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Evaluation of Calcium and Phosphorus content in Scallop Shells (*Placuna placenta*) and Blood Cockle Shells (*Anadara granosa*) from Banyuasin Waters, South Sumatra

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ABSTRACT

Bivalvia is a class in the phylum Mollusca, including blood cockle shell (*Anadara granosa*) and scallop shell (*Placuna placenta*). Both species have become food commodities that are consumed leaving their shells as unused waste. Based on this, research that examines other benefits of shell waste is urgently needed so that shell waste can be optimally explored. In this study, the samples were obtained from the Banyuasin water, South Sumatra in July 2021. The samples were classified into three size categories: *A. granosa* A (> 40 g), B (20-30 g) and C (< 20 g), while *P. placenta* was differentiated into A (> 13 g), B (9-12 g) and C (< 9 g). For the quantitative analysis of the calcium, atomic absorption spectroscopy was used; whereas for phosphorus, UV-Vis spectrophotometry was utilized. The ANOVA, the least significant difference test and the independent sample T-Test were analyzed for statistical analysis. Based on the results, the calcium content in the *P. placenta* was 41.13%, 38.75%, 35.81%, and phosphorus was 0.00054%, 0.00043%, 0.00014%. The *A. granosa* contained calcium with percentages of 45.74, 42.17 and 37.11, while phosphorus recorded values of 0.00049%, 0.00035% and 0.00001%. The ANOVA showed significant differences between sample categories in terms of calcium and phosphorus content (P<0.05). LSD results showed that each category differed significantly. The independent sample T-Test for the calcium of *A. granosa* and *P. placenta* with a Sig-2 tailed value of 0.007 resulted in a significant average difference, while the phosphorus content with a Sig-2 tailed value of 0.544 indicated no significant average difference. Based on this study, both types of shell minerals should be used as an alternative source of calcium and phosphorus.

INTRODUCTION

The Banyuasin Coast is the center of fishing ground for shellfish such as shrimps, crabs, squids, cuttlefish and bivalvia in South Sumatra **(Rozirwan *et al.*, 2019, 2022a, b; Almaniar *et al.*, 2021)**. The location is known to be very fertile with the presence of phytoplankton and other organic nutrients, which play a role in providing food source for the shellfish **(Rozirwan *et al.*, 2021, 2022d; Saputra *et al.*, 2021)**. The bivalvia can be found on soft or rigid substrates **(Goelz *et al.*, 2020; Rozirwan *et al*. 2022c; Rozirwan *et al.*, 2023)**. Indonesian society has not fully utilized the potential of all bivalvia, usually eating only the meat, while the shells are discarded and become waste in the environment **(Mediarman *et al.*, 2021)**. Bivalvia shell contains a high potential of 98% calcium carbonate, while shrimp shells consist of 75% calcium carbonate of the total weight per individual, which can be used optimally **(Asmawati *et al.*, 2018)**. According to **Jeon and Yeom (2009)**, many home and fishing industries in the coastal throw away shells of various types of shellfish, being not used as raw material for their industry.

The high amount of untreated shell waste leaves an unpleasant odor and visual disturbance to the surroundings **(Zain *et al.*, 2022)**. The utilization of shell waste is an interested alternative because it not only reduces the problems associated with fishery waste but also processes fishery waste into value-added products **(Bhattacharjee *et al.*, 2019)**, one of them is utilizing the mineral content contained in the shell. The shell comprises macro minerals such as calcium carbonate, phosphorus, magnesium and others such as carbon (32.73%), Al_2O_3 (14.13%), CuO (9.22%), CaO (34.68%), ZnO (5. 08%) and ZrO_2 (3.03%) and MoO_2 (1.13%) **(Mititelu *et al.*, 2021; District, 2022)**. The mineral content of shells could be used as raw materials in producing value-added compounds, one of which is for food, biomedical, medicines and health supplements **(Terzioğlu *et al.*, 2018)**.

