, using a score sheet of 5-points(1=lowest quality to 5=highest quality) according to (*Klein.,1984 and Penfield and Campbell ,1990*).

Bakery products (bread rolls) were evaluated for: appearance ,color (internal/external),celluniformity,taste,odor and overall acceptability.The results for each characteristic were calculated as mean values, analyzed statistically and tabulated.

Statistical analysis:

The obtained results were analyzed according to the SPSS program. ANOVA test was used to compare results among groups and $P < 0.05$ was considered significant (*SPSS., 1986*).

Results and discussion

Body weight gain and food efficiency ratio (BWG% and FER):

Table 1 indicated significant differences ($P \leq 0.05$) in BWG% between the negative (-) control group and tested groups. Rats in groups 2, 3 and 4 significantly ($P \leq 0.05$) BWG% differed compared to those of the negative (-) control. While the mean value of BWG% in group 5 recorded non-significant change as compared to the negative (-) control group. BWG% in groups 2, 3, 4 and 5 were: 16.52 ± 1.50 ; 21.80 ± 1.22 ; 23.15 ± 1.48 and 30.61 ± 1.12 , respectively vs. 41.05 ± 1.43 for the negative (-) control. Also BWG% in groups 3, 4 and 5 showed significant variations ($P \leq 0.05$) as compared to the control "group 2" (21.80 ± 1.22 ; 23.15 ± 1.48 and 30.61 ± 1.12 , respectively vs. 16.52 ± 1.50 . Data in this table recorded non-significant changes in BWG% between all treated groups.

The tabulated data revealed that osteoporosis significantly ($P \leq 0.05$) altered the FER in rats. Rats in groups 1, 3, 4 and 5 significantly ($P \leq 0.05$) differed from those of the positive (+) control group. The mean values of FER for groups 1, 3, 4 and 5 were: 0.16 ± 0.137 ; 0.13 ± 0.004 ; 0.14 ± 0.007 and 0.15 ± 0.009 , respectively vs. 0.05 ± 0.003 for the positive (+) control. All treated groups showed non-significant changes in FER as compared to the negative control group.

These results showed a significant decrease ($P \leq 0.05$) in body weight gain and FER of control positive (suffering from osteoporosis) compared to control negative (healthy rat). These results are in agreement with the findings of **Oliveira et al., (2010)** and **Soltan, (2013)**, who reported that glucocorticoids induced a significantly lower weight gain (-9.7%) compared to the control group. Meanwhile, rat's administration diet supplemented with 20% sardine fish recorded a significant increase in final body weight and body weight gain compared to the positive control group. These results are in accordance with the results obtained by **Gouda, (2012)**, who reported that control positive (osteoporotic rats) showed a significant decrease in body weight gain. The decrease in body weight gain may be due to high doses of cortisone and long-term.

These results agreed with **EL- Sayed, (2017)** showed that Rats who were fed on a basal diet containing fish as a source of protein recorded higher feed intake and BWG value than other treated groups. Feed intake and BWG were increased in the negative control group than in the positive control group. On the other hand a treated rat with fish illustrated a significant decrease in BWG% when compared with the negative control group.

Daidj and Lamri-Senhadji, (2021) observed that the sardine by-product oil and sardine fillet oil groups showed similar significant decreases in body weight, weight gain, and food intake in the obese control group. Moreover, obese rats consuming fillet and by-product sardine oils had negative FER values compared with control obese and normal rats; FER ratios indicate the animal's ability to transform energy consumed into body mass (**De Sibio et al., 2013**). Fatty fish such as sardines help the body burn fat in addition to generating a feeling of satiety. This satietogenic effect may explain the decrease in food intake in obese groups consuming sardine oil and explains the negative FER values.

