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A COMPARATIVE STUDY IN THE CALCIUM CONTENT OF THE SHELLS OF OYSTER (*CRASSOSTREA ECHINATA*), GREEN SHELL (*PERNA VIRIDIS*), CAPIZ SHELL (*PLACUNA PLACENTA*), AND NYLON SHELL (*CALLISTA ERYCINA*) FROM PANAY ISLAND, PHILIPPINES

Palma, Claire E. [1,2*], **Mamon, Shieselle Jane B.** [1], **Rubin, Khristine Natalie D.** [1], **Lauron, Jennica Marie B.** [1], **Layawon, Gretchin I.** [1], **Jumayao, Sabrina Kaye G.** [1], **Lumauag, Perry Emmanuel E.** [1], **Rodrigo, Stella Marie D.** [1], **Campos, Jeremay P.** [1], **and Bandiola, Teresa May B.** [3]

[1]*College of Pharmacy and Medical Laboratory Science, University of San Agustin, Iloilo City, Philippines*
[2]*College of Pharmacy, Centro Escolar University, Manila City, Philippines*
[3]*College of Pharmacy, National University, Manila City, Philippines*

ABSTRACT

Objective: The study aimed to compare the calcium content of the shells of oyster (Crassostrea echinata), green shell (Perna viridis), Capiz shell (Placuna placenta), and Nylon shell (Callista erycina) from Panay Island, Philippines.
Methodology: Calcium content of the four shells was determined using potassium permanganate titration and atomic absorption spectroscopy (AAS).
Results: There was no significant difference (p = >5) on the calcium content of the oyster shell, the green shell, the Capiz shell, and the Nylon shell. Also, there was no significant difference (p = >5) between the two methods used in determining their calcium content.
Conclusion: Among the four shells, the Nylon shell showed the highest calcium content in both potassium permanganate titration and AAS. Also, the extraction of calcium content does not vary as to what method was used. This study can be utilized as a reference in formulating calcium-related drug products.

Keywords: Calcium content, shells, oyster, green shell, Capiz shell, Nylon shell.

INTRODUCTION

Calcium is a nutrient that is essential for many functions in human health. It is the most abundant mineral in the body with 99% found in teeth and bone and only 1% in serum. Research has shown that adequate calcium consumption can help reduce the risk of fractures, osteoporosis, and diabetes in some populations. The dietary requirements of calcium and other collaborative nutrients vary slightly across the globe (Beto, 2015).

According to the Recommended Energy and Nutrient Intakes (RENI) developed by the Food and Nutrition Research Institute of the Department of Science and Technology (FNRI-DOST) of the Philippines in 2002, calcium requirement differs according to population groups and sex. The male and female adults, 19 – 64 years of age need 750 milligrams (mg) per day of calcium, while those sixty five years old and over need 800 mg per day. Fish was shown to be the primary source of calcium in the Filipino diet, followed by rice and cereals, vegetables and milk and milk products (Philippine Information Agency, 2012).

Seashells are notable as natural source of calcium for food and other similar products (Malu et al, 2009).The adult mollus can shell is a remarkably stable organo-mineral bio composite, in which the calcium carbonate mineral makes up 95–99% (Kocot et al, 2016). In the oyster shells, the high calcium carbonate content (80-95%) gives the potential use as raw material for several products (Alvarenga et al., 2012). In the Philippines, the calcium carbonate from Philippine green mussel shells was shown to be useful as extender in the manufacture of flat latex paints (Musico, 2007). Yet, special attention should be devoted to the disposal or processing of seashells (Alvarenga et al., 2012).

This study has utilized waste products of various seashells as potential recyclable material to reduce pollution since they do not easily decompose.

