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Differences in Calcium Carbonate, Collagen and BMP-2 between *Lutjanus campechanus* Extract and *Brachyura Sp.* Extract

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ABSTRACT

Calcium is a mineral the body needs to maintain bone density and prevent musculoskeletal diseases. One of the basic ingredients that are easy to find are snapper bones and crab shells. *Lutjanus campechanus* species will be used for snapper bones and *Brachyura species* crab shells will be made into a powder extract, then an assessment will be carried out on each sample to measure calcium carbonate levels using EDTA, collagen levels using PicrosiriusRed, and BMP-2 levels. by using MyBioSource. Furthermore, snapper bone and crab shell extracts which have higher levels of calcium carbonate, collagen and BMP-2 can then be used as raw materials which are more economical for the use of the ingredients contained therein. Thus, it is expected that red snapper bones and crab shells can be used as a source of calcium carbonate, collagen, and BMP-2.

Keywords: BMP-2, Calcium carbonate, Collagen, Crab Shell, Fish Bone

INTRODUCTION

Calcium is a mineral that is crucial in maintaining bone density and plays a role in preventing diseases of the musculoskeletal system.[1] Consumption of calcium-rich foods is effective for preventing or delaying osteoporosis in pre- and postmenopausal women as well as in older men.[2] Daily calcium requirements in humans are influenced by various factors such as age, gender, pregnancy, and so on. Calcium requirements have increased in certain groups, such as the postmenopausal group, post-fracture patients who require higher calcium intake because the fracture healing process involves the process of forming callus which requires more nutrient intake. Therefore, calcium supplements are often given to meet practical calcium needs in certain conditions.[3]

Seeing the high need for vitamin D supplementation, several efforts to find raw materials that are more economical with equivalent content have begun to be studied extensively. Fishbone is a valuable source and component of essential elements to improve health. Fish bones contain calcium (Ca) and phosphorus (P) which make up about 2% (20 g/kg dry weight) of fish.[4] In addition, fish bones are thought to contain compounds that are identical to bone growth factors, one of which is bone morphogenetic protein (BMP) which plays an important role in osteoblast differentiation.[5] One type of fish whose bones may be used as a basic ingredient for making calcium supplements is red snapper. Red snapper bone waste can be used as a basic material for making calcium supplements which is certainly more economical when compared to commercial calcium supplements whose purchase costs,

if roughly calculated, can reach up to around 10 million rupiah per year.[6] In addition to fish bones, crab shells also contain calcium carbonate which is very abundant, and varies according to species.[7] Calcium carbonate can be further processed into calcium hydroxyapatite, which chemically and physically has the same mineral content as human bones and teeth. Abundant raw materials, including crab shells, are one of Indonesia's main export commodities. The use of crabs is generally limited to food and necessities, and usually only the crab meat is taken while the crab shells are usually thrown away. But actually the crab shell contains 15.60% -23.90% protein, 53.70% -78.40% calcium carbonate, and 18.70% -32.20% chitin.[8]

Seeing the potential that is still not fully utilized and seeing the abundance, further research on the benefits of this innovation is expected to provide cross-sectoral benefits in the utilization of red snapper bones and crab shells containing calcium carbonate, collagen and BMP-2.

MATERIALS AND METHODS

The research design used a descriptive-analytic study to compare the levels of calcium carbonate, collagen, and BMP-2 between fish bone extract and crab shell extract. The research sample was taken from the reachable population by purposive sampling.

The procedure for collecting research data samples requires several stages including collecting research samples; determination of eligible subjects based on inclusion and exclusion criteria; manufacture of snapper bone extract and crab shells; perform analysis of Calcium Carbonate, BMP-2, and Collagen on samples; then the data obtained is then processed and analyzed.

The collected data were analyzed using SPSS for Windows version 22.0. Data from the results of this study will be analyzed for the characteristics of the research sample and processed to be presented descriptively, using graphs and tables; normality test with Kolmogorov Smirnoff and data homogeneity test with Levene T test; and Inferential test using paired t-test and logistic regression test.

