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Utilization of Waste Mixed Pangasius Fish Fillet and Pineapple Core to Produce Peptone for Lactic Acid Bacteria Growth Media

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ABSTRACT

Over the last few decades, the quantum of research on probiotic strains has increased rapidly in most regions of the world. The use of probiotics in animal production and human nutrition can solve many problems caused by inadequate antibiotic treatment, growth promoters, disease prevention, and improved management. Market demand for probiotics is increasing worldwide, including Indonesia, where these are mainly imported. This situation renders peptone as a media component for isolation and identification of probiotics or as a media for testing bacteria in microbiological work. Pangasius fillet waste (P) and pineapple core (C) are agro-industrial wastes used as raw materials for peptone production. The purpose of this study was to see how the ratio of P to C affects the output of peptone from agro-industrial wastes for use as a component of laboratory media for the growth of microorganisms. Protein P hydrolysis was carried out directly by mixing C in various ratios (1:1, 2:1, and 3:1) with different incubation times (T), 1 day (T1), 2 days (T2), and 3 days (T3). The P: C ratio of 3:1 with an incubation time of one day (T1) was the best for peptone production, with a yield of 42.88±2.66%, the protein content of 5.68±0.13%, degree of hydrolysis of 90.16±2.28%, and 15 amino acids. The effects of commercial and modified MRS broth media containing peptone on LAB growth were non-significantly different, with an average total LAB of 9.084-9.128 log CFU/mL. In conclusion, peptone extracted from P using crude bromelain from C can be used as a nitrogen source to formulate MRS broth for LAB growth.

Key words: Probiotics, Peptone, Pangasius, Pineapple core, Microorganism.

INTRODUCTION

Peptone is an important source of nitrogen used for microbial growth, as it is a protein hydrolysate containing different amino acids. It contains secondary protein derivatives such as polypeptides, dipeptides and amino acids. Peptone is generally derived from animals such as salmon (Idowu et al. 2019), chicken feathers (Akpor et al. 2019), marine food waste (Vázquez et al. 2020), or plants (Ashaolu 2020). Peptone is the most expensive component of microbiological media, and is widely used for biomass production and biotechnology in the laboratory, as well as in the industry (Tavano 2013).

Currently, the demand for peptone in Indonesia is mostly met through its import at very high prices, which increases every year (Atma et al. 2018). According to the Indonesian Central Statistics Agency, peptone imports in 2019 reached a value of US$ 26,511,097, with a quantity of 6,498,206 kg. In 2020, these imports increased to a value of US$ 28,455,743 and a quantity of 6,498,206 kg (BPS 2021a).

One solution for this problem is to produce peptone by utilizing local raw material sources. Raw materials that can be used to produce peptone in Indonesia mostly utilize local protein sources, such as Pangasius fish. According to BPS (2021b), the Pangasius fish (*Pangasius hypophthalamus*) production in the country reached 27,335 tons in 2019. Pangasius fish is a potential source of proteins necessary for peptone production (Dewita and Syahrul 2015). The meat of this fish contains 15 amino acids, including 9 essential amino acids and six non-essential amino acids (Suryaningrum et al. 2010). Pangasius fish has become a potential raw material for peptone production due to its large-scale production, easy to cultivate, low price, and high protein content. Pangasius fish fillet waste (P), such as meat attached to the frame and skin of P, can be used as raw material to produce peptone by hydrolyzing proteins from P using bromeliad.

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Peptone can be produced by hydrolysis of proteins, using chemical solvents (He et al. 2013; Martins et al. 2014) and enzymes (Haslaniza et al. 2010). Enzymatic hydrolysis reduces the size of the peptides and these peptides become the source of amino acids that have desired functional properties (Chalamaiah et al. 2012). Enzymatic hydrolysis has several advantages such as low cost, easy control, and hydrolysis of proteins at certain positions to produce desired peptides and amino acids. Enzymatic hydrolysis has become the best method for the production of quality peptone (Shu et al. 2018).