Calcium and phosphorus:

Table 2 indicated that rats in groups 1, 3, 4 and 5 had significant ($P \leq 0.05$) differences in serum calcium as compared to the positive (+) control group. The mean values of calcium for groups 1, 3, 4 and 5 were: 11.71 ± 0.12 ; 12.18 ± 0.37 ; 11.74 ± 0.13 and 13.08 ± 0.27 , respectively vs. 8.16 ± 0.11 for the positive (+) control also group 5 recorded significant ($P \leq 0.05$) increase in serum calcium, as compared to the negative (-) control (13.08 ± 0.27 vs. 11.71 ± 0.12 respectively) and no significant differences ($P \leq 0.05$) were recorded of serum calcium between groups 1, 3 and 4.

Also the data revealed that rats in groups 1, 3, 4 and 5 had significant ($P \leq 0.05$) levels of serum phosphorus that differed from those of the positive (+) control group. The mean values of phosphorus for groups 1, 3, 4 and 5 were: 13.40 ± 0.21 ; 12.18 ± 0.37 ; 12.72 ± 0.13 and 12.91 ± 0.17 , respectively vs. 9.38 ± 0.15 for the positive (+) control, also groups 3 showed significant decrease ($P \leq 0.05$) as compared to the negative (-) control (12.18 ± 0.37 ; 12.72 ± 0.13 vs. 13.40 ± 0.21 respectively) and no significant differences ($P \leq 0.05$) were recorded of serum phosphorus between groups 1, 4 and 5.

De Nijs ., (2008) stated that bone loss is one of the most important side effects of glucocorticoid use, even in low doses. The main effect of glucocorticoids on bone is the inhibition of osteoblast function, leading to a decrease in bone formation. Also nongenomic effects (mediated by glucocorticoid interactions with biological membranes, either through binding to membrane receptors or by physicochemical interactions) may have a role in the pathogenesis of glucocorticoid-induced osteoporosis (GIOP). In addition secondary hyperparathyroidism induced by the negative balance of calcium due to inhibition of absorption and increase of excretion is an important systemic mechanism of GIOP (**Kaneko and Kawai ., 2011**).

The anti-inflammatory effects of *n*-3 FAs are well-known. Recently, a promising association between higher *n*-3 FA intake and improved bone turnover markers and bone mineral density (BMD) in humans has been reported (**Coetzee et al., 2007**). One of the mechanisms by which dietary *n*-3 fatty acids reduce bone loss in ovariectomized mice is by inhibition of osteoclast generation and activation (**Matsushita et al., 2008**).

McManus et al., (2010) stated that “seafood is a rich source of both calcium and Vitamin D, important bone-building micronutrients. High intake of sea fish is independently associated with greater bone mass and lower the osteoporosis risk in women, especially those consuming more than 250 grams per week of seafood. An abundance of nutrients can be found in mackerel, which include calcium, potassium, selenium and magnesium. These nutrients help maintain proper function of the body and are important for the heart, bones and teeth, nerves and muscles, and proper metabolic function. Mackerel is a good source of vitamins like niacin (vitamin B3), choline, folate, vitamin E, vitamin D, vitamin A, vitamin K, vitamin C and vitamin B12.

Calcium sources for these supplements include calcium carbonate ores, animal skeletons, seashells, and crustaceans. Therefore, other resources including marine resources, have gained attention due to their high safety and biological activity in recent years (**Martiniakova et al., 2022**). Similar results have been obtained in the study of **Brennan et al. (2017)** using a calcium-rich marine multimineral complex which significantly preserved trabecular bone microarchitecture and slowed the onset of bone loss in comparison with calcium carbonate.

Calcium, vitamin D and parathyroid hormone are basic controllers of bone redesigning (**Lu et al., 2013**). Serum calcium and phosphorus are ordinarily utilized as biochemical markers of bone

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development and building. The abatement in serum levels of calcium and phosphorus in rats experiencing osteoporosis actuated as detailed in this examination was like the past report (**Coxam, 2005**). The diminished serum calcium levels were additionally answered to be because of estrogen inadequacy in rats experiencing osteoporosis initiated (**Choi and Seo, 2013**).

