

PAPER • OPEN ACCESS

The impact of dietary hydrolyzed collagen on bone's calcium deficiency of *Rattus norvegicus*

To cite this article: E J Oetama *et al* 2018 *IOP Conf. Ser.: Earth Environ. Sci.* **187** 012017

View the [article online](#) for updates and enhancements.

You may also like

- [Enhanced osteogenic activity of a poly\(butylene succinate\)/calcium phosphate composite by simple alkaline hydrolysis](#)
Suphakit Arphavasin, Weerachai Singhatanadgit, Patcharee Ngamviriyavong et al.
- [Preparation of colloidal nanoparticles PVA-PHEMA from hydrolysis of copolymers of PVAc-PHEMA as anticancer drug carriers](#)
Mohsen Shahrousvand, Mohsen Hajikhani, Leila Nazari et al.
- [Marine based biomaterial-fish collagen enhances the polarization of human macrophage](#)
Chao Liu and Jiao Sun



ECS
The
Electrochemical
Society
Advancing solid state &
electrochemical science & technology

DISCOVER
how sustainability
intersects with
electrochemistry & solid
state science research

The impact of dietary hydrolyzed collagen on bone's calcium deficiency of *Rattus norvegicus*

E J Oetama^{1*}, A L Juwono², D S Soejoko², and D A Astuti³

¹Graduate Student, Department of Physics, Universitas Indonesia, Depok, 16424, Indonesia

²Researcher and Lecturer, Department of Physics, Universitas Indonesia, Depok, 16424, Indonesia

³Researcher and Lecturer, Department of Nutrition and Feed Technology, Bogor Agricultural University, Bogor, 16680, Indonesia

* email : elisabeth.jeanny@sci.ui.ac.id and ariadne.laksmidevi@ui.ac.id

Abstract. Type 1 Collagen is a protein consisting of polypeptides that constitutes organic structure of bones. During aging, the collagen content will decrease and lead to reduced bone strength as the result of the increased activity of bone resorption by osteoclasts cells. In the current experiment, the rat bones were conditioned into calcium deficiency state with low dietary calcium feeding. The hydrolyzed collagen and regular-sized of calcium phosphate (CaP) were later added into the feeding with certain composition as the intake to restore bone strength from the deficiency state. The quantitation of mineral groups and mineral compositions such as phosphorus (P), calcium (Ca), and magnesium (Mg) in bone matrix structure was conducted using Fourier Transform Infrared (FTIR) and Atomic Absorption Spectrophotometry (AAS). The results showed that the deposited mineral of deficiency state bones of rats fed with hydrolyzed collagen and CaP intake was higher compared to those with CaP intake only, thus indicating that the dietary hydrolyzed collagen intake is essential method to restore the bone strength.

1. Introduction

Bone is a complex and dynamic tissue composed of mineral phase in an organic matrix. This organic matrix constitutes mainly of type I collagen fibrils [1]. The anatomy of long bones such as femur shows two types of constituents, namely cancellous or spongy and cortical as seen in Figure 1. Figure 1 also shows the microstructure, sub-microstructure, nanostructure, and sub-nanostructure of certain parts of bone. As the constituent of osteon, there are network of collagen fibers composed of collagen molecules (mainly type 1 collagen) and hydroxyapatite crystals. The formation of collagen molecules is cross-linked which also contributes into bone strength beside the bone crystals or minerals [2-3].

Aging is the most influencing factor into bone strength characterized by disruption of collagen and calcium phosphate in the structure of bone tissue [4]. During aging, the activity of osteoclast-mediated bone resorption and osteoblast-mediated bone formation becomes imbalance resulting in irreversible bone loss. This condition increases the fragility tendency of bone and fracture risk. One method to prevent this condition from becoming worst is by providing nutrition intake through existence of hydrolyzed collagen as part of food composition. Collagen already considered as important part of



bone but there is still less research about impact of collagen as part of nutritional fed into mineral content of rat bones [2-9]. This current study was conducted using a rat model due to several advantages such as compliment with local ethics, husbandry and low cost of acquisition, as well as the similarities in pathophysiologic responses with humans [10]. The hydrolyzed collagen and CaP intake were provided into rats with calcium bone deficiency as well as CaP intake given into another rats separately in a similar condition. The rat bones were analyzed using Fourier Transform Infrared (FTIR) and Atomic Absorption Spectrophotometry (AAS) to study the mineral contents after the particular nutritional feeding.

