

## Utilization of Hydroxyapatite from Shortfin Scad (*Decapterus macrosoma*) Bones as Calcium Fortificant in Cookies

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**Abstract:** Shortfin scad (*Decapterus macrosoma*) is locally known as selayang, basung and sardine in Malaysia. It is commonly used to make 'keropok lekor' and fish crackers as the main ingredient. The fish's entire body of rounded flesh is considered the main product while head, backbone, trimmings (cutoffs), skin and guts constitute by-products or wastage. Therefore, in order to leverage the potential of its by-products, the present study is aimed to utilize hydroxyapatite extracted from *Decapterus macrosoma* bones as calcium fortificant in cookies. In cookies formulation, wheat flour was replaced with hydroxyapatite at 0%, 6%, 12%, 18% and 24% level. T<sub>24</sub> which was incorporated with 9.5% hydroxyapatite, has demonstrated the highest hardness (3096 g) and calcium content 6.67% (w/w), and appeared darkest in color with L\*, 47.85. As for sensory evaluation, control sample was preferred by panelists for all sensory attributes evaluated.

**Keywords:** Cookies, Hydroxyapatite, Calcium, Fortification

### INTRODUCTION

Cookies can be savoured as a snack. Having a broad range of selections, longer shelf life and low cost make cookies convenient for consumer [1]. Due to the variety in taste, crispiness and digestibility, cookies hold a significant position in snack foods [2].

Accordingly, the wholesomeness of cookies relies on their ingredients [3]. There have been numerous attempts to enhance cookies' nutritional benefits by altering their composition. Many food products have been developed through fortification in recent years as consumers are requesting safe, sensory quality foods. Fortifying calcium in cookies, for example, by adding sesame and dried moringa leaves [4].

In line with recent research trends which focuses on sustainable and renewable materials, present study will utilize hydroxyapatite (HAp) extracted from *Decapterus macrosoma* bones as calcium fortificant in cookies. Shortfin scad (*Decapterus macrosoma*) from the Carangidae family are readily discovered in Malaysia and are locally known as selayang, basung and sardine [5]. Regardless of being able to be transformed into other low-value products (e.g. fish meal, natural fertilisers), fish bone is underutilized and more commonly being disposed back into the river or sea. Moreover, discarding fish bone into the river indicates poor waste management that might lead to environmental pollution.

To date, no study has been conducted on the use of waste-based HAp in food use. The results of this project will therefore be enriching and beneficial in order to intensify the use of fish waste for global sustainability. This work is therefore aimed at developing calcium-fortified cookies through the use of HAp derived from *Decapterus macrosoma* bones and exploring their physicochemical and sensory properties.

### MATERIALS AND METHODS

The ingredients needed for making cookies such as wheat flour, butter, castor sugar, eggs, vanilla, baking powder and salt were purchased from local market in Sibul, Sarawak. Analytical grades were used in all chemical reagents.

#### Extraction of Hydroxyapatite from *Decapterus macrosoma* Bones

About 10 kg of *Decapterus macrosoma* bones were obtained from Terengganu. The bones were transferred to container and boiled for 2 hours in 100 °C. The visible impurities and adherent tissues such as fats flesh and scales were removed through tap water prior being transferred to trays and dried in the furnace at 100 °C for 24 hours. The dried fish bones were properly packed and sealed in 150 x 90 mm snap-lock bags (GLAD,

Oneplastic, Malaysia) prior to storage at 10 °C until further use.

The extraction of HAp from the prepared fish bones started off by washing them in tap water, treated with 0.8 M NaCl, followed by soaking in 0.2 M NaOH for five hours at 5 °C, rinsed again in tap water. Subsequently, the treated bones were dried in drying oven at 60 °C overnight. The dried bones were calcined at 900 °C for 3 hours in muffle furnace. The samples were maintained cool naturally in muffle furnace. The calcined bones or known as HAp were ground into powder form by using electric blender (Waring SS-110, Japan). HAp were then sieved to using 100 µm sieve. The ground HAp was then stored in closed container for future use.

### Cookies Preparation

Cookies were prepared by adapting formulation from Quiñones, [6]. The basic formulation employed for cookies was 39.5% wheat flour, 24.6% butter, 22.0% castor sugar, 12.0% eggs, 1.1% vanilla and 0.2% salt as shown in Table 1. The substitution of wheat flour was performed at 6%, 12% 18% and 24% level with hydroxyapatite. Based on preliminary study done, the substitution of wheat flour above 24% level yielded hard texture cookies due to nature of hydroxyapatite which is chemically alike to the mineral component of bones and hard tissues in mammals [7].

slowly introduced and stirred until a velvety dough was achieved. The dough then allowed to rest for 20 minutes and then rolled with a rolling pin to achieve, by visual estimation a uniform thickness of about 1 cm.

