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Bioactive compounds in Argo-industrial waste: A review on the prospects for the application in aquaculture

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Abstract

The agro-industry generates a large amount of waste. This waste represents a source of contamination of soil, air, and bodies of water. This represents a problem for the environment as well as for public health. However, this waste is an important source of bioactive compounds, such as phenolic compounds, terpenes, β -glucans etc. These compounds have certain biological activities that are associated with improvements in health. An approach of using these bioactive compounds as food additives for aquaculture have been addressed, where it is sought that organisms, in addition to growing, preserve their health and become disease resistant. The use of agro-industrial waste as a source of bioactive compounds for aquaculture provide an added value to production chains, reduce pollution, and improve the well-being of organisms through nutrition. The composition of bioactive compounds of agro-industrial wastes and their application in aquaculture is addressed in this paper.

Keywords: Aquaculture, antioxidants, immunostimulants, health hazards, fish

1. Introduction

Population growth and urbanisation have increased the demand for processed foods. This has led to the development of food industries to meet the needs of consumers. Agricultural industries generate a large amount of waste during the collection, storage, transport, and processing of raw materials^[45] which represents environmental pollution. This waste is mainly made up of organic matter and organic matter represents a source of bioactive compounds. A bioactive compound is a substance that has a biological activity. It can affect or can trigger a physiological response on a living organism. The effect may be negative or positive depending of the chemical structure, the dose and the bioavailability of the substance^[25]. However, bioactive compounds are widely recognized for promoting health benefits^[55].

The processing industries of fruits, cereals and vegetables generates large amount of wastes. The husks, bark, seeds, and pomace represent the largest amount of waste, which are made up of a wide variety of bioactive compounds with multiple biological properties such as antioxidant, immunostimulant, antimicrobial, anticancer and prebiotics.

The exploitation of waste for the development of food products with added value could make possible the generation of additional profits. The agricultural industry and scientific community, has made efforts towards the designing of appropriate methods for the extraction and purification of bioactive compounds from waste for the development of functional food.

Currently, the aquaculture sector is in need to find low-cost natural compounds as food additives, which might promote well-being and preserve the nutritional quality of farming organisms, without compromising the environment and the health of consumers. Therefore, the use of plant residues as additives represents a promising alternative. However, these residues require appropriate Characterisation, since some studies report that the use of phytochemical might exert anti-nutritional effects^[20, 47].

2. Bioactive Compounds from Agro-Industrial Waste

There is a wide variety of bioactive compounds found in residues derived from the cultivation and processing of agricultural products. Any part of plants, such as husk, seeds, leaves, roots, and stems, can be considered as a source of bioactive compounds^[65].

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2.1 Phenolic compounds

These are secondary metabolites of plants that are usually esterified or glycosylated (66). These are mainly composed of an aromatic ring (hydrophobic domain) and one or more hydroxyl groups (hydrophilic domain) attached to it. Phenolic acids (hydroxycinnamic and hydroxybenzoic), flavonoid].

Phenolic compounds are known for having several biological properties, such as antioxidants [43], immunostimulants [60], microbiota modulators [15], antibacterial [40], antiparasitic, antiviral [72], anti-inflammatory [19], anticancer [35] and antihypertensives [71] effects.

The antioxidant property of PCs is related to their chemical-structural characteristics. The number and position of hydroxyl groups, the presence of double bonds, and the ability to delocalise electrons determine the ability of PCs to scavenge free radicals and donate hydrogen atoms [72]. Other mechanisms by which PCs can exert their biological activity is by interacting with components of the cell membrane, enzymes, and transcription factors, as well as receptors [18]. For example, PCs provide antioxidant protection to membrane through the interaction of their hydrophilic and hydrophobic domains with the polar head and non-polar chain of lipids bilayer [18]. Antiallergenic and anticarcinogenic activities by Flavonoids is exerted by interacting with membrane raft-associated proteins [61] and decrease superoxide anion production in vascular cells through the inhibition of the translocation of p47phox nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunit in the endothelial cell membrane [71]. PCs exert indirect antioxidant and anti-inflammatory activities by activation of Nrf2 and inhibition of NF- κ B translocation into the nucleus, respectively [27].