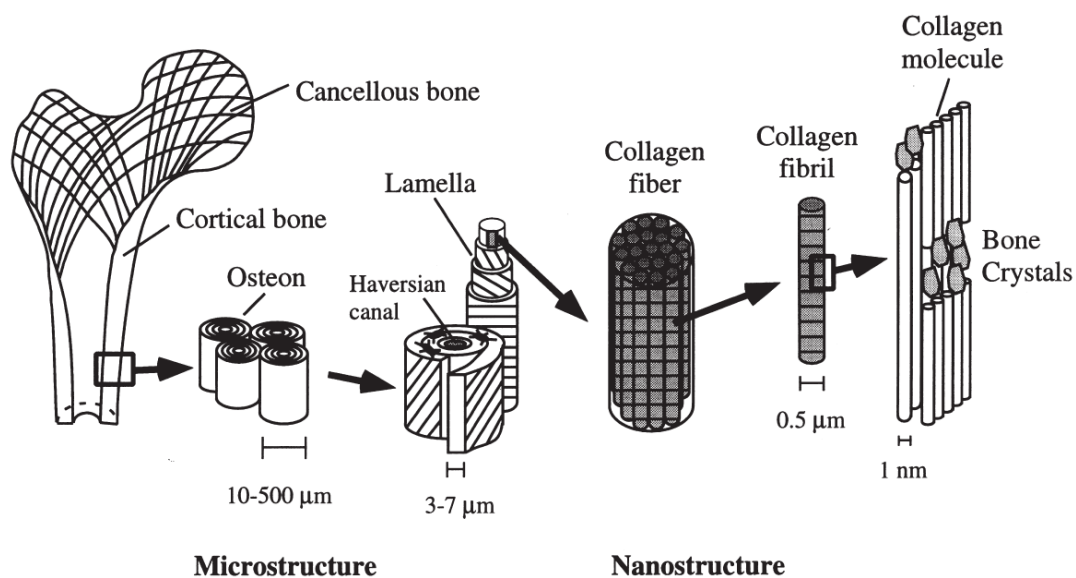


Figure 1. Structures of Bone Matrix [9].

2. Materials

White rats of *Rattus norvegicus* species of Sprague-Dawley at age of eleven months were approved as animal model for the experiment by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia with protocol number 17-08-0841. Nutritional intakes were composed by calcium phosphate, hydrolyzed collagen, sugar flour, rice flour, casein, corn oil, Carboxy Methyl Cellulose (CMC), vitamin mix, calcium free mineral mix, DL-Methionine, and NaCl with different compositions for different treatments.

3. Methods

Rats were nurtured in an appropriate place with average temperature 27.2 °C and average relative humidity 63.3%. During adaptation in new place, rats were fed by standard dietary (S) (commercial product from PT Charoen Pokphand Indonesia Tbk., with a diet phase grower 512) for one week. This group was identified as S dietary rats. After one week adaptation, rats were ready to be fed with deficiency dietary (D) which contained 0.00% of CaP and 0.00% of hydrolyzed collagen (identified as D dietary rats). After several months, the deficiency rats were separated into two groups according to food compositions given to them, one group was fed by hydrolyzed collagen and CaP (HC) as part of the food compositions identified as HC dietary rats and another group was fed by only CaP (NHC) as part of the food compositions identified as NHC dietary rats. Statistically, there were three rats for each of the groups and the food composition for each group can be found in the Table 1. After one month feeding, euthanasia procedures were executed into rats to get the bones to be analyzed.

After the euthanasia procedures, femur and spine bones with surrounding soft tissues were taken for analysis, the samples were heated at the temperature of 60 °C for 24 hours, then soaked into

hydrazinium hydroxide for 7 days to remove surrounding soft tissues. The bones were later soaked with the alcohol for one hour and then rinsed by aquadest three times. After dried in room temperature, the procedure from soaked into hydrazinium hydroxide until put in room temperature was repeated once again. Then, the analysis procedures were conducted into spine using Fourier Transform Infrared (FTIR) to identify functional groups in the infrared spectrum and into femur using Atomic Absorption Spectrophotometry (AAS) to determine the mineral compositions such as phosphorus (P), calcium (Ca), and magnesium (Mg).

Table 1. Rats dietary formulation.

