The paper presents the technology of processing Baltic cod (Gadus morhua callarias) and Atlantic salmon (Salmo salar) backbones – by-products from fish processing – for the production of calcium preparations. The raw material for the process consisted of backbones with remaining muscle tissue, devoid of heads and fins (30 kg of each type of backbones). The processing included the following stages: cutting, preliminary processing in an alkaline environment (soaking in 2 M NaOH), processing with 0.1% citric acid, aroma removal and material disinfection (5% H₂O₂), rinsing with tap water, drying, and grinding the end-product. Calcium preparations from cod (BCP) and salmon (ASP) in the amounts of 1.20 kg and 1.62 kg, respectively, characterized by high calcium content (27.79% in BCP, and 24.92% in ASP) and low protein and fat content (14.20% and 0.25% for BCP, and 10.78% and 0.12% for ASP, respectively) were obtained. The study demonstrated the effectiveness of this technology for production of calcium preparations from fish backbones.

1 Introduction

There were 179.9 thousand saltwater fish and invertebrate catches in Poland in 2011, including 120.6 and 59.1 thousand tons of Baltic fishing and deep-sea fishing, respectively. [1]. Fish processing is currently one of the most dynamically developing food industry sectors in Poland [2]. The magnitude of production in Polish processing plants over the last ten years was within the range of 274–385 thousand tons of fish per year [3,4]. Nearly 70% of this amount of fish is subjected to processing before it enters the market. According to the data of the General Veterinary Inspectorate, 306 processing plants, fulfilling the Union requirements, were registered in Poland by the end of February 2014.

The process of preliminary processing involves numerous tasks, including stunning, grading, slime removal, scale removal, washing, beheading, gutting, cutting of fins, filleting combined with the separation of backbones, and the removal of skin [5]. Numerous by-products are generated during the preliminary processing operation, including heads, backbones, skin, viscera and fins. It is estimated that by-products generated during processing constitute 30–60% of the fish body weight, depending on the processing and fish species. During the process of salmon filleting at a typical automatic line, the fillets constitute 59–63% of fish body weight, while the rest is the by-product which includes backbones (9–15%), heads (10–12%) and fins (1–2%) [6].

By-products from the fish industry are destined for processing or for the landfill. In Norway, in 2009, 77% of fish processing by-products were managed, and 23% were sent to the landfill [7]. The main way to utilize fish by-products is to produce fodder material: meal and fish oil [8] as well as fish hydrolysate (fish silage). The main producer is Norway [9,10]. Fish meal production has increased considerably in recent years, and 25% of the total fish meal mass (1.23 million tons) is produced from fish processing by-products [11]. The main producers are Peru, Chile and China.
By-products contain many valuable compounds including proteins (collagen, enzymes, bioactive peptides, amino acids), fats (fish oil containing omega-3 and omega-6 fatty acids, including EPA and DHA, phospholipids, fat-soluble vitamins, squalene, cholesterol), as well as other important compounds such as taurine and astaxanthin [7,12].

In recent years there has been an intense search for new methods and technologies to obtain bioactive compounds (so-called “value added products”) for food (nutraceutics) from various organic raw materials and for biomedical purposes [10,13-15]. Reports indicate that hard products obtained after the fish filleting process has been completed are used as a source of calcium and phosphorus compounds [16-20]. Fish bone tissue dry matter has a 60−70% mineral content, mainly calcium phosphate and hydroxyapatite [21,22], while calcium content in mineral mass is at a level of 60% [23]. The remaining muscle tissue (meat) should be removed first for backbones obtained after filleting for the production of mineral preparations. For example, backbones obtained after the Atlantic cod is filleted contain 15% of pure bone tissue and as much as 85% meat [24].

There are various technologies of hard fish by-product processing that allow to obtain mineral preparations, mainly calcium. These have been tried on animals to establish the minerals’ bioavailability and biochemical properties [18,25-28].

This study develops the technology of processing of the Baltic cod (Gadus morhua callarias) and the Atlantic salmon (Salmo salar) backbones, by-products of fish processing, and the production of calcium preparations, the latter of which could be applied in the food or pharmaceutical industry as a source of available natural calcium.

2 Experimental procedure

2.1 Materials

We examined fish backbones with remaining muscle tissue, devoid of heads and fins, generated in industrial processing plants at the stage of filleting. The backbones came from two fish species – Baltic cod (Gadus morhua callarias) and Atlantic salmon (Salmo salar). We obtained cod backbones from the company “Fishland” and salmon backbones from “SuperFish”. The obtained raw material was stored at -20°C until the time of processing.

2.2 Processing

Processing took place in the laboratory of Environmental Engineering and Industrial Waste Recycling Department, Poltegor-Institute, on a large laboratory scale. In order to produce the calcium preparations from cod (BCP) and salmon (ASP), the backbones were thawed for 16 h at 20°C. Then we collected representative samples and determined physico-chemical properties of the raw material.

