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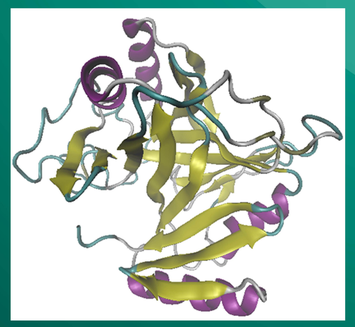

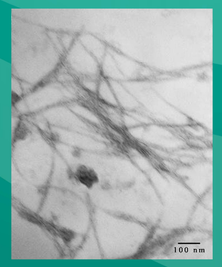

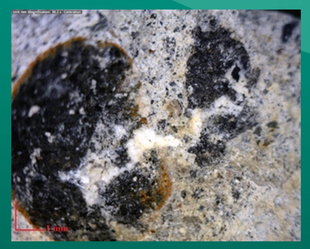

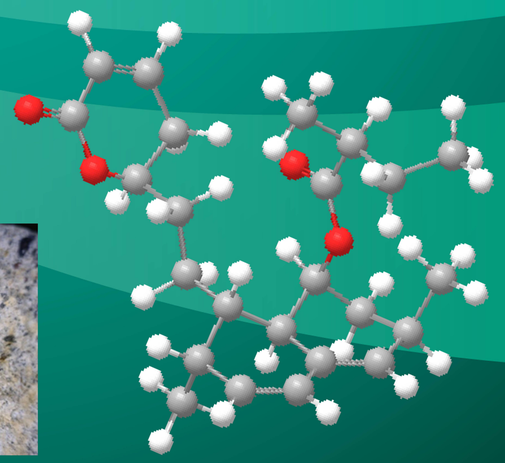

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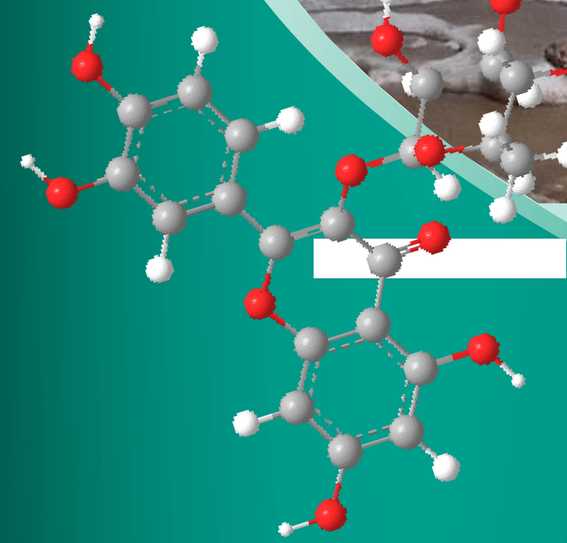
Indonesian Journal on Applied Chemistry



Indonesian Journal of Applied Chemistry | Vol. : 21 | No. : 1 | pp. : 1-55 | Serpong, June 2019 | e-ISSN : 2527-7669

J. Kim. Terap. Indones. e-ISSN: 2527–7669
Vol. 21, No. 1, June 2019

Jurnal Kimia Terapan Indonesia

Indonesian Journal of Applied Chemistry

EDITORIAL BOARD

Jurnal Kimia Terapan Indonesia
Indonesian Journal of Applied Chemistry
Published by Research Center for Chemistry, Indonesian Institute of Sciences

Secretariat:
P2 Kimia LIPI, Gd.452 Building, 1st Floor
Kawasan PUSPIPTEK Serpong, Tangerang Selatan, Indonesia,
Postal Code: 15314 Telp +62 21 7560929, Fax +62 21 7560549
Homepages: http://inajac.lipi.go.id, Email: inajac@mail.lipi.go.id
e-ISSN: 2527-7669

Jurnal Kimia Terapan Indonesia

Indonesian Journal of Applied Chemistry

PREFACE

Preface Greetings to all of honorary Indonesian Journal of Applied Chemistry (J. Kim. Terap. Indones.) readers!

Deepest gratitude from all of us should be conveyed sincerely to Allah The Highest and Most Merciful whom allowed and blessed the publishing of Indonesian Journal of Applied Chemistry (J. Kim. Terap. Indones.) Volume 21 No.1 June 2019. Whom also allowed and blessed this journal to reach all of readers hands, through a systemic supervision by our respectful reviewers.

Indonesian Journal of Applied Chemistry (J. Kim. Terap. Indones.) is a product of our continuous improvements from the originally called Jurnal Kimia Terapan Indonesia (JKTI). In a way to transform into International Journal, several quality improvements had been conducted including (i) all articles were written in English, (ii) close involvement of International reviewers, (iii) adopting Open Access Journal System in submitting, reviewing and publishing articles, (iv) using compact article template and (v) having a registered e-ISSN to accommodate reaccreditation and global indexing process.