Proteolytic enzymes, either from animals (Lukin 2020), plants (Saptarini et al. 2020), or microorganisms (Souza et al. 2015), are commonly used for peptone extraction. Plants as a source of enzymes are generally considered safe (Karahalil 2020).The enzymes often used in the protein hydrolysis are papain (Priatni et al. 2020) and bromelain, derived from pineapples (Golden and Smith-Marshall 2012; Mohan et al. 2016). The pineapple fruit (*Ananas comosus* L) contains a variety of cysteine proteinases; the most prevalent being bromelain, which hydrolytically cleaves the internal peptide bonds in proteins with broad specificity (Hale et al. 2005). Bromelain also has many clinical applications (Manzoor et al. 2016; Chaudhary et al. 2019), and its activity is the highest at pH 3-8 and temperature 30-70ºC (Benefo and Ofosu 2018).

The part of pineapple with relatively high bromelain contents is its core (Chaurasiya and Hebbar 2013). In addition, pineapple core (C) also contains sugars, vitamins, and minerals that can be used as a source of carbon and minerals in the media for microorganisms. Therefore, the scientists have focused on producing peptone from P, using C as a source of crude bromelain without extracting bromelain first. The use of C in the process of making peptone from P provides several advantages. This C is a source of bromelain, which hydrolyzes proteins present in P. The P also contains sugars which serve as an energy source and carbon skeleton for the growth of microorganisms. Other nutrients present in the C are vitamins and minerals that play a crucial role in regulating cell metabolism. The microbial growth medium generally contains a carbon source, peptone, yeast extract, buffer, and sometimes a gelling agent (Akpor et al. 2019). The use of C as a source of bromelain is an attempt to convert waste materials into valuable products.

Peptone production from P and hydrolysis with raw bromelain directly from C is an alternative to simplify the extraction method. Furthermore, this peptone can be used as a component of microorganism growth media, such as lactic acid bacteria (LAB), bacterial pathogens, yeasts, and molds commonly found in humans or animals.

Lactic acid bacteria are widely used in humans and animals as a source of probiotics for food processing (Rossi et al. 2018; Aritonang et al. 2019; Rossi et al. 2021b), and as food preservatives (Rossi et al. 2021a). Lactic acid bacteria are used in animal production systems (Ramos et al. 2020). In addition, probiotics are gaining popularity as a safe and practical alternative to antibiotics for improving productive performance of livestock (Alayande et al. 2020). This rapidly increasing use of probiotics in the food, feed, and health industries has led to increased research on the exploration of natural and local sources of probiotics. As a result, the demand for peptone production has also increased. Therefore, this research was conducted to obtain a source of peptone from raw materials that are abundant and easy to obtain, with the hope that it can help to produce economically cheap local peptone in the country.

The specific objectives of this study were: i) to obtain a comparison of P:C in the production of peptone with the right incubation time (T), ii) to analyze the characteristics of peptone as a source of nutrition for bacterial growth, especially lactic acid bacteria, iii) to formulate media for lactic acid bacteria (LAB), and iv) to compare the total amount of LAB inoculated on modified media, and commercial MRS broth.

MATERIALS AND METHODS

Materials for Peptone Production

For this study, the Pangasius fillet waste (P) was taken from the home industry for processing Pangasius fish fillet. The source of bromelain was the pineapple core (C) obtained from the home industry for making pineapple chips in Kampar Regency, Riau, Indonesia. The test bacteria used were *Lactobacillus plantarum* TMW 1.1623 (Rossi et al. 2018).

Peptone Production

Peptone production was carried out following completely randomized design (CRD) with a two-factor factorial arrangement. The first factor was the ratio of P to C, which consisted of three levels, namely 1:1, 2:1 and 3:1 (w/w), named as PC1, PC2, and PC3, respectively. The second factor was hydrolysis time (T), which also consisted of three levels, namely T1=1 day, T2=2 days, and T3=3 days, with each treatment was repeated three times.

Peptone production was carried out through enzymatic hydrolysis, using natural bromelain contained in the pineapple core. Fresh P was steamed (85ºC) for 15 min then allowed to cool. The C was crushed to produce its pulp. The PC mixture in each treatment was mixed with 0.5mL NaCl and then distilled water was added to this mixture to a volume of 100 mL. Then the mixture was stirred until it was homogeneous and then incubated in a water bath shaker at 55°C for 1, 2, and 3 days. In order to stop hydrolysis by bromelain enzyme, this mixture was immersed in hot water (90°C) for 15 min (Utami et al. 2019). The samples were then centrifuged at room temperature (25ºC) for 30 min at 4500 rpm to separate the supernatant from the precipitate. Protein hydrolysate (peptone) in the form of supernatant was then analyzed for its pH, yield and proteins and tested microbiologically by growing *L. plantarum* TMW 1.1623 bacteria.

