RE-VALORIZATION OF FOOD LOSSES AND FOOD CO-PRODUCTS

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RE-VALORIZATION OF FOOD LOSSES AND FOOD CO-PRODUCTS

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EDITORIAL
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Editorial: Re-valorization of Food Losses and Food Co-products

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Editorial on the Research Topic

Re-valorization of Food Losses and Food Co-products

According to the report on the World State of Food and Agriculture 2019, progress in the fight against food loss and waste, and the reduction of food loss, help to improve the sustainability of the environment, while the reduction of waste benefits food security. According to the FAO, the Food and Agriculture Organization of the United Nations, global food waste represents a third of total food produced for consumption, ∼1.6 billion tons per year, which represents a cost of € 730 million/year. The food groups that stand out for their highest percentages of losses are fruits and vegetables, and roots and tubers, both with a 45% loss. It is followed by fish and marine products with 35%, and cereals with 30%. Both food losses and its co-products are very rich sources of nutrients and other bioactive substances that, once extracted, represent very valuable ingredients for the elaboration of functional and nutraceutical foods (among other products). The extraction and isolation of these compounds allow for their use in other sectors (yogurts and dairy products, juices and beverages, energy bars, etc.), as ingredients that favor certain functions when ingesting them.

The European Parliament's Waste Framework Directive applies a standard prioritization scheme for the recovery of food by-products. In it, the first option is the prevention and reduction of the generation of by-products, and the second is human consumption. Finally, there is the shipment of the by-products to landfill, a solution that cannot be considered as recovery. The continuous development of re-valorization solutions will accelerate the sustainability of the food production and consumption system. This Research Topic highlights the role of integrated teams of research scientists for this purpose. Following the United States Environmental Protection Agency (US-EPA) food recovery hierarchy (**Figure 1**), we invited manuscripts that focus on solutions for re-valorization of food losses and food coproducts, including but not limited to: Prevention and reduction; Human consumption; Bioproducts; Animal feeds; Industrial uses; Energy production; Agronomic uses; and Other ways of disposal.

In total, 5 articles were reviewed and published from authors mainly from America, Asia and Europe. Their topics were mainly about plant foods, but also seafood. In this way, they were centered in the production of food allergy modulators (Bessa et al.), nutraceuticals (Cortés-Ferré et al.), and animal feeds (Kithama et al.), together with approaches on green and sustainable methods (Doria et al.) and also bioconversion and bio-refinery (Venugopal).

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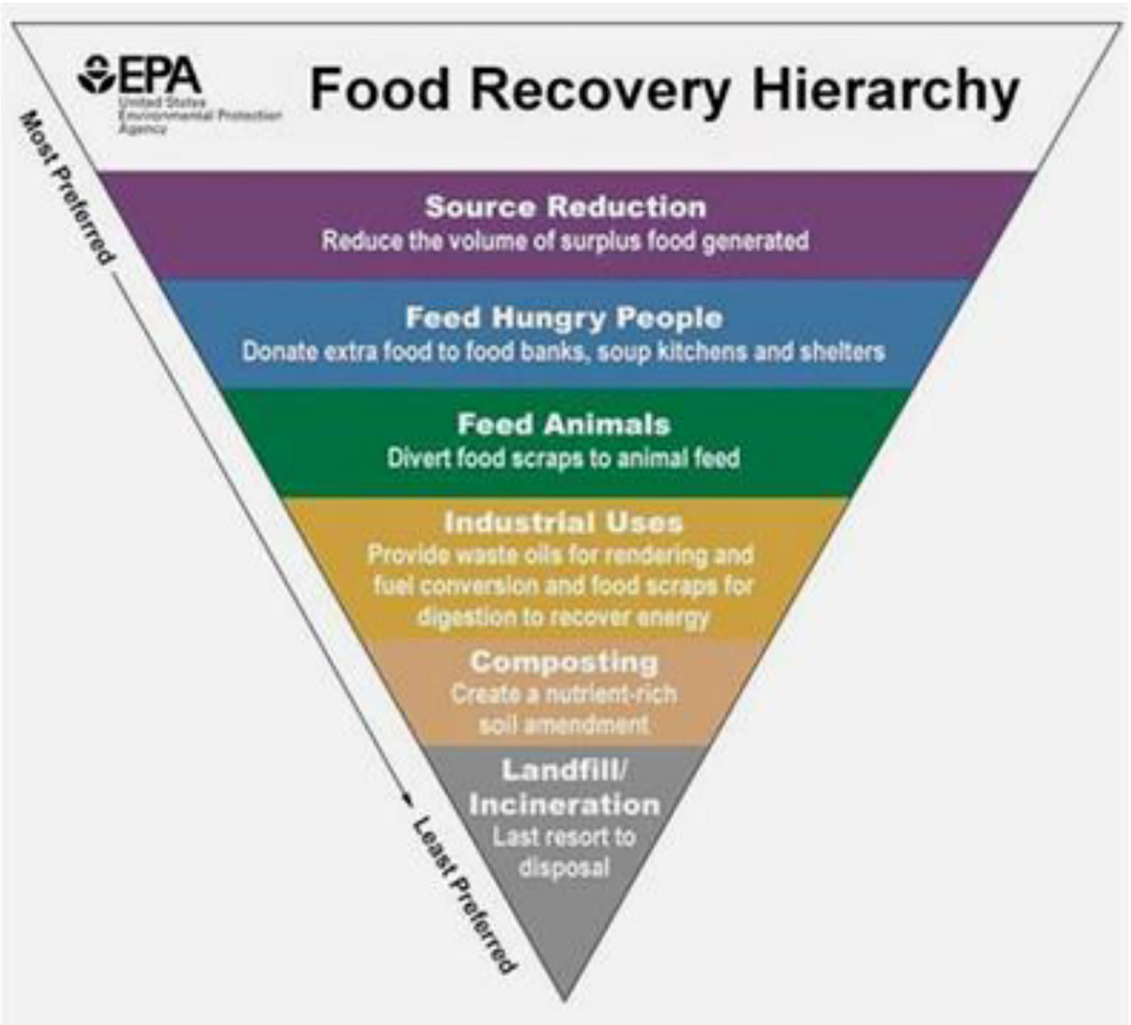

FIGURE 1 | United States Environmental Protection Agency (US-EPA) food recovery hierarchy.

AUTHOR CONTRIBUTIONS

JS-G wrote the first draft. TA, ME, AG-Z, and JX edited it. All authors approved the final version of the manuscript.

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REVIEW
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Recovery of Capsaicinoids and Other Phytochemicals Involved With TRPV-1 Receptor to Re-valorize Chili Pepper Waste and Produce Nutraceuticals

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The hot chili pepper industry represents one of the most important staple foods in Mexico and many Asian countries. Nowadays, large amounts of waste materials are produced from the pepper supply chain that could be used as a source to obtain nutraceuticals. Among the most common and important bioactive compounds contained in pepper residues are the capsaicinoids, which are the responsible of the pungency of the pepper. Capsaicinoids, mainly capsaicin, may ameliorate obesity, gastric disorder, diabetes, cardiovascular diseases, cancer, rhinitis, asthma, immune system diseases, and important viral diseases as the recent COVID-19. The aim of this review is to review the industrial process for the extraction of capsaicinoids ingredients from pepper residues and to examine the relation of the capsaicin and other chili pepper phytochemicals to prevent and treat chronic diseases explained through the key role of the TRPV1 receptor. The extraction and incorporation of these compounds into nutraceutical formulations depend mainly on the development of new methods to improve not only the yield of a particular compound but the validation of the bioactivity and phytochemical characterization.

Keywords: capsaicinoids, capsaicin, TRPV1 receptor, immune system diseases, waste

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INTRODUCTION

The annual production of chili peppers is about 3.2 millions of ton that correspond to the 3.5% of the Mexican agricultural Gross Domestic Product (GDP) (SAGARPA, 2017). Globally, the industry related with chili pepper production increased in other countries where this condiment is relevant for their gastronomy and other cultural purposes. The exportations of dry crushed chili pepper of countries like India, China, or Spain represent 6 billion USD of the global production and correspond to 65% of the chili pepper exportations (Carmona, 2013). Currently in Mexico, chili peppers are the most important cultivars for exportation, where 29.71% of the total production go to international market, mainly USA, Canada, and Guatemala (SAGARPA, 2017). Nevertheless, in order to guarantee this exportation, these products must meet rigorous quality criteria and a big amount of material is discarded. These crop waste materials correspond to 18.4% of the total

national crops, which mainly includes seeds, and incomplete and defective fruits. These pepper wastes could be used as byproducts due to their content of compounds with antioxidant activity, which could be used to produce innovative ingredients (Sandoval-Castro et al., 2017).

In many of fruits and vegetables, only the epithelium or pulp is consumed but substantial amounts of nutraceutical compounds are present in the seeds, peels, and other components as peduncle, loculum or base that are not commonly consumed (Rudra et al., 2015). Extraction processes are the main critical phase to obtain bioactive compounds from waste material. Sample preparation is one of the essential factors to regulate the type and amount of bioactive compounds extracted. Extraction methods can be categorized into two main categories: conventional and innovative techniques (Sagar et al., 2018).

Enzymatic pre-treatment is as a novel and useful way to recover bound compounds from the cell wall and increase their yield of extraction (Sagar et al., 2018). The use of enzymatic treatments coupled to the oil extraction processes such as organic solvent extraction, supercritical fluids extraction, and others improve the yield of extraction resulting into promising high percentages of recovery (Stoica et al., 2016). Enzymatic techniques have been established fundamentally for the extraction of bioactive compounds from different seeds. Different elements including catalyst type, molecular size of plant materials, and the hydrolysis time are observed as main factors for extraction. Enzymatic treatment for extraction is contemplated as an eco-friendly method to extract oils and bioactive compounds because water is used as the solvent instead of organic solvents (Sagar et al., 2018). Cellulases, pectinases, beta-glucosidases, or enzymes cocktails are used to hydrolyze and degrade cell wall components and in consequence release the nutraceutical compounds that are linked to cell wall such as carotenoids, phenolic compounds, capsaicinoids, or other compounds of interest (Walczak et al., 2018).

Pepper seeds contain sterols, saponins and phenolic compounds responsible for the biological activities (Sung and Lee, 2016). Red pepper seed oils are abundant in polyunsaturated fatty acids, bioactive compounds such as polyphenols, tocopherols, and phytosterols (Chouaibi et al., 2019) that have been related with activities described previously and result an interesting field of study. Around 90% of capsaicinoids are located in the pericarp (fruit), which comprises 40% of the pepper, while the other 10% are found in the seeds and then their concentration in the waste parts is 102–154 mg/g while in non-waste material it is 250–450 mg/g of dry material (Oguzkan, 2019). Capsaicinoids have been used in traditional medicine for the treatment of cough, toothache, sore throat, rheumatism, antiseptic, antioxidant and immunomodulator (Sanati et al., 2018). They possess anti-inflammatory effects, antioxidant activity and anti-obesity properties, and have been related to anti-carcinogenic and anti-mutagenic properties that could be exploited as a new nutraceutical approach to the extracts related with chili peppers (Friedman et al., 2019).

Recently, the potential effects of the capsaicinoids under the immune responses have been studied. Grüter et al. (2020) reported that capsaicin exerts an immunomodulatory response on neurilemma cells due to the anti-inflammatory effect provoked by numerous biochemical neural pathways. Nowadays, the worldwide attention is focused in the global pandemic of COVID-19 and the scientific efforts are concentrated on finding alternative treatments to the viral disease, one of the research route is the evaluation of phytochemical compounds that could relieve the symptoms of the SARS-Cov-2 infection.

CHEMISTRY AND BIOSYNTHESIS OF CAPSAICINOIDS

Capsaicinoids (**Figure 1A**) are alkaloid compounds responsible for the pungency associated with consumption of *Capsicum* fruit. Capsaicinoids are highly volatile, pungent, hydrophobic, colorless, and odorless white crystalline powder, with a melting point of 60–65°C and a molecular weight of 305.4 Da (Sharma et al., 2013). The basic chemical structure of these compounds is an acid amide of vanillyl amine combined with branched-chain fatty acids from 7 to 13 carbons in length (Aza-González et al., 2011). Their pungency is mainly related to the benzene ring but also to the acyl chain length (Fattori et al., 2016). Although more than 10 structures exist, the most prominent forms are capsaicin and dihydrocapsaicin, accounting for almost 90% of capsaicinoids (Meghvansi et al., 2010). Nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin are present in lesser amounts (Tewksbury et al., 2006).

A similar group of compounds named capsinoids (**Figure 1B**) also have the beneficial effects of capsaicinoids but without the pungency (Hursel and Westerterp-Plantenga, 2010). Due to their slightly different structure, they stimulate receptors in the intestine but not in the mouth. This is a particularly valuable attribute, as many people struggle to consume capsaicinoids due to their "spiciness" (Luo et al., 2011). In contrast to capsaicinoids, capsinoids are esters of vanillyl alcohol with fatty acids similar to those of capsaicinoids (Fayos et al., 2019). This structural difference could also be responsible for the lower stability of capsinoids and a pungency 1,000 times lower than that of capsaicinoids (Tanaka et al., 2015).

Capsaicinoids are natural compounds related to defense mechanisms of plants against mammalian herbivores and fungi (Tewksbury et al., 2006). Their content in *Capsicum* fruit varieties is largely influenced by genetics, in which the total capsaicinoid content ranges from 720 to 4,360 ppm for cayenne (*Capsicum annuum*) cultivars, 1,110–7,260 ppm for jalapeno (*Capsicum annuum* jalapeño) cultivars, and 5,200–14,000 ppm for habanero (*Capsicum chinense*) cultivars. In addition to genetics, capsaicinoid content is influenced by the fertilization practices, environmental growing conditions, application of bioregulators, and maturity of the fruit (Sharma et al., 2013).

Capsaicinoids are synthetized primarily in the epidermal cells of placental tissue, with low levels found in the pericarp and seeds. L-phenylalanine and L-valine or L-leucine through vanillylamine are capsaicinoids precursors. The proposed pathway for synthesis of the vanillylamine moiety of capsaicinoids from L-phenylalanine is as follows: L-phenylalanine to trans-cinnamic acid by Phenyl Alanine

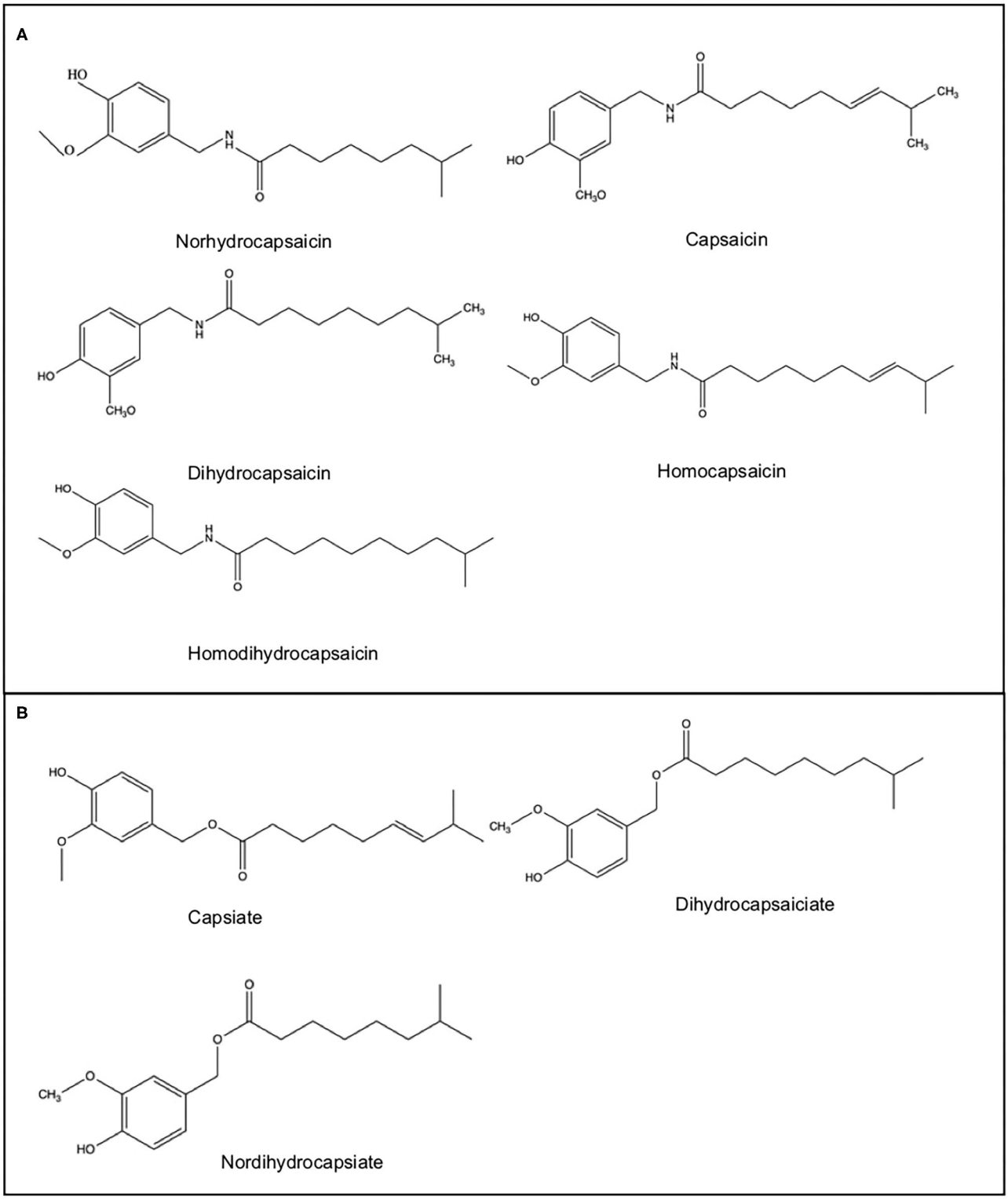

FIGURE 1 | Capsaicinoids **(A)** and capsinoids **(B)** chemical structure.

Ammonialyase (PAL), trans-cinnamic acid to trans-p-coumaric acid by Cinnamic acid-4-hydroxylase (Ca4H), trans-p-coumaric acid to trans-caffeic acid by Coumaric acid-3-hydroxylase (Ca3H), trans-caffeic to trans-ferulic acid by Caffeic acid O-methyl transferase (CoMT), and vanillin to vanillylamine by Amino transferase (AMT) (Kehie et al., 2013). Furthermore, the proposed pathway for the biosynthesis of valine to 8-methyl-6-nonenoic acid is as follows: valine to alpha keto isovalerate by Branched Chain Amino acid Transferase (BCAT), alpha keto isovalerate to isobutyryl CoA, isobutyryl CoA to Malonyl CoA using the enzyme complex of Fatty Acid Thioesterase- Acyl Carrier Protein- Keto Acyl Synthase (FAT-Acl-KAS), Malonyl CoA to 8-methyl-6-nonenoic acid. Finally, vanillylamine and 8-methyl-6-nonenoic acid are condensed by the action of Capsaicin Synthase (CS) to obtain one molecule of capsaicin (Kehie et al., 2013). Commercially, the capsaicinoids are synthetized by the reaction of vanillylamine with 7-methyloct-5-ene1-carboxylic acid chloride.

TECHNOLOGICAL ADVANCES FOR THE EXTRACTION OF CAPSAICINOIDS

Among the different upstream processes, the extraction process is the main critical phase to obtain bioactive compounds from agricultural residues. The sample preparation is critical for the appropriate release of the phytochemicals form the residue matrix, which confers the essential factors to regulate the type and amount of bioactive compounds to extract. The industrial methods (pretreatment and extraction) to obtain capsaicinoids can be categorized into two main categories: conventional and innovative techniques (Sagar et al., 2018).

The conventional methods focus in using organic solvents as the extraction vehicle of capsaicinoids, by the implementation of different techniques such as solvent, hydro-distillation, Soxhlet, and maceration extraction (**Table 1**). Depending on the extraction conditions, the extraction yield, quality, and bioactivity of the extracted compounds is affected. The most extensively used method to recover capsaicinoids is based on the extraction with hexane, which is very toxic and produces residues that could compromise the quality of the compounds (Martins et al., 2017). However, various extraction methods for capsaicinoids have been developed expanding the extraction solvents alternatives to methanol, ethanol, acetonitrile, and water (Lu et al., 2017). The conventional methods, involving organic solvents reach an extraction yield between 70 and 80% of total capsaicinoids and the time of extraction is inversely proportional to the polarity of the solvent (Abdurahman and Olalere, 2016). Another extraction method is the hydro-distillation, which is the simplest technique to obtain capsaicinoids from residues materials but with low yield. Jiang et al. (2013) developed the hydro-distillation with an extraction yield of only 3.1% after 2 h of process. Furthermore, the Soxhlet method is another conventional method broadly used with higher extraction yields. Boonkird et al. (2008) extracted capsaicinoids using ethanol as solvent during 8 h at 78°C to obtain up to 92% of global yield. In another study, Santos et al. (2015) extracted the capsaicinoids from Malagueta pepper (*Capsicum frutescens* L.) using Soxhlet extraction with dichloromethane as a solvent to shift the mixture polarity to 3.1. This solvent modification allowed a recovery of 92.1% of the capsaicinoids. Despite these techniques provide higher extract yield and total content of capsaicinoids, they required high amount of solvents and longtime extractions that increases the cost and limit its applications due to the environmental and health regulations.

Nowadays, due to sustainability consciousness of consumers, industry and governments, modern methods require the minimal use of solvents that could negatively affect the environment. Additionally, there is a growing requirement for new extraction methods with shortened extraction time and reduced organic solvents utilization that not only prevent pollution but also decrease extraction costs. Among innovative technologies for the extraction of bioactive compounds from food by-products are the pressurized liquid extraction, microwave assisted extraction, ultrasound assisted extraction, enzyme-assisted extraction, and supercritical fluid extraction. The selection of extraction method must be fast, low-cost, adaptable, and effective.

The pressurized liquid extraction (PLE) is a technology that required small amounts of solvents, in which depending on the polarity of the target bioactive compounds a variety of green solvents may be used. This process involves the use of elevated temperatures and pressure with the liquid solvent to increase the mass transfer of the solvents within the solid matrix. Barbero et al. (2006), evaluated the use of water, ethanol and methanol as solvents to extract capsaicinoids from different varieties of pepper from Spain using pressurized liquid technologies at 200°C, 100 bar 30 min preheating sample and extraction cycles of 5 min. The methanol extracted the highest content of capsaicinoids (450 μmol/kg), followed by ethanol (400 μmol/kg) and water (250 μmol/kg). In another study, Liu et al. (2013), determined that the optimal conditions of capsaicin (97.1%), dihydrocapsaicin (97.9%), and nordihydrocapsaicin (97.6%) extraction from commercial *Capsicum annuum* were 100°C and 1 cycle of 30 min. Finally, Bajer et al. (2015), used water as solvent for the total recovery of capsaicinoids (28.9 μg/g) at 20 MPa, 200°C, 20 min of static and 10 min continuous extraction. Despite its short time of process, the cartridges used limits the load of residues to process, require the pretreatment of the materials to increase the yields of extraction and further downstream process (evaporation and drying) are required to eliminate the solvents.

Another innovative technology is the microwave assisted extraction (MAE), which is a technology that uses the microwave energy to heat solvents and its increase the diffusivity within the matrix. Similarly, to the PLE, the advantages of this process is to reduce the solvents amounts and time, in which the extraction efficiency depends on medium used to match the low polarity of capsaicinoids (Nazari et al., 2007).

