 e-ISSN 2598-9596

Fermentation Using *Providencia stuartii* and *Salmonella enterica* to Produce N-Acetylglucosamine from Shrimp Shells' Chitin

Yuniwaty Halim[1*], Nabilah binti Chanan Din[1], Lucia C. Soedirga[1]
[1]Food Technology Study Program, Universitas Pelita Harapan, Jl. M.H. Thamrin Boulevard, Tangerang 15811, Indonesia
*Author correspondence: yuniwaty.halim@uph.edu

ABSTRACT

Chitin is a biopolymer that can be extracted from exoskeleton of shrimp shells and other arthropods**.** One of its derivatives is N-acetylglucosamine that is comprised of glucose and amino acid glutamine. N-acetylglucosamine is known for its health effect**,** particularly in the treatment of osteoarthritis symptoms. One of the methods to produce N-acetylglucosamine is by fermentation using chitinolytic microorganisms. The aim of this research was to determine the optimum condition (pH, ratio of bacteria, fermentation time, and temperature) for fermentation using the combination of Providencia stuartii and Salmonella enterica to produce N-acetylglucosamine. The research was conducted at different pH (5, 6, 7, 8, 9) and bacterial ratios (3:1, 1:1 and 1:3). Furthermore, the optimum incubation temperature (30, 37, 40^{o}C) and fermentation time (1, 2, 3 and 4 days) were also evaluated. The results showed the optimum condition was achieved at pH 8 with bacterial ratio of 1:1, incubated at 37^{o}C for 3 days. Under these conditions, N-acetylglucosamine concentration obtained was 87,707.04 ± 906.54 ppm.
Keywords : colloidal chitin; combined fermentation; microbial; N-acetylglucosamine; shrimp shells

INTRODUCTION

Indonesia is one of the leading producers of agricultural and marine products and possesses great potential in the fisheries sector (Amalia *et al.*, 2022). According to BPS (Central Agency of Statistics in Indonesia) (2018), the export value of shrimp in Indonesia has increased by around 10 % each year. The shrimp exported from Indonesia mainly in forms of frozen unshelled shrimp which leaves the shrimp shell as waste or unused by-products. Approximately 50-60% of dried shrimp shells can produce 25% chitin because it contains 25-40% of protein, 45-50% of calcium carbonate and 15-20% of chitin (Marganof, 2003).

Chitin is a biopolymer that is easily found in nature and considered as non-toxic, biodegradable, and biocompatible. Chitin extraction involves two main processes, *i.e.,* demineralization and deproteinization. These processes can be performed biologically using microorganisms or chemically using acids and bases (Rahmawati *et al*., 2012).

Chitin and its derivatives are commonly used in several fields such as medical and health where it can be used as immunoadjuvant and as basic material to make surgical suture (Aiba, 2009). N-

acetylglucosamine is one of the derivatives of chitin that is used to treat the symptoms of osteoarthritis (Wanichpongpan *et al.* 2016), therefore it is often used as a supplement which is commonly consumed by the elderly to treat problems that is associated with the joint (Arbia *et al.,* 2012).

The production of N-acetylglucosamine can be obtained by chemical, enzymatic or microbiological methods. N-acetylglucosamine produced by means of chemical is done with the use of acid or base which results in a relatively fast glucosamine production but can result in low yield and cause acidic waste towards the environment (Wang *et al*., 2008). Enzymatic hydrolysis method requires higher cost, but the utilization of chitinase enzyme results in a higher yield of glucosamine unlike chemical hydrolysis (Pan *et al*., 2011). On the contrary, microbiological method for glucosamine production involves fermentation, particularly using chitinase producing bacteria offer some advantages, such as higher yield (the highest reported was 110 g/L N-acetylglucosamine using recombinant strain *E. coli*), no structure modification, and environmental-friendly. However, a major challenge is that the fermentation method cannot yet be applied on a large scale for commercial production (Liu *et al*., 2013).

Providencia stuartii and *Salmonella enterica* were bacteria isolated from *Penaeus monodon* shrimp shells obtained from previous study done by Hardoko *et al.* (2020). Both bacteria produce chitinase, an enzyme that catalyzes the hydrolysis of insoluble chitin into its oligomeric and monomeric derivatives (Tanaka *et al*., 2001).

Moreover, previous study by Hardoko *et al.* (2020) also reported that *Providencia stuartii* was the bacterium isolated from shrimp shell waste that possessed the highest chitinolytic activity, while Michelle (2019) found that *Salmonella enterica* produced approximately 73,184.74 ± 752.02 ppm of N-acetylglucosamine after three days of fermentation at 37 °C. Therefore, in this study, combined fermentation using these bacteria was expected to produce N-acetylglucosamine more efficiently, in a shorter time, and with a higher yield.

According to Halim *et al.* (2018), several factors influence the N-acetylglucosamine production from fermentation, such as fermentation temperature, pH of the media, and fermentation time. Therefore, this research aimed to determine the optimum pH and bacterial ratio for the combined fermentation of *Providencia stuartii* and *Salmonella enterica* to produce N-acetylglucosamine. Furthermore, this

e-ISSN 2598-9596

research also aimed to determine the optimum fermentation time and temperature for the combined fermentation of *Providencia stuartii* and *Salmonella enterica* in the production of N-acetylglucosamine.