2.2 Terpenes

These compounds are formed by isoprene units (CH) and classified according to the number of isoprene units condensed [34]. Essential oils and carotenoids are terpenes which are characterised by their antioxidant activity. Essential oils exert microbicidal effects [9, 26].

Terpenes biological activities are related to their chemical structure. The antioxidant activity of essential oils is mainly due to the presence of phenolic type components, such as thymol and carvacrol [4]. The antimicrobial effect of essential oils is related to their hydrophobicity. This property allows essential oils to cross the cell wall and cytoplasmic membrane and disorganise the structure of its components. Essential oils are also capable of inhibiting enzymes of energy regulation and synthesis of structural components [28].

2.3 Dietary Fibre (B-glucans)

Dietary fibre is composed of polymers of three or more carbohydrate units that are resistant to the activity of endogenous digestive enzymes, and therefore cannot be hydrolyzed or absorbed by the small intestine [41]. Fibre is classified as insoluble and soluble fibre. Soluble fibres (B-glucans, fructooligosaccharides, galactooligosaccharides, and some pectins) are fermented by the intestinal microbiota and give rise to short-chain fatty acids (acetate, propionate, and butyrate) [14]. In aquaculture, B-glucans are recognized for their immunostimulatory activity [44]. These compounds are polysaccharides made up of glucose units linked by glycosidic bonds. These are found as components of the cell wall of plants and yeasts mainly and also in some species of algae and fungi. The immunostimulatory activity of B-glucans depends on their recognition and binding to membrane

receptors (for example, dectin-1, and CR3).

2.4 Glucosinolates

The glucosinolates are glycosides formed by a β -D-glucopyranose residue linked to a (Z)-N-hydroximosulfate ester by sulfur bridges and an amino acid derivative radical. These compounds are found in all species belonging to the Brassica family, such as canola, broccoli, arugula and mustard [10]. Glucosinolates can be classified based on their amino acid precursor into aliphatic, aromatic, and indole [24, 70].

Glucosinolates and the products derived from their degradation (isothiocyanates) show antioxidant, anticancer and antibacterial activity. Due to their capability of modulating the activity of xenobiotic-metabolizing enzymes (Phase I and Phase I), they triggers the long-lasting antioxidant reactions [66]. On the other hand, the bactericidal activity of the products of the metabolism of glucosinolates has been related to the inhibition of intracellular enzymes responsible for ATP synthesis in pathogenic bacteria [36, 74].

2.5 Saponins

Saponins are amphipathic molecules composed of sugar residues linked to a system of polycyclic rings (sterols and triterpenes) through glycosidic bonds [69]. These compounds are present in plant products, such as agave or legumes [57, 58]. Saponins have immunostimulatory effects [56]. Due to the presence of an aldehyde group at position C19 and C4 of the aglycone [52].

Saponins also exert microbiota modulating effect, which is related to their antimicrobial activity.

Furthermore, saponins can dissociate the cell membrane, and therefore, the flow of extracellular and intracellular components is enabled [31]. The effectiveness of saponins is enhanced against

Gram-positive bacteria, while Gram-negative bacteria are more resistant, possibly due to the presence of the double lipid membrane in the latter [6].

Despite the beneficial properties attributed to bioactive compounds, they might possess anti-nutritional effects due to inhibition of the digestive protease activity and formation of complexes with proteins [17, 49]. Since bioactive compounds might exert beneficial effects on organisms of importance for aquaculture, their use as food additives has been explored. Nevertheless, the effect of these compounds on the metabolism and growth of species is still to be understood.

3. Use of Bioactive Compounds from Agro-Industrial Waste in Aquaculture

Aquaculture is an economic sector that shows a broad growth. Due to the accelerated growth and high demand for aquaculture products, farming has intensified, that is, a greater number of organisms are produced in smaller spaces. Other farming factors, such as poor diet, poor water quality, changes in temperature and pH etc. might cause stress or suppress the immune system of organisms and negatively affect their health condition [39]. These conditions might increase rapid spread of infectious diseases, creating major problem for the aquaculture industry due to the economic losses. Traditionally, antibiotics are used to mitigate this problem, however, their unselective use is responsible for the appearance of antibiotic-resistant bacteria and use of these chemicals is undesirable for the final consumer.