In terms of adaptability at an industrial scale, the ultrasound-assisted extraction (UAE) has been widely studied for the recovery of bioactive compounds from natural products (Esclapez et al., 2011). This technology focus on the cavitation principle, which consist in forming small cavities to release

TABLE 1 | Comparison of different extraction techniques used to recover capsaicinoids and other phytochemicals with potential beneficial effects on health.

Extraction technique	UAE conditions	Extraction Yield	Extraction conditions	Extracted compounds	Evaluated bioactivity	References
Hydro-distillation	–	3.1%	Water, 2 h, 1:20 (water:fruit)	Capsaicin	–	Jiang et al., 2013
Soxhlet	–	92%	Ethanol, 5 h, 78°C, 1:8 (solvent:fruit)	Capsaicinoids		Boonkird et al., 2008
	–	92.1%	Dichloromethane, 6 h	Capsaicinoids	–	Santos et al., 2015
Solvent extraction	–	79.4%	Ethanol, 15 h, 45°C, 1:8 (solvent:fruit)	Capsaicinoids	–	Boonkird et al., 2008
	35 kHz, 600 W	87.4%	Ethanol, 3h, 45°C, 1:8 (solvent:fruit)	Capsaicinoids	–	Boonkird et al., 2008
	400 kHz	Increase in (alpha-glucosidase inhibition by 90% (Ethanol), 92% (Methanol) and 93% (Acetone) when UAE	Ethanol 80%, methanol 80% and acetone 80% (1:4; seeds powder:solvent); 40 min, 40°C	Phenolic compounds, capsaicinoids	Antioxidant, α-glucosidase inhibition	Liu et al., 2020
		–	Room temperature, methanol 95%, 1 week	Chrysoeriol, luteolin-7-O-glucopyranoside, isorhamnetin-7-O-glucopyranoside	Soluble Epoxide Hydrolase Inhibition	Kim and Jin, 2020
	35 kHz, 640 W	–	Water/methanol, 50°C, 20 min, 1:4 (fruit:solvent)	Phenolic compounds, carotenoids, flavonoids, capsaicinoids	Antioxidant, antidiabetic	Sricharoen et al., 2017
Enzymatic Maceration		Increased 17.5% with enzymatic pre- treatment	• 50°C, 18 h, Pectinex AR, Celluclast and combined • Solvent Extraction: Water, 50°C, 18 h	Capsaicinoids, carotenoids	-	de Farias et al., 2020
Enzymatic pre-treatment + Solvent Extraction		Increased 14–22% with enzymatic pre-treatment	• 45–65°C, 30–150 min, pH 4-6. Enzymes: Celluclast, Pectinex Ultra, ViscozymeL, and Protease • Solvent extraction: Acetone and hexane, 1 h	Capsaicinoids	–	Baby and Ranganathan, 2016
		Increased 30-50% with enzymatic pre-treatment	• 60°C, 1 h, cellulase, ViscozymeL, pectinase • Solvent Extraction: Ethanol, 30 min	Phenolic compounds, flavonoids and carotenoids	Antioxidant	Nath et al., 2016
PLE	–	77%	65°C, 10 MPa, ethanol and water, 60 min	Capsiate, phenolic compounds	–	de Aguiar et al., 2020
SFE+PLE	–	91%	• SFE: 50°C, 15 MPa, 120 min • PLE: 65°C, 10 MPa, ethanol and water, 60 min	Capsiate, phenolic compounds	–	de Aguiar et al., 2020
SFE		88%	50°C, 15 MPa, 120 min	Capsiate, phenolic compounds	–	de Aguiar et al., 2020
		92%	10 MPa, 35°C, 90.2 kg/h CO_2 flow rate	Capsaicinoids	–	Rocha-Uribe et al., 2014

(Continued)

TABLE 1 | Continued

Extraction technique	UAE conditions	Extraction Yield	Extraction conditions	Extracted compounds	Evaluated bioactivity	References
	–	88%	60°C, 25 MPa, 120 min	Phenolic compounds, capsaicinoids	Antioxidant	Luiz et al., 2016
	600 W	94%	40°C, 25 MPa, 80 min	Phenolic compounds, capsaicinoids	Antioxidant	Luiz et al., 2016
	–	76.1%	40°C, 15MPa	Capsaicinoids	-	Santos et al., 2015
	360W, 60min	79.4%	40°C, 15 MPa, 150 min	Capsaicinoids	–	Santos et al., 2015
SFE+ microencapsulation		18%	• SFE: 25 MPa, 45°C, 70 min • Microencapsulation: Starch sodium octenylsuccinate, soybean protein isolated, gelatin, maltodextrine, spray drying 120°C	Capsaicinoids	–	Wang et al., 2017
Aqueous two-phase system (ATPS)		95.5%	Ethylene oxide-propylene oxide, pH 4.35, 35°C	Pure capsaicin (50.3% purity)	–	Fan et al., 2017
Tunable Aqueous Polymer-Phase Impregnated Resin (TAPPIR) Extraction		95.82%	Polyethylene glycol (PEG) and 1-ethyl-3-methyl imidazolium acetate ([Emim] [OAc]), 25°C	Pure capsaicin (92%)	–	Lu and Cui, 2019

energy into the matrix/solvent mixture. Sricharoen et al. (2017) found that the UAE produces an oleoresin rich in capsaicinoids, phenolics, carotenoids, flavonoids, and reducing sugars that could have a synergism to induce antioxidant and antidiabetic activity (**Table 1**).The scale-up process is feasible due to the low cost of equipment required and that could be easily integrated into the industry. Furthermore, water could be used as medium of reaction, thus avoiding the use of organic solvents. However, downstream process is required due to the poor selectivity of the extraction process.

Another innovative technology is the enzyme assisted extraction (EAE), which is as a novel and useful way to recover bound compounds from the cell wall and increase their yield of extraction (Sagar et al., 2018). The use of enzymatic treatments coupled to the oil extraction processes such as organic solvent extraction, an novel extraction technologies improves the yield of extraction resulting into promising high percentages of recovery (Stoica et al., 2016). Enzymatic techniques have been established fundamentally for the extraction of bioactive compounds from different seeds. Different elements including catalyst type, molecular size of plant materials, and the hydrolysis time are observed as main factors for extraction. Enzymatic treatment for extraction is contemplated as an eco-friendly method to extract oils and bioactive compounds because water is used as the solvent instead of organic solvents (Sagar et al., 2018). Cellulases, pectinases, beta-glucosidases, or enzymes cocktails are used to hydrolyze and degrade cell wall components and in consequence release the nutraceutical compounds that are linked to cell wall such as carotenoids, phenolic compounds, capsaicinoids, or other compounds of interest (Walczak et al., 2018). The utilization of enzymatic processes (**Table 2**) as a pre-treatment to increase the extraction yield, selectivity, quality, and purity of the capsaicinoids is a novel approach of the green extraction techniques. Baby and Ranganathan (2016) demonstrated that the use of commercial enzymes like Vizcozyme L for the extraction of capsaicinoids from entire fresh green chili peppers (*Capsicum annum* L.) increased by 22% the extraction yield compared with the conventional solvent extraction with methanol (**Table 2**). Nath et al. (2016) use an enzymatic pre-treatment to extract phenolic compounds, flavonoids, and carotenoids from mature fruits of *Capsicum annum* L., the enzymatic activity of pectinase from *Aspergillus niger* spores, Viscozyme L (Beta-glucanase), and cellulase from *Trichoderma reesei* was evaluated through the extraction yield shown that the use of an enzymatic treatment increased 30–50% the yield compared with the control. Enzymatic maceration of fruit pulp has positive results in color and flavor (de Farias et al., 2020). Commercial enzymes such as Pectinex AR (Pectinase), Celluclast 1.5 L (Cellulase) alone or in mixture increased 17.5% the extraction yield and changed the volatile composition of the extract improving the bioactive profile (**Table 2**). Lu et al. (2017) described that the use of an accurate temperature with a proper agitation speed could improve significantly the yield of extraction; using commercial pectinases and carbohydrases increased the extraction yield above 88%

TABLE 2 | Increased yields and phytochemical changes obtained with different enzymes used to release capsaicinoids from chili pepper.

References	Enzymes	Extraction yield	Phytochemical change
Baby and Ranganathan (2016)	• Celluclast 1.5L (Cellulase) • Pectinex Ultra SP.L (Polygalacturonase) • Viscozyme L (Beta-glucanase) • Protease	• Celluclast 1.5 L: ↑20% • Pectinex Ultra SP.L: ↑17.5% • Viscozyme L: ↑22% • Protease: ↑14%	Viscozyme L: increased capsaicin concentration, increasing the pungency of the extracts
Nath et al. (2016)	• Pectinase from *Aspergillus niger* spores • Viscozyme L (Beta-glucanase) • Cellulase from *Trichoderma reesei*	• Control: 30% • Pectinase: 83% • Viscozyme L: 87% • Cellulase: 55%	Increased the phenolic compounds and carotenoids contents and the antioxidant activity compared with control
de Farias et al. (2020)	• Celluclast 1.5 L (Cellulase) • Pectinex AR (Pectinase)	↑17.5%	Changed the volatile composition of the extracts
Gamarra Mendoza et al. (2020)	• Cellulase from *Aspergillus niger* spores	↑ 5 times compared with conventional method	Increased the capsaicin concentration and the pungency of extracts

compared with conventional techniques from dehydrated chili pepper fruits. Gamarra Mendoza et al. (2020) demonstrated that cellulolytic enzymes obtained from *Aspergillus niger* spores increased 5 times the extraction yield of carotenoids and capsaicinoids from *Capsicum baccatum* fruits in contrast with solvent extraction (hexane:methanol) (**Table 2**). The hydrolytic action of the cellulases on the molecular structures of the cellulose in the cell wall improve the extraction of the bioactive compounds compared with the conventional methods. Baby and Ranganathan (2016) and Gamarra Mendoza et al. (2020), proposed that the increase in the capsaicin extraction due the enzymatic treatment is also related to a higher antioxidant activity. However, the EAE ha certain limitations in terms of industrial scale-up processes. Depending on the type of enzyme to use and environmental conditions that affects the enzyme efficiency, may require a high cost of investment.

Finally, the supercritical fluid extraction (SFE) is a green technology that uses elevated pressure and temperatures to reach a critical point, in which the solvent (carbon dioxide CO_2) possess the diffusivity properties of a gas and the solvation power of a liquid. Nowadays, there a number of companies dedicated to install tailor-made industrial equipment's of SFE. For several decades the evaluation of how the different conditions of the SFE influenced into the capsaicinoids extraction yield and the quality of the extracts have generated important data to optimize and standardize these conditions. An interesting consideration in the use of the SFE is the cost of this green technology. de Aguiar et al. (2018) found that the optimal process that assured the minimal cost of manufacturing was reached at 240 min, 50°C and 15 MPa with a cost of 125.41 US$/kg of extract. However, to achieve an optimal process a profound knowledge of the mass transfer mechanism must be carried out and specific technology parts are required, which make this technology more expensive compared to UAS and EAE.

In order to enhance the yield of extraction with SFE, the use of coupled assisted technologies improve the mass transfer through convection and diffusion. Stoica et al. (2016) suggested that SFE might significantly increase the extraction yield of capsaicinoids using the most accurate combination of extraction conditions and the use of assisted technologies. Some of these assisted technologies are the use of ultrasound waves, microwaves, co-solvent extraction, enzymatic pre/during treatment. de Aguiar et al. (2020) showed that the use of SFE and PLE in the same process reduced 1.39 times the cost of manufacturing compared with the methods by separate. This suggests that the use simultaneous techniques could be economically affordable. Santos et al. (2015) improved the yield of extraction up to 77% compared to regular SFE (**Table 1**). The application of ultrasonic waves (US) in SFE processes as an alternative strategy to increase the extraction yield also enhance the quality of the bioactive compounds. Depending on the material matrix, US assisted SFE improved the extraction yield from the regular SFE by 6% (Luiz et al., 2016). In the case of the extraction of capsaicin using US assisted technology, Santos et al. (2015) found that the use of a US frequency of 360 W during 60 min enhanced the extraction yield up to 77% compared to regular SFE. Luiz et al. (2016) suggested that the use of 600 W frequency during 40 minutes improved the total capsaicinoids yield extraction into 45%.

Combining technologies is also relevant to produce stable ingredients with high contents of capsaicin. De Aguiar et al. (2016) applied ultrasound emulsification coupled with SFE to produce high stable emulsions rich in capsaicinoids using the modified food starch Hi-Cap100. Other interesting approach for the use of combined technologies is the use of microencapsulation by high speed homogenization and spray drying assembled with SFE to obtain highly stable capsaicinoids extracts. The process proposed by Wang et al. (2017) consisted on the homogenization of an extract obtained by SFE with starch sodium octenylsuccinate, soybean protein isolate, gelatin, and maltodextrin using high speed stirring and spray drying, giving a highly stable powder with high solubility properties that also provides additional protection for the lipophilic compounds of the capsicum oleoresins. In order to improve the bioavailability of lipophilic compounds in *Capsicum* oleoresins obtained by SFE, nanoemulsification with Tween 80 by coupled high pressure homogenization and ultrasonication produced highly stable

nanoemulsions with good antimicrobial activity (Akbas et al., 2018).

The ultimate stage of the extraction process is the purification of the bioactive compounds. To obtain high purity capsaicin the methods currently used are the high-speed counter-current chromatography (HSCCC) and reversed-phase preparative chromatography but due to the elevated costs of the materials and equipment they are not suitable for the industrial production of pure capsaicin (Goll et al., 2013). Fan et al. (2017), propose the use of the macroporous adsorption resin (MAR) as an inexpensive alternative to obtain pure capsaicin after its extraction with an aqueous two-phase system; recovering 83.7% of the capsaicin with 50.3% of purity in the final product. Lu and Cui (2019) achieved a purity of capsaicin of 92% after Tunable Aqueous Polymer-Phase Impregnated Resin (TAPPIR) Extraction with an extraction yield of 95.82% and suggested that the combination of TAPPIR technology and reverse chromatography as a downstream processing technique is a practical approach for extracting and purifying capsaicin from the capsicum oleoresin.

Beyond extraction yield, the bioactivity of the extracts must be considered since capsaicinoids are extracted along with other phytochemicals such as carotenoids and phenolics compounds. The bioactivity of compounds like capsaicinoids, carotenoids, and phenolic acids from waste materials of chili pepper includes antioxidant activity, α-glucosidase inhibitory activity or anti-inflammatory, and immune regulatory activity (Liu et al., 2020). Sricharoen et al. (2017) found that the UAE produces an oleoresin rich in capsaicinoids, phenolics, carotenoids, flavonoids, and reducing sugars that could have a synergism to induce antioxidant and antidiabetic activity (**Table 1**).

CAPSAICINOIDS IN HUMAN HEALTH VIA TRPV-1 RECEPTOR

Capsaicin selectively binds to the vanilloid receptor subtype 1 (VR1), now on referred to as *TRPV-1*, a member of the superfamily of transient receptor potential ion channels. Friedman et al. (2017) suggested that the synthetic capsaicin analogs are a promising class of non-pungent compounds that mimetic with TRPV-1 receptor and enhance the pro-health properties of capsaicin without the side-effects of the natural compound. TRPV-1 is expressed peripherally in primary afferent nociceptors, and it is physiologically stimulated and sensitized by external factors like heat, protons, and various inflammatory mediators such as prostaglandins, metabolites, and others that make up an "inflammatory broth" (Gerner et al., 2008). New technologies related with painkillers could be either TRPV-1 agonists or antagonists. This breaking opportunity toward the intelligent drug design of TRPV-1 modulators requires a basic understanding of how known ligands interact with TRPV-1 and which are the possible reactions that these drugs could trigger.

TRPV-1 permits ions such as calcium and sodium to pass freely through the membrane of the primary sensory/nociceptive neurons, causing depolarization and excitation and leading to nociceptive responses that are related with analgesic and other kind of reactions (**Figure 2**). Capsaicin exhibits high selectivity for TRPV-1 and, in a very interesting way, it does not activate the other homologous channels related with the TRPV family. In several studies, two open states of TRPV-1 were determined at atomic resolution with either capsaicin or resiniferatoxin/double knot toxin (Yang and Zheng, 2017). A small electron density was observed inside the capsaicin-binding pocket in the capsaicin-bound structure. This provided so far, the most direct evidence of the location of bound capsaicin and could indicate how this cascade of electrons intervenes into the nociceptive responses (Yang et al., 2015). In the closed (apo)state of TRPV-1, an electron density was also observed, indicating that this pocket may be occupied by a lipid molecule in the absence of capsaicin (Yang and Zheng, 2017). Consequently, capsaicin may have to compete with a lipid molecule within a similar structure in order to bind and activate TRPV-1.

Grüter et al. (2020) reported that TRPV-1 and its downstream molecules calcitonin gene-related peptide (CGRP) and Substance P are important for recognition of nociception and thermal inflammatory pain. Additionally, these authors have shown that an augmented expression of TRPV-1 in peripheral nerve terminals mediate thermal nociception in the Fabry disease mouse model. Moreover, previous studies demonstrated the key relation between TRPV-1 and P2Y receptors that are involved in the vasodilation and immune responses. The interaction of TRPV-1 and capsaicin could downregulate the receptor expression and reduce nociceptions that are proper to inhibition of pro inflammatory cytokines and oxidative stress in neurons (Khalid et al., 2019).

Anti-obesity and Gastric Disorders

Capsaicin may induce satiety by increasing the release of hormones from the intestine. In *in vitro* studies about stimulation of TRPV-1 with capsaicin in intestinal enteroendocrine cells, it was found a calcium-dependent release of glucagon like peptide-1 (GLP-1), which is directly related with the mediation of satiety (Szolcsányi and Pintér, 2013). GLP-1 concentration increased after a lunch that contained capsaicin (24 μg/mL of infusion in the lunch) in comparison with a placebo lunch (Van Avesaat et al., 2016). In some cases, the anti-obesity effects of capsaicinoids may be disguised by other factors related to age, sex, ethnic origin, and body composition related with a "desensitization" and a fragile response to capsaicin/TRPV-1 (Van Avesaat et al., 2016). Capsaicin induces satiety in a short amount of time (25 min) and, in consequence, decreases caloric intake, which, in turn, could lead to weight loss.

As a thermogenic compound, capsaicin in a concentration of 6 mg/kg of body weight increases catecholamine secretion from the adrenal medulla. Specific capsaicin-sensitive neurons are similarly affected by the administration of β-adrenergic blockers such the propranolol and metoprolol, drugs used in the treatment of hearth diseases and hypertension at a concentration less than a half that the chemical drugs (**Figure 3**) (Diepvens et al., 2007). In small rodents, after the direct administration (intraperitoneal and intragastric) of capsaicin and other capsaicinoids (135 mg/day), the whole-body energy spending significantly augmented, the adrenosympathetic

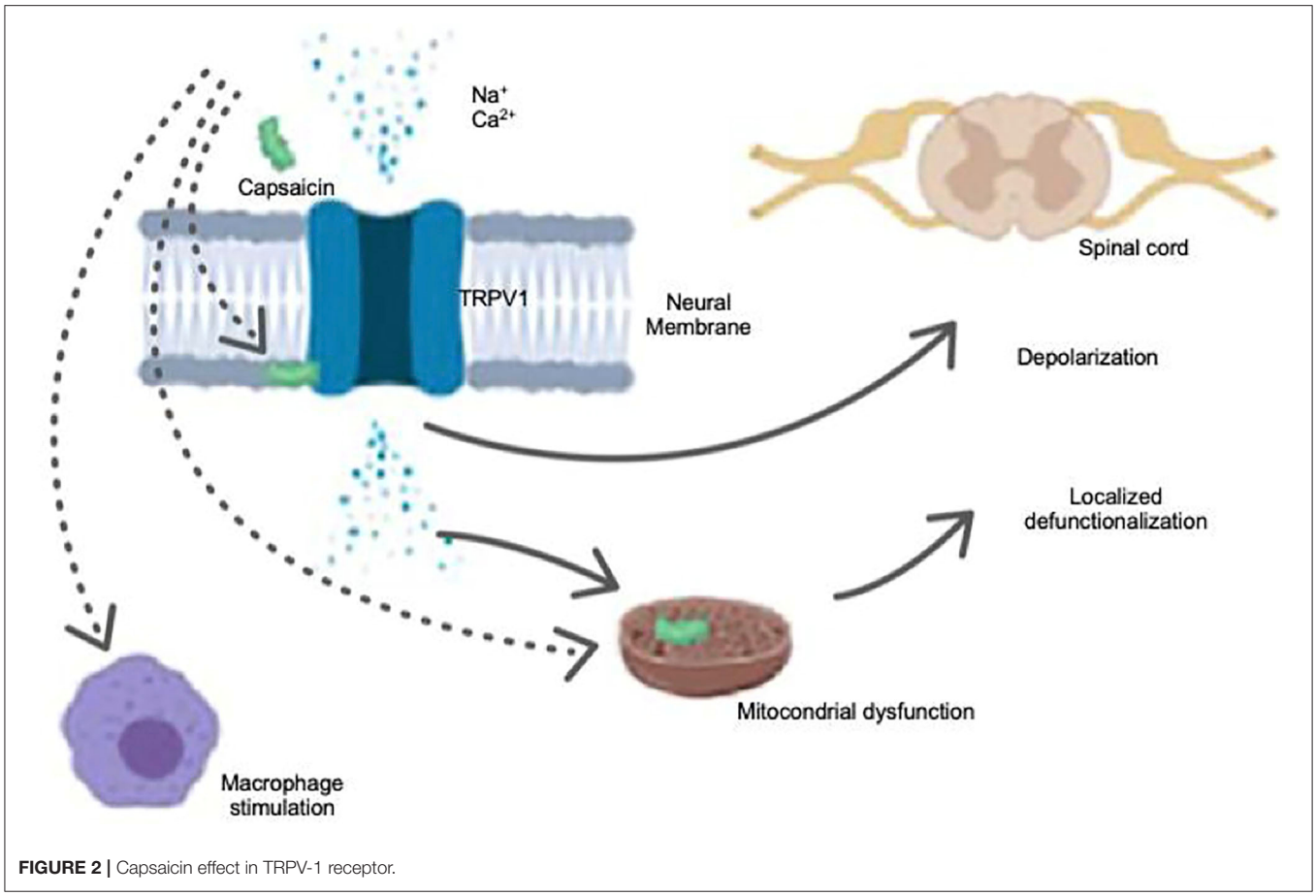

FIGURE 2 | Capsaicin effect in TRPV-1 receptor.

nervous system was activated and by consequence increased the core temperature in 1%. These effects openly impact into the brown adipose tissue (BAT) thermogenesis, producing an anti-obesity property (Saito and Yoneshiro, 2013). Baskaran et al. (2017) demonstrated that feeding capsaicin (1 μM) boosted adipogenic and thermogenic proteins in BAT; these effects were primarily mediated by the activation of SIRT-1/PRDM-16-dependent mechanism and finally produced an anti-obesity effect. N-vanillil oleamide has been proved to increase lipolysis, decrease or inhibit adipogenesis, decrease weight of adipose tissue, modify the size and/or number of adipocytes, increase satiety and inhibit appetite, demonstrating an outstanding affinity toward the vanilloid receptor of subtype 1 (VR1/TRPV-1) (Leung, 2014).