Peptone Characterization

The degree of acidity of the media before and after incubation (AOAC, 2005)was measured using a digital pH meter, with an accuracy of two decimal places. The peptone yield was obtained by calculating the ratio between the final weight produced and the initial weight of the raw material used for making the peptone. Proximate analysis was used to measure quantity of water, protein, fat, and peptone ash contents. The degree of hydrolysis was calculated based on the percentage ratio of trichloroacetic acid (TCA), following Utami et al. (2019). Protein

hydrolyzed into peptone was added with 20% TCA (w/v) with a volume ratio of 1:1 to obtain 10% TCA soluble material. The mixture was allowed to stand for 20 min to allow precipitation and then diluted to a volume of 100mL. The amino acid analysis was carried out through HPLC (Shimadzu Model LC-10A, Japan), which consisted of four stages: the stage of making hydrolysate protein, drying, derivatization, and injection and amino acid analysis (AOAC, 2005).

Microbiological Test

The spread surface plate method was used to determine the total bacteria of *L. plantarum* TMW 1.1623 (Rossi et al. 2021b). The total bacteria calculation was carried out by growing the four indicator bacteria diluted with sterile 0.85% NaCl solution in a ratio of 1:9 into MRS agar medium. A total of 0.1mL of sample from each 10^{-6}-10^{-8} dilution was transferred to a petri dish containing about 15mL of MRS to make it sterile and levelled using a hockey stick. The inoculated petri dishes were then incubated for 24h at 37°C in an inverted position. Petri dishes with the number of colonies from 25 to 250 were selected for colony counting.

Modified MRS Commercial for Lactic Acid Bacteria

Peptone obtained from previous studies was used as component media for *L. plantarum* TMW 1.1623. The treatment modified the commercial MRS broth media using this peptone, soy protein isolate, and yeast extract. The treatments applied in this study were as follows: M1=Commercial medium MRS broth, M2=Modified commercial medium MRS broth peptone from P, M3=Natural Media like MRS broth 1, and M4=Natural Media like MRS broth 2. The detailed composition of LAB media is presented in Table 1.

Statistical Analysis

The data obtained were analyzed statistically using the analysis of variance (ANOVA) technique, following completely randomized design (CRD) with a two-factor factorial arrangement. Duncan's multiple range test was used to assess the significant differences among mean values at a level of 5% (SPSS Version 160).

RESULTS

Chemical Composition of Raw Materials used for Making Peptone

The raw material used for making peptone was Pangasius fish fillet waste (P) had moisture, total crude protein, fat and ash contents of 77.80±019, 20.97±0611, 14.63±0.32 and 1.79±0.22%, respectively. The hydrolysis of proteins (p) required protease enzyme, bromelain, commonly found in pineapples. The nutritional contents of pineapple core (C) in this study were water (83.12%), reducing sugars (14.38%) and crude fiber (3.21%).

Pangasius Peptone Characteristics

The data presented in Table 2 shows that there was an interaction effect between the ratio of P:C and T (days), which was significantly different ($P<0.05$) on the peptone yield, protein content, and degree of hydrolysis (DH) of peptone. The P:C ratio of 3:1 (PC3T1, PC3T2 and PC3T3) resulted in significantly lower ($P<0.05$) peptone yield (40.05±0.94–42.88±2.66%) compared to other treatments. This low yield was due to the hydrolysis process. The raw material had high total solids contents. However, P:C ratio of 1:1 (PC1T1, PC1T2 and PC1T3) showed the highest peptone yield (67.36±3.31-72.41±1.25%) compared to other treatments.