Using a 6 cm diameter cup, the sheeted dough was then cut into circles and baked for 15 min on non-greased aluminum trays at 170 ° C in gas oven equipped with air-circulating fan. All the cookies from each dough were simultaneously baked on different trays. After baking, cookies were left to cool at room temperature (around 24–26 ° C) for 25 min on a cooling rack and then packed in polyethylene bags and kept at room temperature for 3 days in a dry place before chemical and sensory evaluation was conducted.

### Color Analysis

Cookie color measurement was performed using an optical sensor colorimeter based on CIE L \*, a \*, b \* color scheme. L \* values are black to white (0–100), a \* values are positive for redness, and b \* values are positive for yellowness.

### Texture Analysis

Textural analysis was carried out using TA.XT2i SMS Stable Micro Systems Texture Analyzer (Stable Microsystems Ltd., Surrey, England) and adapted the procedure from Budžaki *et al.*, [9]. The three-point

Table 1: Cookies Formulations

Ingredients	Formulation				
	T <sub>0</sub>	T <sub>6</sub>	T <sub>12</sub>	T <sub>18</sub>	T <sub>24</sub>
Wheat flour	39.5%	37.1%	34.8%	32.4%	30.0%
Hydroxyapatite	0.0%	2.4%	4.7%	7.1%	9.5%
Butter	24.6%	24.6%	24.6%	24.6%	24.6%
Castor Sugar	22.0%	22.0%	22.0%	22.0%	22.0%
Eggs	12.0%	12.0%	12.0%	12.0%	12.0%
Vanilla	1.1%	1.1%	1.1%	1.1%	1.1%
Salt	0.2%	0.2%	0.2%	0.2%	0.2%
Baking powder	0.6%	0.6%	0.6%	0.6%	0.6%

\*T<sub>0</sub> - Control, cookies without wheat flour substitution; T<sub>6</sub> - Cookies with 6% wheat flour substitution; T<sub>12</sub> - Cookies with 12% wheat flour substitution; T<sub>18</sub> - Cookies with 18% wheat flour substitution; T<sub>24</sub> - Cookies with 24% wheat flour substitution

Upon modification, the creaming process [3,8] was used to make the cookie dough. In an appropriate bowl with a hand mixer, castor sugar and butter were whisked together for 10 min. Subsequently, in the same bowl, the whisked mixture were beat together with eggs and vanilla for 2 minutes briefly. The sifted wheat flour, ground HAp, salt and baking powder were then

break technique was used to test all cookie formulations. Quantified parameters were hardness (g) — peak breaking force, fracturability (mm) — mean breaking distance that represents a cookie's tendency to fracture when force is applied and work of breaking force (g s) — total curve area that represents the total amount of force required to break the sample.

Measurement employed with the test speed of 3 mm/s and the distance between the two bottoms supports was adjusted to 50 mm.

### Calcium and Phosphorus Determination

Calcium and phosphorus were determined in the cookie samples. Briefly, the samples were first dried in an oven at 105 ° C for 24 hours, ground with a porcelain mortar and pestle 6.4 cm in diameter, then dry-ashed at 550 ° C for 6 hours in a muffle furnace. In 1 M nitric acid (HNO<sub>3</sub>), the ash was subsequently dissolved. The caked, caramel-like mass was then ashed in the same platter again and the second ash dissolved with a few drops of 1 M HNO<sub>3</sub>.

Adapting from Abdel-Moemin, both calcium and phosphorus is determined by flame atomic absorption spectrometry (Atomic Absorption Spectrophotometer 901, GBC Scientific Equipment Pty Ltd., Melbourne, Victoria, Australia) [8]. Wavelengths of 422.7 nm for calcium and 715 nm for phosphorus were used with a 0.2 nm spectral band width. Reagent blanks was used to correct the instrument readings. Calibration standards established by diluting the high standards of pure commercial metal. The standards were developed from the dissolved 1000mg / l standards in 0.1N HNO<sub>3</sub>. A series of working standards was prepared from these standard stock solutions. These standard stock solutions were used to develop a set of working standards. A recovery test for calcium and phosphorus for selected samples was conducted by spiking the analyzed samples with metal standard aliquots and then re-analyzing the samples.