Another potential mechanism related with the anti-obesity effects of the capsaicin is the gut microbiota. Wang et al. (2020), demonstrated that intergastrical administration of capsaicin at concentration of 2 mg/kg of body mass, suppressed obesity due to the increase of the relative abundance of some gut microorganisms such as *Bacteroides, Coprococcus, Prevotella, Akkermansia, Odoribacter,* and *Allobaculum* in obese mice. Additionally, the change in the relative abundances of SCFA-producing bacterial species enhanced acetate and propionate production by 5% compared with control which is beneficial for energy balance. Capsaicin also reduced *Proteobacteria spp.* such as *Desulfovibrio, Escherichia, Sutterella,* and *Helicobacter*, which can produce a chronic, low-grade inflammatory response related with higher risks of obesity (Wang et al., 2020). Previous studies demonstrated that capsaicin at concentrations greater that 10 μg/mL had bactericidal activity and inhibited the growth of certain bacteria among is *H. pylori in vitro* (Jones et al., 2006). Capsaicin administrated in a vehicle at concentration of 4 mM decreased *H. pylori*-induced gastric ulcer in human via topically or g.i., the reduction was related to the diminished IL-8 production and reduced NF-κB activity (Satyanarayana, 2006).

The gastroprotective effects of capsaicin recline in the modulation of the sensory neurons. Co-treatment of capsaicin (10 mg/kg of body mass) and L-nitro-arginine methyl ester (L-NAME, a NOS inhibitor) activated TRPV-1 located at gastric sensory neurons that stimulated the release of CGRP and NO in mice (Fattori et al., 2016). Treatment with capsaicin in mice at a concentration of 1–8 μg/mL of gastric acid level also decreased indomethacin-induced microbleeding (Mózsik et al., 2005).

Diabetes

Capsaicin consumption has a positive impact on the reduction of glucose and insulin levels in humans. Regular daily consumption of 5 mg of capsaicin diminished postprandial hyperinsulinemia

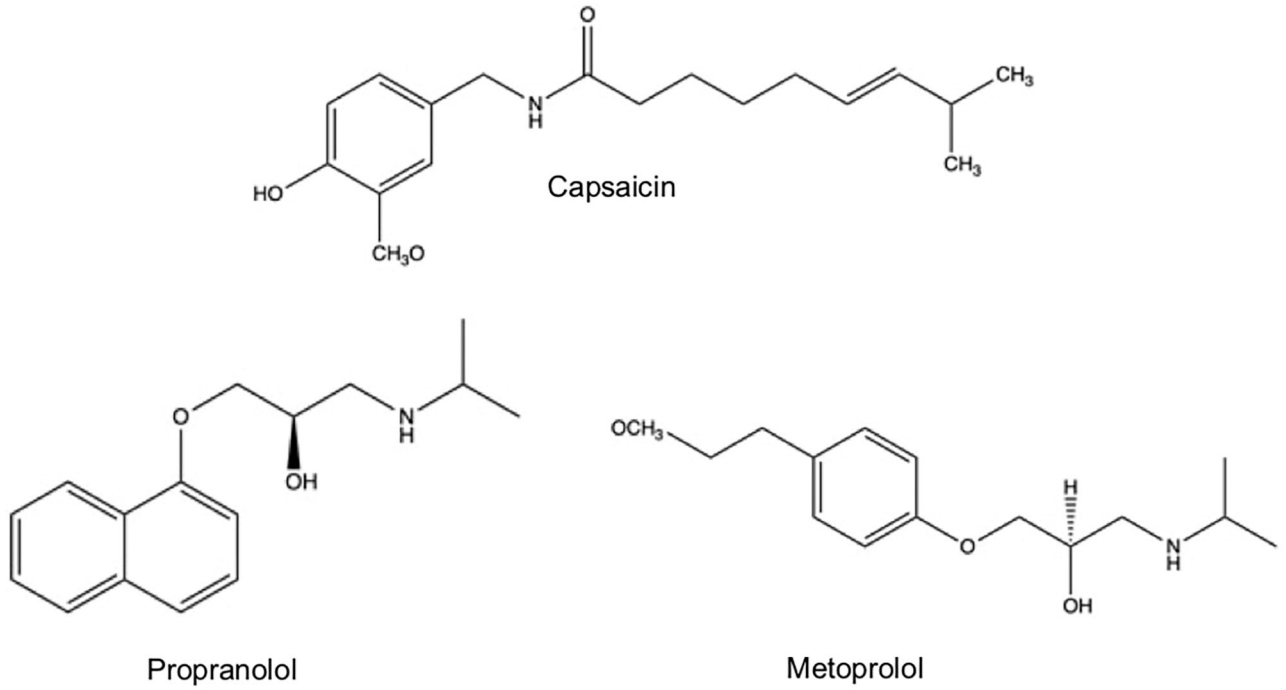

FIGURE 3 | Chemical structure of capsaicin and β-adrenergic blockers: propranolol and metoprolol.

in healthy adults (Yuan et al., 2016). Capsaicin also inhibits adipose tissue inflammatory responses in obesity (Kang et al., 2007). *In vitro* studies reported that capsaicin at a dose of 35 mg/kg of body mass overturns IL-6 and MCP-1 gene expression and protein discharge from adipose tissue and adipocytes; furthermore, dietary capsaicin significantly reduces seven times the adipose tissue macrophages and levels of inflammatory adipocytokines such as TNF-α, MCP-1, IL-6, and leptin and regularizes fasting glucose levels in obese mice (Xu et al., 2017). Obesity-related inflammatory proteins can block insulin signaling.

Recent advances in research have discovered that TRPV-1 receptors play a central role in the development and progression of diabetes type 1 and 2 (Xu et al., 2017). The ablation of TRPV-1 expressing sensory nerves by capsaicin modulates disease development and/or progression. The specific sensory nerves innervating the pancreas are major players in the progress of pancreatitis and islet inflammation and destruction. Capsaicin-induced permanent exclusion of TRPV1-expressing pancreatic sensory neurons decreases islet infiltration, insulin resistance, and β-cell stress in neonatal diabetes-prone non-obese diabetic (NOD) mice (Fattori et al., 2016). Therefore, capsaicin-induced depletion of TRPV1-expressing neurons prevents the development of diabetes in mice that are genetically predisposed to type 1 diabetes. Similarly, in Zucker diabetic fat (ZDF) rats, which are used to study various aspects of human type 2 diabetes, the capsaicin selective eradication of TRPV-1 expressing sensory fibers in the Langerhans islets prevented high plasma glucose levels and improved glucose tolerance and insulin secretion (Fattori et al., 2016).

Some studies demonstrate that resident macrophages use ATP receptors and more probably TRPV-1 receptors to, respectively, monitor beta cell activity and the acidity of the interstitial milieu in the islet. Since capsaicin activates the TRPV-1 activity, it is an important regulator of the islet activities. Identifying the microenvironment helps the macrophage gauge its production of factors that promote islet tissue stability (Yin et al., 2012). Islet macrophages are key actors in autoimmune destruction of the islet caused by type 1 diabetes and have a role in generating inflammation during type 2 diabetes (Weitz et al., 2018). The effects in diabetes are directly connected to the capsaicin-sensitive nerves in blood glucose control and mainly in the type 2 diabetes pathogenesis, since this specific diabetes type show progressive scarring of the pancreas and degenerating of the islet (Gram et al., 2017).

Interestingly, capsaicin also shields mice from the development of type 1 diabetes via TRPV-1 by a possible mechanism related to gut-mediated immune tolerance. Oral administration of 10 μg of capsaicin per kg of body mass could mitigate the proliferation and activation of autoreactive T cells in pancreatic lymph nodes (PLNs), protecting mice from diabetes development (Fattori et al., 2016).

Cardiovascular Disease

Capsaicinoids also have potential beneficial effects on the cardiovascular system. It is well-known that the cardiovascular system is plenty of capsaicin-sensitive sensory nerves, which play

an extensive role in controlling cardiovascular function across the release of numerous neurotransmitters such as CGRP (Calcitonin gene-related peptide), substance P, and others. Capsaicin is able to stimulate the release of CGRP through activating TRPV-1 and consequently it has potential benefits on cardiovascular function (Peng and Li, 2010).

Capsaicin and dihydrocapsaicin inhibit platelet aggregation and the activity of clotting factors VIII and IX (Adams et al., 2009). This specific property intervenes directly to the benefits on the prevention of the onset and the lower incidence of cardiovascular diseases. Capsaicin is capable to insert into the plasma membrane of platelets and to alter membrane fluidity and/or ionic permeability (Panchal et al., 2018). Conversely, it has been discovered that TRPV-1 was indeed present in the human platelets and capsaicin was capable to induce the Calcium cation release from intracellular platelet supplies and successively contributed to ADP and thrombin induced platelet triggering (Harper et al., 2009).

The antioxidant property of capsaicinoids also contributes to their beneficial effects on cardiovascular system. The oxidation of low-density lipoprotein (LDL) is the inducting factor for the development and succession of atherosclerosis (Fattori et al., 2016). Capsaicin at concentration of 15 μg/kg of body mass reduced serum total cholesterol and lipid peroxide level in high-fat-fed rats (Manjunatha and Srinivasan, 2007). Regular ingest of chili fruits for 4 weeks (≈ 3 μg per day) or more augmented the resistance of serum lipoproteins to oxidation in adult men and women (Ahuja et al., 2006). These statements support the antioxidant property of capsaicinoids described above and their potential clinical value on the prevention of cardiovascular diseases, such as atherosclerosis, coronary heart disease, and others.

Anti-cancerogenic

Capsaicin also induces apoptosis in cancer cells and suppress carcinogenesis in some tissues like prostate, colon, skin, breast, lung, and bladder (Wang et al., 2016). In the specific case of prostate cancer, capsaicin may act by two different pathways: primarily, a direct pathway in which capsaicin acts like antagonist of coenzyme Q controlling electron transport. Increase in reactive oxygen species (ROS) induces cell damage and finally apoptosis. Secondly, in the indirect pathway, its interaction with the TRPV-1 receptor leads into the accumulation of Ca^{2+} cations into the cancer cells and consequently produces apoptosis (Wang et al., 2016). It is acknowledged that intracellular Ca^{2+} levels have a substantial effect on cancer cells and TRP channels, and they have imperative roles in intracellular oxidative stress and apoptosis. The upturn of intracellular calcium level leads to an intensification of ROS amount, mitochondrial membrane depolarization and apoptosis in cancer cells (Övey and Güler, 2020).

Furthermore, capsaicin activates the phosphorylation of the STAT3 transcriptors and also activates the pro-survival transcription factor Tyr^{705} p-STAT3 related to antitumoral processes such as apoptosis, mutagenesis, and tumor suppression (Bhatta et al., 2019). Moreover, these studies exposed that the use of capsaicin as complementary treatment could improve the cisplatin ototoxicity activating TRPV-1 and STAT1 with contrasting downstream signaling pathways (Bhatta et al., 2019). Chu et al. (2019) found out that capsaicin exerts a negative effect on cancer cell viability, and induced apoptosis of human melanoma via the activation of cleaved caspase-3 and PARP.

In specific cases such as prostate cancer, capsaicin results in a synergistic effect with Docetaxel activating in a more effective way the AMP-activated kinase that is related with energy homeostasis, blocks cell cycle, induces apoptosis, regulates autophagy and suppresses the anabolic processes required for rapid cell growth (Sánchez et al., 2019). Another line of evidence was presented by Mori et al. (2006) who showed that capsaicin inhibits the activation of the nuclear factor-kappa (NF-k) and tumor necrosis factor-alpha (TNF-α) in prostatic cancer cell lines. Similar evidence of anti-cancer effect has been shown in numerous conditions like gastric cancer (Huh et al., 2011; Wang et al., 2011), colon cancer (Lu et al., 2010), breast cancer (De-Sá-Júnior et al., 2013), lung cancer (Anandakumar et al., 2012), leukemia (Tsou et al., 2006), and hepatocellular carcinoma (Moon et al., 2012). Capsaicin treatment reduced significantly the metastatic load in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice (Venier et al., 2015).

Zheng et al. (2016) demonstrated the activation of TRPV-1 by capsaicin blocked nuclear translocation of proliferating cell nuclear antigen and prove that TRPV-1 and capsaicin could be a target relation for the bladder cancer treatment. Likewise it was demonstrated that capsaicin drastically decreased the cell migration and invasion of cholangiocarcinoma HuCCT1 cells via the inactivation of the NF-κB/p65 signaling pathway and subsequently suppressed the expression of MMP-9. Xu et al. (2018), through the shRNA mediated knockdown of TRPV-1. Besides the role of TRPV-1 to promote the cancer cell apoptosis and avoid the damaged cell proliferation, the vanilloid receptor also possess anti-metastatic effects when it binds with capsaicin.

Anesthetic

Owing to their ability to relieve a variety of human pain disorders, the capsaicinoids (capsaicin mainly) have been used to prevent several clinical conditions related with pain at different scales including postmastectomy syndrome, urticaria, psoriasis, diabetic neuropathy, arthritis, pruritis, contact allergy, postsurgical neuromas, shingles (Herpes zoster), cluster headaches, urological disorders and many others (Howard and Wildman, 2006). Tumors progression occurs in an acidotic environment and this increases the interference of TRPV-1 antagonists that may relieve pain symptoms (Friedman et al., 2019).

Capsaicin interacts with transgerminal nerve endings, which release a neurotransmitter called substance P (neuropeptide with amino acid sequence as follows Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met [RPKPQQFFGLM]). Specifically in arthritis, capsaicin stimulates the release of substance P, after recurrent application, capsaicin drains the neuron of substance P and prevents its reaccumulation (Yang and Du, 2018). In rheumatoid arthritis (RA), capsaicin-sensitive sensory afferents densely innervate the articular capsule and the synovium. A distinctive characteristic of these nerve terminals is their dual nature:

as regular afferents they participate in pain signaling on the central nervous system and they also control the inflammatory reaction by performing as afferents by the liberation of sensory neuropeptides (Borbély et al., 2018).

Capsaicin triggers non-neuronal TRPV-1, which provoke the liberation of interleukin (IL-)8, IL-6, and prostaglandin E2 (PGE2) (Salari et al., 2019). Additionally, inactivation of voltage-gated Na^+ channels and direct pharmacological desensitization of plasma membrane TRPV-1 receptors may contribute to an instantaneous reduction on neuronal excitability and responsiveness. Microtubule depolymerization may interject fast axonal transport. In excessive concentrations of capsaicin than those required to TRPV-1, this antagonist can also reduce mitochondria dysfunction by openly inhibiting electron chain transport (Anand and Bley, 2011).

Capsaicin activates TRPV-1, which also obstructs Piezo proteins, a family of mammalian cation-selective ion channels that react to mechanical stretch (Borbiro et al., 2015). Obstruction of Piezo proteins occurs due to calcium-dependent stimulation of phospholipase Cδ (PLCδ), which depletes phosphoinositides. In fact, injection of phosphoinositides in the cytosol by expurgated inside-out patch clamp decreases rundown inner current of Piezo channels and reverts inactivation (Della Pietra et al., 2020). Therefore, the depletion of these phosphoinositides relates with inhibition of mechanical-stimulation of Piezo channels across inhibition of inward current.

Rhinitis and Asthma

Non-allergic rhinitis (NAR) or idiopathic rhinitis (IR) may be defined as chronic nasal symptoms, such as impediment and rhinorrhea that occur in relation to non-allergic, non-infectious triggers such as change in the weather, exposure to caustic odors or cigarette smoke, and barometric pressure differences (Van Gerven et al., 2014). Intranasal application of capsaicin has potential beneficial effects in this type of diseases, while this application is firstly irritating to the applied area, it can progressively desensitize the sensory neural fibers and decrease nasal hyper-responsiveness (Van Rijswijk et al., 2003). Studies report that the desensitization of sensory nerves with capsaicin relieves the NAR and IR symptoms for up to 9 months (Fattori et al., 2016). A recent study has shown that NAR/IR is related with an amplified expression of TRPV-1 in the nasal mucosa and substance P levels in nasal secretions. Systematic studies discovered that capsaicin employs its therapeutic action by ablating TRPV1-substance P nociceptive signaling pathway in the nasal mucosa (Van Gerven et al., 2014).

Capsaicin Effects as Immuno-Stimulant and COVID-19 Treatment

Capsaicin is directly related with other immune related syndromes. TRPV-1 was described as potential goal in autoimmune diseases as well as a modulator of neuroinflammation. The TRPV-1 receptor is a target for direct immuno-modulatory pathways in immune cells, especially on regulatory macrophages (Pedreiturria et al., 2018; Weitz et al., 2018). Nevius et al. (2012) showed that a capsaicin concentration of 10 μg by oral administration producing IL-10 instead of inflammatory cytokines in an autoimmune diabetes mouse model. This receptor is also known as part of indirectly immunomodulatory pathways when expressed in unmyelinated nerve fibers (Pedreiturria et al., 2018).

Nowadays, the importance of the enhancement of the immune system becomes an important public health care issue due to the SARS-Cov-2 pandemic outbreak. From the immunity point of view, COVID-19 patients have a lower level of lymphocytes, especially Natural Killer (NK) cells. Additionally, a mined immune system revealed the atrophy of spleen and lymph nodes along with the minimal lymphocytes infiltration into the lung lesion that leads the majority infiltration of monocytes and macrophages that conducts to the early endotracheal intubation. Finally, all these processes conclude into a mimicry of hypercoagulability, vasculitis, and multiple organs damage (Zhang et al., 2020).

The newest studies shown that the SARS-Cov-2 virus enters the cell via the angiotensin-converting enzyme-2 (ACE-2) (Prompetchara et al., 2020) and it is identified mostly by Toll-like receptor 7 (TLR7). Activation leads to the production of alpha interferon and the secretion of IL-12 and IL-6 and the production of CD8+ specific cytotoxic T cells that through the CD4+ T cell form the antigen-specific B cells and antibody production (Ahmadpoor and Rostaing, 2020). Giamarellos-Bourboulis et al. (2020), demonstrated that more than one fourth of SARS-Cov-2 patients shown Severe Respiratory Failure (SRF) and later can develop Macrophage Activation Syndrome (MAS), that finally leads into a general immune dysregulation subjugated by low expression of HLA-DR on CD14 monocytes, which is triggered by monocyte hyperactivation, disproportionate liberation of IL-6, and intense lymphopenia. All these combined pathogeneses provoked by the virus proliferation produce, in the majority of the cases, a cytokine storm also called Cytokine Release Syndrome (CRS) and symptoms of sepsis that are the cause of death in 28% of fatal COVID-19 cases. In all these cases the uncontrolled inflammation perpetrates multi-organ damage leading to organ failure, especially of the cardiac, hepatic, and renal systems, that eventually conducts to the death in the great majority of the clinical cases (Shi et al., 2020; Zirui Tay et al., 2020).

An interesting approach by Janda and Iadarola (2020) suggest that TRPV-1 receptor plays an important role in the prognosis of a very specific viral infections such as COVID-19. Some preclinical studies propose that since SARS-Cov-2 is linked to a strong immune response and an immense chain of reactions related with the immune system, the possible inhibition of afferent activity TRPV-1 fibers from the lung and airways could have a beneficial consequence on the compromised lung function. The scoop that could connect the TRPV-1 receptor to the COVID-19 is primarily based on finding a potent TRPV-1 agonist that can down regulate the inflammatory response. According with Nahama et al. (2020), the connection of TRPV-1 expressing innervation combined with the virally focused hyperinflammation in COVID-19 cases may be the root cause of the lethal characteristic of the disease particularly for the mature patients. They propose that interfering TRPV-1 signaling

might decline the severity of the acute respiratory distress syndrome (ARDS) present in COVID-19 patients. Blocking lung sensory neurons signaling might result in inactivation of efferent system decreasing associated inflammation and cytokine storm adverse effects. Suppressing TRPV-1 afferent pathways as part of the treatment for COVID19-related pneumonia/ARDS will not only tackle the inherent pathophysiology of the viral infection and systemic inflammation but would also help to decrease the complications connected with the current gold-standard therapy (mechanical ventilation/oxygen therapy) in severely compromised patients. Silencing TRPV-1 positive nerve fibers could ultimately be additionally of interest to limit the progression of the disease from mild stages to acute respiratory distress.

Since numerous studies demonstrated that the protein responsible of the replication of the virus is the viral protease 3CL-protease and that the inhibition of this enzyme can stop the virus replication in the human body (Chen et al., 2005). This enzyme owns three functional domains where different compounds can bind and inhibit it, stopping the virus replication (Kumar et al., 2020). The major international research about possible treatments against the COVID-19 goes focused to develop or elucidate possible inhibition routes of the 3CL-Protease activity, exploring the use of a numerous group of chemical compounds like azithromycin, oseltamivir, ritonavir, indinavir, remdesivir, and other drugs used in diverse viral diseases as influenza A and B, and HIV (Kadil et al., 2020). Also based on molecular docking analysis, Das et al. (2020) reported a total of 33 compounds including natural products such as capsaicin, to investigate their blocking effects toward 3CL-protease. The capsaicin formed hydrogen bonds with 4 different sites of 3CL-protease (THR190, CYS145, HIS163, and PRO168) with an estimated free energy of binding of −8.15 kcal/mol. In another preliminary study, capsaicin was found to be in the active site and interacted with GLU166 hydrogen bonds but could not exhibit good binding affinity toward target enzyme (Barros et al., 2020).

Furthermore, RNA-dependent RNA polymerase (RdRp), is a key enzyme in virus life cycle for replication and a potential target to find therapeutic agents for COVID-19 (Elfiky, 2020). The molecular docking results of capsaicin with RNA dependent RNA polymerase (RdRp) showed a free energy of binding of −7.3 kcal/mol, similar to remdessivir drug with a value of −9.0 Kcal/mol (Elfiky, 2020). These results suggest that capsaicin may be used as COVID-19 antiprotease drug to prevent the viral replication of SARS-CoV-2. Efficient therapeutic strategies are important to identify drugs for novel viruses that influence the life cycle of viruses by inhibiting the entry and stopping the viral replication or targeting intracellular signal transduction pathways in order to treat the disease or control the further spread of the virus.

The actual fact that capsaicin acts like a TRPV-1 agonist, an anti-inflammatory compound and that could be used as a 3CL-protease and/or RNA-dependent RNA polymerase (RdRp) inhibitor, suggest that the study and subsequent development of a clinical product containing this chili pepper main compound or its derivatives could be promising to the COVID-19 alternative clinical treatment.

CONCLUSIONS

Chili pepper waste product are a potential source of capsaicin and other possible analogs that may interact with TRPV-1 receptor. The industrial and technological approach to the extraction, purification and possible use of these bioactive compounds from waste materials is an important field to focus the scientific efforts. Based on the identification of molecular targets, capsaicin along with other phytochemicals that may be recovered from waste could be incorporated into a new generation of nutraceuticals to motivate a greener production in Mexico and other countries. From the commercial point of view, capsaicin is considered an active ingredient to prevent or treat obesity, gastric disorders, arthritis, asthma, diabetes, and immune system diseases and combinations with other phytochemicals may improve its potential benefits for health.

AUTHOR CONTRIBUTIONS

HC-F, DG-F, and GR-D: compiled, reviewed, and wrote this paper. JG-U: conceived and supervised this work. All authors: have read and approved the final manuscript.

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REVIEW
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The Enzymatic Digestion of Pomaces From Some Fruits for Value-Added Feed Applications in Animal Production

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With the noticed steady increase of global demand for animal proteins coupled with the current farming practices falling short in fulfilling the requested quantities, more attention is being paid for means and methods intended to maximize every available agricultural-resource in a highly sustainable fashion to address the above growing gap between production and consumers' demand. Within this regard, considerable efforts are being invested either in identifying new animal feed ingredients or maximizing the utilization of already established ones. The public preference and awareness of the importance of using waste products generated by fruit-dependent industries (juice, jams, spirits, etc.) has improved substantially in recent years where a genuine interest of using the above waste(s) in meaningful applications is solidifying and optimization-efforts are being pursued diligently. While many of the earlier reported usages of fruit pomaces as feedstuffs suggested the possibility of using minimally processed raw materials alone, the availability of exogenous digestive and bio-conversion enzymes is promising to take such applications to new un-matched levels. This review will discuss some efforts and practices using exogenous enzymes to enhance fruit pomaces quality as feed components as well as their nutrients' accessibility for poultry and swine production purposes. The review will also highlight efforts deployed to adopt numerous naturally derived and environmentally friendly catalytic agents for sustainable future feed applications and animal farming-practices.