In this edition, several astonishing articles from well-known Indonesian Institutions were served. They are: (i) Preliminary Economic Study of ZnO Nanoparticles Production by Sol-Gel Synthesis Method, (ii) Self-Healing Concrete Using Bacteria Calcification from Karst Cave Environment, (iii) Conversion of Hemicellulosa from Kenaf Core Fiber to Xylose through Dilute Sulfuric Acid Hydrolysis, (iv) Phenol Biodegradation and Catechol 2,3-Dioxygenase Gene Sequencing of *Bacillus cereus* IrC2 Isolated from Rungkut Indonesia, (v) The Effect of Acid Hydrolysis Treatment on the Production of Nanocellulose Based on Oil Palm Empty Fruit Bunches, (vi) Characterization of Protease Crude Extract from Indigenous Lastic Acid Bacteria and the Protein Degradation Capacity in Local Tuber and Cereal Paste Flour, (vii) Use of Mg-Al/hydrotalcite Catalyst in Biodesel Production from Avocado Seed Oils: A Preliminary Study.

By this letter, editors would be honored to express our gratitude and appreciation to all reviewers for all of their hard work and kind cooperation in reviewing and improving the quality of articles in this journal. And for sure, to all authors in this journal, your trust and willingness in publishing your articles in this journal are highly appreciated.

As the closing remarks, editors always invite all researcher to publish their articles in Indonesian Journal of Chemistry (J. Kim. Terap. Indones.) in order to spread out their findings and knowledge in applied chemistry related field. To be heard and known by all researcher around the world in the same field. Article manuscripts can be submitted from our official website http://kimia.lipi.go.id/inajac/index.php

Your critics and suggestions were very welcome in the way to support our continuous improvement efforts. Our biggest wish is this journal will convey benefits to all honored readers and contribute to chemistry knowledge. Have a good read!

Serpong, 30 Juni 2019

Editor in Chief

J. Kim. Terap. Indones.
Vol. 21, No. 1, June 2019
e-ISSN: 2527–7669

Jurnal Kimia Terapan Indonesia

Indonesian Journal of Applied Chemistry

TABLE OF CONTENT

 e-ISSN: 2527–7669

ABSTRACT

Preliminary Economic Study on the Production of ZnO Nanoparticles Using a Sol-Gel Synthesis Method

Fikri Aziz Shalahuddin[1*], **Sera Serinda Almekahdinah**[1], **Asep Bayu Dani Nandiyanto**[1]

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J. Kim. Terap. Indones.

Vol. 21, No. 1, June 2019,

pages: 1-6

Keywords:

Zinc Oxide Nanoparticle, Preliminary Economic evaluation, Feasibility study.

Abstract

The economic evaluation is one of the key points in building chemical industries. This paper presented a preliminary economic evaluation of the large-scale production of zinc oxide (ZnO) nanoparticles using the sol-gel method, which is very useful for helping decision whether the fabrication of this material profitable or not. Particularly, the study was done by changing the cost of raw material, which was compared to several economic parameters such as GPM, PBP, and CNPV. The result showed that the project was profitable by increasing raw material cost below 100% from the estimated raw material cost, informing the fact for the prospective fabrication for fulfilling the demand of ZnO nanoparticles..

 e-ISSN: 2527–7669

Self-Healing Concrete Using Bacteria Calcification from Karst Cave Environment

Ananto Nugroho [1, 2] *, Agung Sumarno [1, 3], Luna Nurdianti Ngeljaratan [1], Deni Zulfiana [1], Ni Putu Ratna Ayu Krishanti [1], Triastuti1 [1], Eko Widodo [1]

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J. Kim. Terap. Indones.
Vol. 21, No. 1, June 2019,
pages: 7-13

Abstract

Karst regions in Indonesia have the uniqueness of the landscape and biodiversity. The karst is formed by the dissolution of rocks and the precipitation of mineral. In the cave, there are ornaments of stalactite and stalagmite which are formed by the process of mineral precipitation. We have isolated, screened, and identified the soil bacterium from the cave environment *(Lysinibacillus macroides)*. These bacteria are able to precipitate calcium carbonate and can be developed as a self-healing agent concrete. We investigated the proportions and the properties of mixtures concrete containing lightweight aggregate and volcanic ash impregnated with bacteria. A comparison study was made by concrete cylinders subjected to compressive strength tests with and without the bacteria. It found that the strength of concrete with bacteria decreased by less than 10.56% for 28 days of cured specimens. This study showed that the effects of bacteria on the strength of concrete are not considerable. However, these bacteria are effective to repair in the microcrack less than 0.3 mm.

Keywords:

Self-healing, concrete, bacteria, volcanic ash

 e-ISSN: 2527–7669

Conversion of Hemicellulose from Kenaf Core Fiber to Xylose through Dilute Sulfuric Acid Hydrolysis

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J. Kim. Terap. Indones.

