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Degradation of Milkfish Bone (*Chanos- chanos* Forsskal) with Pure Papain Enzyme Concentration and Heating Time

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aimed to determine the physicochemical characteristics of calcium phosphate derivatives of milkfish bones (*Chanos-chanos* Forsskal) with treatment pure papain enzyme concentration and heating time.
Study Design: The experimental design used was a factorial complete randomized design, namely factor A = hydrolysis with the use of pure papain enzymes and factor B was heating time using a temperature of 60^{o}C, each treatment was repeated 3 times.
Place and Duration of Study: Research was carried out from September 2018 to July 2019, Agroindustry Workshop, and Chemical Laboratory, Department of Fishery Product Processing

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Technology, Pangkep State Agricultural of Polytechnic, Physics Laboratory, Science Center, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Chemistry Laboratory, Makassar State Islamic University, Inorganic Chemistry Laboratory MIPA Lampung State University.

Methodology: Milkfish bones were added with distilled water and pure papain enzyme at pH 8 with a concentration of 4%, 6% and 8%, degraded by heating at 60°C for 6, 8, and 10 hours after extraction, result were then dried in oven at 50°C. After drying, it ground with a bone crusher. The flour was analyzed, namely: physical tests including yield, whiteness degree, proximate test including moisture content, ash content, protein content, fat content, calcium, phosphorus, functional groups using FTIR, crystallinity using XRD, morphology using SEM/EDS.

Results: The results of the analysis of the concentration of pure papain enzyme 8% with a heating time of 10 hours gave the best result, obtained 3.24% moisture content, 83.28% ash content, 10.28% protein, 3.20% fat, 49.30% calcium, 29.17% phosphorus, 7.16% yield, 88.68% whiteness. FTIR analysis has carboxyl groups (OH^-), carbonate groups (CO_3^{2-}) and phosphate groups (PO_4^{3-}). These are the main components of hydroxyapatite formation where the phosphate absorption band spectra (PO_4^{3-}) are at a wave frequency of 403.60 cm^{-1} with an intensity 36.568% is $V_3PO_4^{3-}$ The crystallinity of calcium phosphate derivatives is 74.53% µm hydroxylapatite, 11.94% tricalcium phosphate µm, 13.53% dicalcium phosphate µm. Morphology of Calcium Phosphate Derivative Compounds using XRD, which showed a degree of crystallinity of 73.04% and a crystal size of 4.717 µm.

Conclusion: The result of the analysis obtained by the combination treatment of pure papain enzyme concentration of 8% with a heating time of 10 hours are the best of the other treatment.

Keywords: Bone; calcium phosphate derivatives; milkfish; pure papain enzyme.

1. INTRODUCTION

Calcium is an important mineral for bone health. Lack of calcium interferes with bone growth and impedes the normal functioning of the body. Insufficient calcium intake is obtained from the food consumed so that the body can withdraw calcium stored in the bones.

Utilization of milkfish bone as a source of calcium and phosphorus is an alternative in order to provide a food source rich in calcium and phosphorus, in the form of calcium phosphate compounds, creatinine phosphate compounds and hydroxyapatite compounds. One of the sources of calcium and phosphorus is the utilization of fish bone which have been wasted or made into animal feed, it's just that they have to go through a processing technology process. absorbed 50% of the total calcium consumed [1]. Milkfish bone meal has a calcium mineral content of 14.16% [2].

One alternative to maximize calcium absorption is by forming nanocalcium [3]. In addition to calcium, a mineral element is also needed in the human body, namely phosphorus, which is 1% of body weight. The high number of cases of bone damage that occurs encourages researchers in the field of medicine to make artificial bone. As material technology has developed, hydroxyapatite has been found, which has a structure similar to that of human bone [4,5]. Hydroxyapatite (HAp) is one of the most widely used apatite groups in the medical field due to its biocompatible and osteoconductive properties. Hydroxyapatite (HAp) has the structural formula $Ca_{10}(PO_4)_6(OH)_2$.