MATERIALS AND METHODS

Collection and Preparation of Materials

The shells of oyster were collected from Tatoy's Manokan and Seafood at Villa, Iloilo City, Panay Island. The green shells were obtained from Allan's Talabahan at Oton, Iloilo City, Panay Island. The Capiz shells were gathered from the side vendors along Oton, Iloilo City, Panay Island. And lastly, the Nylon shells were collected from Ocean City Restaurant at General Luna Street, Iloilo City, Panay Island.
The four species of shells were authenticated by Dr. Stephanie S. Pimentel of the School of Fishery and Marine Sciences, Aklan State University. Then, the shells were washed and cleaned using distilled water and were oven-dried for three hours at 135 ^{o}C. The green shell, the Capiz shell and the Nylon shell were ground using mortar and pestle while the oyster shell was ground using a hammer. The shells were further ground until fine powders using a granulator. They were then sieved (mesh #40) to equalize particle size and were classified according to their species.

Moisture Content Test

The oven was regulated to 135 ^{o}C. Two grams of samples were weighed into low covered dishes (50 mm diameter and 40 mm deep). With the covers removed, the dishes and the covers were placed in the oven as quickly as possible and then were dried for two hours. The covers were placed on the dishes and were transferred to a desiccator. They were allowed to cool. After cooling, they were weighed and water was calculated as the loss in weight.

Organoleptic Test

The shells were observed according to their appearance, odour, and texture. Watch glass was used as the container of the ashes.

Total Ash Content

Two grams of each sample were weighed in porcelain crucibles and were placed in a temperature-controlled furnace. The temperature of the furnace was maintained at 600 ^{o}C for two hours. After that, the crucibles were placed in a desiccator and were cooled. They were then weighed immediately and were expressed in percentage.

Permanganate Titration

Two grams of each finely ground samples were weighed into porcelain dishes and were ignited in a furnace to carbon-free ash, but fusing was avoided. The residue was boiled in 40 mL HCl (1:3 v/v) and few drops of concentrated HNO_3. It was transferred to 250-mL volumetric flask, cooled, diluted to volume, and mixed thoroughly. 25 mL clear liquid were taken using a pipette into a beaker was diluted to approximately 100 mL, and then 2 drops of methyl red were added. When the endpoint was reached, an intermediate brownishorange color was shown by the addition of NH_4OH drop wise to pH of 5.6. If overstepped, HCl was added using a dropper until it turned orange. 2 more drops of HCl were added to the solution. The color was pink (pH 2.5-3.0). It was diluted to approximately 150 mL and was brought to boiling. 10 mL hot saturated 4.2% solution of $(NH_4)_2C_2O_4$ were slowly added with constant stirring. If red color changes to orange or yellow, HCl was added drop wise until the color changed back to pink. Then, it was allowed to stand overnight for the precipitate to settle.

The supernatant was filtered through quantitative paper and was washed thoroughly with NH_4OH. The paper or crucible with precipitate was placed in original beaker, and a mixture of 125 mL H_2O and 5mL H_2SO_4 was added. It was heated to 70 degrees and was titrated with 0.1N $KMnO_4$ to slight pink. The presence of paper caused the color to fade in a few seconds. The blank was used to correct the color change and percentage of Calcium was calculated.

Atomic Absorption Spectroscopy (AAS)
The solution of a sample was fed into the head of the burner. A flexible capillary tube connected the solution to the nebulizer. At the tip of the capillary tube, the solution was nebulized and broken down into small drops. The larger drops fell out and were drained off while the smaller ones were vaporized in the flame. A radiation coming from the light source passed through the vaporized sample. A wavelength of 422.17 nm specific for calcium was used. The monochromator selected the specific light for which was absorbed by the calcium atoms. The selection of specific light allowed the determination of the calcium atoms. The light selected by the monochromator was directed unto the detector. The amount of radiation the calcium absorbed was compared with the calibration curve (10 standard curves were used) and this enabled the calculation of calcium concentration in the four shell samples.

Statistical Tools
Statistical tools namely mean, one-way analysis of variance (ANOVA), t-test, and two-way ANOVA were used in the study to tabulate and tally the data gathered. All computations were done using the Statistical Package for Social Sciences (SPSS).