Bivalvia shell have the highest mineral content in terms of calcium and phosphorus. According to the percepective of **Ferraz *et al.* (2019)**, about 60% of the total mass of bivalvia is the shell, and calcium carbonate can reach 95%. Phosphorus is a mineral that is as important as calcium **(Karnkowska, 2005)**. Phosphorus is an essential element for all life, being a structural and functional component of all organisms in bone, cartilage and the exoskeleton **(Nystrand *et al.*, 2016)**. Phosphorus in bivalvia shells is in the form of calcium phosphate **(John, 2016)**. Calcium phosphate is an essential material for ion exchangers, adsorbents, etc...

The waste of blood cockle shells and scallop shells has the potential to be used to make calcium and phosphorus powder. It should be known that the shell powder have a reasonably good taste for food and various benefits for the treatment of osteopenia. The calcium content in *A. granosa* shell can be considered high compared to *P. placenta* shell. *A. granosa* shell contains 98% **(Awang-Hazmi *et al.*, 2007)**. This is supported by the *A. granosa* shell having high calcium ranging from 98%. *P. placenta* shells are a type of bivalvia that can grow up to 16cm and can be found in waters between 0.5 & 100m. Bivalvia shells have potential added value since the shells contain high levels of minerals, carbohydrates and vitamins. The calcium content in the shell is 98- 99%. **(Aydin & Kalemtas, 2021)**. Calcium content is classified as good, considering the minerals are almost the same as those needed for bone and teeth preparation **(Suryaputra *et al.*, 2013)**.

A comparative study of the calcium and phosphorus content of the samples; namely, *P. placenta* shell and *A. granosa* shell, needs to be carried out to analyze the potential benefits of the powder as a substitutional raw material in the future. The relatively high potential of mineral content in bivalvia shells will be followed by an increase in the amount of processing and utilization of shell waste. Based on this, it is necessary to carry out further research related to the high mineral content in bivalvia shells to be provided in the food and health industries.

MATERIALS AND METHODS

1. Sample description

The samples were obtained from Sungsang Village, South Sumatera previously collected from the Banyuasin waters. Samples were identified based on morphological characteristics, as seen in Fig. (1) according to the identification book **(Carpenter & Niem, 2001)**.

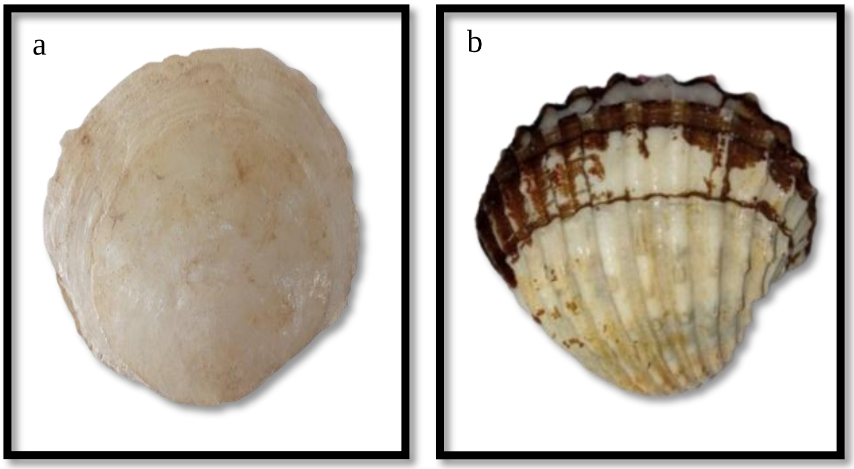

Fig. 1. Top morphological view of bivalvia shell, (a) *P. placenta* shell (b) *A. granosa* shell

P. placenta shell lives at the bottom of the water with a sandy to a muddy substrate. This species eats by filtering out tiny particles. *P. placenta* shell was primarily found in the tropics area **(Li and Ortiz, 2013).** The *A. granosa* shell is white covered with periostracum with a yellow to blackish brown color; it is transparent, round, and brownish white **(Yaqin & Fachruddin, 2018)**. *P. placenta* shell has a flat, thin shape with a width of up to 8cm. The outer surface of shell is smooth and slippery, while on the inside, there are radial serrations along the edge of the shell **(Li & Ortiz, 2013)**.

