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Yeast Hydrolysate Enzymatic (YHE) as Degradation Result Using Pineapple's Bromelain as Preparation Material of Microbiology Culture Media

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YHE was much needed for various purposes in the word and tapioca waste could be an environmental problems, as a potential pollutant in the plant neighborhood. This research aims to produce *Yeast Hydrolysate Enzymatic* (*YHE*) by using tapioca industrial waste which degraded enzimatically with pineapple's bromelain and apply it as preparation material of microbiological culture media. The steps we have done include: (1) preparation of tapioca waste and producing pineapple's bromelain enzyme, (2) producing YHE using tapioca waste by chemical hydrolysis with pineapple's bromelain, (3) characterisizing YHE that consist of: chemical, physical, and microbiological test. YHE in this study is processed in several methods: (1) autolysis, (2) lysis using 10% NaCl and 9% alcohol, (3) bromelain hydrolysis, (4) bromelain hydrolysis and encapsulation with gum, and (5) YHE import (commercial) that is processed by autolysis. Data are analyzed descriptively. The result of this study show that YHE of bromelain hidrolysis and encapsulation with gum has the best physical quality: smelled sour and the powder texture is soft and not sticky; but the result of microbiological test has less quality compare to YHE as the result of bromelain enzyme hidrolysis. Conclusion this research is the comparison of YHE as the result of bromelain hidrolisis has the highest value (N-α amino)/(NT) among other YHE products, that is 0.84. Therefore, YHE utilization as microbiological media, in this case for *Lactobacillus bulgaricus*, is recommended to use the result of bromelain enzymatic hydrolysis. Tapioca waste that is used as growth medium is suggested for YHE is 1.5%.

Keywords: Solid Waste Tapioca, Hydrolysis, Yeast Hydrolysate Enzymatic (YHE).

1. INTRODUCTION

Yeast hydrolysate enzymatic (YHE) is a yeast extract that is enzymatic processed. YHE utilization is very diverse, used to meet the needs of both domestic and abroad, which are as a culture medium (microbiological culture media, cell culture media and tissue culture media), as a basic ingredient of pesticides and bio-fertilizer very beneficial in agriculture. Moreover, microbiology laboratories, biotechnology laboratories and several other labs is very dependent on the yeast extract in the manufacture of culture media that had been imported from another country at a price which is relatively expensive. Technological innovations for promoting new products developments has developed demand in cosmetic and pharmaceutical industry. Hydrolysed yeast extract is used in shampoos, conditioners and skin moisturizers due to conditioning properties. Thus YHE production is an opportunity to meet domestic demand. YHE can be made from yeast, a living organism classified as fungus. To reproduce yeast needed source of energy derived from sugars or carbohydrates. Various natural sources that contain of high carbohydrate content, such as rice, corn, cassava, and even a lot of waste of agricultural products or industrial products that still have a relatively high carbohydrate content. Another example of industrial waste that still keep high carbohydrate and less economic value is tapioca industrial waste.

Identifying biomass of tapioca waste as follows: carbohydrate content is 59.67%, protein is 0.88%, crude fiber is 30%, the water content is 20.33%.[1] Therefore tapioca industrial waste which still contains high carbohydrates is potentially used as a raw material for the production of YHE. it is rich in fiber lignocellulotic, so it is still possible used as raw material for yeast growth. These compounds must be hydrolyzed into the simple particle that can be utilized by yeast directly. The complex carbohydrates in the form of starch can be degraded by enzymes amylase and will produce glucose. Lignocellulotic fibers can be hydrolyzed enzymatically by enzymes lignosellulase.

Yeast that has grown successfully be processed through hydrolysis. Yeast is rich in protein, lipids, RNA, vitamins and minerals. Protein is an essential molecule for all living things. This compound is made up of amino acids linked by a peptide bond and form a variety of complex structures.[5] This protein must be

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degraded into simpler components, that is peptone and various amino acids which can be exploited easily by cells for growth, through enzymatic hydrolysis process. There are several ways to hydrolyze proteins, using acid, base, or may use enzymes or microbes. Chemical and enzymatic hydrolysis were reported most effective. When hydrolysis using enzymes, it will obtain the product known with Yeast Hydrolysate Enzymatic (YHE). When processed by autolysis the product is known as autolyzed yeast.[4] It was reported that the hydrolysis process for the production of yeast extracts could use some enzymes, that is glucanase, protease, nuclease, and deaminase. It depends on the designation of the resulting yeast extracts and raw materials used, one of which is bromelain.

