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REVIEW

Antimicrobial Compounds from Crustaceans and Their Applications for Extending Shelf-Life of Marine-Based Foods

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Abstract

Antimicrobial-resistant microorganisms have become a major challenge for public health and food industries because of their fast adaptability and slow response to synthetic antimicrobials. Bioactive compounds from marine sources exert various biological roles including antioxidant, antimicrobial, anti-inflammatory, anti-hypertensive, and anticancer properties. Their advantage as an antimicrobial compound is gradually be exploited, particularly in marine-based foods (MBFs), which are highly perishable since they are abundant in proteins, lipids, and other nutrients. Also, the growing demand for fresh products with prolonged shelf-life is making the MBFs industry to urgently seek the effective methods for preservation of fresh or refrigerated MBFs. Crustaceans, which are invertebrates, are valuable source of essential nutrient based on their richness in protein, carbohydrate, minerals, lipids, and vitamins. Additionally, the by-product from the processing of crustaceans could be used as an alternative source of antimicrobials, which can be employed in MBFs as natural preservatives. This review therefore revisited the recovery of antimicrobials compounds such as antimicrobial peptides, carotenoids, and chitosan derivatives from crustaceans. The uses of these crustacean antimicrobials in extending the shelf-life of MBFs are also discussed.

Introduction

Living organisms captured from the marine environment and used as food are regarded as seafood or other known as marine-based foods (MBFs) (Hosomi, Yoshida, & Fukunaga, 2012). MBFs may consist of several species of mollusks, fish, echinoderms, seaweed or macroalgae, crustaceans, which are rich sources of fat, protein, vitamins and minerals (Olatunde, Benjakul, & Vongkamjan, 2018). The requirement of these aforementioned nutrients for normal body functions and their beneficial effects on the human physiological functions as well as the brain and nervous system have been emphasized (Liao & Chao, 2009). MBFs are considered as essential in human diet based on dietary analysis, (Hu et al., 2009; Richards & Trinkaus, 2009). The awareness of the nutritional benefits of MBFs has brought about an increase in the rate of consumption. In 2017, the per capita consumption of MBFs worldwide was 20.5 kg, indicating an increase from 19.9 kg recorded in 2014 (Ye et al., 2017). Microbial spoilage in MBFs, associated with changes in both chemical and sensory properties as well as safety has continued to pose the threat in seafood industries (Gram & Huss, 1996).

According to WHO (2013), the treatment and/or control of diseases, spoilage, poisoning or infections caused by microorganisms, particularly those showing resistance to regular antibiotics/drugs is regarded as a public health goal and a major challenge to food

industries. The decreasing therapeutic effect of conventional antimicrobials is associated with the improper use and inappropriate dose of these compounds, which allow the microorganisms to develop the resistance toward them over time (León-Calvijo et al., 2015). Over the years, attention has been geared towards the searching of antimicrobial compounds, particularly from natural sources, which can be applied as a substitute for controlling both pathogenic and spoilage microorganisms. Several antimicrobial compounds from natural sources, particularly from marine organisms and plant, have shown excellent inhibition against antibiotic-resistant microorganisms (Mahlapuu, Håkansson, Ringstad, & Björn, 2016).

Crustaceans including lobster, crab, shrimp, crayfish, and krill contributed majorly to the total aquaculture production (FAO, 2018). Economically, crustaceans are highly important across the globe (FAO, 2014; Gonçalves & de Oliveira, 2016). For instance, global shrimp production, currently at roughly 6 MMT (million metric tons), is expected to increase by 4.8% between the years 2016 and 2019 (Gulzar & Benjakul, 2019). Asia is one of the most prominent consumers and producers of shrimp (Anderson, Valderrama, & Jory, 2013). In recent years, Thailand among the Asian countries has held a major share (Gulzar & Benjakul, 2019; Raju & Benjakul, 2020). For instance, annual production of shrimp has reached 4.8 million tonnes in 2016 (FAO, 2018). By-products are generated during the processing of crustaceans, which can be further transformed to other products with varying bioactivities and increased value (Gulzar & Benjakul, 2019; Sinthusamran, Benjakul, Kijroongrojana, Prodpran, & Agustini, 2018). Thus, this review gathers the recent information on the antimicrobial compounds from crustaceans with major focus on the extraction and bacterial inhibitory activities. The applications of these antimicrobials in extending the shelf-life of MBFs are also discussed.

Microbial Spoilage in Marine-Based Foods

Spoilage in MBFs is primarily due to the proliferation as well as metabolism of microorganisms, particularly specific spoilage microorganisms (SSOs), which were responsible for the production of unwanted compounds, e.g. ketones, alcohols, organic acids, biogenic amines, histamine, aldehydes, sulphides and putrescine (Kuley et al., 2017). Microorganisms derived the energy used for their growth and produce various metabolites from substances having low-molecular weight such as small peptides, free amino acids and sugars available in the fish muscle (Olatunde et al., 2018). Also, the enzymes produced by some bacteria including psychrotolerant Enterobacteria, *Aeromonas* spp, *Vibrio* spp and *Shewanella putrefaciens* have been associated with the formation of trimethylamine (TMA) mediated by the reduction of trimethylamine oxide (TMAO), which contributes greatly to fishy odor in MBFs (Lidbury, Murrell, & Chen, 2014). Enzymatic and bacterial breakdown of nucleotides such as inosine monophosphate or inosine has been reported to produce hypoxanthine in MBFs (Varlet & Fernandez, 2010; Visciano, Schirone, Tofalo, & Suzzi, 2012). Bitter taste in MBFs has been attributed to the combined production of TMA and hypoxanthine (Tikk et al., 2006).

Several factors such as type of seafoods and products, preservation methods, packaging material, storage condition, atmosphere, and salt content, etc. influence the growth of SSOs in MBFs (Gram & Dalgaard, 2002). However, geographic location, method of fishing, and the fishing ground are factors determining bacterial microbiota of fishes (Ghaly, Dave, Budge, & Brooks, 2010). Based on the different abilities of bacteria to tolerate preservation or treatment, the microflora of MBFs may vary (Bekaert, Devriese, Maes, & Robbens, 2015). In general, psychrophilic bacteria are mainly associated with spoilage in refrigerated or chilled MBFs.

Most SSOs responsible for spoilage in MBFs are Gram-negative (G-) bacteria such as *Pseudomonas*, *Shewanella putrefaciens*, *Aeromonas*, *Flavobacterium, Moraxella*, *Acinetobacter*, *Photobacterium*, and *Vibrio* (Sivertsvik, Jeksrud, & Rosnes, 2002). SSOs including *Photobacterium phosphoreum, Shewanella* and *Pseudomonas* were found as the main spoilage bacteria in MBFs (Gram & Dalgaard, 2002). *Pseudomonas* and *Shewanella* were predominant spoilage microorganisms in Asian sea bass slices packaged in air and that packaged under argon based modified atmosphere packaging (MAP) (Olatunde, Benjakul, & Vongkamjan, 2019). *Pseudoalteromonas spp., Luteimonas spp., Psychrobacter spp.*, *Aliivibrio spp.* and *Pseudomonas spp.* were identified as the main SSOs responsible for spoilage in Norway lobster (*Nephrops norvegicus*) (Bekaert et al., 2015). Reynisson et al. (2009) reported *Photobacterium phosphoreum* as the predominant SSOs in North-Atlantic cod (*Gadus morhua*) stored at refrigerated temperature.

Microflora of MBFs is constantly changing during extended storage or transportation/ processing, which allows Gram-positive (G+) bacteria to cause the spoilage (Al Bulushi, Poole, Barlow, Deeth, & Dykes, 2010). Lactic acid bacteria (LAB) and *P. phosphoreum* were also responsible for fish spoilage (Dalgaard, 2000). Spoilage in MBFs was attributed to G+ bacteria, such as *Staphylococcus*, *Corynebacterium*, *Micrococcus*, *Clostridium*, *Bacillus*, *Streptococcus,* and *Brochothrix thermosphacta* (Fall, Leroi, Cardinal, Chevalier, & Pilet, 2010). Therefore, both G- and G+ bacteria contribute to the spoilage of MFBs.