The previous results were in line with **Soltan, (2013)** who showed that all treatments showed a significant increase ($p \leq 0.05$) in serum calcium and phosphorus. These results may be due to sardine fish having higher calcium content and being easily absorbed by the body. **Larsen et al. (2000)** reported calcium from fish would be easily absorbed by the body and the intake of small fish with bones could increase calcium bioavailability. **EL- Sayed, (2017)** observed that fish meat caused a significant increase ($P \leq 0.05$) in serum Ca and P, when contrasted with the positive control group.

Hemmattiet al., (2018) announced that cuttlebone is a natural compound with a high level of CaCO_3 , it tends to be utilized and figured by various fillers as a marine natural anti-Osteoporosis. **Negm, (2018)** showed that feeding rats with powder of cuttlefish bone caused a significant increase ($P \leq 0.05$) in serum Ca and P, when contrasted with the positive control group. The author of this study recommended using powder of cuttlebone for postmenopausal women suffering from osteoporosis.

Bone mineral density (BMD) and bone mineral content (BMC):

Table 3 revealed that osteoporosis significantly ($P \leq 0.05$) altered the bone mineral density (BMD) and bone mineral content (BMC) in female rats.

Rats in groups 1, 3, 4 and 5 had bone mineral density (BMD) that differed significantly ($P \leq 0.05$) from those of the positive (+) control. Mean values of bone mineral density (BMD) for groups 1, 3, 4 and 5

were: 0.181 ± 0.010 ; 0.122 ± 0.021 ; 0.138 ± 0.005 and 0.163 ± 0.007 , respectively vs. 0.076 ± 0.011 for the positive (+) control.

Also rats in groups 1, 4 and 5 had bone mineral content (BMC) that differed significantly ($P \leq 0.05$) from those of the positive (+) control. Mean values of bone mineral content (BMC) for groups 1, 4 and 5 were: 0.232 ± 0.004 ; 0.211 ± 0.008 and 0.228 ± 0.006 , respectively vs. 0.201 ± 0.012 for the positive (+) control. But no significant differences ($P \leq 0.05$) were found between groups 2 and 3 for bone mineral content (BMC).

The Glucocorticoid has harmful effects on bonedensity (**SedoSarkis et al., 2012**). Glucocorticoid induced osteoporosis leads to a suppression of bone formation by decreasing the number and function of osteoblast and induced bone loss (**DiMunno and DelleSeide, 2006**). There are many reported decreasing BMD and BMC in osteoporosis disease (**Wu et al., 2001 and Matsumoto et al., 2002**). These results are in agreement with the findings of **Soltan, (2013)** showed that the mean BMD and BMC of osteoporotic rats fed a diet supplemented with 20 %sardine fish. The increases in BMD and BMC due to increases in calcium and phosphorus in the diet lead to increases in osteoblast cells which lead to increases in bone formation. The previous results were in agreement with **Farina et al., (2011)** observed that Fish consumption may protect against bone loss. Also, **Luo et al., (2015)** showed that salmon calcitonin treatment was effective in delaying the process of disc degeneration in OVX rats. **Negm, (2018)** showed that bone mineral (content and density) significantly ($P \leq 0.05$) increased in rats fed a basal diet and supplemented with powder of cuttlefish bone.

Sensory evaluation for bread rolls:

Table 4 showed significant differences ($P \leq 0.05$) in the appearance of bread rolls between the control group and tested groups (except for bread rolls of sibiafish (group 4). values for appearance in groups 2 and 3 were: 2.50 ± 0.38 and 2.73 ± 0.29 , respectively vs. 4.65 ± 0.10 for the control but no significant differences were found between bread rolls of salmon fish(groups 2) and bread rolls of sardine fish(groups 3).