Keywords: fruit pomaces, exogenous enzymes, fermentation, digestion, microbiota

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INTRODUCTION

Plant-based feed additives are becoming popular in livestock and poultry production chains. Thus, the use of fruit-processing by-products such as pomaces, generated by the industry in millions of tons annually around the globe, could form an integral part of evolving, sustainable, and circular agricultural economies. The use of such pomaces could also be part of the efforts aiming at addressing many of the rising challenges facing modern human societies due to climate-associated changes including the worrying diminish of natural resources. Apart from the historically witnessed trend of promoting the use of such by-products in animal feeding (in order to reduce the environmental burdens resulting from large-scale waste accumulation), a growing interest in

creating value-added products/applications is beginning to be materialized. The principle here is to use such fiber/polyphenols-rich raw products in combination with enzymes (in targeted digestions and fermentations) in order to generate value-added end commodities. The two factors that control the outcomes of the above goal(s) are: **(1)** the chemical composition of the starting materials/substrate(s) and **(2)** the digestion and fermentation processes themselves with their varying parameters (including the enzymes being used, nature and source of pomaces and their endogenous microbiota, pH, presence or absence of oxygen, etc…).

The use of enzymes is not a completely novel idea as intact microbial products (yeast and bacteria containing active wild-type enzymes) have been conventionally used by many industries in the past (and are still today) to enhance food and feed products. The same trend prevails today but with a slight advantageous skew toward using purified or recombinant enzymatic preparations due to numerous empirical considerations that will be discussed in the following sections.

In recent years, there has been increased research efforts aiming to incorporate many abundantly-available fruit pomaces in animal feed (Das et al., 2020). As it shall be seen in subsequent sections, these pomaces have been shown to possess many important bioactives at varying concentration ranges and functionalities including antimicrobial activities against foodborne pathogens such as *Salmonella enterica* for example (Das et al., 2019). More novel applications are being explored currently through enzymatic or microbial interventions where the final products of interest are not limited to the active polyphenolic-rich fraction but also other nutritional and industrial factors. A very good example of these later interests, is the most recent study that explored using red grape pomace as a fermentation medium in combination with *Lactobacillus plantarum* subsp. *plantarum* (ATCC #14917) and *Bacillus subtilis* subsp. *subtilis* (ATCC #6051) cultures to aid in chitin and chitosan extraction, both used as feed supplements (Hirano et al., 1990; Hossain and Blair, 2007; Nuengjamnong and Angkanaporn, 2018; Reddy et al., 2018) from shrimp wastes in a replacement of earlier traditional chemical extraction approaches (Tan et al., 2020). In the above model, red grape pomace replaced glucose (to support the bacterial growth) resulting in recovery levels of chitin and chitosan that were comparable (or surpass) other commercial chitin preparation protocols.

In general, the emerging trend of enhancing pomace usage through enzymatic treatments indicates the possibility of developing many future opportunities that will be discussed in later sections of this review. Furthermore, and while the authors agree that other physical means to enhance pomaces and/or the recovery of bioactive compounds from fruit pomaces {such as the supercritical CO_2 extraction of active ingredient/oils (Squillace et al., 2020) as well as freeze-drying approaches coupled with double-emulsions (water-in-oil-in-water) loading of pomace extracts (Eisinaite et al., 2020)} do exist, enzymatic approaches seem to offer a green sustainable strategy. Hence the current review will focus on such bioconversion procedures for value-added feed applications of pomaces in mainly poultry and swine.

ADVANTAGES AND DISADVANTAGES OF ENZYME-ASSISTED BIOCONVERSIONS OF POMACES

The possibility to produce high-quality exogenous enzymes is among the top technologies that positively impacted the animal feed industry in the past four decades. The incorporation of commercial enzymatic preparations in animal feed is now widely acceptable by farmers as well as end-users alike. No one would imagine the global animal feed industry operating nowadays without the powerful capabilities of fiber-degrading enzymes or without the phytases and amylases that are used routinely (Kiarie et al., 2016a, 2019; Alagawany et al., 2018).

The biggest advantage of feed enzymes is their specificity. Enzymes are not like any other physical or chemical treatments (i.e., heating and acidification for example) that target feed nutrients and compounds in a universal non-discriminatory fashion. Enzymes are rather very specific in their nature and only target certain chemical groups and bonds without affecting other components within the feed-matrix. This increases their efficiency from one side while decreasing any negative off-target effects (if any) when used with animal feed (Bhatia et al., 2020; Jatuwong et al., 2020).

The second appealing factor of using enzymes in the feed industry is their high acceptance rates by average consumers. These catalytic molecules are perceived as part of refined green-technologies and if the source organism was a GRAS (Generally Recognized As Safe), then the approval process by both regulatory agencies and end-consumers could be generally less complicated, in comparison to enzymes that are obtained by other approaches (such as synthetic biology approaches). The importance of having both consumer-acceptance and the regulatory agencies approval concomitantly cannot be overemphasized for any genuine applications (DeRuiter and Dwyer, 2002).

Among the critical issues related to enzyme usages are the technical difficulties. In fact, the preparation of enzymes with an acceptable purity, stability and sufficient catalytic power are challenging. In the past, suppliers had to start with kilograms (or tons in some cases) of raw materials before delivering milligrams of ready-to-use enzymes. Thus, the utilization of enzymatic-preparations was very expensive and extremely unpredictable with susceptibility to purification-dependent variations in performance from batch to batch. Indeed, the cost of such preparations was among the biggest concerns of farmers toward enzymes-usage as it added up to the total costs of feeding. When the difference between profitability and non-profitability melts down to few cents or pennies per ton of used feed, any extra cost(s) need to be carefully considered and planned ahead of time. With the introduction and refinement of exogenous-enzymes biotechnologies using optimized host microorganisms (bacteria, yeasts or fungi), the process is becoming increasingly manageable and robust. While the cost of discovering novel functional enzymes (the research and development phase more precisely) is still considerably high and requires collaborative efforts of many specialized experts (Howard et al., 2003; Monciardini et al., 2014), the actual costs

involved in producing stable enzymatic-preparations (the large-scale production phase) are becoming very reasonable nowadays. Furthermore, the encapsulation and lyophilization of enzymes as well as solid-matrix immobilization techniques reached a pinnacle stage in performance aiding in achieving stable and potent enzymatic preparations continuously (Gifre et al., 2017; Ibrahim et al., 2019).

Most commonly and when purified enzymes are inaccessible or are very expensive to use, fermentation-protocols can be implemented to enhance pomace attributes for animal feed application. The fermentation process is looked at here as a way of harnessing the power of endogenous fungal- or bacterial-enzymes to: (1) naturally degrade the fibrous contents of pomace and release its active polyphenol compounds, (2) convert or transform some compounds to their more active forms, and finally, (3) make use of the involved microbial inoculum (either bacterial, fungal, or yeast) metabolites in combination with the targeted polyphenolics to enhance animal feeding outcomes (performance).

The presence of certain enzymatic co-factors such as reduced nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) as well as, adenosine triphosphate (ATP) and pyrroloquinoline quinone (PQQ) within the intact microbial hosts (Cutlan et al., 2020) is another advantage for microbial fermentations over purified enzymatic preparations. The host-organism is capable in this case of replenishing such co-factors cheaply and compared to using purified enzymatic preparations where the cofactor(s) need to be constantly supplied, the in-host conversions are more cost-efficient.

The recalcitrance of certain plant contents in pomaces to natural degradation makes them more appealing targets for microbial bioconversions than enzymatic treatments (Troncozo et al., 2019). Raw pomaces (grape pomace for example which results from juice and spirits industries) have significant negative environmental impacts due to their water-retention capacity and their high content of lignins which breakdown to generate hydroxylated and methoxylated monoaromatic compounds associated with strong phytotoxic activities (Troncozo et al., 2019). To reduce the environmental concerns connected to the above compounds, many treatments have been suggested in the past including drying or using them as an extraction source of beneficial compounds such as γ-linolenic acid and carotenoids (Dulf et al., 2019) among many other treatments. Thus subjecting pomaces to microbial fermentations looks the most feasible approach when it comes to processing the large amounts of pomaces generated annually.

The ability of using enzymes with pomaces faces another hurdle that emerges from the unique ability of pomace bioactives [including polyphenols and tannins (Hemingway and Laks, 2012)] at certain concentration to influence (or even inactivate) the functionality of used enzymatic preparations either through specific or non-specific interactions (Martinez-Gonzalez et al., 2017; Yildirim-Elikoglu and Vural, 2019). Many reports showed the ability of certain polyphenolics to negatively influence the functions of natural or industrial enzymes in a similar fashion to their ability to negatively impact microbial growths (Gonçalves and Romano, 2017; Ramos-Pineda et al., 2019). For example, pectinases involved in the enhancement (aroma and color) or clarification of many beverages and juices are reported to be inhibited by apple flesh or pomace (Negoro, 1972). An insoluble complex compound of pectinase and inhibitors is formed to influence the enzyme's activity. The recent further characterizations of such a inhibitory activity highlighted tannic acid and (its derivatives) as a possible cause of the above insoluble complex formation (Negoro, 1972). Similarly, the inhibitory effect of polyphenolic compounds from purple and red potato cultivars on activities of α-amylase, α-glucosidase, and aldose reductase are well-established (Kalita et al., 2018). Anthocyanins were recently categorized as non-competitive inhibitors of such enzymes. In contrast, phenolic acids behaved as non-competitive inhibitors of aldose reductase and as mixed inhibitors of α-amylase and α-glucosidase, respectively (Kalita et al., 2018).

Last but not the least, animal feeds preparation encompasses most often a pelleting step (heating, mechanical mixing and shearing, drying…) which together can adversely affect the involved enzymes and reduce their detectable activity. Such influential factors need to be considered carefully before incorporating any digestive enzymes that are not heat-resistant in the processing of animal feed (Kiarie and Mills, 2019). The same observation concerns the used enzyme's ability to withstand and function under the low pH values of animal digestive systems. In such cases, an encapsulation step might be beneficial.

The above mentioned issues open the door for a variety of optimization studies (**Figure 1**) that need to carefully consider the composition of used pomaces, their polyphenolic content, enzyme(s) being utilized, and finally the precise usage condition(s) for each unique feeding application to obtain optimum activities while minimizing the chances of unintended inhibition(s). Some of the above issues can also be addressed though protein engineering (Kuddus, 2018; González-Domínguez et al., 2019; Sharma et al., 2019). In fact, and referring to the pectinases example stated above, the industrial enhancement and optimization of such enzymes led to the selection of numerous pectinases with broad activity ranges that can function even at high polyphenol concentrations, diverse pH-values and/or processing temperatures with some being currently commercialized. The same approach is true in regard to engineering feed enzymes to render them more heat resistant or pH stable (Rigoldi et al., 2018). As shown on **Figure 2,** the *Aspergillus fumigatus* phytase (Xiang et al., 2004) was engineered through a single mutation of lysine to alanine at position 68 (K68A) leading to a noticeable decrease in its pH optima (with phytic acid as substrate) by 0.5 to 1.0 units with no change or even a slight increase in enzyme's maximum specificity (Tomschy et al., 2002).

THE FATE OF FRUIT POMACE BIOACTIVES WITHIN THE ANIMALS' DIGESTIVE SYSTEMS

In order to use exogenous enzymes to treat pomaces, it is important to understand what happens to pomaces and

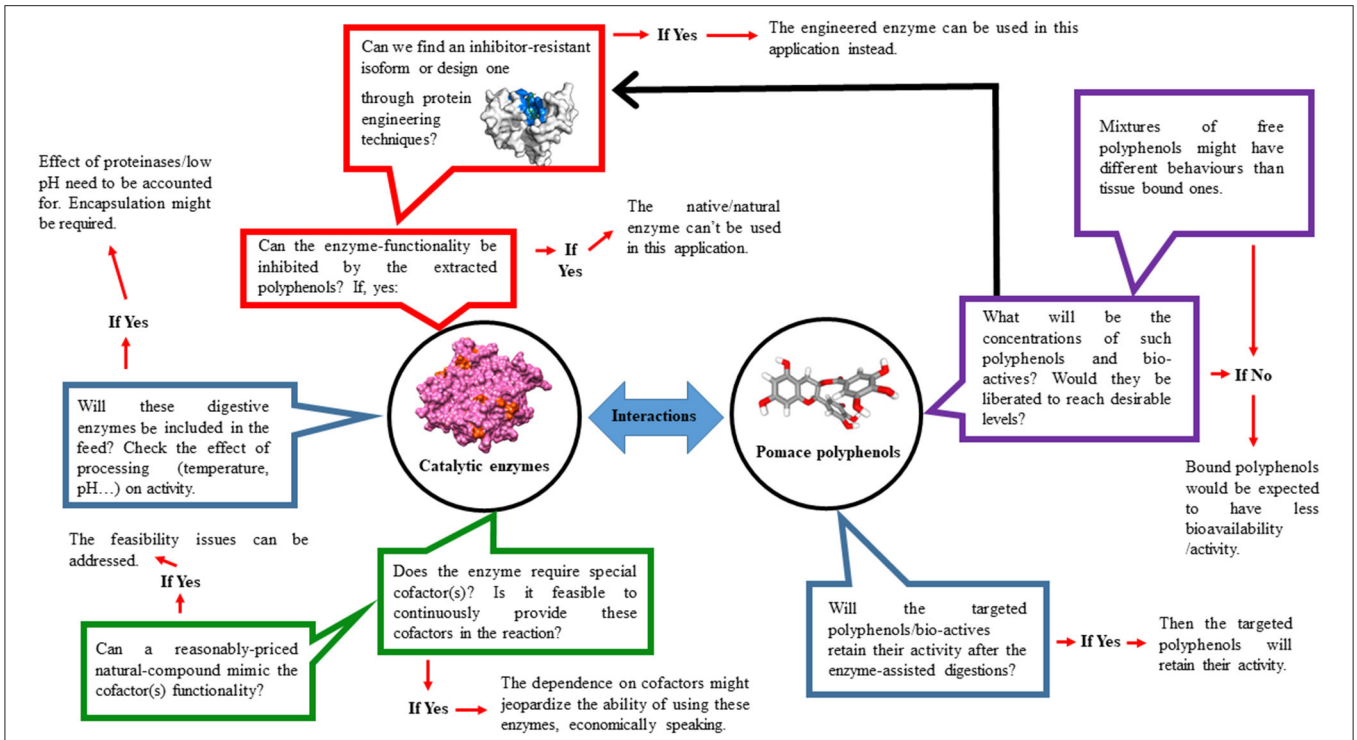

FIGURE 1 | Optimization studies are critically needed when using enzymes and fruit pomaces as animal feed additives. Multiple overlapping factors (pomace composition, its polyphenolic content, enzyme mixes being implemented….) affect the performance of pomaces polyphenols and must be carefully considered for satisfactory and reproducible outcomes.

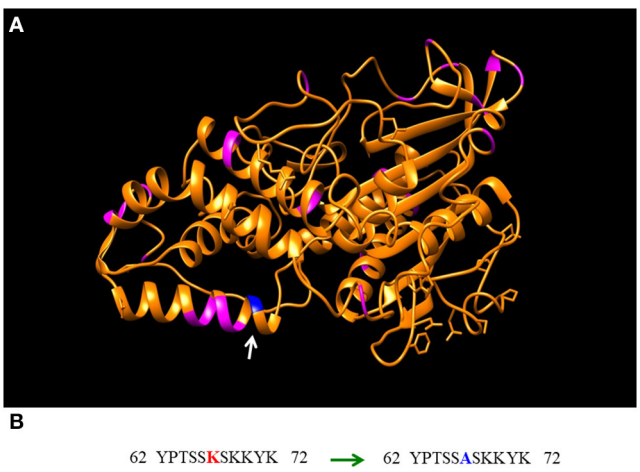

FIGURE 2 | Amino acids sequence (442 residues) and the three-dimensional structure of *Aspergillus Fumigatus* phytase (accession #1QWO_A) identified an important lysine residue (dark blue) in comparison to all other available lysines (magenta) **(A)**. Mutation of this residue (lysine "K" 68 to alanine "A") **(B)**, leads to a noticeable decrease of pH optima by at least 0.5 to 1.0 units.

their final enzymatic digestion products within the animal's digestive systems.

Pomace has been defined as the by-products of fruits after pressing for juice generated by processing industries. It includes the flesh, peels, stems (or parts of stems), and seeds (Ross et al., 2017; Waldbauer et al., 2017). The chemical characterization demonstrates the presence of over 4,000 metabolites and bioactive compounds in many plants and their fruit pomaces. In the following sections, we will discuss a few selected ones that are commonly encountered in animal feeding applications.

Cranberry and Blueberry Pomace

The characterization of pomaces from American cranberry (*Vaccinium macrocarpon*) and wild blueberry (*Vaccinium angustifolium*) by Ross et al. (2017) revealed a wide range of minerals, phenolics, carbohydrate, lipids, and proteins. The detected minerals in these two berries' fruit pomaces included calcium, potassium, iron, copper, zinc, magnesium, manganese, and phosphorus among others, while their specific enriched phenols included tannins, anthocyanins, tartaric esters, and flavanols (Ross et al., 2017), whose amount is mainly abundant in the fruit's peel.

Despite some anti-nutritive properties of tannins in terms of reducing iron absorption, protein digestibility hence growth performance, various studies demonstrated their potential as anti-carcinogen, antibacterial, antioxidant and immune-modulator agents among others (Chung et al., 1998; Kumar Ashok and Upadhyaya, 2012). Ethanolic extracts of above mentioned cranberry and wild blueberry pomaces were enriched in phenolics, tartaric esters, and antioxidant activities compared to original pomaces (Ross et al., 2017). Feeding broiler chicken

with cranberry and blueberry pomaces and their extractives, induced metabolic changes in the bird's blood including a significant increase in the levels of plasma quinic-acid suggesting that these products can reduce oxidative stresses and improve metabolic functions against reactive oxygen species' and their associated-damages in chickens (Das et al., 2020).

Apple Pomace

The characterization of apple pomace revealed that it contains about 43.6% dietary fibers (Sato et al., 2010), mono and di-saccharides such as sucrose, arabinose, galactose and fructose (Gabriel et al., 2013), triterpenoids (Almeida-Trasviña et al., 2014), polyphenolic compounds (Vrhovsek et al., 2004), volatile compounds such as glycerol esters and aldehydes (Madrera and Valles, 2011), non-volatile acids such as malic, citric and quinic acids (Chapman and Horvat, 1989), proteins (7.1%), in addition to minerals such as sodium, potassium, calcium, phosphorus, magnesium, iron, manganese, zinc, and copper (Pieszka et al., 2015). Polyphenols in apple are predominantly found in the peel fraction, where they are bound to pectin and other cell-wall structures. This was demonstrated through measuring the quantity of polyphenols including catechins, proanthocyanidins, hydroxycinnamates, flavonols, dihydrochalcones, and anthocyanins in the juice and in the pomace, with the pomace showing higher polyphenolic concentrations (Vrhovsek et al., 2004; Marag et al., 2015). The biological activities and benefits associated with apple pomace consumption (and its derivative compounds) have been documented (Sato et al., 2010; Gabriel et al., 2013; Almeida-Trasviña et al., 2014). Apple pomace fibers were reported to improve the digestion and metabolism in animals through their prebiotic actions and the promotion of the growth of beneficial bacteria in the gut. This in turn enhances the peristaltic movements of the bowel, mediation of homeostasis of cholesterol and triglycerides, which then improves the cardiovascular health together with triterpenoids which enhance the bioactivity of the nitric oxide synthase enzyme (Beermann et al., 2018). Moreover, plasma from lambs fed a fattening diet containing ∼10% apple pomace, demonstrated a higher antioxidant activity (Rodríguez-Muela et al., 2015). Wistar rats fed native apple pomace, showed an increased superoxide dismutase (SOD) activity in their red blood cells, demonstrating further the potential of apple's pomace bioactives in mitigating oxidative stresses (Juśkiewicz et al., 2011). In broiler chickens, Colombino et al. (2020) elucidated the importance of apple polyphenols in improving the gut microbiota composition of birds, specifically increasing the α-diversity as compared to blackcurrant and strawberry diets that were also used in these experiments. Moreover, the authors did hypothesize that since the broilers' histomorphometric indices were not negatively affected by the fruit pomaces, then the latter do not hamper gut development and nutrient absorption. Another study in turkey poults by Juskiewicz et al. (2016) utilized apple, blackcurrant and strawberry pomaces at 5% inclusion levels to show a significant decrease in sucrose and maltase mucosal activity in the small intestine of birds fed blackcurrant and strawberry, but not in the apple group. This could be partially due to the varying levels of fibers content in these three pomaces that could influence enzymes activities.

Grape Pomace

Grape pomace has been suggested also as an animal feed additive to take advantage of its proteins, lipids, non-digestible fibers and minerals, in addition to its non-phenolic vitamins such as β-carotenoids and tocopherols that are known to exhibit antioxidant properties (Silva Soares et al., 2018). Despite its rich content in potential nutritionally-important phytochemicals, it is only in the present times that the necessity of fully characterizing of grape-pomace compounds and their nutritional influence has been realized (Makris and Kefalas, 2013). Earlier characterizations demonstrated a wide array of polyphenolic compounds such as catechins, stilbenes, saponins, flavanols, anthocyanins, phenylpropanoids and derivatives of *p*-coumaric and benzoic acids (Makris and Kefalas, 2013). Further studies showed the anthelmintic, ovicidal and antilarval activities and the stimulatory potential of grape saponins and tannins (up to a certain level, beyond which the tannins would have anti-nutritive effects due to their strong binding to proteins and macromolecules and complex formation) (Silva Soares et al., 2018). The above phytochemicals reflected grapes' pomace potential to inhibit the hatching, growth and movement of free-living *Haemonchus contortus* worms. In a more recent study (Chedea et al., 2018), extracts from grape were used in pre-weaned piglets that are usually susceptible to physico-chemical damages to their enteric systems associated with the weaning-process stress. Polyphenols derived from grape extracts showed an anti-inflammatory effect within the small intestines of these piglets hence minimizing villus erosion and atrophy. Moreover, the antioxidant potential of grapes significantly increased the levels of glutathione peroxidase and SOD activities in the duodenum and colon sections of the digestive system. The authors suggested possible relationships between the above positive effects with grape pomace polyphenolics including gallic acid, hydroxycinnamic acid, anthocyanins, catechins, and epicatechins (Chedea et al., 2018). In chicken, studies by Goñi et al. (2007) explored the use of grape pomace at 5, 15, and 30 g/kg inclusion levels with vitamin E [α-tocopheryl acetate (200 mg/kg)] in a corn-soybean basal diet. They reported that grape pomace was able to increase the anti-oxidative capacity of the administered diets, birds' excreta, and meat during storage, with the latter having a higher oxidative stability at the highest pomace inclusion level. Additionally, these authors (Goñi et al., 2007) found that liver vitamin E concentrations were significantly high to warrant the consideration of alternatively using grape pomace flavonoids to increase vitamin E in chicken which could reduce costs associated with this vitamin in diet.