Antimicrobial Compounds

An antimicrobial is an agent that can inhibit the proliferation of microorganisms. They could either be bacteriostatic (prevent/retard the bacteria growth or metabolism) or bactericidal (inactivate/kill bacterial cell), depending on its source, concentration, and

efficiency (Olatunde et al., 2018). The mechanisms of antimicrobial compounds against both G+ and G- bacteria can be varied. However, the sensitivity between G+ or G- bacteria toward antimicrobial compounds differs. G+ bacteria show more resistivity towards antimicrobial compounds in comparison with G- bacteria. This is mainly owing to the several layers of peptidoglycan in the outer membrane of G+ bacteria as compared to the single layer of peptidoglycan in G- counterpart (Abdollahzadeh, Rezaei, & Hosseini, 2014). In general, the major mechanism of several antimicrobial compounds is their interaction with the outer membrane of a microbial cell, forming pores or blocking membrane ion gradients, consequently leading to cell death (Moravej et al., 2018). However, mechanism or mode of action of different antimicrobial compounds can be differentiated toward the target microorganisms.

Microorganisms are developing the resistance to synthetic antimicrobial compounds (Mantravadi, Kalesh, Dobson, Hudson, & Parthasarathy, 2019), therefore posing as worldwide threats for food preservation and safety (Hayashi, Bizerra, & Da Silva Junior, 2013). To circumvent this challenge, attention has been recently shifted to natural antimicrobials, which have shown promising bacteriostatic or bactericidal impacts. Medicinal plants, marine, and terrestrial organisms, including bacteria and fungi have been demonstrated as potential natural sources of antimicrobial compounds with high activity (Hayashi et al., 2013).

Antimicrobial Compounds in Crustaceans

Several compounds in crustaceans such as bioactive peptides, chitosan and its derivatives as well as carotenoids/ astaxanthin have been proven to have high antimicrobial properties against several microorganisms.

Antimicrobial Peptides and Extracts

Over the years, attention has been geared towards the development of antimicrobials, particularly from natural sources, with no evidence of inducing resistance in pathogenic microorganisms. As a consequence, those natural antimicrobials could be used as an alternative for bacteria inactivation. Among the natural antimicrobial agents, antimicrobial peptides (AMPs) have shown great potential in preventing damages caused by antibiotic-resistant microorganisms (Mahlapuu et al., 2016). AMPs, also known as host-defense peptides (HDP), are present in all forms of life, in which several forms are reported in archaea (4), fungi (13), bacteria (200), vertebrates and invertebrates (2159) and plants (343) (Kumar, Kizhakkedathu, & Straus, 2018). AMPs are short-chain peptides with positive charge and varying amino acid sequence. The amphipathic fragment of proteins isolated from plant or animal kingdom, showed the promising antimicrobial properties (León-Calvijo et al., 2015). G+ bacteria, G- bacteria, fungi, parasites, and viruses have been inhibited by AMPs (Bahar & Ren, 2013).

The selection of AMPs in clinical treatments or foods is largely due to their speed of microbial inactivation action, high selectivity, and difficulty of microorganisms in developing resistance against them (Bradshaw, 2003). AMPs are sometimes regarded as HDP (Riedl, Zweytick, & Lohner, 2011), anionic antimicrobial peptides/proteins (Harris, Dennison, & Phoenix, 2009), cationic amphipathic peptides (Groenink, Walgreen-Weterings, van't Hof, Veerman, & Nieuw Amerongen, 1999), α-helical antimicrobial peptides (Huang, Huang, & Chen, 2010), cationic host defense peptides (Brown & Hancock, 2006), cationic AMPs (Bradshaw, 2003). Commercially, many AMPs, particularly produced from microorganisms such as bacteriocins, ambicin (nisin), polymixin B and gramicidin S have been applied for clinical purposes (Yang, Lin, Sung, & Fang, 2014) or food treatment (Olatunde et al., 2018), in which high microbial inhibition is attained. However, they are associated with the high cost of production, and low yield (Juturu & Wu, 2018), To circumvent these limitations, attention has been shifted to AMPs produced from marine sources, particularly from their processing by-products.

Marine animals are a good source of AMPs such as pardaxin, misgurin, cathelicidins, defensins, hepcidin, NK-lysin, piscidin with very high promising activities (Shin et al., 2017). The production of peptides from by-products of marine-based food processing is generally carried out by chemical (acid and alkaline), enzymatic and microbial hydrolysis (Harnedy & FitzGerald, 2012). Enzymatic hydrolysis has been commonly used as the fast method for producing peptides with varying bioactivities including antimicrobial activity. Proteins are gradually cleaved by proteases from different sources (Figure 1). Due to the differences in specificity toward proteinaceous substrate, the resulting peptides can be varied. Additionally, the antimicrobial activity of AMPs can be different. Ennaas, Hammami, Beaulieu, and Fliss (2015) reported varying antimicrobial activities of AMPs derived from Atlantic mackerel via enzymatic hydrolysis with Neutrase, Protamex, papain, and Flavourzyme against *Escherichia coli* MC4100 and *Listeria innocua* HPB13. Different fractions of AMPs extracted from Nile tilapia (*Oreochromis niloticus)* by-product, which had leucine, lysine, glutamic acid, arginine, aspartic acid, alanine and glycine as the most dominant amino acids, showed varying antimicrobial properties against *Edwardsiella tarda, Yersinia ruckeri,* and *Bacillus megaterium* (Robert et al., 2015). The presence of residual chemicals and generation of some toxic substances in AMPs produced using chemical hydrolysis has limited the application of such AMPs for food application in spite of the fast process (Olatunde et al., 2018).

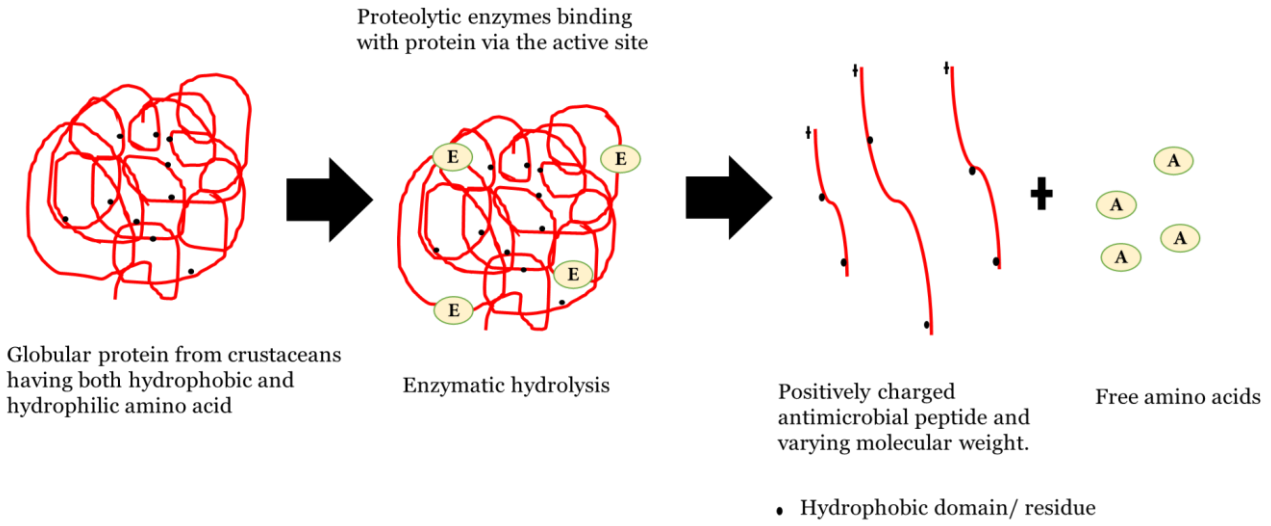

Figure 1. Enzymatic production of antimicrobial peptides from crustaceans

Antimicrobial Properties

Attention has been geared towards the antimicrobial properties of AMPs extracted from several sources as well as secondary metabolites (bacteriocins and antibiotics) generated during the fermentation of different food matrices (Linares et al., 2017). AMPs from crustacean processing byproducts have been rarely studied, as compared to other bioactivities such as antioxidants, anti-inflammatory and anticancer. Generally, AMPs have <50 amino acids (AAs) with a molecular weight <10 kDa, in which half of the AAs are hydrophobic in nature (Najafian & Babji, 2012; Zamora-Sillero, Gharsallaoui, & Prentice, 2018). Because of the charge and size of AMPs, they interact rapidly with negatively charged domain of G+ and G- bacteria cells and inactivate them (Zhang, Zou, Manchu, Zhou, & Wang, 2008). The applications of AMPs have gained massive interests in treating illness and diseases as compared to conventional antibiotics due to their high rate of bacterial inactivation and abilities to inhibit antibiotic-resistance microorganisms (Najafian & Babji, 2012).

