Use of Waste Salmon Bones as a Biomaterial

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***Abstract*— In the presented study; Hydroxyapatite (HA) used in many areas such as filling of cavities, bone tissue treatments, chin-face, orthopedic and dental surgeries, was obtained from waste salmon fish bones. Instead of producing chemically in a laboratory environment, hard tissue waste of natural resources was used. As trace elements such as magnesium, zinc, and strontium in the structure of natural resources support bone formation, waste salmon fish bones, which are a natural source, were preferred as raw materials. Other advantages include being easy to access raw materials, cheap and environmentally friendly. HA was obtained from salmon bones by the thermal calcination method. The obtained pure salmon hydroxyapatites were sintered at different temperatures, and the effect of changing sintering temperature on the density, microhardness, compressive strength, and elasticity module in the material was investigated. Crystal phase analysis of salmon hydroxyapatite powder and thermal analysis up to a certain temperature were made. MTT cytotoxicity test was performed to measure whether the materials were toxic. This study has the potential to contribute to the development of biomaterial studies for bone repair.**

Keywords —biomaterial; hydroxyapatite; mechanical properties; waste Salmon fish bones.

I. INTRODUCTION

Worldwide, large amounts of fish and shellfish (more than 91 million tons) are hunted every year, but approximately about 50-60 % is consumed by humans, while the rest is waste (Boutinguiza ve diğerleri, 2012). There is no commercial practice to evaluate these wastes and they are by-products with no value. These wastes create environmental pollution and health risks. Studies are carried out to recycle these wastes, which are seen as a harmful and worthless by-product, for the production of Hydroxyapatite (HA) for use in commercial and biomedical applications. Hydroxyapatite, which has the chemical component ($Ca_{10}(PO_4)_6(OH)_2$), is the main inorganic component of bone, which is the most remarkable among calcium phosphates. Cortical (compact) bone consists of 69% by weight calcium phosphate (CaP) minerals, 22% organics, and 9% water. CaP ceramics are used in bone tissue treatments and coatings because of their chemical and crystallographic similarities to hard tissues such as bones and teeth. There are many methods for synthesizing CaP ceramics such as radio frequency plasma spray, sol-gel, hydrolysis, mechanochemical precipitation method. As an alternative to these methods, there are natural resources such as cattle, goat, pig, shell, and fishbone [1].

In this study, the thermal calcination method was applied to obtain HA from waste salmon fish. The cytotoxicity and mechanical properties of the material at different sintering temperatures were examined and the results were evaluated.

II. MATERIAL & METHODS

A. Material

Waste salmon fish bones were used in the study to obtain Hydroxyapatite.

B. Methods

Waste salmon fish bones were boiled in 100 °C water for 1 hour to get rid of the skin. Cleaned bones were treated with 1% sodium hydroxide (NaOH) solution to get rid of protein, lipids, and other organic impurities and then washed with pure water dried. The dried and dehumidified bones were calcined at 800 °C for 3 hours in a special oven. Figure 1 shows the cleaned and calcined fish bones. Bones were ground in a centrifugal ball mill, then were screened through a 63 µm screen and sHA powders were obtained. According to British Standards 7253 [2], sHA powders were pressed with 350 MPa uniaxial press and cylindrical pellets of 11 mm height and 11 mm diameter were obtained. Pellets were sintered in Nabertherm LHT 02/17 oven at 1000, 1100, 1200, and 1300°C for 4 hours.

Vickers microhardness (HV) measurements were performed on the Shimadzu HMV-2T device with a load of 200 g and a 20 second standby time. Measurements were made on 3 different samples and averaged. The Devotrans device was used for Compression testing (2 mm/min displacement). The elasticity module was calculated with the stress-strain graphic data on the same device. The density of the samples was measured by the Archimedes method.

Crystal phase analysis of nonsintered pure sHA powders were analyzed by X-ray diffraction (XRD, PANALYTICAL X'PERT PRO) with CuKa radiation. XRD analysis was obtained in a 2θ range of 10-90° with a step size of 0,02°.