POLYPHENOLS ABSORPTION, TRANSPORT, AND BIOAVAILABILITY

While absorption and transport of Polyphenols in gut is a controversial topic, recent studies conducted in human subjects have shown that once ingested into the gut, about 5 to 10 per

cent of the monomeric and dimeric polyphenols are absorbed by passive diffusion into the enterocytes (Corrêa et al., 2019). Upon this absorption, the aglycones are bio-transformed in the enterocytes and also in the liver hepatocytes with the resultant metabolites distributed to various organs in the body before excretion in the urine. The more complex and larger polyphenols (oligomeric and polymeric such as tannins) reach the large intestine where they are degraded by some microbes resulting in generation of less complex compounds. The gut microbial transformation of these polymeric structures include C-ring cleavage, demethylation, decarboxylation, and dihydroxylation (Corrêa et al., 2019). Whilst still in the gut, the above bioactive or bio-transformed compounds exert their effects based on many factors where bioavailability and absorbability stand on the top of such factors. This bioavailability of polyphenols is influenced by many physicochemical factors such as polarity, molecular mass, type of polyphenols present and their digestibility by the gut enzymes and in turn their absorption (Ozdal et al., 2016). Bio-accessible nutrients are defined as the fraction that is released from the food matrix into the bloodstream. According to Parada and Aguilera (2007) this part of the nutrients reaching the bloodstream is more important than the total amount of nutrients excreted. Furthermore, many beneficial bioactive compounds are present in biological forms that are usually not fully available hence a disruption of the food matrix (and the structures holding such food/feed components) to make them more accessible is a must.

The role of enzymes in enhancing nutrient's bio-accessibility becomes a critical aspect during the *in vivo* digestion and plays a key role in the bioavailability of numerous pomace bioactives. As the major bioactive compounds found in pomaces (such as those of cranberry, blueberry, apple and grape) are polyphenols described as large heterogeneous molecules with a distinguishing hydroxylated phenyl moiety (Ozdal et al., 2016); this review will focus briefly on their digestion and absorption. Scalbert and Williamson (2000) reported that many polyphenols pass through the gastrointestinal system without being absorbed to end up in the hindgut to be bio-transformed by colonic microbiota into different metabolites. These metabolites engineer a symbiotic relationship within the gut leading to the inhibition of pathogenic microbial populations while supporting beneficial bacterial proliferation and ameliorating the host's health. The microflora living in this symbiotic relationship with the polyphenols are mainly bacteria, archaea and eukaryotes (Williamson and Clifford, 2010). Researchers postulated that the three main genera of bacteria that form about 90% of the gut bacterial microflora of chicken are *Bacteroides*, *Clostridium* and *Eubacterium* while genera of *Fusobacterium*, *Peptostreptococcus*, and *Bifidobacterium* being fewer but of significant importance (Iqbal et al., 2020).

Many of the functional and antimicrobial compounds identified in pomaces (such as resveratrol for example) were subjected to stability studies in the past and their fate was tracked during numerous processing steps (Bertelli et al., 1998). As mentioned above, about 5 to 10% of polyphenols are absorbed in the small intestine (Manach et al., 2005; Faria et al., 2014).

In terms of their nature and structures, polyphenols can be categorized into two groups: flavonoids and non-flavonoids phenolics. The structure of flavonoids consists of two benzene rings (A and B), linked together by a heterogeneous pyrone C ring (**Figure 3**). This group contains compounds such as flavonols, flavones, flavanones, flavone C-glycosides, isoflavones, flavanols, and anthocyanins. These phenolics have very varied structural differences, which influence their metabolism, interaction with gut microbiota, absorption, and assimilation (Silva Soares et al., 2018). In comparison, the non-flavonoid phenolics are more heterogeneous in nature and extend in structure from the simple benzoic acid to more complex compounds such as tannins and lignans (Wu et al., 2009; Ozdal et al., 2016). The latter group encompasses compounds such as phenolic acids, stilbenes, and lignans, which are reported in abundance in cranberries and grapes pomaces. Resveratrol is an example of a stilbenoid that is of great importance in gut health, as it is bio-transformed by gut microbiota to dihydroresveratrol, 3,4′-dihydroxy-trans-stilbene, and 3,4′-dihydroxybibenzyl (lunularin) (Bode et al., 2013).

In the hindgut the majority of biotransformation products from polyphenolics include: benzoic acids (C6-C1), protocatechuic acid, phenylacetic acids (C6-C2), and 3-(3'-hydroxyphenyl)-propionic acid; which are easily absorbed into blood, taken to tissues, and thereafter excreted via the kidneys (Williamson and Clifford, 2010). **Table 1** shows various bio-transformed metabolites of some of the most common flavonoids and non-flavonoid-type phenolics found in pomaces.

The catabolism of pomace bioactives is dependent on the host's enzymatic systems and colonic microflora. Bound polyphenols are usually released from the food/feed matrixes. After absorption and uptake in the liver, aglycones and glucosides become conjugated through glucuronidation and sulphation processes and part is returned to the small intestine (Chen et al., 2018). The portion of the polyphenols reaching the colon will be de-conjugated by microbial enzymes such as glucuronidases and sulphatases. This enables the re-uptake or catabolism of the aglycones and glucosides into simple compounds such as hydroxyphenylacetic acids (flavonols), hydroxyphenyl-propionic acids (flavones and flavanones), phenylvalerolactones, and hydroxyphenylpropionic acids (flavanols) (Ozdal et al., 2016). **Figure 4** shows the fate of two flavonoids: flavone *C*-monoglucosides and flavone *C*-multiglycosides, where the former is de-glycosylated into smaller metabolites and then excreted via the colon, while the latter is absorbed in the small intestine and either re-circulated via the liver or excreted via the kidneys. Other processes in which polyphenols are bio-transformed by gut microflora include deglycosylations, esterifications, methylations, hydrolysis and hydroxylation reactions (Chedea et al., 2018).

CURRENT *IN VITRO* POMACE ENHANCEMENT APPROACHES

Enzymatic digestions and/or microbial fermentations introduce fundamental changes in pomace chemical composition, physical structure, or both. Many recent studies have investigated such

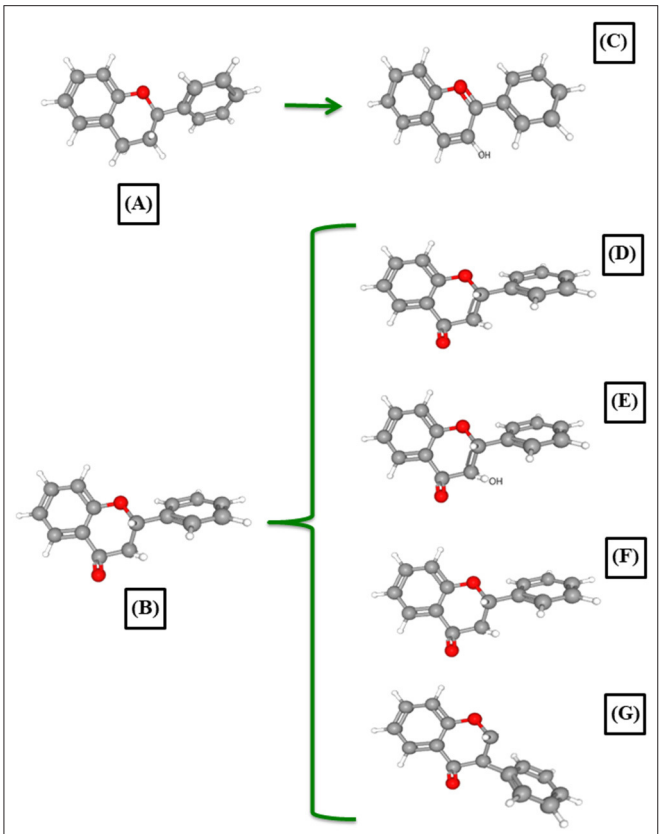

FIGURE 3 | The diverse structure and classification of major plant flavonoids: **(A)** flavan nucleus; **(B)** 4-oxo-flavonoid nucleus; **(C)** anthocyanidins; **(D)** flavones; **(E)** flavonols; **(F)** flavanones; and **(G)** isoflavones. The presented polyphenols within the mentioned figure are simplified, but show the complexity and diversity of major plant flavonoids. More in-depth depiction of polyphenols was published most recently (Hassan et al., 2019).

changes trying to evaluate the final products and tracking specific compounds throughout the entire valorization process. Various factors (including the fruit variety, vintage, and processing techniques) influence the enzyme- and fermentation-induced changes and the overlap between these factors is substantial.

Apple Pomace

The high carbohydrates contents of certain pomaces (such as apple pomace) aids in incorporating them as fermentation substrates in multiple microbial processes for the production of useful bio-compounds such as organic acids, enzymes, ethanol, pigments, and single-cell proteins. Recently, the biomass degradation efficiency of an enzymes-mix targeting apple pomace was demonstrated at 20–30% solid loading with desirable digestion and fermentation end-products of glucose, fructose, and ethanol; respectively (Magyar et al., 2016). Similarly, another study compared the effects of acetic acid and pectinase pre-treatments with cellulase prior to apple pomace at 10% hydrolysis resulted in the highest pectin and fermentable sugar yields (Luo and Xu, 2020). Another study reported that the enzymatic hydrolysis of apple pomace can be optimized at room temperature using un-buffered systems with maximum production of galacturonic acid, glucose, arabinose and galactose achieved through treating apple pomace with Novozyme, Viscozyme and Celluclast (Gama et al., 2015).

In a recent study, pectin extraction from apple pomace with endo-xylanase and endo-cellulase enzymes resulted in 19.8 and 15% extraction efficiency, respectively, when enzymes were used individually whereas the utilization of both enzymes simultaneously led to lower pectin mass and extraction efficiencies. The study also reported that the pectin extracted with different enzymes have varying mass and chemical composition (Wikiera et al., 2016). Finally, apple pomace was used as a substrate for the production of multiple carbohydrases by utilizing the brown rot fungus *Rhizopus delemar* F2 in solid-state fermentations (SSF). Higher yields of cellulose (18.20 U g−1), xylanase (158.30 U g−1), pectinase (61.50 U g−1), and amylase (21.03 U g−1) were achieved when a microwave pre-treatment was coupled with SSF of apple pomace (Pathania et al., 2018).

Grape Pomace

One recent study reported the fate of polyphenolics in grape skins and seeds after a fermentative maceration step (Guaita and Bosso, 2019). Polyphenolics composition of skins, seeds and pomaces from four fresh grapes varieties namely Albarossa, Barbera, Nebbiolo, and Uvalino were investigated after the fermentative maceration. Despite a varietal dependent polyphenolics composition, the differences between cultivars in the polyphenolic profile disappeared after the fermentative maceration (Guaita and Bosso, 2019). Grape pomace was used recently as a fermentation and growth substrate in the cultivation of two *Pleurotus ostreatus* and *P. pulmonarius* to study the influence of different fermentation modes on the consumption of phenolic compounds, the production of mycelial mass and enzymes. Maximum biomass values (∼0.5 g/g) were obtained within the submerged cultures while laccase (a polyphenol oxidase belonging to the blue oxidase family and one of the important lignocellulolytic enzyme) production was induced in solid-state fermentations (26.247 U/g). Both *P. ostreatus* and *P. pulmonarius* strains were able to degrade up to 79% of the total phenolic content, regardless the fermentation/culture conditions (Papadaki et al., 2019). From the three enzymes used in the above study, Novoferm was reported to induce the strongest effect on phenolics release from grape waste, followed by Pectinex Ultra and Celluclast. Similarly, both cellulase and gluco-amylase were used to extract polyphenols from grape pomace. The addition of cellulase increased the total polyphenolic contents to 41.05 ± 1.07 mg ChAE/g, with the optimal concentration being around 0.25 mg/mL, whereas the addition of gluco-amylase exhibited little effect (Kabir et al., 2015). A water-soluble extract containing high polyphenol contents (12%), peptides, carbohydrates, and lipids was obtained from grape pomace using an endo-protease mixture at 20% solid loading. This extract exhibited anti-inflammatory properties *in vitro* as it decreased pro-inflammatory factors and inhibited excessive microglial activation (Rodríguez-Morgado et al., 2015). In a recent study, the ultra sonication with 0.5M KOH combined with a mixed enzymes treatment provided the highest lignin

TABLE 1 | A shortlist of the most encountered pomace active compounds and their fate within the digestive system.

Flavonoids	Compound	Metabolites	References
Flavonols	Quercetin Phloroglucinol Myricetin trihydroxylation	2-(3,4-dihydroxyphenyl)acetic acid, 2-(3-hydroxyphenyl)acetic acid, and 3,4-dihydroxybenzoic acid 3-(3,4-dihydroxyphenyl)propionic acid, and 3-(3-hydroxyphenyl)propionic acid 2-(3,5-dihydroxyphenyl)acetic acid, 2-(3-hydroxyphenyl)acetic acid, and 2-(3,4,5-trihydroxyphenyl)acetic acid	Rechner et al., 2004
Flavones and Flavanones	Rutinosides (6-*O*-α-l-rhamnosyl-d-glucosides) Neohesperidosides (2-*O*-α-l-rhamnosyl-d-glucosides)	3-(3,4-dihydroxyphenyl)propionic acid 3-(3,4-dihydroxyphenyl)propionic acid	Rechner et al., 2004
Flavone *C*-Glycoside	Orientin	Phloroglucinol	Zhang et al., 2007
Flavanols	Catechin and epicatechin	5-(3′,4′-dihydroxyphenyl)-γ-valerolactone, 3-(3-hydroxyphenyl)propionic acid, and 3-hydroxyhippuric acid, 5-(3′-hydroxyphenyl)-γ-valerolactone	Déprez et al., 2000; Rios et al., 2003
Anthocyanins	Cyanidin Delphinidin	Protocatechuic acid Gallic acid	Williamson and Clifford, 2010
Lignans	Pinoresinol Secoisolariciresinol	Enterolactone and enterodiol	Bowey et al., 2003
Stilbenes	*trans*-Resveratrol	Dihydroresveratrol, 3,4′-dihydroxy-trans-stilbene	Folmer et al., 2014
Phenolic acids	Hydroxycinnamate ferulic acid	3-(3′-hydroxyphenyl)propionic acid	Andreasen et al., 2001

degradation activity with a yield of 13% along with the release of many commercially-important polyphenols in substantial amounts (Karpe et al., 2017). Similarly, polyphenolic compounds of wet and dried red grape pomace (*Vitis vinifera L.*) were extracted utilizing refined enzymatic digestions with a celluclast enzyme providing the highest yield of extracted polyphenols (Ferri et al., 2016).

The solid-state fermentation (SSF) seems to be preferred because of the high concentration and stability of the obtained products, its low operational costs, and the low catabolic repression of involved microbes (Hölker and Lenz, 2005). An extraction of grape pomace, where the commercial enzymes Cellubrix, Neutrase, and Viscozyme were used in combination, resulted in a 20–45% increase in the yields of soluble solids and 25–65% increase in phenols. Maximum phenolic yields were achieved by treating distilled Cabernet Sauvignon pomace with 30 g/kg of the enzyme mixture (Costoya et al., 2010). A different study investigated the production of hydrolytic enzymes through SSF by a mutant strain *Aspergillus niger* 3T5B8 using grape pomace and wheat bran combinations or solely wheat bran. The addition of grape pomace resulted in more diverse fermentation compounds with a higher proanthocyanidins content and higher antioxidant potential (Teles et al., 2019).

Berry Pomace

An aqueous enzyme-assisted (carbohydrases and proteases) extraction procedure to recover lipophilic compounds (essential fatty acids, tocols, phytosterols, and ellagitannins) and polyphenols from raspberry (*Rubus idaeus L.*) was developed (Ferri et al., 2016). Under the reported optimized conditions [1.2 units of thermostable alkaline protease, pH 9, 60°C, and 2 h hydrolysis], more than 38% of the total lipophilic content was recovered within the aqueous phase. The recovery of polyphenols and antioxidant activity was 48 and 25%, respectively, and higher than the ones obtained through extractions with a methanol-acetone-water mixture (Saad et al., 2019). The outcomes of the above experiments showed the possibility of using enzymes to overcome the drawbacks of organic solvent use for bioactive polyphenols recovery and the ability of developing eco-friendly and cost-effective alternatives to recover such compounds (Saad et al., 2019). Furthermore, using enzymes presents an economic feasibility compared to more safe extraction procedures such as supercritical carbon dioxide. Studies compared the use of pressurized liquid extractions with supercritical carbon dioxide to enzymatic assisted extractions from solid state fermentation in recovering bio-functional compounds from blackberry pomace (Kitryte et al., 2020). The consecutively applied treatments at optimized parameters yielded lipophilic fractions containing polyunsaturated fatty acids (linoleic 64.1%, α-linolenic 12.9%) while recovering up to 29.1 mg gallic acid and 168.7 mg Trolox equivalents from pomace antioxidants. This study also demonstrated that enzyme-assisted extractions (a commercial multi-enzymes complex, Viscozyme L, with a strong pectolytic activity and a wide range of carbohydrases, including arabanase, cellulase, β-glucanase, hemicellulase, and xylanase) could recover more valuable pomace constituents compared to solvent-based extractions as evidenced by a significantly lower quantities (0.5–0.7 mg/g) of anthocyanins obtained in the solvent-based extraction systems (Kitryte et al., 2020). Moreover, as pomace particle size decreases from 500 to 1,000 μm to <125 μm, the yield of phenolic compounds increased by 1.6 to 5 times (Landbo and Meyer, 2001).

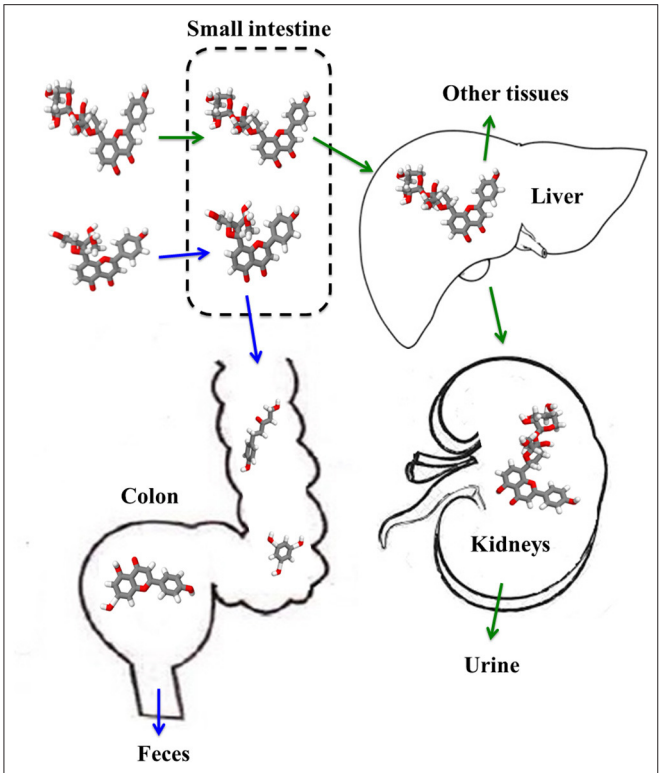

FIGURE 4 | Biotransformation of flavone *C*-monoglucosides and flavone *C*-multiglycosides into different flavonoid aglycones, monomers and dimers in various organs and tissues, and their subsequent excretion. The depicted pathway though simplified, does not reflect the complexity of polyphenols absorption, transport, and bioavailability.

Orange and Tomato Pomace

The production of pectinases by *Aspergillus niger* was facilitated by SSF with orange pomace substrate. The maximum activity of pectinases produced occurred at 45–55°C and the maximum enzyme productivity occurred at 70% moisture content and a C/N ratio of 10 (Mahmoodi et al., 2017). The production of laccase, xylanase and protease were achieved through SSF on tomato pomace with white-rot fungi *P. ostreatus* and *Trametes versicolor*. Significant laccase and protease activities were detected: up to 36 U g−1 and 34,000 U g/1 dry matter, respectively (Iandolo et al., 2011). Using orange pomace as substrate in the process of SSF, *Paecilomyces variotii* was able to produce tannase and phytase simultaneously. High tannase and phytase activities being 5,000 U/gds after 96 h and 350 U/gds after 72 h, respectively were obtained. In addition, the antioxidant capacity of orange pomace increased around ten-fold as a result of SSF (Madeira et al., 2012).

In essence, laboratory experiments definitely show promising outcomes and strong justification for using enzymes and/or fermentation to enhance the extraction efficiency of polyphenols from pomace. More examples are listed in **Table 2**; **Figure 5** (Sunna and Antranikian, 1997) as reviewed in below sections dealing with the *in vivo* models of pomace usage in combination with enzyme(s) supplementation for animal feeding applications.

Finally, the use of enzymes should be evaluated individually and case by case based on the desired final product(s) as well as the surrounding factors of the treatment (such as berry composition for example). In a separate study, the effect of enzyme-treatments before juice pressing on the anthocyanin composition of *Aronia melanocarpa* (black chokeberry) pomace was evaluated. Cold maceration of frozen berries with no enzyme showed the highest concentrations of anthocyanin in pomace (Vagiri and Jensen, 2017). Similarly, no effect was observed with direct enzyme addition to anaerobic digestion of olive pomace in order to enhance methane production; however, methane production increased 71% compared to the control after a pre-treatment of enzymatic maceration (Donoso-Bravo et al., 2016). Later and after optimization, using immobilized laccase on porous nanocomposite of Fe3O4@SiO2@KIT-6 for delignification and phenol extraction generated a degradation rate of lignin and phenol of 77.3 and 76.5%, respectively (Amin et al., 2018).

THE *IN VIVO* UTILIZATION OF ENZYMATIC-ENHANCEMENT SYSTEMS

Enzymes are ubiquitous catalysts of biochemical reactions and pathways (Bourlieu et al., 2020). The exogenous enzymes used in the animal industry are biotechnological products derived from microorganisms such as bacteria and fungi; together with the endogenous microbial enzymes they degrade the cell walls of ingested feed to release nutrients/bioactives (Bedford and Schulze, 1998; Slominski, 2011; Kiarie et al., 2013). Apart from their help in enzymatic degradation of feed, the hindgut microbes are also an important source of vitamins as well as acting as a biological deterrent to the growth and colonization of the gut by pathogenic bacteria (Coates, 1987).

Poultry Production

Enzymes in poultry diet have been used to increase nutrients utilization and improve digestibility (Kiarie et al., 2014, 2015a, 2017; Gallardo et al., 2017; Sanchez et al., 2019). They influence the absorption of nutrients and produce nutrients for specific bacterial populations through their actions (Bedford and Cowieson, 2012; Kiarie et al., 2013, 2019). The classes of enzymes commonly used in poultry include phytase, carbohydrases (xylanase, cellulase, α-galactosidase, β-mannanase, α-amylase, and pectinase), and proteases (Kiarie et al., 2013). It has been reported that enzymes such as xylanase which is well-accepted in poultry diets, especially those containing wheat, can reduce digesta viscosity, increase nutrient digestion and passage rate, and modify gut microbiota in broilers (Nian et al., 2011; Kiarie et al., 2014, 2017; Munyaka et al., 2016). As shown in **Figure 5,** a heteroxylan polysaccharide would not have all its various xylosidic or side-chain substrates' linkages accessible to the xylan degradaing enzymes. Thus, specific xylanase enzyme such as xylosidase, glucoronidase, endoxylanase, acetyl xylan esterase and arabinofuranosidase are required. Moreover, these enzymes work in a synergistic way allowing the hydrolytic action of Acetyl xylan esterase in the same heteroxylan to

TABLE 2 | Examples of exogenous enzymes used to enhance fruit s pomaces' quality as animal feed additives.