Ingredient	D	NHC	HC
	w/w %		
Sugar Flour	49.00	41.50	41.90
Rice Flour	25.00	30.00	29.50
Casein	18.00	21.00	14.00
Hydrolyzed Collagen	0.00	0.00	7.00
Corn Oil	3.50	2.50	2.50
CMC	3.00	3.00	3.00
CaP	0.00	0.50	0.60
Vitamin Mix	0.50	0.50	0.50
Ca Free Mineral Mix	0.50	0.50	0.50
DL-Methionine	0.30	0.30	0.30
NaCl	0.20	0.20	0.20
TOTAL	100.00	100.00	100.00

D : Deficiency dietary

NHC : CaP content of dietary

HC : Hydrolyzed Collagen and CaP content of dietary

4. Results and Discussion

Analysis data of the rats' dietary food content is available in Table 2. The analysis including mineral composition (calcium, phosphorus, and magnesium), dry matter, ash, fat, protein and crude fiber. Table 2 shows that D sample has lowest Ca content compared to other samples, as this sample was used to induce calcium deficiency state of rats. Although standard dietary (S) has higher Ca content, however, the administration was only conducted during the adaptation period. Others dietary food (NHC and HC) were found suitable as food, since these two types of food improved the calcium deficiency state due to higher Ca content compared to that D sample (Table 1 and 2). Protein content of HC were higher than D and NHC (Table 2), suggesting that the collagen content (Table 1) support cells regeneration process that further increase bone mineral content [11-12].

Table 2. Food content analysis for four groups of treatments.

Sample Code	Ca	P	Mg	Dry Matter	Ash	Fat	Protein	Crude Fiber
w/w %								
S	1.35	0.48	0.14	9.89	5.94	8.75	19.45	2.02
D	0.28	0.04	0.02	93.35	2.05	2.01	15.80	0.33
NHC	0.94	0.60	0.14	90.89	2.57	2.01	16.96	0.00
HC	0.84	0.24	0.01	89.87	2.45	2.38	18.50	0.22

S : Standard dietary

NHC : CaP content of dietary

D : Deficiency dietary

HC : Hydrolyzed collagen and CaP content of dietary

The AAS results of femur mineral analysis is shown in Table 3. Our data indicates that the Ca content in bones sample of S dietary rats was 74.63 w/w % and used as the reference for the control condition. The Ca content in femur were 40.76 w/w %, 74.25 w/w %, and 72.29 w/w % for rats fed with D, HC, and NHC dietary foods, respectively. The HC dietary rat had higher Ca content in femur rather than NHC dietary rat, but similar with Ca content of that femur of S dietary rat. The higher results of the mineral deposit (Ca) in bones of HC dietary rat compared to NHC dietary rat indicated the occurrence of calcium deficiency has already improved into normal conditions following the treatment of the particular dietary food.

The Ca and protein contents in HC rats were 37.78% and 4.88% lower, respectively, than that S dietary food. Meanwhile, the Ca and protein contents in NHC rats were 30.37% and 12.80% lower, respectively, compared to S dietary (Table 2). Such conditions suggest that HC dietary was rich in protein but poor in Ca compared to NHC dietary, contrarily to that HC dietary. After HC and NHC dietaries fed into rats with calcium deficiency state, the deposited Ca in bones from HC and NHC dietary rat were 0.51% and 3.14 % lower than S dietary rat, respectively. This data suggests that HC dietary rat had more Ca content in bones than the NHC dietary rat. Although the HC dietary had lower Ca content than the NHC dietary, Ca content in bones of HC dietary rat was higher than NHC dietary rat. This result indicates that the presence of hydrolyzed collagen as protein with adequate calcium intake increased bones calcium content.

Table 3. Ca, P, and Mg contents in femur samples.