Identification of shells referred to in the book of FAO entitled “The Living Marine Resources of Western Central Pacific” **(Carpenter & Niem, 2001)**. The sample of shellfish with as much as 1,000g was then grouped into three categories based on size. Each category contained three individuals, so there were nine individuals for each type of shellfish. The size categories in this study were *P. placenta* shell A (> 40 g), B (20-30 g), and C (< 20 g), and *A. granosa* shell A (> 13 g), B (9-12 g) and C (< 9 g).

2. Sample preparation

The categorized shellfish samples were separated from their organs, feces and shells. The following process was boiling the clam shells, which aimed to release the remnants of meat still attached to the shell. The clam body was separated from the shell for 15 minutes at the required temperature of less than 100°C. For the process of hydrolysis, samples were put with 1 N NaOH solution on a hotplate at 50ºC for 3- 4h **(Suryawanshi *et al.*, 2019)** to remove organic matter in the clam shells. Shell powder was dried in an oven for 6– 8h at a temperature of 60°C. The sample was placed in aluminum foil, and then the drying process was carried out in an oven at 121ºC for 1- 2h, after which the sample was mashed to obtain shell powder.

3. Sample destruction

Destruction of clam shells for testing calcium content used the wet destruction method with 1g of shell powder sample, and then 5mL of HNO_3 was added and left to stand for 1- 2h. Furthermore, the outcome was put on a hot plate stirrer for 4h at a low temperature and deposited for one night. Then, 0.4 mL of H_2SO_4 was added and stirred again for one hour. A mixture of 2- 3 drops of $HClO_4$: HNO_3 in a ratio of (2: 1) was marked by a light yellow color change. The sample was transferred and added to 2mL of distilled water and 0.6mL of HCl. The sample was filtered for 15 minutes using Whatman No. 42 filter paper in a 100mL volumetric flask. The digested samples were analyzed by an atomic absorbance spectrophotometer (AAS) at a wavelength of 422.7nm with the Shimadzu AA-7000 series **(Untailawan & Wijaya, 2021)**.

Destruction of shells for calcium content analysis used P Bray 1 destruction, where the sample was weighed as much as 2g and put into an Erlenmeyer. 1 N ammonium fluoride solution was prepared by dissolving 3.7g of NH_4F with H_2O in 100mL, then 5 N HCL solution was prepared by dissolving 20.2mL of HCl and H_2O into 500mL. Bray 1 solution dissolved 30mL of 1 N NH_4F solution, and 5mL of 5 N HCl was added to H_2O. Furthermore, a solution of 5 N sulfuric acids was prepared with 140mL of concentrated H_2SO_4 BD 1.84 kg/L and H_2O until the volume reached 1000mL. 12 g $(NH_4)6Mo_7O_{24}.4H_2O$ plus 250 mL H_2O. After that, 1.298 g $KSbOC_4H_4O_6$ was added to 100mL of H_2O. For the preparation of phosphate reagent, all the steps were mixed with H_2O to a volume of 2L, adding 1g of ascorbic acid to 200mL (**Friedlein *et al.*, 2019)**, UV-Vis Spectrophotometer wavelength 380 – 780 with the 752AP model was used to analyze the samples.