The mechanisms of antimicrobial properties for AMPs vary, dependent on the size of the peptide, the amino acid sequence, flexibility and hydrophobic residues (Jenssen, Hamill, & Hancock, 2006). The interaction of AMPs with the microbial cytoplasmic membrane is the first mode of action for inactivating the microbial cells (Perez Espitia et al., 2012). However, cationic AMPs can also exhibit antimicrobial activities via modification of the hydrophobicity ratio or the net charge (Sila et al., 2014). Pores are formed on the microbial cells via the interaction between AMPs and microorganisms. Furthermore, these interactions could lead to the blockage of the membrane ion gradients, thereby causing microbial destruction or death (Najafian & Babji, 2012). Microorganisms are also inactivated by the ability of the AMPs in modifying the microbe's cellular metabolisms (Wald, Schwarz, Rehbein, Bußmann, & Beermann, 2016). The different modes of antimicrobials action by AMPs have been summarized in Figure 2.

AMPs from crustaceans have been studied. Zhao, Yin, Liu, and Cao (2013) purified AMPs from protein hydrolysate (PH) obtained from Antarctic krill using Protamex. The peptide had a molecular weight (MW) ranging from 245 to 709 Da and inhibited the proliferation of *Staphylococcus aureus* [minimum inhibition concentration (MIC) = 5.0 mg/mL]. The mechanism of action was attributed to the rupture of the outer membrane of bacteria, increased cell membrane permeability and the loss of intracellular substances, which led to the death of *S. aureus* (Zhao et al., 2013). G+ bacteria, including *Listeria innocua* CECT 910, *Lactobacillus helveticus* DSM 20075, and *S. aureus* CECT 240, and G- bacteria including *Escherichia coli* CECT 515, *Citrobacter freundii* ECT 401, and *Pseudomonas fluorescens* CECT 4898, were inhibited by PH derived from shrimp processing by-products prepared with the aid of an enzyme produced from *Enterococcus faecalis* DM19 and heated in the presence of glucosamine (800 mg) at 100 °C (Djellouli, López-Caballero, Arancibia, Karam, & Martínez-Alvarez, 2019). Some indigenous AMPs in the marine animal also have antimicrobial activity. Imjongjirak, Amparyup, and Tassanakajon (2011) identified 2 novel peptides (GRPSp and arasin-likeSp) from the hemocytes of *Scylla paramamosain*, which showed potent inhibition against *Vibrio harveyi*, *Aerococcus viridans, V. anguillarum,* and *Micrococcus luteus*.

The extract from crustacean is another source of antimicrobials peptides with varying properties and activities. Hemolymph extracted from male and female brachyuran crabs, *Liagore rubromaculata,* possessed strong antibacterial activity against *Salmonella typhi, V. cholerae, E. coli, Ent. Faecalis, K. oxytoca,* and *Proteus vulgaris*; the antimicrobial activity was highest toward *Proteus vulgaris* and *Ent. faecalis* for female and male crabs, respectively (Priya, Ravichandran, & Jawaharlal, 2014). B. Chen et al. (2015) reported that sphistin

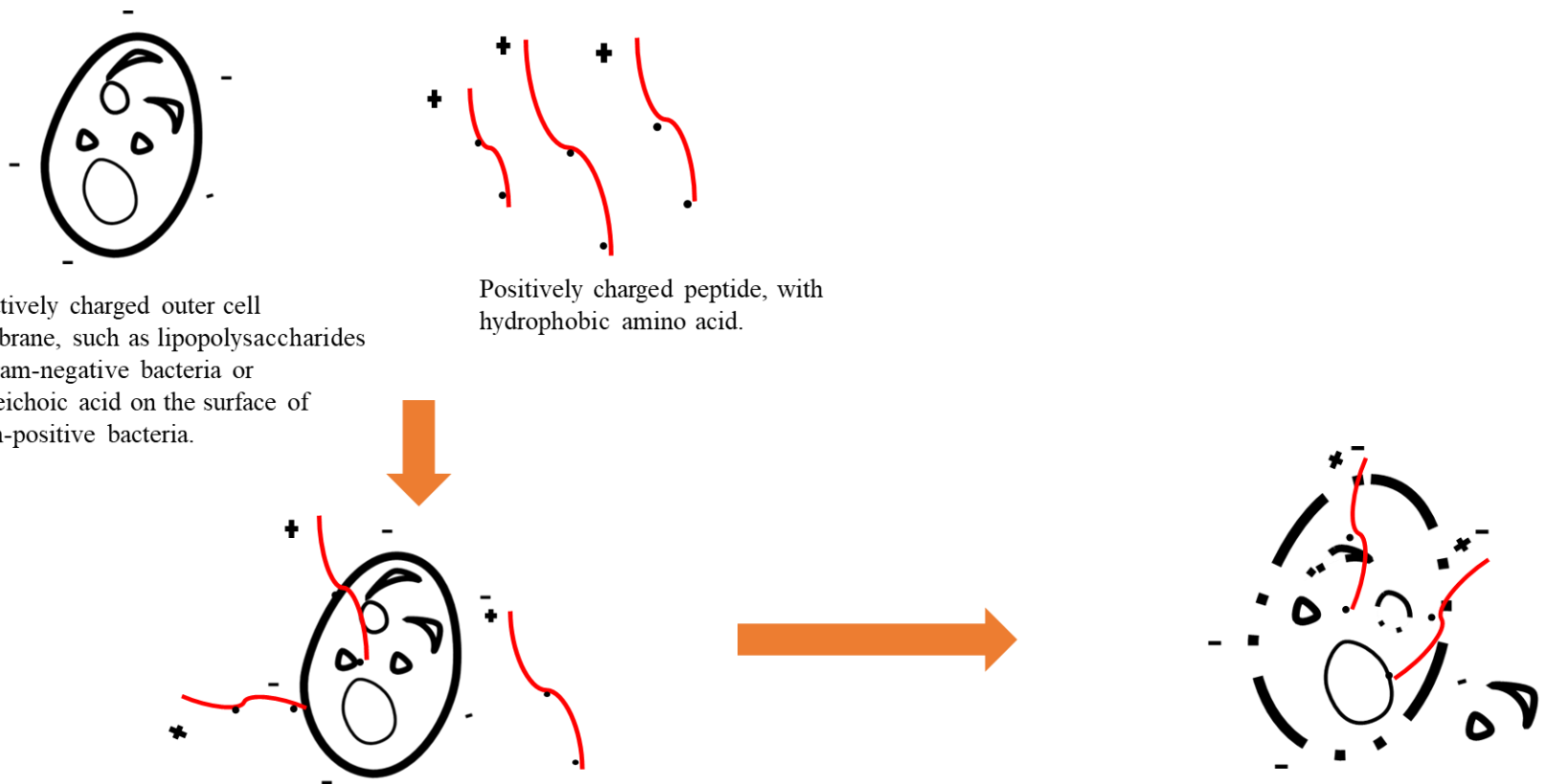

Figure 2. Mechanism of microbial inactivation by antimicrobial peptides

derived from the N-terminus of crab histone H2A found in the hemolymph of *Scylla paramamosain* showed great inhibition towards a number of G- and G+ bacteria, in which inhibition was highest for *Micrococcus luteus, Micrococcus lysodeikticus* Fleming, *Corynebacterium glutamicum, S. aureus*, *B. subtilis, P. fluorescens, Shigella flexneri,* and *P. stutzeri.* Also, sphistin was able to inhibit yeast (*Pichia pastoris and Candida albicans*). The mechanism of action was attributed to the loss of the cellular contents and disruption of the cell outer membrane induced by sphistin (B. Chen et al., 2015).

