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## Document heading

# Calcium and phosphorus determination in bones of low value fishes, *Sardinella longiceps* (Valenciennes) and *Trichiurus savala* (Cuvier), from Parangipettai, Southeast Coast of India

Logesh AR\*, Pravinkumar M, Raffi SM, Kalaiselvam M

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai–608502, India

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## ABSTRACT

**Objective:** To investigate calcium and phosphorus levels in the bones of low value fishes *Sardinella longiceps* (oil sardine) and *Trichiurus savala* (ribbon fish). **Methods:** Bones and skeletal remains were subjected to alkaline–alcohol treatment for specified period of time and were then dried and pulverized. Calcium and phosphorus levels were determined by both volumetric and instrumental methods using atomic absorption spectroscopy. **Results:** Volumetric analysis of calcium and phosphorus were 28.98% and 14.2% in oil sardine; 24.2% and 11.6% in ribbon fish respectively. Atomic absorption spectroscopy analysis of calcium and phosphorus were 32.73% and 17.2% for oil sardine and 27.17% and 10.83% for ribbon fish respectively. Protein level was 4.82% in oil sardine and 3.97% in ribbon fishes. **Conclusions:** The findings of the present study revealed that sardine bones are rich in calcium and will be utilized for the production of calcium powder to treat osteoporosis.

## 1. Introduction

India is bestowed with multispecies multisector marine fishery resources. Oil sardine fishery represented mainly by *Sardinella longiceps* Val. (Clupeidae) (*S. longiceps*) forms the mainstay of Indian marine fisheries and contributes nearly one third of the total marine fish production of India in productive years<sup>[1]</sup>. Oil sardines are coastal, pelagic, tropical schooling fish and recent data shows that it contributes to 12.4% of 3.16 million tons of total marine fishery production of India<sup>[2]</sup>.

Fish bone, the non–edible part separated after removal of muscle proteins from the skeletal frame, is a valuable store house of different health–promoting components. The organic component of fish bone, which accounts for 30% of the material, is made up of collagen<sup>[3]</sup>. Therefore, fish bone is considered as an excellent source for the isolation

of collagen and gelatin<sup>[4]</sup>. Fish bone consists of 60%–70% of inorganic substances and is mainly comprised of calcium phosphate and hydroxyapatite. Fish bone is a potential source of calcium but limited studies have been conducted so far on the bioavailability of calcium from fish bone and its potential applications.

Calcium is one of the essential mineral required for human health. Bones and teeth contribute approximately 99% of total body calcium and rest is found in the blood. Calcium plays a vital role in various physiological activities such as nerve conduction, muscle contraction, and blood clotting. When the blood calcium level drops in the body, it will be replaced from the bones so as to maintain the level. Deficiency of calcium will lead to deformity of bones called osteoporosis. Calcium is generally obtained from the diet and it is severely deficient in most of regular diets. Therefore, it is essential to have calcium along with the food as a supplement. Several calcium–fortified products and tablets are mushroomed in the market and demands for these products are growing at a faster price.

Most of these products are tagged higher price that makes it difficult for the economically downtrodden to utilize it. Paucity of raw materials and complicated methodologies

\*Corresponding author: Logesh AR, Doctoral Research Student, Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai – 608502, India.

Tel: +91 9443 654 597

E-mail: [marinefungi@gmail.com](mailto:marinefungi@gmail.com)

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involved in its production are two of the major reasons for keeping its price to higher end despite of commercial motive.

Investigations pertaining to the bioavailability of calcium from other resources which are relatively cheaper and are available round the year is found wanting. In this backdrop, an attempt has been made in the present study so as to check the availability of calcium in the bones and skeletal remain of low value fishes such as *S. longiceps* (Oil sardine) and *Trichiurus savala* (*T. savala*, Ribbon fish).

## 2. Materials and methods

### 2.1. Sample collection

Low value fishes such as *S. longiceps* (Oil sardine) and *T. savala* (Ribbon fish) were procured from the fish landings in Parangipettai, Tamil Nadu (latitude 11°24' N, longitude 79°46' E).

### 2.2. Methods

The fishes were degutted and tissues were removed. The skeletal remains of oil sardine and ribbon fishes were taken separately. Bones of each fish (1 000 g) were transferred to 1 000 mL beaker separately and bones were treated with different concentration of sodium hydroxide and ethanol with different immersion time. In treatment I, bones were immersed in 0.5 mol/L sodium hydroxide for 20 h and then in 40% (v/v) ethanol solution for 15 h. In treatment II, bones were immersed in 1.0 mol/L sodium hydroxide for 30 h and then in 60% (v/v) ethanol solution for 10 h. Orthogonal experiment was conducted to analyze the yield. At the end, bones subjected to alkaline–alcohol treatment were washed with demineralized water and kept in an oven for about 2 h at 80 °C for drying.

The bones were then pulverized into finer particles using a pestle and mortar. Final yield of the bone powder of each experiment was recorded. Volumetric analysis of bone powder was conducted for calcium and phosphorus determination following the method of Huang[5]. The powder was further analyzed by atomic absorption spectroscopy (Varian–VAS–0091) for calcium and phosphorus levels. Protein content was determined by micro–Kjeldahl method[6] and fat content was determined by Soxlet extraction method.