Vol. 21, No. 1, June 2019,

pages: 14-22

Keywords:

kenaf core waste, hemicellulose, xylose, hydrolysis, sulfuric acid

Abstract

Kenaf (*Hibiscus cannabinus*) is a lignocellulosic plant that is usually utilized as a fiber source for sack production. The core from kenaf fiber has not been utilized yet in Indonesia, therefore it is still considered as a waste. Hemicellulose from kenaf core can be hydrolyzed to xylose through dilute sulfuric acid hydrolysis in high temperature. Hydrolysis in this study was done by using autoclave at 121℃ and 10% (m/v) biomass: acid ratio for 15 and 45 minutes with a variation on acid concentration (2%, 4%, and 6% v/v). Xylose concentration in the hydrolyzate tends to increase with higher acid concentration and longer heating time. 6% (v/v) sulfuric acid concentration and 45 minutes of heating time produce the highest xylose concentration (20.53 gr/L) and yield (86.50%).

 e-ISSN: 2527–7669

Phenol Biodegradation and Catechol 2,3-Dioxygenase Gene Sequencing of *Bacillus cereus* IrC2 isolated from Rungkut Indonesia

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J. Kim. Terap. Indones.

Vol. 21, No. 1, June 2019,

pages: 23-30

Keywords:

Bacillus cereus IrC2, catechol 2,3-dioxygenase gene, phenol degradation

Abstract

Phenol is toxic organic compounds that harmful to humans, mammals, and disrupt the aquatic environment, especially higher-organisms in a fresh-water environment. The biodegradation method using bacteria to degrade hazardous chemical and detoxify wastewater is an effective and efficient method. Bacillus cereus IrC2 isolated from sludge in an industrial wastewater treatment plant in Rungkut – East Java, Indonesia has been examined for the ability to degrade phenols in a minimal salt medium. Bacillus cereus IrC2 is Gram-positive bacterium. This bacterium is motile, rod-shaped and its nucleotides sequence of 16S rRNA gene has been sequenced and can be accessed in GenBank with accession number MK511840. Bacillus cereus IrC2 is capable to use phenol up to 400 ppm as the sole carbon source to grow for 48 hours incubation. Phenol degrades 96% from initial concentration. Degradation of phenol was calculated by colorimetric method using 4-aminoantipyrine reagent and confirmed by GC-MS analysis. The aerobic degradation of phenol pathways consists of three steps; in the first step, two hydroxyl groups are inserted into aromatic ring and catalyzed by mono or dioxygenase to produce dihydroxy aromatic compounds which are mostly catechols. Catechol enters the next step of aromatic ring cleavage catalyzed by catechol 1,2-dioxygenase and/or catechol 2,3-dioxygenase. The catechol 2,3-dioxygenase gene of Bacillus cereus IrC2 has been amplified by PCR and cloned into pTA2 vector. The cloned plasmid (pTA2-catE) was transformed into E. coli DH5α and selected blue-white colonies. The insert sequence was determined by Sanger deoxy sequencing method. The catechol 2,3-dioxygenase gene nucleotides sequence of Bacillus cereus IrC2 was submitted into GenBank with accession number MK561609.

J. Kim. Terap. Indones.
Vol. 21, No. 1, June 2019

e-ISSN: 2527–7669

The Effect of Acid Hydrolysis Treatment on The Production of Nanocellulose Based on Oil Palm Empty Fruit Bunches

Athanasia Amanda Septevani[1,*], Dian Burhani[1], Yulianti Sampora[1], Yenni Apriliany Devy[1], Gita Novi Ariani[1], Sudirman[2], Dewi Sondari[3], Khairatun Najwa Mohd Amin[4]

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J. Kim. Terap. Indones.

Vol. 19, No. 1, June 2019,

pages: 31-37

Keywords:

Oil Palm Empty Fruit Bunches, Nanocellulose, Acid Hydrolysis, Cellulose Nanocrystal, Single Crystal

Abstract

Nanocellulose has been known as promising reinforcing material in various polymer based product resulted in remarkable improvement in mechanical and thermal properties. Hence, studies to date have developed and explored various sources of biomass to produce nanocellulose. This study aims to synthesize and fully characterize nanocellulose obtained from abundantly available oil palm empty fruit bunches via two different methods which are strong (H_2SO_4) and mild acid (H_3PO_4) hydrolysis at 50 $^{\circ}$C for 3.5 hours. Based on the morphological study using Transmission Electron Microscopy, rod-like nanocellulose was obtained using strong acid hydrolysis while mild acid hydrolysis produced long filament shape. X-Ray diffraction analysis showed that the degree crystallinity of nanocellulose produced from strong acid hydrolysis was higher, which is 96% than that of mild acid hydrolysis recorded with 86%. While the sulphuric acid hydrolysis usually produces lower thermal stability than that of other types acid hydrolysis, surprisingly, in this study, the thermal stability of nanocellulose from strong acid hydrolysis was relatively similar to mild acid hydrolysis due to the formation of single crystal structure affording unique characteristic of the obtained nanocellulose.

 e-ISSN: 2527–7669

Characterization of Protease Crude Extract from Indigenous Lastic Acid Bacteria and the Protein Degradation Capacity in Local Tuber and Cereal Paste Flour

Tatik Khusnati, Nanda Sabbaha Nur Kasfillah, Vilya Syafriana, Resti Sofia Zahara, Padmono Citroreksoko, Sulistiani and Trisanti Anindyawati

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J. Kim. Terap. Indones.