RESULTS AND DISCUSSION

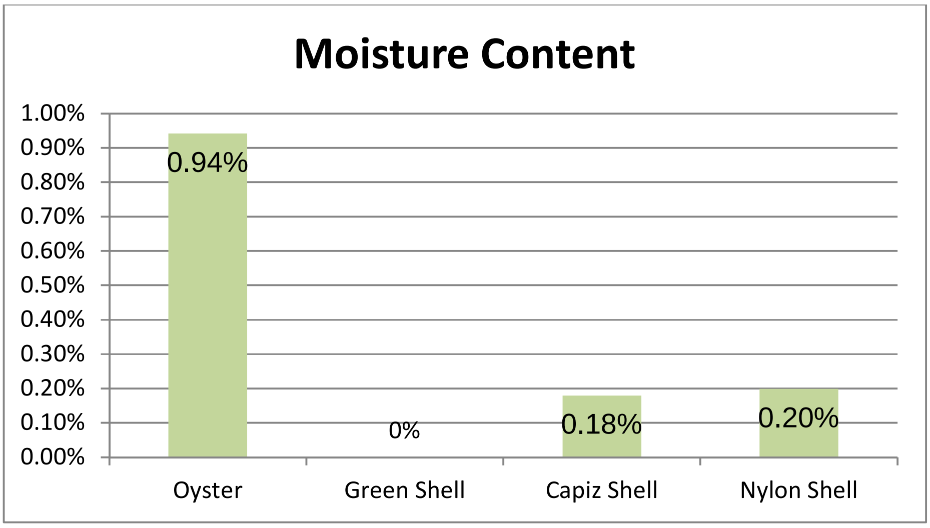

Figure 1. Moisture Content

Figure 1 shows that the oyster shell has the highest moisture content of 0.94% which means that it is the most susceptible to microbial growth or contamination. Next to oyster is the Nylon shell with 0.20% followed by the Capiz shell with 0.18%. Both are susceptible to microbial contamination also but to a lesser extent. The green shell with 0% shows to have no moisture content which implies that it is not susceptible to microbial contamination and can be a good raw material for product formulation.

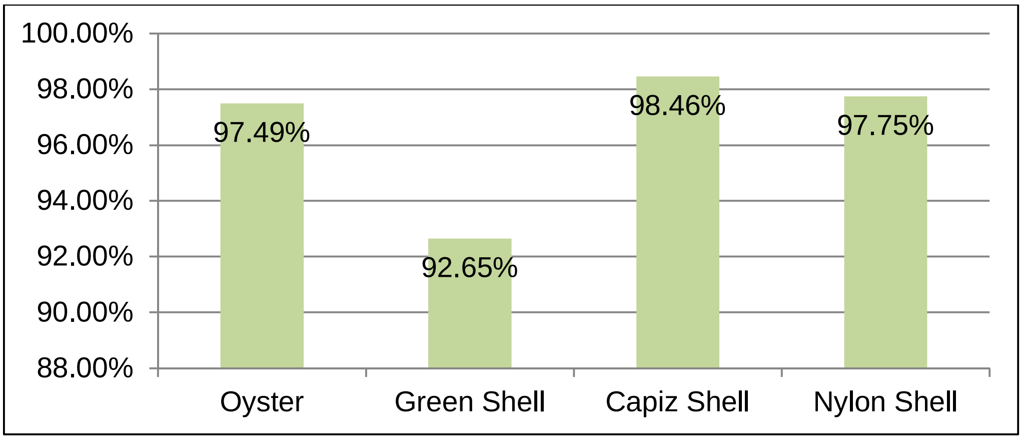

Figure 2. Ash Content

Figure 2 shows that the Capiz shell has the highest ash content of 98.46% which indicates that it is the purest shell among the four samples. It is followed by the Nylon shell with 97.75%, the oyster shell with 97.49%, and the green shell with 92.65%, respectively.

