DOI: http://dx.doi.org/10.33846/hn60205
http://heanoti.com/index.php/hn

RESEARCH ARTICLE
URL of this article: http://heanoti.com/index.php/hn/article/view/hn60205

An Analysis of Characteristics and Inhibition of Vaname Shrimp Chitosan on *Streptococcus mutans*

I Gusti Agung Ayu Dharmawati[1(CA)], Nyoman Mastra[2], Ni Nyoman Dewi Supariani[3], I Wayan Juniarsana[4]
[1(CA)]Department of Medical Laboratory Technology, Poltekkes Kemenkes Denpasar, Indonesia; ayu_dharmawati@yahoo.com (Corresponding Author)
[2]Department of Medical Laboratory Technology, Poltekkes Kemenkes Denpasar, Indonesia; nyoman_mastra@yahoo.co.id
[3]Department of Dental Health, Poltekkes Kemenkes Denpasar, Indonesia; dewisupariani66@gmail.com
[4]Department of Nutrition, Poltekkes Kemenkes Denpasar, Indonesia; wyjuniarsana9@gmail.com

ABSTRACT

Indonesia is a maritime country with abundant marine products, but the processing of marine animal waste has not been a concern, the shell contains chitosan as a multifunctional biopolymer. Chitosan is a proven biomaterial that is non-toxic, biocompatible, and biodegradable compared to other polymers. The purpose of this study was to find out the content of Vaname shrimp chitosan and its inhibition against *Streptococcus mutans* bacteria. An experimental study was used, with a post test only control group design with the following treatments: control, 16 mg chitosan, 20 mg chitosan. Inhibitory power data (milli meter) was tested with *One Way Anova*. The research was carried out at the RAN Denpasar Laboratory, the Laboratory of the Faculty of Nutrition (Public Health, Airlangga University, Faculty of Pharmacy, Chemistry, Unair), UPT Analytical Laboratory Unud, and Microbiology Laboratory Unair. Results of this study indicated the content of carbohydrates, nitrogen, water, ash, protein, fat and the degree of deacetylation. Chitosan has an inhibitory effect on *Streptococcus mutans* bacteria. The quantity and quality of chitosan in Vaname shrimp, mets the requirements specified by Protan Biopolymer and was useful as an antimicrobial.

Keywords: chitosan; vaname shrimp; characteristics; inhibition

INTRODUCTION

Indonesia was a maritime country with abundant marine products, but the processing of waste extraction from marine animal shells such as crustaceans is still not utilized. So far, crustaceans are only used for their flesh, while the shell containing chitosan is not used properly. Bali as a tourism destination has prepared many supporting facilities for the advancement of tourism with the presence of restaurants to prepare the culinary needs of tourists, including dishes in the form of shrimp. To meet the supply of shrimp, restaurant entrepreneurs cooperate with shrimp farmers, so that many shrimp farmers have emerged in Bali[(1)]. Chitosan is an extract from the skin of hard-skinned animals such as shrimp and crabs. Chitosan is a chitin-derived biomaterial that is still being developed today, because it is proven to be non-toxic, biocompatible, and biodegradable compared to other polymers, so chitosan is very useful in the biomedical field[(2)]. Chitosan is a derivative compound from the deacetylation of chitin which is widely contained in marine animals such as shrimp and crabs, chitosan is a multifunctional biopolymer because it contains three reactive functional groups, namely amino acids at C2, primary hydroxyl groups at C3 and secondary at C6, causing chitosan to have chemical reactivity. tall one. Chitosan also has superior properties with an LD_{50} (Lethal Dose 50%, which is the dose threshold in 50% of the sample) of 16gr/kg BW[(3)].

