Biostimulants and Their Extraction from Food and Agro-Based Industries

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Abstract

The bioactive compounds present in the waste and by-products of the food and agro-based industries, can be utilized for the valorization of the unutilized biomass generated by the food and agriculture industries. Several classes of organic wastes generated by food and agro-based industries consist of various active ingredients which have an utmost potential to be exploited as biostimulants. Biostimulants can be of natural or synthetic origin, and are used to treat the seeds, plants, and/or soil. Biostimulants are known to improve the abiotic stresses tolerance of the plants and thereby improve the quality and yield of the produce. The availability of huge quantity and diverse types of agricultural and food processing waste provide us with an opportunity to test a vast range of biostimulant substances in order to expand the possible ways for their valorization. Number of conventional techniques are being utilized and researched for the extraction and development of biostimulants from the waste biomass generated by the food and agro-based industries. This utilization, if scaled up efficiently, will not only provide a strategy of utilization of agro-industrial waste but will also help in reduction of agro-chemical usage in an eco-friendly way.

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7.1 Introduction

Biostimulants can be of natural or synthetic origin that can be used to treat seeds, plants, or soil, influence the plant growth by improving the abiotic stresses tolerance, and simultaneously improve the quality and yield of the produce (Ana and Lucia 2019).Various researchers have defined Biostimulants differently. Biostimulants are the preparations that are composed of substances or some beneficial microbes, which increase the abiotic stress tolerance, enhance the nutrient uptake and nutrient efficiency, thereby improving the crop yield and quality (Przybysz 2014). Yakhin et al. (2017) defined biostimulants as biological-formulated products that result in the improved plant productivity due to their multiple constituent composition. These biostimulants comprise plant growth regulators, plant protective compounds, essential plant nutrients, amino acids, phenolic compounds, sugars, sterols, etc. (Puglia et al. 2021).

Initially, the categorization of biostimulants was attempted by Filatov (1951), who had suggested four groupings of biogenic stimulants. Followed by that, biostimulants have been vastly classified by various researchers and sources. A list of 15 biostimulants and their 59 components was compiled by Karnok (2000). Ikrina and Kolbin (2004) systematized the patent literature and categorized the raw materials used to derive biostimulants into nine categories. In 2008, another researcher, Basak suggested a biostimulants classification based on origin and mode of action of the active ingredient. He also suggested that they can be grouped on the basis of number of components in formulations—single or multiple. The scientific rationale of classification was pioneered by Du Jardin (2012a), in which he considered eight classes of biostimulants, but subsequently he reduced them to seven classes, wherein he explicitly excluded microorganisms from his categorization, as microorganisms are already categorized as biopesticides and plant hormones sources. Another classification based on the action of the biostimulants on the physiological responses of the plant has been proposed by Bulgari et al. (2015). One additional categorization is based on different origins and characteristics of the primary sources from which the biostimulants are derived such as plant and animal protein hydrolysates, sea weed and vegetal extracts, humic and fulvic substances, and a few microorganisms.

Biostimulants hold a high potential to stimulate plant growth, increase the stress-tolerance, and positively influence the crop yield, through regulating or modifying the plant's physiological processes. When applied appropriately, the biostimulants provide potential benefits to the growth and development of the crops and also act on the physiological processes of the plants to cope with the salinity, water stress, and toxicity of elements (Du Jardin 2015; Couto et al. 2012). Biostimulants have a direct or indirect response on the plant productivity. In a direct response, the plants or soils respond directly to the biostimulant application, whereas, in an indirect response, the biostimulant impacts the soil and plant microbiome with consequent effects on the plant productivity (Yakhin et al. 2017). The biomass with potential biostimulant activity has active ingredients that can be categorized into a range of molecules, including amino acids (Colla et al. 2017), phytohormones (Letham and Palni 1983; Werner and Schmülling 2009; Pacifici et al. 2015), polyamine (Fuell et al. 2010), etc. Biostimulants are efficient even in small concentrations in enhancing the nutrition efficiency, crop quality traits, abiotic stress tolerance, regardless of its nutrients content. According to Yaronskaya et al. (2006), these biostimulating substances have effects similar to those of the plant growth hormones such as auxins, gibberellins, and cytokinins.

