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synthesis of spinel-hydroxyapatite composite utilizing bovine bone and Q

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[Crystals] Manuscript ID: crystals-1526077 - Paymer



Julia Ding <julia.ding@mdpi.com> to Gerald, MDPI, Julia, Alma, Crystals, Agus, Gerald, Ganang, Deni 👻

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Manuscript ID: crystals-1526077 Type of manuscript: Article Title: Synthesis of spinel-hydroxyapatite composite utilizing bovine bone and beverage can

Authors: Agus Pramono *, Gerald Ensang Timuda *, Ganang Pramudya Ahmad Rifai, Deni Shidqi Khaerudini *

Thank you in advance for your cooperation.

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We look forward to hearing from you soon.

Kind regards, Ms. Julia Ding E-Mail: julia.ding@mdpi.com

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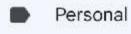


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Re: [Crystals] Manuscript ID: crystals-1395829 - Authorship Change Form 😕 🔤



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Crystals <crystals@mdpi.com> to me, Agus, Fatah, Deni, Anistasia 🝷

Dear Dr. Pramono,

We hope you are well. The revised manuscript was well received. We noticed that the authorship was changed. As per our policy, please sign the Authorship Change Form (see attachment). After that, we will process your manuscript further soon. Many thanks for your great support.

Looking forward to your reply.

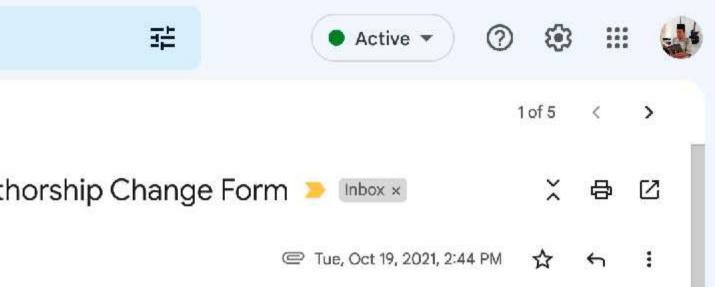
Best regards, Richard Li Managing Editor, MDPI Wuhan

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crystals@mdpi.com to agus.pramono, gerald.ensang.timuda, ganangpramudya, deni.shidqi.khaerudini, billing, we Dear Authors, We are pleased to inform you that your article "Synthesis of Spinel-Hydroxyapatite Composite Utilizing Bovine Bone and Beverage Can" has been published in Crystals as part of the Special Issue Mineralogical

Crystallography Volume II and is available online:

Abstract: https://www.mdpi.com/2073-4352/12/1/96 HTML Version: https://www.mdpi.com/2073-4352/12/1/96/htm PDF Version: https://www.mdpi.com/2073-4352/12/1/96/pdf The meta data of your article, the manuscript files and a publication certificate are available here (only available to corresponding authors after login):

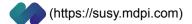
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(/user/manage_accounts)	Туре	Article
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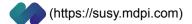
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					-	w 48: "has" been reported; 3: …this is probably related	
		previous sections is repea	ted - e>	: The HAp	was extrac	are several places where in ted from bovine bones; the p ferent sieve sizes were used	process

Regarding substrate, I have 4 observations:

- 1. From what the final part of the introduction and later results and discussions present, the composite is made out of MgO-Al₂O₃-MgAl₂O₄-HAp and in 2.2., it is stated that Al and Mg were used as the metal component in the composite. I would advise reformulations wherever metallic components of the composites appear. At a first glance, it misleads the reader.
- 2. At 2.3. Self-propagating Intermediate-temperature Synthesis (SIS), it is not obvious how can the heat propagation from one specific surface (instead of from all surfaces) induce a more homogenous heating (I advise a reference here); in regard to this observation, it is somehow unclear what benefits come from the SIS processing compared to conventional heating, microwave heating, hydrothermal processing and why this technique was selected. More data from the literature is required here.
- 3. Considering the fact that the main compound of this work contains a relatively large amount of AI (10%), as a form of Al₂O₃ or MgAl₂O₄, a reader will find it necessary to find a more comprehensive justification for the use of AI in a HAp composite, more than the fact that Al₂O₃ poses no harmful effect on the human body. What benefit does it bring towards HAp? How does the literature describe the effect of different contents of Al/Al₂O₃/MgAl₂O₄ upon a HAp composite?
- 4. In the results and discussion section, it is stated that AI is the metal used in the composite as a reinforcement to improve the mechanical properties of the HAp ceramics. Although initial X-ray analysis was performed on the HAp obtained from bovine bones, there is no compaction pressure nor hardness performed on the reference sample, required to evaluate the effect that AI has in reinforcing the ceramic. A reference sample without AI/Mg is needed here.

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The results report are reproducible?

Comments:

Authors

Paragraph 2.3 (pag. 2)

we ask you to explain the process described in more detail

Fig. 2 (pag. 3)

The figure shows the XRD of the bovine tooth used, with details on the crystallographic planes 211, 112 and 300. Usually the signal of the 300 is lower than the other two indicated. Can the author explain why?

In table 2 (reported on page 5) the tests performed are reported, clearly indicated in the following fig. 3.

The following figures (figs. 4 and 5 on page 6) show only 4 XRDs relating to some tests carried out.

Is it possible to also evaluate the other XRDs carried out on the other tests not shown?

Fig. 4: This figure shows how by applying different experimental parameters different results are obtained.

Figure 4b, for example, shows how there is no Al2O3 signal, the phase present and visible in Figure 4a.

Figure 4b also shows how in this test there is less presence of the Al signal, and how the HAp shows a double signal at the diffraction maximum 211.

Could this result be linked to a different degree of crystallinity of the hydroxyapatite obtained in example 4b compared to that relating to the diffractogram 4a?

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Isn't that a value (the degree of crystallinity) that we can introduce into this discussion?

Figs 6 and 7: introduce bar scales. You don't read well

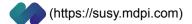
Conclusions: only one thing is not clear to me: do Al and Mg also enter the crystal structure of HAp or do they only serve to attribute chemical-physical properties to the composite described?

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Ø ✓User Menu Crystals (https://www.mdpi.com/journal/crystals) (ISSN 2073-4352) Journal Home (/user/myprofile) Manuscript ID crystals-1457676 Manage Accounts Article Type (/user/manage accounts) Change Password Title Synthesis of spinel-hydroxyapatite composite utilizing bovine bone and beverage can (/user/chgpwd) Authors Agus Pramono *, Gerald Ensang Timuda *, Ganang Pramudya Ahmad Rifai, Deni Shidgi Edit Profile (/user/edit) Khaerudini * Logout (/user/logout) Spinel-based hydroxyapatite composite (SHC) has been synthesized utilizing bovine bones as Abstract the source of the hydroxyapatite (HAp) and beverage cans as the aluminum (AI) source. The bovine bones were defatted and calcined in the air atmosphere to transform them into ✓ Submissions Menu hydroxyapatite. The beverage cans were cut and milled to obtain fine AI powder and then sieved to obtain three different particle mesh size fractions: +100#, -140#+170#, and -170#. The SHC Submit Manuscript was synthesized using the self-propagating intermediate-temperature synthesis (SIS) method at (/user/manuscripts/upload) 900 oC for 2 h with (HAp : AI : Mg) ratio of (87 : 10 : 3 wt.%) and various compaction pressure of 100, 171, and 200 MPa. It was found that the mechanical properties of the SHC are influenced **Display Submitted** by the AI particle size and the compaction pressure. Smaller particle size produces the tendency Manuscripts of increasing the hardness and reducing the porosity of the composite. Meanwhile, increasing (/user/manuscripts/status) compaction pressure produces a reduction of the SHC porosity. The increase of the hardness is **Display Co-Authored** also observed by increasing the compaction pressure except for the smallest AI particle size (-170#), where the hardness instead becomes smaller. Manuscripts (/user/manuscripts/coauthored) **English Editing** The coverletter for this review report has been saved in the database. You can safely close (/user/pre english article/status) this window. **Discount Vouchers** (/user/discount voucher) Authors' Responses to Reviewer's Comments (Reviewer 2) Invoices (/user/invoices) LaTex Word Count Author's Notes Thank you for your comment. The supplementary information has been carefully improved (/user/get/latex word count) according to your suggestion.

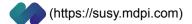
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Comments and Suggestions for Authors The supplementary material needs to be substantially improved with extensive editing of the English language and style.

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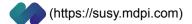
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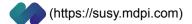
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Suggestions for Authors Authors Network and the products have constant quality, applicable in biomedicine.

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(/user/manage_accounts)	Туре	Article							
Change Password (/user/chgpwd)	Title	Synthesis of spinel-hydroxyapatite composite utilizing bovine bone and beverage can							
Edit Profile (/user/edit)	Authors	Agus Pramono * , Gerald Ensang Timuda * , Ganang Pramudya Ahmad Rifai , Deni Shidqi Khaerudini *							
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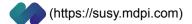
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	Are the	results clearly presented?	()	()	(x)	()			
	Are the conclusions	supported by the results?	()	()	(x)	()			
	Comments and Suggestions for	The authors responded th				e			

Suggestions for Authors Authors Ne additions responded that this study is in a very 'carry study': They should enalacterise the prepared materials by different methods, not inly by XRD! They have to be sure that the preparation methods are reproducible and the products have constant quality, applicable in biomedicine.

Submission Date26 October 2021Date of this review18 Nov 2021 12:36:12

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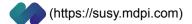
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	Are the conclusions	supported by the results?	()	ı	()	(x)	()
	Comments and Suggestions for Authors	for the quality is indispensable. There are a lot of questions regarding this research work:						-
		1. the Hap obtained from	bovir	ıe	bone was i	not enough	h charc	terized (e. g. Fig 2!)
		2. how the authors insur				•		•
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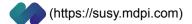
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	Comments and Suggestions for Authors	s for evaluating mechanical properties in relation to the arming agent. There are severa						
		For the form, the English I	anguag	e should be	e improved;	ex:		
					-	w 48: "has" been reported; 3: …this is probably related		
		previous sections is repea	ted - e>	: The HAp	was extrac	are several places where in ted from bovine bones; the p ferent sieve sizes were used	process	

Regarding substrate, I have 4 observations:

- 1. From what the final part of the introduction and later results and discussions present, the composite is made out of MgO-Al₂O₃-MgAl₂O₄-HAp and in 2.2., it is stated that Al and Mg were used as the metal component in the composite. I would advise reformulations wherever metallic components of the composites appear. At a first glance, it misleads the reader.
- 2. At 2.3. Self-propagating Intermediate-temperature Synthesis (SIS), it is not obvious how can the heat propagation from one specific surface (instead of from all surfaces) induce a more homogenous heating (I advise a reference here); in regard to this observation, it is somehow unclear what benefits come from the SIS processing compared to conventional heating, microwave heating, hydrothermal processing and why this technique was selected. More data from the literature is required here.
- 3. Considering the fact that the main compound of this work contains a relatively large amount of AI (10%), as a form of Al₂O₃ or MgAl₂O₄, a reader will find it necessary to find a more comprehensive justification for the use of AI in a HAp composite, more than the fact that Al₂O₃ poses no harmful effect on the human body. What benefit does it bring towards HAp? How does the literature describe the effect of different contents of Al/Al₂O₃/MgAl₂O₄ upon a HAp composite?
- 4. In the results and discussion section, it is stated that AI is the metal used in the composite as a reinforcement to improve the mechanical properties of the HAp ceramics. Although initial X-ray analysis was performed on the HAp obtained from bovine bones, there is no compaction pressure nor hardness performed on the reference sample, required to evaluate the effect that AI has in reinforcing the ceramic. A reference sample without AI/Mg is needed here.