The sample size can be calculated using the formula:

$$n = 2\left[\frac{(Z\alpha + Z\beta)Sd}{d}\right]^2$$

where n is the number of samples for each group, s is the average standard deviation of the two groups, X1 is the mean value of the treatment group, X2 is the mean value of the control group, X1-X2 is the desired clinical difference, α is the error rate I (set 0.05), Zα is set at 1.96, β is the error level II (set at 2), and Zβ is set at 0.824. From the calculation using the sample size formula above, with a drop out of 10%, it is obtained that the number of samples is 16 samples from each group, so the total sample required is 32 samples.

RESULTS

Characteristics of Collagen in Crab Shells and Snapper Bones

The average collagen level based on measurements using PicrosiriusRed on crab shells was 73.88 ± 3.68 .tg/100mg while for snapper bones was 48.76 ± 2.49 .tg/100mg. Based on the independent T-test, the results obtained were p <0.001 so that the collagen levels in the crab shells were significantly higher than the collagen levels in the snapper bones.

Table 1. Characteristics of Collagen in Crab Shell and Fish Bone

	Crab Shell (n=20)	Fish Bone (n=20)	p
Collagen content (µg/100 mg), mean ± SD	73.88 ± 3.68	48.76 ± 2.49	< 0.001

Characteristics of BMP-2 in Crab Shells and Snapper Bones

The average BMP-2 level based on measurements using MyBioSource on crab shells was 35 ± 10.20 .tg/100mg while for snapper bones was 21.7 ± 10.12 .tg/100mg. Based on the independent T-test, the results obtained were p <0.001, so the BMP-2 content of crab shells was significantly higher than the BMP-2 levels of snapper bones.

Table 2. Characteristics of BMP-2 in Crab Shell and Fish Bone

	Crab Shell (n=20)	Fish Bone (n=20)	p
BMP-2 content (µg/100 mg), mean ± SD	35 ± 10.20	21.7 ± 10.12	< 0.001

Characteristics of Calcium Carbonate in Crab Shells and Snapper Bones

The average calcium carbonate level based on measurements using Ethylenediamino Tetracetic Acid Dissodic (EDTA) on crab shells was 65.3 ± 6.00 .tg/100mg while on snapper bones was 35.9 ± 4.56 .tg/100mg. Based on the independent T-test, the results obtained were p <0.001 so that the calcium carbonate levels of crab shells were significantly higher than the calcium carbonate levels of snapper bones.

Table 3. Characteristics of Calcium Carbonate in Crab Shell and Fish Bone

	Crab Shell (n=20)	Fish Bone (n=20)	p
Calcium carbonate content (µg/100 mg), mean ± SD	65.3 ± 6.00	35.9 ± 4.56	< 0.001

DISCUSSION

Red snapper bone extract has lower collagen, BMP-2, and calcium carbonate levels when compared to crab shell extract, and is statistically significant. (crab shells 73.88 ± 3.68 µg/100mg, snapper bones 48.76 ± 2.49 µg/100mg, p <0.001; Levels of BMP-2 crab shells 35 ± 10.20 µg/ 100 mg, snapper bones 21.7 ± 10.12 µg/100 mg, p <0.001; EDTA calcium carbonate content of crab shells 65.3 ± 6.00 µg/100 mg, snapper bones 35.9 ± 4.56 µg/100mg, p<0.001). This is the first research to compare those two, which are abundantly available in Indonesia.

Nevertheless, the amount of collagen in fish bone found in this research was lower from what Nguyen found [9], which showed the amount of 14.27g/ 100g. This might be attributed to the living condition of the fish in Indonesia water, and different measuring technique.

Bone Morphogenic Proteins (BMPs) play an important role in vertebrate biology, important roles as morphogens during embryonic development and as bone inducers, which were first recognized for their osteogenic properties. [1] There has never been a research to measure the amount of BMP-2 in crab shells. Yet, our study showed that there is higher amount of BMP-2 found in crab shells compared to the red snapper bone – highlighting its tremendous potential for future use in medical and orthopaedic surgery field.

