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# Chemical composition, mineral content and amino acid and lipid profiles in bones from various fish species

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**Abstract**

The chemical composition, content of minerals and the profiles of amino acids and fatty acids were analyzed in fish bones from eight different species of fish. Fish bones varied significantly in chemical composition. The main difference was lipid content ranging from 23 g/kg in cod (*Gadus morhua*) to 509 g/kg in mackerel (*Scomber scombrus*). In general fatty fish species showed higher lipid levels in the bones compared to lean fish species. Similarly, lower levels of protein and ash were observed in bones from fatty fish species. Protein levels differed from 363 g/kg lipid free dry matter (dm) to 568 g/kg lipid free dm with a concomitant inverse difference in ash content. Ash to protein ratio differed from 0.78 to 1.71 with the lowest level in fish that naturally have highest swimming and physical activity. Saithe (*Pollachius virens*) and salmon (*Salmo salar*) were found to be significantly different in the levels of lipid, protein and ash, and ash/protein ratio in the bones. Only small differences were observed in the level of amino acids although species specific differences were observed. The levels of Ca and P in lipid free fish bones were about the same in all species analyzed. Fatty acid profile differed in relation to total lipid levels in the fish bones, but some minor differences between fish species were observed.


*Keywords:* Amino acids; Chemical composition; Fatty acids; Fish bone; Lipids; Minerals

## 1. Introduction

Bones are mainly regarded as rich in minerals such as calcium and phosphorus and collagen proteins, but some special carbohydrate and lipids are also found (Johns, 1977). The bone tissue is mainly built up of an organic extra cellular matrix covered with hydroxyapatite ($Ca_5(PO_4)_3OH_2$). Bone tissue is an important depot for storage of calcium and phosphates and is essential in the regulation of plasma concentrations of these minerals (Nordin, 1976). Due to the physiological importance of calcium and phosphorous in the soft tissues, calcium and phosphate present in the bones may be relocated to other tissues when the dietary supply do not meet the requirement. Bones constitute a significant part of the fish; approximately 10–15% of total fish biomass are bones from the head and vertebrae. Knowledge of the chemical composition of fish bones is limited, but may be interesting for several reasons. In a recent study dried fish bones were used as feed ingredient in diets for cod, showing a positive effect on growth and feed efficiency compared to traditional diets (Toppe et al., 2006). Fish meal produced from fish by-products or whole fish contains about 10% minerals, especially high in calcium and phosphorus, and represent an important source of minerals when included in feed. The digestibility of minerals from fish meal shows great variation, but is generally low, and the presence of phytic acid from vegetable protein sources may further decrease the availability. The level of available phosphorus in feeds for aquaculture might be limited for optimal growth and fish health (Nordum et al., 1997). However, processing of fish bones may improve the availability of the present minerals (Aksnes, unpublished). Fish bones represent a significant part of the cut offs from the filleting industry and a better utilization of this raw material for various applications is a matter of great scientific interest. Bone minerals for human health products may be of interest as well as the protein from the collagen bone matrix (Liaset et al., 2003; Kim and Mendis, 2006).

Information about the chemical composition of fish bones is therefore of interest for several reasons. It is important in

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understanding the physiological role of bone tissue, and for evaluating the potential of processing bone rich by-products from the fish processing industry. Bones from whole fish and cut offs could be used as a raw material for health products and as ingredient in feeds for aquaculture (Toppe et al., 2006). The aim of the present work is to obtain more information about the differences in chemical composition in bones from various fish species with special focus on fish species of interest for aquaculture, either as farmed fish or as a potential raw material for fish feed.

## 2. Materials and methods

### 2.1. Biological material

Thirteen different samples of fish were collected and stored on ice or at −20 °C before being processed to separate the bones from the remaining fish. The samples represented eight different species, including three samples of saithe, three samples of salmon and two samples of herring. The fish samples represent different locations, seasons or size (Table 1). All the samples were collected from coastal areas of western Norway and the Norwegian sea. The salmon, trout, mackerel, herring (small) and saithe were purchased from local seafood retailers. The cod was farmed at local facilities at Austevoll, Bergen. The remaining three samples were fish used for industrial processing; blue whiting, herring (big) and horse mackerel caught in the North Sea. Further details of the samples are shown in Table 1.