Enzymes are protein that function as catalysts for chemical reactions in biological systems. Enzymes have high catalytic power. Enzymes can increase the speed of reactions up to one million times faster than reactions without enzymes. Enzyme molecules also have a certain level of specificity towards the substrate of the reaction they catalyze [6]. The ability of an enzyme to catalyze a reaction is illustrated by its activity. The papain enzyme is a proteolytic enzyme that is able to hydrolyze proteins into amino acids or peptides. The activity of the papain enzyme only decreased by 20% when heated to 70^{o}C for 30 minutes at a pH of 7.0 [7]. Papain enzyme has a sulfhydryl functional group and is able to hydrolyze peptide bonds in the amino acids lysine and glycine. The optimum temperature for papain ranges from 50–65^{o}C, and the optimum pH is between 5 – 7. The optimum pH range for papain ranges from 5-7.5 and is stable at 40-60°C [8]. The proper temperature and length of time for the hydrolysis of the papain enzyme to produce edamane juice

with the best chemical and physical characteristics is the hydrolysis temperature of 60^{o}C for 2 hours [9]. Papain activity increases with increasing temperature from 40^{o}C to 60^{o}C. Maximum activity is achieved at 60^{o}C, which is 14.39755 x 10 5 U/g, thus 60^{o}C is the optimum temperature for the papain enzyme [10]. Utilization of the papain enzyme as much as 4% is able to remove the lime layer on the sand sea cucumber and does not cause the black layer of skin on the back of the sea cucumber to be damaged [3].

2. MATERIALS AND METHODS

2.1 Materials

The material used are milkfish bones (*Chanos-chanos* Forsskal) obtained from the processing of Milkfish bone extract UKM Mentari Citra Lestari, Pangkajene and Kepulauan Regency, technical papain enzymes, 1.5 N NaOH. Materials used in the analysis include n-Hexane and Ethyl acetate, DPPH solution 0.2% material.

The tool used in the processing procedure are boiling pots, stoves, blenders, heating tubes, autoclaves, extraction equipment, digital scales, packaging, mill machines, 100 mesh size sieve, basins, choppers, stirrers, filter cloths, vacuum filtration, filters, furnaces , vacuum oven, hot plate, balance, set of proximate analytical units. Instruments for the analysis used: whiteness meter, Scanning Electron Microscopy (SEM/EDS), Fourier Transform Infrared (FTIR), and X-ray Diffraction (XRD).

2.2 Methods

Milkfish bones were added with distilled water and pure papain enzyme at pH 8 with a concentration of 4%, 6% and 8%, degraded by heating 60°C for 6, 8, and 10 hours after extraction result were then dried in an oven at 50°C. After drying, ground it with a bone crusher. The flour was analyzed, namely: physical tests including yield, whiteness degree, proximate test including moisture content, ash content, protein content, fat content, calcium, phosphorus, functional groups using FTIR, crystallinity using XRD, morphology using SEM/EDS.

2.3 Experimental Design

The experimental design used in this study was a completely randomized factorial design.

2.4 Analysis Data

The research data were analyzed using analysis of variance using SPSS software

3. RESULTS AND DISCUSSION

3.1 Characteristics of Milkfish Bone Meal (*Chanos-chanos* Forsskal)

Characteristics of milkfish bone meal (*Chanos-chanos* Forsskal). With the treatment of 8% pure papain enzyme concentration with a heating temperature of 60^{o}C for the best 10 days, an analysis of water content, ash content, protein content, fat content, calcium content, phosphorus content, yield content, degree of whiteness, size of calcium phosphate crystals was carried out.

The value of the water content in the treatment of 8% pure papain enzyme concentration with a heating time of 10 hours, the average is 3.24% means that the longer the heating the lower the water content produced this is because the water evaporates freely (released) during heating and drying. The result of the research the difference in water content is influenced by the type of fish bone, drying method, and degradation method. This value is still within the standard range set by SNI 01-3158-1992 for bone meal moisture content (maximum 8 percent), but higher than the water content of bone meal produced by International Seafood of Alaska (ISA), which is 3.4% and [11] by 3.3%. According to [12] drying time, and drying temperature and surface area of the material can affect the moisture content of a material, the method of drying and the surface area of the material, as well as the container in which the material is dried affect the water content.

The value of ash content in the treatment of 8% pure papain enzyme concentration with 10 hours of heating time was 83.28 percent, the higher the ash content obtained, the higher the enzyme concentration, the faster and greater the activity of enzymes to break down protein into amino acids. The result of this study are almost the same as the result of [1] ranging from 95.93 to 69.37%. The value of this study was lower than the ash content obtained in tuna fish bone hydroxyapatite, which was 99.83 [13] percent, research on belida fish bone ash content ranged from 83.94 to 76.79% [14], research on tuna bone ash content ranged 77.87-53.43% [15], on the other hand it is higher than the ash content obtained in commercial flour produced by ISA [16], namely 33.0%., 56.51-26.37% [17].