We cut thawed backbones (Figs. 1 and 2) into pieces about 10 cm long, weighed 30 kg (CAS PB 60 scales) of each kind of raw material (cod and salmon), and placed them in a container (volume of 170 dm³) enabling easy separation of solids from liquids (a 5 mm sieve).

The scheme of the examined process is presented in Fig. 3. In order to dissolve the remaining muscle tissue,
we treated the by-products with 2 M NaOH at room temperature (time of soaking, 48 h for Baltic cod and 24 h for Atlantic salmon). We obtained bone materials after the alkaline processing stage, rinsed them with tap water in a sieve, and then, in order to remove the dissolved proteins, fat and aroma, and for material disinfection, treated them with the following solutions:
- 0.1% citric acid (C_{6}H_{8}O_{7}xH_{2}O analytical grade), bone material to citric acid ratio of 1:5 (w/v), 0.5 h;
- 5% hydrogen peroxide (H_{2}O_{2} technical grade), bone material to H_{2}O_{2} ratio of 1:5 (w/v), twice for 1 h.

The investigated processing end-products, the bone preparations, were rinsed with water (approx. 15 min.), filtered with a sieve, dried in a dryer (WAMED SUP-200W, 220 dm^3) at 60°C until a minimum of 90% dry matter was obtained (Figs. 4 and 5), weighed (RADWAG APP 25/C/2 scales) and ground (POLYMIX PXMFC90D grinder). The granulation of the final product was 0–2.5 mm. We then examined the final calcium preparations – BCP (Fig. 6) and ASP (Fig. 7). Physico-chemical properties of preparations are shown in Tables 3 and 4.

### 2.3 Physico-chemical analyses

We performed physico-chemical analyses, including the determination of dry matter, crude ash, total protein, crude fat as well as calcium and phosphorus content, in the Laboratory of the Department of Animal Nutrition and Feed Management, Wroclaw University of Environmental and Life Sciences. We examined dry matter content with the gravimetric method in a laboratory dryer (temperature 105°C, drying time 6 h), and crude ash in a muffle furnace (porcelain pots, temperature 600°C, combustion time 8 h). We determined total protein content using the Kjeldahl method after the mineralization of samples in concentrated H_{2}SO_{4}, in the presence of a catalyst (Kjeltec-2300v Apparatus, Tecator). The raw fat was determined by the extraction method (petroleum ether) on Buchi apparatus (B-811). In order to determine calcium (Ca) content, we subjected the samples to wet mineralization (HNO_{3}) in a MARS microwave furnace, and performed analyses using an AA-240 FS spectrometer (VARIAN). Phosphorus (P) content was determined using the photometric method (Specol-11 apparatus, Carl Zeiss Jena).

We determined the content of mineral components (Na, K, Fe, Mn, Zn, Cu) and undesirable elements (Cd, Pb, As, Hg, F) in the BCP and ASP preparations. The analyses were performed in the Chemical Laboratory of Multi-elements Analysis, Wroclaw University of Technology. We examined fluorine using the potentiometric method with an ion-selective electrode of Orion company (Thermo Electron Corporation, Germany). The other elements were examined using the ICP-OES technique (inductively coupled plasma optical emission spectrometry) on a Vista MPX spectrometer (Varian, Australia) and the ICP-MS technique (inductively coupled plasma mass spectrometry) on an XSeries2 spectrometer (Thermo Scientific, Germany) after mineralization in a microwave furnace ( Milestone, MLS-1200 MEGA). We analyzed mercury content in the samples in the Laboratory of Biotechnology in Poltegor-Institute using atomic absorption spectrometry with the technique of mercury vapor amalgamation on the automatic mercury analyzer AMA 254 (Altec).
3 Results and discussion

Cod and salmon backbones constituted the raw material for the processing and contained 21.58 and 34.65% dry matter, 5.91% and 4.09% ash, 15.27 and 18.02% protein, and 0.76 and 12.30% fat, respectively (Table 1). The values characterizing cod backbones are consistent with those obtained by Skierka et al. [29]. Ramirez [30] reported similar results to the present study relating to the basic components of the Atlantic salmon backbones (35.7% dry matter, 15.2% fat, 14.1% protein, 6.4% ash).

Table 1: Characteristics of the process of saltwater fish backbones processing on calcium preparations.

| Raw material               | Mass of raw material [kg] | Yield of cleaned and filtered bone product after decontamination and disinfection stage [kg] | Yield of final product after drying and grinding [kg] | Yield of final product [kg/100kg of raw material] |
|----------------------------|---------------------------|--------------------------------------------------------------------------------------------|------------------------------------------------------|--------------------------------------------------|
| Baltic cod backbones       | 30                        | 3.26                                                                                       | 1.20                                                 | 4.0                                              |
| Atlantic salmon backbones  | 30                        | 3.88                                                                                       | 1.62                                                 | 5.4                                              |

Figure 4: Bone product from cod backbones after processing and drying.