The present statistics predict that global production and consumption of all types of food commodities is likely to continue to escalate (FAO and OECD 2018). The industrial waste streams and by-products of food and agro based industries consist of various active ingredients that are of utmost potential for the extraction of biostimulants. The extraction of biostimulants from the waste and by-products of food and agro-based industries provides a good opportunity for the valorization of the waste produced in hefty amounts. Food and agro-based industries generate various classes of source material that are composed of protein hydrolysates, chitin and chitosan, and other biostimulants. Protein hydrolysates manufactured from hydrolyzed protein-rich waste generated in food industry are commonly used biostimulants (Dieterich et al. 2014). Thus, the extraction of biostimulants not only provides a means of valorization of the organic wastes and by-products generated from Food and Agro-based industries but is also helpful in waste management. not only provides a means of extracting Organic wastes are the promising sources for production of biostimulants.

7.2 Biostimulants from Food and Agro-Based Industries

While developing a biostimulant, the selection of the biomass resource is imperative. Plants, animals, microorganisms, seaweeds are the prime sources of the biostimulants (Table 7.1). Apart from the several primary sources of biostimulants, the waste generated from food and agro-based industries are undoubtedly the potential significant sources for biostimulant development. With the escalating demand for healthy food, the food and agro-based industries are flourishing to meet that demand, thereby generating huge amount of waste and by-products during food production and processing. The scale of biomass production substantially varies with the type of crop, source of food, and it's processing. Among various challenges that have to be overcome in agriculture and food production systems, waste management is one of the major on the list. The extraction of biostimulants from the industrial waste and by-products of food and agro-based industries renders a good opportunity for valorization as well as for waste management. However, when these raw materials are being used, a basic knowledge of their biochemical

Table 7.1 Examples of biostimulants extracted from different wastes and by-products of agro- and food-based industries

Biostimulant	Source	Reference
Protein hydrolysates (PH)	By-product extracts (rapeseeds, apple seeds, rice husks)	Donno et al. (2013)
Protein hydrolysates (PH)	Waste biomass from tomato plants	Baglieri et al. (2014)
Protein hydrolysates (PH)	By-products of carob (Carob germs)	Parrado et al. (2008)
Chitin	By-products of the mushroom industry	Tao et al. (2004)
Biostimulant extract	Viticulture waste (wine-shoot aqueous extracts)	Sánchez-Gómez et al. (2017a, b)
Protein hydrolate	Pea and tomato residues	Colla et al. (2014)
Fish PHs	Fish skin	Chalamaiah et al. (2012)
Chitin and chitosan	Shrimp waste	Khanafari et al. (2008)
Biostimulant extract	By-products extracts (lemon, fennel, barley grains)	Chehade et al. (2017)
Biostimulant extract	Seaweed extract	Billard et al. (2014)
Biostimulant extract	Plant waste such as leaves, seeds, and exudates of *Amaryllidaceae*, *Brassicacae*, *Ericaceae*, *Fabaceae*, *Fagaceae*, *Moringaceae*, *Plantaginaceae*, *Poaceae*, *Rosaceae*, *Solanaceae*, *Theaceae*, *Vitaceae.*	Yakhin et al. (2014); Parrado et al. (2008); Apone et al. (2010); Ertani et al. (2014); Colla et al. (2014); Yasmeen et al. (2014); Ugolini et al. (2015)

characteristics such as preservation of the specific bioactive ingredient is a prerequisite (Povero et al. 2016). Further in this section, various source materials from plant and animal origins that are considered to be wastes and by-products in the food and agro-based industries such as protein hydrolysates, chitin and chitosan, and others will be discussed.