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The results report are reproducible?

Comments:

Authors

Paragraph 2.3 (pag. 2)

we ask you to explain the process described in more detail

Fig. 2 (pag. 3)

The figure shows the XRD of the bovine tooth used, with details on the crystallographic planes 211, 112 and 300. Usually the signal of the 300 is lower than the other two indicated. Can the author explain why?

In table 2 (reported on page 5) the tests performed are reported, clearly indicated in the following fig. 3.

The following figures (figs. 4 and 5 on page 6) show only 4 XRDs relating to some tests carried out.

Is it possible to also evaluate the other XRDs carried out on the other tests not shown?

Fig. 4: This figure shows how by applying different experimental parameters different results are obtained.

Figure 4b, for example, shows how there is no Al2O3 signal, the phase present and visible in Figure 4a.

Figure 4b also shows how in this test there is less presence of the Al signal, and how the HAp shows a double signal at the diffraction maximum 211.

Could this result be linked to a different degree of crystallinity of the hydroxyapatite obtained in example 4b compared to that relating to the diffractogram 4a?

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Isn't that a value (the degree of crystallinity) that we can introduce into this discussion?

Figs 6 and 7: introduce bar scales. You don't read well

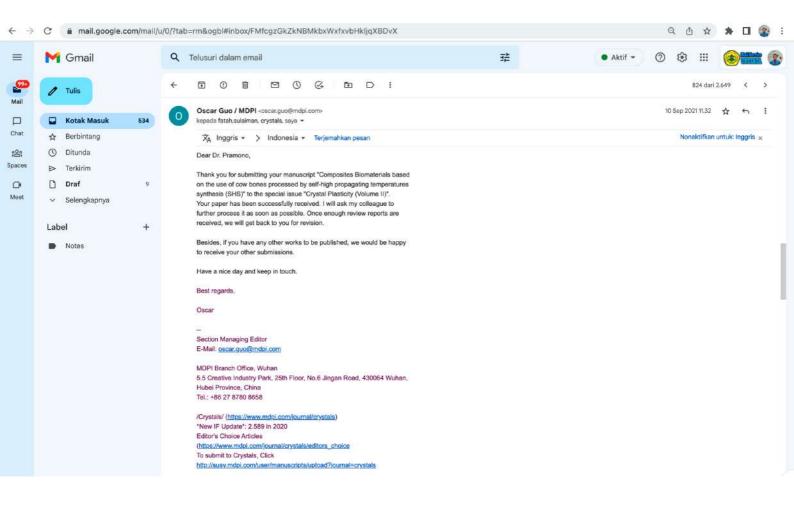
Conclusions: only one thing is not clear to me: do Al and Mg also enter the crystal structure of HAp or do they only serve to attribute chemical-physical properties to the composite described?

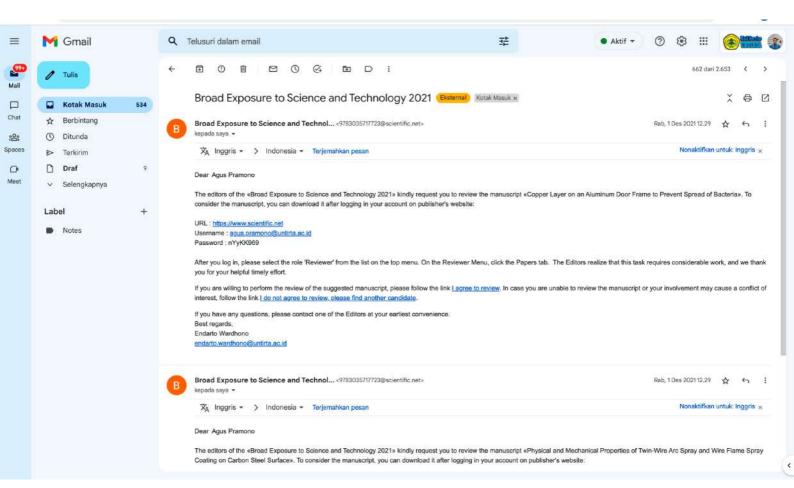
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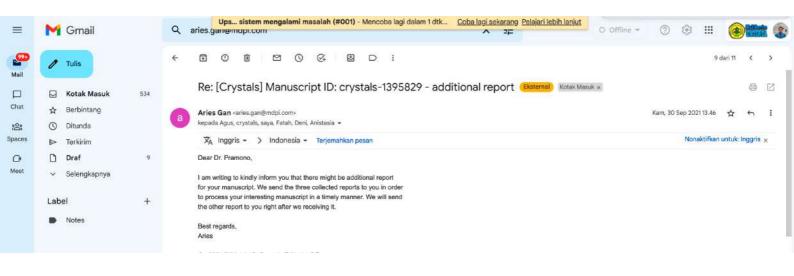
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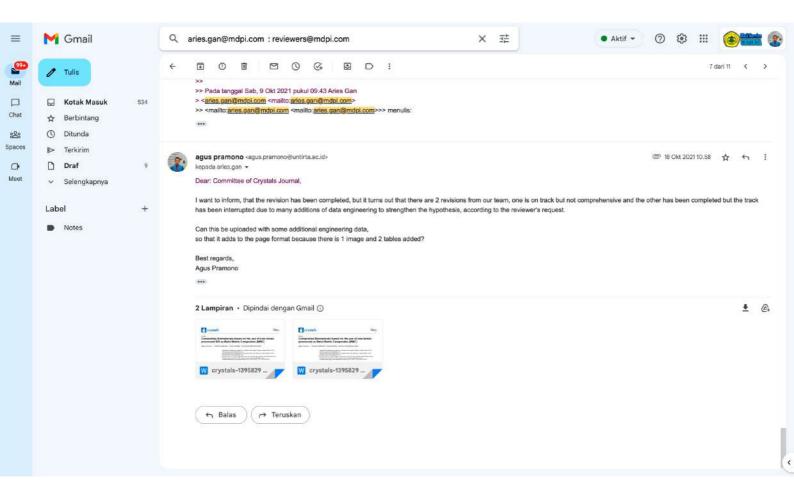


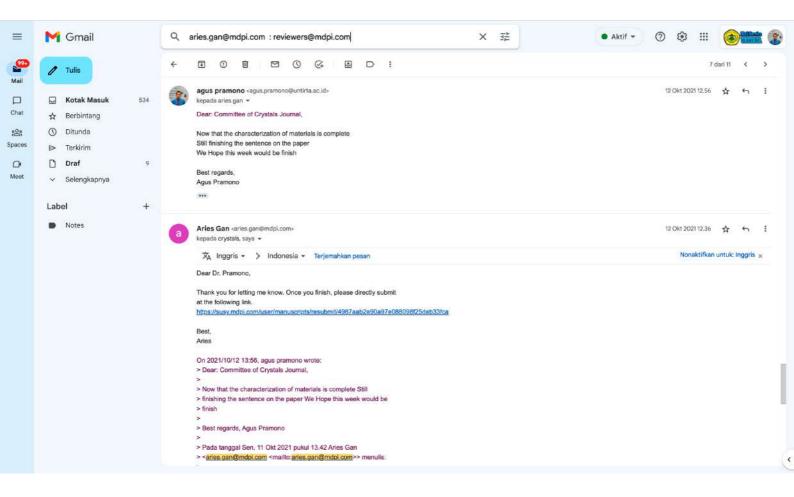


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		agus pramono -agus pramono@untirta.ac.id>kepada aries.gan * Dear: Crystal Journal Committee Currently, I am still completing a fairly long revision, after the revision is complete we will definitely send the manuscript and uple share the learn's performance here. so we also need to speed up the revision. Thank you for the notification Best regards. Agus Pramono Image: Completing a fairly long revision is complete we will definitely send the manuscript and uple share the learn's performance here. so we also need to speed up the revision. Thank you for the notification Best regards. Agus Pramono Image: Completing a fairly long revision Image: Completing a fairly long revision, after the revision is complete we will definitely send the manuscript and uple share the learn's performance here. so we also need to speed up the revision. Thank you for the notification Best regards. Agus Pramono Image: Completing a fairly long revision Image: Completing a fairly long revision <td< th=""><th>Jum, 8 Okt 202115,16 🖈 🕤 ead it. May I know when the deadline for the last revision, considering we</th></td<>	Jum, 8 Okt 202115,16 🖈 🕤 ead it. May I know when the deadline for the last revision, considering we