Chitosan and its Derivatives

Chitin (β-(1-4)-poly-N-acetyl-D-glucosamine) is mainly prepared from the exoskeletons of crustaceans (Kandra, Challa, & Jyothi, 2012), although chitin with varying amounts has been reported in numerous insects, mushrooms, fungi and worms (Arcidiacono & Kaplan, 1992). It occurs naturally as the ordered microfibril and serves as the main structural component in the exoskeletons of crustacean (Elieh-Ali-Komi & Hamblin, 2016). Due to high porosity, biodegradability, predictable degradation rate, non-toxicity to cells, bio-absorbability, biocompatibility, and structural integrity, the applications of chitin in various fields have gained more attention (Elieh-Ali-Komi & Hamblin, 2016; Kandra et al., 2012). Four major steps, which include 1) demineralization using EDTA, HCl or formic acid, 2) deproteinization using proteases or NaOH, 3) decolorization using solvent and 4) treatment with an oxidizing agent such as sodium hypochlorite, chlorine or hydrogen peroxide or their combination are involved during the extraction of chitin (Lodhi et al., 2014). However, due to its insolubility, it cannot be widely used and its exploitation is limited.

Chitosan (β-(1-4) linked 2-amino-2-deoxy-β-D-glucopyranose), which is prepared by the deacetylation of chitin using either chemical process (under strong alkaline condition) or enzymatic hydrolysis (chitin deacetylase) is the most widely used derivative of chitin (Elieh-Ali-Komi & Hamblin, 2016; Islam, Bhuiyan, & Islam, 2017). Chitosan (*N*-deacetylated derivative of chitin) is formed when acetamide group in chitin is converted by the aforementioned processes into primary amino groups (Islam et al., 2017). However, deacetylation is not a complete process as deacetylated chitin or chitosan still contains few acetamide groups (Dutta, Dutta, & Tripathi, 2004). The deacetylation process used determines the distribution of acetyl groups as well as the degree of deacetylation (DD) of chitosan. These two factors influence the reactivity, biodegradability, solubility and other properties of the resultant chitosan (Islam et al., 2017; Lodhi et al., 2014; Rinaudo, 2006). Chitosan is insoluble in water due to its high MW, however it can be dissolved in slightly acidic condition (1% formic or acetic acid), which is associated with the protonation of the NH_2 group of the D-glucosamine repeat unit at the C-2 position (Rinaudo, 2006). Chitosan has been widely modified chemically, enzymatically, and physically to augment its applicability and solubility (Olatunde et al., 2018).

The production of chitooligosaccharide (degree of polymerization of ≤20 and an average MW of <3900 Da) and glucosamine via the hydrolysis of chitosan has gained increasing interest, due to the improved properties of those derivatives. Several methods including enzymatic methods using non-specific enzymes (lipase, protease, lysozyme, amylase cellulose, etc.) or specific enzymes (chitinase, chitosanase, etc.), chemical methods (hydrogen peroxide oxidation, acid hydrolysis), electrochemical methods or physical methods (ultrasonic treatment, ultraviolet radiation and microwave treatment) have been employed for the production of chitooligosaccharide (COS) (Liang, Sun, & Dai, 2018). Fully deacetylated COS and partially acetylated COS with MW between 0.2 and 1.2 kDa were produced during the enzymatic hydrolysis of chitosan using neutrase and chitinase from *Trichoderma harzianum*, respectively (Santos-Moriano et al., 2019).

Antimicrobial Properties

Chitosan and its derivatives possess antibacterial properties against various spoilage and pathogenic microorganisms. Chitosan and its derivatives generally exhibit bacteriostatic rather than bactericidal activities (Goy, Britto, & Assis, 2009). Several models for the antimicrobial action of chitosan and its derivative have been proposed. Those include 1) the interaction between chitosan molecules (dissolved in acidic solution), which is positively charged and microbial cell membranes, which is negatively charged, mediated by electrostatic forces between negatively charged residues and protonated NH_3^+ groups, presumably by competing with ions, particularly Ca^{2+} for electronegative sites on the membrane surface (Goy et al., 2009; Olatunde et al., 2018), 2) the invasion of chitosan into the nuclei of the microorganism, thereby leading to inhibition of protein synthesis and messenger ribonucleic acid (mRNA) (Sebti, Martial-Gros, Carnet-Pantiez, Grelier, & Coma, 2005), and 3) the suppression of spore via their binding to essential nutrients and the chelation of metals (Figure 3) (Goy et al., 2009). *S. aureus* of clinical origin is inhibited by low MW chitosan (119.48 kDa), prepared by chemical hydrolysis (thermo-alkaline hydrolysis) of chitin dissolved in 1% acetic acid (Escárcega-Galaz, López-Cervantes, Sánchez-Machado, Brito-Zurita, & Campas-Baypoli, 2017). Unmodified chitosan and chitosan modified with different flavonoids such as flavanols, flavonols, flavones, flavanones, and isoflavones inhibited the proliferation of *B. subtilis, S. aureus, K. pneumonia,* and *P. aeruginosa.* Greater activity was recorded for flavonoid-functionalized chitosan as compared to the unmodified counterpart (Sousa, Guebitz, & Kokol, 2009).

Low MW chitosan (5.06 kDa, DD=90%) had higher inhibition against *Salmonella typhimurium, E. coli,* and