The biostimulants of plant origin can be extracted from the plant waste such as seeds, leaves, roots, exudates from families such as *Brassicacae, Amaryllidaceae*, *Ericaceae*, *Fabaceae*, *Fagaceae*, *Rosaceae*, *Poaceae*, to name a few (Naumov et al. 1993; Yakhin et al. 2014; Parrado et al. 2008; Apone et al. 2010; Ertani et al. 2014; Colla et al. 2014; Yasmeen et al. 2014; Ugolini et al. 2015), generated in food and agro-based industries. According to Food and Agriculture organization (2019), the inedible plant residues generated from agro- and food-based industries are annually recorded as being approximately 250 million tons. These huge wastes are rich in lipids, carbohydrates, and aromatic molecules that can be used to extract biostimulants.

Protein hydrolysates (PH) are an important class of biostimulants that are produced from the protein hydrolysis found in by-products generated from the food and agriculture industries based on the food from plant and animal origins (Colla et al. 2014). Protein hydrolysates (PH) of plant origin are considered to be more eco-friendly and are generally developed or extracted through enzymatic hydrolysis. This results in the mixture of amino acids and peptides of different lengths (Colla et al. 2015a, b). Recently, the protein hydrolysates developed from organic wastes have gained popularity as plant biostimulants. Different sources such as the extracts from the by-products of rapeseeds, apple seeds, and rice husks (Donno et al. 2013), waste biomass from tomato plants (Baglieri et al. 2014) have been used for the production of biostimulants. The carob germs, which are the high-protein by-product obtained during treatment of carob fruit, have also been explored for the production of amino acids and proteins using enzymatic process (Parrado et al. 2008). Parrado et al. (2007) also studied the effect of enzymatic extract of PHs derived from vegetable by-products, along with the phytohormone, on the anthocyanin levels in grapes.

Another class of biostimulants that have potential applications in a number of industries such as food, cosmetics, and industrial processes is Chitin, which is a biopolymer derived from crustaceans' shells. Chitosan, which is the deacetylated form of chitin, also holds a great potential as a biostimulant. These can be derived from the food and agro industry wastes (Olsen et al. 2014). The physico-chemical and biological properties of chitin and chitosan are determined by the ratio of each monomer in the polymer chain (Pichyangkura and Chadchawan 2015). Chitosan is a bio-waste product that is much cheaper as compared to other biopolymers. The sources of chitin are majorly marine animals' and crustacean residues, but can also be obtained from insects and microorganisms. The chitosan has antibacterial and antifungal properties, along with mucoadhesivity, atoxicity, and biocompatibility (Santos et al. 2020).These biostimulants can be derived from the industries generating mycelial waste and mushroom industry. White et al. (1979) were pioneer in isolating chitosan from fungal mycelium, which was followed by many other researches (Elsoud and El Kady 2019). Crude fungal chitin on fresh basis (0.65–1.15%) was yielded by Tao et al. (2004); this anticipated the potential of the by-products of mushroom industry for the development of biostimulants.

One other attractive sector of agriculture that produces an enormous quantity of waste is viticulture. The waste generated by viticulture is responsible for environment problems related to its disposal (Sánchez-Gómez et al. 2017a, b). Approximately, 1.4–2.0 tons per hectare of vine-shoot waste is generated annually (Peralbo-Molina and Luque de Castro 2013) that can be utilized for the production of biostimulants owing to their high content of phenolic compounds (Garcia Martinez et al. 2021). Sánchez-Gómez et al. (2017a, b) used some extraction methods to obtain several vine-shoot aqueous extracts which then they tested on *Leptinotarsa decemlineata*, *Lactuca sativa*, and *Lolium perenne*. A few other studies also have addressed the utilization of residues of vine-shoot. Water extract from the shoots of vine, when applied to the grapevine itself, has been reported to improve the wine quality and aroma, volatile and phenolic compound content (Pardo-García et al.

2014a, b). Garcia Martinez et al. (2021) have also reported an increase in the alcohol content and grape yield.