MATERIALS AND METHOD

Materials and Equipment

The materials used in the experiment were shrimp shells (*Penaeus monodon*) obtained from PT. Wirontono Baru located at Ancol Jakarta Utara, *Providencia stuartii* and *Salmonella enterica* culture obtained from previous study of Hardoko *et al.* (2020), distilled water, 1 M hydrochloric acid (HCl) "Merck", 3.5% sodium hydroxide (NaOH) "Merck", 37% hydrochloric acid (HCl) "Merck", ethanol "Smartlab", 1 M sodium hydroxide (NaOH) "Merck", 5 N sodium hydroxide (NaOH) "Merck", Nutrient Agar (NA) "Merck", Nutrient Broth (NB) "Merck", Monopotassium phosphate (KH_2PO_4) "Merck", Dipotassium phosphate (K_2HPO_4) "Merck", Magnesium sulfate ($MgSO_4.7H_2O$) "Merck", Bromocresol Purple (BCP) indicator, tartaric acid "Merck", sodium phosphate, 0.8% ninhydrin, Coomassie Brilliant Blue G-250 "Merck", 85% Bovine Serum Albumin (BSA) "Sigma Aldrich", Methylene Blue (1:5), and N-acetylglucosamine standard "Sigma Aldrich".

The equipment used were analytical balance "Mettler Toledo", dry blender "Philips", sieve shaker 60 mesh "Retsch", Miller "Fomac", centrifuge "Medical Instruments MPW-223e", UV-Vis spectrophotometer "Thermo Scientific Genesys 10S UV-Vis", FTIR spectroscopy, furnace "Thermolyne 48000" and autoclave "Hirayama", and glasswares.

Procedures

Isolation of Chitin from Shrimp Shells

Chitin preparation was done according to Halim *et al.* (2018). *Penaeus monodon* shrimp shells were cleaned, dried under the sun for 2 days, size-reduced using blender and miller, and was further sieved using 60-mesh siever. The shrimp shells powder obtained was analysed for its moisture content (AOAC, 2005), ash content (AOAC, 2005), yield (Dompeipen *et al.,* 2016) and protein content using Bradford method (Nielsen, 2009 with modification).

Afterwards, shrimp shells powder was subjected to demineralization process where the powder was soaked with 1 M HCl (ratio 1:10 w/v) and heated at 75^{o}C for 2 hours. The mixture was then cooled down, neutralized until pH 5 and dried at 50^{o}C for 24 hours using a cabinet dryer. The demineralized powder was then undergone

e-ISSN 2598-9596

deproteinization process where the powder was soaked with 3.5% NaOH (ratio 1:10 w/v) and heated at 75ºC for 2 hours with constant stirring. Then, the mixture was cooled down, neutralized until pH 7and dried using a cabinet dryer at 50ºC for 24 hours. The isolated chitin was analysed for its moisture content (AOAC, 2005), ash content (AOAC, 2005), yield (Dompeipen *et al.,* 2016), degree of deacetylation (Benhabiles *et al.,* 2012), and protein content using Bradford method (Nielsen, 2009 with modification).

Colloidal Chitin Preparation

10 grams of chitin was mixed with 37% HCl (ratio 1:14 w/v) and stirred continuously for 2 hours at room temperature until chitin fully dissolved. 500 mL ethanol was added to mixture and 5N NaOH was added until reach pH 7. Then the mixture was centrifuged for 5 minutes at 4000 rpm. The precipitate obtained was the colloidal chitin (Setia, 2015 with modification).

Chitinolytic Assay for *Providencia stuartii* and *Salmonella enterica*

Selective media for chitinolytic activity assay was prepared according to the formulation in Table 1.

Table 1. Formulation for selective media

Ingredient	Amount (g/L)
Nutrient Agar	20.0
Colloidal chitin	5.0
KH_2PO_4	0.3
K_2HPO_4	0.7
$MgSO_4.7H_2O$	0.5

Source: Setia (2015)

The selective media was then added Bromocresol purple (BCP) indicator, boiled, cooled down and added with 10% tartaric acid until pH 4.7 was reached. The media was then sterilised in an autoclave for 15 minutes at 121ºC. To perform the assay, the media was poured into Petri dish and left to solidify. A 5 mm diameter well was made and 60µL of culture was poured and incubated at 37ºC for 24, 48 and 72 hours. According to Halim *et al.* (2018), chitinolytic activity was indicated by the formation of purple color on the clear zones, while no color change indicates the absence of chitinolytic activity. Therefore, chitinolytic index was determined by measuring the diameter of the purple zone around the well using a Vernier calliper. The larger the purple zone, the higher the chitinolytic index of the bacteria (Agrawal and Kotasthane, 2012).

Determination of Optimum pH and Bacterial Ratio of *Providencia stuartii* and *Salmonella enterica*

In this research, the fermentation using combination of *Providencia stuartii* and *Salmonella enterica* was done using

e-ISSN 2598-9596

different pH and bacterial ratio. The fermentation media itself was prepared according to the formulation in Table 2.