Likewise, the Interior color, Exterior color and Cells uniformity of bread rolls followed the same pattern as those of the appearance. The data also showed significant differences ($P \leq 0.05$) in the taste of bread rolls between the control group and all tested groups .values for taste in groups (2,3 and 4) were: 2.00 ± 0.25 ; 2.39 ± 0.27 and 3.36 ± 2.69 , respectively vs. 4.57 ± 0.11 for the control but no significant differences were found between groups 2 and 3.

Likewise, the odor and total acceptance of bread rolls followed the same pattern as those of the taste.

The bread rolls of Sibia fish powder had a higher appearance, interior color, exterior color, cells uniformity, taste, odor and total acceptance than the bread rolls of salmon and sardine fish powder.

Table (1): Effect of Sibia, Sardine and Salmon fish on body weight gain and food efficiency ratio (FER) in female rats with (BWG) osteoporosis.

Parameters Groups	BWG%	FER
G1: Control (-ve)	41.05 ^a ±1.43	0.16 ^{ab} ±0.137
G2: Control (+ve)	16.52 ^c ±1.50	0.05 ^c ±0.003
G3: 5% Salmon	21.80 ^{bc} ±1.22	0.13 ^{ab} ±0.004
G4: 5% Sardine	23.15 ^{bc} ±1.48	0.14 ^{ab} ±0.007
G5: 5% Sibia	30.61 ^{ab} ±1.12	0.15 ^b ±0.009

Values are expressed as means ± SD

Values at the same column with different letters are significantly different at P≤0.05.

Table(2): Effect of Sibia, Sardine and Salmon fish on levels of serum calcium and phosphorus in female rats with osteoporosis.

Parameters Groups	Ca mg/dl	P mg/dl
G1: Control (-ve)	11.71 ^b ±0.12	13.40 ^a ±0.21
G2: Control (+ve)	8.16 ^c ±0.11	9.38 ^c ±0.15
G3: 5% Salmon	12.18 ^b ±0.37	12.18 ^b ±.37
G4: 5% Sardine	11.74 ^b ±0.13	12.72 ^{ab} ±.13
G5: 5% Sibia	13.08 ^a ±0.27	12.91 ^a ±0.17

Values are expressed as means ± SD

Values at the same column with different letters are significantly different at P≤0.05.

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Table (3): Effect of Sibia, Sardine and Salmon fish on bone mineral density (BMD) and bone mineral content (BMC) infemale rats with osteoporosis.

Parameters	BMD g/cm ²	BMC g/cm
G1: Control (-ve)	0.181 ^a ±0.010	0.232 ^a ±0.004
G2: Control (+ve)	0.076 ^f ±0.011	0.201 ^c ±0.012
G3: 5% Salmon	0.122 ^d ±0.021	0.209 ^c ±0.009
G4: 5% Sardine	0.138 ^c ±0.005	0.211 ^b ±0.008
G5: 5% Sibia	0.163 ^b ±0.007	0.228 ^{ab} ±0.006

Values are expressed as means ± SD

Values at the same column with different letters are significantly different at P≤0.05.

Table (4): Effect of Sibia, Sardine and Salmon fish on Sensory evaluation for bread rolls.

	Appearance (5)	Interior color (5)	Exterior color (5)	Cells uniformity (5)	Taste (5)	Odor (5)	Total Acceptance (5)
G1: Control	4.65±0.10 ^a	4.78±0.09 ^a	4.57±0.13 ^a	4.23±0.18 ^a	4.57±0.11 ^a	4.55±0.13 ^a	4.63±0.17 ^a
G2: Salamone	2.50±0.38 ^b	2.65±0.32 ^b	2.36±0.34 ^b	3.05±0.35 ^b	2.00±0.25 ^c	2.26±0.32 ^c	2.34±0.31 ^c
G3: Sardine	2.73±0.29 ^b	2.76±0.20 ^b	2.65±0.25 ^b	3.26±0.28 ^{ab}	2.39±0.27 ^c	2.15±0.21 ^c	2.31±0.24 ^c
G4: Sibia	4.62±0.15 ^a	4.36±0.20 ^a	4.50±0.15 ^a	4.13±0.14 ^a	3.36±2.69 ^b	3.36±0.28 ^b	3.76±0.23 ^b

Values are expressed as means ± SD

Values at the same column with different letters are significantly different at P≤0.05.