Enzyme	Class	Pomace	Activity/ Target(s)	Suggested concentration	Host	Outcomes	References
Viscozyme® L (cellulolyticmixture)	Hydrolase	Grape *(Vitis vinifera* var. Shiraz) pomace	Valorize grape pomace	100 g/kg of pomace; 1 g/kg of enzyme	*Aspergillus* sp.	Ameliorated anti-nutritional effects of tannins and fiber	Kumanda et al., 2019
Avizyme® 1,505 (enzyme complex) and tannin acyl hydrolase	Hydrolase	Grape (*Vitis vinifera* var. Cencibel) pomace	Hydrolyze the polymeric structures into smaller catechins	5% of pomace; 500 ppm of enzyme	xylanase: *Trichoderma reesei* ATCC 5588 subtilisin: *Bacillus subtilis* ATCC 2107 amylase: *B. amyloliquefaciens* ATCC 3978.	Reduced the digestibility of total polyphenols and protein; promoted a lower digestibility of the monomeric and dimeric catechins	Lima et al., 2014
Avizyme® 1,505 (enzyme complex) and tannin acyl hydrolase	Hydrolase	Grape (*Vitis vinifera* var. Cencibel) pomace	Improve the extraction of phenols	5% of pomace; 500 mg/kg of enzyme complex, 500 mg/kg of tannase	xylanase: *Trichoderma reesei* ATCC 5588 subtilisin: *B. subtilis* ATCC 2107 amylase: *B. amyloliquefaciens* ATCC 3978	Increased the amount of total polyphenol released in intestine; did not improve the stability to meat lipid oxidation	Chamorro et al., 2015
Avizyme® 1,502	Hydrolase	Dried tomato pomace	Increase digestibility of pomace	30 g of pomace; 20 mg of enzyme		Reduced the loss of dry matter, nitrogen and energy from broiler cockerels; improved the coefficient of total tract apparent digestibility of dry matter, nitrogen and metabolizable energy values of dried tomato pomace	Mansoori et al., 2008

release of acetic acid, which in turn improves the accessibility of the xylan polysaccharide to endoxylanase (Wong et al., 1988; Sunna and Antranikian, 1997). Proteases release amino acids while improving gut development of broilers, increase their bodyweight and improve feed efficiency (Wang et al., 2016). These authors also reported that broilers fed carbohydrases showed a decreased ileal *C. perfringens* of 15-day-old broilers, higher levels of plasma zeaxanthin at d 22 and higher levels of plasma lutein at d 15 and 22 (Wang et al., 2016).

Slominski (2011) suggested that the main action of enzymes, specifically carbohydrases, is breaking down of high molecular weight polysaccharides into monosaccharides, oligosaccharides and low molecular weight polysaccharides. Exogenous enzymes such as phytases (Bedford and Cowieson, 2012) and proteases (Cowieson and Roos, 2016) have been shown to have beneficial effects on the integrity of the gastro-intestinal system by improving the integrity of intestinal mucin and enterocyte tight junctions integrity as well as by reducing inflammatory activities in the gut, and undigested substrates that could in turn become substrates for pathogenic bacteria and thus improving the general and gut health of the bird. Microbial enzymes have similar beneficial effects of synthesis of short chain fatty acids for energy utilization, protein breakdown and immune support (Bedford and Cowieson, 2012; Kiarie et al., 2013). However, the challenge with microbes is in maintaining a balance between beneficial and pathogenic microflora populations. Thus, this balance could be achieved by the use of exogenous enzymes for *in vivo* uniform movement of substrate(s) from the fore gut to the hind gut (Kiarie et al., 2013; Cowieson and Kluenter, 2019). The modalities in which exogenous enzymes have an effect *in vivo* on digestive health, immunological health, physiological health and microbiological health has been well-described. In terms of digestive health, Cowieson and Kluenter (2019) indicated that it is not important to use exogenous enzymes in very young broiler chicks as a way to improve nutrient utilization because their intestine accounts for a bigger proportion of their body mass and their body mass is not too demanding physiologically-speaking from the intestine; but this changes after day 14 when the birds are growing bigger and the intestine and pancreatic tissue diminish in proportion to the metabolic weight. When it comes to physiological health, Cowieson and Kluenter (2019) highlighted the importance of the physical well-being of the intestine, with regard to the integrity of the enterocytes, villi length and brush border integrity, mucin production and also microbial stability. Cowieson et al. (2004) demonstrated that phytase in the diet reduced the loss of sialic acid, a mucoprotein component, that is exacerbated by ingestion of feed rich in phytic acid, and thus phytase degraded the phytate and prevent this loss. Further research by Fernandez et al. (2000) showed that the addition of xylanase to wheat based diets of broilers also increased the production of sulfates mucins, reduced the numbers of *Campylobacter jejuni* in the ceca while increasing the digestibility of ingested nutrients along with reducing the loss of proteins by interfering with the endogenous protein flow. **Table 3** lists examples of well-established usages of natural and recombinant enzymes in the feed industry.

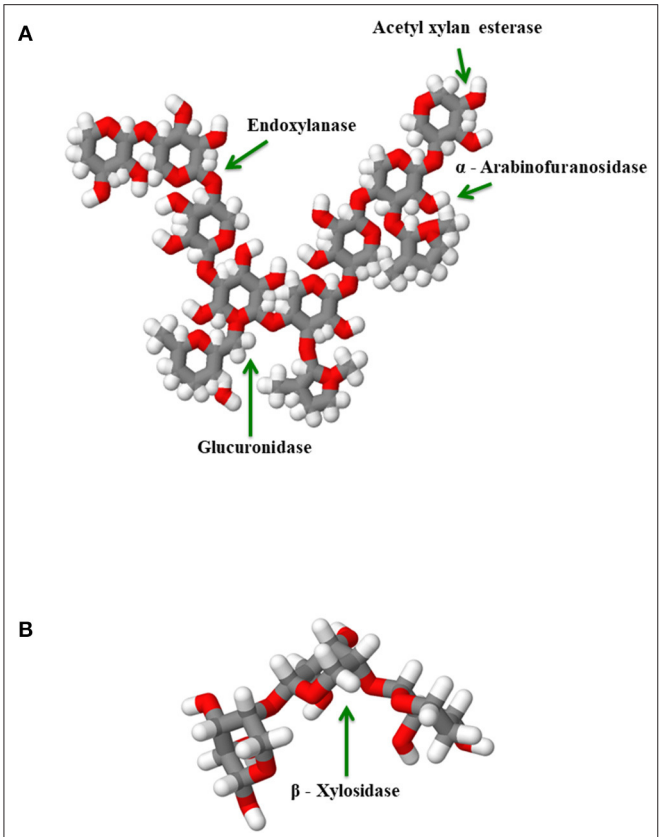

FIGURE 5 | Degradation of xylan polyssacharide and xylooligosaccharide to produce α-arabinofuranose, α-glucuronic acids among other acetyl groups (Wong et al., 1988): **(A)** Xylan degradation by xylanolitic enzymes to produce α-arabinofuranose and α-glucuronic acid, **(B)** β-xylosidase enzyme hydrolytically degrading a xylooligosaccharide.

Exogenous enzymes in broiler diets can induce shift of the site of digestion from the posterior to more proximal sections of the gut (Bedford and Cowieson, 2012), resulting in what is termed as the "starvation of the hind gut microbes" from getting important substrates. With reduced substrates for beneficial microbes to metabolize, their proliferation and population reduces, and this puts the colon and large intestine at the risk of proliferation of pathogenic microflora. Addition of xylanase to wheat and maize based broiler diets leads to production of xylo-oligomers, which are more readily fermentable in the posterior gut (Kiarie et al., 2009a, 2014, 2017; Singh et al., 2012). These xylo-oligomers are thought to influence the release of the hormone peptide YY, which is involved in bowel movement and increase the residence time of ingesta to be acted upon by microflora. Moreover, and in addition to the above activities, enzymes such as lysozymes cleave the bond between *N*-acetylglucosamine and *N*-acetylmuramic acid of the peptidoglycan in the bacterial cell wall, leading to bacterial death (Cowieson and Kluenter, 2019). Lysosomes are thought to be important host's antibacterial defense factors.

Lastly, with regard to the immunological considerations, enzymes alter the microbiome which in turn influences the host immunity, intestinal integrity and development (Bedford and Cowieson, 2012). For example, xylanase was shown to have a substantial effect in mucin production, increasing the density of goblet cells, and phytase aided reduction of sialic acid loss. Non-starch polysaccharide (NSP) degrading enzymes (NSPases) have been shown to increase mucin production in poultry (Fernandez et al., 2000). Products that elicit enzymatic activities, such as mannan-oligosaccharide derived from yeast cell walls, have been shown to directly influence the immune systems of production birds by activating mononuclear cells (such as macrophages). Dietary mannan-oligosaccharides was reported to increase the humoral response in poultry, following vaccination against Newcastle disease virus and Gumboro (Oliveira et al., 2009).

Swine Production

Generally, the benefits of using exogenous feed enzymes have not been profound in swine compared to poultry due to various differences between these two species. These differences include gastrointestinal anatomy, characteristics of digesta, the digestive capacity, the chemical environment of the gut, and transit time in the gut (Ravindran and Son, 2011; Owusu-Asiedu et al., 2012; Kiarie et al., 2013, 2016a; Ndou et al., 2015; Walsh et al., 2016; Rho et al., 2018).

With pigs having a longer intestinal length, the ingesta have a longer mean retention time (MRT) of 4-6 h and the nutrients are better extracted. This differs with poultry, which have shorter gut, and hence have shorter MRT of about 30 min to an hour in the gizzard and small intestine, respectively (Ravindran and Son, 2011). Additionally, pigs consume lots of water during feeding improving thus, the viscosity of the digesta, especially from NSP diets like wheat, barley and rye. In contrast, poultry, whether, broilers, layers or turkeys, have a crop where-in the exogenous enzyme will become activated before it reaches the more acidic sections of the gut (Dierick, 1994). The exogenous enzyme's optimal pH value determines whether the enzyme can work in less acidic conditions like the crop or more acidic conditions in the proventriculus and further down the gut. The longer MRT in pigs and their large colon and caecum provide for a higher proliferation of bacteria in these gut regions and translates to better opportunities for microbial fermentation of fibers to produce volatile fatty acids than in poultry. Nonetheless, at the foregut, the proliferation of pathogenic bacteria may cause disturbances in the animal and potentially a state of disease. This large proliferation of gut microbiota is sometimes touted as a reason for lesser exogenous enzyme action in swine, compared to poultry. Nonetheless, the use of exogenous enzymes have shown better performance results in young pigs with various studies (Sudendey and Kamphues, 1995; Kiarie, 2008; Kiarie et al., 2008a, 2009a,b, 2015b; Agyekum et al., 2015) showing the effects of the enzymes in reducing the negative effects of viscosity in wheat and barley based diets. Xylanases are the predominant enzymes used in these diets due to the fact that the main NSPs found in wheat and barley are the arabinoxylans.

Mechanisms behind the efficacy of exogenous enzymes used in swine is complex and need to be established. Several factors related to these complexities include the potential of enzymes to change gut microflora, enzyme combinations and sources of

TABLE 3 | Examples of well-established natural and recombinant enzymes used in the feed industry.

Enzyme[1]	Activity/Target(s)	Suggested concentration	Host	Target animal	Outcomes	References
Xylanase 50,316 (VR007)	Hydrolyze the glycosidic linkages of xylans in feed	2,000 mg/kg/day	*Pseudomonas fluorescens* BD50316	Swine, Broiler	Increase nutrient digestibility	Van Dorn et al., 2018
Phytase 50,104 (VR003)	Hydrolyze phytic acid to release phosphate	2,000 mg/kg/day	*P. fluorescens*	Swine, Broiler	Increase digestibility of phytate	Krygier et al., 2014
Xylanase	Hydrolyze the glycosidic linkages of xylans in feed	10 IU/g	*B. subtilis subsp.subtilis* JJBS250	Swine, Broiler	Enhance liberation of reducing sugars	Alokika and Singh, 2018
Xylanase	Release xylooligosaccharides from xylan	1,200 IU/kg	*Hericium caputmedusae*	Swine, Broiler	Reduce pathogenic infection of broilers	Zhang et al., 2018
Ronozyme WX (xylanase)	Degrade arabinoxylans	200 FXU/kg	Aspergillus oryzae	Swine	Increase sow feed intake and nutrient digestibility	Zhou et al., 2018
Endofeed W (xylanase)	Reduce non-starch saccharides (NSPs)	500 mg/kg	Aspergillus niger	Broiler	Increased the efficiency of energy utilization for protein	Nourmohammadi et al., 2018
β-glucanase; β-xylanase	Degrade β-glucans	0.1 g/kg	*Trichoderma reesei*	Swine	No effect on digestive health and animal performance	Clarke et al., 2018
6-phytase	Hydrolyze phytic acid to release phosphate	4,000 U/kg	*Aspergillus oryzae*	Broiler	Improve P digestibility and retention of low P diets	Pekel et al., 2016
endo-1,4-β-xylanase	Degrade soluble and insoluble xylans	800 U/kg		Broiler	No effect on growth performance; increased ileal digestible energy, Ca and P retention	
endo-1,4-β-xylanase	Hydrolyze non-starch saccharides (NSPs) and influence intestinal microbial composition	1,875, −5625 XU/kg	*Trichoderma citrinoviride*	Broiler	Decreased ileal digesta viscosity, improved apparent ileal digestibility of nutrients and microflora balance; reduced odor	Liu and Kim, 2017

[1]*All listed enzymes belong to the hydrolase class.*

feed. It is also very critical to understand the role of enzymes in gut physiology and its influence on the chemical and hormonal aspects of the gut (Dierick and Decuypere, 1996; Kiarie et al., 2013, 2016a; Agyekum et al., 2016).

THE PHYSIOLOGICAL CAPACITY OF POMACES-DEGRADING ENZYMES TO SUPPORT ANIMAL PRODUCTION

Characterization of fruit pomaces that are destined for animal feeding applications is important due to the varying compositions of such additives. For example, characterized cranberry and blueberry pomaces are usually reported with total carbohydrate contents of 88.78 and 84.91%, respectively (Ross et al., 2017). Similarly, apple pomaces have carbohydrate contents of 84.7%, of which 5.6% is starch on average (Perussello et al., 2017) and encompassing dietary fibers, hemicellulose and pectins.

Complex polysaccharides within the pomace need to be solubilized in the hindgut for proper fermentation, which results in the production of short chain fatty acids that increase energy utilization in the animal. Breaking down carbohydrate-protein bonds in pomaces is also vital to improve amino acid availability for absorption/utilization by the animal. Furthermore, when the NSP are enzymatically hydrolyzed in the hindgut, it results in prebiotic products that ameliorate gut health in chickens and pigs (Kiarie and Nyachoti, 2008; Kiarie et al., 2008b, 2009b, 2010; Slominski, 2011). Some feed components such as pectins, heteropolysaccharides that are composed of galacturonic acid and methoxyl esters, does also interact and bind some of the polyphenols that are found in apple pomace.

Poultry

In formulating pomace-based poultry diets, the pomace ingredients would be formulated together with other ingredients such as corn, soybean meal and wheat that could result in an increase in the NSPs. In the grains, the main NSPs are cellulose and non-cellulosic polysaccharides. In corn, the NSPs are comprised of non-cellulosic polysaccharides such as arabinoxylans and β-glucans; in soybean it consists of arabinans, arabinogalactans, galactans, galactomannans, mannans, and pectic polysaccharides and in wheat the non-cellulosic polysaccharides are more water soluble and viscous β-glucans and arabinoxylans that are notorious for making ingesta doughy and for interfering with digesta movement (Slominski, 2011). This creates problems of sticky fecal droppings in poultry housings and also the impeded digesta does have an effect in reducing the performance and health of the birds (Graham and Åman, 1991).

Therefore, this necessitates the use of NSP degrading-enzymes such as β-glucanases and xylanases, which would aid in better

energy and protein utilization of the birds. Due to the complexity of the different NSPs in the diet derived from the varied feed ingredients including pomace, the best approach would be to use a cocktail of multi-carbohydrase enzymes that would induce a better cell-wall breakdown and hydrolysis of the NSPs. The use of multi-carbohydrase enzymes with canola meal has been shown to improve nutrient and energy utilization and fiber (NSP) solubilization for effective hind gut fermentation in broiler chicks (Gallardo et al., 2017). This breakdown is associated with degradation of the anti-nutritive factors in feed (Kiarie et al., 2016b) and according to Slominski (2011) it also leads to the release of starch, fats, proteins and minerals hence improves energy utilization. The use of a cock-tail of enzymes instead of a single pure enzyme(s) is beneficial for pomaces since pomaces are structurally complex and the nutrients and bio-phenols in these pomaces may not exist in simple entities but rather as complexes of proteins, fats, carbohydrates and fibers (Ravindran, 2013). Various studies of using enzymes in poultry feed (Karimi et al., 2013; Gallardo et al., 2017) showed the beneficial effects of their use. **Table 4** shows the growth performance of broiler chickens and turkeys that were fed corn-soybean meal-based diets supplemented with different cocktail of enzymes.

In broilers, studies in the past three decades showed that this disruption of cell-wall matrix and release of encapsulated nutrients greatly leads to the improvement of growth performance of birds and some researchers including Acamovic (2001) have suggested that NSPs from wheat could act as a physical entrapment that prevent or slow down the endogenous enzymes to reach their substrates. Other experiments showed that the intact NSPs cell walls when tracked microscopically encapsulated starch, and that the use of NSP-depolymerizing enzymes released these nutrients for absorption and utilization by birds (Bedford and Autio, 1996).

The efficacy of multiple multi-carbohydrase enzymes to improve nutrient utilization and growth performance in broilers was determined (Meng et al., 2005). Using wheat, soybean meal, canola meal and peas as substrates, the authors showed that the body weight gain of birds reared on enzyme-supplemented diets was higher than in control diets. The combination of enzymes C+P+XG+MC produced the highest improvement in the broilers specific parameters: 4%, 9%, 144kcal/kg, 8%, and 8 units for starch and protein digestibility, apparent metabolizable energy, weight gain, and feed-to-gain ratio, respectively (Meng et al., 2005).

Swine

As is with the poultry diets, pigs are fed mainly grain-based diets around the world making therefore, carbohydrase enzymes pivotal in swine production. The viscosity of digesta again comes up since these cereal-based diets contain high molecular weight and soluble NSPs, which have a negative effect on the digesta movement. It was demonstrated that digesta dry matter flows of piglets and growers, from the fore stomachs to the hind stomach were significantly increased in diets with added exogenous enzymes amylase, β glucanase and xylanase, due to a reduction of viscosity (Sudendey and Kamphues, 1995).

Exogenous enzymes have also been suggested to influence or stimulate feed intake in piglets, which could contribute to higher daily live weight gains than diets without enzymes (Haberer et al., 1997).

It has been reported that exogenous xylanases break down fermentable cecal substrates in the pig's hind gut leading to products that influence enteroglucagon

TABLE 4 | Growth performance of broiler chickens and turkeys fed corn-soybean meal based diets with or without different cocktail of enzymes [adapted from Slominski (2011)].

Enzyme preparations	Trial length (days)	Bodyweight gain (g/bird per day)			Feed conversion ratio (g of feed/g of gain)			References
		PC[1]	NC[2]	NC + enzyme	PC[1]	NC[2]	NC + enzyme	
Broilers:								
Xylanase–β-glucanase	1–42	79.1	79.2	79.1	1.60[a]	1.65[b]	1.58[a]	Cowieson et al., 2010
Xylanase–β-glucanase	1–42	58.6	58.2	58.1	1.87	1.87	1.88	West et al., 2007
Xylanase–amylase–protease	1–38	52.4	50.5	51.7	1.73	1.80	1.79	Yu et al., 2007
Xylanase–amylase–protease	1–21	32.9	31.4	33.6	1.38	1.48	1.40	Cowieson and Ravindran, 2008
Turkeys:								
Xylanase–amylase–protease	1–56	90.2	90.4	91.3	1.87	1.89	1.85	Troche et al., 2007
Improvement over NC (%)				2.1			2.1	
Improvement over PC (%)				0.7			−0.6	

[a,b] *Means within the same row but with no common superscripts differ significantly ($P < 0.05$).*
[1] *Positive control (PC) diets.*
[2] *Negative control (NC) diets.*

levels, which in turn influence gastrointestinal motility and emptying (Bedford and Schulze, 1998). This loop creates a faster flow rate of digesta than in diets without xylanase, which could be a reason for the increased intake, and nutrient availability in the small intestine, even though further studies are needed to ascertain this physiologic capacity.

CONCERNS AND PERSPECTIVES

The antimicrobial benefits of fruit pomaces have been widely researched. However, these products still need to be fully adopted to exploit their promising bioactive molecules. Various products of pomaces such as non-dialyzable extracts of cranberries have been demonstrated, with yet to be determined mechanisms, to have humoral immune response in poultry with phagocytic and bactericidal effects on *Staphylococcus aureus*, whilst also having antioxidant activity (Islam et al., 2017).

In humans, cranberry juice has been shown to reduce *E. coli* bacterial adhesion to the urinary tract and *Helicobacter* to the gastric mucosa, an occurrence that has not been replicated in other pomaces such as blueberry, grape, apple and orange (Johnson-White et al., 2006). On the other hand, grape pomace has been touted for its antioxidant properties inhibiting the lipid oxidation of fish, turkey meat (Pazos et al., 2005), and in rats it has been shown to diminish lipid peroxidation of liver, kidneys and lungs tissues. Despite the present evidence showing that it had no impact on growth performances of chicken, it has been demonstrated that grape pomace extracts interacted with intestinal microflora to reduce the number of undesirable bacteria such as Clostridia, while increasing the population of positive ones such as *Lactobacillus* and *Bifidobacterium* (Papadopoulou et al., 2005). The pleiotropic effects of pomace seem to be linked to their numerous chemical contents many of which still need to be identified and characterized in future research.

However, with a limited number of studies showing a depressed growth performance in birds fed with grape seed extracts for instance, attributed probably to high tannin levels (Hughes et al., 2005), a question on the optimization of these products does linger in parallel to the side effects of the known and unknown anti-nutritive factors that might be present in fruit pomaces. Alienable literatures suggest that the beneficial effects of pomaces to both animal- and human-nutrition are forthcoming but are inconclusive awaiting more optimization and standardization. As studies progress toward microbiota-microbiome research, more focus needs to be applied on identifying the relationships between beneficial and pathogenic bacteria under -phytochemicals feeding, their synergy and/or discordance in the gut function and immune status of the host. Investigating the role of other factors such as genetic, epigenetic, environmental, management, diet, etc. that could improve the cost benefits of pomace will provide a broad and in-depth understanding of future research directions as well as what type of questions need to be addressed first.

CONCLUSIONS

Overall, this review provides insights on the power of enzyme-assisted fermentation-to enhance fruit pomaces bioactive and nutrient contents for animal (specifically poultry and swine) feeding applications. The growing number of purified fungal and bacterial enzymes and theirs recombinant-products make their usage more practical and economically appealing for both farmers and producers alike. Certainly, there are many technical issues that still exist and which need to be addressed. Many research and development efforts are scrutinizing the conditions surrounding the bioactive-compounds production from pomaces, the chemical and structural targets of the involved enzymes within the utilized pomaces, the molecular targets of the released bioactives within the animal's tissues and organs, and finally animal-responses and the overall impacts of such harnessed bioactives on animal health and performance. However, it is a matter of time before pomaces (still viewed as a burden within some industries) take their proper place within the animal production chain and start to get funneled into more sustainable green solutions within the circular economies. This matter of time, so to say, shall give research and development the space to continue working on fractions and sub-fractions of pomace bioactives that will be more cellular level targeted in their actions. With different pomaces producing varied bioactives, research and development would enable to extract the beneficial pomace components and possibly use them either singly or in combination, as feed additives. Despite that most of previously reported research efforts had focused merely on the industrial usage of pomaces within the ethanol industry rather than animal feed applications and while the ethanol production and processing differs fundamentally from feed operations, lessons learned from that industry (which rely heavily on the usage of commercially available biomass/fiber-degrading enzymes) can give many insights in regard to future usage of pomaces and enzymes in the feed industry.