Table 2 Formulation for fermentation media

Ingredient	Amount (g/L)
Chitin	20.0
$KH_2PO_4.3H_2O$	1.0
$MgSO_4.7H_2O$	0.5
$NaNO_3$	1.0
Glucose	20.0
Distilled water	Until 1.0 L

Source: Saskiawan and Handayani (2011)

To adjust the pH, the fermentation media were added with either 1 M NaOH or 1 M HCl along with buffer pH to achieve the desired pH which were pH 5, 6, 7, 8 and 9. The media was then sterilised using an autoclave at 121ºC for 15 minutes. The media was then inoculated with different ratio of *Providencia stuartii:Salmonella enterica*. which were 3:1, 1:1 and 1:3. the media was then incubated at 37ºC for 24 hours (Ulfa, 2016 with modification). This fermentation time and temperature was chosen because it is the optimum fermentation temperature and incubation time for *Providencia stuartii* and *Salmonella enterica* (Keerthirathne *et al*., 2016). Furthermore, the fermentation results were then analyzed for its *N*-acetylglucosamine content.

Determination of Optimum Incubation Time and Temperature

The pH of media and bacterial ratio in this stage were set according to the optimum pH and the bacterial ratio obtained from the previous stage of research. Fermentation media was prepared according to Table 2. The media was incubated at different temperatures which were 30, 37 and 40ºC. Meanwhile, incubation time was 1, 2, 3, and 4 days4, (Ulfa, 2016 with modification). The sample obtained which was *N*-acetylglucosamine was measured using spectrophotometer at 324 nm.

Analysis of N-acetylglucosamine

The analysis of N-acetylglucosamine was done according to Ulfa (2016) and Halim *et al.* (2018). There were three steps done for measurement of N-acetylglucosamine, consisting of preparation of standard curve, extraction of N-acetylglucosamine from the sample, and quantification of *N*-acetylglucosamine.

The standard curve of N-acetylglucosamine was prepared by diluting N-acetylglucosamine to concentration 2000, 4000, 6000, 8000 and 10000 ppm. 4 mL of each concentration added with 0.5 mL of 0.8% ninhydrin and 0.5 mL buffer phosphate pH 7. These standard solutions were heated in water bath for 15 minutes at 95ºC and measured using spectrophotometer at 324 nm. Moreover, the blank used in this measurement was distilled water added with 0.5 mL of 0.8% ninhydrin and 0.5 mL buffer phosphate pH 7.

e-ISSN 2598-9596

To extract the N-acetylglucosamine, each fermentation sample was heated in water bath at 75ºC for 45 minutes, filtered using Buchner funnel and centrifuged for 15 minutes at 5000 rpm. The supernatant obtained was diluted 10x.

To quantify the concentration of N-acetylglucosamine, 4 mL of diluted mixture was added with 0.5 mL of ninhydrin and 0.5 mL of buffer phosphate pH 7. The mixture was then heated for 15 minutes at 95ºC in a water bath and was measured with spectrophotometer at 324 nm to obtain its absorbance value. The concentration of N-acetylglucosamine was then calculated based on the standard curve.

Data Analysis

All data obtained in this research were analyzed statistically using Univariate Analysis and Duncan Test for Post Hoc analysis. The software used for data analysis was SPSS version 25.

RESULTS AND DISCUSSION

Characteristics of Shrimp Shells Powder

The chemical composition of the shrimp shell powder obtained in this research can be seen at Table 3. The moisture content obtained was below 10% and lower compared to previous research by Halim *et al.* (2021), in which the moisture content of shrimp shells powder obtained was 9.99 ± 0.18%. According to Steele (2004), moisture content of dried sample must be below 10% to increase shelf life and to prevent microbiological contamination. Furthermore, the lower moisture content may be due to the longer drying time applied in this research, which was 2 days. This result also indicated that the drying process was sufficient.

Table 3 Chemical composition of shrimp shell powder

Parameter	Content (%)
Moisture content (wet basis)	5.61 ± 0.49
Ash content (wet basis)	37.24 ± 0.54
Protein content	11.60 ± 0.20

Based on Table 3, the ash content of the shrimp shell powder was 37.24 + 0.54%. According to Sillanpaa and Ncibbi (2017), ash content of shrimp shell waste is around 30-50%, therefore the result obtained was in accordance with previous study. Moreover, the protein content of shrimp shells powder obtained in this research (11.60 + 0.20%) was also comparable to previous research by Hossain *et al*. (2014) which was 12.73%.

Characteristics of Isolated Chitin

The chitin was isolated from shrimp shells powder through demineralization and deproteinization process. The chemical composition of isolated chitin can be seen at Table 4. The result obtained was compared to SNI 7948:2013.

e-ISSN 2598-9596

Table 4 Chemical composition of isolated chitin

Parameter	Content (%)	SNI Requirement (%)*
Moisture content (wet basis)	6.45 ± 0.29	Max 12
Protein content	0.07 ± 0.02	Max 5
Ash content (wet basis)	0.24 ± 0.02	Max 5
Degree of deacetylation	26.58	10-65
Yield (dry basis)	40.42 ± 1.01	-

Source: *BSN (2013)

Moisture content of chitin obtained in this research was higher than Sanusi (2014) which was 5.22%. This is because Sanusi (2014) used higher drying temperature, *i.e.,* 80^{o}C for 24 hours. However, drying at lower temperature of 50^{o}C for 24 hours that was done in this research resulted in moisture content lower than 12%. Therefore, the isolated chitin was still in accordance with SNI requirements.

