The abstracts encapsulate selected MS theses of graduate students in the Department of Biochemistry and Molecular Biology, College of Medicine, University of the Philippines Manila.

Preparation and Characterization of Chitosan from the Exoskeleton of Mantis Shrimp *(Orastoquilla nepa)* and Shrimp *(Penaeus monodon)* and A Preliminary Study on their Hypocholesterolimic Activity in Rabbits

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Chitosan was prepared from the exoskeleton of mantis shrimp *(Orastoquilla nepa)* and shrimp *(Penaeus monodon),* by a process involving decalcification of the shells followed by deproteination with sodium hydoxide solution and reduction using sodium borohydride in hot, concentrated alkali solution. The product obtained was characterized using spectroscopic, physical, and chemical methods. Infrared spectroscopy revealed that the chitosan from the two sources had a broad -OH peak, an N-H peak, a sharp C-O peak, and a weak C=O peak. Confirmation of the structure of the monomeric unit of the prepared polysaccharides were done using ^{1}H and ^{13}C NMR spectroscopy. ^{1}H NMR spectroscopy gave four broad peaks while ^{13}C NMR spectroscopy gave six peaks. Integration of the ^{1}H NMR spectra gave hydrogen ratios of 5:1:5:1 which corresponds to the total number of hydrogens in each monomeric unit of the polysaccharide. The six peaks in the ^{13}C NMR spectra indicates the six carbon atoms in each monomeric unit. Chemical analyses of the chitosan isolates using titrimetric analysis showed that the deacetylation degree for the mantis shrimp chitosan was 94.63 % while that for shrimp chitosan was 94.03 %. Spectrophotometric determination of protein showed that mantis shrimp chitosan contained an average of 1.38 % protein while shrimp chitosan gave an average of 1.47 %. Viscosimetry results showed that shrimp chitosan has a greater molecular weight than mantis shrimp chitosan . The conformation of the chitosan isolates from the two sources in solution were also affected by the type of solvent used. The study showed that there was a shift in molecular conformation from spherical to globular as the ionic atmosphere of the solution was increased. When the prepared chitosan from the two sources were supplemented in the diets of rabbits previously made hypercholesterolemic, both polysaccharides exhibited plasma lowering effect that was significant as early as nine days after supplementation. The exact mechanism for the lowering of plasma cholesterol was not taken into account in this study.

Isolation, Purification and Characterization of an Anti-*Staphylococcus aureus* Compound from the Philippine Red Macro Algae *Ceratodictyon spongiosum* and its Sponge Symbiont *Haliclona cymaeformis*

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This study aims to isolate and purify the anti-S.aureus compound from the Philippine red macro algae Ceratodictyon spongiosum and its sponge symbiont Haliclona cymaeformis through a bioassay-guided purification scheme. The bioassays performed to determine antimicrobial activity were TLC bioautography assay and agar cup diffusion assay. The sample was homogenized and extracted with methanol, then modified Kupchan partitioning was performed. The Kupchan chloroform fraction exhibited the most potent activity and interesting TLC profile suggesting the presence of nitrogenous compounds upon development with chlorine-o-toluidine test. The chloroform fraction was subjected to C 18 flash column chromatography (FCC) and three successive gradients of High Performance Liquid Chromatography (HPLC). These methods led to the isolation and purification of the compound 201-1-1-1. The MS-ESI spectrum revealed a base peak of 1110.7 m/z. Infrared analysis suggested the presence of amide bonds and amines, which was verified by the bicinchoninic acid assay, and subsequent ninhydrin and chlorine-otoluidine test. The ^{1}H NMR analysis detected the presence of protons adjacent to alkanes, hydroxyl, amines and amides. The isolate 201-1-1-1 exhibited anti-S.aureus activity and is non-cytotoxic against normal mammalian AA8-CHO cells.

Preliminary Studies in some Aspects of Carotenoid Metabolism of Tiger Shrimp *(Penaeus monodon Fabricius)*

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Studies on some aspects of carotenoid metabolism of black tiger shrimp *(Penaeus monodon* Fabricius) were conducted. The reactions of carotenoid metabolizing enzyme C-3 and C-4 monooxygenase were optimized. The Enzyme Activity Factor (EAF) was used which allowed identification of four possible reactions· in the betacarotene-astaxanthin pathway, namely: a) oxygenation, b) reductive, c) reductiveoxygenation and d) reductive cleavage. An extraction solvent containing 50 roM TrisHCl buffer at pH 7.4 with 1.15% KCl and 1 Mm EDTA was found optimal for the reactions. Hepatopancreas

homogenates from live-postmolt and live-starved shrimp samples exhibited oxygenation reaction with the former having thrice activity as the latter. Homogenate coming from fresh-chilled sample showed reductive-cleavage reaction. Oxygenation and reductive reactions occurred in incubation temperature range of 27-31°C while reductive-cleavage reaction occured below 27°C or above 31°C. Incubation pH of 4-6 showed oxygenation reaction, while pH 7 and 8-10 showed reductive-oxygenation and reductive-cleavage, respectively.

The use of ^{14}C-label1ed astaxanthin. and unlabelled beta-carotene to probe enzyme activity in various reaction mixtures demonstrated enzyme mediation in both oxygenation and reductive reactions. Oxygenation reaction involving C-3 and C-4 monooxygenase was not found to require NADPH/NADH in contrast to reductive reaction. The presence of beta-carotene promoted oxygenation and reductive reactions.

Studies on effects of hypoxia demonstrated carotenoid depleting effect mediated through enhanced fecal carotenoid release, promotion of reductive-cleavage reaction, elevated hepatopancreas carotenoid levels and increased hemolymph serum protein levels. Formation of beta-doradexanthin monoester possibly coming from astaxanthin monoester was detected.

Subjection to three consecutive hypoxia challenges resulted to enhanced fecal carotenoid release and elevated hemolymph serum protein levels every after hypoxia challenge. Induction of BSX syndrome was achieved in 1-2 weeks. Posthypoxia, BSX positive shrimps contained lower total carotenoid content in different tissues like those of farmed blue shrimps.

Carotenoid supplementation during hypoxia challenge altered the carotenoid depleting effect of hypoxia. Beta-carotene supplementation (15 ppm) caused temporary suppression of carotenoid fecal release which is reversed in succeeding hypoxia challenges as well as decreased reductive-cleavage activity of hepatopancreas enzymes. Astaxanthin supplementation (20 ppm) caused initial delays in fecal carotenoid release response up to two hypoxia challenges. Regardless of carotenoid supplementation, hemolymph serum protein levels remain elevated after hypoxia challenge. Carotenoid supplementation did not totally prevent BSX development in shrimps subjected to hypoxia challenge.

Carotenoid supplementation in BSX-affected commercial ponds reduced BSX incidence with more apparent effect achieved with astaxanthin (45%) than beta-carotene. (16%). Total carotenoid content of the different tissues were higher at post-supplementation while hemolymph serum protein levels were low. Total correction of BSX-incidence was not achieved in two weeks of carotenoid supplementation.

The results obtained predicted the existence of an astaxanthin pool consisting of astaxanthin (A), astaxanthin monoester (AM), and astaxanthin diester (AD) which can be mobilized to different tissues and participate in various metabolic processes. As the physiological saturation point of the A-AM-AD pool is maintained, a slow carotenoprotein formation predisposes fecal clearance of carotenoids in excess of the saturation limit. The occurrence of BSX syndrome can be explained by the effect of hypoxia on the astaxanthin pool directly via conversion of astaxanthin monoester to betadoradexanthin monoester, and indirectly through involvement of energy and amino acid metabolism.