Sample Code	Ca	P	Mg
	w/w %		
S	74.63	14.02	2.92
D	40.76	1.58	13.64
NHC	72.29	13.93	4.01
HC	74.25	13.38	4.14

S : Control rat with S dietary

NHC : Deficiency rat with NHC dietary

D : Deficiency rat with D dietary HC : Deficiency rat with HC dietary

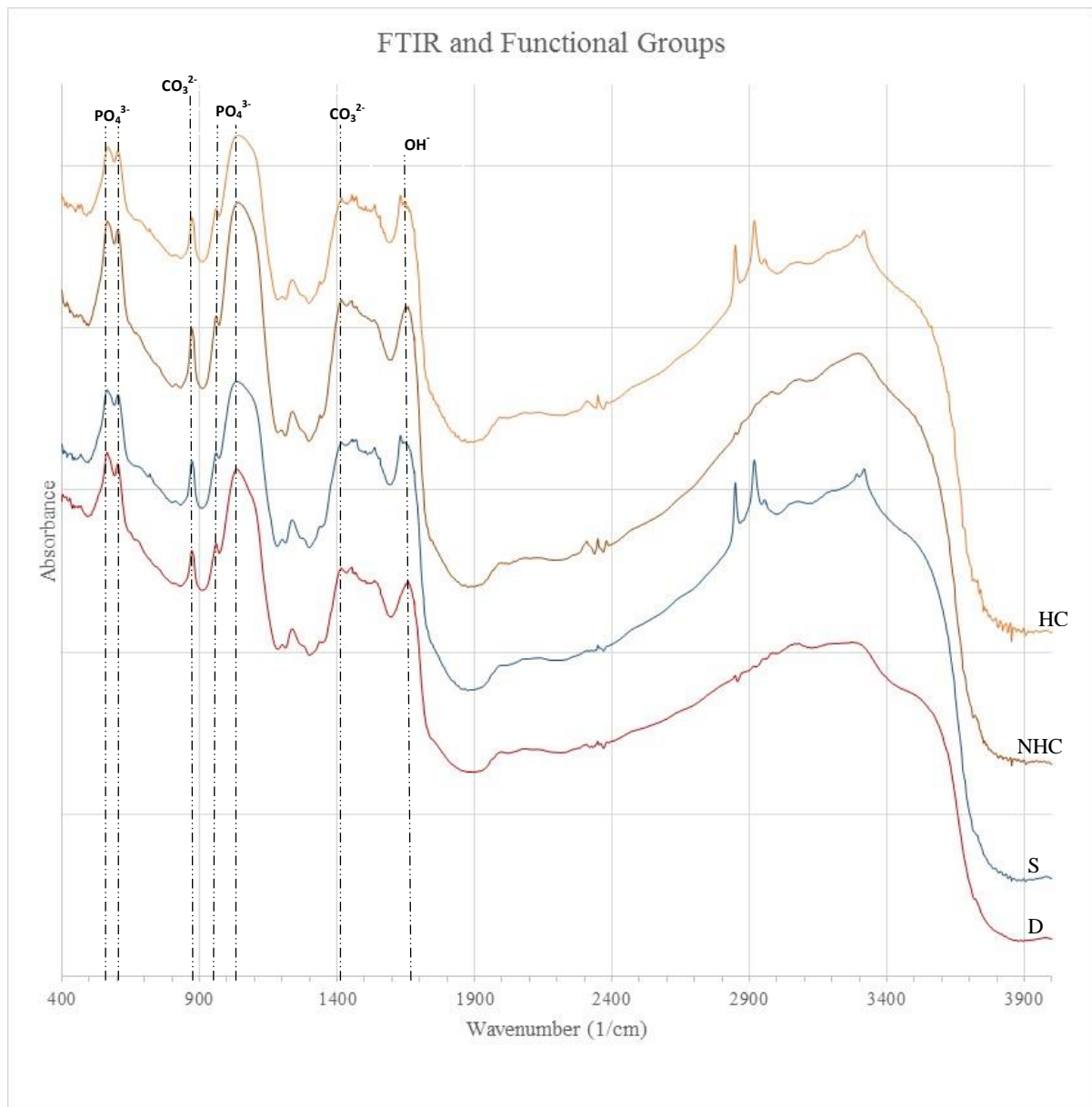


Figure 2. FTIR spectra from bones samples of D dietary rats, S dietary rats, NHC dietary rats, and HC dietary rats.