Bromelain is an enzyme that is classified as the protease. The role of proteases in the manufacture of yeast extract is hydrolyzed proteins into simpler compounds that can be directly utilized by living organisms, such as yeast. Many factors affect the enzymatic hydrolysis process, such as enzyme concentration, pH, temperature, time of incubation.[5] These factors will affect the resulting yeast extract products.

There are 3 main methods for the production of YE, that are autolysis, plasmolysis, and hydrolysis.[13] Enzymatic hydrolysis can be facilitated through the initial process that is plasmolysis, a plasma discharge process of the yeast cell wall. Plasmolysis did by adding a solution of salt (NaCl) or organic solvents (ethyl acetate or isopropanol). Moresi et al.[14] state that maximum production of yeast-whey protein extracts was achieved when the endogenous enzymes of ground cells are induced by 5% w/v salt addition. At this stage, the dead cells but do not inactivate the enzyme. The addition of plasmolysis agents can also kill bacterial contaminants. Conditions that suitable for this process is a temperature of 58 °C, pH 5.5 for 40–48 hours. It will provide the acquisition of 65%. If using isopropanol 0.5% (v/v) then used a lower temperature and for 5 hours. Autolysis did digest the contents of the cell. This process is done by enzymes of the yeast's own. This process is influenced by several factors, among which is the temperature. The ideal temperature for autolysis depending on the species/strains of yeast and the desired result 50 °C for 24 hours is ideal for autolysis.[10] Autolysis process can be done by adding the enzyme from the outside, that is the yeast, the lysozyme, and protease (papain) 0.04% (w/w).[6, 10] The use of protease able to increase yeast extract acquisition of approximately 5%. This process is carried out at a temperature of 55 °C, pH 5.5 for 20 hours.[4] Protease is a proteolytic enzyme, an enzyme that can break down or break down proteins. Proteases that used in industrial are most from microorganism cells. Traditionally, the industry uses enzymes from plants and animals, such as papain protease of a plant, ficin, bromelain of pineapple.[8] Pasteurization and sterilization are done in the creation of YHE, that for to kill the vegetative cells of bacteria and inactivate the protease papain and yeast. When this process is done at high temperatures, for example at a temperature of 115 °C for 20 minutes, then will occur reaction Mallard which is produced brownish color.

There for a study is very much needed in order to optimize the quality of products produced. To determine the quality of YHE which has been produced, we need a step product characterization, comparison with existing products, one of commonly used is a product of YHE (import) named commercial product. As a side note, the effectiveness of yeast extract that has been produced by enzymatic also serves as microbiological culture media (mold growth and bacterial growth media). Composition a yeast extract with malt media in consecutive: water content, protein ($N \times 6.25$), Sodium chloride, ash (including NaCl), and Carbohydrates are as follows at 27%, 44%, 10%, 13%, and 6%. Vitamin B (mg per g of extract) are 20–70 Thiamine, Riboflavin 55–100, 12–16 Pyridoxine, Niacin 250–700. Amino acid (out of total protein ($N \times 6.25$) after hydrolysis is as follows: Isoleucine (4.7%), Leucine (7.0%), Methionine (1.5%), Phenylalanine (3.8%), Threonine (0.5%), tryptophan (1.7%), Valine (6.0%), Alanine (7.2%), arginine (1.7%), Cystine (10.2%), Glutamic (11.5%), Glycine (5.5%), histidine (2.3%), Proline (5.0%), Serine (4.5%), Tyrosine (3.0%).[4] By considering the composition the chemical, then YHE lets applied as a culture medium microbiological.

Based on the above background has been conducted research that aims to produce *Yeast Hydrolysate Enzymatic* (*YHE*) by using tapioca industrial waste which degraded enzimatically with pineapple's bromelain and apply it as preparation material of microbiological culture media. In this research using tapioca waste for produce yeast.