The protein contents of the peptone solution ranged from 3.28±0.17 (for PC1T3) to 5.68±0.13% (for PC3T1), the difference was significant ($P<0.05$). This difference in protein content was due to the difference in the P:C ratio in each treatment. The PC3 treatment at each time (T1, T2, and T3) of hydrolysis resulted in higher protein content (5.08±1.56–5.68±0.13%) than other treatments. These results were parallel to the DH of peptone. The PC3 treatment at each T1, T2, and T3 had the DH of 90.16±2.28, 91.54±2.96 and 93.63±2.10%, respectively.

Hydrolysis of P protein by direct mixing to C was carried out with different ratios, namely 1:1, 2:1, and 3:1 (w/w). The results showed that the first day of incubation at the same P:C ratio always showed a lower DH than the other hydrolysis times. The DH increased significantly, reaching 93.63±2.10% DH in the PC3T3 treatment, when the ratio of P to C was 3:1 with a hydrolysis time of 3 days (Table 2).

As has been shown in Fig. 1, there was no interaction effect between P:C ratio and T on total LAB. The total LAB varied from 8.59to 9.18 log CFU/mL. This application of P peptone as medium for LAB (Fig. 2) showed that the interaction between P:C ratio and T treatments affected the pH of peptone before and after incubation. The pH values before and after incubation were around 4.45–6.27 and 3.60–4.63, respectively. The PC3T3 treatment had the highest pH of 6.25 ($P<0.05$) compared to the pH of the other treatments.

The Amino Acid of Pangisius Peptone

In previous stage research, the best peptone was produced in the PC3T1 treatment. This treatment used a ratio of P:C as 3:1 with an incubation time of 1 day (T1). Results of the amino acid composition of liquid peptone from P are shown in Table 3. This peptone contained 15 amino acids; among these, the highest amino acid content was recorded for glutamic acid (6.04%) and the lowest amino acid content was for histidine (0.51%).

The Growth Rate of Lactic Acid Bacteria on Modified MRS-Broth Media

The three modified MRS-broth media (M2, M3, and M4) had the same pH as the commercial MRS-broth medium (M1) before LAB were inoculated. The pH of the medium after incubation of *L. plantarum* TMW 1.1623 for 24 hours at 37°C was influenced ($P<0.05$) by the composition of the bacterial growth medium (Table 4). Commercial medium (M2) had the lowest pH after incubation (3.77±0.01) compared to the pH in other three media (M1, M3 and M4). The pH decreased from 6.5 to 3.80, 3.77, 4.53, and 4.25, respectively for *L. plantarum* TMW 1.1623 on M1, M2, M3, and M4. Table 4 also shows that the effects of treatments; MRS broth media (M1, M2, M3 and M4), either commercial MRS broth or modified MRS broth containing peptone, were non-significantly different on the growth of LAB. Total LAB in M1, M2, M3 and M4 treatments were 9.09±0.12, 9.03±0.17, 9.13±0.07 and 9.08±0.21 log CFU/mL, respectively.

Table 1: Composition of media in each treatment in 1000mL

Materials	Treatments			
	MRS broth		Natural Media	
	M1	M2	M3	M4
Peptone from Pegasus protein (mL)	Commercial MRS	Modified commercial	250	250
Soy protein isolate hydrolyzate (mL		MRS using peptone	250	250
Yeast extract (mL)			50	0
Soy sprout extract (mL)			400	450
Tomato extract (mL)			20	20
Sucrose (g)			30	30

M1=Commercial medium MRS broth, M2=Modified commercial medium MRS broth peptone from Pangasius fish fillet waste, M3=Natural Media like MRS broth 1, and M4=Natural Media like MRS broth 2.

Table 2: Characteristics of peptone produced from mixing waste Pangasius fillet and pineapple core as a source of bromelain and nutrition

Treatments	Yield (%)	Protein (%)	Degree of hydrolysis (%)
PC1T1	71.07±1.85d	4.99±0.48c	72.99±0.84a
PC1T2	67.36±3.31d	4.77±1.39bc	78.09±0.56b
PC1T3	72.41±1.25d	3.28±0.17a	72.21±0.83a
PC2T1	55.73±2.89c	5.17±1.10c	81.83±2.46c
PC2T2	48.58±2.42b	3.40±1.18ab	82.21±2.10c
PC2T3	57.63±1.36c	4.65±0.79bc	84.56±1.34c
PC3T1	42.88±2.66a	5.68± 0.13c	90.16±2.28d
PC3T2	42.58±2.06a	5.14±1.26c	91.54±2.96d
PC3T3	40.05±0.94a	5.08±1.56c	93.63±2.10d

P=Pangasius fish fillet waste, C=Pineapple core, and T=hydrolysis time (days). The values are expressed as the mean ± standard deviation of triplicate determinations. Within the same column, different alphabets show statistically significant difference (P<0.05).

