# THE EFFECT OF YTTRIA-STABILIZED ZIRCONIA (YSZ) ADDITION ON THE SYNTHESIS OF BETA-TRICALCIUM PHOSPHATE (β-TCP) FROM BIOGENIC HYDROXYAPATITE

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**Abstract**. The utilization of β-tricalcium phosphate (β-TCP) in medical field has been on demand for their excellent biocompatibility, bioactivity and solubility properties. This material can be produced through degradation of hydroxyapatite (HA) at high temperature. In this research, biogenic hydroxyapatite from fish scales (FsHA) was selected as pre-cursor for the synthesis of β-TCP. The effect of yttria-stabilized zirconia (YSZ) addition on degradation of FsHA into β-TCP have been investigated. Different amount of YSZ ranging from 5 to 15 wt% were mixed with FsHA and ball milled into fine powder. The mixture was then sintered at temperature of 1200 °C. The materials were characterized using X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM). The results from FTIR, XRD and SEM-EDX analyses proved that the presence of β-TCP in the sintered sample at 1200 ºC. The percentage of β-TCP was calculated ranging from 17 to 21 % as analyzed from XRD pattern. It was found that the FsHA/YSZ composite with 5 wt% of YSZ addition produced the highest composition of β-TCP with smallest crystallite size at the ideal sintering temperature of 1200 °C. The results also shown that the FsHA was not fully converted into β-TCP during sintering. Furthermore, XRD analysis also clearly shown the presence of another phase known as calcium zirconate ($CaZrO_3$) phase. The triphasic calcium phosphate (BCP) has high potential to be used as biomaterials for bone fillers applications.

**Keywords:** Fish scale hydroxyapatite (FsHA), yttria-stabilized zirconia (YSZ), sintering temperature, calcium zirconate (CaZrO3)

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## Introduction

The excellent properties of hydroxyapatite (HA) for its biocompatibility and bioactivity are undeniably gaining attention in medical field as this material resembles the mineral component of the bone and teeth [1]. Current research utilized the HA extracted from natural source i.e fish scale of the tilapia through the hydrolyzation process denoted as fish scale hydroxyapatite (FsHA). The usage of FsHA is more ideal than the HA produced synthetically in the aspect of cost and chemicals intervention, as it is also made the best usage of the aquatic waste. The FsHA also enhance its credibility from the perspective of HALAL status over the usage of HA that is extracted from the mammals such as porcine and bovine [2].

HA tend to decompose into other calcium phosphate products such as tetracalcium phosphate (TTCP) when introduced to high temperature. At an even higher temperature, TTCP can be decomposed further into tricalcium phosphate (TCP) and calcium oxide (CaO) [3]. Different types of TCP can be produced at certain temperature and condition i.e α-TCP and β-TCP. The formation of β-TCP through the decomposition of HA by sintering method improved the degradation rate of HA. This will spread the demand of HA application which is not limited for the bone filler only but also for the bone tissue engineering template materials. At the certain sintering temperature and condition, the HA was not completely decomposed resulted the formation of β-TCP phase, thus forming biphasic calcium phosphate (BCP). BCP contain both HA and β-TCP phases which make them adequately resorbable, osteoinductive, optimally soluble and maintaining its biocompatibility as compared to pure HA [4].

It is crucial to be concern regarding the ideal sintering temperature and method in order to synthesize β-TCP without completely decompose HA. In this research, the conventional sintering method using pressure less furnace has been utilized. Previous study reported that HA degraded up to 56 % into other types of calcium phosphate such as TCP after sintered at 1250 °C [5]. Another research stated that approximately 5 % TCP were generated when HA being sintered at 1200 °C even without any addition of zirconia [6]. On the other hand, other researcher confirmed that YSZ contributes numerously in the synthesize of TCP [7]. The presence of small percentage of YSZ in HA matrix was able to decompose HA at a relatively lower temperature [8,9], thus reducing the cost of β-TCP production. The decomposition of HA/YSZ composites to β-TCP is correlated to the addition amount of YSZ [10]. None of the research have been reported to synthesis β-TCP from FsHA as starting material, thus this research was done to observe the decomposition of FsHA into of β-TCP with the presence of 5 to 15 wt% of YSZ at sintering temperature of 1200 °C.