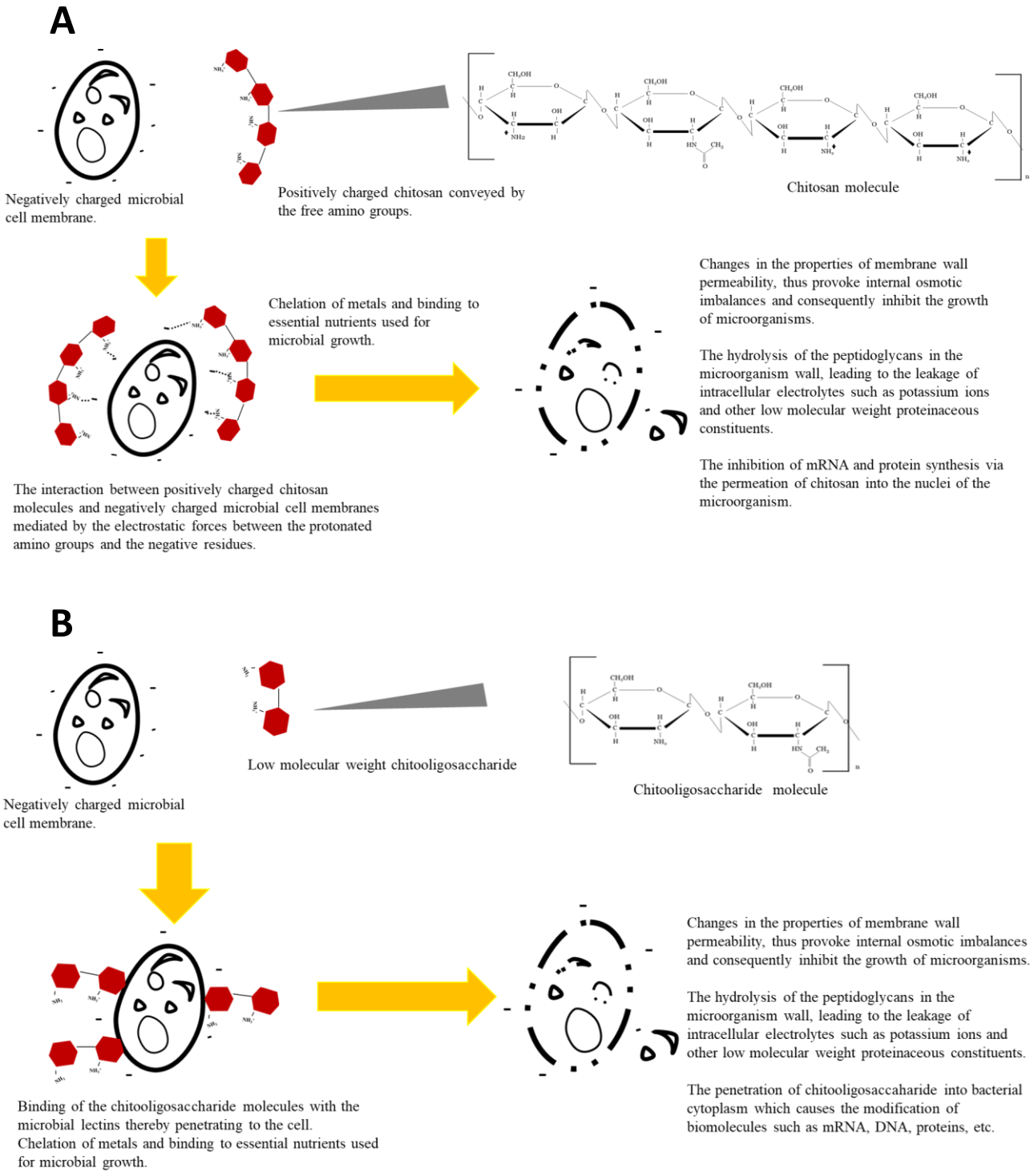

Figure 3. Mechanism of microbial inactivation by chitosan (A) and chitooligosaccharide (B)

Salmonella enteritidis as compared to high MW counterpart (14.3 and 41.1 kDa, DD=90%) (Laokuldilok et al., 2017). Nevertheless, Batista, Dantas, Santos, and Amorim (2011) documented the antimicrobial properties of high MW chitosan (CSH) and medium MW chitosan (CSM) with DD of 85% and 90.43%, respectively, dissolved in acetic acid (0.25%), against *Lactobacillus casei* ATCC 7469, *S. aureus* 319U and *E coli* ATCC 25922. Although the increased activity was attained with higher chitosan concentration, CSM exhibited greater inhibition when compared to CSH. The antibacterial action of CSM against *E. coli* was the result of the damage of the cell wall membrane and the loss of cellular components (Batista et al., 2011). The proliferation of *Escherichia coli* (NCTC 9001) and *Staphylococcus aureus* (NCTC 8532) was inhibited by chitosan with different MW (628, 591 and 107 kDa,

having DD in the range of 80-85%), which was all dissolved in 1% (v/v) acetic acid (Fernandes et al., 2008).

The antimicrobial properties of modified chitosan have also been demonstrated. G+ bacteria (*S. aureus* RCMB 010028 and *Streptococcus pneumonia* RCMB 010010), G- bacteria (*E. coli* RCMB 010052), and fungi (*Candida albicans* RCMB 05036, *Geotricum candidum* RCMB 0509, and *Aspergillus fumigatus* RCMB 02568) were inhibited markedly by Schiff base modified chitosan (acrylonitrile) as compared to unmodified chitosan (Sabaa, Elzanaty, Abdel-Gawad, & Arafa, 2018). Chitosan modified by grafting borneol 4-formylbenzoate using a stable Schiff base bond, in which the process yielded borneol-modified chitosan (BMC) inhibited the growth of *E. coli*, *B. subtilis*, and *Aspergillus niger* (Xin et al., 2020). *Pyricularia grisea, Fusarium oxysporu,* and *Alternaria solani* were all inhibited by chitosan nanoparticles, which was prepared by the addition of anionic proteins isolated from *Penicillium oxalicum* culture to chitosan solutions (Sathiyabama & Parthasarathy, 2016). Y. Chen et al. (2016) documented enhanced antimicrobial properties against *Staphylococcus aureus* ATCC6538 and *Escherichia coli* ATCC25922 for chitosan modified with sulfobetaine and quaternary ammonium. The antimicrobial properties of native chitosan against *P. aeruginosa, E. coli, B. cereus, S. aureus, Salmonella sp.,* and *Candida albicans* were boosted after chitosan was modified with indole-3-carboxaldehyde and 4-dimethylaminobenzaldehyde using the Schiff base method (Hassan, Omer, Abbas, Baset, & Tamer, 2018).

COS is another chitosan derivative with a wide range of bioactivities, including antimicrobial activity (Liaqat & Eltem, 2018). COS has been demonstrated to have antimicrobial activity and can be applied with ease due to its high water solubility. Fernandes et al. (2010) documented that low MW COS (<3 and <5 kDa) had a greater inhibitory effect against G- bacteria (*K. pneumonia, E. coli, and P. aeruginosa*) than high MW chitosan (628, 591 and 107 kDa). Lower inhibition efficacy was reported for G+ bacteria (*S. epidermidis* and *S. aureus*), which could be owing to the more rigid cell membrane of G+ bacteria. COS with a degree of polymerization (DP) of 4 and DD of 90%, produced from the hydrolysis of chitosan using chitosanase from *Pseudomonas* CUY8, subdued the proliferation of various microbial strains including *E. coli* ATCC 1150, *S. aureus* ATCC 6538P, *B. subtilis* KCTC 1028 and *Streptococcus lactis* KCTC 1950 as well as several fungi such as *Mucor circinelloides* ATCC 1216*, Saccharomyces cerevisiae* ATCC 4126, *Penicillium charlesii* ATCC 20841, *Rhodotorula bacarum* ATCC 7025, *Aspergillus niger* ATCC 1015 and *Rhizopus apiculatus* ATCC 11996 (Wang et al., 2007). Fernandes et al. (2008) stated that 2.5% solution of COS with DD in the range of 80–85%, and MW of <5 and <3 kDa, inhibited the growth of *Escherichia coli* (NCTC 9001) and *Staphylococcus aureus* (NCTC 8532). COS (MW= 8.54 kDa-8.62 kDa) produced by the enzymatic hydrolysis of chitosan (720 kDa, 86.5% DD) using crude chitinase extracted from *Astrosphaeriella sp.* BUSK 55-1 had higher antimicrobial activity against *P. aeruginosa, E. coli, B. subtilis,* and *S. aureus* as compared to that manufactured using crude chitinase extracted from *Oxydothis sp*. BUSK43-2 (Pilantanapak, Waiprib, Eadtem, & Panbangred, 2017). Table 1 summarizes the antimicrobial activity of chitosan, COS, as well as their derivatives.

Astaxanthin

Astaxanthin (ASXN) is a class of carotenoids, which is extracted from crustaceans (Mezzomo & Ferreira, 2016). In crustaceans, ASXN (3,3′-dihydroxy-β,β′-carotene-4,4′-dione) is the major lipophilic carotenoid, which is classified as xanthophyll (Hussein, Sankawa, Goto, Matsumoto, & Watanabe, 2006). The recovery of ASXN from shrimp (Asghar, Ali, Mozhgan, Parisa, & Tayebehe, 2016), crawfish (Pu, Bankston, & Sathivel, 2011) and crab (Coral-Hinostroza & Bjerkeng, 2002) have been demonstrated.

Different solvents including chloroform, methanol, acetone, hexane etc. have been used to extract ASXN from crustaceans. ASXN (0.90 mg/g oil) was found in oil extracted from the hepatopancreas of the Pacific white shrimp cephalothorax when the mixture of isopropanol and hexane (1:1, v/v) was used as the extracting medium (Takeungwongtrakul & Benjakul, 2016). Hooshmand, Shabanpour, Moosavi-Nasab, and Golmakani (2017) documented the varying amounts of carotenoids from by-products of blue crab (*Portunus pelagicus*) and shrimp (*Penaeus semisulcatus*) using hexane, isopropyl alcohol, acetone, the mixture of hexane and acetone (1:1 v/v) and the mixture of hexane and isopropyl alcohol (1:1 v/v) as the extraction media, in which the highest yields (6.63 and 61.32 µg/g for blue crab and shrimp, respectively) was recorded in the extract using acetone. ASXN (72.42 µg/g) was recovered from pink shrimp (*Parapenaeus longirostris*) shells using acetone as the extracting medium (Sila et al., 2013). Gómez-Estaca, Calvo, Álvarez-Acero, Montero, and Gómez-Guillén (2017) documented the extraction of lipid having ASXN content of 7 mg/g from shrimp waste (*Litopenaeus vannamei*) using ethyl acetate.