### 2.2. Processing

Before processing, the frozen samples where thawed overnight at 15 °C. One sample of fish was processed at a time, consisting of minimum 5 fish, and a total mass of at least 4.5 kg. The fish was gutted, filleted and gills were removed. As much as possible of the remaining meat was removed by scraping the backbone with a small knife. A casserole of 20 L was filled with 15 L of water and the bones and heads were put into the boiling water. The temperature of the water then dropped to 80–85 °C. After simmering for 2.5 min, the bones and heads were taken out, and the remaining meat on the bones could easily be removed by using running cold water. For the largest fish samples (salmon, trout, saithe and cod) the heads were allowed to simmer for an additional minute, allowing all the meat to coagulate and subsequently easily be removed.

The clean bones where then frozen at −20 °C and lyophilized (final plate temperature 24 °C). The pressure in the freeze dryer was equalized to atmospheric pressure with nitrogen gas, to avoid oxidation of components in the samples. The dried samples were then ground in a Retsch mill (<1 mm filter bags).

### 2.3. Analysis

Crude protein ($N \times 6.25$) was determined by the Kjeldahl method (ISO 5983-1997), moisture gravimetrically after drying for 4 h at 105 °C (ISO 6496-1999) and ash after combustion for 16 h at 550 °C (ISO 5984-2002). Phosphorus was determined by a spectrometric method (ISO 6491-1998) and calcium by atomic absorption spectrometry (ISO 6869-2000). The remaining mineral analyses were done by ICP-MS. Samples for analyses of total amino acids were hydrolyzed in 6 M HCl for 22 h at 110 °C and analyzed by HPLC using a fluorescence technique for detection (Cohen and Michaud, 1993). Total lipid was determined both by Soxhlet extraction (AOCS, Ba 3-38) and the Bligh and Dyer method (Bligh and Dyer, 1959). Fatty acid analyses were carried out by AOCS Official Method (Ce 1b-89). All analyses were carried out in duplicate samples of at least 5 fish each. Data are given as mean±standard deviation. Where analyses of sample parallels differed significantly, analyses were repeated. All analyses, except ICP-MS analyses were run at Fiskeriforskning, Bergen. Eurofins Norway arranged for the ICP-MS analyses. Proximate analyses (except Bligh and Dyer method) and analyses on phosphorus are accredited analyses. Analytical data were subjected to a one-way analysis of variance (ANOVA) using SYSTAT 5.0 for

Table 1
Fish samples used for analyses of fish bones

| Species | Latin name | Origin in Norway | Date of catch | Number of fish | Average fish mass (g/fish) |
|---|---|---|---|---|---|
| Cod[ab] | *Gadus morhua* | Austevoll | 20 July 2004 | 5 | 1566 |
| Saithe1[bd] | *Pollachius virens* | Sotra | 10 September 2004 | 6 | 1230 |
| Saithe2[bd] | *Pollachius virens* | Nordhordland | 18 October 2006 | 6 | 1270 |
| Saithe3 (big)[bd] | *Pollachius virens* | Nordhordland | 11 October 2006 | 6 | 2714 |
| Blue whiting[c] | *Micromesistius poutassou* | North Sea | 28 June 2003 | 65 | 69 |
| Salmon1[abd] | *Salmo salar* | Frøyfjord | 5 July 2004 | 6 | 2495 |
| Salmon2[abd] | *Salmo salar* | Sekkingstad | 11 October 2006 | 6 | 2709 |
| Salmon3[abd] | *Salmo salar* | Brandasund | 18 October 2006 | 6 | 2714 |
| Trout[abd] | *Salmo trutta* | Sognefjord | 8 July 2004 | 6 | 2567 |
| Herring1 (small)[b] | *Clupea harengus* | Hardangerfjord | 11 July 2004 | 75 | 63 |
| Herring2 (big) [c] | *Clupea harengus* | North Sea | October 2004 | 14 | 369 |
| Mackerel[b] | *Scomber scombrus* | Hardangerfjord | 18 July 2004 | 15 | 326 |
| Horse mackerel[c] | *Trachurus trachurus* | North Sea | November 2004 | 12 | 429 |

[a]Farmed.
[b]Stored on ice before processing.
[c]Stored at −20 °C before processing.
[d]Gutted.