According to the Household Health Survey conducted that dental and oral disease is the 6th highest disease complained of by Indonesian people[(4)]. This is evidenced by the presence of 60% of the Indonesian population still experiencing dental and oral diseases[(5)]. Patients with dental caries in Indonesia have a prevalence of 50-70% with the most sufferers being children under five[(4)]. Dental caries is an infection of the teeth caused by *Streptococcus mutans* bacteria which causes demineralization of the tissue, causing localized damage to the tissue. Dental caries begins with the formation of dental plaque which is tightly attached to the tooth and gingival surfaces and has a large enough potential to cause disease in the hard tissues of the teeth[(6)]. This situation is caused by plaque containing various kinds of bacteria with various metabolic products. The previously study also reported that using chlorhexidine and povidone iodine as mouthwash to suppress the development of plaque-forming bacteria. Chlorhexidine not only has bactericidal and bacteriostatic effects, but also has side effects, namely discoloration of the teeth and tongue and impaired taste after every rinse[(7)].

The aim of current study was to conducting chitosan-based research as a product for dental health care. Therefore, the researchers tried to make chitosan from shrimp shells and tested the quality, quantity and inhibition of *Streptococcus mutans* bacteria contained in chitosan of Vaname shrimp produced by shrimp farmers in Bali.

METHODS

The research method was an experimental study, with a post test only control group design with control treatment, 16 mg chitosan, 20 mg chitosan (dissolved with 1% acetyl acetate). Chitosan of Vaname shrimp produced by shrimp farmers were collected from local area of Denpasar district-Bali, and authenticated by an expert from Universitas Udayana, Bali.

Population of this study was *Streptococcus mutans* bacteria, sample size was taken by *simple random sampling* technique (using the Federer formula). Tested on *Streptococcus mutans* bacteria (equivalent to 0.5 Mc Farland) which was cultured on MHA (Mueller Hinton Agar) by diffusion method. Inhibitory power data (milli meter) was tested with *One Way Anova*. The research was carried out at the RAN Denpasar Laboratory, the Laboratory of the Faculty of Nutrition (Public Health, Airlangga University, Faculty of Pharmacy, Chemistry, Unair), UPT Analytical Laboratory Unud, and Microbiology Laboratory Unair.

This study obtained ethical clearance from the Health Polytechnic of Denpasar, Ministry of Health Denpasar. The time of the study began on April 1st to August 31st, 2021.

RESULTS

Qualitative and Quantitative Test of Vaname Shrimp Chitosan

Table 1. Quality and quantity test results on vaname shrimp chitosan ponds in Bali

Parameters	Result
Carbohydrat (%)	68.54
Nitrogen (%)	3.07
Water (%)	10.75
Ash (%)	0.69
Protein	29.24
Fat	6.18
Degree of Deacetylation	77.60

The Chitosan content of Vaname shrimp in the form of carbohydrates, nitrogen, water, ash, protein, fat and the degree of deacetylation that meets the requirements of Protan Biopolymer.

The Inhibition Test of White Shrimp Chitosan against *Streptococcus mutans*

Table 2. Mean inhibition of chitosan against *Streptococcus mutans* in each group

Group	n	Mean ± SD	*p*
Control	5	0.000 ± 0.00	
Chitosan 16 mg	5	13.720 ± 0.94	0.000
Chitosan 20 mg	5	16.880 ± 0.64	

Analysis by the One Way Anova Test, * significant at p <0.05

There was a significant difference in inhibition between treatment groups (p <0.05). The average inhibition against *Streptococcus mutans* showed that 20 mg Chitosan had the highest inhibitory power.

Table 3. Mean inhibition of chitosan against *Streptococcus mutans* bacteria between groups

Group	Mean diff.	p
Control – Chitosan 16 mg	13.720	0.000
Control – Chitosan 20 mg	16.880	0.000
Chitosan 16 mg - Chitosan 20 mg	3.160	0.003

Analysis by the One Way Anova Test, * significant at p <0.05

There were significantly different inhibition between control group with 16 mg and 20 mg Chitosan (p<0.05). There was a significant difference in inhibition between 16 mg and 20 mg Chitosan (p <0.05).