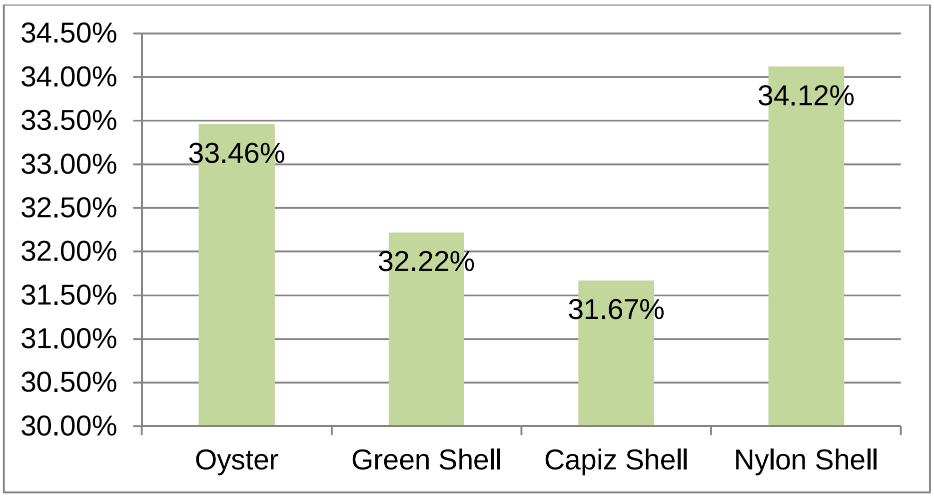

Figure 3. Calcium Content Determination Using Permanganate Titration

Figure 3 shows that the Nylon shell has the highest calcium content of 34.12% compared to the three samples followed by oyster shell with 33.46%, the green shell with 32.22%, and the Capiz shell with 31.67%, respectively. This shows that the Nylon shell can be the best source of calcium in making different calcium-related dosage forms.

Figure 4 shows that the Nylon shell has the highest calcium content with 39.76%, followed by the oyster shell with 34.64%, the green shell with 32.47%, and the Capiz shell with 28.12%, respectively. This result implies that the Nylon shell can be the best source of calcium in the formulation of different calcium-related dosage forms.

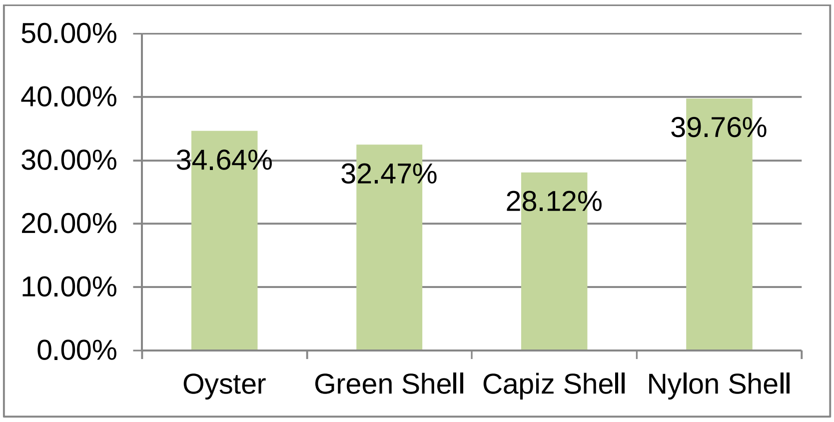

Figure 4. Calcium Content Determiantion Using AAS

Table 1. Organoleptic Examination

Characteristics	Oyster	Green Shell	Capiz Shell	Nylon Shell
Appearance	fine powder	flakes	coarse powder	coarse powder
Color	white	greyish	white	yellowish– white
Odour	mild salty odour	odourless	salty odour	salty odour
Texture	rough	coarse	smooth	Smooth

Table 1 shows that the oyster shell is a white and rough powder with a mild salty odor, the green shell as grayish and course flakes with no odour, the Capiz shell as white and smooth powder with a salty odor, and finally, the Nylon shell as yellowish-white and smooth powder with a salty odor.