The protein content was measured for isolated chitin to determine the efficacy of deproteinization process. According to Table 4, the protein content of isolated chitin obtained in this research was lower than Isa *et al*., (2012), Islam *et al.* (2016), and Halim *et al.* (2018) which were 4.16%, 3.33%, and 2.16%, respectively. This result shows that the deproteinization process in this research was more efficient to remove to the protein from shrimp shells powder.

Furthermore, the ash content was accounted for in order to see the effectivity of the demineralization process that was done on the shrimp powder. A lower ash content indicates that the chitin contains fewer minerals, showing a higher level of purity. Based on Table 3, ash content obtained in this research was lower compared to previous research done by Isa *et al.* (2012), which was about 5.6% and comparable to Halim *et al.* (2018), which was about 0.67%. However, this result shows that the demineralization process in this research was more efficient to remove to the protein from shrimp shells powder.

Degree of deacetylation (DD) is measured to ensure that demineralization and deproteinization process resulted in chitin and not chitosan, which is the deacetylated form of chitin. According to Arbia *et al.* (2012), chitin had a DD value of less than 50%, while chitosan had a DD value of higher than 50%. According to Table 3, DD value obtained in this research was 26.58% which is within the range of DD set by SNI (10-65%).

Furthermore, the yield of isolated chitin obtained in this research (40.42 + 1.01%) was similar to the study done by Alabaraoye *et al*. (2017), which was 40.89%. According to Kaimudin *et al*. (2016), there are 40-60% chitin (dry basis) found in the exoskeleton of shrimp. Therefore, it can be stated that the result obtained was in accordance with previous study.

Chitinolytic Activity

Chitinolytic index for both *Providencia stuartii* and *Salmonella*

e-ISSN 2598-9596

enterica were measured to ensure that these bacteria possess the chitinolytic activities. The chitinolytic index of *Providencia stuartii* and *Salmonella enterica* can be seen at Table 5.

Table 5 Chitinolytic index

Bacteria	Chitinolytic Index		
	Day 1	Day 2	Day 3
Providencia stuartii	1.77 ± 0.03	2.17 ± 0.09	1.43 ± 0.12
Salmonella enterica	1.52 ± 0.15	1.89 ± 0.16	1.36 ± 0.02

According to Table 5, *Providencia stuartii* had higher chitinolytic index compared to *Salmonella enterica,* with the highest activity was observed after 2 days of fermentation. This result is in accordance with the result obtained by Hardoko *et al.* (2020) which stated that *Providencia stuartii* had the highest chitinolytic index (4.46) out of all the bacteria isolated from shrimp shell powder. The bacteria with the highest chitinolytic index were *Providencia stuartii* because have chitinase enzyme type A, C1 and chitobiase which makes them able to break down chitin (Hardoko *et al.,* 2020).

On the other hand, *Salmonella enterica* is categorised as lysozyme-like domain when it comes to their chitinase enzyme (Frederiksen *et al.,* 2013). This means that *S.enterica* does not actually have chitinase but instead have lysozymes or bifunctional chitinases. Therefore, the breakdown of chitin by lysozymes becomes less efficient.

Effect of Different pH and Bacterial Ratio of *Providencia stuartii* and *Salmonella enterica* on N-acetylglucosamine Production

The result of fermentation using different pH and bacterial ratio of *Providencia stuartii* and *Salmonella enterica* can be seen in Figure 1. Statistical analysis using Univariate analysis showed that different pH and bacterial ratio significantly affected the concentration of N-acetylglucosamine produced from fermentation ($p<0.05$) Moreover, the interactions between pH and bacterial ratio also significantly affected the concentration of N-acetylglucosamine produced from fermentation ($p<0.05$).