## Materials and Methods

### *Materials*

The main materials used in this research was fish scale hydroxyapatite (FsHA) derived from the Tilapia fish scales and yttria-stabilized zirconia (YSZ) purchased from MAJU Saintifik Sdn Bhd, Selangor, Malaysia. The commercial HA and β-TCP were purchased from Sigma-Aldrich and Fluka, respectively as the comparative materials from those of laboratory synthesized.

### *Preparation of Composite*

The FsHA/YSZ composites were prepared using the wet milling technique. The mixture of 5, 10 and 15 wt% of YSZ into FsHA making up dry powder of 100 g and addition of 1000 ml of distilled water were milled in the jar mill for 48 hours. This way, the mixture of FsHA and YSZ were able to spread homogenously [7]. The homogenized slurries were then dried in the oven at 60 $^{o}$C for 24 hours. The FsHA/YSZ mixture were then sintered at temperature of 1200 $^{o}$C in Nabertherm furnace at the heating rate of 5 $^{o}$C per minute.

### *Characterization of Composite*

The sintered composites were then characterized by X-Ray Diffraction (XRD) analysis (Model: Rigaku MiniFlex) with the usage of voltage and current was 40 kV and 15 mA, respectively, scanning from 3$^{o}$ to 80$^{o}$ with the rate of 3.0$^{o}$ per minute. The analysis on the functional group of the FsHA/YSZ composites was carried out by Fourier-transform infrared spectroscopy (FTIR) analysis (model Nicolet, USA). The spectra were recorded from 4000 $cm^{-1}$ to 400 $cm^{-1}$. The morphology of the sample was analyzed using field emission scanning electron microscope (FESEM) (model Hitachi, SU8020, Japan). The micrograph obtained from the samples were used to determine the microstructure of the sample. In this study, energy dispersive x-ray analysis (EDX) was conducted to determine the element present in the composites. The results from EDX analysis gave the information about the calcium (Ca) and phosphate (P) compositions in the composite sample**.**

## Results and Discussion

The XRD patterns for FsHA/YSZ composites is presented in Figure 1(a) along with the commercial HA and β-TCP for the correlation purposes. Meanwhile, Figure 1(b) shows the phase identification of FsHA, β-TCP and calcium zirconate ($CaZrO_3$) in the FsHA/YSZ composites. The analysis was made on the 2θ value ranging from 20$^{o}$ to 60$^{o}$ as shown on the spectrums. The identification of major peaks for HA is in parallel with the peaks position at 26.02$^{o}$, 33.02$^{o}$, 39.91$^{o}$ and 50.58$^{o}$. Meanwhile, the peaks position for β-TCP were identified at 27.84$^{o}$, 31.06$^{o}$, 32.48$^{o}$, 34.40$^{o}$ and 53.02$^{o}$. These findings were similar to the results which reported by Ananth et. al. [10].

The XRD patterns of commercial HA and β-TCP shown in Figure 1(a) were good matched with the JCPDS standard for HA (JCPDS No. 9–432) and β-TCP (JCPDS No. 9–169) [11]. From the XRD analysis, all composites have shown peaks that corresponds to both HA and β-TCP compounds, however, it was observed extra peaks at 30.42$^{o}$ which correlated to the $CaZrO_3$ compound. The present of $CaZrO_3$ compound was also reported by other researchers when the mixture of HA and $ZrO_2$ were sintered at high temperature [4,12]. This can be explained by the formation of $CaZrO_3$ phase in the solid state as the results of diffusion of $Ca^{2+}$ ions into $ZrO_2$ structure by mass transfer mechanism starts from about 900 ◦C to 1500 ◦C [12].

From Figure 1, the intensity of $CaZrO_3$ observed to be the highest at the FSHA/YSZ with 10 wt% YSZ followed by 15 wt% YSZ and 5 wt% YSZ. Rapacz-Kmita et al., reported that the formation of $CaZrO_3$ with the decomposition of HA into TCP and denoted that it obstructed the phase transformation of HA ceramic [13]. The formation of TCP and $CaZrO_3$ were also agreed as reported by Towler et. al. [14].