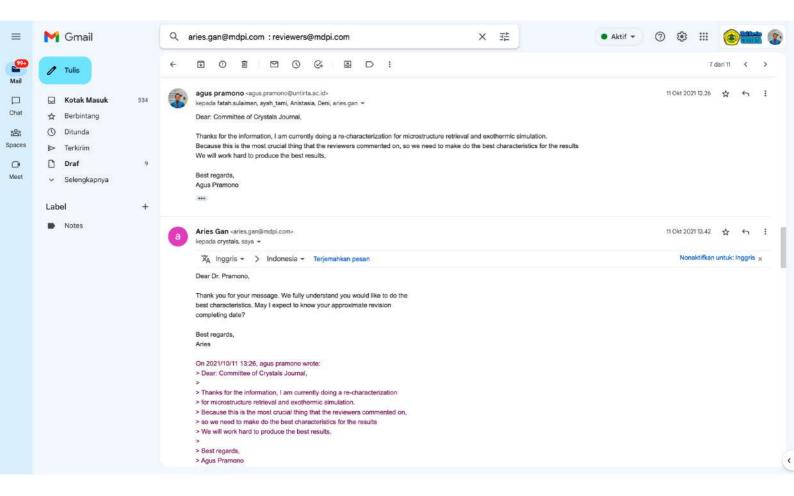
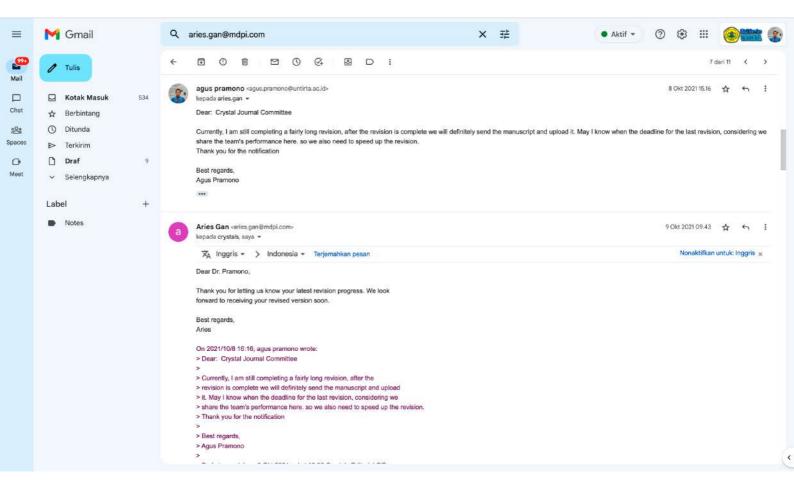
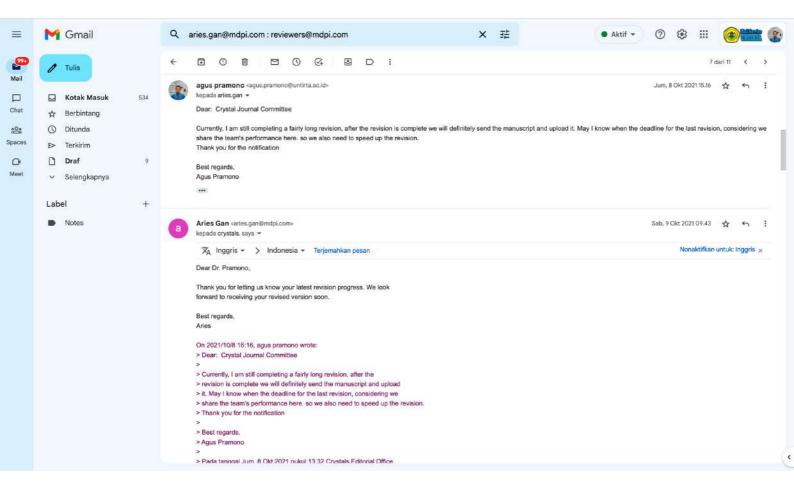
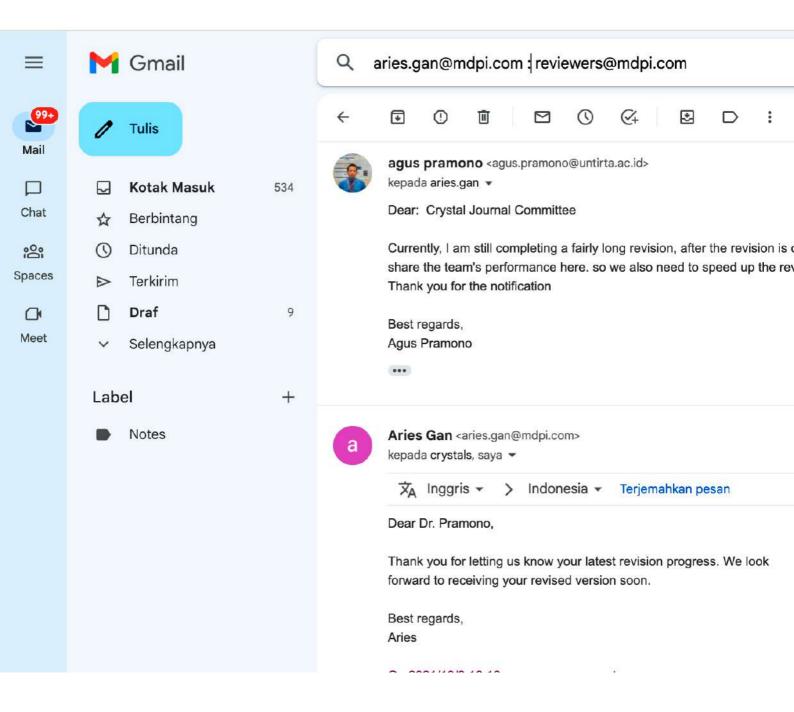
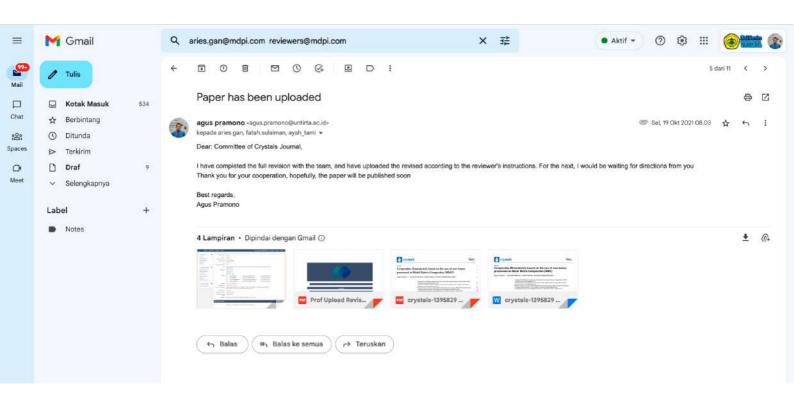


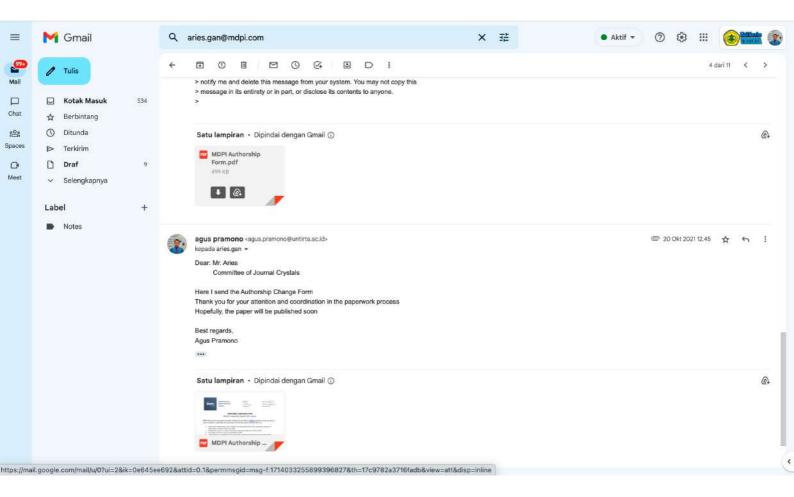
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 	Mail Chat Spaces	▶ Kotak Masuk 534 ☆ Berbintang 534 ③ Ditunda 534 ▶ Terkirim 9 ↓ Selengkapnya 9 Label +	 Authors: Agus Pramono *, Fatah Sulaiman, Deni Shidqi Khaerudini, Anistasia Milandia * Received: 10 September 2021 E-mails: agus.pramono@untifta.ac.id>, fatah.sulaiman@untifta.ac.id <mailto.gaus.pramono@untifta.ac.id>, fatah.sulaiman@untifta.ac.id</mailto.gaus.pramono@untifta.ac.id> <mailto.gata.sulaiman@untifta.ac.id>, fatah.sulaiman@untifta.ac.id</mailto.gata.sulaiman@untifta.ac.id> <mailto.gata.sulaiman@untifta.ac.id>, fatah.sulaiman@untifta.ac.id</mailto.gata.sulaiman@untifta.ac.id> <mailto.gata.sulaiman@untifta.ac.id< li=""> Submitted to section: Alloys and Compounds, https://www.mdpi.com/journal/crystals/special_issues/crystal_plasticity_2 https://www.mdpi.com/journal/crystals/special_issues/crystal_plasticity_2 https://www.mdpi.com/wow.gata.sulaiman https://www.mdpi.com/wow.gata.sulaiman https://www.mdpi.com/wow.gata.sulaiman https://www.mdpi.com/wow.gata.sulaiman https://www.mdpi.com/wow.gata.sulaiman </mailto.gata.sulaiman@untifta.ac.id<></mailto.gata.sulaiman@untifta.ac.id<></mailto.gata.sulaiman@untifta.ac.id<></mailto.gata.sulaiman@untifta.ac.id<></mailto.gata.sulaiman@untifta.ac.id<></mailto.gata.sulaiman@untifta.ac.id<></mailto.gata.sulaiman@untifta.ac.id<></mailto.gata.sulaiman@untifta.ac.id<></mailto.gata.sulaiman@untifta.ac.id<></mailto.gata.sulaiman@untifta.ac.id<></mailto.gata.sulaiman@untifta.ac.id<></mailto.gata.sulaiman@untifta.ac.id<>								