e-ISSN 2598-9596

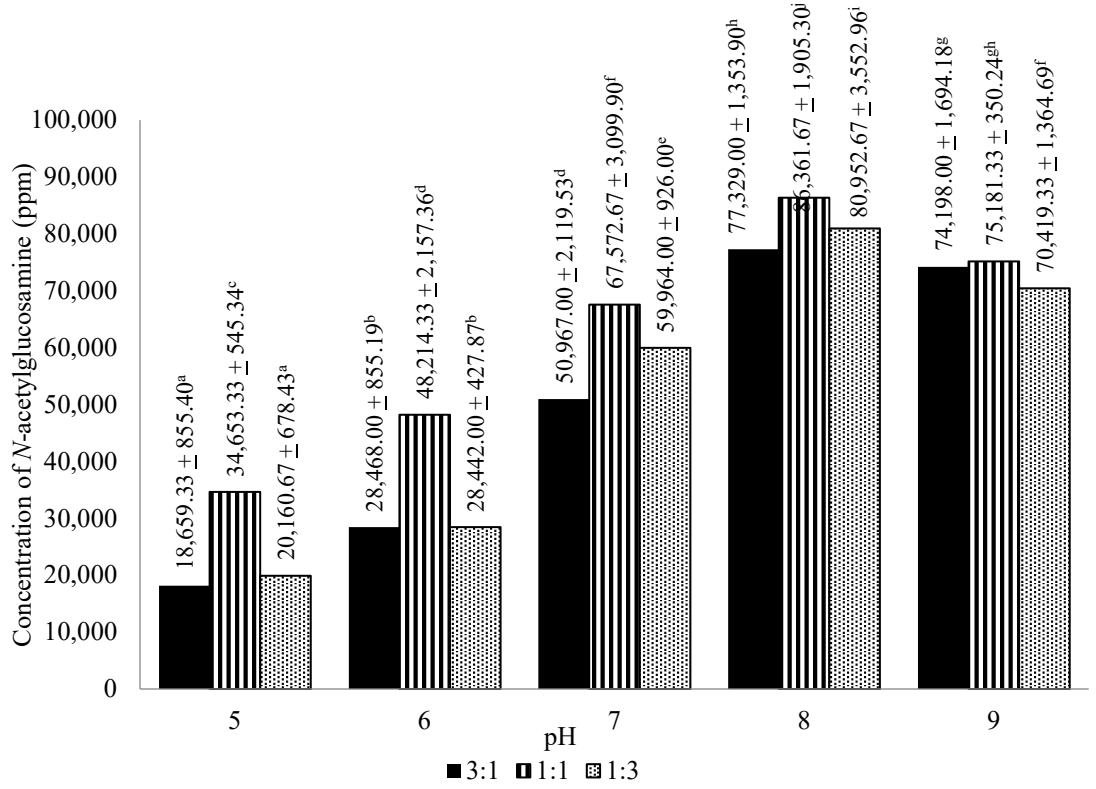

Figure 1. The effect of bacterial ratio and fermentation pH on production of *N*-acetylglucosamine
Note: The different notations (letters) indicating a significant difference ($p<0.05$)

Figure 1 shows that the lowest *N*-acetylglucosamine production is at fermentation pH of 5 with bacterial ratio of 1:1, which was 34,653.33 ± 545.34 ppm, meanwhile the highest N-acetylglucosamine produced was obtained at pH 8 with bacterial ratio of 1:1, *i.e.*, 86,361.67 ± 1,905.30 ppm. Figure 1 also shows that the N-acetylglucosamine production increases as the pH increases until reaches the highest point of pH 8 and then decreases at pH 9. According to Podolak *et al.* (2010), *Salmonella enterica* can grow at a wide range of pH from 5.2 to 9.5. Meanwhile, *Providencia stuartii* can grow at pH ranging 6-9 (Khatib *et al.,* 2017). Therefore, the decrease of N-acetylglucosamine production at pH 9 may be due to partial deacetylation of chitin to chitosan (Younes and Rinaudo, 2014). Partial deacetylation of chitin can occur at high pH (>9) and exposure to heat (>70ºC) for a long time (>24 hours) (Cleo *et al.*, 2013). In this research, the exposure to heat might happen during sterilization of media prior to fermentation process. Therefore, less chitin in the fermentation medium means fewer resources for the bacteria to degrade, resulting in reduced N-acetylglucosamine production at pH 9.

Furthermore, the bacterial ratio that obtained the highest N-acetylglucosamine production was ratio 1:1 (*Providencia stuartii:Salmonella enterica*). These

findings indicate a potential synergistic interaction between *Providencia stuartii* and *Salmonella enterica*. However, *Salmonella enterica* replicates faster compared to *Providencia stuartii* because *Salmonella enterica* reaches optimum cell growth after 8 hours compared to *Providencia stuartii* after 18 hours (Nguyen *et al.,* 2016). However, the plausible reason for bacterial ratio 1:1 (*Providencia stuartii:Salmonella enterica*) to produce the highest N-acetylglucosamine may be because although *Salmonella enterica* replicates faster, they possess lower chitinolytic activity compared to *Providencia stuartii* as can be seen on Table 5.

Effect of Different Fermentation Time and Temperature on *N*-acetylglucosamine Production

The production of N-acetylglucosamine using different fermentation time and temperature was performed using fermentation media with pH 8 and ratio between *Providencia stuartii* and *Salmonella enterica* of 1:1. The result of fermentation using different incubation time and temperature can be seen in Figure 2.

Statistical analysis using Univariate analysis shows that different fermentation time and temperature significantly affected the production of N-acetylglucosamine ($p < 0.05$). Moreover, the interactions between fermentation time and temperature also significantly affected the N-acetylglucosamine production.

Figure 2 shows that the highest N-acetylglucosamine production was obtained at fermentation temperature of 37ºC for 3 days which was 87,707.04 ± 906.54 ppm. This is because according to Oscar (2008), *Salmonella enterica* optimum incubation temperature is at 37ºC. *Salmonella enterica* can still grow at temperature 40ºC but with a longer lag time and will die when the temperature exceeds 50ºC (Hudson *et al.,* 2011). Meanwhile, *Providencia stuartii* optimum incubation temperature is also at 37ºC (Khatib *et al.,* 2017). Furthermore, the growth of *Providencia stuartii* increases when temperature increases up until the optimum temperature (37ºC) and decreases when temperature is higher than 37ºC (Ayangbenro, 2017).