Table 3: Amino acid composition of peptone and the other test peptones (%)

Sr. No	Amino Acids	Liquid peptone	Liquid Peptone Nurilmala et al. (2018)	Dried peptone Setijawati et al. (2020)	Meat Pratama et al. (2018)
1	Alanine	2.17	2.30	5.79	1.15
2	Arginine	1.95	1.55	5.86	1.32
3	Aspartic acid	3.43	3.27	5.47	1.98
4	Phenylalanine	1.98	2.03	4.73	0.86
5	Glycine	3.69	4.04	19.05	0.81
6	Glutamic acid	6.04	6,27	10.05	3.28
7	Histidine	0.51	0.49	1.30	0.45
8	Isoleucine	1.75	1.84	4.91	1.06
9	Leusin	2.81	2.72	8.57	1.66
10	Lisin	2.27	2.30	5.46	1.72
11	Methionine	0.74	0.75	0.76	0.61
12	Serin	1.09	1.21	4.77	0.79
13	Tirosin	0.82	0.84	1.01	0.69
14	Threonine	1.07	1.05	5.91	0.82
15	Valine	1.91	1.95	6.84	1.06

Table 4: pH of the medium and total lactic acid bacteria in commercial and other media

Medium	pH before incubation	pH after incubation	Total LAB Log CFU/mL
M1	6.5	3.80±0.01b	9.09±0.12
M2	6.5	3.77±0.01a	9.03±0.17
M3	6.5	4.53±0.01d	9.13±0.07
M4	6.5	4.25±0.01c	9.08±0.21

M1=Commercial medium MRS broth, M2=Modified commercial medium MRS broth peptone from Pangasius fish fillet waste, M3=Natural Media like MRS broth 1, and M4=Natural Media like MRS broth 2. The values (mean±SD) within the same column, different alphabets show statistically significant difference (P<0.05).

DISCUSSION

Chemical Composition of Raw Materials for Making Peptone

The composition of fish products and by-products varies depending on nutrition, fish size, sex, age, environment, and season (Petricorena 2015); the results of the chemical analysis of pangasius fillet waste obtained in this study are almost the same as previous studies (Dewita and Syahrul 2015). Previous research has looked at the proximate content of the tuna head (*Euthynnus affinis*), which comprises protein, moisture, fat, and ash contents of 19.30, 68.79, 7.01 and 4.77%, respectively (Khoddami 2012). Proximate analysis of Pangasius catfish heads had lower (11.72%) protein content (Setijawati et al. 2020) than 20.97% protein content observed in this study.

On the other hand, body composition varies widely from one species to other. Based on the protein content, material used in this study was suitable as a source of peptone. Several previous studies generally used raw materials containing relatively high protein for making peptones, such as Tilapia fish (Deraz et al. 2011), sheep wool protein (Taskin et al. 2016), chicken feather (Akpor et al. 2019), meat and soy protein (Utami et al. 2019). Considering high protein and low-fat contents of catfish, it is considered suitable for use as raw material for making peptone.