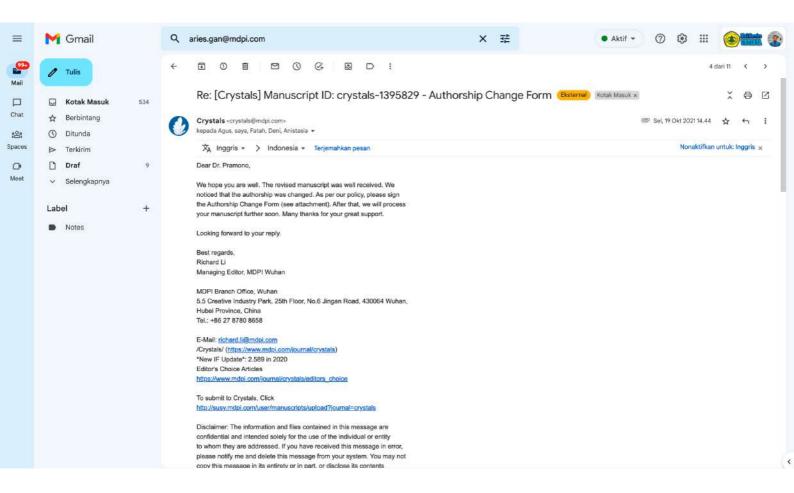


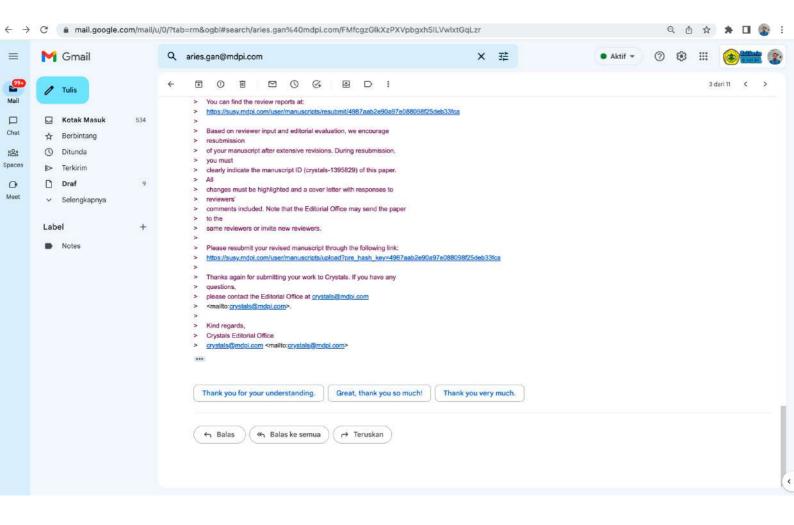




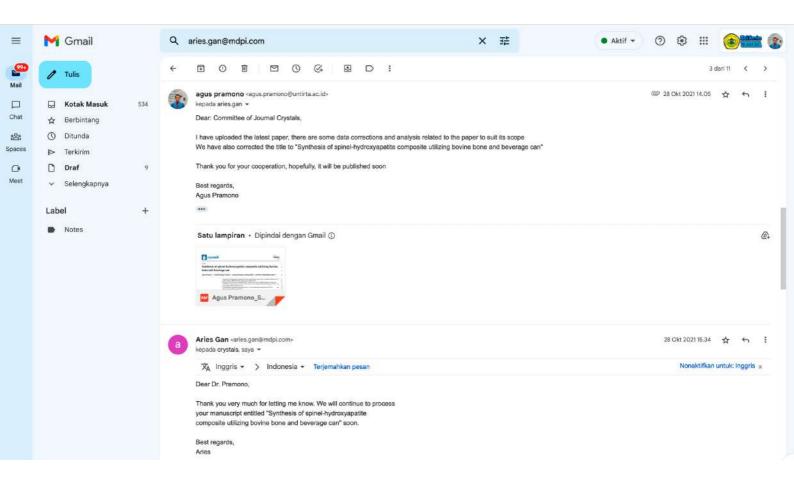








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		> Type of manuscript: Article		
		> Title: Composites Biomaterials based on the use of cow bones		
		 pro-cessed as Metal Matrix Composites (MMC) 		
		> Authors: Agus Pramono *, Anistasia Milandia *, Fatah Sulaiman, Deni		
		> Shidqi > Khaerudini		
		 Received: 10 September 2021 		
		> E-mails: agus.pramono@untirta.ac.id		
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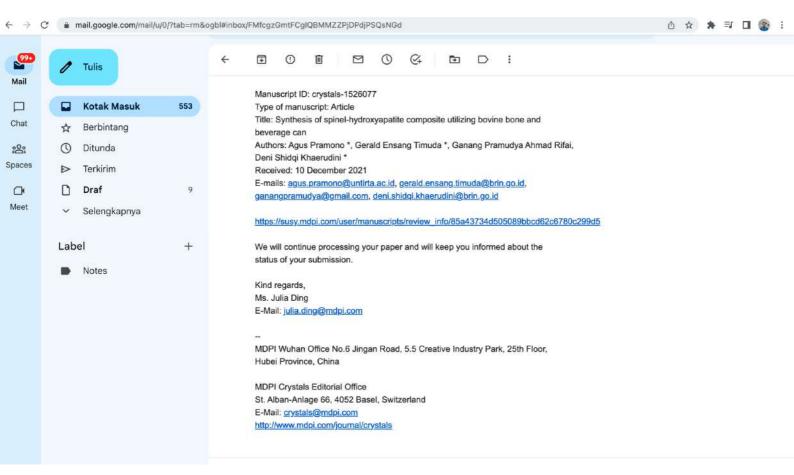
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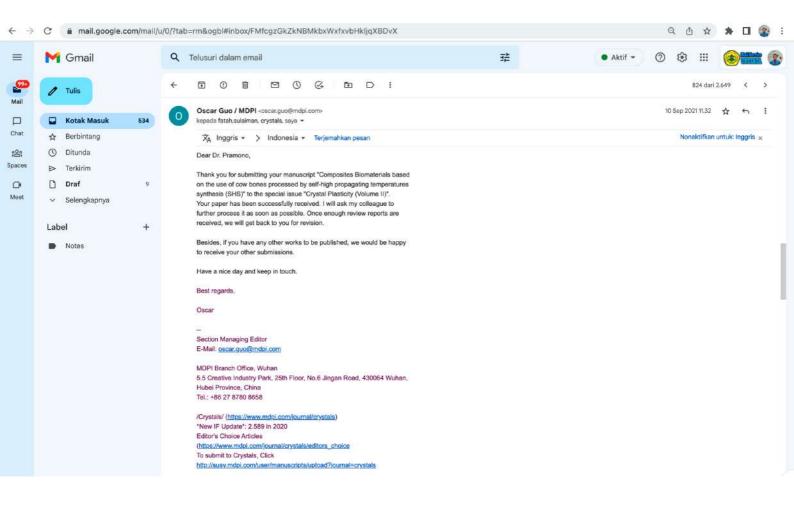
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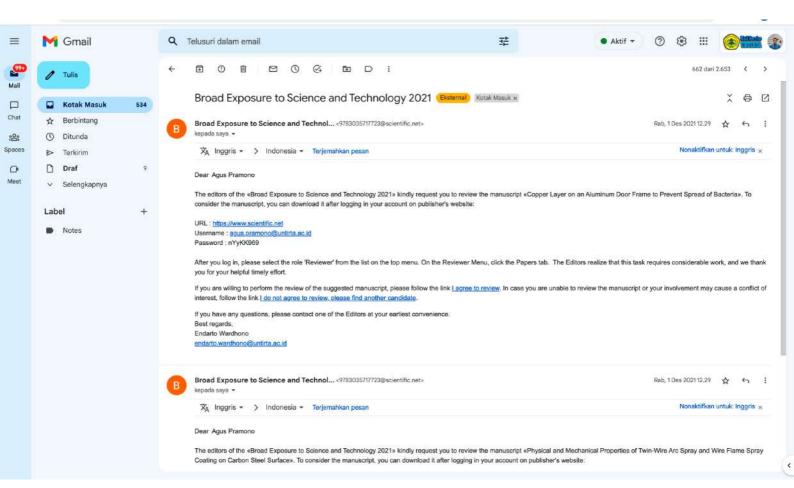
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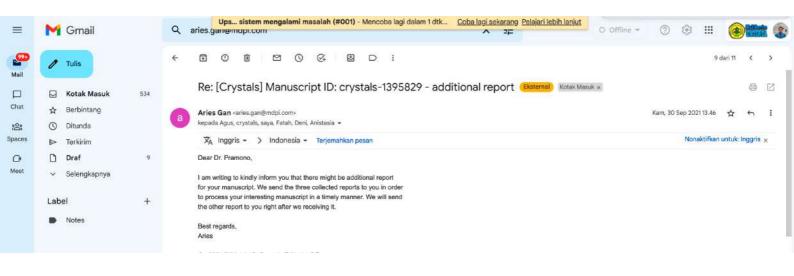
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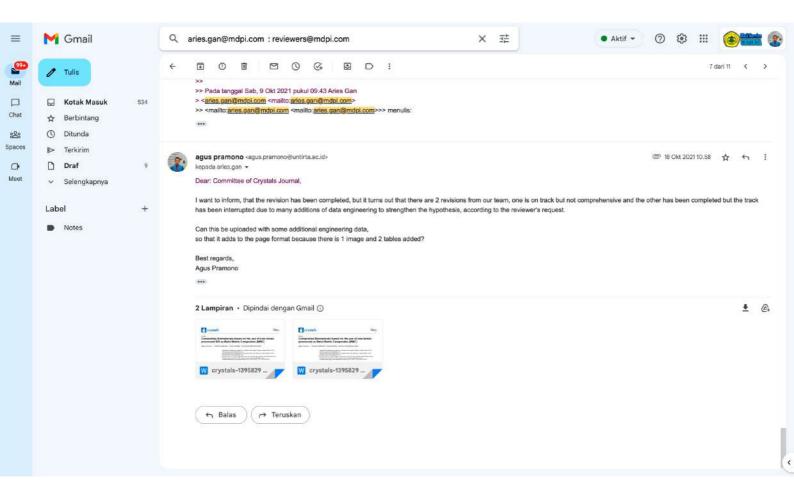