Figure 2 also shows that the production of N-acetylglucosamine was the highest at day 3. It is because both bacteria have a stationary phase up to three days (Rychlik *et al*., 2002; Kurmasheva *et al.,* 2018). Therefore, the production of N-acetylglucosamine decreased after 4 days of fermentation because both bacteria have undergone the death phase. Another possible reason is the limited availability of chitin substrate after 4 days of fermentation.

e-ISSN 2598-9596

Furthermore, the N-acetylglucosamine produced in this study was higher than that reported by Michelle (2019), who used *Salmonella enterica* in the fermentation and obtained 73,184.74 ± 752.02 ppm of N-acetylglucosamine after 3 days of incubation, but lower than that reported by Halim *et al.* (2023), who used *Providencia stuartii* in the fermentation and obtained 97,722.67 ± 391.07 ppm after 4 days of fermentation. These results suggest that combined fermentaion using both bacteria was more effective in enhancing N-acetylglucosamine production from shrimp shells' chitin.

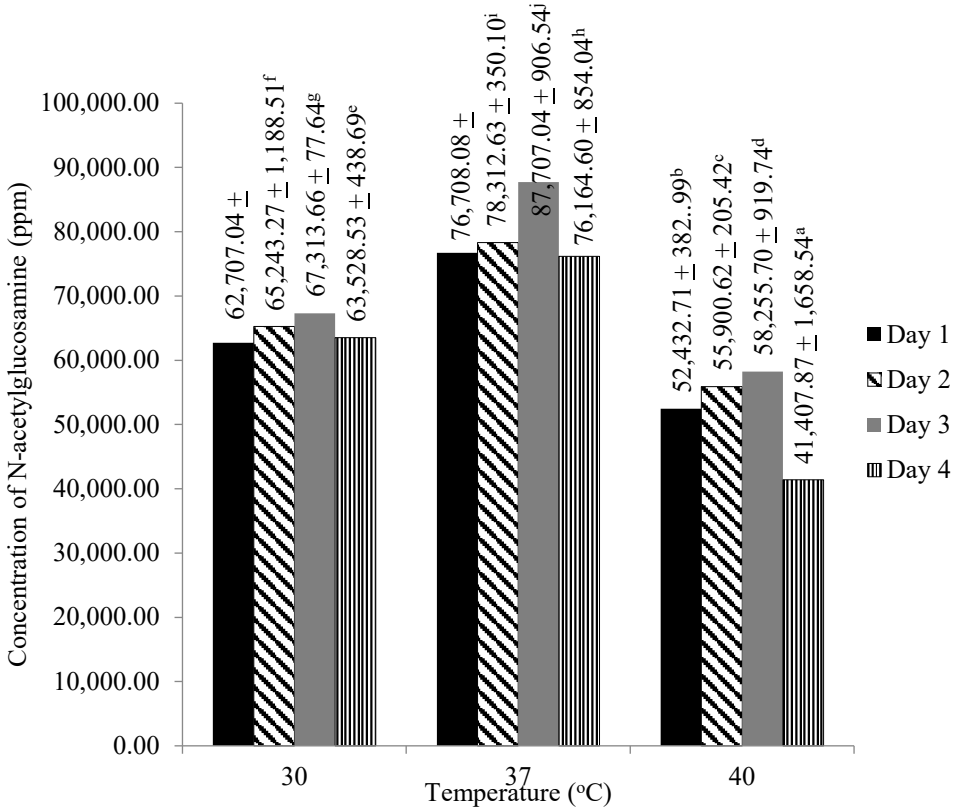

Figure 2. The effect of fermentation time and temperature on production of *N*-acetylglucosamine
Note: The different notations (letters) indicating a significant difference ($p<0.05$)

CONCLUSION

The optimum condition for N-acetylglucosamine production from shrimp shells' chitin through combined fermentation using *Providencia stuartii* and *Salmonella enterica* was achieved in a fermentation medium with pH 8, using ratio between *Providencia stuartii* and *Salmonella enterica* of 1:1, with fermentation temperature of 37°C for 3 days. These fermentation condition co yielded N-acetylglucosamine at the concentration of 87,707.04 ± 906.54 ppm.

ACKNOWLEDGMENT

The authors would like to thank Microbiology Laboratory and Food Quality

Control Laboratory at Universitas Pelita Harapan for providing the necesarry facilites.

REFERENCES

Agrawal, T., & Kotasthane, A. S. (2012). Chitinolytic assay of indigenous *Trichoderma* isolates collected from different geographical locations of Chhattisgarh in Central India. *Springerplus, 1*(1): 65-73. https://doi.org/10.1186/2193-1801-1-73

Aiba, S. (2009). Chemical and enzymatic modification of chitin and chitosan towards functional materials. In Lambertus A. M., van den Broek, C., & Boeriu, G. (Eds). *Chitin and Chitosan: Properties and Applications* (pp. 1-18). Institute for Biological Resources and Functions.

Alabaraoye, E., Achilonu, M., & Hester, R. (2017). Biopolymer (chitin) from various marine seashell wastes: isolation and characterization. *Journal Polymer Environment, 26*(7), 120-132. https://doi.org/10.1007/s10924-017-1118-y

Amalia, R., Rejeki, S., Widowati, L. L., & Ariyati, R. W. (2022). The growth of tiger shrimp (*Penaeus monodon*) and its dynamics of water quality in integrated culture. *Biodiversitas, 23*(1), 593-600. https://doi.org/10.13057/biodiv/d230164

AOAC. 2005. *Official Methods of Analysis*. The Association of Official Analytical Chemists, Gaithersburg.