4. Statistical analysis

4.1 determination of yield value data

The clam shell samples were then calculated to compare the clamshell powder with the intact shells. The result value was calculated based on the following formula of **Indriani and Wardhani (2022)**:

$$Yield\ value = \frac{Wt}{Wo}\ x\ 100\%$$

Information:

Wt = The final weight of clam shell powder (g)
Wo = The initial weight of the clam shell (g)

4.2 Determination of calcium and phosphorus content

The calcium content was measured by an atomic absorption spectrophotometry (AAS), which was then calculated based on the following formula **(Hasibuan *et al.*, 2019)**:

$$\text{Concentration mg/kg} = \frac{\text{C} \times \text{V}}{\text{W}}\text{Fp}$$

Information:

C = Sample concentration ($mg.L^{-1}$)
V = Sample volume (L)
Fp = Dilution factor
W = Weight sample (kg)

Calculation of the phosphorus content in the previous shellfish sample was performed in ppm units using the conversion in % form with the following formula **(Morris *et al.*, 2019)**:

$$\text{P2O5 content} = \text{curve (conc)} \times \frac{extract\ (mL)}{sample\ (g)} \times Fp \times \frac{142}{190} \times Abs$$

Information:

Fp = Dilution factor
$\frac{142}{190}$ = PO_4 to P_2O form conversion factor
Abs = Absorbance reading

The statistical data analysis used in this study was one-way ANOVA. The one-way ANOVA was analyzed using XLSTAT 2022 **(Fitria *et al.*, 2023)**. If the results obtained were significantly different, it would be continued by the least significant difference test. The independent sample t-test of the IBM SPSS v.26 programs was used in this study **(Hasibuan *et al.*, 2019)**.

RESULTS

1. Yield value

The yield value is one of the essential parameters to determine the economic value and effectiveness of a process that is carried out. The *P. placenta* and *A. granosa* shell yield data in this study are presented in Fig. (2).

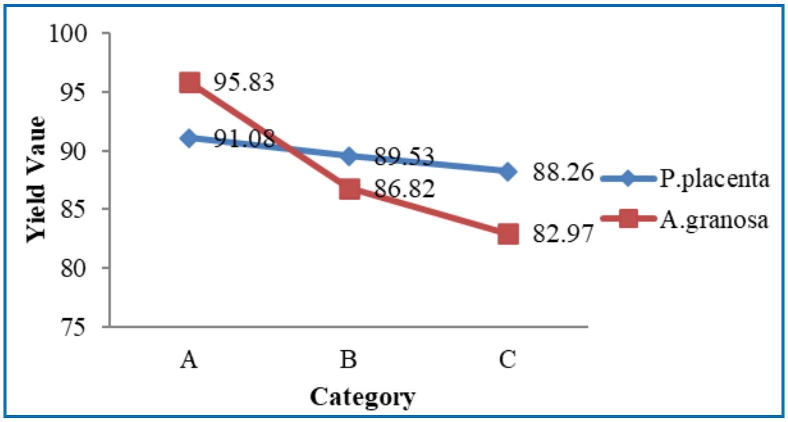

Fig. 2. Average of yield value

Based on Fig. (2), for the yield value obtained in category A, *A. granosa* shell was around 95.68 %, category B was 86.82 %, and category C was around 82.97 %. In addition, the yield of *P. placenta* shell with the average percentage of yield value for category A was around 91.08 %, category B was 89.53 %, and category C was 88.26 %.

2. Calcium content of *P. placenta* shell and *A. granosa* shell

Analysis of the calcium content of the two samples was obtained from atomic absorption spectrophotometry (AAS) readings with the flame method. The average calcium content in *A. granosa* shell and *P. placenta* shell with categories A, B, and C in this study is presented in Fig. (3).