2. METHOD OF THE RESEARCH

2.1. Preparation of Materials

Preparation include: preparation of, baker's yeast that contain yeast, and bromelain enzyme preparation (including the extraction and protease's isolation of pineapples. Preparation of tapioca waste biomass included: neutralizing, drying, milling and sieving passes 100 mesh sieve, after that done gelatinization. Gelatinization is done by dissolving 25 grams of tapioca starch waste into aquadest as much as 100 mL distilled water and heating at 100 °C temperature until gel form and waited until cool. Subsequently entering Textan (cellulase and xylanase) enzymes as much as 0.5 ml into the gel and incubating for 24 hours and generating waste hydrolyzate which will be used for YHE production (Agustini, 2015). Preparation of bromelain done a way the pineapple was grinded using a domestic juicer without water or buffer and the contents were centrifuged at $8000 \times g$ at 4 °C for 30 min. The supernatant was cooled to 4 °C and the proteins were precipitated using ammonium sulfate 40%. The salt was slowly added to the extract under constant agitation and, after the complete salt addition, it remained under stirring for 30 min at 4 °C. The resulting suspension was centrifuged at $8000 \times g$ at 4 °C for 15 min. The precipitate is a crude extract of bromelain which ready to be used in the production of YHE.[9]

2.2. Yeast Hydrolysate Enzymatic (YHE) Production

500 grams of Baker's yeast is added to 500 ml media that contain the tapioca's waste hydrolyzate and 12,5% sucrose (Agustini, 2015). Yeast is placed in an incubator at the temperature of 37 °C for 5 days. In this step will be autolysis, the next step is to conduct plasmolysis by adding 10% technical NaCl and 9% technical alcohol of the media's total volume, then incubated for 2 days at a temperature of 50 °C.[10] Yeast is cooled, decanted, and washed with warm distilled water to desalinate it, then centrifuged. The precipitate containing yeast is taken and enzymatic hydrolyzed by adding the crude extract of pineapple's bromelain, incubated at a temperature of 37 °C for 24 hours. The result of enzymatic hydrolysis is YHE which ready to be encapsulated using gum, then slurry made powder, and characterized.

2.3. Production of YHE Powder

The slurry was concentrated to 15% w/v of total solids content in an 80 °C water bath. The concentrate was subsequently spray-dried in a pilot scale spray drier (Buchi) using an inlet air temperature of 180–190 °C, an outlet air temperatures of 80–85 °C, a feeding rate of 30 ml min^1, an air flow rate of 80 m^3 h^1, and an air pressure of 0.005 bar. The resulting powder was stored in a jar at 4 °C, and ready to characterized.[11]

2.4. YHE Characterization

Characterization of YHE on the study include chemical test, microbiological test, and physical test. Chemical test: Total nitrogen (NT) using Makro-Kjedahl's methods by 6.25;[2] α-amino nitrogen (N-α) using Amino acid analyzer (17 kinds of amino acids); pH (2% in the solution) using a pH meter; total carbohydrates using Luffscoorl methods;[2] water contents;[2] Vitamin: thiamin, riboflavin, pyridoxine and niacin) using HPLC. Microbiological test results are seen from the total aerobic mesophilic flora (TPC). In this research using *Lactobacillus bulgaricus*. While physical tests are from color, smell and texture. The results of this characterization compared with commercial YHE.

3. RESULTS AND DISCUSSION

Prior to use, tapioca's waste needs to be prepared by doing gelatinization. Enzymatic hydrolysis process was preceded by the preparation process of the substrate, in this case, waste of tapioca industry. The second preparation was gelatinization, a process of forming a gel that begins with swelling the starch granules due to water absorption. This process was carried out to facilitate the access of enzymes to hydrolysis, starch degradation or other components, which is lignocellulolytic fiber. In the gelatinization process, a substrate will be produced and ready to be hydrolyzed by enzymatic. Gel produced from this gelatinization then hydrolyzed by enzymatic, (in this research) using α-amilase and lignocellulose. The time required for incubation in hydrolysis is 48 hours. α-amilase is also called 1,4-alpha-D-glucan4-glucanohydrolase, catalyzed enzymes capable of bonding 1,4-α-D-glycocidic polysaccharides containing three or more units of D-glucose linked by the α-1,4 bond. This enzyme hydrolysis will produce a mixture of maltose, maltotriose, and oligosaccharides consisting of 6–8 glucose units. This enzyme has an important role in initiating degradation of amylum (polysaccharides).[3, 12] Lignocellulase is an enzyme