Table1. Characteristics of milkfish bone meal (*Chanos-chanos* Forsskal) with the treatment of pure papain enzyme concentration of 8% with a heating temperature of 60°C for 10 hours

Component	Pure Papain Enzyme Concentration 8% with a Heating Temperature of 60°C for 10 Hours
Water content	3.24%
Ash Content	83.28%
Protein Content	10.28%
Fat level	3.20%
Calcium Levels	49.30%
Phosphorus Levels	29.17%
yield	7.16%
White Degrees	88.68%

The protein content of the pure papain enzyme concentration degradation treatment sample with a long heating time was 10.28%. The low protein content is caused by the longer the heating the lower the protein content produced, this is due to the higher concentration of the pure papain enzyme and the longer the heating time at the optimum concentration, the faster and greater the decomposition of protein compounds into amino acid compounds, including amino acids containing minerals. The protein content obtained was lower than the protein content obtained by ISA [16], which was 34.20%, and almost the same as research [18], 16.90% and 11.08% [11].

The result of the analysis of fat content in the treatment of pure papain enzyme concentration, heating time, was 3.20%. The higher the concentration of the papain enzyme and the longer the heating time, the fat breaks down into fatty acids. Most of the fat in the bone is in the form of lipoprotein which are a combination of lipid and protein molecules. The Indonesian National Standard (SNI 01-3158-1992) that the fat content for fishmeal for quality I is 3% wb. Referring to these standards, the fat content of milkfish bone meal resulting from the treatment of pure papain enzyme concentrations with prolonged heating is included in quality II. The results of the fat content of this study were almost the same as the fat content of tilapia bone meal, which was 5.82% [19], the fat content of tuna fish meal was 4.13% [20], the fat content of yellow tuna meal was 3.86% [15], and the result this study is higher than the result of catfish flour research, namely 2.09% [21].

The result of the analysis of calcium level in the treatment of pure papain enzyme concentration, heating time, was 49.30%. Calcium level in the treatment of pure papain enzyme concentration with this long heating time were higher than tilapia fish bone calcium level ranging from 22.56-18.70% [1], sardine fish bone calcium level ranging from 32.73-18.91% [22]. This study is almost the same as the result of research on tuna bone meal calcium level ranging from 39.24 to 23.72% [20].

The result of analysis of phosphorus content in the treatment of pure papain enzyme concentration, heating time, is 29.17% the higher the concentration of pure papain enzyme the higher the phosphorus content, this is due to the decomposition of inorganic compounds into simple compounds, namely minerals including phosphorus. This value is below the standard range of phosphorus content values. Stipulated by SNI for fish bone meal is 30% (quality I) and is above 20% (quality II), based on SNI the result of this study are at quality II. The result of phosphorus level in the treatment of pure papain enzyme concentration and heating time were higher than the phosphorus content of tilapia bone meal 12.05-8.86% [1], the phosphorus content of yellow finned tuna bone meal [15] ranged from 23.31- 14.58% [15].

The yield measurement result in the study of pure papain enzyme concentrations with a long heating time were 7.16%. Peptide compounds decompose again into amino acids, eventually turning into ammonia, CO_2 and H_2O. Likewise, long heating will decompose all organic and inorganic components found in bones along with heating, making the mineral form purer. The yield value obtained was almost the same as that of tilapia bone, namely 5.84% [9].

The result of the analysis of the degree of whiteness of pure papain enzyme concentration with a heating time of 88.68%. The tendency for the degree of whiteness produced increases as

the pure papain enzyme concentration increases at the 8% limit as well as the heating time increases, this is due to the color of the pure enzyme itself being white as well as some pure white minerals including white calcium. The whiteness of milkfish bone meal resulting from the treatment of pure papain enzyme concentrations with this long heating time is higher than the whiteness of tuna fish bones ranging from 74.8 to 59.3% [20].