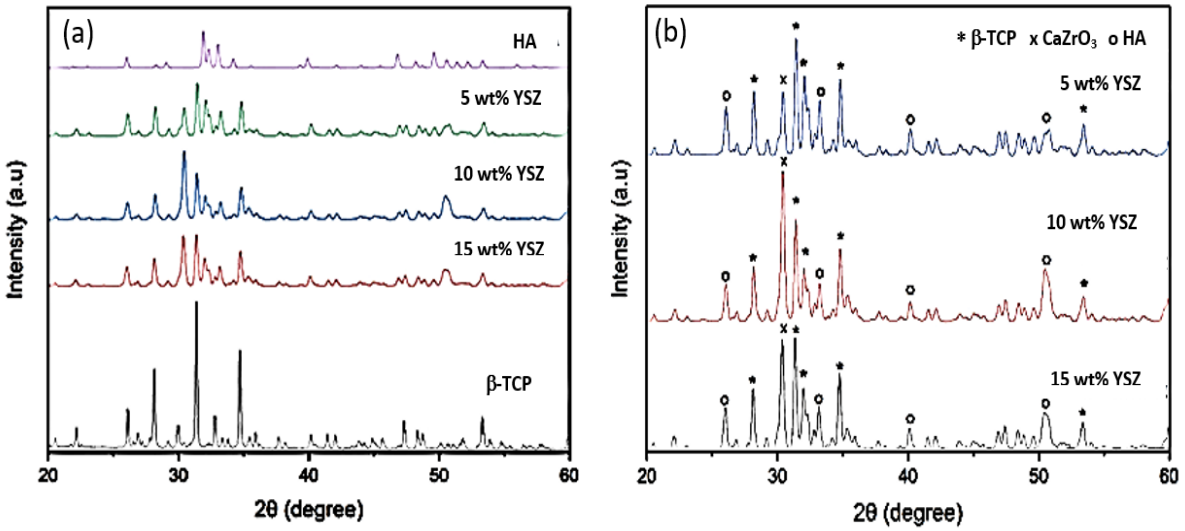


**Figure 1:** XRD patterns of (a) the correlation between commercial HA and β-TCP with FsHA/YSZ composites (5, 10 and 15 wt% YSZ) and (b) the phase identification of HA, β-TCP and $CaZrO_3$ in the FsHA/YSZ composites.

Since all samples composed of HA, β-TCP and $CaZrO_3$, the determination on the percentage of β-TCP in the composites has been made in order to dictate the effect of YSZ addition on the synthesis of β-TCP. The percentage of β-TCP were determined using the formula as follows:

$$\text{Percentage of } \beta\text{-TCP} = \frac{\text{Area of } \beta\text{−TCP crystalline peaks}}{\text{Area of all peaks}} \times 100 \quad (1)$$

The FsHA/YSZ composite with the addition of 5 wt% YSZ was found to generate the highest percentage of β-TCP with 21.9 %. Meanwhile the 10 wt% and 15 wt% YSZ produced 17.5 % and 19.8 % of β-TCP, respectively. Previous studies utilizing the synthetic HA reported that the addition of 5 wt% zirconia produced 17 % of TCP with the same sintering temperature [6]. On the other hand, another researcher specified that HA was completely decomposed into β-TCP with addition of 10 wt% zirconia [14]. Their report was contradicted with current finding as HA is still present in the FsHA/YSZ composite with the addition of 10 wt% YSZ after sintering at 1200 $^{o}$C. This was probably due to natural HA from fish scales (FsHA) has different thermal properties as compared to synthetic HA.

The crystallite size of the sintered FsHA/YSZ composite can be calculated using the Scherrer equation [15] and presented in Figure 2. The FsHA/YSZ composite with 5 wt% YSZ produced the smaller crystallite size (23.57 nm) followed by 15 wt% and 10 wt% at 23.7 nm and 24.90 nm respectively. The small size of crystallites is able to reduce the sintering temperature, thus it will enhance the decomposition of FsHA into β-TCP at lower temperature [16]. The percentage of crystallinity of β-TCP and the crystallite size are summarized in Table 1. Therefore, the composition of 5 wt% of YSZ is the best choice to produce higher composition of β-TCP.