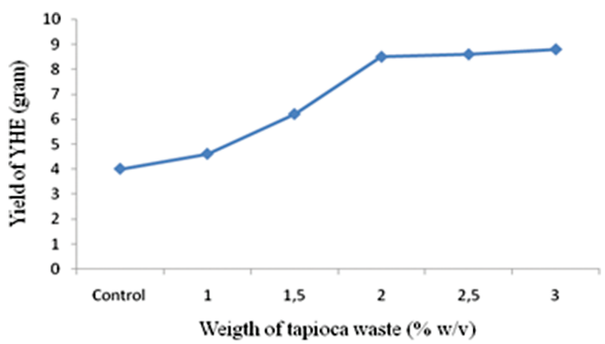

Fig. 1. Picture about the weight of YHE from fermentation in tapioca waste percentage treatment.

Table I. Data about pH of YHE products.

	Samples	pH
1	YHE autolysis	7
2	YHE NaCl lysis	7
3	YHE bromelain hydrolysis	5
4	YHE bromelain hydrolysis encapsulated with gum	7
5	Commercial YHE	6

that contributes in degrading or hydrolyzing lignocellulose. Lignocellulose is a term commonly used to describe a material containing (primarily) lignin, cellulose and hemicellulose. Hemicellulose and cellulose degraded by lignocellulase enzymes and produces monosaccharides (simple sugars) that can be used for yeast growth media. The results of this enzymatic hydrolysis are called hydrolysate, ready to be used as a medium for the growth of yeast fermentation.

The next step was the enzymatic hydrolysis process which was preceded by the process of substrate preparation. In this case, we use tapioca flour. The preparation of both wastes was by conducting gelatinization, the process of gel forming that begins with starch granule swelling due to water absorption. This process was carried out to facilitate the access of enzymes to hydrolysis, starch degradation, and other components, which was lignocellulolytic fiber. Gelatinization will produce the substrate that will be ready to be hydrolyzed enzymatically. The gel that was obtained from this process is hydrolyzed enzymatically by using α-amilase and lignocellulose. Incubation phase in hydrolysis needs 48 hours. Lignocellulase is an enzyme that contributes in degrading or hydrolyzing lignocellulose. Lignocellulose is a term commonly used to describe a material containing (primarily) lignin, cellulose and hemicellulose. Hemicellulose and cellulose degraded by lignocellulase enzymes and produces monosaccharides (simple sugars) that can be used for yeast growth media. The results of this enzymatic hydrolysis are called hydrolysate, ready to be used as a medium for the growth of yeast fermentation. The next step is the fermented centrifugation and the precipitate obtained is YHE. This study begins by producing

Table II. Analysis result of α-amino nitrogen (N-α).

Chemical test of amino acid	YHE autolysis (%)	YHE NaCL lysis (%)	YHE bromelain hydrolysis (%)	YHE bromelain hydrolysis encapsulated with gum (%)	Commercial YHE (in %)
Isoleucine	1.29	1.10	1.23	1.10	1.80
Leucine	1.98	1.54	1.72	1.53	3.00
Methione	0.43	0.32	0.37	0.33	0.60
Phenylanine	1.36	1.00	1.14	1.02	2.00
Threonine	1.83	1.25	1.41	1.27	1.10
Alanine	1.56	1.26	1.40	1.31	4.40
Agrinine	1.41	0.99	1.10	1.03	1.40
Cystine	0.09	0.03	0.04	0.33	0.20
Glutamic	2.56	2.58	2.27	2.25	0.20
Glycine	1.34	1.04	1.16	1.15	1.00
Histidine	0.60	0.46	0.50	0.49	0.40
Proline	0.99	0.78	0.87	0.80	0.80
Serine	1.64	1.04	1.18	1.11	1.30
Tyrosine	0.98	0.72	0.81	0.70	3.00
Lisin	2.19	1.97	2.14	2.00	1.90
Aspartat	2.05	2.05	2.24	2.06	1.60
Total	21.32	18.14	20.07	17.36	24.70
(N-α)/(NT)	0.75	0.65	0.77	0.71	0.51

Note: Data source of commercial YHE (advanced bio-processing, 2006).

Table III. Analysis result of vitamin B.