Vol. 21, No. 1, June 2019,

pages: 38-44

Keywords:

Degradation protein, protease, L. plantarum B110, L. satsumensis EN 38-32, paste flour of local tuber and cereal

Abstract

Protease hidrolyzed protein in flour in order to more digest by human ulcer. *Lactobacillus plantarum* B110 and *Lactobacillus satsumensis* are indigenous lactic acid bacteria that produce protease. The objective of this research is to characterization of protease crude extract from indigenous lactic acid bacteria and the protein degradation capacity in local tuber and cereal paste flour. Tuber and cereal flour used were purple sweet potato (*Dioscorea alata*), cassava (*Manihot esculenta*), rice (*Oryza sativa*), corn (*Zea mays*) and wheat (*Triticum*) as comparison. Protease activity was tested by Horikoshi method (1971) and protein degradation was by formol titration. Research results showed that optimum activities and stabilities of *Lactobacillus plantarum* B110 were at pH: 7.5, 45^{o}C and pH:5.0-8.0, 35-50^{o}C, while that *L. satsumensis* EN 38-32 were at pH: 7.0, 40^{o}C and pH:6.0-8.0, 20-45^{o}C. Increases in protein degradation capacity of the paste flour additional proteases crude extract from *L. plantarum* B110 were 0.0838% (purple sweat potato), 1.3299% (cassava), 0.5834% (corn), 0.7499% (rice) and 1.5551% (wheat as comparison); while that *L. satsumensis* EN 38-32 were 0.20% (purple sweet potato), 0.32% (cassava), 0.87% (corn), 1.17% (rice). Based on increases in protein degradation capacity, protease crude extract from *L. plantarum* B110 and *L. satsumensis* EN 38-32 were sequently better to hidrolyze protein of cassava and rice paste flour than that other tuber and cereal.

 e-ISSN: 2527–7669

Use of Mg-Al/hydrotalcite Catalyst in Biodiesel Production from Avocado Seed Oils: A Preliminary Study

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J. Kim. Terap. Indones.

Vol. 21, No. 1, June 2019,

pages: 45-54

Keywords:

Avocado seed oils, biodiesel, Mg-Al/hydrotalcite catalyst, renewable and green fuels

Abstract

Biodiesel production from avocado seed oils has been carried out using the heterogeneous catalyst of Mg-Al/hydrotalcite. Transesterification process was conducted by varying temperature reaction and oil-methanol molar ratio. The reaction temperature was 30, 40, 50, and 60°C, whereas the oil-methanol molar ratio was 1:3, 1:6, 1:9, and 1:12, respectively. The as-synthesized Mg-Al/hydrotalcite catalyst was characterized using X-ray diffraction and FTIR. Meanwhile, the biodiesel was analyzed their density, viscosity, water content, and ^{1}H-NMR analysis. The results showed that optimum condition in biodiesel production was the oil-methanol molar ratio of 1:6 at a reaction temperature of 60°C for 60 minutes and catalyst quantity of 2% yielding biodiesel conversion percentage was approximately 15.90%. However, these preliminary findings showed that Mg-Al/hydrotalcite was able to convert the avocado seed oils into biodiesel even if still need further analysis and research so that produces a higher percentage of biodiesel conversion.

J.Kim.Terap.Indones. 21(1),
pp. 38 – 44, June 2019

e-ISSN:2527-7669

Characterization of Protease Crude Extract from Indigenous Lactic Acid Bacteria and the Protein Degradation Capacity in Local Tuber and Cereal Paste Flour

Tatik Khusnati[1*)], Nanda Sabbaha Nur Kasfillah[2], Vilya Syafriana[2], Resti Sofia Zahara[3], Padmono Citroreksoko[3], Sulistiani[1] and Trisanti Anindyawati[4]

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ARTICLE INFO

Article history:

Received date :1 April 2019

Revised date : 19 May 2019

Accepted date:19 June 2019

Available online at : http://inajac.lipi.go.id/

Keywords:

degradation protein, protease, L. plantarum B110, L. satsumensis EN 38-32, paste flour of local tuber and cereal

Abstract

Protease hidrolyzed protein in flour in order to more digest by human ulcer. *Lactobacillus plantarum* B110 and *Lactobacillus satsumensis* are indigenous lactic acid bacteria that produce protease. The objective of this research is to characterization of protease crude extract from indigenous lactic acid bacteria and the protein degradation capacity in local tuber and cereal paste flour. Tuber and cereal flour used were purple sweet potato (*Dioscorea alata*), cassava (*Manihot esculenta*), rice (*Oryza sativa*), corn (*Zea mays*) and wheat (*Triticum*) as comparison. Protease activity was tested by Horikoshi method (1971) and protein degradation was by formol titration. Research results showed that optimum activities and stabilities of *Lactobacillus plantarum* B110 were at pH: 7.5, 45°C and pH:5.0-8.0, 35-50°C, while that *L. satsumensis* EN 38-32 were at pH: 7.0, 40°C and pH:6.0-8.0, 20-45°C. Increases in protein degradation capacity of the paste flour additional proteases crude extract from *L. plantarum* B110 were 0.0838% (purple sweat potato), 1.3299% (cassava), 0.5834% (corn), 0.7499% (rice) and 1.5551% (wheat as comparison); while that *L. satsumensis* EN 38-32 were 0.20% (purple sweet potato), 0.32% (cassava), 0.87% (corn), 1.17% (rice). Based on increases in protein degradation capacity, protease crude extract from *L. plantarum* B110 and *L. satsumensis* EN 38-32 were sequently better to hidrolyze protein of cassava and rice paste flour than that other tuber and cereal.