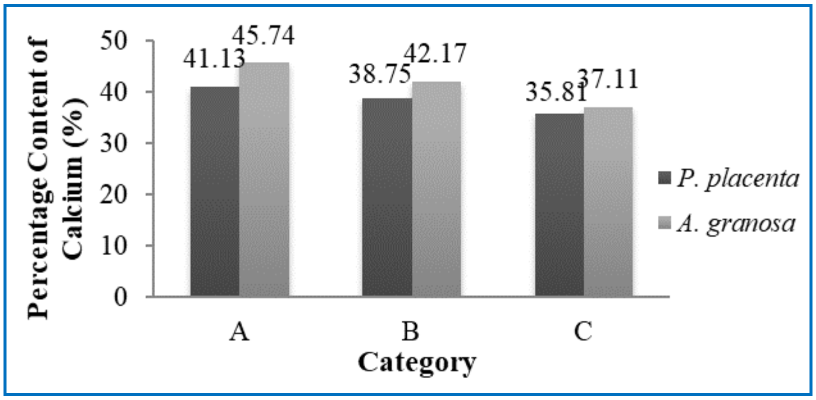

Fig. 3. The calcium content of clam shell

The study's results on the calcium content of *P. placenta* shell based on Fig. 3 showed that sample category A was around 41.13%, category B was around 38.75%, and category C was 35.81%. While, the *A. granosa* shell in category A was 45.74%, category B was 42.17%, and category C was around 37.11%. Overall, it was found that the highest calcium content was recorded in category A for the two types of shellfish.

3. Phosphorus content of *P. placenta* shell and *A. granosa* shell

The analysis of phosphorus content for two shellfish samples was obtained from the readings of UV-Vis spectrophotometry. Phosphorus content in *P. placenta* shell and *A. granosa* shell with categories A, B, and C can be seen in Fig. (4).

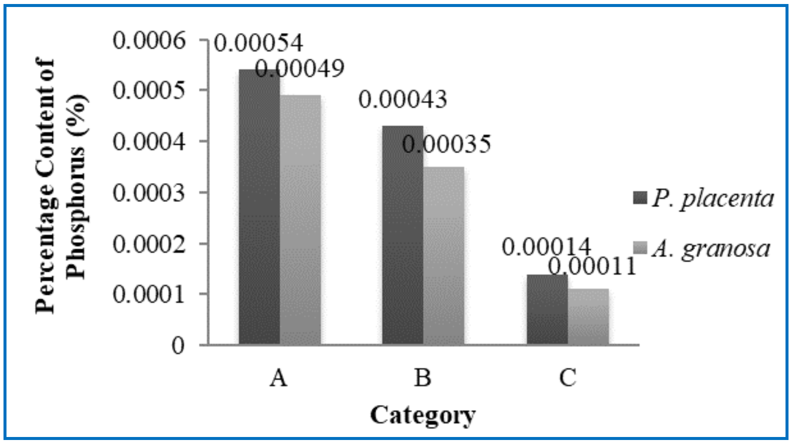

Fig. 4. Phosphorus content of clam shell

Based on Fig. (4), the phosphorus content of *P. placenta* shell in sample A reached 0.00054%, category B was 0.00043%, and category C was 0.00014%. Meanwhile, the phosphorus content of *A. granosa* shell in category A was 0.00049%, category B was 0.0035%, and category C was 0.00011%. Based on the overall results obtained, the phosphorus content in this study was classified as very low, ranging from 0.00014-0.00054%.

4. Comparison of calcium and phosphorus content

A comparison of calcium and phosphorus in *P. placenta* shell and *A. granosa* shell was tested to determine the significant difference in the size of the shells on the calcium and phosphorus content produced. The ANOVA test results are presented in Table (1). The results of the ANOVA test showed a significant effect between the size of the *P. placenta* shell with calcium and phosphorus content. The LSD test was conducted to find of the differences in each category of samples, as presented in Table (2).

Table 1. ANOVA test of calcium and phosphorus content of *P. placenta* shell and *A. granosa* shell

Shell type	Test parameters	F Count	F Table		Notation
			5%	1%	
P. placenta	Calcium	18.01524	5.14	10.92	**
	Phosphorus	253.021	5.14	10.92	**
A. granosa	Calcium	24.03264	5.14	10.92	**
	Phosphorus	1273.88	5.14	10.92	**

Note: *Real effect, ** Influence is very real.