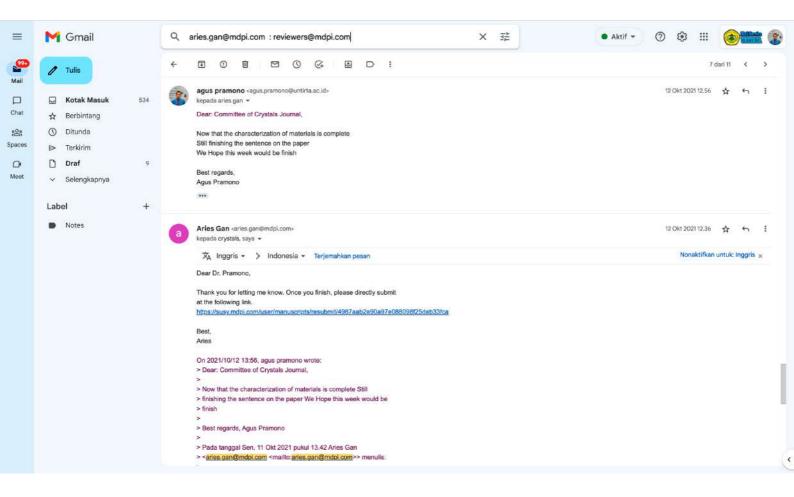


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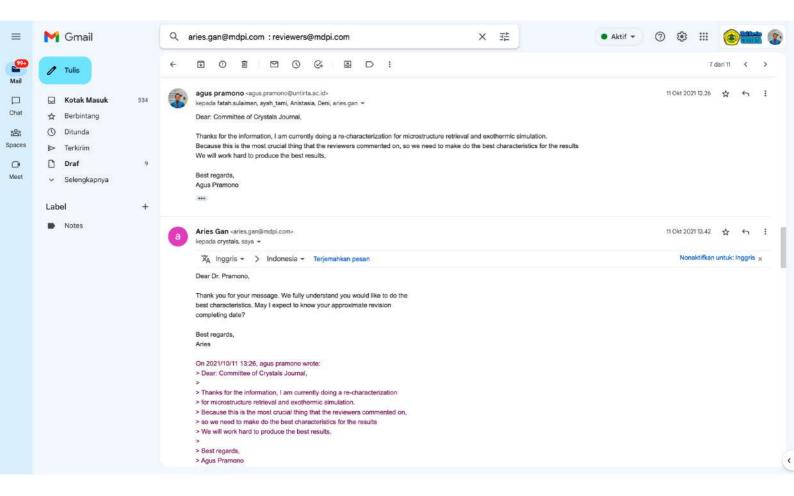
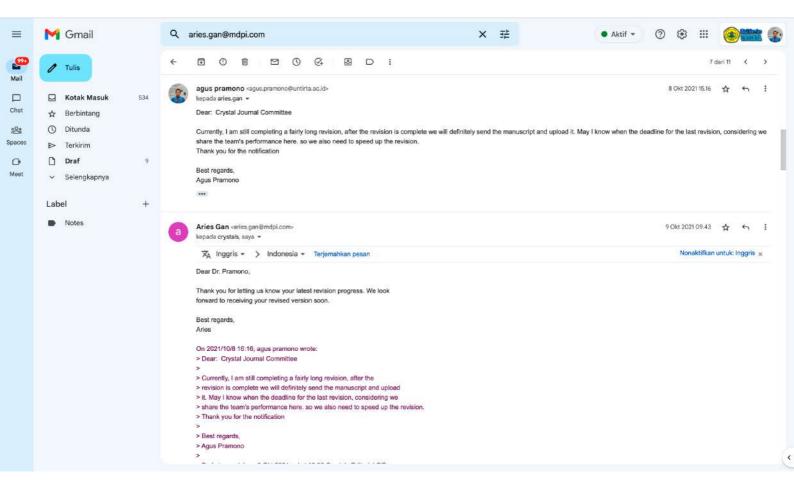
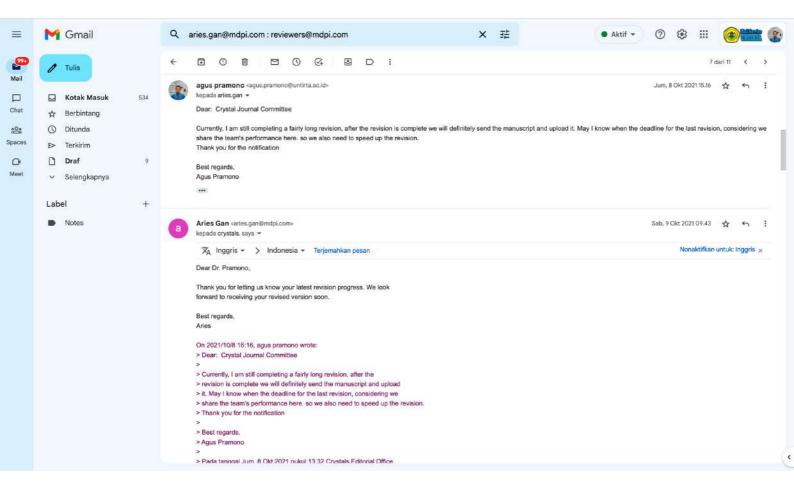
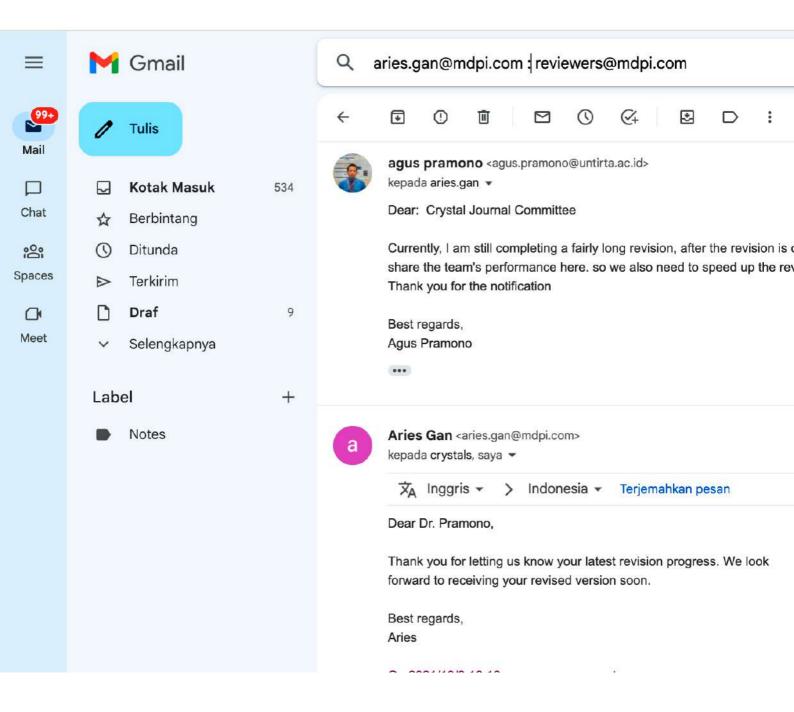
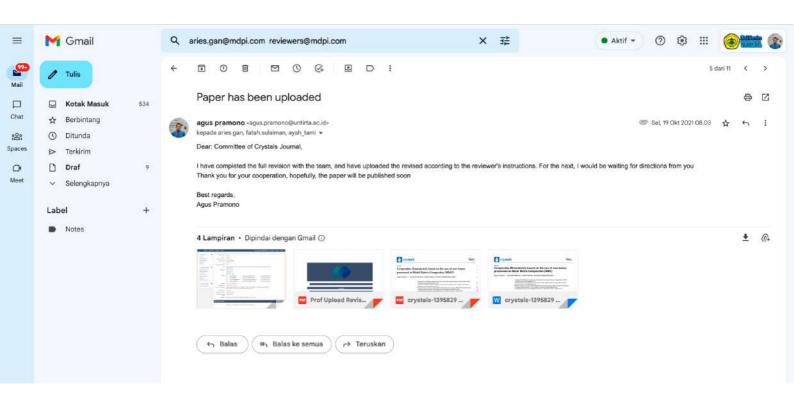


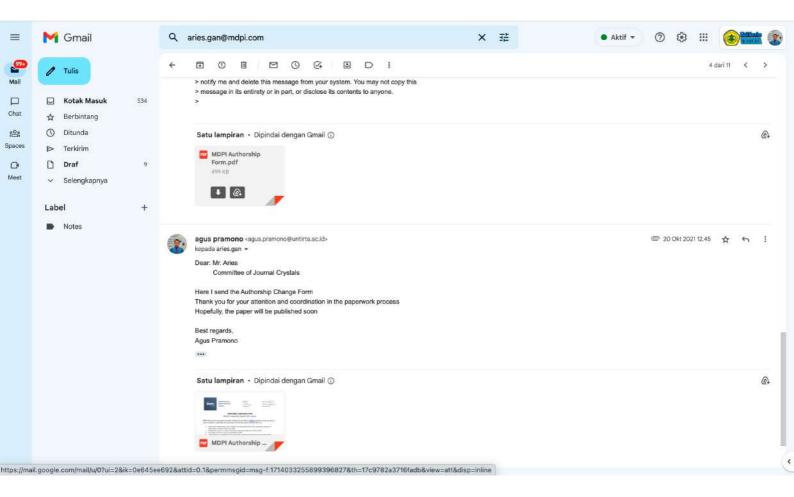
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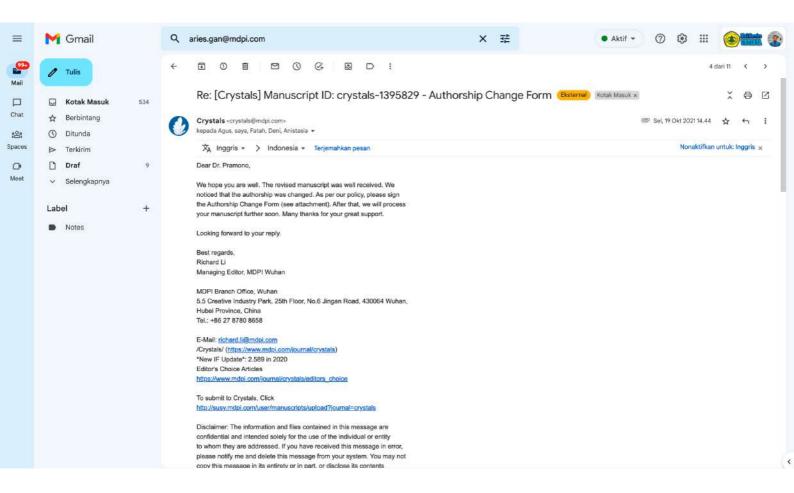


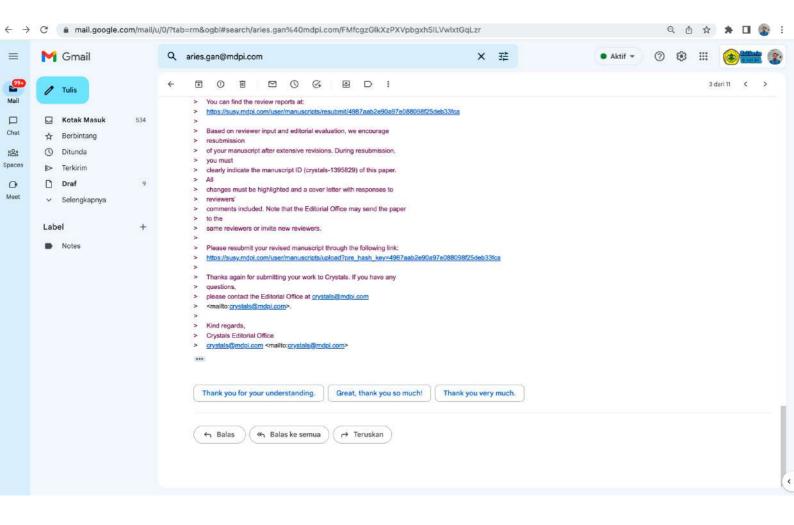




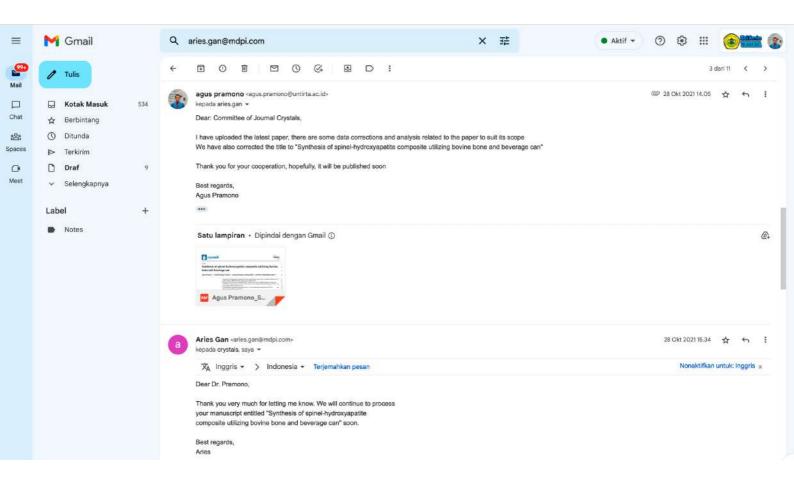








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		> Shidqi > Khaerudini		
		 Received: 10 September 2021 		
		> E-mails: agus.pramono@untirta.ac.id		
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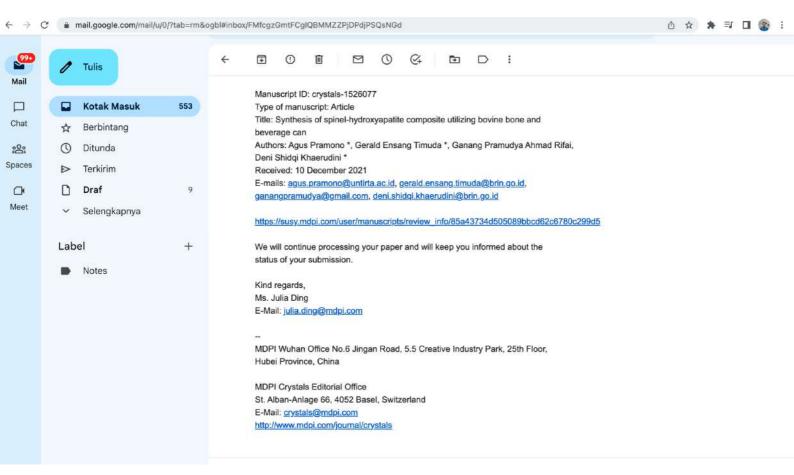
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Synthesis of spinel-hydroxyapatite composite utilizing bovine bone and beverage can