The recovery of ASXN from crustaceans using oil as the extraction medium has been documented. Hooshmand et al. (2017) demonstrated the recovery of carotenoids from by-products of blue crab (*Portunus pelagicus*) and shrimp (*Penaeus semisulcatus*) using sesame oil, sunflower oil, soy oil and rice bran oil as the extracting media, in which sunflower oil had the highest yields (0.21 and 4.03 µg/g for blue crab and shrimp, respectively). Regardless of the oil used, recovery of carotenoids from shrimp and blue crab by-product was higher when solvent was used (Hooshmand et al., 2017). The yield of ASXN recovered from by-product of shrimp (*Farfantepenaeus subtilis*) waste, which was previously dried at different temperatures (50-70°C) before extraction with palm olein, was decreasing with

Table 1. Antimicrobial properties of chitosan and its derivatives from crustaceans

Chitosan/its derivatives	Method of preparation	Target microorganisms	Findings	References
Chitosan	Chitosan prepared from shrimp shell chitin using 20 M NaOH. Chitosan was dissolved in 1.5 % acetic acid.	*Aspergillus niger* and *Aspergillus oryzae.*	Chitosan had higher antifungal activity when compared with the standard drug of Fluconazole.	Rajalakshmi, Krithiga, and Jayachitra (2013)
	Chitosan (DD=94.32%, MW=1052.93 g/mol) produced from shrimp waste chitin deacetylated with 60% NaOH. Chitosan was dissolved in 1M acetic acid.	*Staphylococcus aureus* (ATCC 25923), *Candida albicans, and Aspergillus niger.*	All bacteria and fungi tested were inhibited.	Kurniasih and Dewi (2018)
	Commercial chitin and chitosan dissolved in 1% acetic acid.	*Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Bacillus subtilis, Methicillin-Resistant Staphylococcus aureus, Staphylococcus aureus,* and *Micrococcus lutes.*	Proliferation of all tested bacteria was inhibited. Activity was similar to the standard drug of Fluconazole.	Gumgumjee, Shiekh, and Danial (2018)
	Chitosan prepared from chitin extracted from *Podophthalmus vigil* shell waste using 40% NaOH. Chitosan was dissolved in 1% acetic acid.	*Salmonella typhii, Pseudomonas auergunosa, Staphylococcus aeureus, Escherichia coli, Klebsiella pneumonia, Bacillus substilis. Aspergillus flavus, Aspergillus fumigatus, Candida albicans, and Penicillium citrinum.*	Antibacterial and antifungal activities were influenced by concentration.	Prabu and Natarajan (2012)
	Chitosan prepared from crab (*Carcinus mediterraneus*) shell chitin and shrimp (*Penaeus kerathurus*) using 12.5 M NaOH having DD of 78 and, 88 %, respectively and MW of 6120 and 17030 g/mol, respectively. Chitosan was dissolved in 0.1% acetic acid.	*Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 19430, *Klebsiella pneumonia* ATCC 13883, *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* (ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* ATCC 4698, *Fusarium solani*, *Fusarium oxysporum*, *Aspergillus niger*, *Alternaria solani*, and *Botrytis cinerea*.	Antimicrobial activity against all tested organisms was high. Crab chitosan had higher antibacterial and antifungal properties as compared to shrimp chitosan.	Hajji, Younes, Rinaudo, Jellouli, and Nasri (2015)
	Chitosan prepared from chitin recovered through enzymatic deproteinization of the Norway lobster (*Nephrops norvegicus*) processing by-products. Chitosan was dissolved in 0.1% acetic acid.	*Salmonella enterica* ATCC 43972, *Escherichia coli* ATCC 2592, *Micrococcus luteus* ATCC 4698, *Klebsiella pneumoniae* ATCC 13883, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus thuringiensis* ATCC 10792, *Listeria monocytogenes* ATCC 43251, *Salmonella typhinirium* ATCC 19430, *Enterobacter sp. Fusarium solani, Alternaria solani, Botrytis cinerea, and Aspergillus niger. T.*	Antimicrobial activity was increased with increasing concentration.	Sayari et al. (2016)
	Chitosan-M (C-M) and chitosan-C (C-C) obtained by enzymatic and alkaline treatment, having MW of 19,800 and 5.820 g/mol, respectively. Chitosan was dissolved in 0.1% acetic acid.	*Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 13883), *Salmonella typhi, Staphylococcus aureus* (ATCC 25923), *Micrococcus luteus* (ATCC 4698), *Bacillus cereus* (ATCC 11778) *and Enterococcus faecalis* (ATCC 29212).	C-C had a slightly higher antimicrobial activity than C-M.	Younes et al. (2014)
	Chitosan (DD=80%) prepared from *Ceriodaphnia quadrangular* Ephippia chitin using 50% NaOH. Chitosan was dissolved in 1% acetic acid.	*Bacillus subtilis* RSKK 244, *Listeria monocytogenes* ATCC 7644, *Lactococcus garvieae, Streptococcus agalactiae* Pasteur Inst. 55118, *Vibrio alginolyticus, Yersinia enterocolitica* NCTC 11175, *Salmonella enteritidis* RSKK 171, and *Candida albicans* ATCC 10231.	Chitosan showed high antimicrobial activities against the human and seafood pathogens tested.	Asan-Ozusaglam et al. (2016)