Arbia, W., Arbia, L., Adour, L., & A. Amrane, A. (2012). Chitin extraction from crustacean shells using biological methods – a review. *Food Technology Biotechnology, 51*(1), 12-25.

Ayangbenro, A. S. (2017). Biodegradation of natural bitumen by *Providencia stuartii* isolated from heavy oil contaminated soil. *Global NEST Journal, 19*(2), 353-358. https://doi.org/10.30955/gnj.002148

Badan Pusat Statistik (BPS). (2018). *Statistik Perusahaan Perikanan 2017.* Retrieved May 5, 2019 from https://www.bps.go.id/publication/2018/10/22/78d5e2542ad793f3fbe4ecc1/statistik-perusahaan-perikanan-2017.htm

Benhabiles, M. S., Salah, R., Lounici, H., Drouiche, N., Goosen, M. F. A., & and Mameri, N. (2012). Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste. *Food hydrocolloids, 29*(1), 48-56. https://doi.org/10.1016/j.foodhyd.2012.02.013

Cleo, T. P., Vilela, J., & Airoldi, C. (2014). The effect of chitin alkaline deacetylation at different condition on particle properties. *Procedia Chemistry, 9,* 220-225. https://doi.org/10.1016/j.proche.2014.05.026

Dompeipen, E. J., Kaimudin, M., & Dewa, R. P. (2016). Isolasi kitin dan kitosan dari limbah kulit udang. *Majalah Biam, 12*(1), 32-38.

Frederiksen, R. F., Paspaliari, D. K., Larsen, T., Storgaard, B. G., Larsen, M. H., Ingmer, H.. Palcic, M. M., & Leisner, J. J. (2013). Bacterial chitinases and chitin-binding proteins as virulence factors. *Microbiology, 159*, 833-847. https://doi.org/10.1099/mic.0.051839-0

Halim, Y., Hardoko, & Christy, A. (2018). Optimum conditions for N-acetyl glucosamine production from *Penaeus monodon* shrimp shells by solid state fermentation using *Trichoderma virens. Asian Journal of Microbiology, Biotechnology, and Environmental Sciences, 20*(4), 1081-1088.

Halim, Y., Tantradjaja, S. F., Hardoko, & Handayani, R. (2021). Immobilization of Providencia stuartii cells in papaya

trunk wood for N-acetylglucosamine production from Pennaeus vannamei shrimp shells. Jurnal Ilmiah Perikanan dan Kelautan, 13(2), 208-221. http://doi.org/10.20473/jipk.v13i2.28011

Halim, Y., Handayani, R., Ayu, N., Lamtoro, S. T., & Hardoko. (2023). Penentuan kondisi fermentasi dalam produksi N-asetilglukosamin dari kulit udang menggunakan bakteri *Providencia stuartii. Jurnal Sains dan Teknologi Pangan, 8*(5), 6604-6617.

Hardoko, Josephine, C., Handayani, R., & Halim, Y. (2020). Isolation, identification and chitinolytic index of bacteria from rotten Tiger shrimp (*Penaeus monodon*) shells. *AACL Bioflux, 13*(1), 360-371.

Hossain, M. S., & Iqbal, A. (2014). Production and characterization of chitosan from shrimp waste. *Journal Bangladesh Agriculture University*, *1*(12), 153-160. https://doi.org/10.3329/jbau.v12i1.21405

Hudson, J. A., Olsen, L., & Cook, R. (2011). Maximum Growth Temperatures of foodbourne pathogens and appropriate temperatures for hot holding. Publications Logistics Officer, Wellington.

Isa, M. T., Ameh, A. O., Gabriel, J. O., &Adama, K. K. (2012). Extraction and characterization of chitin from Nigerian sources. *Leonardo Electronic Journal of Practices and Technologies*, *21*, 73-81.

Islam, S. Z., Khan, M. & Noswad, A. K. M. A. (2016). Production of chitin and chitosan from shrimp shell wastes. *Journal Bangladesh Agriculture University, 14*(2), 253-259. https://doi.org/10.3329/jbau.v14i2.32701

Kaimudin, M., & Leounupun, M. F. (2016). Karakterisasi kitosan dari limbah udang dengan proses bleaching dan deasetilasi yang berbeda. *Majalah Biam, 12*(1), 1-7.

Keerthirathne, T. P., Ross, K., Fallowfield, H., & Whiley, H. (2016). A review of temperature, pH and other factors that influence the survival of *Salmonella* in mayonnaise and other raw egg. *Pathogens, 5*(63), 1-11. https://doi.org/10.3390/pathogens5040063

Khatib, M. E., Tran, Q. T., Nasrallah, C., Lopes, J., Bolla, J. M., Vivaudou, M., Pages, J. M, & Colletier, J. P. (2017). *Providencia stuartii* forms biofilms and floating communities of cells that display high resistance to environmental insults. *PLOS One*, *12*(3), 1-17. https://doi.org/10.1371/journal.pone.0174213

Kurmasheva, N., Vorobiev, V., Sharipova, M., Efremova, T., & Mardanova, A. (2018). The potential virulence factors of *Providencia stuartii*: motility, adherence, and invasion. *BioMed Research International, 2018*, 1-8. https://doi.org/10.1155/2018/3589135

Liu, L., Liu, Y., Shin, H. D., Chen, R., Li, J., Du, G., & Chen, J. (2013). Microbial production of glucosamine and *N*-acetylglucosamine: advances and perspectives. *Applied Microbial Biotechnology, 97*(14), 6149-6158. https://doi.org/10.1007/s00253-013-4995-6

Marganof, P. (2003). Potensi limbah udang sebagai penyerap logam berat (timbal, kadmium dan tembaga) di perairan [Bachelor Thesis]. Institut Pertanian Bogor, Bogor, Indonesia.