Therefore, there is a need to seek alternative options to reduce the problems of disease occurrences by increasing the

antioxidant and immune responses in organisms. These bioactive compounds have been shown to have multiple properties, such as to promote growth and improve the health of aquatic organisms by reducing oxidative stress and stimulating the immune system, which ultimately provides resistance to diseases.

3.1 Bioactive Compounds as Antioxidants

In aquaculture, there is enough information on the use of bioactive compounds from medicinal plants, as food additives, to increase the antioxidant response and counteract the effects of oxidative stress (lipid oxidation and loss of nutritional quality). In this regard, bioactive compounds from plant residues have been poorly explored.

Corn, rice, wheat and sorghum are among the most consumed cereals in the world. A significant amount of residue is generated from the cereal processing industry. This waste could be used for the development of functional foods with antioxidant activity. Catap *et al.* [11] reported that the dietary administration of corn silk extract (Zen mays) lowered the level of lipid peroxidation in the liver of Nile tilapia (*Oreochromis niloticus*) under paracetamol-induced oxidative stress. Corn silk is an important source of flavonoids such as luteolin, formononetin, maizine, and apigenin. In general, these compounds are recognised for neutralising reactive oxygen species (ROS) and modulating antioxidant enzyme activities [50]. Therefore, it was suggested that flavonoids improve the antioxidant response in Nile tilapia liver under induced stress.

Lee *et al.* [38] indicated that the dietary inclusion of residues from the distillation of sorghum (200 g/kg), increases the antioxidant activity and delays the oxidation process of low-density lipoproteins in the plasma of the Lysa (*Mugil cephalus*). In sorghum the bioactive compounds are phenolic acids (Caffeic, ferulic and chlorogenic acids) and flavonoids (apigeninidin, luteolinidin and naringenin), which have been directly related to its antioxidant activity [67]. Therefore it was concluded that the efficacy of sorghum to increase antioxidant activity and delay the oxidation of low-density lipoproteins in the plasma of *M. cephalus* is attributed to the ability of phenolic compounds to neutralise free radicals.

Waste from fruit processing is also one of the main sources of bioactive compounds important to aquaculture. Giri *et al.* [22] evaluated the effect of the dietary inclusion of banana peel (*Musa acuminata*) (10, 30, 50 and 70 g/kg) at different feeding times (30 and 60 days) on the formation of MDA (malondialdehyde) and the activity of SOD (superoxide dismutase), GPx (glutathione peroxidase) and CAT (catalase) in rohu (*Labeo rohita*) liver, infected with *Aeromonas hydrophila*. Fish feed including banana peel (50 g/kg and 70 g/kg) showed a significant decrease in MDA levels in both feeding times. SOD and CAT activity increased in fish fed 50 g/kg of banana peel during 60 days of feeding, while GPx activity showed an increase after 30 days of feeding fish the banana peel (30, 50, and 70 g/kg). The authors concluded that the diversity of bioactive compounds presented in the banana peel, such as phenolic acids, flavonoids and carotenoids, is responsible for improving the hepatic antioxidant response in rohu.

Furthermore, Vicente *et al.* [65] evaluated the effect of orange peel fragment (OPF), as a food additive, on the antioxidant enzyme activity of Nile tilapia subjected to heat/dissolved oxygen-induced stress. In the study, fish were fed diets with different inclusion of OPF (0, 0.2, 0.4, 0.6, and 0.8%) for 70

days. At the end of the feeding trial, fish were subjected to stress conditions (32 °C/2.3 mg/L dissolved oxygen) for three days. Before stress, SOD, CAT, and GPx activities were higher in the non-supplemented group. Nevertheless, after stress, OPF supplementation increased SOD, CAT, and GPx activities. The increase in the antioxidant enzyme activities in Nile tilapia liver could be associated with the presence of hesperidin, a flavonoid, in the orange peel fragments [96]. This flavonoid upregulates the Nrf2 gene expression which improves antioxidant enzyme activity, therefore minimizing oxidative stress [51].