### Sensory Evaluation

Affective testing, or known as hedonic test method, was used to measure the extent of liking or disliking of a product. In this study, the sensory properties of cookies determined using hedonic test method. About 30 untrained panelists were involved in this sensory evaluation. Drinking water was used for the mouth

rinsing. The cookies have been evaluated in terms of the colour, odour, flavour, texture and overall acceptability. The scales of rating range from dislike extremely (1) to like extremely (9).

### Statistical Analysis

Results attained in this study are expressed as mean ± standard deviation of 3 independent replicates. One-way analysis of variance (ANOVA) and Tukey's Range Test (SPSS software program, Version 25.0, SPSS Inc., Chicago, IL, USA) was used to determine significant difference at the level of p-value<0.05.

## RESULTS AND DISCUSSION

### Color of Cookies

The effect of HAp incorporation on colour of cookies is visualized as in Table 2. The findings indicate that the increasing percentage of HAp used results in generally reduced lightness (L\*) of cookies. In agreement with Suriya *et al.*, [10], Chauhan *et al.*, [11] and Cheng and Bhat [12], this may be caused by increment in a\* and b\* of cookies. As been reported by Cheng and Bhat [12] in their research, yam cookies appeared darker in color due to the reduction in lightness and augmentation in both redness and yellowness value.

Both Maillard reaction caramelization process are responsible for the color of cookies during baking process. In present study, the independent variables were the percentage of HAp utilized to substitute wheat flour. As such, across the level of substitution from 6% to 24%, percentage of wheat flour used was reduced. Cutting the percentage of wheat flour has resulted in the decreased sugar and protein content in cookies and consequently affected browning reactions. In contrast to Chevallier *et al.*, [13], the whiteness of cookies was negatively proportional to the protein content. This theory suggested, as protein and sugar content were reduced, browning reactions somehow slowed down, thus the cookies appeared less dark. However, in

Table 2: Color Properties of Cookies

Formulation	Color Parameter		
	L*	a*	b*
T0	65.24 ± 1.57 <sup>d</sup>	3.66 ± 0.13 <sup>a</sup>	15.06 ± 0.677 <sup>a</sup>
T6	56.09 ± 0.51 <sup>c</sup>	4.09 ± 0.02 <sup>b</sup>	16.96 ± 0.43 <sup>ab</sup>
T12	54.89 ± 0.84 <sup>bc</sup>	4.33 ± 0.05 <sup>bc</sup>	16.64 ± 0.59 <sup>ab</sup>
T18	52.00 ± 1.19 <sup>b</sup>	4.52 ± 0.21 <sup>c</sup>	17.69 ± 0.84 <sup>b</sup>
T24	47.85 ± 1.27 <sup>a</sup>	5.10 ± 0.11 <sup>d</sup>	22.08 ± 1.28 <sup>c</sup>

\*Mean with different superscript within the same column are significantly different at P<0.05.

\*T<sub>0</sub> - Control, cookies without wheat flour substitution; T<sub>6</sub> - Cookies with 6% wheat flour substitution; T<sub>12</sub> - Cookies with 12% wheat flour substitution; T<sub>18</sub> - Cookies with 18% wheat flour substitution; T<sub>24</sub> - Cookies with 24% wheat flour substitution

present study, the reduction of wheat flour in cookies' formulation contributed to the declination in lightness of cookies. This might be due to the color of HAp which was graying white in color, resulted in darker color perceived.

### Texture of Cookies

The hardness and fracturability of the cookies are shown in Table 3. Substitution of HAp powder in the cookies formulation eventually will result in significant changes to the textural quality of the cookies. Substitution of wheat flour with HAp powder at 24% level showed the highest hardness. The lowest hardness was noticeable in the control where no HAp powder is

mineral form has significantly increased the hardness of cookies. As HAp is naturally dense, it suppressed the expansion of cookies, resulted in more compact cookies, which ultimately necessitated higher energy to break the cookies into pieces during mastication. In line with Nawaz *et al.*, [16], incorporation of fish bone decreased the expansion in fried snacks.