## 3. Results

*S. longiceps* and *T. savala* bones were taken as raw materials and were subjected to alkaline–alcohol treatment. The yield obtained after orthogonal experiment were detailed (Figure 1). The maximum yield was obtained in treatment II with comparatively higher yield for oil sardine [(7.0±0.2)%].

Calcium and phosphorus were analyzed by volumetric methods and atomic absorption spectrometry and were detailed in Table 1. Among the two orthogonal experiments, treatment II resulted in higher levels of calcium (32.73%) and phosphorus (17.2%) for oil sardine and 27.81% and 10.83% for ribbon fish. From the results, it is inferred that treatment II was found to procure comparatively higher levels of calcium and phosphorus in both the fish samples. Further, bone powder underwent treatment II was analyzed for protein, fat and water content and the results are expressed in Figure 2.

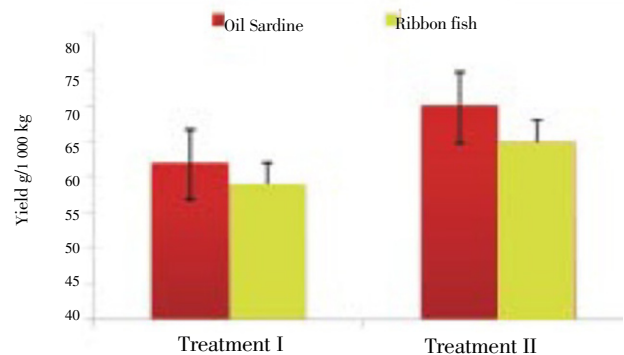


Figure 1. Yield of bone powder obtained between the treatments (values in g/1000g of raw bones).

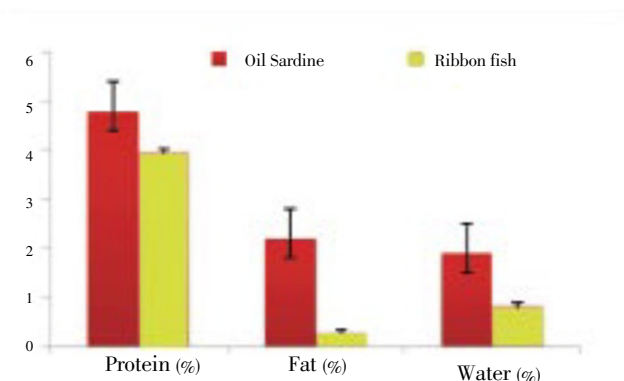


Figure 2. Percentage wise details of protein, fat and water for bone powder obtained from treatment–II.

Table 1

Levels of Calcium and phosphorus obtained in AAS and volumetric methods (%).

Species	Treatment No	AAS		Volumetric methods	
		Calcium	Phosphorus	Calcium	Phosphorus
Oil Sardine	1	26.39	16.57	25.64	9.61
Oil Sardine	2	32.73	17.2	28.98	14.2
Ribbon fish	1	19.33	7.21	18.91	7.36
Ribbon fish	2	27.81	10.83	24.2	11.6

## 4. Discussion

Jiancong *et al.* have conducted the orthogonal experiment on haddock (*Melanogrammus aeglefinus*) fish bones with different concentrations and immersion times in sodium

hydroxide and ethanol, and they recorded maximum yield of 23.7% in the experimental setup with 1 mol/L sodium hydroxide for 30 h and 40% (v/v) ethanol for 15 h[7]. However, in the present investigation, the bone powder obtained from sardine bones exhibited higher calcium content than that obtained from haddock bone and was purely whitish without any odor. Changhu *et al.* estimated about 38.27% of calcium and 17.73% of phosphorus from the Pollack fish bones (*Pollachius pollachius*) and the ratio of calcium/phosphorus being 2:1, which is similar to that of human bones[8]. The results of the present findings showed a similar trend in the case of sardine bones under treatment II. Xu investigated the calcium powder from the freshwater fishes through the process of heating, saponification and degrease and degumming obtained comparatively less results[9]. Osteoporosis is dreadful skeletal disease, formed due to calcium deficiency characterized by low bone mass, increase of bone fragility and susceptibility to fracture that leads to irreversible deterioration of bone structure.

Osteoporosis is becoming widespread in recent years due to calcium malnourishment. From the results of present investigation, it is quite evinced that the bones of low value fish like *S. longiceps* are rich in calcium; it should be recommended for human consumption either directly or indirectly by making use of it as a raw material for the production of calcium tablets. Since oil sardines are landed in large quantities throughout the year and are available at cheaper price, sustainable and judicious utilization of its skeletal remains for the production of calcium tablets are warranted.

From the results, it is proved that oil sardine bones have high amount of calcium. Both volumetric and atomic absorption spectrometry analysis proved that treatment II was found to be an excellent method for the extraction of calcium and phosphorus. From the present findings, it is safe to conclude that sardine bones serve as an excellent calcium storehouse and it will be utilized for the production of calcium tablets.

## Conflict of interest statement

We declare that we have no conflict of interest.

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