Figure 5: Bone product from salmon backbones after processing and drying.

Figure 6: Final product BCP-calcium preparation from cod.

Figure 7: Final product ASP- calcium preparation from salmon.
The processing allowed us to obtain 1.20 kg of ready calcium preparation from cod (BCP) in the form of a white powder, and 1.62 kg of calcium preparation from salmon (ASP) in the form of a yellow powder, from 30 kg of each kind of raw material, which is the equivalent of 4.0 kg of cod calcium preparation and 5.4 kg of salmon calcium preparation per 100 kg of raw material. The preparations were devoid of aroma, and microbiological examinations did not demonstrate the presence of bacteria or fungi in either preparation [31,32]. Presumably, sodium hydroxide and hydrogen peroxide solutions applied in the process caused their microbiological decontamination. This is a significant issue for longer storage, as well as food applications or fodder purposes. It is important to note that a high amount of wastewater was generated during the processing of investigated preparations which contained mainly organic compounds. An analysis of wastewater composition will require further investigations to find an effective method of utilization.

The calcium preparations BCP and ASP were characterized by high dry matter (94.03% and 94.66%) and ash content (70.15% and 73.28%), as well as low fat (0.12% and 0.25%) and protein content (10.78% and 14.20%) (Table 3). Alkaline processing using NaOH caused considerable reduction in protein and fat levels, but did not eliminate them entirely from the products obtained. Calcium and phosphorus contents in the cod preparation were 27.79% and 13.40%, respectively, and were higher than in salmon preparations (24.92% and 12.50%, respectively). The ratio of Ca/P was 1.99 for the BCP preparation and 2.07 for the ASP preparation – similar to the Ca/P ratio of human bones (2:1).

Similar results relating to the content of water (2.46%), protein (14.81%) and ash (75.83%), and insignificantly higher fat content (5.82%), were obtained by Hemung [33] in the powder from tilapia bones, which were manufactured using the alkaline processing method (0.8% NaOH, 90°C) for 1 h. Huo et al. [34] obtained insignificantly higher results, compared with the present study: the content of dry matter (96.2%) and fat (0.44%), comparable content of calcium (27.8%) and phosphorus (12.2%), and a near three-fold lower protein content at an average level of 3.67%, in the preparation of bones from the haddock (in the Gadidae family) obtained using the hydroxide-ethanol method.

The processing of the ribbon fish (Trichiurus savala) backbones using 1 M sodium hydroxide and 60% ethanol produced a powder of raw bones in the amount of 65 g/1000 g, characterized by protein, fat and water content of 4%, 0.3% and 0.8%, respectively, as well as a calcium and phosphorus content of 27.81% and 10.83%, respectively [28]. Results similar to those presented here in relation to the calcium content were obtained by Techochatchwal et al. [25] in the preparation of the Nile tilapia (Tilapia nilotica) bones. Xavier et al. [35] produced a bone powder of raw materials in the amount of 70 g/1000 g, containing 31.98% calcium and 18.5% phosphorus, in the process of Fringescale sardinella (Sardinella fimbriata) backbone processing using 1 M NaOH and 60% ethanol. Changhu et al. [36] noted Ca and P concentrations of 38.27% and 17.73%, respectively in Pollack fish bones (Pollachius pollachius), with a calcium/phosphorus being 2:1, which is similar to that of human bones. Brown powder containing 31.8% calcium was obtained in the process of whiptail hake (Macruronus novaezelandiae) bone processing using an enzymatic method with application

**Table 2:** Basic physico-chemical parameters of the fish backbones used as raw material in the processing (%).

| Raw material                  | Content [%] |
|------------------------------|-------------|
|                              | Dry matter  | Total protein | Raw fat | Raw ash |
| Atlantic salmon backbones    | 34.45       | 18.02         | 12.30   | 4.09    |
| Baltic cod backbones         | 21.58       | 15.27         | 0.76    | 5.91    |

**Table 3:** Physico-chemical parameters of the final calcium preparations.

| Calcium preparation          | Content [%] |
|------------------------------|-------------|
|                              | Dry matter  | Total protein | Raw fat | Raw Ca | Raw P |
| Calcium preparation from     | 94.66       | 10.78         | 0.12    | 70.15  | 24.92 |
| Atlantic salmon (ASP)        |             |               |         | 12.50  |       |
| Calcium preparation from     | 94.03       | 14.20         | 0.25    | 73.28  | 27.79 |
| Baltic cod (BCP)             |             |               |         | 13.40  |       |