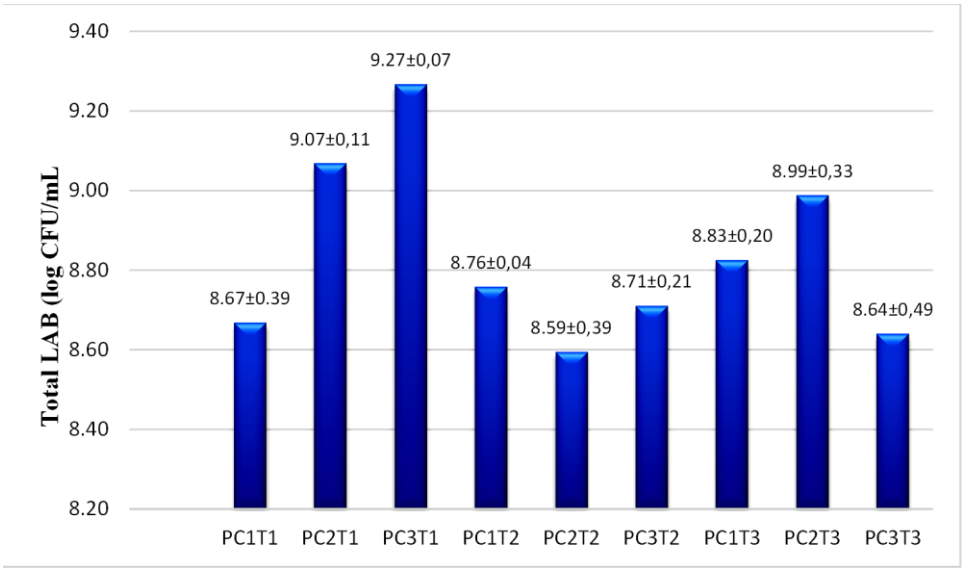

Fig. 1: Total LAB (log CFU/mL) on peptone for the growth of lactic acid bacteria: P= Pangasius fish fillet waste, C= Pineapple core, PC1= ratio P:C= 1:1, PC2= ratio P:C= 2:1 and PC3= ratio P:C= 3:1, T= Incubation time T1= 1 day, T2= 2 days, and T3= 3 days. The values are expressed as the mean ± standard deviation (n=3).

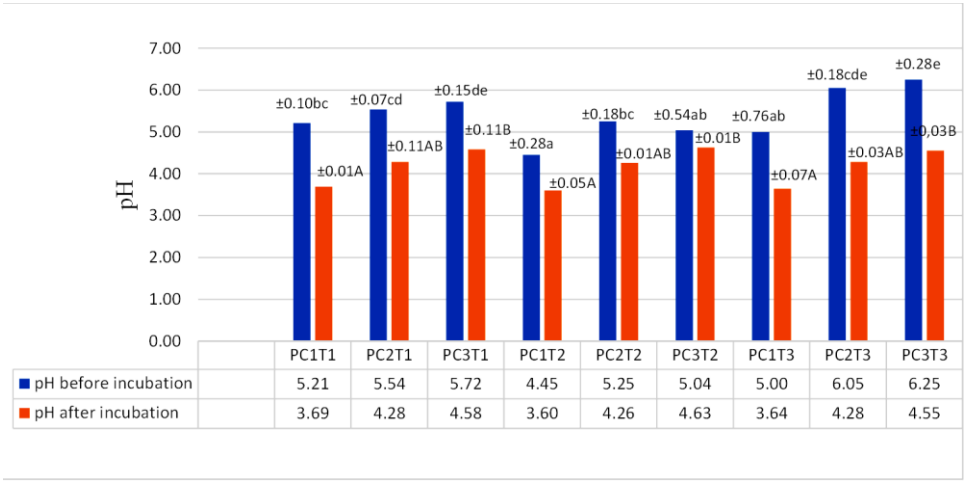

Fig. 2: pH before and after incubation on peptone for the growth of lactic acid bacteria: P= Pangasius fish fillet waste, C= Pineapple core, PC1= ratio P:C= 1:1, PC2= ratio P:C= 2:1, and PC3= ratio P:C= 3:1, T= Incubation time T1= 1 day, T2= 2 days, and T3= 3 days. The values are expressed as the mean ± standard deviation of triplicate determinations. Within the same bar, different alphabets show statistically significant difference (P<0.05).

The protein hydrolysis process required protease enzyme, bromelain, commonly found in pineapples (Sanewski et al. 2018). Pineapple core is a part of the pineapple fruit that is usually discarded from the pineapple processing industry. This core, in addition to containing sugars, vitamins and minerals, also contains relatively high bromelain. Bromelain is most often obtained from ripe pineapple, however, core, bark, and litter can also be used (Crestani et al. 2010). The nutritional contents of pineapple pith in this study were: water (83.12%), reducing sugars (14.38%), and crude fiber (3.21%).