### Calcium and Phosphorus Content of Cookies

As presented in Table 4, cookies formulation in which wheat flour was substituted at 24% level with HAp exhibited the highest calcium content. Increasing trend was obviously observed as across the level of substitution from 6% to 24% level. Conforming to

Table 3: Textural Properties of Cookies

Formulation	Texture Parameter	
	Hardness	Fracturability
T0	2101.74 ± 247.71 <sup>a</sup>	27.82 ± 0.23 <sup>a</sup>
T6	2328.17 ± 413.89 <sup>b</sup>	29.60 ± 0.65 <sup>bc</sup>
T12	2647.05 ± 298.32 <sup>c</sup>	28.36 ± 0.83 <sup>ab</sup>
T18	3027.19 ± 619.30 <sup>d</sup>	30.94 ± 0.15 <sup>c</sup>
T24	3096.68 ± 631.54 <sup>d</sup>	29.82 ± 0.84 <sup>c</sup>

\*Mean with different superscript within the same column are significantly different at P<0.05.

\*T<sub>0</sub> - Control, cookies without wheat flour substitution; T<sub>6</sub> - Cookies with 6% wheat flour substitution; T<sub>12</sub> - Cookies with 12% wheat flour substitution; T<sub>18</sub> - Cookies with 18% wheat flour substitution; T<sub>24</sub> - Cookies with 24% wheat flour substitution

being used in the cookies baking.

The findings presented in Table 4.2, indicated that the substitution of wheat flour with HAp has significantly increased the hardness of cookies. The higher percentage of HAp, the harder cookies produced. In this study, HAp was extracted from fish bone by using sintering method or calcination process, where the cleaned bones were heated at a very high temperature (5000 °C). As supported by Hariani and Said [14], by subjecting fish bones to immense temperature, the mechanical properties of extracted HAp were greatly affected, such as the increase of hardness. Calcining in presence of oxygen will remove organic materials, leaving only non-organic material displaying a high degree of crystallinity [15], which ultimately result in increased hardness. Therefore, the substitution of wheat flour with HAp which presents as

Abdul-Moemin [8], calcium content determined was directly proportional to the amount of fish bone incorporated into cookies. Furthermore, fish bone is rich in calcium, phosphate, and carbonate [17,18] where such minerals could be extracted in the form of HAp.

Therefore, the higher amount of HAp added, the higher content of calcium was determined in cookies. Likewise, the phosphorus content in cookies demonstrated increasing trend in parallel with level of substitution and could be observed highest in T<sub>24</sub>. One might be able to predict the phosphorus (phosphate) content in a sample by knowing the calcium content and divide it with 1.67. The value of 1.67 is the molar ratio of Ca/P found in HAp derived from natural sources [18].

Table 4: Textural Properties of Cookies

Formulation	Calcium (g/100 g)	Phosphorus (g/100 g)
T0	0.08	0.16
T6	1.77	1.06
T12	3.04	1.84
T18	4.31	2.59
T24	6.67	4.01

\*Mean with different superscript within the same column are significantly different at  $P < 0.05$ .

\*T<sub>0</sub> - Control, cookies without wheat flour substitution; T<sub>6</sub> - Cookies with 6% wheat flour substitution; T<sub>12</sub> - Cookies with 12% wheat flour substitution; T<sub>18</sub> - Cookies with 18% wheat flour substitution; T<sub>24</sub> - Cookies with 24% wheat flour substitution

### Sensory Evaluation

Sensory attributes evaluated by panelists were color, taste, mouthfeel, crispiness and overall acceptability. A 9-point of hedonic scale was used to rate each attribute ranging from score 1 (extremely dislike) to score 9 (extremely like). The samples were served within one week after production. The mean scores of each attribute were presented in the Table 5. 30 untrained panelists consisted of students and staff of University College of Technology Sarawak involved in this sensory evaluation of cookies.

treated samples. This parameter exhibited declining trend as the percentage of HAp used was increased.

It implies that the addition of HAp into cookies formulation had significantly reduce the taste acceptability. Based on the study conducted by Tyagi *et al.*, [19], the taste of cookies is affected by the ingredients used. It indicates that, in spite of the nature of HAp which presents in the form of tasteless powder, it somehow gives unpleasant taste to the cookies. Chaudari and Fanion [20] suggested that the incorporating in demand nutrients into food may influence sensory characteristics including taste,