Figure 2 shows the FTIR spectra from bones samples of S dietary rats as standard and 3 types of treated samples. The FTIR spectra indicates the presence of PO_4^{3-} peaks at 571 cm^{-1} , 606 cm^{-1} , 963 cm^{-1} , 1044 cm^{-1} and CO_3^{2-} peaks at 876 cm^{-1} and 1424 cm^{-1} respectively at the typical wave number of phosphate groups (PO_4^{3-}) and carbonate groups (CO_3^{2-}) in the range of $500\text{--}1200\text{ cm}^{-1}$ [13]. The existence of phosphate groups and carbonate groups represent the presence of calcium phosphate in the form of apatite carbonate. In Figure 2, besides phosphate groups and carbonate groups, hydroxyl group (OH^-) were also found at 1661 cm^{-1} . This result also agrees well with the range of hydroxyl group (OH^-) wave numbers from $1600\text{--}1700\text{ cm}^{-1}$ [14]. Figure 3 shows how to measure Splitting Factor (SF) of phosphate groups. SF for phosphate groups in the range of $500\text{--}650\text{ cm}^{-1}$ wavenumber were 2.03, 2.01, 2.01, and 2.00 for D dietary rat, S dietary rat, NHC dietary rat, and HC dietary rat respectively. From the data, the different of SF between treatments was not significant. Higher number of splitting factor

means the increasing of crystallinity [15]. The correlation of lower splitting factor from FTIR data and the higher content of Ca from AAS results indicate that rat bone fed with HC dietary shows the best bone conditions compared to other groups.

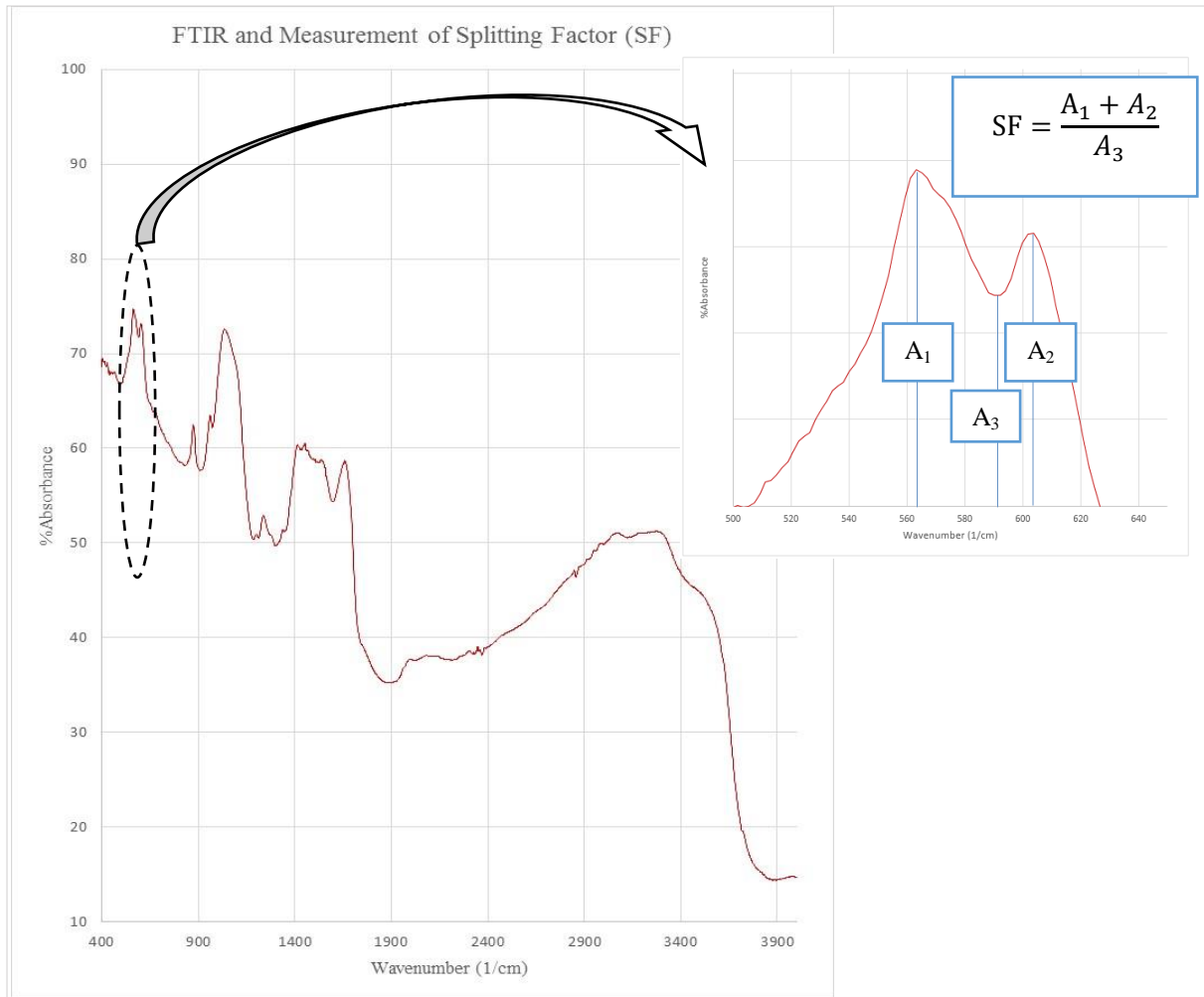


Figure 3. FTIR spectra and measurement of splitting factor [15].