Agus Pramono 1,*, Gerald Ensang Timuda 2*, Ganang Pramudya Ahmad Rifai 1, and Deni Shidqi Khaerudini 2-

- ¹ Department of Metallurgical Engineering, Sultan Ageng Tirtayasa University, Jl. Jendral Soedirman KM. 3, Cilegon, Banten, 42435, Indonesia; agus.pramono@untirta.ac.id (A.P.); ganangpramudya@gmail.com (G.P.A.R.)
- Research Center for Physics, National Research and Innovation Agency (BRIN), Building 442 Kawasan PUSPIPTEK, Tangerang Selatan, Banten, 15314, Indonesia; deni.shidqi.khaerudini@brin.go.id (D.S.K.); gerald.ensang.timuda@brin.go.id (G.E.T.)
- Correspondence: agus.pramono@untirta.ac.id (A.P.); deni.shidqi.khaerudini@brin.go.id (D.S.K.); gerald.ensang.timuda@brin.go.id (G.E.T.)

Abstract: Spinel-based hydroxyapatite composite (SHC) has been synthesized utilizing bovine 13 bones as the source of the hydroxyapatite (HAp) and beverage cans as the aluminum (Al) source. 14 The bovine bones were defatted and calcined in the air atmosphere to transform them into hydrox-15 yapatite. The beverage cans were cut and milled to obtain fine Al powder and then sieved to obtain 16 three different particle mesh size fractions: +100#, -140#+170#, and -170#. The SHC was synthesized 17 using the self-propagating intermediate-temperature synthesis (SIS) method at 900 oC for 2 h with 18 (HAp: Al: Mg) ratio of (87:10:3 wt.%) and various compaction pressure of 100, 171, and 200 MPa. 19 It was found that the mechanical properties of the SHC are influenced by the Al particle size and 20 the compaction pressure. Smaller particle size produces the tendency of increasing the hardness and 21 reducing the porosity of the composite. Meanwhile, increasing compaction pressure produces a re-22 duction of the SHC porosity. The increase of the hardness is also observed by increasing the com-23 paction pressure except for the smallest Al particle size (-170#), where the hardness instead becomes 24 smaller. 25

Keywords: hydroxyapatite composite, spinel, beverage cans, bovine bones

1. Introduction

G.E.; Rifai, G.P.A., Khaerudini, D.S Synthesis of spinel-hydroxyapatite composite utilizing bovine bone and beverage can. Crystals 2021, 11, x. https://doi.org/10.3390/xxxxx

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Hydroxyapatite is an important biomedical implant material. It is the natural bone's 29 inorganic component [1], has a good osteoconductivity, biocompatibility, and bioactivity, 30 thus makes it easily incorporated into the bones [1-3]. However, the utilization of chemi-31 cally synthesized hydroxyapatite for hard tissue replacement is limited because it is ex-32 pensive while large quantity of material is required [3]. Furthermore, synthesized hydrox-33 yapatite has different physicochemical properties (such as strength and chemical compo-34 sition) than the natural one, leading to lower biological activity [4,5]. Therefore, extracting 35 hydroxyapatites from biological materials has been considered to provide a cheaper and 36 up-scalable alternative synthesis route, and to obtain physicochemical properties as close 37 as those of the natural hydroxyapatite. Several biological materials have been reported as 38 the source of the hydroxyapatite, such as bovine bone [3,6], fish scale [4,7], snail shell [2,8], 39 and eggshell [9,10]. Bovine bone can be considered as a biological waste that needs to be 40 recycled. Therefore, its processing into hydroxyapatite is economically and environt-41 mentallyenvironmentally beneficial. 42

To further improve the mechanical properties of hydroxyapatite, it can be synthe-43 sized into a composite with metal/alloys [11–14], metal oxides [5,15–17], or polymer [1,18]. 44 The composite of hydroxyapatite (SHC) with a single metal element such as in Mg/HAp 45

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system was considered to combine both advantages of biocompatibility and biodegrada-46 bility in Mg and bioactivity in HAp [11,14]. Improvement of the mechanical properties 47 such as hardness and compression strength haves been reported in the Mg/HAp compo-48 site compared to pure HAp [14]. However, the Mg/HAp system is more prone to corrosion 49 than the alloy matrix [11]. Therefore, addition of other metal as an alloying component 50 was considered to improve the corrosion properties, such as in Mg-Sn/HAp [11] or Mg-51 Ca/HAp [13]. Similarly, composite of HAp with metal oxides such as ZrO₂, TiO₂, Al₂O₃ 52 [5], or MgO [17] were considered as the reinforcement to improve the HAp's mechanical 53 stability [5]. Furthermore, bio-inert material such as the Al₂O₃ poses no harmfull effect on 54 human body [5] 55

In this study, the composite of HAp with two metal components, Al and Mg, is reported. The HAp source is provided by the recycledrecycled bovine bone. The Al source is supplied from recycled baveragebeverage cans which was reported to contain 93 – 97% of Al [19–21]. The metal components are prone to oxidation due to the presence of oxygen in the environtmentenvironment during heating process thus lead to formation of a unique-HAp-MgO-Al₂O₃-MgAl₂O₄/HAp compositespinel (MgAl₂O₄) based composite. The introduction of MgAl₂O₄ has been reported to improve the phase stability of HAp during sintering [16]. The effect of Al particle size and compaction pressure on the me chanical properties of the composite is discussed.

2. Materials and Methods

2.1. Bovine Bones Treatment

Bovine bones (<u>leg part</u>) were used as the hydroxyapatite (HAp) source material and were obtained from local markets in Jakarta, Indonesia. The bones were defatted by twice boiling for 2 h followed by washing under running water, then dried <u>in-below</u> the <u>sun-Sun</u> for 8 h. The dried bones were then cut into small pieces by the grinder and calcined at 850 °C for 2 h. Afterward, it was disk milled for 20 s to obtain the HAp powder.

2.2. Aluminum Cans Treatment

Aluminum (Al) and magnesium (Mg) were used as the metal component in the composite. In this study, the Al was provided from recycled beverage cans. The cans were square cut to 1×1 cm² then disk milled for 15 min with the on:off interval time of 1:2 (min/min), producing Al powder. Its purity content was then characterized by the X-ray fluorescence (XRF, S2 PUMA Bruker). The powder was then sieved with the sieve sizes of 100#, 140#, and 170# to obtain three different particle size fractions of +100#, -140#+170#, and -170#, respectively.

2.3. Self-propagating Intermediate-temperature Synthesis (SIS)

A mixture of HAp, Al, and Mg (Merck, 99% purity) powders with the ratio of (87:10 82 : 3 wt.%) was prepared using a shaker mill for 10 min. The mixture was then pelleted with 83 different compaction pressures of 100, 171, and 200 MPa. The 9 different compositions of 84 pellets were prepared from the variation of Al particle sizes and compaction pressures. 85 Afterward, each pellet was put in the SIS vessel which was then heated at 900 oC for 2 h. 86 The SIS vessel is specifically designed so that when undergoing heat treatment in the fur-87 nace, the heat propagation can only come from one specific surface instead of from all 88 surfaces in the conventional heat treatment case. In this way, the heat propagation and 89 distribution in the sample became more controllable and homogenous. More detailed in-90 formation about SIS method is presented in the Supplementary Information. 91

After cooling down, the hydroxyapatite-composite (SHC) samples in the SIS vessel 92 were taken out then polished by sandpaper and characterized using Archimedes porosity 93

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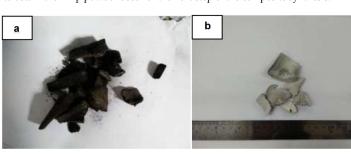
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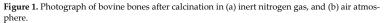
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3. Results and Discussions

The hydroxyapatite composite (SHC) synthesized in this study consists of hydroxy-100 apatite (HAp), aluminum (Al), and magnesium (Mg) as the bio-ceramic matrix, reinforce-101 ment, and wetting agent, respectively. The HAp was extracted from the bovine bones. The 102 needs the bones to be defatted, dried, cut, calcined, and milled to obtain the HAp 103 pro powder needed for the SHC synthesis. Figure 1 shows the visual appearance of the defat-104 ted bovine bones after calcination at 850 °C. It can be seen that in an inert condition, the 105 bones looked burnt and charred (Figure 1a), indicating the formation of carbon instead of 106 the desired HAp. This is because heating in a low or no oxygen atmosphere can lead to 107 pyrolysis resulting in carbonization of the bones [22]. On the other hand, the bones cal-108 cined in the air atmosphere have a white color (Figure 1b), indicating the formation of the 109 desired HAp [3,23]. The air-calcined bones also lose their previous stickiness feels, indi-110 cating the removal of the remaining fat unable to be removed from the previous defatting 111 process. The XRD of the air-calcined bones is shown in Figure 2 where a HAp phase with 112 100% purity is observed. The XRD graphspatterns of other HAp from different batches 113 are shown in the Supplementary Information, showing similar pattern results indicating 114 a good reproducibility. The air-calcined bones were then crushed in the ring disk milling 115 to obtain the HAp powder used for the next step of the composite synthesis. 116

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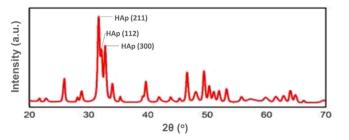


Figure 2. X-ray diffractogram of the <u>HAp-based</u> bovine bones after calcination in air atmosphere.