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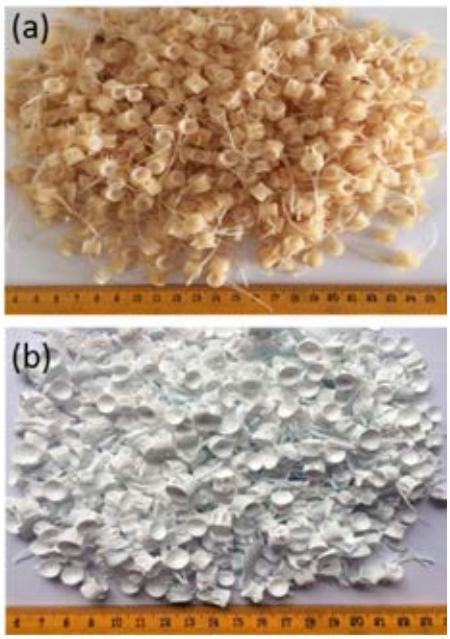

Fig. 1: Salmon fish bones; a) cleaned, b) calcinated

MTT cytotoxicity tests have been made to measure whether the material is toxic. First, the conditioned medium was prepared to understand any possible toxic effect induced by possible ionic leach-out products from the samples into the medium. For this aim, 5 mL fresh medium (DMEM) was added in tubes with a piece (□0,05 g) of tested sHA, which were kept in an incubator. After 1 day, 3 days, and 7 days, the conditioned medium (or simulated body fluid (SBF)) [3] was extracted and later used in cytotoxicity tests. MTT assays were performed in 96-well plates. Saos-2 cells (about 105cells per well) were seeded onto the 4h UV sterilized sHA and (96-well plates) incubated for 72 h. Cell viability was measured by determining mitochondrial NADH/NADHP–dependent dehydrogenase activity, which resulted in the cellular conversion of the 3-(4, 5-dimethylthiazol-2-gl)-5-(3-carboxymethoxylphenyl)-2-(4-sulfophenyl-2H) tetrazolium salt into a soluble formazan dye. After 72 hours, supernatants were removed, and 10µL 3- {4, 5-dimethylthiazol-2yl}-2,5-diphenyl-2H-tetrazolium-bromide (MTT- 5mg/mL- Sigma) solution was added to each well. Following incubation at 37 oC for 3,5h and kept dark in a humidified atmosphere at 5% CO2 in the air. MTT was taken up by active cells and reduced in the mitochondria to insoluble purple formazan granules according to Mosmann's study [4]. Subsequently, the supernatant was discarded, and the precipitated formazan was dissolved in dimethyl sulfoxide (100µL per well), and the optical density of the solution was evaluated using a microplate spectrophotometer (Kayto RT-2100 □C) at a wavelength of 570nm.

III. RESULTS AND DISCUSSION

The TGA and DTA analysis of sHA's reaching 1300°C is shown in Figure 2. The water and organic part in salmon fish bones have already been removed from the structure during the calcination process at 800°C. The fact that the weight of the salmon fish bones decreased to 2 in 3 after the calcination process supports this situation. In Figure 2 (a), no obvious bending point was observed on the TGA graph of sHA. When sHA was sintered at a temperature as high as 1300°C, a very low amount of weight loss, such as 1.31%, occurred. This confirms that sha is free of organic parts and water and is in the pure form [5].

Figure 2 (b) shows the DTA analysis of sHA up to 1300°C. The peaks at 1124.7 and 1151.4 °C in the graph show that new phases are formed.