(control)



(Sibia)

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(Sardine)



(Salmon)

References

AOAC (Association of official chemists) international(2005):

Official methods of analysis of AOAC international, 18th Ed.,AOAC international Gaithersburg, Maryland, USA.

Brennan O.; Sweeney J.; O'meara B.; Widaa A.; Bonnier F.; Byrne H. J. and O'Brien F. J., (2017):

A natural, calcium-rich marine multi-mineral complex preserves bone structure, composition and strength in an ovariectomised rat model of osteoporosis. *Calcified Tissue International.*; 101(4): 445-455.

Calderon-Garcia J. F.; Moran J. M.; Roncero-Martin R.; Rey-Sanchez P.; Rodriguez-Velasco F. J. and Pedrera-Zamorano J. D., (2013):

Dietary habits, nutrients and bone mass in Spanish premenopausal women: the contribution of fish to better bone health. *Nutrients.*; 5(1): 10-22.

Chapman D.G.; Gastilla R. and Campbell J. A., (1959):

Evaluation of protein in foods: 1- A Method for the determination of protein efficiency ratio. *Can. Journal Biochem. Phy.*;37(4).6). Pp:679-686.

Choi M. J. and Seo J. N., (2013):

Effect of taurine feeding on bone mineral density and bone markers in rats. In *Taurine.*; 8: (pp. 51-58). Springer, New York, NY.

Chuamani P., (2010):

Calcium Enrichment in Crispy Snacks by Using Fish Bone Powder. Suratthani: RachaphatSurathani University.

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Coetzee M.; Haag M. and Kruger M. C., (2007):

Effects of arachidonic acid, docosahexaenoic acid, prostaglandin E2 and parathyroid hormone on osteoprotegerin and RANKL secretion by MC3T3-E1 osteoblast-like cells. *The Journal of nutritional biochemistry.*; 18(1): 54-63.

Coxam V., (2005):

New advances in osteoporosis nutritional prevention. *Medecine Sciences: M/S.*; 21(3): 297-301.

Daidj N. B. B. and Lamri-Senhadji M., (2021):

Hepatoprotective and Anti-Obesity Properties of Sardine By-Product Oil in Rats Fed a High-Fat Diet. *Preventive Nutrition and Food Science.*; 26(3): 285–295.

De Nijs R. N. J., (2008):

Glucocorticoid-induced osteoporosis: a review on pathophysiology and treatment options. *Minerva medica.*; 99(1): 23-43.

**De Sibio M. T.; Luvizotto R. A. M.; Olimpio R. M. C.;Correˆa C. R.;
Marino J.; de Oliveira M. and Nogueira C. R., (2013):**

A comparative genotoxicity study of a supraphysiological dose of triiodothyronine (T3) in obese rats subjected to either calorie-restricted diet or hyperthyroidism. *PLoS One.*;8(2): e56913.

Di Munno O. and DelleSedie A., (2006):

Glucocorticoid-induced osteoporosis and rheumatic diseases. Pathogenesis, prevention and treatment. *Reumatismo.*; 58(1): 11-21.

El-Merzabani M.; El-Aaser A. and Zakhary N., (1977):

A new method for determination of inorganic phosphorus in serum without deproteinization. *J ClinChemClinBiochem.*; 15:715-718.

El-Sayed Mahmoud EL-Serawy E.,(2017):

Effect of garlic oil and fish on glucocorticoid-induced osteoporosis in rats. *The Egyptian Journal of Specialized Studies.*; 5(16): 43-63.