Microorganisms can use the sugar content (14.38%) in pineapple core as a carbon source for their growth. Most microorganisms need organic carbon derived from carbohydrates, e.g., monomers, dimers, and polymers (Adams and Nout 2001). Therefore, in this study, C served as a source of bromelain, as well as a carbon source. In addition, mixing C pulp directly with P simplified and shortened the time of making media for microbial growth. Media are usually produced by first making the required components, such as peptone uses bromelain to hydrolyze proteins (Utami et al. 2019), glucose sources, and minerals which are then added to produce a growth medium.

Pangasius Peptone Characteristics

The differences in yield and characteristics of the peptone produced were caused by differences in the P:C ratio and the hydrolysis time of P protein to produce peptone. The 3:1 ratio of P:C resulted in lower yield (P<0.05) compared to the yield in other treatments. This low yield for PC3T1, PC3T2, and PC3T3 was due to high total solids mostly contained in C. This high total solid content contributed to the high fiber content in C. According to Ketnawa et al. (2012), C is a pineapple waste that produces bromelain with a relatively high fiber content.

The protein contents of the peptone solution ranged from 3.28 to 5.69%. The differences in protein contents were due to the differences in the P:C ratio in each treatment. These findings are comparable to the DH of this peptone. The PC3 treatment at T1, T2, and T3 showed the DH of 90.16, 91.54, and 93.63%, respectively. Hydrolysis of P protein by direct mixing to C was carried out with different ratios, namely 1:1, 2:1, and 3:1 (w/w). This difference also implied that the ratio of protein and protease enzymes was also different. The enzymatic reaction was carried out at 50°C with different incubation times (T), namely 1, 2 and 3 days. The results showed that the first day of incubation at the same P:C ratio always showed a lower DH than the other incubation times. The DH increased significantly, reaching 93.63% in the PC3T3 treatment, namely the ratio of P to C was 3:1 with a hydrolysis time of 3 days (Table 2). The DH continued to increase up to 3 days of incubation. This DH indicates that

crude bromelain derived from C could hydrolyze proteins in P. The higher the dissolved nitrogen concentration, the longer would be the incubation time. The results of this study are almost in line with those of Ovissipour et al. (2010), Utami et al. (2019), Lima et al. (2020) and Priatni et al. (2020). These workers have found that when the incubation period increases, DH also increases.

This halal media uses peptone from P, hydrolyzed by direct mixing without extracting bromelain from the pineapple core (C), containing sufficient nutrients to support the growth of *L. plantarum* TMW 1.1623. Total *L. plantarum* TMW 1.1623 was almost the same as the total *L. plantarum* TMW 1.1623 present in commercial media (Fig. 1). The growth of *L. plantarum* TMW 1.1623 was almost similar to that observed by Utami et al. (2019), who used halal media to grow *L. plantarum*. Peptones produced from cod viscera, according to Horn et al. (2005), are potential industrial media materials for *L. plantarum* and other food microorganisms.

This application of P peptone showed that the interaction between P:C ratio and T affected the pH of peptone before and after incubation. The pH values before and after incubation were around 4.45–6.27 and 3.60–4.63, respectively. Before incubation, the pH media differences were due to the ratio of P and C. The PC3T3 treatment showed significantly higher pH before incubation (6.25; $P<0.05$) compared to other treatments, except for the pH in the PC3T2 treatment ($P>0.05$). Because of this observation, peptone was produced by mixing three parts of P and one part of C, with an extraction time of 3 days (T3). An almost similar pattern in the pH was seen after the media was incubated with *L. plantarum* TMW 1.1623. As shown in Fig. 2, the pH of the peptone after incubation was lower than the pH before incubation in the same treatment. The media inoculated with *L. plantarum* TMW 1.1623 and incubated for 24 hours at a temperature of 36ºC had a low pH, ranging from 3.69 to 4.55. This decrease in the pH of the medium after incubation was caused by the activity of LAB, which fermented sugars present in the media, which produced lactic acid and decreased the pH of the media (Salminen and Wright 2011).