Chemical test	YHE autolysis	YHE NaCL lysis	YHE bromelain hydrolysis	YHE bromelain hydrolysis bromelain encapsulated with gum	Commercial YHE
Thiamine	0.0295	0.0270	0.020	0.015	0.020–0,070
Riboflavin	0.0071	0.0069	0.580	0.500	0.055–0,100
Pyridoxine	0.0079	0.0070	0.069	0.065	0.012–0,016
Niacin	10.270	9.880	9.700	8.990	0.250–0,700
Total	10.315	9.9210	10.307	9.570	0.337–0,886

Note: Vitamin B (mg extract per gram) → measurement using HPLC.

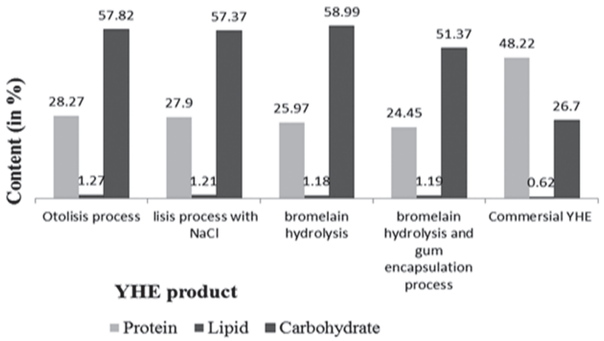

Fig. 3. Concentration level of protein, fat, carbohydrates and water in YHE (in %).

YHE with tapioca waste variations at: 1%, 1.5%, 2%, 2.5% and 3%. The result of the treatment as seen in Figure 1.

The production process of this research carried out in the stage, starting from autolysis, lysis using 40% NaCl and 80% of technical alcohol (plasmolysis), enzymatic hydrolysis using bromelain, enzymatic hydrolysis using bromelain then encapsulated using gum. Each YHE characterization includes chemical test, microbiological test, and organoleptic test. The results of this characterization compared with commercial (imported products).

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3.1. Chemical Test

The chemical test includes Total nitrogen (NT); α-amino nitrogen (N-α); pH (2% solution) using universal pH; fat; total carbohydrate; water level; and vitamin: thiamine, riboflavin, pyridoxine, and niacin). Figure 1 above states the chemical test results in the form of concentration levels of protein, fat, carbohydrates and water in YHE (in %).

Based on the data presented in Table I, it is known that YHE with the highest protein levels is commercial YHE, which is 48.22%. YHE with the highest carbohydrate levels is the result of bromelain hydrolysis with 58.99%, and the highest fat levels are the result of autolysis, which amounted to 1.27%. Table I above states pH measurement results of each product.

Based on the analysis of pH, dissolved YHE at 2% distilled water has a pH between 5–7. The measurement analysis result of α-amino nitrogen (N-α) is presented in Table II.

Based on analysis of α-amino nitrogen (N-α), it was known that the highest (N-α) is the result of autolysis, which is 23.70. The value of (N-α)/(NT) between YHE result of autolysis almost equal with hydrolysis bromelain YHE result, which is 0.84 for autolysis and 0.83 for bromelain hydrolysis. Therefore, analysis result about the contents of Vitamin B in each product is presented in Table III.

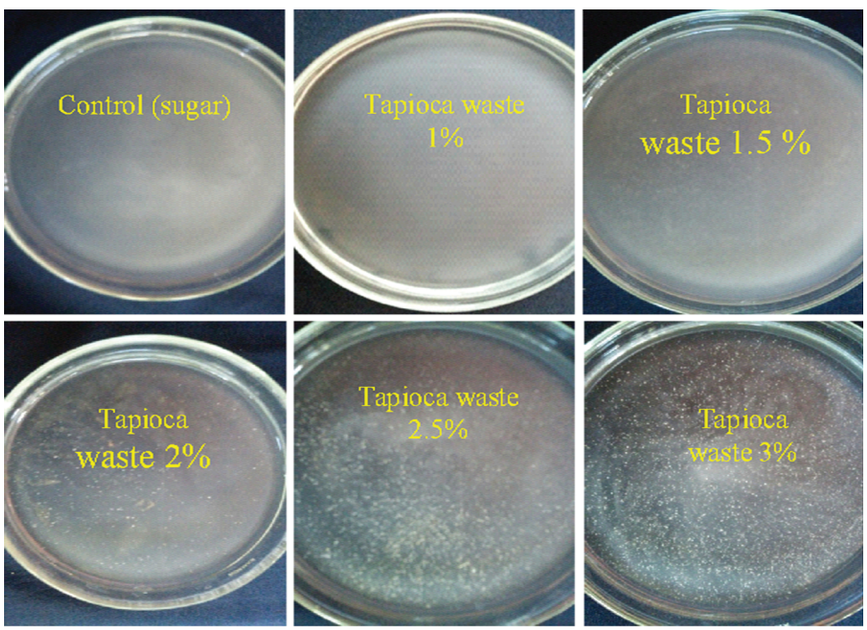

Fig. 2. YHE display the percentage of tapioca waste treatment variations.