Fan F.; Xue W. Q.; Wu B. H.; He M. G.; Xie H. L.; Ouyang W. F. and Chen Y. M., (2013):

Higher fish intake is associated with a lower risk of hip fractures in Chinese men and women: a matched case-control study. *PloSone.*; 8(2): e56849.

Farina E. K.; Kiel D. P.; Roubenoff R.; Schaefer E. J.; Cupples L. A. and Tucker K. L., (2011):

Protective effects of fish intake and interactive effects of long-chain polyunsaturated fatty acid intakes on hip bone mineral density in older adults: the Framingham Osteoporosis Study. *The American journal of clinical nutrition.*; 93(5): 1142-1151.

Gao Z.; Yang L.; Huang F.; Xiong A.; Zhou N.; Zhou L.; Li K.; Deng J. ;Li K.; Liu W.; Chen Y.; Luo W. and Nie H., (2013):

Effects of different extracts of kanggushu on osteoporosis in model rats and the underlying mechanisms. *Chin. J. Integr. Med.*; (19):844–852.

Gindler M. and King J., (1972):

Chemical method for determination of calcium in serum. *Am J ClinPathol.*; 58:376.

**Omnia G. Refaat, Hany G. El-Masry , Eman S. Ibrahim
And Ola A. Abd El-Khalek**

Gisslen W., (2007):

Professional cooking.7thEd.,JohnWiley&Sons, Inc, USA. pp:
963-944.

Gouda S.M.H., (2012):

Effect of flaxseeds, pumpkin seeds and sesame seed on bone
of rats suffering from osteoporosis. PhD Thesis, Nutrition &
Food Science, Home Economy Department, Faculty of
Education, Ain Shams University, Egypt.

**HemmattiA.;MostoufiA.; Shakiba N.; Khrosgani Z. and
Memarzade S.,(2018):**

Preparation of the edible supplement product of calcium-D in
form of tablet from powder of sepia skeleton (cuttlebone) and
investigation of its physic-chemical properties. World Family
Medicine. ; 16(3):158-165.

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.

**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
(Theragrachalcogramma) backbone by pepsinolytic
hydrolysis. *Process Biochemistry.*; 41(9): 2097-2100.

Kaneko K. and Kawai S., (2011):

Mechanisms and therapeutics of glucocorticoid-induced
osteoporosis. *Nihon RinshoMen'ekiGakkaiKaishi= Japanese
Journal of Clinical Immunology.*; 34(3): 138-148.

Kim S. K. and Mendis E., (2006):

Bioactive compounds from marine processing byproducts—a review. *Food Research International.*; 39(4): 383-393.

Klein B., (1984):

The experimental study of food. University of Illinois, USA, pp: 82.

Larsen T.; Thilsted S. H.; Kongsbak K. and Hansen M., (2000):

Whole small fish as a rich calcium source. *British Journal of Nutrition.*; 83(2): 191-196.

Liao J.; Li Q.; Wu T.; Hu B.; Huang L.; Li Z.; Zhao W.; Zhang M. and Zhong S., (2003):

Effects of prednisone on bone mineral density and biomechanical characteristics of the femora and lumbar vertebrae in rats. Institute of Clinical Anatomy, First Military Medical University, Guangzhou 510515, China. *Di Yi Jun Yi Da Xue Xue Bao.*; 23(2):97-100.

Liaset B.; Julshamn K. and Espe M., (2003):

Chemical composition and theoretical nutritional evaluation of the produced fractions from enzymic hydrolysis of salmon frames with Protamex™. *Process Biochemistry.*; 38(12): 1747-1759.

Lu M.; Farnebo L. O.; Bränström R. and Larsson C., (2013):

Inhibition of parathyroid hormone secretion by caffeine in human parathyroid cells. *The Journal of Clinical Endocrinology & Metabolism.*; 98(8): E1345-E1351.