Table 5: Sensory Acceptability of Cookies

Sensory Attribute	Formulation				
	T <sub>0</sub>	T <sub>6</sub>	T <sub>12</sub>	T <sub>18</sub>	T <sub>24</sub>
Color	8.77 ± 0.73 <sup>a</sup>	8.27 ± 0.64 <sup>a</sup>	5.57 ± 0.97 <sup>b</sup>	4.37 ± 0.85 <sup>b</sup>	2.77 ± 0.43 <sup>c</sup>
Taste	8.93 ± 0.25 <sup>a</sup>	7.53 ± 0.51 <sup>ab</sup>	6.43 ± 0.50 <sup>b</sup>	5.17 ± 0.59 <sup>bc</sup>	3.43 ± 0.50 <sup>c</sup>
Mouthfeel	9.00 ± 0.00 <sup>a</sup>	7.53 ± 0.51 <sup>b</sup>	5.13 ± 0.35 <sup>c</sup>	3.63 ± 0.67 <sup>d</sup>	1.87 ± 0.35 <sup>e</sup>
Crispiness	8.80 ± 0.41 <sup>a</sup>	7.43 ± 0.50 <sup>b</sup>	5.60 ± 0.50 <sup>c</sup>	4.30 ± 0.47 <sup>c</sup>	2.07 ± 0.79 <sup>d</sup>
Overall Acceptability	8.50 ± 0.51 <sup>a</sup>	7.63 ± 0.49 <sup>b</sup>	5.63 ± 0.49 <sup>b</sup>	4.10 ± 0.40 <sup>c</sup>	2.17 ± 0.70 <sup>d</sup>

\*Mean with different superscript within the same row are significantly different at  $P < 0.05$ .

\*T<sub>0</sub> - Control, cookies without wheat flour substitution; T<sub>6</sub> - Cookies with 6% wheat flour substitution; T<sub>12</sub> - Cookies with 12% wheat flour substitution; T<sub>18</sub> - Cookies with 18% wheat flour substitution; T<sub>24</sub> - Cookies with 24% wheat flour substitution

Referring to the Table 5, the effect of HAp incorporated on color acceptability of cookies demonstrated declining rating as across the level of substitution from 6% to 24%. It indicates that lighter color of cookies was more preferable among panelists.

This finding is supported by the result from color analysis (Table 2) where the lightness L\* of cookies was negatively correlated with percentage of HAp used. Likely, Abdul-Moemin [8] suggested reduced color acceptability along with augmentation in percentage of Nile tilapia bones in cookies.

As for the taste acceptability of cookies, there were significant difference between control sample and

appearance, texture and other attributes as these eventually constitute perceived product quality.

The mouthfeel acceptability of cookies was indirectly proportional to the percentage of HAp used. The addition of HAp into cookies formulation significantly reduced mouthfeel acceptability. This finding could be justified with the fact that HAp present in the form of crystalline grains [21]. This physical characteristic of HAp resulted in distinctive sandiness in cookies. The results is supported by Engelen *et al.*, [22], suggesting that the addition of particles with larger size increased the sensation of roughness attributes in vanilla custard dessert, perceived as sandiness or grittiness.

Table 5 also represents the negative correlation between crispiness acceptability and percentage of HAP used. According to Pereira *et al.*, [23], the crispiness of the cookies is directly correlated to the hardness of the cookies. Nevertheless, panelists had rated control sample as the best for its crispiness. It indicates, hardness of cookies over 2101 g yielded unacceptable crispiness for panelists.

Control sample recorded the highest score for overall acceptability. Generally, overall acceptability exhibited decreasing trend as the percentage of HAP added increased. According to Stone *et al.*, [24], overall acceptability necessitates the panelists rate the product as a whole without direction to any sensory aspects that are considered important. However, in addition to the significant differences observed, the declined mouthfeel and crispiness acceptability might also lessen the overall acceptability. According to Devereux *et al.*, [25], Texture is one of the key attributes of food quality, where lower texture scores may lessen overall acceptability.

### CONCLUSION

Conclusively, cookies fortified with calcium by utilizing HAP extracted from *Decapterus macrosoma* bones was successfully produced. In terms of colour analysis, as across level of substitution from 6% to 24%, L\* decreased along with the increased a\* and b\*. The hardness and fracturability of treated cookies demonstrated increasing trend as the amount of HAP used was escalated, ranging from 2328 – 3096 g for the former and 29.6 – 30.94 mm for the latter. Similar trend was observed in calcium content. As expected, both calcium and phosphorus content was found highest in sample T<sub>24</sub> as this sample had highest percentage of HAP. Meanwhile, as across the substitution level from 6% to 24%, the results from sensory evaluation has demonstrated declined trend in all sensory attributes analyzed; color, taste, mouthfeel, crispiness and overall acceptability.

It is recommended to discover other methods of comminution to reduce the particle size of HAP into nano-sized particles to enhance sensory qualities of cookies such as texture and mouthfeel characteristics. Besides, it is highly suggested to determine microbiological properties of the cookies incorporated with HAP from *Decapterus macrosoma* bones as well as the absorption rate of calcium in human through bioavailability study.

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