AUTHOR CONTRIBUTIONS

MD (the principal investigator), YH, and MK conceptualized the review. MD and EK contributed resources, reviewed and edited the manuscript. MK, YH, and KG wrote the paper. MD provided overall guidance and mentorship, throughout the scope of this review. All authors read and approved the final manuscript.

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REVIEW
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Valorization of Seafood Processing Discards: Bioconversion and Bio-Refinery Approaches

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The seafood industry generates large volumes of waste. These include processing discards consisting of shell, head, bones intestine, fin, skin, voluminous amounts of wastewater discharged as effluents, and low-value under-utilized fish, which are caught as by-catch of commercial fishing operations. The discards, effluents, and by-catch are rich in nutrients including proteins, amino acids, lipids containing good proportions of polyunsaturated fatty acids (PUFA), carotenoids, and minerals. The seafood waste is, therefore, responsible for loss of nutrients and serious environmental hazards. It is important that the waste is subjected to secondary processing and valorization to address the problems. Although chemical processes are available for waste treatment, most of these processes have inherent weaknesses. Biological treatments, however, are environmentally friendly, safe, and cost-effective. Biological treatments are based on bioconversion processes, which help with the recovery of valuable ingredients from by-catch, processing discards, and effluents, without losing their inherent bioactivities. Major bioconversion processes make use of microbial fermentations or actions of exogenously added enzymes on the waste components. Recent developments in algal biotechnology offer novel processes for biotransformation of nutrients as single cell proteins, which can be used as feedstock for the recovery of valuable ingredients and also biofuel. Bioconversion options in conjunction with a bio-refinery approach have potential for eco-friendly and economical management of seafood waste that can support sustainable seafood production.

Keywords: seafood by-products, seafood waste treatment, valorization, bioconversions, bio-refinery, marine biotechnology

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INTRODUCTION

Global food production is growing significantly to meet rising consumer demands. The seafood industry, a major segment of the food industry, provides finfish and shellfish of choice to consumers worldwide. Marine seafood includes finfish (pelagic, anchoveta, pollock, tuna, herring, mackerel, whiting, and others), and shellfish, which include crustaceans such as shrimp, krill, crab, lobster, and, mollusks, consisting of bivalves (mainly mussels, oysters, clams, and scallops), cephalopods (squid and cuttlefish), and gastropods (mainly abalone and snails). In 2018 global

seafood production was 178.5 million tons (MT), consisting of 96.4 MT of capture fisheries (FAO, 2020). Of the 71.9 MT of finfish, the most popular marine species were anchoveta (7 MT), followed by Alaska pollock (3.4 MT), skipjack tuna (3.2 MT), herring (1.8 MT), and blue whiting (1.7 MT). Aquaculture production was 82.1 MT in 2018 consisting of finfish, mollusks, and crustacea at 7.3, 17.3, and 5.7 MT, respectively. Seafood amounting to 67.1 MT consisting of marine and farmed species including shrimp, prawns, salmon, mollusks, tilapia, catfish, sea bass, sea bream, and others constituted international trade. It is projected that utilization of fishery products for human consumption will reach 204 MT by the year 2030. It should be noted that percentage of fish stocks within biologically sustainable levels in 2017 was only 65.8% against 90% in the year 1990 (FAO, 2020). This suggests efforts by stakeholders for sustainability of the seafood resources are necessary.

SEAFOOD PROCESSING DISCARDS AND EFFLUENTS

The processing of food generates enormous amounts of waste, which include both solid discards as well as wastewaters containing portions of food being processed, which are released as process effluents. It has been estimated that, on average, about one-third of food produced globally, amounting to 1.3 billion tons, is wasted (Gustavsson et al., 2011). In the case of seafood, all the species harvested are not adequately used as food. Consumers prefer only a few select seafood items. A significant portion of the total harvest, therefore, remains unused or poorly used due to inherent problems related to unattractive color, smaller size, and high fat content. These result in a sizeable amount of catch being treated as by-catch, which is usually a combination of several species, particularly from tropical shrimp fisheries, and is unused or poorly used as food (Venugopal and Shahidi, 1998). Gustavsson et al. (2011) observed that food loss and waste for the whole fisheries sector amounted to 35% of global catches; 9–15% of these losses arise from by-catch.

The seafood industry processes about 80% of total harvest into chilled, frozen, smoked, dried, fermented, or marinated products. The centralized pre-processing operations, which include beheading, de-shelling, skinning, gutting, removal of fins and scales, fileting, washing, and others, leads to significant amounts of solid wastes and wastewater as effluents. The waste, on a wet weight basis, constitutes as high as 50% of whole shellfish, such as shrimp, krill, and crab. Shrimp waste contains about 70% head and 30% shell (Yan and Chen, 2015). Argentine red shrimp (ARS) is a highly popular shellfish, the industrial processing of which yearly yields 18,000 MT of shell waste, which is responsible for environmental pollution and ecological imbalances in Argentine Patagonia (Cretton et al., 2020). Lobster processing generates 50–70% of the shellfish as by-products such as heads, shells, livers, and eggs, which annually amounts to more than 50,000 MT (Nguyen et al., 2017). India produces up to 80,000 MT of shellfish waste (Chandrasekharan, 2015). Discards from finfish constitute 25–50% of the raw material, and is comprised of entrails, heads, skeletal frames, skin, scales, and viscera. Processing of freshwater fish such as trout, carp, pike, and bream generates 40–60% of the fish as waste (Venugopal, 2006). Love et al. (2015) reported that during the period 2009–2013 about 47% of the edible seafood supply was not available in the US for human consumption. This also included 16–32% of the harvest discarded as by-catch. In Europe, for each ton of seafood consumed an almost equal amount is estimated to be discarded as waste. The processing of shrimps and crabs in the EU alone results in more than 100,000 MT of shell waste each year (Sieber et al., 2018). Further, it is cautioned that large amounts of new fish biomass will be generated in European ports following the Landing Obligation Guidelines issued by the EU (Uhlmann et al., 2019).

The seafood industry, apart from solid discards, generates voluminous amounts of wastewater as process effluents as a result of operations such as washing, chilling, blanching, fileting, cooking, marination, and others. It has been estimated that ∼10–40 m^3 water is required for processing each ton of raw seafood (Arvanitoyannis and Kassaveti, 2008). One of the largest herring-processing factories in Europe, with an annual production of about 50,000 MT, releases ∼1,500 m^3 of wastewater daily (Steinke and Barjenbruch, 2010). Surimi production, which involves repeated washing of fish mince, uses more water than canning, curing, or freezing (Park, 2013). Water requirements for farmed production per metric ton of fish range from 1.5 to 6 m^3 for generic fish (Hall et al., 2011).

Problems Associated With Seafood Discards and Effluents

Loss of Nutrients

Seafood items are known to be rich in nutritionally valuable proteins, essential fatty acids, particularly long chain n-3 polyunsaturated fatty acids (omega-3 PUFA), mainly eicosa pentaenoic acid (EPA, C20:5; n-3) and docosa hexaenoic acid, (DHA,C22:6; n-3), and vitamins and minerals (James, 2013; Venugopal and Gopakumar, 2017; Venugopal, 2018). Analysis of the compositions of more than 40 types of seafood processing discards showed that the discards have average contents of 60% proteins, 19% fat, and 22% ash, as shown in **Table 1** (Islam et al., 2004). Shrimp head waste, on a dry weight basis, contains up to 65% proteins, 21% ash, and 18% chitin (Yan and Chen, 2015). Comparative dry-basis proximal analysis of shells of Argentine red shrimp (ARS) and southern king crab (SKC) showed both had 19–20% chitin. However, there were significant differences in contents of proteins and ash (18 and 48% for SKC and 26 and 55% for ARS, respectively). Analysis of shell and heads of ARS showed the highest lipid content (11%), with 5 mg and 158.8 μg of n-3 PUFAs and carotenoids per g, respectively. This suggested potential for recovering n-3-PUFAs and carotenoids from the ARS waste (Cretton et al., 2020). The raw heads, shells, and tails of Northern pink shrimp and spotted shrimp have crude proteins, which were rich in aspartic acid, glutamic acid, phenylalanine, lysine, and arginine. Their lipid contents ranged from 9.3 to 11.6%, while the contents of calcium, phosphorus, sodium, and magnesium were of 3,000, 400, 270, and 100 mg%, respectively. The contents of free amino acids (taurine, threonine, leucine,

TABLE 1 | Major components of seafood processing discards.

Nutrients	Composition
SOLID DISCARDS[a]	
Crude protein (%)	57.9 ± 5.3
Fat (%)	19.1 ± 6.1
Crude fiber (%)	1.2 ± 1.2
Ash (%)	21.8 ± 3.5
Calcium (%)	5.8 ± 1.3
Phosphorous (%)	2.0 ± 0.6
Potassium (%)	0.7 ± 0.1
Sodium (%)	0.6 ± 0.1
Effluents*	
Total suspended solids*	27–1,201 mg/ml
Ammoniacal nitrogen	50 mg%
Nitrate nitrogen	50 mg%
Phosphate	95 mg%
BOD_5*	179–276 mg/ml
COD*	458–1,717 mg/ml

[a]*Adapted from Islam et al. (2004). The values represent average values of 43 samples on dry weight basis, corrected to single decimal. *Typical values for effluents released from a seafood processing plant (Jamieson et al., 2017).*

tryrosine, and phenylalanine) of the processing by-products and edible parts were 2.0 and 1.7 g%, respectively (Heu et al., 2003). Lobster processing waste is responsible for appreciable loss of nutrients. Lobster liver may contain up to 41% proteins, on a dry weight basis, while its head contains meat up to 20% of the shellfish weight (Nguyen et al., 2017).

The characteristic features of seafood processing effluents are contents of total suspended solids (TSS), fats, oils, and grease (FOG), and pigments and minerals. The TSS includes proteinous matter (myofibrillar proteins, collagen, gelatin, enzymes, soluble peptides, and amino acids) in soluble, colloidal, or particulate form (Islam et al., 2004). Ching and Ghufran (2017) reported 2.2% total solids (consisting up to 550 mg% TSS and 260 mg% dissolved solids), 50 mg% each of ammonia and nitrate nitrogen, and up to 100 mg% of phosphate typical effluent. Tuna processing effluents contained TSS, fat, chemical oxygen demand (COD), and biochemical oxygen demand (BOD) at 1,570, 450, 11,100, and 6,600 mg per lg, respectively (Achour et al., 2000). These losses deprive the consumers of significant amounts of nutrients. It has been estimated that annual seafood discards in the US represent a loss of about 208 billion g of proteins and 1.8 trillion mg of n-3 PUFA (Love et al., 2015). These indicate that processing effluents, in addition to solid discards and by-catch, contribute to losses of nutrients.

Environmental Impacts

The food system has generally been considered a threat to the environment. The seafood industries consider huge volumes of by-catch, solid waste, and effluents a burden because of their potential to become environmental hazards. The industry dumps enormous amounts of by-catch in the ocean, while good amounts of solid wastes are disposed off as landfill or subjected to incineration. Ocean dumping causes reduced oxygen levels at the ocean bottom, burial or smothering of living organisms, and introduction of disease to the ecosystem of the sea floor (US EPA, 2017). Nguyen et al. (2017) observed that the disposal of lobster processing costs annually upward of about $7.5 million, and also presents an environmental burden to the lobster processors. Composting and ensilage of waste has been practiced, but they have limitations because of their longer process time, higher costs, and emission of volatile organic compounds. Anaerobic decomposition of seafood in landfill causes formation of methane (CH_4), ammonia (NH_3), and hydrogen sulfide (H_2S), which are detrimental to the environment (Xu et al., 2018). Landfill contributes to climate change, about 10 times larger than other waste disposal options, while composting has the largest impact on carcinogens (Gao et al., 2018). The TSS and FOG of process effluents are responsible for high BOD and COD values, indicative of their adverse influence on the oxygen balance and, in turn, the flora (Gonzalez, 1995). Furthermore, shortage of drinking water, eutrophication (growth of unwanted biota), biotic depletion, algal blooms, habitat destruction, water acidification, disease outbreaks, and extensive siltation of corals are other environmental hazards (Hall et al., 2011). The environmental problems and loss of nutrients associated with seafood process effluents have been pointed out recently (Venugopal and Sasidharan, 2021).

Measures for Sustainable Seafood Processing

Food sustainability demands optimal uses of resources for maximum benefits, including economic viability. The challenges of sustainable seafood processing are linked to reducing environmental pollution, conservation of water, and prevent losses of nutrients. Improving waste utilization is essential for a sustainable industry to prevent or minimize the environmental impact (López-Pedrouso et al., 2020). The problems of environmental hazards and nutrient losses facing the seafood industry can be addressed by measures such as selective trawling to reduce by-catches, appropriate treatments of wastes and effluents, and valorization of wastes by recovery of useful ingredients. The major advantages with respect to waste treatments are reduction of environmental hazards, conservation of water, and isolation of commercially valuable ingredients and improvement of the economy. In view of seafood-related environmental hazards, a need for a biological solution for the disposal of the seafood processing discards has been recognized (Pal and Suresh, 2016). Failure in these efforts not only leads to loss of potential revenues but also increases the cost of waste disposal and also public health problems (Etemadian et al., 2021). The recent United Nations Conference on Sustainable Development acknowledges the global importance of food losses and food waste. The Conference aims to halve per capita global food waste and reduce food losses along production and supply chains, including post-harvest losses, by 2030 (FAO, 2020).

Conventional Processes for Waste Valorization and Their Limitations

Conventional processes for valorization of seafood discards and effluents are based on chemical and physical methods. These

processes invariably have several limitations. For instance, the process of chitin extraction from crustacean shells involves initial alkali treatment generally with 5 M sodium hydroxide for deproteination followed by hydrochloric acid treatment for demineralization, which involves decomposition of calcium carbonate in the shells. Alkali treatment can result in the hydrolysis of chitin and also partial deacetylation of chitin. The process is also corrosive. Further, it requires large volumes of fresh water to wash off alkali and acid from the treated shells, releasing harmful wastewater (Mao et al., 2017; Yadav et al., 2019). Alkali extraction of proteins can lead to the loss of certain amino acids (Venugopal, 2006). Similarly, the traditional solvent extraction of fish oil can cause its oxidation, which is rich in unsaturated fatty acids. de Oliveira et al. (2016) extracted oil from tuna by employing chemical refining. The process consisted of degumming, neutralization, washing, drying, bleaching, and deodorization. Although chemical refining was successful, temperature and chemical reagents favored the removal of PUFA from the oil. In view of these drawbacks, interest in alternate green processes is growing. Biological processes can have minimal environmental impacts, be cost-effective and safe, and have minimum adverse impacts on the properties of the isolated components. These processes, therefore, offer an economic and versatile way to transform and concentrate waste and wastewater into valuable products.

Biotechnology holds promise for novel waste treatment and resource recovery processes, diversification of value-added products, and in quality assurance (Pleissner and Lin, 2013; Chandrasekharan, 2015). Such processes are supported by novel green techniques for industrial recovery of biomolecules from seafood by-products and discards. The extraction efficiency can vary highly depending on the food matrix, the target compounds, and methods of extractions. Therefore, the choice of green extraction technique depends essentially on the matrix and the target compound features (Bruno et al., 2019). Such approaches can also lead to a bio-based economy (Puyol et al., 2017). This article will examine potentials of biological processes depending on bioconversions of components in seafood discards, including effluents. Prospects of algal biotechnology and bio-refinery approaches will also be discussed.

BIOCONVERSION REACTIONS FOR VALORIZATION OF FOOD WASTES

Bioconversion reactions are biological methods, which are carried out to initially detach food components from their matrices. The detached components can be recovered and purified by suitable techniques. Two major bioconversion processes have been recognized, which employ either microorganisms or externally added enzymes. These help detach the components from the food matrices. Both microbial and enzymatic processes are environmentally friendly, cost-effective, and safe, unlike most conventional chemical extraction processes. Microbial fermentation of the waste results in the production of hydrolytic enzymes by the organism, which causes bioconversions of the food components. Exogenous enzymes, on the other hand, cause a direct release of the components from the food matrix. A number of microorganisms, particularly lactic acid bacteria (LAB), have been used for fermentation. The use of algae is a novel approach in this respect. The algae-induced bio-transformations help not only with the treatment of wastewater but also production of nutrient-rich biomass, which is useful for a variety of applications. In addition to microbial and enzymatic methods, biophysical processes such as modifications of pH and temperature can favor the release of components such as proteins and lipids from the food matrices. **Figure 1** depicts a schematic representation of the bio-conversion processes for valorization of seafood discards. These aspects will be discussed in detail.

Microbe-Mediated Bioconversions

The microbe-mediated bioconversion is termed as fermentation, which is safe, environmental-friendly and energy savvy. The process makes use of live microorganisms (bacteria, fungi, mycelium, or microalgae) to convert raw materials into products with desired qualities. Fermentation can be traditional, biomass or precision types. Traditional fermentation has been practiced for a few centuries. Since the 1980s biomass fermentation has emerged in the food industry for the production of cell mass for further use as sources of enzymes, flavors, food, biomaterials, therapeutics, fuels and in recent times, as sources of alternative proteins to develop cultivated meat formulations. Precision fermentation is intended to produce specific functional ingredients using tailor-made microbial hosts (GFI, 2020). Fermentation by lactic acid bacteria (LAB) has been used is a popular method for the development of fermented fishery products (Anihouvi et al., 2012). The efficiency of lactic acid fermentation depends on the type of organism, inoculums size, initial pH and pH attained during fermentation. The lactic acid formed during sugar breakdown creates low pH, which, in turn, suppresses growth of spoilage causing microorganisms and enhances activity of acid proteases, optimally acting on seafood proteins, many of which remain bound to chitin, lipids and carotenoids.

The bioremediation using microorganisms and their aggregates is recognized to be an efficient low-cost green process. The technology of microbial conversion also provides a potential way to isolate and exploit compounds of biotechnological potential (Wang et al., 2019). The microorganisms used for the purpose may be aerobic, anaerobic, or facultative including bacteria, fungi, and protozoa. Microbial fermentation processes can be under solid state, submerged or liquid state, anaerobic, batch, continuous, or fed batch conditions. The process is influenced by factors such as the nature of the starter culture, time, pH, and substrate composition. Fed-batch is a commonly used means for the production of microbial biomass, ethanol, organic acids, antibiotics, vitamins, enzymes, and other compounds in which the culture medium is added continuously or in pulses to reach the maximum volume. The advantages of fed-batch over the conventional batch operation include a higher biodegradation rate, higher productivity, higher dissolved oxygen in the medium, and decrease in fermentation time (Chandrasekharan, 2015; Puyol et al., 2017).

Microbial growth results in the production of hydrolytic enzymes such as proteases, lipases, and chitinases. Proteases and chitinases cause demineralization, deproteination, and

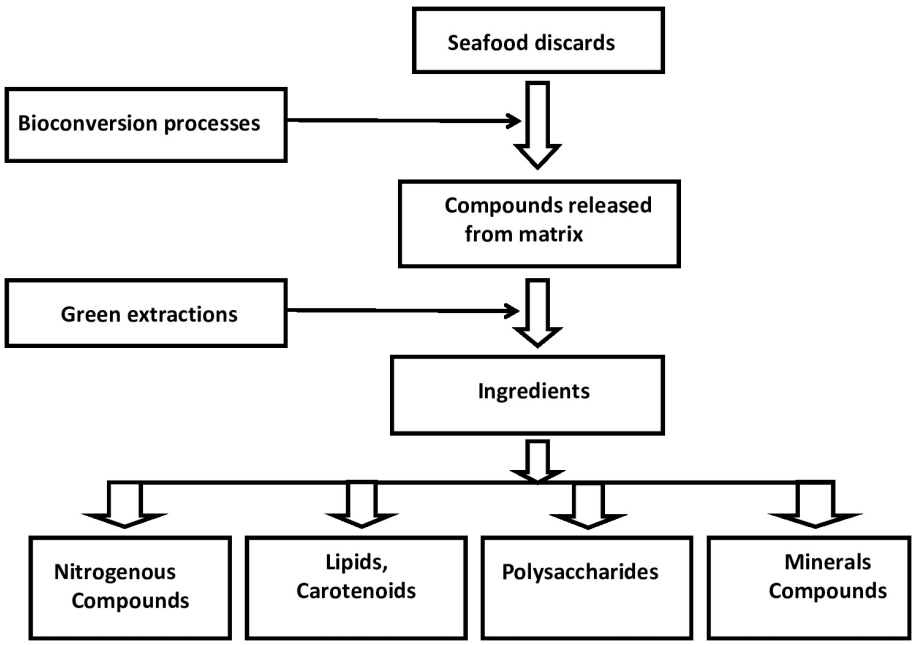

FIGURE 1 | Schematic representation of bioconversion for valorization of seafood discards and the major classes of compounds that can be extracted from seafood processing discards.

proteolysis in the substrate. Chitinases catalyze the cleavage of the β-1,4-O-glycosidic linkages in chitin. Lipases function as triacylglycerol hydrolases and also catalyze synthesis of ester compounds. Fish fermentation, which is traditionally used to increase fish shelf-life, results in the formation of bacteria metabolites of interest. Lactic acid bacteria (LAB) have been used for a long time for the development of fermented fishery products (Anihouvi et al., 2012). Fermentation by LAB produces lactic acid; the low pH enhances acid proteases which act on proteins bound to chitin, lipids, and carotenoids. Fermentation can be applied for the production of cell mass, enzymes, flavors, food additives, and a range of other high value-added products. For example, fermentation of shrimp shell waste by symbiotic LAB such as *S. thermophilus, L. acidophilus,* and *L. bulgaricus* rapidly decreased pH to about 4.2 and promoted the removal of calcium and protein, with 91.3% calcium, 97.7% protein, and 32.3% carotenoid removed from shrimp waste after 168 h fermentation activated acid proteases gave bioactive peptides of size between 1,000 and 10,000 Da (Shan et al., 2011). LAB -induced fermentations bring about diversity into foods, make otherwise inedible foods products edible, enhance nutritional value, decrease toxicity, preserve food, and decrease cooking time and energy requirements. The technology is safe, environmentally friendly, and does not consume much energy.

Microbe-assisted bioconversions are ideal for the bioremediation of seafood processing waste and production of aquafeed and fertilizer. Applied to fish by-products, fermentation gives rise to quality protein hydrolysates and oil and produces antioxidant compounds (Marti-Quijal et al., 2020). Fish offal and a mixture of sawdust and wood shavings in equal proportions were subjected to composting by placing them in an open structure with passive aeration. Solid state fermentation converted the waste into a highly nutritive fertilizer with a nitrogen content as high as 12% (Wang et al., 2019). Dried skipjack tuna waste (red meat, gills, viscera, fins, etc.) was mixed with 25% wheat flour and fermented with *L. plantarum* and *B. licheniformis* for 14 days. The proximate analysis showed significant changes in the composition of *L. plantarum*. The fermented product can be used as a nutritive aquafeed ingredient (Hena et al., 2009). Conversion of fish waste to liquid fertilizer was achieved with mixed microorganisms, resulting in about 28% degradation of fish waste. The product was stable against putrefaction for 6 months at ambient temperature (Dao and Kim, 2011). The microbe-assisted aerobic bioprocess of aquaculture solid waste for 15 days at 35 °C and at pH 6.0–6.5 maximized nitrogen bioconversion in the form of ammonium ions (NH_4^+) (Khiari et al., 2019). Fermentation is a viable alternative to chemical treatment for the extraction of collagen (Song et al., 2021).