**Omnia G. Refaat, Hany G. El-Masry , Eman S. Ibrahim
And Ola A. Abd El-Khalek**

Luo Y.; Zhang L.; Wang W. Y.; Hu Q. F.; Song H. P. and Zhang Y. Z., (2015):

The inhibitory effect of salmon calcitonin on intervertebral disc degeneration in an ovariectomized rat model. *European Spine Journal.*; 24(8): 1691-1701.

Luu P. H. and Nguyen M. H., (2009):

Recovery and utilization of calcium from fish bones byproducts as a rich calcium source. *Journal of Science and Technology-Vietnam Academic of Science and Technology.*; 47(6): 91-103.

Malde M. K.; Bügel S.; Kristensen M.; Malde K.; Graff I. E. and Pedersen J. I., (2010b).

Calcium from salmon and cod bone is well absorbed in young healthy men: a double-blinded randomised crossover design. *Nutrition & metabolism.*; 7(1): 61.

Malde M. K.; Graff I. E.; Siljander-Rasi H.; Venäläinen E.; Julshamn K.; Pedersen J. I. and Valaja J., (2010 a):

Fish bones—a highly available calcium source for growing pigs. *Journal of animal physiology and animal nutrition.*; 94(5): e66-e76.

Martiniakova M.; Babikova M.; Mondockova V.; Blahova J.; Kovacova V. and Omelka R., (2022):

The Role of Macronutrients, Micronutrients and Flavonoid Polyphenols in the Prevention and Treatment of Osteoporosis. *Nutrients.*; 14(3): 523.

Matsumoto H.; Jiang G. Z.; Hashimoto T.; Kuboyama N.; Yamane J.; Nonaka K. and Fujii A., (2002):

Effect of organic germanium compound (Ge-132) on experimental osteoporosis in rats: the relationship between transverse strength and bone mineral density (BMD) or bone mineral content (BMC). *International Journal of Oral-Medical Sciences.*; 1(1): 10-16.

Matsushita H.; Barrios J. A.; Shea J. E. and Miller S. C., (2008):

Dietary fish oil results in a greater bone mass and bone formation indices in aged ovariectomized rats. *Journal of bone and mineral metabolism.*;26(3):241-247.

McManus A. and Merga M., (2010):

Seafood: Nutritional gold for seniors. *Australasian Medical Journal.*; 13(3): 855-859.

Negm S. H., (2018):

The possible protective role of powder cuttlefish bone, crabshell and eggshell on osteoporotic rats. *Journal of Food and Dairy Sciences.*; 111-121.

Nemati M.; Huda N. and Ariffin F., (2017):

Development of calcium supplement from fish bone wastes of yellowfin tuna (*Thunnusalbacares*) and characterization of nutritional quality. *International Food Research Journal.*; 24(6): 2419-2426.

North L.; Labonte D.; Oyen M.; Coleman M.; CaliskanH.andJohnstonR.,(2017):

Interrelated chemical-microstructural nano-mechanical variations in the structural units of the cuttlebone of *Sepia officinalis*. *APL MATERIALS.*; 5, 116103.

**Omnia G. Refaat, Hany G. El-Masry , Eman S. Ibrahim
And Ola A. Abd El-Khalek**

**Oliveira M. L.; Bergamaschi C. T.; Silva O. L.; Nonaka K. O.;
Wang C. C.; Carvalho A. B. and Lazaretti-Castro M.,
(2010):**

Mechanical vibration preserves bone structure in rats treated with glucocorticoids. *Bone.*; 46(6): 1516-1521.

Penfield M. and Campbell A.M.,(1990):

Experimental food science,3rd Ed., Academic press,Inc.London.pp:33-34.

**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. *Songklanakarin J. Sci. Technol.*; 28(2): 327-335.

Sandnes K.; Pedersen K. and Hagen H., (2003):

Kontinuerligenzymprosesseringavferske marine biprodukter Trondheim. Norway. *Stiftelsen RUBIN.*; 28.