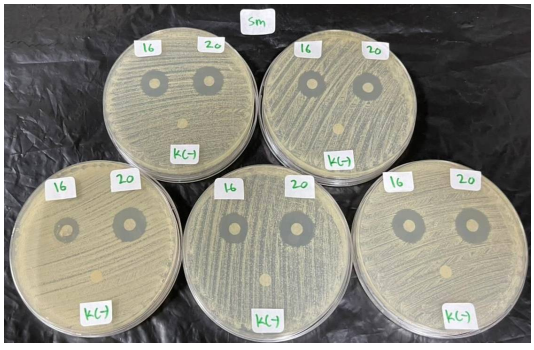

Figure 1. Inhibition zone diameter of control group with 16 mg and 20 mg chitosan

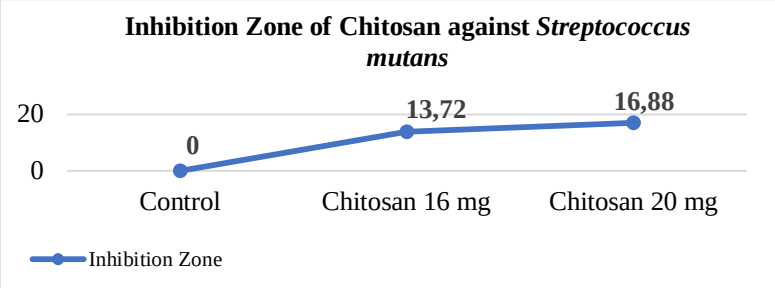

Figure 2. Inhibition zone differences of control group with 16 mg and 20 mg chitosan

DISCUSSION

The presence of carbohydrates, nitrogen, water content, ash content, protein, fat, and the degree of deacetylation showed in Table 1. The carbohydrate content in this study was 68.54, still lower than the results of previous study on the Characteristics of Chitosan from Waste Windu Shrimp, which was 81.39%, while from the results of the carbohydrate content of shrimp chitosan was 74.12% [8,9]. The nitrogen content of this Chitosan was 3.07%. This result meets the quality standard of chitosan as determined by Protan Biopolymer, which was 5%. Nitrogen content in chitosan determines the nature of chitosan to interact with other groups. The higher concentration of NaOH and temperature at the time of deacetylation, the nitrogen content tended to be smaller[10].

The water content in this study was 10.75 (Table 1), still higher than the quality standard determined by Protan Biopolymer for chitosan, which was 10%. The water content produced was influenced by drying process, the length of drying carried out, amount of chitosan being dried and surface area where chitosan was dried[11]. The ash content showed a result of 0.69% which meant that the ash content obtained in this shrimp chitosan has met quality standard of chitosan. Where according to Protan Biopolymer is < 2%. The mineral removal process was influenced by the stirring process. The constant stirring process caused uniform heat so that the solvent (HCl) can bind minerals perfectly[12].

Table 1 showed the protein content obtained in this study was 29.24 lower than the results of previous study of 32.03[13]. The fat content in this study was 6.18 which was still higher than the results of of previous study on the Characteristics of Chitosan from Waste Windu Shrimp, which was 3.13%[8]. Meanwhile, the fat content in shrimp shells of 11.9 ± 1.4%. High fat content was influenced by high acid levels during demineralization and high alkaline levels during deproteinization. The higher concentration of solution is expected to be more capable of denaturing proteins, fats, pigments and organic content and releasing minerals in the material[8].