Beta-glucans from mushroom (*Pleurotus pulmonarius*) stalk waste (MSW) have also been explored as antioxidants in aquaculture. Ahmed *et al.* [3] evaluated the use of hot water extracts

(HWE) from MSW as an additive in fish feed and determined the effect on growth performance and the *in vivo* antioxidant status of Nile tilapia. When the HWE from MSW, rich in B-glucan content (20.05 + 0.44%), was added to the diet (10 g/kg), SOD and CAT activities in the liver and kidney were enhanced. The authors mentioned that the effect of Beta-glucan on SOD and CAT activity might help to prevent the deleterious effects of ROS on organisms. It was also evaluated that whether the oxidative stress caused by pH fluctuations in Nile tilapia provide protection by B-glucans present in HWE of MSW. Administration of 5 g/kg and 10 g/kg under pre-stress conditions increased SOD and CAT activities in the liver and kidney respectively. Nevertheless, the activity of these enzymes was reduced in liver and kidney samples due to pH changes (5.5 and 10.5). The authors concluded that the supplementation of B-glucans from MSW in the diet for tilapia, enhanced the antioxidant enzyme activities *in vivo*, which led fish to reduce stress for pH fluctuations and therefore show a normal growth.

3.2 Bioactive Compounds as Modulators of the Immune System and Resistance to Infections

The use of immuno stimulants to control aquaculture diseases emerges as an alternative to the use of antibiotics. Phenolic compounds are a group of phytochemicals used in aquaculture as food additives due to their potential as immuno stimulants. During wine processing PCs from grape seed is usually discarded. The effect of grape seed extracts (*Canosina Nero di Troia Vitis vinifera*) on the immune response of juveniles of *Dicentrarchus labrax L* was evaluated by Magrone, Fontana, Laforgia, Dragone, Jirillo and Passantino [42]. In the study, the authors reported that the incorporation of 0.1 and 0.2 g/kg of phenolic extract in feed for *D. labrax*, had reduced the levels of interleukins (IL-1 β and IL-6) in the intestine, while the concentration of Interferon (IFN- γ) in the spleen was increased. The effect of PCs on the levels of cytokines could be due to the modulation that they exert on the NF- κ B factor (nuclear factor kappa light-chain-enhancer of activated B cells), which regulates the expression of several cytokines [53]. It is found that, the number of melanomacrophage centers (MMCs) also increased. These results showed that the diet with polyphenols reduces intestinal inflammation by reducing the levels of proinflammatory cytokines, while the increase in interferon expresses a more powerful adaptive immune response. Another aspect is that the increase in the number of MMCs, which contain melanin, is associated with protective functions against pathogens [2].

Likewise, Arciuli *et al.* [5] evaluated the effect of PCs extracted from grape seeds on the activity of MCs, dopa-

oxidase, and peroxidase in commercial size fish of *D. labrax*. The administration of 0.2 g/kg in the diet of *D. labrax* increased the activity of dopa-oxidase and peroxidase. These enzymes participate in the synthesis of melanin. The presence of the melanin in fish is associated with protective functions against pathological or stress conditions [12]. From the above mentioned studies, it can be concluded that the addition of PCs from grape seeds increases melanin levels in fish. Since the presence of melanin pigment is associated with the resistance of organisms against pathogens thus PCs could be an option to improve the health status of fish.

Hoseinifar *et al.* [29] indicated that the dietary administration of olive waste cake (OWC) (0.5, 2.5, and 5.0 g/kg of feed), increased weight gain (WG) and specific growth rate (SGR), and decreased the feed conversion ratio (FCR) in rainbow trout (*Oncorhynchus mykiss*). In the study it was also reported that the dietary inclusion of 2.5 g/kg and 5.0 g/kg OWC feed increased total Ig concentration and mucosal lysozyme activity, also up-regulated relative expression of gut IL-8 gene. While, the supplementation of 2.5 g/kg OWC increased the serum lysozyme activity. The immunomodulatory effects of OWC are related to the presence of PCs (hydroxytyrosol, tyrosol, caffeic, p-coumaric and vanillic acids, and lutein and lignans) and vitamin E previously identified in olive [33]. Phenolic compounds extracted from olive oil processing waste have also been used in combination with other plant extracts rich in PCs for instance, chestnut. In this regard, Hoseinifar *et al.* [30] evaluated the effect of dietary supplementation of a mixture of PCs extracted from olive mill wastewater (OMWW) and chestnut wood (CW) (9:1, OMWW: CW) in concentrations of 0.5, 1.0, and 2.0 g/kg of feed, on the innate immune response of convict cichlid (*Amatitlania nigrofasciata*). The authors reported that mucus total protein levels and lysozyme activities increased in fish fed OMWW: CW. They also found that the supplementation of 2.0 g/kg of OMWW: CW increased serum total protein and total Ig levels, as well as peroxidase and radical scavenging activities. The effect of PCs extracted from OMWW: CW (0.5, 0.1, and 2.0 g/kg) on growth performance and innate immune response also have been evaluated in common carp (*C. carpio L.*) [32]. In this study authors reported that supplementation with OMWW: CW increased skin mucus total proteins and Ig levels and lysozyme, peroxidase, and radical scavenging activities in this species. Serum total Ig levels increased in fish fed 0.1 y 0.2 g/kg of OMWW: CW. Besides, PCs from OMWW: CW improved growth (WG and SGR) and feed utilisation (FCR) in common carp.