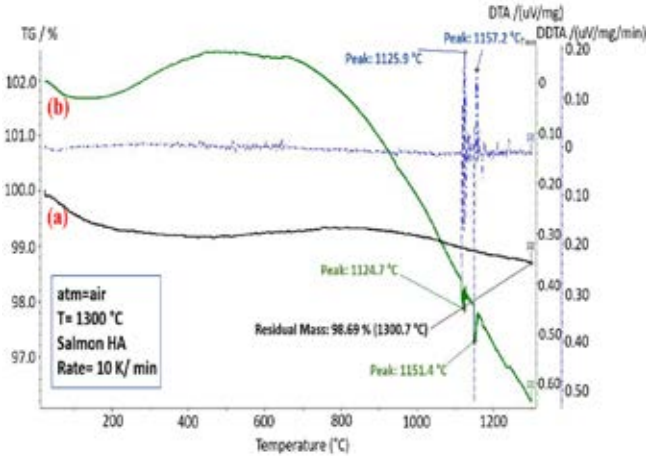

Fig. 2: Thermal analysis of sHA; (a) TGA and (b) DTA

Figure 3(b) gives the XRD graph of pure sHA and Figure 3(a) gives the XRD graph of commercial hydroxyapatite [6]. Compared to the XRD data crystal structures are similar, as can be seen from the peaks in the graph. The peaks at the angles 32.89, 33.0, 39.77, 47.19, and 50.44 in the sHA XRD graph show the 98-005-2691 hydroxyapatite structure according to the JCPDS code number. The peaks at angles 27.96, 31.16, and 34.55 in the graph show the 98-008-2984 tricalcium phosphate (TCP) structure according to the JCPDS code number.

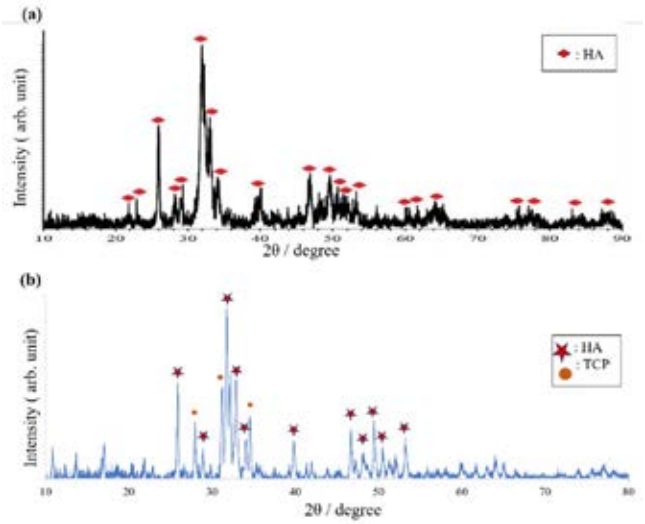

Fig. 3: X-ray diffraction graph (a) commercial HA, (b) pure sHA powder

The pellets were sintered at different temperatures and the effect of temperature on density, microhardness, compressive strength, and elastic modulus was investigated. Figure 4 shows the effect of different sintering temperatures on density and microhardness. Increasing sintering temperature caused an increase in density in the samples. By sintering the samples, the pores decreased and a more compact structure was formed by increasing the density value from 2.01 ± 0.01 to 2.96 ± 0.03g/cm^3. A similar relationship can be established between microhardness and sintering temperature. The microhardness values of the samples, which became intense with the increase of sintering temperature, increased. When the sintering temperature rises to 1100 °C, the increase in hardness is remarkable. As mentioned in previous studies, hardness depends on the porosity and secondary phases formed [7].

Figure 5 shows the effect of increasing sintering temperature on compressive strength and modulus of elasticity. The lowest compression value occurred at 1000°C sintering temperature, where the density was about 2 g/cm^3. The average compressive strength (116.00 ± 31.16) of the samples sintered at 1100 °C gave the highest value. This compressive strength value is better than the samples sintered at 1200 and 1300 °C. This situation can be attributed to the harder and more brittle structure of the sintered samples at high temperatures[8]. The modulus of elasticity shown in Figure 5(b) is the highest, with 633 MPa at 1100°C, as in compressive strength values.

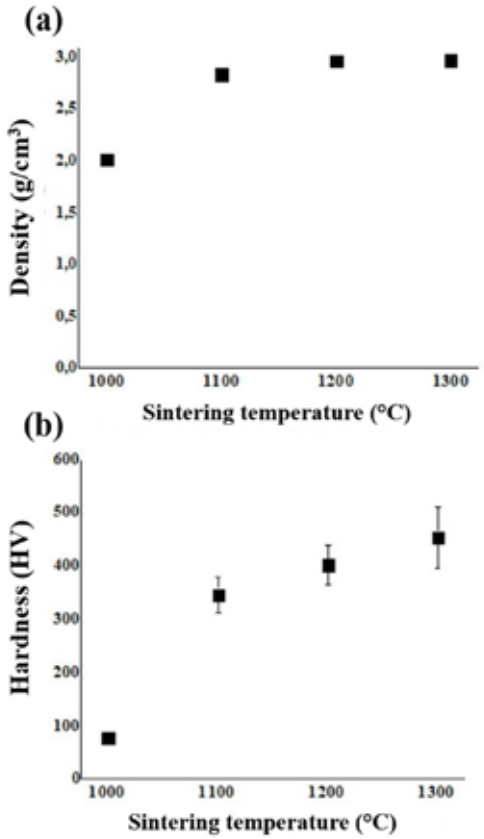

Fig. 4: The effect of sintering temperature on the (a) density, (b) hardness of sHA