These biostimulants extracted from agro food-based industries have proven effects on the various parameters of plant growth. The hydrolyzed extract developed from the residues of rice husks, apple seeds, and rapeseed was tested for its effect on the growth and quality parameters of Kiwi. An increased fruit weight, ascorbic acid, and antioxidant activity in kiwi was recorded resulting due to the presence of auxins, gibberellins, and cytokinins in the extract (Donno et al. 2013). Colla et al. (2014) tested a protein hydrolate derived from residues of pea and tomato on maize crop and recorded an increase in the shoot length, SPAD index, total biomass, nitrogen content, and induced rooting. Chehade et al. (2017) created a biostimulant extract from fennel, lemon, and brewer's spent grain residues and recorded the effect of the product on the metabolic and agronomic performance of *Solanum lycopersicum*. This biostimulant product was reported to increase the nutrient content, vitamin C content, shoot growth, dry matter, and in *Solanum lycopersicum*. The maize waste was tested for Trans-zetain and other phytohormones by Choez et al. (2014) in order to develop a biostimulant. The residues of blueberry fruit, red grape skin material and hawthorn leaf extracts were studied for their biostimulative effects on maize crop by Ertani et al. (2016) and it was reported that they increased the protein content, chlorophyll, and nitrogen uptake by the crop.

Biostimulants of animal origin are also gaining pace and can comprise protein hydrolysates and amino acids derived from wastes and by-products from animal-based food industries (Mladenova et al. 1998; Rodríguez-Morgado et al. 2014), and chitin and chitosan derived from insects (Sharp 2013). Another animal-based industry that generates vast amounts of waste is the fishing industry. The fishing industry produces enormous amounts of exoskeletons of crustaceans (crab, shrimp, and lobster) that account for 5.9 Mt. global production, out of which 35–45% is discarded (Sharp 2013). Head, skin, fins, frames, etc., consists of over 60% of the biomass of the fish by-product (Chalamaiah et al. 2012). Fish skin is a source of collagen and gelatine and also can be used for extracting protein hydrolysates (Chalamaiah et al. 2012). By-products such as fish head, viscera, muscle, bone, frame, liver, and egg (Chalamaiah et al. 2012) are rich in fats, proteins, amino acids, antioxidants, and have already been valorized as food or feed and are now being explored as plant biostimulants (Halim et al. 2016). Another source of protein hydrolysates is the keratin-rich feather waste (Korniłłowicz-Kowalska and Bohacz 2011). High amounts of feathers of chicken are discarded in poultry slaughterhouses that are the most common keratin waste and have the potential for development of protein hydrolysates (Korniłłowicz-Kowalska and Bohacz 2011). Owing to the biostimulating properties of protein hydrolysates to increase crop quality and quantity, they are now widely accepted in agriculture (Colla et al. 2017). The protein hydrolysates generated by the enzymatic hydrolysis of plant proteins are more preferred over those produced from animal by-products for sustainable farming. Preferring plant-based PHs also at the same time avoids the possible risk of pathogens contamination.

In addition to protein hydrolysates, another class of biostimulants generated by agro-food industries of animal origin is chitin and chitosan. In many countries, seafood is a popular choice of animal protein. Seafood industry generates about 10^6 tons of waste per annum, which is either composted or is converted into animal feeds or fertilizers that are generally low-value commodities (Schmitz et al. 2019). According to Muñoz et al. (2018), annually 2000 tons of chitosan is extracted from the residues of crab shell and shrimp, which can be utilized efficiently for the chitin extraction. The conversion of chitin to chitosan is done by a simple process of deacylation (Nessa et al. 2010). The crustaceans (crabs, shrimps, lobsters, and krill) are the major sources of chitin, out of which shrimp waste is the most significant source of chitin for commercial use (Yadav et al. 2019). Khanafari et al. (2008) used both chemical and microbiological methods of extraction to extract chitin and chitosan from waste of *Penaeus semisulcatus*.

Apart from this, in recent years, several other types of by-products have been reported to enhance plant production and food quality. The pruning process of vineyards generated vine shoots that are rich in volatile, phenolic, and mineral compounds, which, when applied to grapevine, acted as a biostimulant as well as a foliar fertilizer (Sánchez-Gómez et al. 2017a, b). Chehade et al. (2017) reported that the by-products extract from fennel, lemon, and barley imparted biostimulating effect on the fruit quality and yield in tomato. Another study by Ertani et al. (2013b) reported that the by-products extract of castor oil, rapeseed, and flaxseed show biostimulating activity in plantlet growth of maize. The nutrient by-products generated by aquaculture are also reported to have biostimulatory effects. There is a lack of firm evidence to determine whether the effects are due to the added primary nutrients (Nicoletto et al. 2018) or due to the role of microorganisms and dissolved organic matter in water (Delaide et al. 2016).