Terpenes, mainly essential oils, have also been studied for their promoter effect of the immune response. It has been reported that the use of essential oils to enhance the immune system response in aquaculture species is a potential alternative to the use of antibiotics [13]. Peels obtained from citrus processing are a good option as a source of essential oil to use them as additives in aquaculture foods [59]. In this context, Acar *et al.* [1] evaluated the dietary effect of essential oils obtained from orange peel (*Citrus sinensis*) on the growth of Mozambique tilapia (*Oreochromis mossambicus*) and its resistance against the pathogen *Streptococcus iniae*. The fish were fed a control diet, which does not contain essential oils, and three experimental diets (1, 3, and 5 g/kg) for 12 weeks, after which time the fish were challenged by infection with *S. iniae*. Fish fed essential oils, increased lysozyme, and MPO activities. Besides, the addition of essential oils in 1, 3, and 5 g/kg increased fish survival by 48.33%, 46.67%, and 58.33%,

respectively. Limonene, a phenolic monoterpene present in the orange peel essential oil, has antibacterial properties and could be responsible for these effects. In general, the results of this study demonstrated that the inclusion of essential oils in diets for tilapia improves the immune response of the fish and therefore may have the potential to be used as antibiotic substitutes. Likewise, Baba *et al.* [7] evaluated the effect of essential oils obtained from the lemon peel (*Citrus limon*) on the immune system and resistance against *Edwardsiella tarda* in Mozambique tilapia. Fish fed 5 g/kg and 7.5 g/kg of lemon peel essential oil increased the levels of the immunohematological parameters, such as the nitroblue tetrazolium (NBT), the number of white cells, hematocrit, and the activity of lysozyme and myeloperoxidase (MPO). After the feeding trial the tilapias were subjected to infection by *E. tarda*. Fish fed the control diet showed 80% of mortality percentage, while those fed 5, 7.5, and 10 g/kg of essential oils reduced this percentage to 36.6%, 51.6%, and 58.3%, respectively. All the parameters evaluated in this study are important to determine the immune response of the fish. Particularly, lysozyme and MPO have an important role in the elimination of pathogenic microorganisms. Limonene is the main component of the essential oils of lemon peel (54.4%). This compound exerts antimicrobial activity due to its ability to destabilize the bacterial cell membrane [8]. The authors suggest that the addition of 5 g/kg of essential oil of lemon peels into the diet, exerts an immunostimulatory effect and increases the resistance of Mozambique tilapia against pathogenic bacteria. From these results it can be concluded that citrus peel essential oils are a natural and safe alternative for the formulation of food for aquaculture species. B-glucans have been widely studied as immunostimulants for organisms of interest in aquaculture, such as shrimp and Nile tilapia [55, 68]. B-glucans evaluated are usually obtained from fungi or yeasts [44]. Chirapongsatongkul *et al.* [12] recently evaluated the potential of B-glucans obtained from split gill mushroom (*Schizophyllum commune*) cultivation waste as immune stimulant. The authors obtained a crude glucan extract from mycelium containing spent mushroom substrate (SMS) of the *S. commune* to stimulate immune system of Nile tilapia. Fish were injected with 100 µg/mL of glucan extract and after six hours they were challenged with *Aeromonas veroni*. Fish treated with the crude glucan extract showed an increase in immune parameters (Ig, lysozyme) and up-regulation in the expression of cytokine genes (INF- α , IL-1 β , and NF-KB) related to the immune response. Glucan extract treatment also increased the survival rate of Nile tilapia infected with *A. veronii*. These results demonstrate the possibility of use of crude glucan extracts from mushroom cultivation waste to improve the immune response in tilapia.