3.2 Functional Group Characterization Of Milkfish Bone Meal (*Chanos-chanos* Forsskal) Enzymatically

FTIR analysis showed that the milkfish bone meal sample was identified as having a component/group consisting of a carboxyl group (OH^-), a carbonate group (CO_3^{2-}) and a phosphate group (PO_4^{3-}) (Fig. 1). These three groups are the main components in the formation of hydroxyapatite. The treatment of pure papain enzyme concentration of 8% with a heating time of 10 hours can be seen in Fig. 1, where the spectra of the phosphate absorption band (PO_4^{3-}) are at a frequency or wave number of 403.60 cm^{-1} with an intensity of 36.568%, which is $V_3PO_4^{3-}$ (asymmetric stretching vibration), 560.63 cm^{-1} (29.024%), 601.91 cm^{-1} (31.551%) both of which are $V_4PO_4^{3-}$ (asymmetric bending vibrations), 1030.29 cm^{-1} (27.321%) are $V_3PO_4^{3-}$ (asymmetric stretching vibrations). There are four apatites carbonate, where there is one type A (AKA), namely at 1560.28 cm^{-1}(38.842%) the $Ca_{10}(PO_4)_6CO_3$ compound is formed because the carbonate ion (CO_3^{2-}) replaces the hydroxyl position (OH^{1-}) in hydroxyapatite $Ca_{10}(PO_4)_6CO_3$, while the three included type B carbonate apatite (AKB), namely 1458.15 cm^{-1} (39.526%), 1419.57 cm^{-1} (41.187%), and 872.14 cm^{-1}(45.310%) were $Ca_{10}(PO_4)_3(CO_3)_3(OH)_2$ where a carbonate ion (CO_3^{2-}) replaces one of the positions of a phosphate ion (PO_4^{3-}) in hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$. [23,24].

The content of phosphate groups (PO_4^{3-}) in the sample treated with 8% pure papain enzyme concentration with a heating time of 10 hours is indicated by visual readings of the spectral peaks detected at wave number 1030.29 cm^{-1}, also at wave number readings 403.60-601.91 cm^{-1}. The detection of a phosphate group (PO_4^{3-}) has similarities with a study conducted by Mondal et al. (2012) [19] the formation of a phosphate complex (PO_4^{3-}) at wave numbers ranging from 1,000 cm^{-1} – 1,100 cm^{-1} with a V_3 pattern (asymmetric stretching vibration) (1030.29 cm^{-1}), and at wave number 576.30 cm^{-1} with a V_4 pattern (bending asymmetry vibrations are in the pattern (403.60 cm^{-1} and 601.79 cm^{-1}).

3.3 X-Ray Diffraction (XRD) of Calcium Phosphate Derivatives of Milkfish Bone Flour (*Chanos-chanos* Forsskal)

The profile of the result of the XRD (X-ray Diffraction) analysis was adjusted to the data of JC PDS (Joint Committee on Powder Diffraction Standard) characterization was carried out to determine the phase formed in the sample, the degree of sample crystallinity and the sample crystal size. The compound that formed in the sample was calcium phosphate ($Ca_4P_2O_9$) in crystalline and amorphous form. The crystalline phase that formed and dominated the sample was hydroxylapatite, $Ca_5(PO_4)_3(OH)$. The result of XRD analysis showed that the milkfish bone meal sample was identified to contain hydroxylapatite , tricalcium phosphate, and dicalcium phosphate, where the most abundant hydroxyalapatite compounds form complexes with the formula hydroxyapatite compound [$Ca_5(PO_4)_3(OH)$] The presence of an amorphous phase in milkfish bones indicates that the formation of stable crystals of apatite is preceded by the formation of non-apatite crystals [25,26]. The crystallinity of calcium phosphate derivatives in the treatment of pure papain enzyme concentration was 8% with a heating time of 10 hours µm hydroxylapatite was 74.53%, µm tricalcium phosphate was 11.94%, µm dicalcium phosphate was 13.53% (Fig. 2).

3.4 Morphology of Calcium Phosphate Derivatives of Milkfish Bone Meal (*Chanos-chanos* Forsskal)

Morphological analysis using SEM on samples treated with 8% pure papain enzyme concentration with 10 hours of heating time at 100 times magnification showed that the sample surface morphology had the size of calcium phosphate granules of milkfish bone meal which were relatively small flakes fine, dense, slightly dark in color, these results supported by the result of analysis using XRD, which showed a degree of crystallinity of 73.04% and a crystal size of 4.717 µm (Fig. 3).

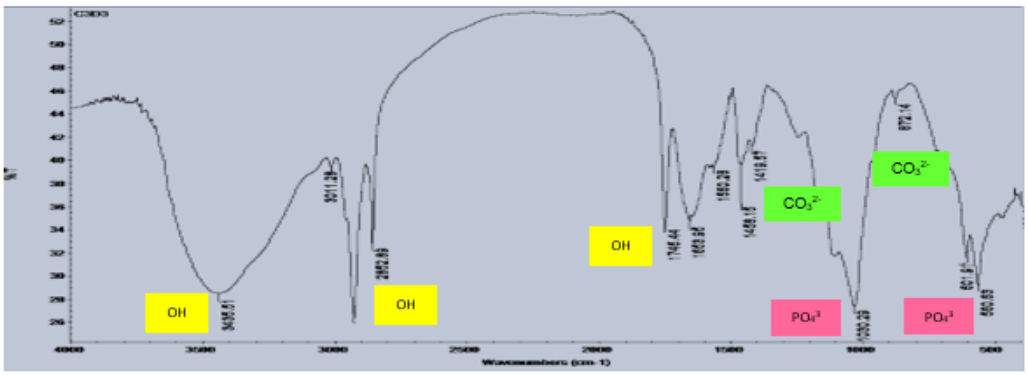

Fig. 1. Results of functional group analysis of calcium phosphate in milkfish bone meal (*Chanos-chanos* Forsskal) treated with 8% pure papain enzyme concentration with a heating time of 60 °C for 10 hours