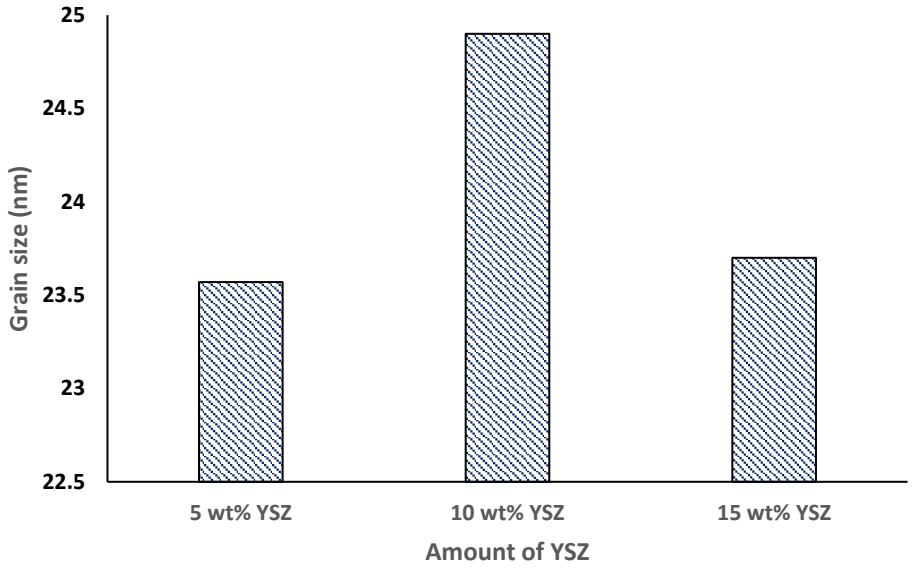


**Figure 2:** The crystallite (grain) size of FsHA/YSZ composites with 5, 10 and 15 wt% YSZ.

**Table 1:** The percentage of β-TCP and the crystallite size.

| Amount of YSZ added | Percentage of crystallinity of β-TCP | Crystallite (grain) size |
|---|---|---|
| 5 wt% | 20.90 % | 23.57 nm |
| 10 wt% | 17.52 % | 24.90 nm |
| 15 wt% | 19.81 % | 23.70 nm |

Figure 3 presents the FTIR spectra of FsHA/YSZ composites with different composition of YSZ addition (5, 10 and 15 wt%). From FTIR spectra the absorption peaks correspond to the functional groups of the HA and β-TCP composites which sintered at 1200 °C. The peaks appeared at wavenumber 1019.7 $cm^{-1}$ for 5 wt% YSZ shifted to higher wavenumber to 1020.3 $cm^{-1}$ and 1021.6 $cm^{-1}$ for 10 and 15 wt% YSZ respectively which represent the anti-symmetric stretch of $PO_4^{3-}$ functional groups [17,18]. The asymmetric bridge of P-O stretching mode can be observed at the band of 945.6 $cm^{-1}$ [10]. The band obtained at 600.0 $cm^{-1}$ is referred to the bending mode of O-P-O mode [19]. The sharp peaks at the band 544.3 $cm^{-1}$ to 545.6 $cm^{-1}$ for all FsHA/YSZ composites represented the symmetric vibration of $PO_4^{3-}$ denoting the presence of high crystallinity β-TCP [20]. The presence of YSZ could be detected at the split weak bands at 400.0 $cm^{-1}$ to 525.0 $cm^{-1}$ [21]. Meanwhile, the band at 423.6 $cm^{-1}$ for 15 wt% YSZ indicated the Zr-O stretching mode [22].

The morphology analysis of the sintered FsHA and FsHA/YSZ composites is shown in Figure 4. It can be seen that, the rough surface with less porosity of FsHA samples but there are smooth and high porosity matrix of sintered FsHA/YSZ composite. From the results, it indicated that addition of YSZ up to 15 wt% significantly increased the porosity of the FsHA matrix with the highest intensity is shown by 15 % composition of YSZ. The results obtained in this research supported by Ferreira et. al. finding as they studied the modification of synthetic HA and YSZ [7].