Chitosan/its derivatives	Method of preparation	Target microorganisms	Findings	References
Modified chitosan (MC)	Chitosan dissolved in 1% (v/v) acetic acid and the chitosan-sugar complex from six types of sugar (glucose, fructose, lactose, arabinose, maltose, and galactose). MC was dissolved in water.	*Escherichia coli* (TISTR 780), *Pseudomonas aeruginosa* (TISTR 781), *Staphylococcus aureus* (TISTR 1466) and *Bacillus cereus* (TISTR 687).	Chitosan-sugar complex had a higher potential to act as a better antimicrobial, when compared to chitosan alone.	Mahae, Chalat, and Muhamud (2011)
	Chitosan (DD=67.58- 95.19%, MW= 949.95-4467.05 kDa) from shrimp chitin at three particle sizes (20, 40 and 60 mesh) by deacetylating with different concentrations of NaOH solution (30%, 40% and 50%) under microwave irradiation for 10 min. MC was dissolved in 1% acetic acid.	*Salmonella typhimurium* ATCC 14028 and *Escherichia coli* ATCC 25922.	Inhibitory effects differed, depending on the types of chitosan and the tested bacteria with greater antimicrobial activity against *Salmonella typhimurium* than *Escherichia coli.*	Mahdy Samar, El-Kalyoubi, Khalaf, and Abd El-Razik (2013)
	Chitosan/hydroxypropyl methylcellulose film-forming hydrosols hydrolyzed from low MW chitosan (DD= 75%–85%) with cellulase at two concentrations (0.05% and 0.1%). MC was dissolved in water.	*Pseudomonas fluorescens* PCM2123, *Yersinia enterocolitica* PCM1889, *Bacillus cereus* PCM2003, *Staphylococcus aureus* PCM1932.	Antibacterial activities were dependent on cellulase addition and hydrolysis time of polysaccharide solution.	Zimoch-Korzycka, Bobak, and Jarmoluk (2016)
	Chitosan with classical deacetylation (CDC) and ultrasound-assisted deacetylated chitosan (UDC) with DD of 73.68% and 83.55%, respectively. All chitosans samples were dissolved in 1% acetic acid.	*Staphylococcus aureus ATCC, Escherichia coli ATCC, Pseudomonas aeruginosa ATCC, Klebsiella pneumonia ATCC, Candida albicans ATCC, Candida parapsilosis ATCC.*	Antimicrobial activities were increased with augmenting the degree of deacetylation. UDC exhibited higher activity as compared to CDC.	Hafsa et al. (2016)
	Chitosan (MW=1000 kDa, DD= 80.23%) obtained by 50% NaOH treatment of chitin without and with modification by ultraviolet or ozone (UV/ozone) at different times. All chitosan were dissolved in 1% acetic acid.	*Staphylococcus aureus* ATCC 29213*, Bacillus cereus, Bacillus subtilis* ATCC6633, *Escherichia coli* ATCC 25922*, Pseudomonas aeruginosa* ATCC27953*, Aspergillus Niger, Candida albicans* NRRL Y-4773*, Candida tropicalis and Rhizopus.*	No differences in the antibacterial properties of unmodified and modified chitosan were attained.	Ragab et al. (2018)
Chitooligosaccharides (COS)	Chitin extracted from shrimp sell waste with MW of 338 kDa and DD of 35%), chitosan (DD= 80% Mw= 12kDa) prepared from chitin using 50% NaOH, N-acetyl chitooligosaccharides (chitin hydrolyzed with HCl) and chitooligosaccharides (chitosan hydrolyzed with HCl). Chitin, chitosan, and chitooligosaccharides were dissolved in cultured bacteria suspension	*Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 43300*, Bacillus subtilis, Bacillus cereus, Escherichia coli* ATCC 25922*, Pseudomonas aeruginosa* ATCC 27853*, Salmonella typhimurium, Vibrio cholerae, Shigella dysenteriae, Prevotella melaninogenica* and *Bacteroides fragilis.*	Chitin exhibited a bacteriostatic effect on *E. coli, Vibrio cholerae, Shigella dysenteriae*, and *Bacteroides fragilis*. Chitosan exhibited a bacteriostatic effect on all bacteria tested, except *Salmonella typhimurium*. Chitosan oligomers exhibited a bactericidal effect on all bacteria tested.	Benhabiles et al. (2012)
	Chitosan oligomers produced by hydrolysis of chitosan with nitrous acid ($NaNO_2$ +CH_3COOH). Chitosan was prepared from shrimp shell waste using 50% NaOH. COS was dissolved in 1% acetic acid	*Enterobacter aerogen* NCDC 106, *Enterococcus faecalis* NCDC 119, *Escherichia coli* NCDC 134, and *Staphylococcus aureus* NCDC 109	Tested microorganisms were inhibited but higher inhibition against *Enterococcus faecalis* was noticeable.	Varun et al. (2017)
	Chitooligosaccharides with different Mw prepared > 100kDa, 100 to 10 kDa, 10 to 1 kDa and <1 kDa by enzymatic hydrolysis of chitosan (MW= 300 KDa) using immobilized pepper chitosanase.	*Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Saccharomyces chevalieri, Macrophomina phaseolina,* and *Aspergillus niger*	Antimicrobial activities depended on the type of the microorganism and not on MW.	El-Sayed, Omar, El-Sayed, and Shousha (2017)
	Chitooligosaccharides (MW= 5.1, 14.3, and 41.1 k Da) prepared from commercial chitosans with 80% and 90% DD using papain. COS was dissolved in 0.25% acetic acid solution	*Escherichia coli, Staphylococcus aureus, Salmonella enterica* serovar Typhimurium *and Salmonella enterica* serovar Enteritidis	All chitooligosaccharides inhibited the tested microorganisms, however higher activity was reported for *Escherichia coli.*	Laokuldilok et al. (2017)

augmenting drying temperatures. These was attributed to the isomerization of ASXN to the cis form caused by light, oxidation as well as high temperature (Silva, Rodrigues, Silva, & Rodrigues, 2018). Flaxseed oil was used as the extracting medium for the recovery of ASXN in the by-product of crawfish (*Procambarus clarkii*). The recovered ASXN was then microencapsulated in sodium caseinate by spray drying method. ASXN content of 13.76 mg/g powder was recorded in the microencapsulated ASX powder (Pu et al., 2011).

Carotenoids, especially ASXN has also been extracted from crustaceans using enzymes hydrolysis, in which carotenoids are released during the hydrolysis or breakdown of carotenoprotein complex (Olatunde & Benjakul, 2020). Papain trypsin and alcalase combined with sunflower oil was used for the extraction of carotenoids from *Penaeus indicus* processing by-product. Higher content of carotenoids (24.4-28.6 µg/g sample) was recorded for the extraction with enzymes in comparison with the control (23.7 µg/g sample) (Sachindra & Mahendrakar, 2011). Senphan, Benjakul, and Kishimura (2014) found an augmenting carotenoprotein content from shrimp shell with increasing enzymes concentration as well as hydrolysis time when proteases from hepatopancreas of Pacific white shrimp was used. Trypsin from tuna spleen showed promising potential for extracting carotenoprotein from Pacific white shrimp (Poonsin et al., 2018). Sowmya, Rathinaraj, and Sachindra (2011) demonstrated the recovery of carotenoprotein for shrimp (*Penaeus monodon*) heads using protease isolated from the same species.

Antimicrobial Properties

ASXN extracted from prawn (*Penaeus monodon*) using high-pressure processing (238 MPa for 16.29 min) with a mixture of methanol and acetone (3:7 v/v) possessed better antibacterial properties against *B. subtilis, S. aureus,* and *Ent. aerogenes E. coli* when compared to ASXN extracted with the solvent only (Irna, Jaswir, Othman, & Jimat, 2017). Suganya and Asheeba (2015) reported that *E. coli* isolated from spoiled milk and rotten meat was inhibited by ASXN extracted from three spotted, spiny king and blue crab using a dimethyl sulfoxide and acetone solvent (1:3, v/v) mixture as extracting medium. Although the antimicrobial activities of carotenoids, especially ASXN, from crustaceans, are very promising, there is limited information on *in vitro* antimicrobial properties or mode of action of ASXN for microbial inhibition.

Applications of Antimicrobial Compounds from Crustaceans in Extending the Shelf-Life of MBFs

Among all the antimicrobial compounds from crustaceans, chitosan and its derivatives have been widely used for assuring the safety of MBFs as well as shelf-life extension. Chitosan is mainly used as a coating solution for MFBs. Chitosan (1% w/v) dissolved in lactic acid (1% v/v) acid and glycerol (0.1% v/v) used as coating for liquid smoked Nile tilapia (*Oreochromis niloticus*) fillet significantly reduced both psychrotrophic and mesophilic bacteria counts, in which the shelf-life of 30 days was found in comparison with 10 days recorded for the control (without coating) during the storage at 4 ºC (da Silva Santos et al., 2017). Silver carp slices treated with chitosan (2% w/v) dissolved in glacial acetic acid, retained its good quality characteristics with low oxidation as well as low microbial load in comparison with the untreated counterpart during frozen storage (−3 °C) for 30 days (Fan et al., 2009). Chamanara, Shabanpour, Khomeiri, and Gorgin (2013) reported a lower total psychrotrophic count for butterfly-shaped rainbow trout (*Oncorhynchus mykiss*) coated with 2% chitosan (w/v) prepared in 1% acetic acid as compared to the untreated counterpart throughout refrigerated storage at 4 ºC for 15 days. Increases in TVC, *Vibrio parahaemolyticus*, *V. cholerae, V. alginolyticus* and total coliform counts in tilapia (*Oreochromis niloticus*) fillets were lower for those treated with 2% shrimp chitosan (prepared in 1% acetic acid) in comparison with the control during iced storage for 21 days (Chaparro-Hernández et al., 2015). Atlantic salmon (*Salmo salar*) coated with chitosan extracted from lobsters (1% w/v dissolved in 1% v/v lactic acid) had lower bacterial load as compared to the untreated counterpart during storage at 0 ºC for 18 days (Souza et al., 2010).