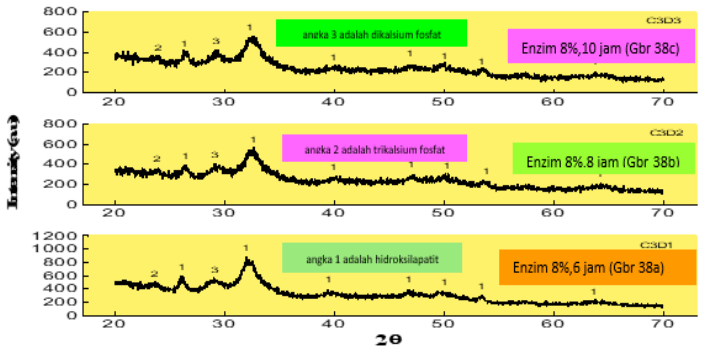

Fig. 2. X-Ray Diffraction patterns in the treatment of pure papain enzyme concentrations with variations in heating time of 60°C, where the number one is µm hydroxylapatite, the number two is µm tricalcium phosphate, and the number three is µm dicalcium phosphate

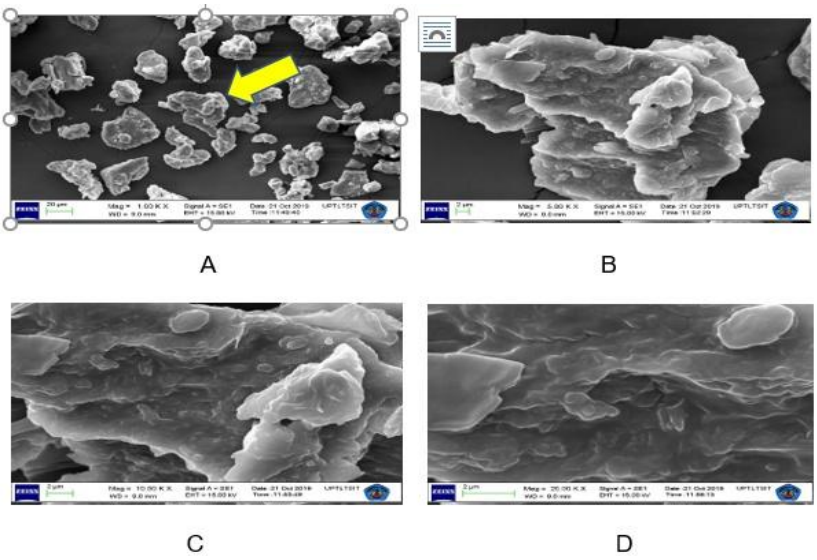

Fig. 3. Results of morphological analysis of milkfish bone meal (*chanos-chanos* forsskal) with old treatment concentration of pure papain enzyme 8% with heating time 60 °c for 10 hours (A = magnification 100 x , B = magnification 500 x. C = magnification 10,000 x and D = magnification 20,000 x)

4. CONCLUSION

The result of the analysis of the treatment of pure papain enzyme concentration of 8% with a heating time of 10 hours gave the best result. Milkfish bone in this study obtained 3.24% moisture, 83.28% ash, 10.28% protein, 3.20% fat, 49.30% calcium, 29.17% phosphorus, 7.16% yield, degree of whiteness 88.68%, FTIR analysis has a phosphate absorption band spectra (PO_4^{3-}) at a frequency or wave number of 403.60 cm^{-1} with an intensity of 36.568% is $V_3PO_4^{3-}$ (asymmetric stretching vibration), 560.63 cm^{-1} (29.024%), 601.91 cm^{-1} (31.551%). XRD analysis identified hydroxylapatite, tricalcium phosphate and dicalcium phosphate content, the crystallinity of calcium phosphate derivatives was 74.53% µm hydroxylapatite, 11.94% µm tricalcium phosphate, 13.53% µm dicalcium phosphate and the morphology of calcium phosphate derivatives using XRD, showed a degree of crystallinity of 73.04% and a crystal size of 4.717 µm.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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