I. INTRODUCTION

Protease can be used to hidrolyze protein in tuber and cereal flour to peptide in order to more digest by human ulcer. Lactic acid bacteria, such as: *Lactobacillus plantarum* S31 and *Lactobacillus delbrueckii* subsp. *lactis* CRL 581produced protease (Budiarto *et al*., 2016; Villegas and Brown, 2014)

The protease activities in hidrolyzing protein in tuber and cereal flour were affected by the type of the bacteria producing protease (Sawant and Nagendran, 2014; Thiele *et al*., 2002). Furthermore, the different type of tuber and cereal flour caused the different concentration of protein hidrolyzed by microbial proteases in those two flour (Li *et al*., 2012; Adeniji, 2013).

The protein in tuber and cereal flour were hidrolyzed by the microbial protease activities to simple compounds of peptides (Endo and Okada, 2005; Ganzle *et al*., 2008). The peptides produced depend on the type of tuber and cereal flour used.

e-ISSN:2527-7669

Some species of Lactic acid bacteria (LAB) had ability to produce protease, such as: *Lactococcus lactis, Lactococcus cremoris* and *Lactococcus garviae* (Adi and Guessas, 2016), *Lactococcus lactis* 1598, *Streptococcus thermophillus* t3D1, *Lactobacillus lactis* 1043 and *L. delbrueckii* subsp. *bulgaricus* b38, b122 and b24 (Atanasova *et al*., 2014). However, LAB species of *Lactobacillus plantarum* B110 and *Lactobacillus satsumensis* EN38-32 producing protease which have potency to degrade protein in local tuber and cereal flour haven't been known yet.

The objective of this research was to characterization of protease crude extract from indigenous lactic acid bacteria and the protein degradation capacity in local tuber and cereal paste flour

2. EXPERIMENTAL

2.1. Production of protease (Tennalli *et al*., 2012 modified)

The 2 % inoculum culture was poured into 50 mL media of nutrient broth with addition of 1% casein and it was incubated at temperature 37°C for 48 hours. The production media was then centrifuged at 3500 rpm for 15 minutes. Supernatant found was protease crude extract.

2.2. Optimization of protease activity in various pH (Tennalli *et al*., 2012 modified)

Optimization of protease activity was conducted in pH 4.5. 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. The activity test was conducted at temperature: 37ºC and incubation time for 10 minutes. The highest protease activity in certain pH was stated as optimum protease activity.

2.3. Optimization of protease activity in various temperatures (Tennalli *et al*., 2012 modified)

Optimization of protease activity was conducted in various temperatures: 20, 25, 30, 35, 40, 45, and 50ºC. The activity test was conducted in optimum pH, with incubation time for 10 minutes. The highest protease activity in certain temperature was stated as optimum protease activity.

2.4. Stability of protease in various pH (Eijsink *et al*., 2005; Moran *et al.*, 2012)

Stability of protease in various pH were conducted by measuring protease relative activities in pH 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0. The activity test was conducted in optimum temperature with incubation time for 60 minutes. Protease relative activities with values: ≥ 50% showed that those activities were in stable condition.

2.5. Stability of protease in various temperatures (Eijsink *et al*., 2005; Moran *et al.*, 2012)

Stability of protease in various temperatures was conducted by measuring protease relative activities in temperatures: 20, 25, 30, 35, 40, 45 and 50ºC. The activity test was conducted in optimum pH with incubation time for 60 minutes. Protease relative activities with value: ≥ 50% showed that those activities were in stable condition.

2.6. Production of tuber and cereal paste flour additional protease

Tuber and cereal flour used were purple sweet potato (*Dioscorea alata*), cassava (*Manihot esculenta*), rice (*Oryza sativa*), corn (*Zea mays*) and wheat (*Triticum*) as comparison. One (1) gram for each flour from wheat, purple sweet potato, cassava, rice, and corn was soluted in 10 mL aquadest, heated at temperature 70ºC with agitation. Heating was conducted up to paste flour formed. Each supernatant (1 unit crude protease) from *L. plantarum* B110 and *L. satsumensis* EN 38-32) was added into 10 mL paste flour and incubated in *shaker incubator* with 100 rpm, at temperature 40ºC, for 24 hours. Paste flour treated was then analyzed its protein degradation.

e-ISSN:2527-7669

2.7. Protease activity (Horikoshi modified, 1971)

Protease activity test was conducted by method of Horikoshi (1971) modified. The 0.2 mL protease was poured into reaction tube, added 0.4 mL 2% casein and 0.4 mL buffer phosphate 0,05 M pH 8. The mix was incubated at temperature 37 ºC for 10 minutes, added 1 mL 20% TCA and homogenized. Incubation was continued at temperature 37ºC for 10 minutes, and solution was centrifuged by rotation 3500 rpm for 5 minutes. Controle was made. One unit protease was defined as the amount of mL protease needed to produce 1 µmol tirosin every minute with casein as substrate.