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Al is the metal used in the composite here as a reinforcement to improve the mechan-123 ical properties of the HAp bio-ceramic. In this study, the Al source was taken from recy-124 cled beverage cans. The recycling process includes cutting the cans and then crushing 125 them in the ring-disk milling. After milling, the purity of the crushed powder was charac-126 terized using XRF (Table 1). The Al content is at 91.90 ± 1.50 %, which is slightly smaller 127 than those reported in the literature (about 93-97% [19-21]). This may come from the paint 128 coating of the can's exterior which was not removed before being recycled. For application 129 as the reinforcement in the composite, the Al powder was further sieved to obtain a finer 130 and homogenous particle size. Three different sieve sizes were used: 100#, 140#, and 170#, 131 correspond to particle sizes of +100#, 140#+170#, and 170#, respectively. The smaller the 132 particle size is supposed to give a better homogeneity of the particle's distribution, better 133 bonding among the composite's components, and an increase of the composite's density 134 [24]. Therefore, it is a crucial factor that determines the quality of the composite and is 135 further studied in this report. 136

Table 1. XRF elemental composition of the Al powder recycled from the beverage can.

Elements	Percentage (%)	Elements	Percentage (%)
Al	91.90 ± 1.50	Ni	0.06 ± 0.02
Mn	3.20 ± 0.40	Zr	0.03 ± 0.03
Si	2.80 ± 0.80	Ti	0.02 ± 0.09
Fe	1.39 ± 0.19	Zn	0.01 ± 0.01
Cu	0.62 ± 0.12		

For the hydroxyapatite-composite (SHC) synthesis, the HAp, Al, and Mg powders138(87:10:3 wt.%) were homogenously mixed which were then pelleted before being heated139inside the SIS vessel. The compaction pressure is an important parameter in the SIS140method [25]. In this study, three different compaction pressures are studied: 100, 171, and141200 MPa. 9 SHC samples are studied to evaluateHere, -the effect of these variations of the142Al particle size and the compaction pressure on the properties of the SHC is studied.143

Table 2 and Figure 3 shows the effect of these variations on the hardness and porosity 144 of the SHC produced. The largest hardness (44.92 HV) is obtained by the smallest particle 145 size (-170#). And, tThe largest porosity (34.36%) is produced by the biggest particle size 146 (+100#). Both with the same compaction pressure of 100 MPa. On the other hand, the 147 smallest hardness (8.36 HV) is produced by the biggest particle size (+100#, with the com-148 paction pressure of 100 MPa). And Meanwhile, the smallest porosity (19.82 %) is obtained 149 by the smallest particle size (+170#, with the compaction pressure of 200 MPa). In general, 150 for the same value of compaction pressure, there is a tendency that the smaller the Al 151 particle size produces larger hardness and smaller porosity of the composite. This is in 152 accord with the expectation that the smaller particle size can be homogenously distributed 153 in the mixture and increase the composite's density [24], which is shown by the smaller 154 porosity and larger hardness. However, if evaluating the value of each Al particle size 155 group, the tendency is not consistent for all cases. For most of the cases, the larger the 156 compaction pressure tends to give a larger hardness and a smaller porosity. The exception 157 is for the smallest size-fraction (+170#) where the larger the compaction pressure instead 158 gives the smaller hardness (dashed lines in Figure 3a). The trend tendency of the porosity, 159 on the other hand, is more consistent (Figure 3b). The reason for the deviation of the hard-160 ness trend is unconfirmed at the present but might be related to the formation of the ox-161 ides, which will be discussed later. The largest hardness obtained in this study (44.92 HV) 162 is close to those of the human teeth dentin's (46 - 64 HV [26,27]) and human cortical bone's 163 (40.38 HV [28]). However, its porosity is at 31.12 % while the human cortical bone's one 164

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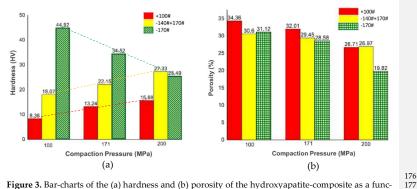
needs to be less than 30% [29]. Some samples fulfilled the porosity criteria for the cortical165bone in this study such as (-140#+170#) 171 MPa and (-170#) 171 MPa with the porosity of16629.45 and 28.58 %, respectively, but their hardness values are much lower than the criteria167(at 22.15 and 34.52, respectively). However, judging the trend of the hardness and porosity168in the -170# group, it can be projected that the criteria of hardness at around 40 HV with169the porosity of less than 30% is possible to be obtained using a compaction pressure be-170tween 100 and 171 MPa.171

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Al Particle Size-Fraction	Compaction	Hardness (HV)	Porosity (%)
	Pressure (MPa)		
+100#	100	-8.36	34.36
	171	13.2 4	32.01
	200	15.68	26.71
-140#+170#	100	18.07	30.60
	171	22.15	29.45
	200	27.33	26.97
-170#	100	<u>44.92</u>	31.12
	171	<u>34.52</u>	28.58
	200	25.49	19.82



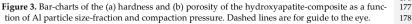
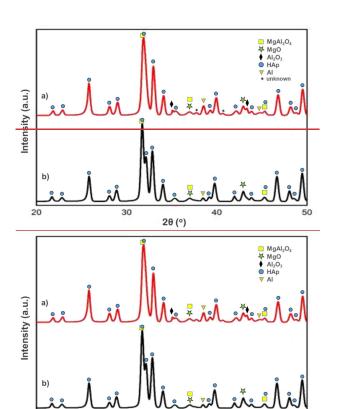


Figure 4 shows the XRD of the SHC made by the compaction pressure of 100 MPa179with the Al particle size-fraction of -170# and +100#. Both samples show the formation of180oxides in the form of magnesia (MgO), and spinel (MgAl2O4) beside the hydroxyapatite.181The formation of the oxides is because the SIS heating was performed in a non-vacuum182furnace and without flowing an inert gas too. Therefore, there was air trapped in the SIS183vessel which allows oxidation of the metal components. However, for the Al particle size-184

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fraction of -170#, there is an extra alumina (Al2O3) peaks detected (Figure 4a). This indi-185 cates that in the case of smaller Al particle size (-170#), the oxidation of the Al metal into 186 alumina is easier compared to those of spinel. On the other hand, for bigger Al particle 187 size (+100#), the oxidation of Al metal is more favorable toward into the spinel formation, 188 as shown by the presence of Al and spinel peaks but in the absence of alumina (Figure 189 4b). Alumina is a known bioceramics for medical applications, such as in dental applica-190 tions (i.e. orthodontic brackets, dental implants, fixed prostheses, and bone cement filler) 191 [30], or joint replacements [31]. The presence of alumina has also been reported to increase 192 the hardness of a composite [32]. The presence of alumina might be responsible for the 193 increase of the hardness observed from 8.36 to 44.92 HV for +100# and -170#, respectively. 194



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Figure 4. XRD of the hydroxyapatite-composite made by compaction pressure of 100 MPa and Al198particle size-fraction of (a) -170# and (b) +100#.199

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Another possibility of the increase of hardness is coming from the increase of crystallinity200of the HAp [33]. The HAp peak at (112) in the +100# sample is relatively taller compared201to the -170# one, indicating its higher crystallinity. The higher the HAp crystallinity in-202stead produce smaller hardness value in this study, opposite as those reported by Ref.203

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[33]. This indicates that the effect of Al₂O₂ formation on the composite's hardness is more dominant.

Figure 5 shows the XRD of the hydroxyapatite-composite made using the smaller Al 206 particle size-fraction (-170#) with different compaction pressures of 100 and 200 MPa. As 207 explained above, for 100 MPa case, the peaks of alumina and Al metal can be observed 208 (Figure 5b). However, increasing the pressure to 200 MPa completely transform Al metal 209 into the MgAl₂O₄ spinel, as shown in the absence of both alumina and Al metal peaks 210 (Figure 5a). This explains the decrease of the hardness of the 200 MPa sample compared 211 to the 100 MPa one (25.49 and 44.92 HV for 200 and 100 MPa samples, respectively), even 212 though the higher pressure supposes to increase the density (as shown by the smaller 213 porosity in Table 2 and Figure 3b) which eventually increase the hardness. This indicates 214 that for the hydroxyapatite composite made of smaller Al particle size (-170#) in this 215 study, the presence of alumina is crucial in increasing its mechanical properties (hence the 216 hardness). The HAp peak at (112) of the 200 MPa sample is relatively taller than those of 217 100 MPa sample, indicating its higher crystallinity. Again, like in the above case, the 218 higher HAp crystallinity instead produces smaller hardness, indicating that the 219 effect of Al2O3 formation on the hardness is more dominant. 220

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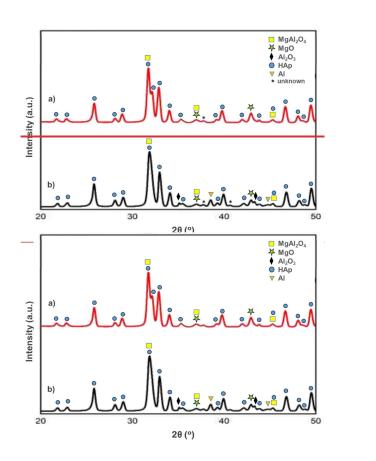
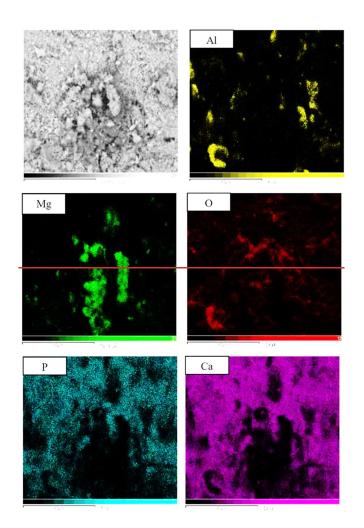


 Figure 5. XRD of the hydroxyapatite-composite made using Al particle size-fraction of -170# and compaction pressure of (a) 200, and (b) 100 MPa.
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FigureFigures 6 and 7 show the SEM-EDS pictures of the SHC made by compaction226pressure of 100 MPa and Al size-fraction of -170# and +100#, respectively. It can be seen227that for each casescase, the Ca and P have almost similar distribution, indicating the for-228mation of the hydroxyapatite. The Ca/P ratio for the two samples, calculated from the229elemental composition derived from the EDS measurements (Table 32), are almost similar230at 1.47 and 1.46 for -170# and +100#, respectively, slightly smaller than those of a pure231hydroxyapatite at 1.67 [34].232



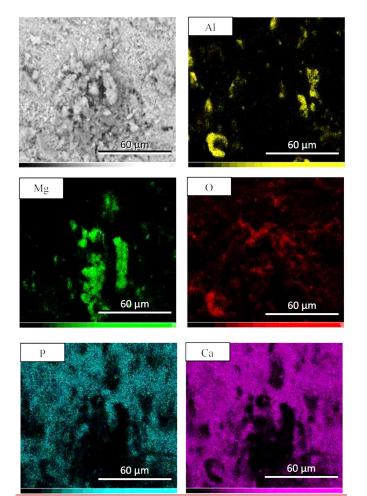
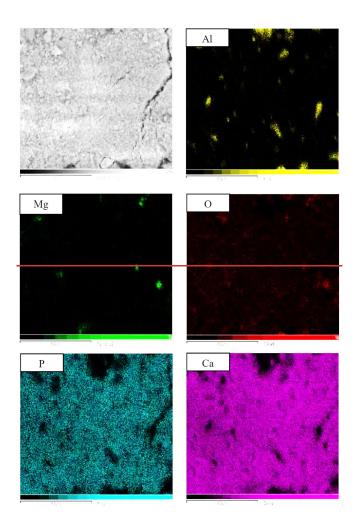


Figure 6. SEM-EDX pictures of the hydroxyapatite composite made by Al size-fraction of -170# with compaction pressure of 100 MPa.