Table 2. LSD test results of calcium and phosphorus content of *P. placenta* shells and *A. granosa* shell

Category	*P. placenta*		*A. granosa*	
	Calcium	Phosphorus	Calcium	Phosphorus
A	41.13 ± 1.86^{a}	0.00049 ± 0^{a}	45.74 ± 1.86^{a}	0.00054 ± 0^{a}
B	38.75 ± 0.06^{b}	0.00035 ± 0^{b}	42.17 ± 0.06^{b}	0.00043 ± 0^{b}
C	35.81 ± 1.66^{c}	0.00011 ± 0^{c}	37.11 ± 1.66^{c}	0.00013 ± 0^{c}

The results of the LSD test in Table (2) show the average value in each category of *P. placenta* shell samples and *A. granosa* shell. Significant differences were detected between categories A, B and C, as indicated by different letter notations. This could be caused by the size of the sample affecting the amount of calcium and phosphorus content. The group A category produced a high calcium content in *P. placenta* shell ranging from 41.13% and *A. granosa* shell at 45.74%. In comparison, phosphorus was classified as low in *P. placenta* shell at 0.00049% and *A. granosa* shell at 0.000054%.

Based on the independent sample T-Test, $P > 0.05$ was obtained in both cases, and the data were distributed in a distinctive and homogeneous manner. Significant differences in calcium and phosphorus content in the two samples are presented in Table (3).

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Table 3. Independent sample t-test of calcium and phosphorus content average of *P. Placenta* shell and *A. granosa* shell

No.	**Variances**		**t-test for Equality of Means**		
			Sig. (2-tailed)	**Mean difference**	**Std. error difference**
1	Calcium	Equal variances assumed	0.007	-3.11111	1.57983
2	Phosphorus	Equal variances assumed	0.544	-0.00005111	0.000082

Table (3) shows no significant differences in average calcium content between *P. placenta* shell and *A. granosa* shell. This was evidenced with the sig (2-tailed) value; it was obtained at 0.007. The value was smaller than 0.05. In addition, this could be shown from the mean difference -3.11111% in the calcium content of the two shell samples.

DISCUSSION

A. granosa samples were classified as filter feeders, living on sandy mud substrates at depths of 10 to 20m **(Theerachat *et al.,* 2020)**. The *A. granosa* shells are round, thick and jagged based on morphological observations. *A. granosa* shell can grow reaching a length of 35mm in less than 2 years **(Harith *et al.,* 2016)**. According to **Hendra *et al.* (2020)**, *A. granosa* shell has a white color covered with periostracum with a yellow to blackish brown color. The growth lines on the outer shell represent annual and seasonal patterns **(Mirzaei *et al.,* 2014)**. The age of the shell can be seen from the hardness and ring shape **(Richardson, 1987)**.

Based on the results obtained, the yield value of *P. placenta* shell and *A. granosa* shell in this study was relatively high compared to previous studies, such as freshwater clam shells at a length of 13–14 centimeters (56.25%) **(Rahayu *et al.,* 2015)**. The yield value of *A. pleuronectes* shells obtained in the Brebes area was 62.30% **(Agustini *et al.,* 2011)**. The difference in results obtained for each type and size of the shell is assumed to affect the amount of calcium content; the higher the calcium, the heavier, longer and thicker the shell. Biominerals such as calcium carbonate were found in the shells of mollusks **(Huang *et al.,* 2018)**. These shells are made of crystals of calcite and aragonite **(Ramnath *et al.,* 2018)**. The shells have high potential sources of minerals such as calcium **(Fitriyana *et al.,* 2019)**. Biologically, the mineral content of mollusca shells is influenced by environmental factors, such as temperature, pH, and food availability **(Grenier *et al.,* 2020)**. The high calcium carbonate content in bivalvia shells can be seen in the hardness **(Akbar *et al.,* 2019)**. The more complicated the shell, the higher the calcium carbonate content. The length of the shells, which ranges from 7-8cm requires a lot of minerals in the water for their growth period, thus the calcium content in the shells is high **(Rahayu *et al.,* 2015)**.