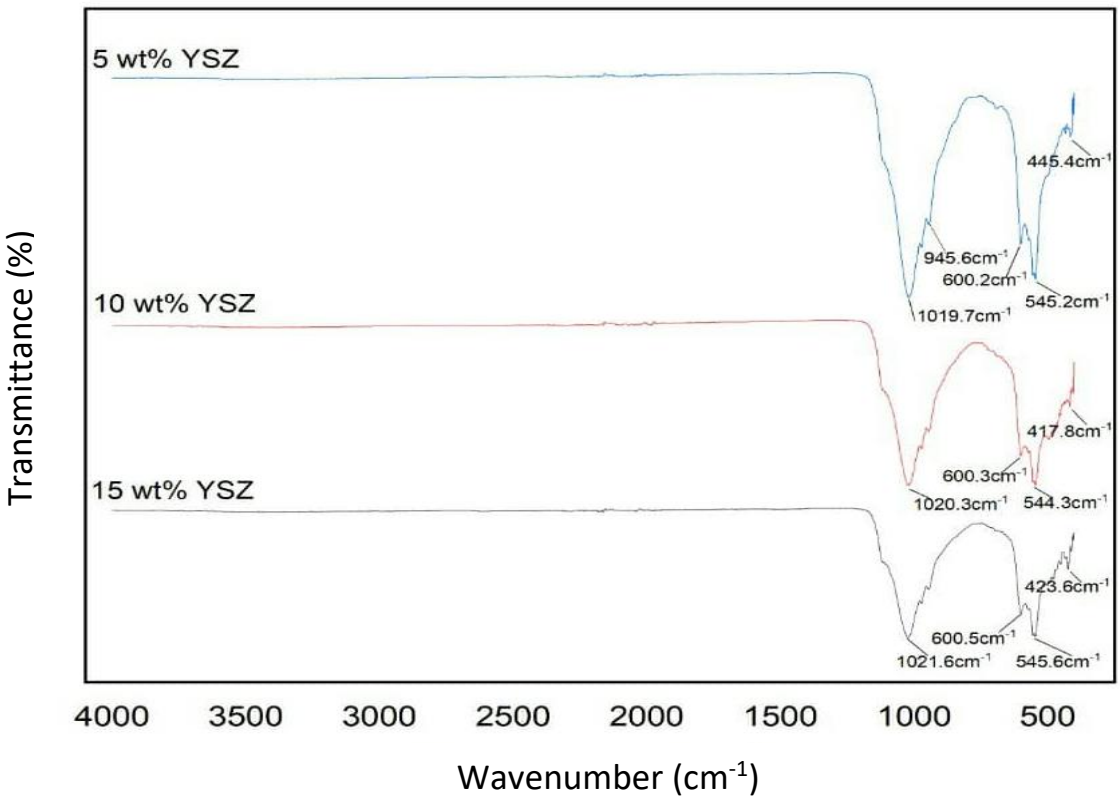


**Figure 3:** Overlaid FTIR spectra of FsHA/YSZ composites at various composition of YSZ

The formation of porous structure within the FsHA matrix is due to dehydroxylation within the matrix. It was reported, the presence of YSZ within the FsHA matrix decreased the decomposition temperature, so that decompose into a mixture of TCP and CaO. The CaO is then reacted with $ZrO_2$ phase resulting in the formation of calcium zirconate ($CaZrO_3$) according to the following reaction [23]:

$$Ca_{10}(PO_4)_6(OH)_2 + \text{Y-}ZrO_2\ (t) \rightarrow 3\beta\text{-}Ca_3(PO_4)_2 + \text{CaO-doped Y-}ZrO_2\ (c)/\text{Y-doped }CaZrO_3 + H_2O$$

From EDX analysis, the FsHA showed Ca/P ratio of 1.67, whereas sintered FsHA/YSZ (90:10) and FsH/YSZ (85:15) showed the Ca/P ratio of 1.78 and 1.87, respectively. The Ca/P results are closed to the theoretical value of HA (1.66) and β-TCP respectively.