The degree of deacetylation indicated the percentage of acetyl groups that can be removed from chitin to produce chitosan. The high degree of deacetylation indicated that the acetyl group contained in chitosan is low. The less acetyl groups in chitosan, the interaction between ions and hydrogen bonds from chitosan will be stronger. The quality standard of chitosan set by Protan Biopolymer is 70%. The degree of deacetylation of chitosan was 77.60 which met the quality standard of chitosan. That the degree of deacetylation is influenced by the high concentration of NaOH and the temperature used during deproteinization, thus facilitating the termination of the acetyl group during deacetylation[10]. Chitosan from shrimp shells had the highest levels of deacetylation, solubility, viscosity and yield[14]. So it can be concluded that shrimp chitosan was superior to crab and squid chitosan. Utilization of shrimp shell waste for the production of chitin and chitosan will provide more economic and biological value, and can reduce environmental pollution.

Table. 2 showed the inhibition test of Vaname shrimp chitosan against *Streptococcus mutans* showed a significant difference in inhibition between the control group and the treatment group, namely 16 mg and 20 mg chitosan ($p=0.000 / p<0.05$). The average inhibition (Table 3) shown from the results of in vitro studies proves control (0.000 ± 0.00) that chitosan with 16 mg (3.720 ± 0.9420) and 20 mg has the largest diameter of inhibition (16.880 ± 0.64). Administration between 16 mg and 20 mg showed a significant difference in inhibition ($p=0.003/p<0.05$) with a mean difference of 3.160 mm. This proves that Vaname shrimp chitosan had an increasing inhibitory power with increasing concentration.

This study is in accordance with the research of previous study which examined shrimp chitosan as an antimicrobial against *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Escherichia coli bacteria*[15]. This study showed the ability of shrimp chitosan to inhibit these bacteria. Similarly, previous studies have proven the ability of chitosan to inhibit the growth of *Propionibacterium acne* bacteria as the cause of acne at a concentration of 0.125%[16]. Chitosan has potential as an antibacterial because it contains lysozyme enzymes and aminopolysaccharide groups that can inhibit bacterial growth. Lysozyme is an enzyme that can destroy bacterial cell walls, causing lysis of bacteria. Besides that, chitosan also has a negatively charged polycation which can inhibit the growth of bacteria[17]. The antibacterial ability of chitosan is caused by the presence of an amine functional group (-NH2) which has a very strong positive charge which can attract negatively charged amino acid molecules that form proteins in microbes[18]. The antibacterial activity of shrimp chitosan, showed almost the same results as this study[19]. The study was conducted on Gram positive and Gram negative bacteria, namely *Staphylococcus aureus* and *Salmonella paratyphi* in vitro which showed the presence of inhibition with diameters

between 16mm and 14mm. This condition is supported by the statement which states that chitosan antimicrobials are influenced by the degree of deacetylation and molecular weight. The antimicrobial activity of chitosan was caused by the interaction of positively charged molecules of chitosan with negative charge components on the walls and membranes of bacterial cells, there by disrupting normal cell metabolism. The polycation of chitosan will bind to the negative charge on the surface of the bacteria, causing leakage in the cells and forming molecular aggregation groups. The more chitosan absorbed, the more changes in the structure and permeability of bacterial cells. This condition is supported by the results of previous research proving that shrimp chitosan has an MIC (Minimum Inhibitor Concentration) between 0.1% and 1% and an effective inhibitory power on Gram negative and Gram positive bacteria[20].

CONCLUSION

The results of quality and quantity test of Vename shrimp shell chitosan, in general, from the ash content, water, nitrogen and degree of deacetylation have met the Chitosan quality standard set by Protan Bioplomer. Shrimp shell chitosan as a result of this study also still contains levels of fat, protein, carbohydrates as nutritional ingredients. Shrimp Chitosan can be used as a natural-based antimicrobial agent (with the highest inhibitory power).