The phosphorus content in the shells produced by each category is different. In addition, the sample size of each shell affects dramatically the amount of mineral content contained in shellfish, such as calcium and phosphorus. The phosphorus content in the *P. placenta* shell and *A. granosa* shell is higher than calcium content. This is supported by other studies on freshwater clam shells of 0.081% phosphorus **(Nurjanah & Wardhani, 2010)**. The presence of mineral elements in water such as phosphorus is very important for shellfish **(Khan *et al.,* 2020)**. Phosphorus in the bivalvia shell is phosphorus in the form of phosphate with a low content of about 1-2 %. The phosphorus content in the shells is calcium phosphate **(Kalesaran *et al.,* 2018)**. Shell contains 12 macro and microelements, such as calcium (Ca), carbon (C), magnesium (Mg), sodium (Na), phosphorus (P), potassium (K), ferrum (Fe), copper (Cu), nickel (Ni), zinc (Zn), boron (B) and silica (Si) **(Awang-Hazmi *et al*., 2007)**. Based on the measurement results of the phosphorus content of the two samples, the phosphorus value was smaller than the calcium content in the shells.

The difference between calcium and phosphorus content in each size category in the two types of shell was based on the ANOVA **(Park, 2009)**. Based on the data obtained in testing, the data distribution is expected and homogeneous. These data mean a very substantial difference between calcium and phosphorus content in *P. placenta* shell and *A. granosa* shell with different weight categories.

Based on the negative mean difference in calcium content results, the average calcium content in *A. granosa* shell has a higher value than in *P. placenta* shell. The more negative the value, the higher the calcium content of the *P. placenta* shell. If the mean difference is positive, then the calcium content of *P. placenta* shell is higher than that of *A. granosa* shell.

The results of phosphorus content showed that there was no significant difference in the average phosphorus content between *P. placenta* shell and *A. granosa* shell. It was known from the value (sig 2-tailed) that the value obtained was 0.544, which was greater than the value of 0.05. There was no significant difference in the average phosphorus content of the two samples. The difference in the average of each of the contents in the two samples was detected. The results of calcium analysis were qualitative data, where the percentage of the resulting calcium content value is not absolute. This is because the analysis carried out is an analysis that only describes the composition of bivalvia shells based on estimates **(Hermita *et al*., 2017)**.

The shell is the largest constituent of the shell composition with a coverage of 60–76% of the total weight. The size of the shell depends on the type and size of the body itself and is influenced by the living habitat of the bivalvia. Shell is a mineral source material coming usually from marine animals in the form of shells that have been milled and have significant carbonates. Bivalvia shell calcium is around 38%. The protein content of shellfish flour is 2- 3%, and the calcium content is 30- 40%. The clamshell contains several layers made of calcium carbonate that was formed by the deposition of crystals of this salt on the organic matrix of the protein conchiolin **(Khaing *et al*., 2019)**.

Calcium and phosphorus were two elements related to each other in shells. Based on the study's results, it was demonstrated that, both *P. placenta* shell and *A. granosa* shell could be used as an alternative source of calcium and phosphorus, which has a high enough calcium content for plastics, building materials, and etc.. **(Silva *et al*., 2019)**. Calcium carbonate in shells is used in polymer applications **(Hamester *et al*., 2012)**. Besides reducing wastewater pollution, the rest of the shells can help the economy of the surrounding community.

CONCLUSION

A. granosa shell shells have an average calcium and phosphorus content greater than *P. placenta* shell. This is due to differences in habitat and the quality of the shells used, but both can be used as natural sources of calcium and phosphorus. Comparing the calcium content of *P. placenta* shell and *A. granosa* shell, a significant average difference was determined. Meanwhile, the phosphorus content of the two samples did not have a significant average difference.

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