Crab shell is a natural resource that is rich in calcium carbonate ($CaCO_3$). Several calcium derivatives such as hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$), biphasic calcium phosphate (BCP) and tricalcium phosphate (TCP, $Ca_3(PO_4)_2$) which are obtained from natural resources, are actively used in dentistry and orthopedic surgery. [7] Calcium phosphate is also used as a substitute for synthetic bone grafts and is incorporated into bone implant coatings due to its biocompatible, bioactive, biodegradable and osteoconductive properties. The emergence of CaP from natural resources which has a structure and morphology similar to human bone provides benefits in the treatment of bone diseases and for the regeneration of damaged organs and tissues.[10] Our study showed a similar

amount of calcium carbonate found in the literatures.

CONCLUSION

Based on this research, crab shells have a higher nutritional value and can then be considered as a basic ingredient for innovative supplement products. Further research is needed regarding the differences in the absorbability of calcium, collagen, and BMP-2 between snapper bones and crab shells and the differences in the effectiveness of increasing calcium, collagen, and BMP-2 between extracts of snapper bones and crab shells.

Declaration by Authors

Ethical Approval: Approved

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Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. Carreira, A. C., Lojudice, F. H., Halcsik, E., Navarro, R. D., Sogayar, M. C., & Granjeiro, J. M. Bone morphogenetic proteins: Facts, challenges, and future perspectives. (2014). Journal of Dental Research. https://doi.org/10.1177/0022034513518561
2. H Aihanuwa, U.-E., Chukwujekwu, K., & Chukwuemeke, C. Bone Morphogenetic Proteins an Update and Review. (2017). Tropical Journal of Natural Product Reseach. https://doi.org/10.26538/tjnpr/v1i1.2
3. Straub, D. A. Calcium supplementation in clinical practice: A review of forms, doses, and indications. (2007). In Nutrition in Clinical Practice. https://doi.org/10.1177/0115426507022003286
4. P. H. Luu, & Nguyen, M. H. Recovery and Utilization of Calcium from Fish Bones by Products as A Rich Calcium Source.oor, An Introduction to Signal Detection and Estimation. (2009). New York: Springer-Verlag, 1985, ch. 4.
5. Darmanto, Y. S., Agustini, T. W., Swastawati, F., & Al Bulushi, I. The effect of fish bone collagens in improving food quality. (2014). International Food Research Journal.
6. Liao, W. L., Lu, H. H., Huang, S. K., Wu, J. L., Huang, J. H., & Lin, E. C. Study of growth and body composition of red snapper Lutjanus erythropterus fed diets containing Escherichia coli expressing recombinant tilapia insulin-like growth factor-I. 2008. Fisheries Science. https://doi.org/10.1111/j.1444-2906.2008.01533.x.
7. Asphama, A. I., Amir, F., Malina, A. C., & Fujaya, Y. (2015). Habitat Preferences of Blue Swimming Crab (Portunus pelagicus) Species Complex. Aquacultura Indonesiana, 16(1). https://doi.org/10.21534/ai.v16i1.10
8. Raya, I., Mayasari, E., Yahya, A., Syahrul, M., & Latunra, A. I. (2015). Shynthesis and Characterizations of Calcium Hydroxyapatite Derived from Crabs Shells (Portunus pelagicus) and Its Potency in Safeguard against to Dental Demineralizations. International Journal of Biomaterials, 2015. https://doi.org/10.1155/2015/469176
9. P. H. Luu, & Nguyen, M. H. (2009). Recovery and Utilization of Calcium from Fish Bones by Products as a Rich Calcium Source. J Sci Technol 47 (6):91–103.
10. Abdul Hanan, M. R., Nasution, A. K., Hussain, R., & Saidin, S. (2018). Fabrication of poly (Lactic-co-glycolic acid)/calcium phosphate bone cement composite: Synthesization of calcium phosphate from crab shells. Jurnal Teknologi, 80(4), 103–109. https://doi.org/10.11113/jt.v80.11525

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