Biostimulants have also been derived from the microorganisms such as bacteria, yeasts, and fungi. The biostimulant preparations of microorganism origin may be composed of microorganisms and their metabolites. The establishment of the concept of biostimulant preparations based on microorganisms and their metabolites was first reported by Xavier and Boyetchko (2002). Biostimulants are also commonly extracted from various species of algae (mostly seaweeds) (Crouch and van Staden 1993; Aremu et al. 2015). In the process of producing food, fine chemicals, alginate, agar, etc. from the seaweeds, significant amounts of waste are produced, which is generally discarded in the landfills. The waste generated from seaweed is rich in the compounds responsible for promoting the growth of the plant and yield, root development, seed germination, and abiotic stress resistance (Katuzewicz et al. 2018).

7.3 Modes of Action of Biostimulants from Organic Waste

The diversity of source materials and various technologies of extraction makes it difficult to determine a shared mode of action for all classes of biostimulants. The varied characteristics and properties of raw materials used for the biostimulant development and the diverse composition of biostimulant products renders it difficult to identify the exact component(s) accountable for the biostimulating activity and what is the mode of action (Parađiković et al. 2011).

Biostimulants do not substitute, but rather supplement the products used for plant protection and nutrition such as pesticides and fertilizers. Their mode of action is different to that of pesticides and fertilizers, as they do not directly affect the pests, and their mode of operation is also different from that of fertilizers (http://www.biostimulants.eu). According to Brown and Saa (2015), the biostimulants that originate from food and agro-industrial wastes, macro and micro-algae and living microbial cultures are extremely complex, attributing to the multiple components involved resulting into biostimulant products that are poorly characterized and has a limited understanding of its mode of action.

Different categories of biostimulants are known to positively impact the plant productivity through improvement in the physiological processes of the plants (photosynthesis, phytohormone modulation, senescence, nutrients and water uptake) (Khan et al. 2009). Biostimulants are also responsible for activation of genes responsible for abiotic stress resistance, altered plant phenology and architecture (Sharma et al. 2012). In a study reported by Wozniak et al. (2020), under drought conditions, the damage measured by electrolyte leakage of *Arabidopsis thaliana* leaf tissue was reduced by fermentation metabolites present in the biostimulants. Some reports suggest that many biostimulants increase the assimilation of carbon, nitrogen, and sulfur and improve photosynthesis, stress responses, and ion transport, thereby improving the plant productivity (Jannin et al. 2012; Khan et al. 2009; Parađiković et al. 2011). Most of the biostimulants are known to impart the protective effect against biotic as well as abiotic stresses. This protective property has been linked to a reduction of stress-induced reactive oxygen species followed by activation of the plants' antioxidant defense system or elevated levels of phenolic compounds (Ertani et al. 2011, 2013a).

To understand the mode of action of biostimulants, a multidisciplinary and systematic approach is required, where the technologies from the different fields such as biology, genomics, and chemistry need to be considered together (Povero et al. 2016). After the application to the plants, the stages of the plants need to be considered such as tissue penetration, reaction with the plant metabolites, binding to metabolic enzymes, and impact of the compound on the plant's physiology and gene expression modulation (Yakhin et al. 2016).

For a better insight into modes and mechanisms of biostimulant action, Yakhin et al. (2017) have categorized the biostimulant's action on plants into the stages such as (a) penetration of biostimulants into the plant tissues followed by its translocation and transformation; (b) gene expression, plant signaling, and the regulation of hormonal status; and (c) metabolic processes and integrated whole plant effects. The PHs penetrate into the tissues through the diffusion via membrane pores, which is an energy-dependent process (Kolomazník et al. 2012; Parrado et al. 2008). Solubility of the biostimulant is an important property for its efficiency. It must be readily soluble in water and other solvents. Sometimes, to overcome the solubility and lipophilicity, surfactants and other additives may be required (Pecha et al. 2012).