2.8. Protein degradation (U.S.P., 1989 in Nilsang *et al*., 2005).

Protein degradation was tested by formol titration. Sample of 10 mL treated paste flour was added phenolphthalein, and neutralized by NaOH 0,1 N solution. The 10 mL of 37 % Formaldehyde was added into the solution, titrated by standard solution of NaOH 0,1 N up to the colur change to pink. The protein degradation was then calculated as % Nitrogen x Fc (Conversion Factor).

3. RESULTS AND DISCUSSION

Research results showed that the values of protease activities of *Lactobacillus plantarum* B110 in various pH 4.5-8.0 were in the range 0.4488 – 0.8995 U/mL (Table 1) and in various temperatures: 20-50ºC were 0.4415 - 1.0357 U/mL) (Table 2) with relative activities at pH 4.5-8.0 were in the range 47.56 – 100% (Table 3) and at temperatures: 20-50ºC were 40.57 - 100% (Table 4).

The optimum protease activity of *Lactobacillus plantarum* B110 was reached at pH: 7.5 (0,8995 U/mL)(Table 1) and temperature: 45ºC (1.0357 U/mL) (Table 2) with stabilities at relative activity ≥ 50% were at pH 5.0-8.0 (Table 3) and temperatures: 35-50ºC (Table 4).

Table 1. Protease Activity from *L. plantarum* B110 in Various pH

pH	Protease Activity (U/mL)
4.5	0.4488 [a]
5.0	0.4966 [a]
5.5	0.5408 [b]
6.0	0.7432 [c]
6.5	0.7542 [c]
7.0	0.8848 [d]
7.5	0.8995 [d*]
8.0	0.6567 [bc]

Table 2. Protease Activity from *L.plantarum* B110 in Various Temperatures

Temperature	Protease Activity (U/mL)
20	0.4415 [a]
25	0.4893 [a]
30	0.5545 [a]
35	0.5868 [a]
40	0.6070 [a]
45	1.0357 [c*]
50	0.7910 [b]

Table 3. Protease Relative Activity from *L. plantarum* B110 in Various pH

pH	Protease Activity (U/mL)	Protease Relative Activity (%)
4.5	0,4230	47.56
5.0	0,4893	55.01
5.5	0,5390	60.60
6.0	0,7082	79.62
6.5	0,7229	81.27
7.0	0,8701	97.82
7.5	0,8895	100
8.0	0,6457	72.59

Table 4. Protease Relative Activity from *L. plantarum* B110 in Various Temperature

Temperature	Protease Activity (U/mL)	Protease Relative Activity (%)
20	0.4157	40.57
25	0.4304	42.01
30	0.4525	44.16
35	0.6052	59.07
40	0.6144	59.97
45	1.0246	100
50	0.7744	75.58

e-ISSN:2527-7669

The values of protease activities of *L. satsumensis* EN 38-32 in various of pH 4.5-8.0 were in the range 0.2032 – 0.4574 U/mL (Table 5) and in various temperatures of 20-50ºC were 0.2385 – 0.7214 U/mL) (Table 6) with relative activities at pH 4.5-8.0 were in the range 34.10 - 100% (Table 7) and at temperatures: 20-50ºC were 42.92 - 100% (Table 8).

Table 5. Protease Activity from *L. satsumensis* EN 38-32 in Various pH

pH	Protease Activity (U/mL)
4.5	0.2032 [a]
5.0	0.2482 [a]
5.5	0.3383 [b]
6.0	0.3834 [d]
6.5	0.3737 [cd]
7.0	0.4574 [e*]
7.5	0.3480 [bc]
8.0	0.3222 [b]

Table 6. Protease Activity from *L. satsumensis* EN 38-32 in Various Temperatures

Temperature	Protease Activity (U/mL)
20	0.2385 [a]
25	0.3222 [b]
30	0.4896 [c]
35	0.5637 [c]
40	0.7214 [d*]
45	0.3866 [b]
50	0.3576 [b]

Table 7. Protease Relative from *L. satsumensis* EN 38-32 in Various pH at 60 minutes incubation tim

pH	Protease Activity (U/mL)	Protease Relative Activity (%)
4.5	0.0430	34.10
5.0	0.0478	37.90
5.5	0.0575	45.60
6.0	0.0773	61.30
6.5	0.0870	68.99
7.0	0.1261	100.00
7.5	0.0795	63.05
8.0	0.0816	64.71

Table 8. Protease Relative Activity from L. satsumensis EN 38-32 in Various Temperature at 60 Minutes Incubation Time

Temperature (ºC)	Protease Activity (U/mL)	Protease Relative Activity (%)
20	0.0510	54.31
25	0.0596	63.47
30	0.0666	70.93
35	0.0778	82.85
40	0.0939	100.00
45	0.0687	73.16
50	0.0403	42.92

The optimum protease activity of *L. satsumensis* EN 38-32 was reached at pH: 7.0 (0.4574 U/mL) (Table 5) and temperature: 40ºC (0.7214 U/mL)(Table 6), with stabilities at relative activities ≥ 50% were at pH 6.0 - 8.0 (Table 7) and temperatures: 20 - 45 ºC (Table 8).