**Table 4:** Macro and microelements and undesirable elements content in the final calcium preparations.

| Calcium preparation          | Content [%] | Mg | Na | K  | Cu | Fe | Mn | Zn | As | Cd | Pb | Hg | F  |
|------------------------------|-------------|----|----|----|----|----|----|----|----|----|----|----|----|
| Calcium preparation from Atlantic salmon (ASP) | 0.66 | 1.00 | 0.027 | 3.9 | 11 | 53 | 57 | 1.1 | 0.19 | 0.29 | 0.0014 | 61 |
| Calcium preparation from Baltic cod (BCP)       | 0.66 | 1.23 | 0.030 | 6.2 | 24 | 28 | 50 | 1.3 | 0.16 | 0.39 | 0.0018 | 41 |
of a preparation named Allzyme FDP [16]. Application of the enzymatic preparation Protamex for enzymatic hydrolysis of Atlantic salmon backbones (Salmo salar L.) produced 25 kg of fresh mass of bone fraction from 200 kg of raw material, characterized by dry matter content of 41.5% (10.4 kg d.m. of bones), while the content of protein, fat, ash, as well as Ca and P, in bone fraction dry matter was 33%, 9.6%, 51.4%, 16.7% and 10.5%, respectively [6]. Malde et al. [27] performed enzymatic processing of Atlantic cod bones (Gadus morhua) using papain (6000 NFPU/mg), and Atlantic salmon (Salmo salar) using protease. The calcium preparation from cod backbones contained 67.8% ash, 26.6% protein and < 0.2% fat, while dry matter calcium and phosphorus contents were 26.1% and 15.1%, respectively. The preparation from salmon backbones contained 55.5% ash, 36% protein and 3.0% fat, while dry matter calcium and phosphorus contents were 20.8% and 15.6%, respectively.

The content of microelements in the BCP and ASP preparations (Table 4) were 1.00 and 1.23% for Na, 0.46 and 0.66% for Mg, and 0.027 and 0.030% for K. A higher content for these elements was characteristic for the BCP preparation. Different results of sodium and potassium contents in fish bone preparation manufactured using the enzymatic method were obtained by Malde et al. [37] – contents of these elements for the salmon preparation were 0.19% d.m. and 1300 mg kg⁻¹ d.m., respectively, and 0.21% d.m. and 30 mg kg⁻¹ d.m. for cod. The content of magnesium in the salmon preparation was similar (0.41% d.m.), while the magnesium content for cod was two-fold lower (0.32%) compared to the results obtained in this study. Microelement content (Table 4) in the ASP preparation were generally lower than in the BCP, except for Mn and Zn concentrations. The investigated preparations contained the highest amounts of arsenic (1.1 and 1.3 mg kg⁻¹) and lead (0.32 and 0.29 mg kg⁻¹), and the lowest concentrations of cadmium (0.11 and 0.17 mg kg⁻¹) and mercury (0.0014 and 0.0018 mg kg⁻¹) regarding the toxic element content (Table 4). The Fluorine ion content was 41 and 61 mg kg⁻¹, and higher in the ASP. The concentrations of Cd, Hg and Pb did not exceed the acceptable values established by the Commission Regulation (EU) no. 1881/2006 from December 19, 2006 (with further changes EC no 629/2008) [38] and the Decree of the Ministry of Agriculture and Rural Development of February 6, 2012 [39]. The content of the toxic elements (As, Cd, Pb) determined by Malde et al. [37] in the preparation of cod and salmon bones produced using the enzymatic method was insignificantly lower than those observed in this study; only Hg content in the salmon bone preparation was higher (0.01 mg kg⁻¹ d.m.). A considerably higher content of mercury, was noted in Baltic cod and Atlantic salmon bones after their mechanical cleaning from remaining muscle tissue (0.039 mg kg⁻¹ d.m. and 0.024 mg kg⁻¹ d.m., respectively) compared to this study [40]. Liaset et al. [6] observed a higher content of arsenic (2.2 mg kg⁻¹ d.m.) in the bone fraction of Atlantic salmon backbones, compared to the present study.

5 Conclusions

The study demonstrated the effectiveness of the above-described technology for the production of calcium preparations from cod and salmon backbones, due to a high content of dry matter, ash and calcium, as well as a low content of protein and fat in the preparations obtained. The investigated method is easy to apply on an industrial scale, since no expensive equipment was used.

Despite the fact, that the concentration of toxic elements (As, Cd, Pb and Hg) in the investigated preparations was not above acceptable levels set for food materials (including diet supplements), it is important to emphasize that it could be increased in by-products from the fish industry. Thus, the content of such elements should be monitored to avoid intoxication.

Further studies are essential when considering the application of the investigated preparations in the manufacturing of diet supplements, foods materials for particular nutritional purposes, or special fodder additive components.

The investigated method of calcium preparation production using the technology of saltwater fish by-product processing has been submitted to the Polish Patent Office [41].

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