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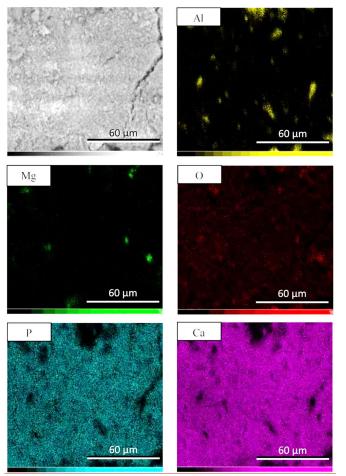


 Figure 7. SEM-EDS pictures of the hydroxyapatite composite made by Al size-fraction of +100# with compaction pressure of 100 MPa.
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posite made by Al size-fraction of -170# and +100# with the compaction pressure of 100 MPa.						
Elements	at.%		-			
Elements	-170#	+100#				
			-			

Table 12. Elemental composition derived from the EDS measurements of the hydroxyapatite com-242 pc

Elements	at.%			
Elements	-170#	+100#		
0	66.75	66.60		
Mg	2.47	2.12		
Al	2.46	4.22		
Р	11.45	10.20		
Ca	16.87	14.86		
Total	100.00	100.00		

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4. Conclusions We have successfully synthesized spinel-based hydroxyapatite composite (SHC) using bovine bones and beverage cans as hydroxyapatite and aluminum (Al) metal sources, respectively, using self-propagating intermediate-temperature synthesis (SIS) method. Aluminum particle size and compaction pressure play important roles in determining the mechanical properties of the SHC. Decreasing the Al particle size tends to increase the hardness and reduce the porosity of the SHC. Increasing compaction pressure also tends to decrease the porosity of the SHC. On the other hand, for hardness value, tendency of it to increase as the compaction pressure is increased only apply for the bigger Al particle sizes. For the smallest Al particle size, the higher the compaction pressure instead decrease the hardness value. This is probably related with to the presence of the alumina in the smaller pressure which contribute to the improvement of the composite's mechanical properties. Author Contributions: Conceptualization, Deni Shidqi Khaerudini; Data curation, Gerald Ensang Timuda; Formal analysis, Ganang Pramudya Ahmad Rifai and Deni Shidqi Khaerudini; Funding acquisition, Agus Pramono; Investigation, Ganang Pramudya Ahmad Rifai and Deni Shidqi Khaerudini; Methodology, Agus Pramono and Deni Shidqi Khaerudini; Supervision, Agus Pramono, Gerald Ensang Timuda and Deni Shidqi Khaerudini; Validation, Agus Pramono, Gerald Ensang Timuda and Ganang Pramudya Ahmad Rifai; Visualization, Agus Pramono; Writing - original draft, Gerald Ensang Timuda; Writing - review & editing, Agus Pramono and Deni Shidqi Khaerudini. Funding: This research was initiated by Hibah Penelitian Kementrian Pendidikan dan Kebudayaan, part of being funded by Penelitian Terapan Unggulan Perguruan Tinggi (PTUPT), with contract number: B/03/UN43.9/PT.00.03/2020. Acknowledgments: Authhors acknowledge the Mechanical Materials Metallurgical Laboratory I and II, Engineering Faculty of Sultan Ageng Tirtayasa University and Research Center for Physics, National Research and Innovation Agency (BRIN) for research facilities. Institutional Review Board Statement: Not applicable. Informed Consent Statement: Not applicable. Data Availability Statement: Data is contained within the article. Conflicts of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. Wu, S.; Wang, J.; Zou, L.; Jin, L.; Wang, Z.; Li, Y. A Three-Dimensional Hydroxyapatite/ Polyacrylonitrile Composite Scaffold Designed for Bone Tissue Engineering. RSC Adv. 2018, 8, 1730-1736. Sari, Y.W.; Rudianto, R.P.; Nuzulia, N.A.; Sukaryo, S.G. Injectable Bone Substitute Synthesized from Mangrove Snail Shell. J. Med. Phys. Biophys. 2017, 4, 115-121. Odusote, J.K.; Danyuo, Y.; Baruwa, A.D.; Azeez, A.A. Synthesis and Characterization of Hydroxyapatite from Bovine Bone for Production of Dental Implants. J. Appl. Biomater. Funct. Mater. 2019, 17, 2280800019836829. Pon-On, W.; Suntornsaratoon, P.; Charoenphandhu, N.; Thongbunchoo, J.; Krishnamra, N.; Tang, I.M. Hydroxyapatite from Fish Scale for Potential Use as Bone Scaffold or Regenerative Material. Mater. Sci. Eng. C 2016, 62, 183–189. Vignesh Raj, S.; Rajkumar, M.; Meenakshi Sundaram, N.; Kandaswamy, A. Synthesis and Characterization of Hydroxyapatite/Alumina Ceramic Nanocomposites for Biomedical Applications. Bull. Mater. Sci. 2018, 41, 1-8. Budiatin, A.S.; Gani, M.A.; Samirah; Ardianto, C.; Raharjanti, A.M.; Septiani, I.; Putri, N.P.K.P.; Khotib, J. Bovine

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Response to Reviewer 2 Comments

The present work is aimed at synthesizing a spinel-based hydroxyapatite composite and evaluating mechanical properties in relation to the arming agent. There are several aspects that would require attention, regarding both form and substrate:

Point 1: For the form, the English language should be improved; ex:

Row 40: bovine bone that "needs" to be recycled; Row 48: "has" been reported; Row 54: "harmful"; Row 227: ...play important "roles"...; Row 233: ...this is probably related "to" the presence..

Response 1: Thanks for your remarks. It is modified according to the remarks.

Point 2: Still regarding form, at 3. Results and discussions there are several places where info from previous sections is repeated - ex: The HAp was extracted from bovine bones; the process needs the bones to be defatted, dried, cut ...; Three different sieve sizes were used: 100#, 140#, and 170# etc.

Response 2: Thank you very much for your comments. We have deleted and rephrase sentences in the Section 3 which contain info in the previous section.

Regarding substrate, I have 4 observations:

Point 3: 1. From what the final part of the introduction and later results and discussions present, the composite is made out of MgO-Al2O3-MgAl2O4-HAp and in 2.2., it is stated that Al and Mg were used as the metal component in the composite. I would advise reformulations wherever metallic components of the composites appear. At a first glance, it misleads the reader.

Response 3: Thank you for your remarks. Now, the term of "MgO-Al2O3-MgAl2O4-HAp" has been reformulated into "HAp-spinel (MgAl₂O₄) based composite".

Point 4: 2. At 2.3. Self-propagating Intermediate-temperature Synthesis (SIS), it is not obvious how can the heat propagation from one specific surface (instead of from all surfaces) induce a more homogenous heating (I advise a reference here); in regard to this observation, it is somehow unclear what benefits come from the SIS processing compared to conventional heating, microwave heating, hydrothermal processing and why this technique was selected. More data from the literature is required here.

Response 4: Thank you very much for your comment. Now, the detail of the SIS method is presented in the Supplementary Information section.

Point 5: 3. Considering the fact that the main compound of this work contains a relatively large amount of Al (10%), as a form of Al2O3 or MgAl2O4, a reader will find it necessary to find a more comprehensive justification for the use of Al in a HAp composite, more than the fact that Al2O3 poses no harmful effect on the human body. What benefit does it bring towards HAp? How does the literature describe the effect of different contents of Al/Al2O3/MgAl2O4 upon a HAp composite?

Response 5: Thank you very much for your comment. The addition of Al leads to the formation of Al₂O₃ which can improve the hardness of the composite (10.1007/s12034-018-1612-4). And, together with Mg, they lead to the formation of the MgAl₂O₄ which can improve the phase stability of the HAp during sintering (doi: 10.1111/jace.13556). This explanation has been added in the introduction section.

Point 6: 4. In the results and discussion section, it is stated that Al is the metal used in the composite as a reinforcement to improve the mechanical properties of the HAp ceramics. Although initial X-ray analysis was performed on the HAp obtained from bovine bones, there is no compaction pressure nor hardness performed on the reference sample, required to evaluate the effect that Al has in reinforcing the ceramic. A reference sample without Al/Mg is needed here.

Response 6: Thank you very much for your comment. Indeed, we already did make the samples without addition of Al or Mg, however they were always break after heating. So, the addition of Al and Mg is crucial in the reinforcing the composite.

Response to Reviewer 3 Comments

Hydroxyapatite for medical purposes needs to be very well characterised. A rigorous control of the quality is indispensable. There are a lot of questions regarding this research work: **Point 1:** 1. the Hap obtained from bovine bone was not enough charcterized (e. g. Fig 2!)

Response 1: Thank you very much for your comment. In Figure 2 we present the XRD of the heattreated bovine bone as the HAp source, which we already shown and explained in the text that it consisted of 100% HAp according to the XRD analysis. We consider that it is enough for this stage, since this is not yet the final product of this study, for it will be further used to synthesize the spinel-HAp composite.

Point 2: 2. how the authors insure the constant and same quality of Hap from bovine bone?

Response 2: Thank you very much for your comment. We have produced several batches of HAp from bovine bones obtained from the same source (the same local market), especially in this work we use the leg part of the bovine bone, which have relatively similar XRD pattern. Therefore, we are sure that the method is reproducible in producing HAp for spinel-HAp composite synthesis. There is a possibility that different result might be produced from different source of bovine bone, however, it is beyond the scope of the current study.

Point 3: 3. On Fig. 4 there or "unknown" peaks! It is not allowed in a research paper! For medical purposes the raw materials and the products need to be well characterised and the preparation method to be reproducible!

Response 3: Thank you very much for your comment. We have removed the label "unknown" in the XRD graphs according to your suggestion. We appreciate your remark about well characterization and reproducible product. We notice that there are various characterizations to elaborate further prior to implementation of biomedical application, such as in vivo study and clinical trial. However, this study is still at a very early stage, and those points are beyond the scope of the current study.