Table IV. Total calculation result of aerobic mesophilic flora that are grown in various media containing yeast and calculations using TPC method (in CFU).

	Sample name	CFU
1	Yeast enzymatic hydrolysis	304×10^{10}
2	Yeast imported products	1×10^{10}

Notes: • Yeast used is 1% of dry weight (1 gram/100 liquid media). • Incubation carried out at temperatures of 37 °C for 48 hours. • Media used MRS.

Based on the analysis, it is known that the contents of vitamin B (thiamine, riboflavin, pyridoxal and niacin) has the highest result in YHE autolysis, which was 10.3145 mg/g.

3.2. Microbiological Test Can be Seen from the Total of Aerobic Mesophilic Flora (TPC)

Figure 2 above states the microbiological test results in dilution 10^{-7} up to 10^{-10}. Total calculation result of aerobic mesophilic flora that are grown in various media containing YHE and calculations using TPC method is presented in Table IV, and in this experiment use *Lactobacillus bulgaricus*.

Based on the data presented in Table IV, it can be concluded that YHE which yield microbial growth, in this case, *Lactobacillus bulgaricus* was YHE by enzymatic processed using pineapple bromelain. The use of YHE 1% dry weight (1 gram/100 liquid medium), incubation at a temperature of 37 °C for 48 hours was capable of producing colonies 304×10^{10} CPU (calculation using TPC method). Data show that the YHE processed with enzymatic hydrolysis using bromelain generate colony growth better than those using a commercial product (imported products). The products of commercial product (imported products) can only produce 1×10^{10} CFU. Physical characteristics (color, smell, and texture). Table V below states the physical characteristics of various products of YHE.

Based on the data presented in Table V, it could be concluded that the best physical characteristics in terms of smell and texture is YHE hydrolysis bromelain encapsulated with gum: sour smell and texture of the powder is not sticky. The better color is from commercial YHE. The results showed that YHE with the highest protein levels was commercial YHE imported products), which is 48.22%. This percentage is higher than YHE results of autolysis, plasmolysis (lysis with NaCl), and bromeain hydrolysis encapsulated with gum. YHE processed by autolysis, causing less of protein lost during processing, in addition YHE derived from brewery yeast while this research is using baker's yeast. Despite having lower protein level, hydrolysis bromelain YHE was better than market YHE when used as medium for the growth of *Lactobacillus bulgaricus*. Possibly because of bromelain hydrolysis YHE has high carbohydrates level used for carbon source. Based on the data, pH YHE bromelain was a pH of 5, therefore it was advisable to add NaOH gradually to reach 7 when used as a medium for microbiological growth.

Table V. Physical characteristics of YHE product.

	Products	Color	Smell	Texture
1	YHE autolysis	White	Sour	Powder soft and sticky
2	YHE NaCL lysis	White	Sour	Powder soft and sticky
3	YHE bromelain hydrolysis	White	Sour	Powder soft and sticky
4	YHE bromelain hydrolysis and encapsulation with gum	White, a bit browny	Sour	Powder is soft but not sticky
5	YHE (commercial product)	Yellow	Stink	Powder is soft but not sticky

4. CONCLUSION

Based on the results of the study, concluded that YHE of bromelain hidrolysis and encapsulation with gum has the best physical quality: smelled sour and the powder texture is soft and not sticky; but the result of microbiological test has less quality compare to YHE as the result of bromelain enzyme hidrolysis. The comparison of YHE as the result of bromelain hydrolisis has the highest value (N-α amino)/(NT) among other YHE products, that is 0.84. Therefore, YHE utilization as microbiological media, in this case for *Lactobacillus bulgaricus*, is recommended to use the result of bromelain enzymatic hydrolysis. Tapioca waste that is used as growth medium is suggested for YHE is 1.5%.

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