The observations that the biostimulants induce the genes and benefit the plant productivity only under abiotic and biotic stress complicates the pursuit for mode of action even more.

The role of signaling molecules in how a plant responds to the environment has been an active research area. The signal transmission process involves (a) synthesis of signaling molecules (ligands); (b) their translocation, binding to receptors; (c) the resulting cellular responses; and (d) degradation of the signaling molecules (Zhao et al. 2005; Wang and Irving 2011). When the signaling molecule binds to its receptor, it activates the secondary messengers that further leads to consecutive cellular responses.

Biostimulants that have shown to affect the hormonal status of the plants have been developed from complex organic materials, seaweeds, humic substances, antitranspirants, etc. (Du Jardin 2012a). The crude extracts of lower and higher plants have also been demonstrated to have the same effect (Kurepin et al. 2014). It has been reported that biostimulants might induce the de novo synthesis of plant hormones in the treated plants (Jannin et al. 2012). On the other hand, in many biostimulants, compounds such as polysaccharides, glycosides, amino acids, etc. are present that might act as activators/precursors of endogenous plant hormones (Parađiković et al. 2011). Thus, the biostimulants derived from microorganisms, algae, higher plants, animal and humate-based raw material could therefore be responsible for the hormone-like effects or hormonal actions.

Chitin and chitosan are used for the improvement of crop resistance to abiotic stress and pathogen attack, attributing to its potential to induce plant defense mechanisms and initiate the stress response pathways. The chitin molecule activates a key plant defense enzyme, Phenylalanine ammonia-lyase (PAL), that is responsible for accumulation of phenolic compounds in the plants, including papaya (*Carica papaya* L.), sweet basil (*Ocimum basilicum* L.), and sunflower (*Helianthus annuus* L.) (Pichyangkura and Chadchawan 2015). Chitosan is responsible for abiotic stress gene regulation through phytohormone regulators such as abscisic acid (ABA), jasmonic acid (JA), and phosphatidic acid (PA) (Pichyangkura and Chadchawan 2015). A proteomic approach study by Lucini et al. (2018) revealed that chitosan was responsible for phenylpropanoids' biosynthesis in grapes.

7.4 Extraction of Biostimulants from Food and Agro Waste

A number of techniques are being used for using the by-products and wastes generated from agro and food industries in order to extract and prepare biostimulants. These techniques include extraction, cultivation, fermentation, processing and purification, hydrolysis, and high-pressure cell rupture treatment (Halim et al. 2016). The final biological properties, the final composition, and commercial use of the biostimulant greatly depends upon the techniques that are used for extracting or developing the biostimulants and the raw material used (Traon et al. 2014). A number of studies have focussed on valorizing the food and agro-industrial waste considering their high content of high value molecules into the plant biostimulants. They have used various techniques for the extraction and development of the biostimulant extract that is rich in phytohormones, amino acids, protein, and phenolic compounds (Puglia et al. 2021). Sometimes, if different production methods and different sources are used, the result can be a mixture of components.

For producing a commercial product that is uniform, frequently the extracts are utilized rather than the raw biomass through a standardized manufacturing process (Michalak and Chojnacka 2014). The processes being opted for the production of many commercial products are decided and driven by the marketing demands, rather than the target to optimize the efficacy of the commercial biostimulant. According to Lötze and Hoffman (2016), the biostimulants that are derived from same raw materials are usually marketed as similar products, but can significantly vary in terms of their composition as well as efficiency. Craigie (2011) mentioned that the biostimulants produced from the same species of the seaweed extract, are rarely equivalent. Most of the manufacturers do not disclose the process/technique of production of biostimulant, as it is a trade secret (Traon et al. 2014).