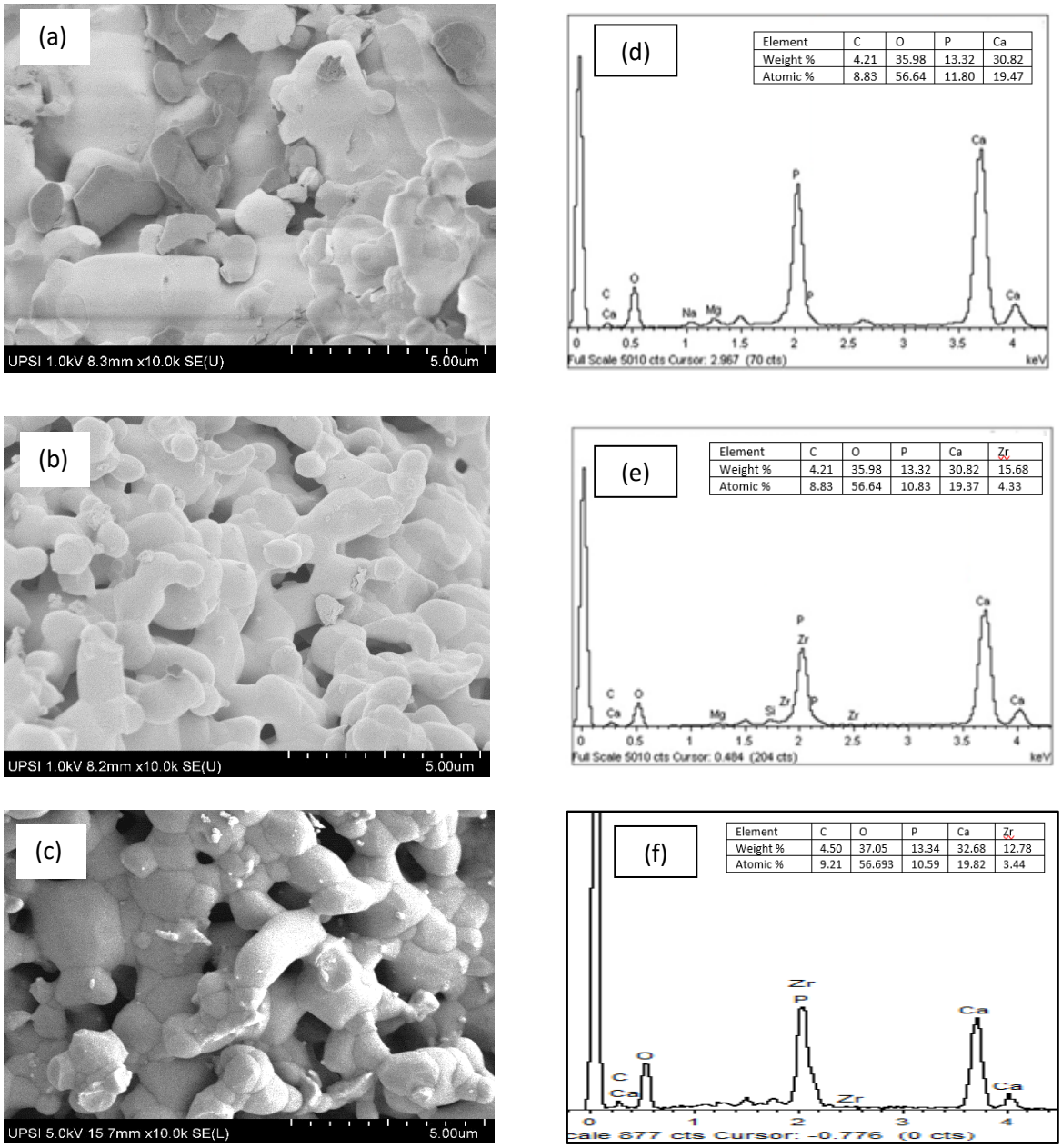


**Figure 4:** FESEM micrographs and respective EDX spectrum of (a) FsHA, (b) FsHA/YSZ (90:10) and (c) FsHA/YSZ (85:15) after sintering 1200 ºC. The respective EDX spectrum is shown in (d), (e) and (f), respectively.

## Conclusions

Sintering of fish scales hydroxyapatite (FsHA) with addition of 5, 10 and 15 wt% of YSZ at 1200 ºC was able to produce about 21.9%, 17.5% and 19.8% of β-TCP, respectively without completely degrading of FsHA. The results from XRD and FTIR analyses also revealed the presence of $CaZrO_3$ phase in all composite samples will gave advantage in mechanical properties. It was found that the FsHA/YSZ composite with 5 wt% YSZ produced the smallest crystallite size at 23.57 nm after sintering at 1200 ºC. The triphasic calcium phosphate (BCP) produced in this research has high potential to be used as biomaterials for bone fillers applications.

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## Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

## Disclosure of Conflict of Interest

The authors have no disclosures to declare.

## Compliance with Ethical Standards

The work is compliant with ethical standards.

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