Cereals, such as oats, barley, and wheat are a rich source of B-glucans [47] and might be used as additives in diets for fish. Udayangani *et al.* [62] evaluated the effect of B-glucans from the endosperm of oat grains on the immune response of zebrafish larvae against *E. tarda*. Once the larvae hatched, they were kept in solution for three days with two concentrations of B-glucan (100 and 500 µg/mL). Afterward, the larvae were exposed to the pathogen *E. tarda* and the expression of cytokines, lysozyme, and survival percentage were determined. Treatment with 500 Mg/mL of B-glucan significantly increased the up-regulation of the expression of lysozyme and cytokine genes (TNF- α , IL-1 β , IL-10, and IL-12) related to the immune response, as well as the survival rate. Therefore, B-glucans have potential in the aquaculture

industry as promoters of the immune system for larval stages of fish.

3.3 Bioactive Compounds as Modulators of the Intestinal Microbiota

The modulation of the intestinal microbiota through the use of prebiotics (Inulin, galactooligosaccharides and xylooligosaccharides) has received significant attention due to the growing need to replace probiotics due to their high purchasing value, reduce the incidence of infectious diseases, improve the health status of aquaculture organisms, and increase crop production and profitability. Inulin is the most widely used prebiotic in aquaculture [62]. The use of this prebiotic increases the lactic acid bacteria population in the gut of surubies (*Pseudoplatystoma sp.*) and beluga sturgeon (*Spindle spindle*) and decreases *Vibrio* spp. in turbot (*Psetta maxima*).

Although there are few studies on the use of prebiotics obtained from agro-industrial wastes, some research has been conducted on prebiotics extracted from cereals, such as wheat. In this regard, Geraylou *et al.* [21] evaluated the effect of dietary inclusion of wheat bran arabinoxylans (20 g/kg and 40 g/kg), on the composition of the intestinal microbiota of Siberian sturgeon (*Acipenser baeri*). The authors reported that fish fed 20 g/kg and 40 g/kg of arabinoxylans showed an increase in the relative abundance of Eubacteriaceae, Clostridiaceae, Streptococcaceae, and Lactobacillaceae and in Bacillaceae, respectively. Besides, dietary inclusion of wheat bran arabinoxylan oligosaccharides (20 g/kg) modulated the growth of *Lactococcus* sp., *Lactobacillus* sp., *E. budayi*, and several species of the genus *Clostridium*. Furthermore, it has been reported that arabinoxylan oligosaccharides from wheat bran suppress the growth of *Aeromonas* sp., *Citrobacter freundii*, and *Escherichia coli* and increase the content of short-chain fatty acids (acetate and butyrate) in the intestine of Siberian sturgeon [124]. In both studies, the increase in the abundance of the mentioned bacteria is because they possess enzymes (endo-1,4- β -xylosidases, α -L-arabinofuranosidases, β -xylosidases, α -glucuronidases, and feruloyl esterases) with capacity to ferment the prebiotics evaluated [23]. It is concluded that wheat bran prebiotics have an impact on the composition of the intestinal microbiota and that the increase in the abundance of lactic acid bacteria and short-chain fatty acids could provide health benefits of Siberian sturgeon.

The lack of information on the use of PCs extracted from plant residues is a field worth exploring, especially since it is currently known that PCs provide beneficial effects on human health through the increase of bacterial populations beneficial and short-chain fatty acid content [42].

4. Conclusions

Agro-industrial wastes have heavy environmental impact on disposal. Its potential reuse in aquaculture could represent a sustainable approach to recycling nutrients. There is little research aimed to the importance of agro-industrial waste and its use as a source of bioactive compounds to incorporate them into aquaculture food. The activity of bioactive compounds, such as phenolic compounds, terpenes and β -glucans, depends on their chemical structure, the source, the doses, and if it is isolated or in presence of other compounds, as well as the species used for the study. Therefore, these compounds are required to be evaluated in different aquatic organisms of commercial interest, such as shrimp, tilapia, white snook, snapper and others, to determine their biological

effect in terms of antioxidant, immunostimulant, or microbiota modulator. The above reveals that there is a need and huge opportunity of research and development to use these waste products.

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