For the production of the protein hydrolysates from the by-products and wastes of food and agriculture industries, chemical and enzymatic protein hydrolysis are the most preferred methods (Colla et al. 2014). The technique/process to be used for the protein hydrolysis depends on their source of origin. For example, proteins from animal origin such as horns, feathers, beaks, wool are keratin rich and thus are usually hydrolyzed through acidic or alkaline treatment, often keratinase enzymes of bacterial origin are also used (Pasupuleki and Braun 2010). On the other hand, the animal waste and by-products from whey, intestines, casein and meat, rice, pea, cottonseed, etc. are hydrolyzed by general enzymatic or microbial processes (Dieterich et al. 2014; Pasupuleki et al. 2010). The protein hydrolysates originated from the plants are generally extracted by enzymatic hydrolysis that produce a blend of peptides and amino acids of varying lengths and low salinity (Colla et al. 2015a, b).

The protein hydrolysates derived from organic wastes through acid and alkaline hydrolysis have been widely endorsed as efficient biostimulants for plant. Acid hydrolysis has an advantage of being cost efficient as compared to the alkaline hydrolysis. The complete hydrolysis of proteins through alkaline hydrolysis method is done at a high temperature with the help of chemical agents, viz., sodium, calcium, or potassium hydroxide (e.g., 4 mol/L for 20 h). Alkaline hydrolysis obtains both soluble and insoluble fractions (Dai et al. 2014). The chemical properties of protein hydrolysates produced majorly depend on the source of protein and the production process (Colla et al. 2015a, b).

A novel technique, i.e. Cavitation-assisted extraction (CAE) is also an alternative to the conventional methods such as reflux, percolation, maceration using organic solvents. CAE process adds to the "(a) increase in temperature and pressure resulting into high mass transfer rate; (b) improved diffusion and implosion of agitating bubbles; (c) enlargement of pores; and (d) production of exceedingly reactive free radicals aiding cell disruption" (Panda and Manickam 2019). Xu et al. (2015) have described the effects of pulse electric field on dairy protein extraction. His study reported the cell disruption and extraction of β-LG band. In another recent study by Ghosh et al. (2019), 78 ± 8 mg/mL protein content was generated by the waste meat using high voltage. Fermentation has also been mentioned as an effective method of obtaining hydrolysates from underutilized fish protein by-products (Ruichang et al. 2021).

During the chitin extraction, the extraction conditions determine the various characteristics of purified chitin like degree of deacetylation, molecular weight, purity, and polydispersity index (Yadav et al. 2019). The chemical extraction method has three basic steps (a) an alkaline solution deproteinization; (b) an acid solution demineralization; and (c) discoloration (Garcia et al. 2019). All these steps have an impact on the final chitin product and its physicochemical properties (Ali et al. 2018; Küçükgülmez 2018).

Although the chemical extraction methods are considered uneconomical and detrimental to the environment, known to unfavorably affect the chitin properties, still it is the most common method on the commercial scale. To overcome these disadvantages, biological extraction method is seeking interest due to its economic viability and safer treatment. The biological extraction methods are still limited to laboratory scale only. Tao et al. (2004) used alkali treatment and acid reflux to extract chitinous material from alkali insoluble material and examined it's purity, crystallinity, degree of acetylation (DA), and yield.

The yield was recorded as 0.65–1.15% on a fresh basis. Chitin and chitosan were also extracted through alkali-acid treatment by Khanafari et al. (2008) from *Penaeus semisulcatus* waste collected from a shrimp processing landing center, and the yields recorded were 510 and 410 mg/g, respectively.

7.5 Conclusion

The global agriculture and food production industry is under pressure for meeting the escalating demand of human food, animal feed, and energy production. This leads to the generation of huge amounts of waste and by-products that ultimately find their way into the environment. Finding alternative ways to make use of such material is imperative. In this context, the valorization of the waste produced in food and agro-waste industry into the development of a high value product such a biostimulants. Biostimulants (PB) have their applications in agriculture and are a heterogeneous, complex, and varied group of substances. Biostimulants regulate and modify the plant's physiological processes in order to stimulate their growth, to impart stress resistance and to increase plant yield. Biostimulants can be categorized into those from plant and animal origins, protein hydrolysates, chitin and chitosan, etc. Various techniques are being opted for extraction and development of the biostimulants such as extraction, cultivation, fermentation, processing, purification, hydrolysis, and high-pressure cell rupture treatment. The utilization of the waste generated by the agro and food industry will not only help in valorization and waste management but also provide a substitute for the reduction of agrochemicals.

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