5. Conclusion

The results from both AAS and FTIR analysis of rat bones indicate that rats fed with dietary food composed by hydrolyzed collagen and calcium phosphate and rats fed with dietary composed by calcium phosphate improved the calcium deficiency state into normal state, as shown by the presence of PO_4^{3-} (peaks: 571 cm^{-1} , 606 cm^{-1} , 963 cm^{-1} , 1044 cm^{-1}) and CO_3^{2-} (peaks : 876 cm^{-1} and 1424 cm^{-1}). The rats fed with dietary composed by hydrolyzed collagen and calcium phosphate for one month showed higher calcium content than rats fed with dietary composed by calcium phosphate only. Therefore, the presence of hydrolyzed collagen with adequate calcium content could improve the calcium deficiency state of bone.

Acknowledgment

This study was financially supported by “PITTA grant” for 2017 from Universitas Indonesia.

References

- [1] Bala Y, Farlay D, and Boivin G 2012 Bone mineralization : from tissue to crystal in normal and pathological contexts *Osteoporosis Int.*
- [2] Eyre D R and Wu J-J 2005 Collagen cross-links *Topics in Current Chemistry* **247** 207-229
- [3] Saito M and Marumo K 2009 Collagen cross-links as a determinant of bone quality : a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus *Osteoporosis Int.* **21** 195-214
- [4] Wang X, Shen X, Li X, and Mauli Agrawal C. 2002 Age-related changes in the collagen network and toughness of bone *Bone* **31** (1) 1-7
- [5] Takeda S, Park J-H, Kawashima E, Ezawa I, and Omi N. 2013 Hydrolyzed collagen intake increases bone mass of growing rats trained with running exercise; *J. Int. Soc. Sports. Nutr.* **10** 35
- [6] Garnerio P 2015 The role of collagen organization on the properties of bone *Calcif. Tissue Int.* **97** 229-240
- [7] Ferreira A M, Gentile P, Chiono V, and Ciardelli G 2012 Collagen for bone tissue regeneration *Acta Biomaterialia* **8** 3191-3200
- [8] Nair A K, Gautieri A, Chang S-W, and Buehler M J 2013 Molecular mechanics of mineralized collagen fibrils in bone *Nature Communications* **4** 1724
- [9] Rho J-Y, Kuhn-Spearing L, and Zioupos P 1998 Mechanical properties and the hierarchical structure of bone *Medical Engineering & Physics* **20** 92-102
- [10] Lelovas P P, Xanthos T T, Thoma S E, Lyritis G P, and Dontas I A 2008 The laboratory rats as an animal model for osteoporosis research *Comparative Medicine* **58** (5) 424-430
- [11] Yang J, Shi P, Tu M, Wang Y, Liu M, Fan F, and Du M 2014 Bone morphogenetic proteins : Relationship between molecular structure and their osteogenic activity *Food Science and Human Wellness* **3** 127-135
- [12] Heaney R P and Layman D K 2008 Amount and type of protein influences bone health *Am. J. Clin. Nutr.* **87** (suppl) 1567S-1570S
- [13] Figueiredo M M, Gamelas J A F, and Martins A G 2012 Characterization of bone and bone-based graft materials using FTIR spectroscopy *Infrared Spectroscopy – Life and Biomedical Sciences* ed T Theophile (Croatia : InTech) p 315-338
- [14] Miculescu F, Stan G E, Ciocan L T, Miculescu M, Berbecaru A, Antoniac I 2012 Cortical bone as resource for producing biomimetic materials for clinical use *Digest Journal of Nanomaterials and Biostructures* **7** (4) 1667-1677
- [15] Surovell T A and Stiner M C 2001 Standardizing infra-red measures of bone mineral crystallinity : an experimental approach *Journal of Archaeological Science* **28** 633-642