To increase the efficacy of antimicrobial compounds from crustaceans, several compounds, particularly polyphenols have been incorporated in combination. Li, Li, Hu, and Li (2013) documented a shelf-life of 20 days for red drum (*Sciaenops ocellatus*) fillets coated with chitosan (1.5% w/v) dissolved in acetic acid (1% v/v) combined with either tea polyphenols (0.2% w/v) or grape seed extract (0.2% w/v) as compared to the 12 days reported for the control (without coating). Large yellow croaker (*Pseudosciaena crocea*) coated with chitosan (1.5% w/v) in acetic acid (1% v/v) combined with either tea polyphenol (0.2% w/v) or rosemary extract (0.2% w/v) had a shelf-life of 20 days as compared to the control (12 days) during storage at 4ºC (Li et al., 2012). Ramírez-Guerra et al. (2018) reported that the shelf-life of Sierra (*Scomberomorus sierra*) fillets packaged in edible chitosan film (prepared by dissolving 1% w/v chitosan in 1% v/v acetic acid and 0.5% v/v glycerol) incorporated with 0.3% (v/v) ethanolic tomato plant extract was extended by at least 5 days when compared to the control. Overall, the rate of change in the total viable count (TVC) was lower in fillets coated with chitosan incorporated with tea polyphenols, followed by fillet coated with chitosan only and tea polyphenol only, lastly in the control (Ramírez-Guerra et al., 2018). Indian salmon fillets coated with 1) 1.5% chitosan + 30% lime juice, + 10% gelatin, 2) 1.5% chitosan + 30% garlic extract + 10% gelatin, and 3) 10% gelatin solution had shelf-life of 16, 16, and 8 days, respectively, compared to 4 days

Table 2. Recent applications of chitosan and chitosan in combination with other preservatives in retarding microbial proliferation in MBFs during refrigerated storage

Chitosan and its derivatives	Marine based foods	Findings	References
Chitosan (0–2.0% w/w)	Surimi gel made from African catfish (*Clarias gariepinus*)	The addition of 1.5–2.0% (w/w) chitosan inhibited the proliferation of bacteria in catfish surimi gels during the refrigerated storage at 4 ºC for 20 days	Amiza and Kang (2013)
Chitosan solution (5.0 g/l) prepared in 1% acetic acid.	Aquacultured tilapia (*Oreochromis niloticus*)	A shelf-life of 6 days was observed for untreated tilapia fillets, while a shelf-life of 12 days was observed for chitosan-treated samples	Cao, Liu, Yin, and Wu (2012)
Chitosan coating solution (1 and 2% w/v in 1% acetic acid)	Indian oil sardine (*Sardinella longiceps*) fillets	The shelf-life of fillets treated with 1 and 2 % chitosan was extended to 7 and 9 days, respectively during storage at 4 ºC as compared to the control (5 days).	Mohan, Ravishankar, Lalitha, and Srinivasa Gopal (2012)
Chitosan coating solution (2% w/v in 1% acetic acid)	Rainbow trout (*Oncorhynchus mykiss*)	Total mesophilic and psychrophylic counts were lower in hot smoked vaccum packed fillets treated with chitosan as compared to the control group during refrigerated storage at 4 °C for 24 days	Yağin and Büyükyörük (2017)
Chitosan coating solution prepared with 2% (w/v) chitosan in 1% v/v acetic acid incorporated with 0.2% (w/v) black pepper essential oil 1.5% (v/v)	Common carp (*Cyprinus carpio*)	Changes in aerobic plate count, psychrophilic bacteria count, lactic acid bacteria, and Enterobacteriaceae bacteria counts were lowered in crap treated with chitosan and black pepper essential oil as compared to that treated with chitosan only and the control.	Moosavi-Nasab et al. (2016)
Chitosan coating solution prepared with 1% (w/v) chitosan in 1% v/v acetic acid and 0.2% (w/v) antioxidant of bamboo leaves.	Silver carp (*Hypophthalmicthys molitrix*)	TVC was lower in treated samples throughout refrigerated storage at 4 ºC for 24 days as compared to the untreated counterpart.	Wenjiao, Yongkui, Pan, and Yuwen (2013)
Chitosan coating solution prepared with 2% (w/v) chitosan in 1% v/v acetic acid combined with 1.5% cinnamon essential oil	Rainbow trouts (*Oncorhynchus mykiss*) fillets	The shelf-life of fillets treated with chitosan only and those treated with chitosan and essential oil was extended by twice that of the control during refrigerated storage at 4 ºC for 16 days.	Ojagh, Rezaei, Razavi, and Hosseini (2010)
Chitosan-based edible coatings prepared by dissolving 3% chitosan in water incorporated with garlic oil at 0.5, 1.0 and 1.5%	Deepwater pink shrimps (*Parapenaeus longirostris*)	Coating treatment, treatment, and the combined effect of treatment and period influenced the antibacterial efficacy of chitosan. The shelf of shrimp treated with chitosan was extended by 3 days at 4 ºC storage.	Aşik and Candoğan (2014)
Chitosan (2% w/v) prepared in 1% acetic acid incorporated with *Thymus vulgaris* essential oil (1% w/v).	Butterfly-shaped rainbow trout (Oncorhynchus mykiss)	The lower total psychrotrophic count was observed as compared to that treated with chitosan only and the control. The shelf-life was extended to more than 15 days during refrigerated storage at 4 ºC.	Chamanara et al. (2013)
Chitosan (2% w/v) recovered from shrimp prepared in 1% acetic acid incorporated with carvacrol at different concentrations (0.125 and 0.25% w/v).	Tilapia (*Oreochromis niloticus*)	Lower increases in total viable, *Vibrio parahaemolyticus*, *V. cholerae, V. alginolyticus* and total coliform counts in fillets were observed during ice storage of 21 days. Efficacy was increased with augmenting concentrations of carvacrol in chitosan solution.	Chaparro-Hernández et al. (2015)

recorded for the control (without any treatment) (Thaker, Hanjabam, Gudipati, & Kannuchamy, 2017). Indian mackerel (*Rastrelliger kanagurta*) wrapped in chitosan film (prepared by dissolving 1.5% w/v chitosan in 1% v/v acetic acid) incorporated with 5% v/v *Garcinia atroviridis* had lower increases in pH and TVC as compared with the fillets wrapped only with chitosan throughout refrigerated storage at 4°C for 6 days (Zaman, Lin, & Phing, 2018).

Aşik and Candoğan (2014) reported that shrimps coated with 1% chitosan (w/v) dissolved in 1% acetic acid in conjunction with garlic oil (1.5%) had a shelf-life of 15 days as compared to 5 days found for the control. Lower microbial loads were noted in deep-water pink

shrimp wrapped with chitosan film prepared by dissolving 1.5% chitosan (w/v) in combination with orange peel essential oil (0.5–2.0%) as compared to that wrapped with films prepared by 1.5% chitosan only and the unwrapped counterpart during refrigerated storage at 4 ºC (Alparslan & Baygar, 2017). Aşik and Candoğan (2014) reported the reduced microbial proliferation in shrimps coated with 3% chitosan (w/v) combined with garlic oil (up to 1.5%), which prolonged the shelf-life by 5 days.

Antimicrobial compounds from crustaceans, particularly chitosan and its derivatives, have been included in the formulation of several products, for retarding the microbial proliferation during storage. For example, the shelf-life of restructured fish products from pangasius (*Pangasianodon hypophthalmus*) surimi incorporated with 0.75% chitosan was extended to 17 days as compared to that without chitosan (10 days) during refrigerated storage at 4ºC (Jeyakumari et al., 2016). Recent applications of chitosan and derivatives, alone and in combination with other preservatives for retarding microbial proliferation in MBFs are presented in Table 2.

Conclusion and Future Prospectus

Antimicrobial compounds can be extracted from crustaceans, which could serve as natural preservative in many food and food products. The recovery of these antimicrobials from the by-product during the processing of crustaceans could lead to value addition, since this discard contains significant quantities of carbohydrates, pigments and structurally diverse proteins, that could serve as substrates for the generation of novel antimicrobials or act an antimicrobial themselves. Antimicrobial peptides, carotenoids as well as chitosan and its derivatives showed promising potential as a natural antimicrobial compound, which is been increasingly preferred over antibiotics because of being nontoxic and posing no health concerns. These aforementioned compounds have also been successfully produced and applied in different MBFs. However, their application in MBF is not fully exploited. Therefore, future research must be directed toward the application of antimicrobials from crustaceans, particularly peptides, carotenoids or COS directly or along with the coating, or other technologies for extending the shelf-life of MBFs. Also, screening and identification of novel antimicrobials from crustaceans processing by-products should be researched extensively.

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