**SedoSarkis K. M.; Medeiros PinheiroV. ;LuuaSzejnfeld. and L.
Araujo Martini., (2012):**

High bone density and bone health. *Endocrinology Nutrition.*; 59(3): 207-214.

**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.

Soltan S. S., (2013):

The effects of skimmed milk, soybean flour and sardine fish powder on osteoporotic female rats. *Middle East J Sci Res.*; 15: 984-97.

SPSS., (1986):

"Statistical package for social science". version 11. SPSSInc., II. U.S.A.

Stevanato F. B.; Almeida V. V.; Matsushita M.; Oliveira C. C.; SouzaN. E. and Visentainer J. V., (2008):

Fatty acids and nutrients in the flour made from tilapia (*Oreochromis niloticus*) heads. *Food Science and Technology.*; 28(2): 440-443.

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(1-4): 636-645.

Toppe J.; Albrektsen 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: Biochemistry and Molecular Biology.*; 146(3): 395-401.

Wu J.; Wang X. X.; Takasaki M.; Ohta A.; Higuchi M. and Ishimi Y., (2001):

Cooperative effects of exercise training and genistein administration on bone mass in ovariectomized mice. *Journal of Bone and Mineral Research.*; 16(10): 1829-1836.

Xia W. B.; He S. L.; Xu L.; Liu A. M.; Jiang Y.; Li M. and Cummings S. R., (2012):

Rapidly increasing rates of hip fracture in Beijing. *China Journal of Bone and Mineral Research.*; 27(1): 125-129.

Zhao Z.; Liang Z. and Ping G., (2011):

Macroscopic identification of Chinese medicinal materials: traditional experiences and modern understanding. *J Ethnopharmacol.*; 134:556–564.

تقييم تأثير التدعيم بأسمك السيبيا والسردين والسلمون كمضادات لهشاشة
العظام على الخصائص العامة لخبز الفينو

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الملخص العربى

تدعيم الأطعمة الرئيسية بشكل عام يعتبر طريقة فعالة لتوفير المتطلبات اليومية لمجموعة من الفيتامينات والمعادن. تهدف الدراسة الحالية الى معرفة مدى قبول خبز الفينو المدعم بعظام الأسماك للوقاية من هشاشة العظام. تم عمل أربع عينات من خبز الفينو باستخدام مسحوق عظم السمك بنسبة 5% من إجمالي المكونات كما يلي: العينة الأولى وهي العينة الضابطة والعينة الثانية التي تحتوي على مسحوق عظام سمك السلمون بنسبة 5% والعينة الثالثة والتي تحتوي على مسحوق عظام سمك السردين بنسبة 5% ، والعينة الرابعة التي تحتوي على مسحوق عظم سمك السيبيا بنسبة 5%. ثم تم التقييم الحسي لهذه العينات من حيث المظهر واللون الداخلي واللون الخارجي والمسام والطعم والرائحة والقبول العام. أظهرت النتائج أن خبز الفينو المضاف إليه مسحوق عظام سمك السيبيا أقرب إلى العينة الضابطة في الخواص باستثناء الطعم والرائحة والقبول العام. وتم دراسة تأثير اسمك السيبيا والسردين والسلمون على كالسيوم وفسفور الدم ايضا بالإضافة الى قياس محتوى وكثافة العظام من الأملاح المعدنية وظهرت النتائج أن الثلاث انواع من الأسماك وخاصة السيبيا كانت لها نتيجة ايجابية بالإضافة الى زيادة كاملة فى الكالسيوم والفسفور وفى محتوى وكثافة الاملاح المعدنية فى العظام. وفي الختام ، الثلاثة أنواع من الأسماك خاصة سمك السيبيا احدثت تراجع للفئران من حدوث هشاشة العظام وذلك لمحتواها من فيتامين د والكالسيوم والفسفور لذلك فهي مفيدة للبشرية.

الكلمات المفتاحية : سيبيا ، سردين ، سلمون ، بريدنيزون اسيتات ، هشاشة العظام.