The different optimum activities and stabilities between protease from *Lactobacillus plantarum* B110 and *L. satsumensis* EN 38-32 were due to the different species of both lactic acid bacteria producing protease. It has been reported that the different species of bacteria may have resulted in the different characteristics of protease produced (Eijsink, 2005; Hayek and Ibrahim, 2013, Naidu, 2011, Sulthoniyah *et al*., 2015). The optimum temperature and pH of protease *B. subtilis* were in the range from 40ºC to 50ºC and pH 8, respectively (Naidu, 2011), while that *Lactococcus* species were 37ºC and pH 7.2 (Addi and Guessas, 2016).

The protein degradation concentration of the tuber and cereal paste flour with addition of *L. plantarum* B110 protease crude extract were 1.2570% (purple sweat potato), 1.8077 (cassava), 1.9305% (rice), 1.7506 (corn), and 1.5551% (wheat as comparison)(Table 9).

The increases of protein degradation concentration of the tuber and cereal paste flour additional *L. plantarum* B110 crude protease were 0.0838% (purple sweat potato), 1.3299% (cassava), 0.7499% (rice), 0.5834% (corn), and 1.5551% (wheat as comparison)(Table 9).

The increasing of protein degradation concentration from the treated cassava paste

e-ISSN:2527-7669

flour was highest than that the other local tuber and cereal paste flour (Table 9).

Table 9. Protein Degradation Concentrations of Local Tuber and Cereal Paste Flour With and Without *L. plantarum* B110 EN 38-32 Protease

Tuber Paste Flour	Protein Degradation Concentration (%)		Increase of Protein Degradation (%)
	Control*	Sample**	
Wheat	1.2771	2.8322	1.5551
Purple Sweet Potato	1.1732	1.2570	0.0838
Cassava	0.4778	1.8077	1.3299*
Rice	1.1806	1.9305	0.7499
Corn	1.1672	1.7506	0.5834

Notes :
* Paste flour without addition of *L. plantarum* B110 protease
** Paste flour with addition of *L. plantarum* B110 protease

The protein degradation concentration of the tuber and cereal paste flour additional *L. satsumensis* EN 38-32 crude protease were 0.7300 (purple sweat potato), 0.7900 (cassava), 2.1900% (rice), 1.9600 (corn), and 1.5551% (wheat as comparison)(Table 9).

The increases of protein degradation concentration of the tuber and cereal paste flour additional *L. satsumensis* EN 38-32 crude protease were 0.20% (purple sweet potato), 0.32% (cassava), 0.87% (corn), 1.17% (rice) and 1.83% (wheat as comparison)(Table 10)

The concentration increase of protein degradation of the treated rice paste flour was highest than that the other local tuber and cereal paste flour (Table 10)

The different increases of protein degradation concentration between the tuber (purple sweet potato and cassava) and cereal (corn and rice) paste flour with wheat paste flour (as comparison) additional crude proteases from *L. plantarum* B110 and *L. satsumensis* EN 38-32 were due to the the different protease activities from those two lactic acid bacteria in protein hidrolysis of those two flour. It has been reported that the protease activities from the different bacteria species may have resulted in the different hidrolysis of flour protein (Adeniji, 2013; Gupta *et al*., 2002; Sawant and Nagendran, 2014). The LAB of *Lactococcus lactis* 1598, *Streptococcus thermophilus* t3D1, *Lactobacillus lactis* 1043 and *L. delbrueckii* subsp. *bulgaricus* b38, b122 and b 24 had high proteolytic activity in the formation of peptides with molecular weight between 5 and 10 kDa (Atanasova *et al*., 2014)

Table 10. Protein Degradation Concentrations of Local Tuber and Cereal Paste Flour With and Without *L. satsumensis* EN 38-32 Protease

Tuber Paste Flour	Protein Degradation Concentration (%)		Increase of Protein Degradation (%)
	Control*	Sample**	
Wheat	1.2771	3.8322	1.5551
Purple Sweet Potato	0.5300	0.7300	0.2000
Cassava	0.4700	0.7900	0.3200
Rice	1.0200	2.1900	1.1700*
Corn	1.0900	1.9600	0.8700

Notes :
* Paste flour without addition of *L. satsumensis* EN 38-32 protease
** Paste flour with addition of *L. satsumensis* EN 38-32 protease

4. CONCLUSION

The characterization of protease crude extract from indigenous lactic acid bacteria and its protein degradation capacity in local tuber and cereal paste flour showed that the optimum activity and stability of proteases from *Lactobacillus plantarum* B110 were at pH: 7.5, 45ºC and pH:5.0-8.0, 35-50ºC, while that *L. satsumensis* EN 38-32 were at pH: 7.0, 40ºC and pH:6.0-8.0, 20-45ºC. The maximum increase of protein degradation concentration of the tuber and cereal paste flour (with wheat paste flour as comparison) additional proteases crude extract from *L. plantarum* B110 was at cassava with value of 1.3299%, while that *L. satsumensis* EN 38-32 was at rice with 1.1700%. Based on the increases of protein degradation concentration, proteases crude extract from *L. plantarum* B110 and *L. satsumensis* EN 38-32 were sequently better to hidrolyze protein of cassava and rice

paste flour than that the other tuber and cereal paste flour.