Table 2. Significant Difference Between AAS and Permanganate

Process	N	M	T	df	Two-tail sig (0.05)
AAS	8	33.747791	0.543	14	0.602
Permanganate	8	32.618001			

Table 2 shows that there is no significant difference between AAS and permanganate methods. Therefore, the extraction of calcium content does not vary as to whether AAS or potassium permanganate method is used.

Table 3. Significant Difference Among the Shells

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	109.965	3	36.655	3.188	.063
Within Groups	137.964	12	11.497		
Total	247.929	15			

Table 3 shows that the calcium contents of the four kinds of shell have no significant differences, $\underline{F}$ (3,32) = 3.188, $\underline{p}$ = 0.063. The probability of 0.063 is greater than 0.05 indicating that the calcium contents of the four shells do not vary.

Table 4 reveals that based on the total mean, the Nylon shell has the highest calcium content of 36.94%. It is followed by the oyster shell with a calcium content of 34.05% and the green shell with a calcium content of 31.85%. On the other hand, the Capiz shell has the lowest calcium content of 29.89%.

Table 4. Comparison of the Calcium Contents

Process	Shell	Mean	N	Std. Deviation
AAS	Oyster	34.63910	2	5.2699254
	Green Shell	32.47435	2	6.9860029
	Capiz Shell	28.11665	2	2.3390385
	Nylon Shell	39.76105	2	.8848027
	Total	33.74779	8	5.6492801
Permanganate	Oyster	33.46090	2	1.1955761
	Green Shell	31.22030	2	1.7203908
	Capiz Shell	31.66835	2	1.4405886
	Nylon Shell	34.12245	2	1.1402097
	Total	32.61800	8	1.6657483
Total	Oyster	34.05000	4	3.1932048
	Green Shell	31.84732	4	4.2165002
	Capiz Shell	29.89250	4	2.5923586
	Nylon Shell	36.94175	4	3.3603950
	Total	33.18289	16	4.0655402

In the potassium permanganate titration process, the Nylon shell has the highest calcium content with a mean of 34.12%. It is followed by the oyster shell with a mean calcium content of 33.46% and is followed by the green shell with a mean calcium content of 31.22%. Additionally, the Capiz shell has the least mean calcium content of 31.67%.

In the AAS process, the Nylon shell has the highest calcium content with a mean of 39.76%. It is followed by the oyster shell with a mean calcium content of 34.64% and is followed by the green shell with a mean calcium content of 32.47%. The Capiz shell has the least mean calcium content of 28.12%.
Statistical data shows that the Nylon shell has the highest calcium content on both processes. Also, the results on the calcium content on both processes do not vary.

Table 5. Test of Between-Subjects Effects

Dependent Variable: Calcium					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Process	5.106	1	5.106	.451	.521
Shell	109.965	3	36.655	3.237	.082
process * shell	42.264	3	14.088	1.244	.356

Table 5 shows that there is no interaction between the processes used in determining the calcium content and the type of shell used, p= 0.356. This means that there is no specific type of process to be used in determining the calcium content for a specific type of shell. Any process will yield the same calcium content using any of the four kinds of shell.

It also shows that there is no significant difference in the calcium content of the four types of shell, p=0.082. This shows that the calcium contents of the four kinds of shell do not vary.

CONCLUSION

It is, therefore, concluded that the Nylon shell has the highest calcium content among the four kinds of shell and can be a good source of calcium in the formulation of various calcium-containing products. Furthermore, both methods of determination showed that there is no significant difference in terms of the calcium content of the shells.

This study also paves way to use various seashells as potential recyclable materials to reduce pollution since they do not easily decompose.

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