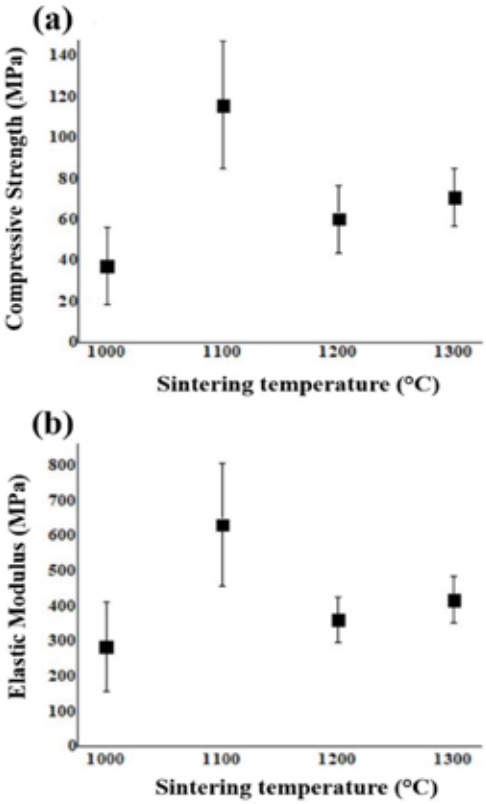

Fig. 5: The effect of sintering temperature on the (a) compressive strength, (b) elastic modulus of sHA

Figure 6 shows the cytotoxicity (MTT) test of sHA's and control group sintered at 1000,1100,1200 and 1300 °C. The samples were compared with the control group and it was observed that it had no cytotoxic. While there was no change in cell proliferation as a result of the test on the first day of the samples, there was an increase in cell proliferation at 1000 °C on the third day. In the seventh-day study, samples at 1300°C gave the best results. Statistically significant results were indicated with (*) and p-value was calculated as $p < 0.05$. It is thought that the samples are in appropriate cytocompatibility in the MTT results and will contribute to the studies in biomedical applications.

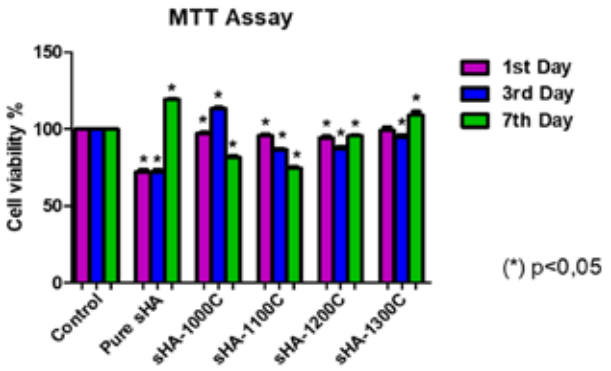

Fig. 6: The cytotoxic effects of sHA-1000°C /1100°C /1200°C /1300°C on viability of Saos-2 cells in 1., 3. and 7. days obtained by MTT assay

IV. CONCLUSION

The evaluation of salmon fish bones, which are seen as waste, as biomaterials is a study that does not have raw material restrictions and requires cheap, low equipment. This evaluation of waste fish bones in this way is an environmentally friendly method that reduces pollution. The XRD crystal phase analysis shows peaks belonging to the hydroxyapatite structure. Fishbone is a nontoxic source for obtaining hydroxyapatite. MTT test results confirm this. It is thought that it will contribute to the studies in this field with the mechanical properties and tests examined.

INFORMATION

The studies were carried out at the Marmara University Nanotechnology and Biomaterials Application and Research Center (NBUAM).

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