REFERENCES

1. Direktorat Jendral Perikanan Budidaya. KKP Encourage Karangasem Bali to Produce Quality Shrimp Parent. 2020.
2. Hartomo BT, Firdaus FG. Utilization of Chitosan Biomaterial in Oral Surgery. B-Dent: J Kedokteran Gigi Universitas Baiturrahmah. 2020;1:63-70.
3. Rupiasih NN, Sumadiyasa M, Windari NP. Utilization of Shrimp Waste (Seawater Lobster) as a raw material for making radiographic waste filter membranes. Research Final Report. Unud. 2013.
4. Kemnkes. Indonesian Health Profile 2010. Ministry of Health of the Republic of Indonesia. 2011.
5. Komariah A. The Effectiveness of Antibacterial Nano Chitosan Against the Growth of *Staphylococcus aureus* (In Vitro). Seminar Nasional XI Penidikan Biologi FKIP UNS. 2014:371-377.
6. Restiana D, Warganegara E. Gum Jatropha (Jatropha) as an Inhibitor of *Streptococcus mutans* Bacteria in Dental Caries. Medical Journal of Lampung University, 2016;1(3):62-67.
7. Mervrayana J, Rahmatini R, Bahar E. J Ke Comparison of the Effectiveness of Mouthwash Containing Chlorhexidine with Povidone Iodine against *Streptococcus.* J. Andalas, 2015:4(1).
8. Cahyono E. Characteristics of Chitosan from Waste Windu Shrimp (*Panaeus monodon*). J Akuatika Indonesia. 2018;3(2): 96-102.
9. Iza MT., Ameh.AO., Gabriel J.O., Adama KK. 2012. Extraction and Characterization of Chitin from Nigeria. *J of practices and Technologyes.* 5(4): 73-81.
10. Sophanodora P, Benjakula S. Convertion an Utilization of from Prown shell. Depelopment of food science and technology in Southest Asia. Procedding at The 8th Asia Good Conference, 1993:17-21.
11. Saleh MR, Abdillah, Suerman E, Basja J, Indriati N. Effect of Temperature, Time and Solvent Concentration on Chitosan Extraction from Frozen Shrimp Processing Waste on Several Quality Parameters of Chitosan. J Pasca Panen Perikanan. 1994;81: 30-43.
12. Zahiruddin W, Ariesta A, Salamah E. Characteristics of Quality and Solubility of Chitosan from Silage Dregs of Windu Shrimp Head (*Panaeus monodon*). Buletin Teknologi Hasil Perikanan. 2008;11(2):140-151.
13. Nadia MLN, Suptijah P, Ibrahim B. Production and Characterization Chitosan Nano from Black Tiger Shrimp with Ionic Gelation Methods. JPHPI. 2014.;17(2).
14. Parhiban F, Balasundari S, Gopalakannan A, Rathnakumar, Felix S. Comparison of the Quality of Chitin and Chitosan from Shrimp, Crab and Squilla Waste. J Current World Environment. 2017; 12(3):672-679.
15. Suherman B, Latif M, Dewi STR. Potency of Vaname Shrimp Skin Chitosan (*L Vaname*) as antibacterial against *S epidermidis, P aeruginosa, P agnes,* and *E coli* by disk diffusion method. Media Farmasi. 2018;14(1).
16. Mariska. Chitosan Isolation from Windu Shrimp Waste (*Paneus monodon*) and Antibacterial Activity Test against *Propionibacterium acne.*Sekolah Tinggi Ilmu Farmasi Makasar. 2012.
17. Riski R. Anti Acne Cream Formulation from Chitosan Nano Particles Tiger Shrimp Shell (*Penaeus monodon*). JF FIK UINAM. 2015;3(4).
18. Sarwano R. Utilization of Chitin or Chitosan as Antimicrobial Material. Pusat Penelitian Kimia LIPI. 2010.
19. Islam MDM, Masum MDS, Mahbub KR. In Vitro Antibactery of Shrimp Chitosan Against *Salmonella Paratyphi* and *Staphylococcus aureus*. J Bangladesh Chemical Society. 2011;24(2):185-190.
20. Junior JCV, Ribeaux DR, da Silva CAA, Takaki GMDC. Physicochemical and Antibacterial Properties of Chitosan Extracted from Waste Shrimp Shells. International J of Microbiology. 2016.