ACKNOWLEDGEMENT

The authors would like to acknowledge to DIPA Priority Project of Research Center for Biology, Deputy of Natural Sciences, Indonesian Institute of Sciences for giving this research funding, and Desy Septiani S.Si. with Khairrunnisa S.Si for their research assistances in Laboratory

REFERENCES

[1] Adeniji, T. 2013. Review of cassava and wheat flour composite in bread making: prospects for industrial application, *the African Journal of Plant Sciences and Biotechnology* 7(1): 1-8.

[2] Adi, N. and Guessas, B. 2016. Characterization of protease activity of *Lactococcus lactis* species Isolated from raw camel's milk, *Journal of Biological Sciences* 16: 215-220.

[3] Atanasova, J., Moncheva, P. and Ivanova, I. 2014. Proteolytic and antimicrobial activity of lactic acid bacteria grown in goat milk, *Biotechnology & Biotechnological Equipment* 28(6): 1073-1078.

[4] Budiarto, B., Mustopa, A. and Idarmawan, T. 2016. Characterization of partially extracellular proteases from bekasam-isolated *Lactobacillus plantarum* S31 and its application to hydrolyze skimmed-milk with antibacterial property, *International Food Research Journal* 23(1): 340-349.

[5] Endo, A. and Okada, S. 2005. *Lactobacillus satsumensis* sp. nov., isolated from mashes of shochu, a traditional Japanese distilled spirit made from fermented rice and other starchy materials, *Journal of Systematic and Evolutionary Microbiology* 55: 83-85.

[6] Eijsink, V., Gaseidnes, S., Borchert, T. and van den Burg, B. 2005. Directed evolution of enzyme stability, *Biomolecular Engineering* 22: 21-30.

[7] Ganzle, M., Loponen, J. and Gobbetti, M. 2008. Proteolysis in sourdough fermentations: mechanisms and potential for improved bread quality, *Trends in Food Science & Technology* 19: 513-521.

[8] Gupta, R, Beg, O.K., Khan, K and Chauhan, B. 2002. An overview on fermentation, downstream processing and properties of microbial alkaline proteases, *Applied Microbiology and Biotechnology* 60(4): 381-395.

[9] Hayek, S. and Ibrahim, S. 2013. Current limitations and challenges with lactic acid bacteria: a review, *Food and Nutrition Sciences* 4: 73-87.

[10] Horikoshi, K. 1971. Production of alkalin enzymes by alkalophilic microorganisms, *Agr. Biol. Chem.,* 35(9): 1407-1414.

[11] Li, S.,Yang, X., Yang, S., Zhu, M. and Wang, X., 2012. Technology prospecting on enzymes: Application, marketing and engineering, *Computational and Structural Biotechnology Journal*, 2(3): 1-11.

[12] Moran, L., Horton, H., Scrimgeour, K. and Perry, M. 2012, *Principles of Biochemistry*. *5th edition.* New Jersey: Pearson Prentice Hall. Page. 170.

[13] Naidu, K. 2011. Characterization and purification of protease enzyme, *Journal of Applied Pharmaceutical Science,* 1(3): 107-112.

[14] Nilsang, S., Lertsiri, S., Suphantharika, M., and Assavanig, A., 2005. Optimization of enzymatic hydrolysis of fish soluble concentrate by commercial proteases, *Journal of Food Engineering,* 70(4): 571–578.

[15] Sawant, R. and, S. 2014. Protease: an enzyme with multiple industrial applications, *World Journal of Pharmacy and Pharmaceutical Sciences,* 3(6): 568-579.

e-ISSN:2527-7669

[16] Sulthoniyah, S. Hardoko. T.M. and Nursyam, H. 2015. Characterization of extracellular protease lactic acid bacteria from shrimp paste, *Journal of Life Science and Biomedicine*, 5(1): 01-05.

[17] Thiele, C., Ganzle, M. and Vogel, R. 2002. Contribution of sordough *Lactobacilli*, yeast, and cereal enzymes to the generation of amino acids in dough relevant for bread flavor, *Cereal Chem.,*79: 45-51.

[18] Tennalli, G., Udapudi, B. and Naik., P. 2012. Isolation of proteolytic bacteria and characterization of their proteolytic activity, *International Journal of Advances in Engineering. Science, and Technology* (*IJAEST*), 2(3): 185-195.

[19] Villegas, J. and Brown, E. 2014. Characterization of the mature cell surface proteinase of *Lactobacillus delbrueckii* subsp. *lactis* CRL 58, *Applied Microbiology and Biotechnology,* 99 (10): 4277-4286.