The Amino Acids of Pangisius Peptone

In this study, the best quality peptone was produced in the PC3T1 treatment. This treatment comprised a P:C ratio of 3:1, with an incubation time of 1 day (T1). This peptone contained 15 amino acids, consisting of eight essential amino acids including: histidine, threonine, methionine, valine, phenylalanine, isoleucine, leucine, and lysine. There were seven non-essential amino acids including: alanine, arginine, aspartic acid, glutamic acid, serine, glycine, and tyrosine (Table 3). Glutamic acid was the most abundant amino acid in this peptone, whereas histidine was the least abundant. These results are supported by the findings of Nurilmala et al. (2018). However, Setijawati et al. (2020) reported that glycine was the most abundant amino acid in dry peptone derived from catfish protein. Based on the amino acid composition of peptone samples isolated from Pangasius fish fillet waste in this study, this peptone was an essential substrate for the growth of microorganisms. According to Shirahigue et al. (2018), the amino acids in peptone can help in the rapid growth of microorganisms.

The Growth Rate of Lactic Acid Bacteria on Modified MRS-Broth Media

The three modified MRS-broth media (M2, M3 and M4) used in this study had the same pH (6.5) as that of the commercial MRS-broth medium (M1) before incubation of LAB. The pH of the medium after incubation of *L. plantarum* TMW 1.1623 for 24 hours at 37°C was significantly influenced ($P<0.05$) by the composition of the growth medium (Table 4). Commercial medium (M2) had the lowest pH after incubation compared to the pH in other three media (M1, M3, and M4). This decrease in pH indicated the growth of *L. plantarum* TMW 1.1623 during incubation. Decreased pH from 6.5 to 3.80±0.01, 3.77±0.01, 4.53±0.01, and 4.25±0.01, respectively for *L. plantarum* TMW 1.1623 on M1, M2, M3, and M4 was caused by LAB through fermenting the nutrients present in the media to produce lactic acid.

L. plantarum TMW 1.1623 has been isolated from the solid waste of soymilk production (Aritonang et al. 2017) and used as a probiotic (Rossi et al. 2018). Its culture showed good performance as a starter culture for milk fermentation. Cultures were grown in modified media such as commercial MRS broth. As has been shown in Table 4, modified media containing peptone provided a good nitrogen source for LABs. Besides peptone, soy protein hydroxylate and yeast extract also serve as the nitrogen source for MRS media. Utami et al. (2019) reported that media using peptone from meat and soybeans could also be used as LAB growth media. However, the yield was lower than that of LAB grown on commercial MRS media.

In the present study, *L. plantarum* TMW 1.1623 was grown on media containing the P peptone as a complex nitrogen source with several media formulations (Table 1). The M1 media was commercial MRS broth, M2 was commercial MRS broth modified by replacing beef broth and peptone with P peptone and soy protein isolate. The M3 and M4 were media that contained peptone, sucrose as a carbon source, bean sprout extract and tomato extract as sources of vitamins and minerals. Both these media contained peptone from P, hydrolysed by direct mixing without extracting bromelain first from pineapple cores, and also contained sufficient nutrients to support *L. plantarum* TMW 1.1623 growth. The total *L. plantarum* TMW 1.1623 in these media was almost the same as the total *L plantarum* TMW 1.1623 in the commercial media. The growth of *L plantarum* TMW 1.1623 recorded in the present study is almost the same as that reported by Utami et al. (2019). According to Horn et al. (2005), growth media containing hydrolysed protein from Atlantic cod (*Gadus morhua*) eviscerate produced a growth rate of LAB almost the same as that of LAB grown on commercial MRS media.

Conclusion

Peptone can be extracted from Pangasius fish fillet waste (P) using bromelain enzyme from pineapple core (C). The extraction process directly mixes the two ingredients, namely P and C. With different P and C ratios and varying incubation times, the best P peptone was produced using three parts P and one part C, with an extraction time of 1 day. This P peptone was used as a component of MRS broth media for LAB growth. The effects of commercial and modified MRS broth media containing peptone on the growth of LAB were non-significantly different, with an

average total LAB of 9.084–9.13 log CFU/mL. These findings indicated that peptone extracted by mixing Pangasius fish fillet waste and pineapple core could be a promising alternative for local peptone used as a component of growth media for LAB that can be applied for humans and animal needs.

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Author's Contributions

All authors contributed to the preparation of materials, data analysis, and text writing of this manuscript.

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