Michelle, V. (2019). Production of nN-acetylglucosamine from chitin extracted from shrimp shells by fermentation using *Salmonella enterica* strain LT2 [Bachelor Thesis]. Universitas Pelita Harapan, Tangerang, Indonesia.

Nguyen, H. H., Yi, S. Y., Woubit, A., & Kim, M. (2016). A portable surface plasmon resonance biosensor for rapid

detection of *Salmonella typhimurium*. *Applied Science Converging Technology*. 25(3): 61-65. https://doi.org/10.5757/ASCT.2016.25.3.61

Nielsen, S. 2009. *Food Analysis*. Springer, USA. https://doi.org/10.1007/978-1-4419-1478-1

Oscar, T. P. (2009). Predictive model for survival and growth of *Salmonella Typhimurium* DT104 on chicken skin during temperature abuse. *Journal of Food Protection, 72*(2), 304-314. https://doi.org/10.4315/0362-028X-72.2.304

Pan, S., Wu, S., & Kim, J. (2011). Preparation of glucosamine by hydrolysis of chitosan with commercial α-amylase and glucoamylase. *Journal of Zhejiang University*, *12*(11), 931-934. https://doi.org/10.1631/jzus.B1100065

Podolak, R., Enache, E., Stone, W., Black, D. G., & Elliott, P. H. (2010). Sources and risk factors for contamination, survival, persistence and heat resistance of *Salmonella* in low-moisture foods. *Journal of Food Protection, 73*(10), 1919-1936. https://doi.org/10.4315/0362-028X-73.10.1919

Rahmawati, W., Dian, H., & Husniati. (2012). Produksi kitosan dari bahan baku cangkang udang menggunakan metode kimia dan enzimatis dengan enzim kitin deasetilase [Bachelor Thesis]. Universitas Lampung, Lampung, Indonesia.

Rychlik, I., Martin, G., Methner, U., Lovell, M., Cardova, L., Sebkova, A., Sevcik, M., Damborsky, J., & Barrow, P. A. (2002). Identification of *Salmonella enterica* serovar typhimurium genes suppression in stationary-phase nutrient broth cultures and in the chicken intestines. *Archives of Microbiology, 178*(6), 411-420. https://doi.org/10.1007/s00203-002-0458-7

Sanusi, M. (2004). Transformasi kitin dari hasil isolasi limbah industri udang beku menjadi chitosan. *Marina Chimica Acta, 5*(2), 28-32.

Saskiawan, I., & Handayani, R. (2011). Production of *N*-acetyl-D-glucosamine by submerged fermentation from chitin. *Berita Biology, 6,* 20-28.

Setia, I. N. (2015). Chitinolytic assay and identification of bacteria isolated from shrimp waste based on 16S rDNA sequences. *Advances in Microbiology, 5*, 541-548. http://dx.doi.org/10.4236/aim.2015.57056

Sillanpaa, M., & Ncibbi, C. (2017). *A sustainable bioeconomy: the green industrial revolution*. Springer International Publishing, New York.

Steele, R. (2004). *Understanding and measuring the shelf-life of food*. Woodhead Publishing Limited, Florida. https://doi.org/10.1533/9781855739024

Tanaka, T., Fukui, T., & Imanaka, T. (2001). Different cleavage specificities of the dual catalytic domains in chitinase from the hyperthermophilic archaeon *Thermococcus*. *Journal of Biological Chemistry, 276*(38), 35629-35635. https://doi.org/10.1074/jbc.M105919200

Ulfa, M. (2016). Penentuan kadar glukosamin dari fermentasi kulit udang windu (Penaeus monodon) dengan metode hidrolisis dan pemanasan [Bachelor Thesis]. Universitas Lampung, Lampung, Indonesia.

Wang, W. P., Dua, Y. M., Qiu, Y. L., Wang, X. Y., & Hu, Y. J. (2008). A new green technology for direct production of low molecular weight chitosan. *Carbohydrate Polymers, 74*(1), 127-132. http://dx.doi.org/10.1016/j.carbpol.2008.01.025

Wanichpongpan, P., & Attasat, S. (2016). Optimum conditions for preparation of

glucosamine hydrochloride and glucosamine sulfate from shrimp-shell chitin. *International Journal of Applied Science and Technology, 6*(2), 24-29.

Younes I., & Rinaudo, M. (2014). Chitin and chitosan preparation from marine sources. *Structure, Properties and Application Marine Drugs, 13*(3), 1133-1174. https://doi.org/10.3390/md13031133