Rashid et al. (2018) fermented shrimp-shell powder with *B. cereus* to produce sugar, antioxidant, and DNA protective compounds. The fed-batch biodegradation was operated in a 5-L bioreactor for 96 h according to three time pulse-feeding strategy. On the basis of the equal working volume of 3 l, the fed-batch biodegradation showed a better production of the target compounds than the batch biodegradation, with higher cell density and a shortened biodegradation period. The maximum values of the target compounds were about 0.3 mg per ml of reducing sugar and 92 to 98% antioxidant activities. Fed batch fermentation gave ∼3–12% higher values compared with batch biodegradation.

Microorganisms can treat seafood industry process effluents in reaction systems such as activated sludge, aerobic lagoons, trickling filters, and rotating disc contactors. In the commonly used activated sludge system, the sludge consisting of an optimized, mixed flora of microorganisms degrades the organic materials in the presence of dissolved oxygen, thereby decreasing the BOD of the effluent (Gonzalez, 1995; Choudhury et al., 2010). An aerobic continuous bioreactor treated high saline fish processing wastewater for 8 h, which removed the offensive odor of the effluent (Ching and Ghufran, 2017). Anaerobic digestion (AD), a popular green technology for waste treatment, involves fermentation of the material in the absence of molecular oxygen with the formation of CO_2, hydrogen, and/or acetic acid; reduction of the CO_2 and acetate leads to the production of methane. AD of tuna processing effluents involved a decanter to remove the fats and the TSS, an anaerobic digester, and an activated sludge aerated bioreactor. The integrated system helped with the removal of up to 95% of the COD (Achour et al., 2000). AD of seafood industry effluents in a dissolved air flotation system (DAF) removed organic contents. The process flow consists of the separation of effluent in DAF and treatment of clarified water in a double nitrification-de-nitrification stage. AD of solids separated during the DAF process produces biogas and significantly reduces sludge volume (Fluence, 2019).

Algae-Based Bioconversions

Microalgae such as Chlorella, Spirulina, Dunaliella, diatoms, and cyanobacteria, commonly referred to as blue green algae, are the main algae grown commercially as sources of functional materials in natural foods. These organisms are bestowed with a high growth rate in nutrient media under phototrophic (light and CO_2) conditions. Their digestive actions allow the degradation of organic contents, unused food, and excretory products together with the removal of CO_2, NH_3-N, CO_2, and H_2S, thereby ameliorating environmental pollution (Puyol et al., 2017; Gifuni et al., 2019). The phototrophic algae can be cultivated in open ponds or in closed photo-bioreactors, or heterotrophically in closed systems. One of the major advantages associated with the open ponds is its low production and operating costs. However, the limitations include uneven light availability and distribution within the pond. Heterotrophic cultivation in closed systems eliminates the requirement of light, but heterotrophic culture is prone to contamination by other microbial species (Nigam et al., 2020).

Microalgae are promising agents for bioconversions of food, fishery, and agricultural waste into biomass rich in bioactive compounds (Das, 2015). The algal mass (referred as single cell proteins, SCP) can contain up to 60% proteins, good amounts of oil, and also polysaccharides, minerals, and pigments including chlorophylls, carotenoids, and phycobiliproteins. Stringent nitrogen limitations stimulate algae to produce more lipids, as high as 75% with high n-3 PUFA contents (Stengel and Connan, 2015). SCP has found wide applications as sources of bioactive peptides, plant growth stimulants, animal feeds, food additives, cosmeceuticals, drugs, and as probiotics in aquaculture. SCP can also replace expensive soy meal and fishmeal in animal and aquaculture feeds (Sharma and Sharma, 2017; Caporgno and Mathys, 2018; Smárason et al., 2019). Cultivation of microalgae in wastewater offered the highest atmospheric carbon fixation rate (1.83 kg CO_2/kg biomass) and rapid biomass productivity-−40–50% higher than terrestrial crops (Shahid et al., 2020). Growth of microalgal biomass has been estimated to require 200–1,000 liter of water per kg of dry biomass. This suggests comparable volumes of seafood industry effluents could be treated for producing equivalent amounts of microalgae as SCP (de Farias and Barbera, 2018). Batch cultivation of *Chlorella* sp. in seafood processing water gave a biomass yield of 896 mg per lg (Gao et al., 2018). *Bacillus* sp., *Brevibacterium* sp., and *Vibrio* sp. associated with seaweed (*Ulva* sp.), having a consortium of hydrolytic enzymes including cellulase, protease, and chitinase, degraded crab shells, prawn shells, and fish scales within 4 days in the seawater-based broth. The reducing sugars released during degradation can be used for ethanol fermentation by *Saccharomyces cerevisiae* (Samant et al., 2019). Fermentation of fish media can result in 3 to 4 fold reduction in treatment costs (Vázquez et al., 2020).

Microalgae have the key advantage to produce third generation biofuel, because of its rapid growth and high lipid contents (Shuba and Kifle, 2018; Koyande et al., 2019). In comparison to petroleum diesel, biodiesel is characterized by lower emissions of carbon dioxide, sulfur dioxide, and harmful air pollutants. Oil from SCP is a plausible choice for biofuel. Therefore, cultivation of oleaginous microorganisms can be a promising approach for valorization low-cost organic waste for energy production (Cho and Park, 2018). Seafood discards, including effluents, can be a promising alternative feedstock for the sustainable production of biodiesel and biogas (Jayasinghe and Hawboldt, 2012). Direct transformation of lipidic biomass into biodiesel has also gained attention. Fadhil et al. (2017) produced liquid biofuels and activated carbons by trans-esterification of fish oil with methanol and ethanol using potassium hydroxide as a base catalyst. Fish oil that contains high levels of free fatty acids may require a modified esterification process. The process comprised rapid purification of the oil, followed by methanol esterification at 60°C for 1 h initially under acidic conditions followed by alkaline conditions. The preparation satisfied required standards, in terms of viscosity, flash point and other parameters (Kara et al., 2018). Anaerobic digestion of seafood processing wastewater by Chlorella sp. supports biogas production (Jehlee et al., 2017). These suggest algal technology has potential for valorization of seafood processing discards and effluents.

Enzyme-Assisted Bioconversions

Processing with an enzyme holds enormous potential in waste management. Enzymes can mitigate hazards of conventional chemical transformations for resolution of food waste-related environmental problems, help production of novel compounds, and function as analytical tools for food quality assessment. Enzymes can be included as additional processing aids to conventional processes or can be exclusively used to upgrade existing technologies in seafood processing. The advantages of enzymes are their low energy requirements, safety, and low-cost (Venugopal, 2006; Chandrasekharan, 2015; Fernandes, 2016;

Yang and Yan, 2018). Hydrolases, which include carbohydrases, proteases, and lipases, are popular enzymes in biotechnology,. Enzymatic hydrolysis of protein from aquatic by-products and livestock, poultry, and plants offer novel products with applications in foods, pet feed, pharmaceutical, and other industries (Etemadian et al., 2021). Specific, energy-efficient, and easily controllable enzymatic techniques using proteases, glycoside hydrolases, lipases, transglutaminases, and other enzymes are emerging as bio-processing techniques for seafood processing (Shah et al., 2016; Huang et al., 2017).

DOWNSTREAM PROCESSING FOR THE RECOVERY OF SEAFOOD COMPONENTS

The by-catch and the various seafood processing discards are rich in balanced proteins, collagen, enzymes, lipid, carotenoids such as astaxanthine and β- carotene, polysaccharides including chitin, glycosaminoglicans, and various minerals. These can be recovered by coupling the various bioconversion processes, discussed above, supported by marine biotechnology-based downstream processes. These processes are mostly mild, energy-efficient, safe, and environmentally friendly. Green techniques include pressurized liquid, sub-critical, super-critical, enzyme-mediated, microwave-, and ultrasound-assisted extractions (Muffler and Ulber, 2005; Freitas et al., 2012; Chavez et al., 2013). Membrane bioreactors integrate reaction vessels with membrane separation units for producing materials such as peptides, chito-oligosaccharides, and PUFA from seafood discards (Kim and Senevirathne, 2011).

The past few years have seen notable interests in seafood-derived compounds for varied applications including food, pharmaceutical, agriculture, and other industries. For example, bioactive compounds from lobster processing by-products can be extracted using microwave, ultrasonic, and supercritical fluid extraction. The proteins, chitin, lipids, minerals, and pigments recovered from lobster processing by-products possess several functionalities and bioactivities, useful for their applications in water treatment, agriculture, food, nutraceutical, pharmaceutical products, and biomedicine (Nguyen et al., 2017). The diverse compounds that can be extracted from seafood processing discards can be grouped into four classes, depending upon their chemical nature. The major classes include nitrogenous, lipid, polysaccharide, and mineral-based compounds. Some of the individual compounds under each class are given in **Table 2**. In addition, a multitude of derivatives can also be developed from many of these components. These include bioactive peptides, gelatin, n-3 PUFA, glucosamine, chitosan, and its various derivatives. Detailed aspects of preparations, properties, and applications of components of seafood processing discards will not be discussed here; nevertheless, a very brief mention may be made. Fish proteins and protein hydrolyzates have the potential to be used as a protein supplement, in the fortification of foods, and as sources of bioactive peptides. Fish oil has numerous health benefits and can also be used to impart functional properties to food products. Chitosan and their oligosaccharides are applied as antioxidants, antibacterial and antifungal agents, and functional

TABLE 2 | Major seafood-derived industrial components and nutraceuticals.

Finfish	Shellfish (Crustaceans and mollusks)
Nitrogenous compounds	Nitrogenous compounds
Proteins (myofibrillar)	Proteins
Collagen	Collagen
Gelatin	Gelatin
Protein hydrolyzate	Protein hydrolyzates
Bioactive Peptides	Bioactive peptides
Enzymes	Enzymes
Proteases, Collagenases, Transglutaminases, Chitinases, β-1,3-Glucanase, β-Galacto-sidase, Lysozyme, Catalase, Glutathione, Peroxidase, and others	Lysozyme from crab, scallop, others
Lipid-based:	Lipid-based:
PUFA rich oil	PUFA rich oil
Squalene, Squalamine	Carotenoids
	Astaxanthin
	B-caarotene
Mineral-based:	Polysaccharide-based:
Biological:calcium Phosphopeptides Hydroxyapatite from tuna bone	Chitin, Chitosan, Chitosan derivatives, Glucosamine oligosaccharides, novel polusaccharides from mussel
Polysaccharide-based:	Mineral-based:
Glycosaminoglycan,	Calcium carbonate,
Chondroitin,	Calcium lactate
Dermatan sulfate,	Calcium acetate,
Hyaluronic acid	Small peptides containing Calcium

compounds in food, pharmaceutical, and other industries. Gelatin is used for the purposes of gelling, edible coating, emulsification, and microencapsulation. The reader is referred to a few recent articles for details (Karim and and Bhat, 2009; Yu and Gu, 2013; Venugopal and Lele, 2014; Vidanarachchi et al., 2014; Muxika et al., 2017; Sasidharan and Venugopal, 2019; Shahidi et al., 2019; Ashraf et al., 2020). Nawaz et al. (2020) recently observed a need to focus on bioavailability, interaction with other ingredients, nutritional, biotechnological, and sensorial aspects, and other factors of seafood-derived compounds that can significantly favor valorization of fisheries by-products. The following discussion will focus on bioconversion processes for extraction of major classes of compounds seafood discards and effluents.

Nitrogenous Compounds

Diverse proteins, peptides, amino acids, and their co-products constitute the nitrogenous fraction. Scarcity of nutritional proteins in many parts of the world necessitates novel and economical processes to recover them from unexplored sources (Henchion et al., 2017). Seafood processing discards, having a maximum of about 60% proteins, can be good sources of proteins, which can be recovered while retaining most of its native properties. Bioconversion processes for protein

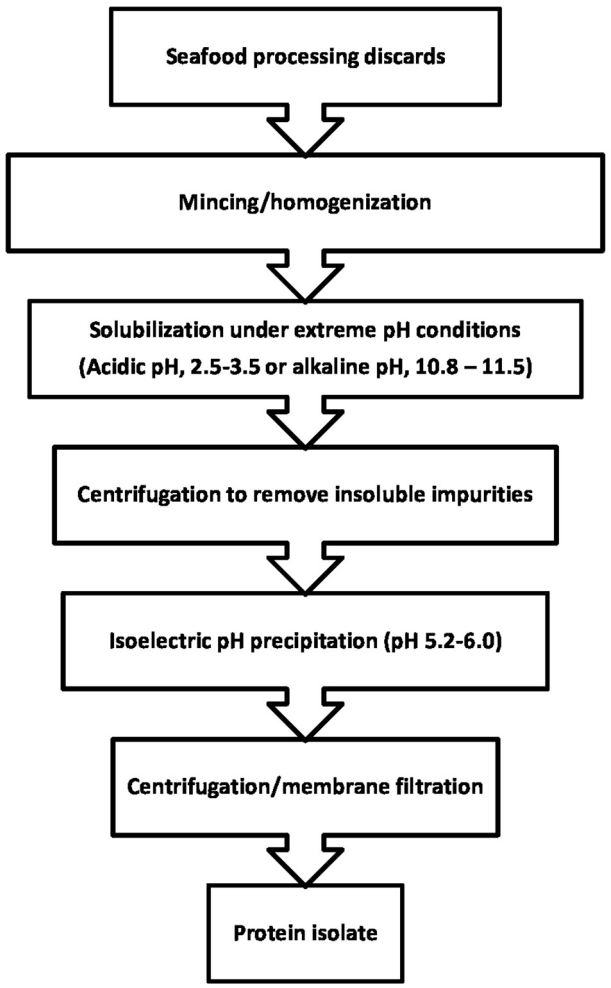

FIGURE 2 | Recovery of proteins from seafood discards by isoelectric solubilization precipitation (Adapted from Hultin et al., 2005).

recovery from the discards have been developed. These make use of biophysical changes that induce their coagulation and precipitation of the macromolecules. These are discussed below.

Isoelectric Solubilization Precipitation (pH Shift Process)

Isoelectric solubilization precipitation (ISP) is a gentle bioprocess. This involves homogenization of underutilized fish or processing discards with either dilute acid (pH 2.5–3.5) or alkali (pH 10.8–11.5). The treatment dissolves sarcoplasmic and myofibrillar proteins, while insoluble impurities such as bone, skin, oil, and membranes are removed. Up to 90% of the dissolved proteins are precipitated by raising the pH of the solution to their iso-electric pH of 5.2–6.0. The proteins are concentrated by centrifugation or filtration. The process, as depicted in **Figure 2**, is ideally performed at 10°C or below, to avoid denaturation of the protein (Hultin et al., 2005). Recovery of proteins by the ISP process can be enhanced by coupling it with high intensity sonication, electro-flocculation, and ultrafiltration. Sasidharan and Venugopal (2019) summarized studies on ISP-based protein recovery from various species of finfish and shellfish, their discards, by-catch, and also process effluents. Some of the fishery sources included mackerel, catfish. rockfish, Pacific whiting, rainbow trout, Atlantic croaker, channel catfish, bullhead catfish, shrimp, crab, mussel, and squid. The fish protein isolates (FPIs) have protein contents of at least 65% and fat below 2%. FPIs differ from conventional surimi, which is a concentrate of fish myofibrillar proteins obtained by repeated washing of fish meat mince (Park, 2013). Unlike surimi, which is only myofibrillar proteins, particularly myosin and actomyosin, FPIs contain sarcoplasmic proteins along with the refined concentrate of myofibrillar proteins. The FPIs generally retain biochemical, nutritional, and functional properties of the native proteins, which make them valuable raw materials for applications such as the development of restructured food products, protein supplements, and bioactive peptides (Sasidharan and Venugopal, 2019).

Apart from myofibrillar proteins, collagen is another protein from marine sources. Marine collagen is a promising biocompatible alternative to mammalian collagen, particularly in biomedical and food applications. Collagen-based novel functional food ingredients contain a nutritional benefit, such as essential and non-essential amino acid, to improve the quality of different food products. It can also be used as a natural antioxidant and texturizing agent that can reduce the utilization of chemical food additives and may be able to fulfill the consumer demands for safe and green food products (Pal and Suresh, 2016).

Collagen and collagen hydrolysate (CH) were recovered from the bone and skin containing residues emerging during the ISP process from silver carp. Isolated collagen maintained their triple-helical structure and was characterized as type I collagen. Pepsin-hydrolysis and sequential hydrolysis by pepsin and trypsin degraded all heavy molecular weight chains of collagen; sequential enzyme treatment yielded a higher degree of hydrolysis. When CH was added to silver carp protein isolate prior to gelation, the gel properties were dependent on the molecular weight of the added CH. More hydrolyzed collagen emerging from sequential hydrolysis improved the water holding capacity of the gel while reducing its breaking force. The results suggest that residue from pH-shift processing of fish can be used for isolation of functionally active collagen and CH (Abdollahi et al., 2018). Tilapia type I collagen is biocompatible and can be used as an effective biodegradable scaffold biomaterial for regenerative medicine (Hayashi, 2020). Gelatin is extracted from collagen generally by pre-treatment with dilute NaOH, followed by swelling with dilute acetic acid and then by warm (45°C) water (Vázquez et al., 2019). Gelatin prepared from collagen of skins and bones of various marine and freshwater fishery sources have good gelling properties. Gelatin extract of big eye tuna skin had glycine, up to 32% of total amino acids, and hydroxyproline together with proline and alanine. Rheological studies revealed Newtonian and shear thickening properties of the gelatin. The tuna gelatin could be useful for the formulation of functional foods and nutraceutical and biomedical applications (Dara et al., 2020).

Mild Acid Induced Gelation

This process makes use of the ability of muscle structural proteins to undergo gelation under mild acidic conditions when water is strongly bound to the protein matrices. The process of extraction involves initial mechanical deboning of by-catch or fish discards such as heads and frames. The meat mince is washed twice with chilled (0–5°C) water, followed by homogenization of the washed mince in equal amounts of fresh chilled water. The pH of the homogenate is lowered to 3.5–4.0 by drop-wise addition of weak acid, such as acetic acid, which induces gelation of the proteins. The gelation process is associated with a fall in viscosity of the homogenate; the viscosity fall can be enhanced by mild heating to 50°C. Proteins in the low-viscous dispersion are highly stable as they cannot be precipitated by heating even at temperatures as high as 100°C. Such thermo-stable dispersions have been prepared from Atlantic herring, Atlantic mackerel, threadfin bream, and shark. Shark meat, however, is an exception. The homogenate of washed shark meat in water exhibited an increase in viscosity during the course of acidification to pH 4 to 5 (Venugopal, 2017). In the case of capelin, thermo-stable dispersion could be prepared without the need for acidification. The thermo-stable protein dispersions prepared from different fishery products can have varied applications, such as preparation of fish protein powder, as protein coating of fresh fish to extent their refrigerated shelf life, preparation of fermented sauce, or for the development of edible packaging (Venugopal, 1997). Meat recovered from by-catch fish can be used for protein dispersion or can be a resource for value added food products such as urimi, sausages, or fermented products, among others (Venugopal and Shahidi, 1998).

Flocculation

Proteins, which are present in suspended or dissolved states in process effluents, can be flocculated and precipitated by food grade polysaccharides such as carrageenan, alginate, and carboxy methylcellulose, which are then subjected to concentration by filtration, sedimentation, and/or centrifugation (Forghani et al., 2020). Proteins from herring industry processing effluents were recovered using electroflocculation (EF) and ultrafiltration (UF). EF and UF recovered up to 80% proteins. The highest protein and fatty acid contents of the effluent were 12.7 and 2.5 g per lg, respectively. Leucine and glutamic acid/glutamine were the dominating amino acids while calcium and magnesium were the dominating trace elements. The proteins had good foaming and emulsifying properties, which make them good functional additives (Gringer et al., 2015). Biomass from cooking wastewaters of snow crab was concentrated by membrane filtration. The concentrate had 59% proteins and contained desirable flavor compounds. The extract can be a natural aroma for the food industry (Tremblay et al., 2020).

Enzymatically Hydrolyzed Proteins

Proteolytic enzymes from various sources, including microorganisms (such as alcalase, flavourzyme, and protamex), animal (colla genase, proteinase, serine-protease, neutrase, and trypsin), and plants (papain, bromelain, and ficin), can extract proteins from seafood processing discards as fish protein hydrolyzates (FPHs). The ideal treatment conditions are incubation temperature, 35–37°C; enzyme to substrate ratio, 1–50; and incubation up to 24 h. The degree of hydrolysis determines the properties of the hydrolyzate, such as solubility, water-holding capacity, emulsification, and foam-forming ability, and the contents and chemical nature of peptides formed. The FPH can be concentrated by spray drying or ultrafiltration. They generally show a beneficial effect on growth performances and feed utilization at low inclusion levels (Chalamaiah et al., 2012; Vijaykrishnaraj and Prabhasankar, 2015). Fish frames without heads from Atlantic salmon and Atlantic cod were treated with commercial proteases for 2 h. Salmon treated with alcalase and cod treated with pepsin yielded 64 and 68% proteins, respectively (Liaset et al., 2003). Proteins, together with chitin and astaxanthin, were extracted from shrimp using enzymatic treatment with alcalase and pancreatin. Alcalase was more efficient than pancreatin, which increased recovery of proteins from 57.5 to 64.6% and of astaxanthin from 4.7 to 5.7 mg astaxanthin per 100 g of dry waste, at a degree of hydrolysis of 12%. An increase in the DH from 6 to 12% resulted in 26% to 28% protein recovery (Routray et al., 2019). Alcalase hydrolysis of the industrial waste from *Xiphopenaeus kroyeri* shrimp allowed 65% protein recovery in the form of hydrolysates (Holanda and Netto, 2006).

Bioactive peptides are specific protein fragments that can have high nutraceutical potentials and may be able to address important public health issues like obesity, stress, hypertension, and more. Such peptides have been produced from hydrolyzates of several fish and shellfish. Their potential functions include antimicrobial, antiviral, antitumor, antioxidative, antihypertensive, cardioprotective, anti-amnesiac, immunomodulatory, analgesic, antidiabetic, antiaging, appetite-suppressing, and neuroprotective activities (Chalamaiah et al., 2012; Vijaykrishnaraj and Prabhasankar, 2015). These activities are related to the sequence, composition, and type of amino acids in the peptides. FPHs can be used as a source of bioactive peptides with potentials for use as functional food ingredients industry. Tonon et al. (2016) prepared protein hydrolysate from the shrimp cooking effluents by enzymatic hydrolysis and ultrafiltration. The hydrolyzate prepared at 75°C and at pH of 9.0 have essential amino acids that can satisfy people's recommended daily needs. The preparation had significant antioxidant activities. Hypertensive and antioxidant peptides were prepared by enzymatic hydrolysis of proteins from cuttlefish wastewater. The proteins were initially concentrated by ultrafiltration (Amado et al., 2013). Pepsin soluble collagen (PSC) was enzymatically hydrolyzed, and the resultant hydrolysates were ultrafiltrated and characterized. Electrophoretic patterns showed the typical composition of type I collagen, with denaturation temperatures ranging between 23 and 33°C. In terms of antioxidant capacity, results revealed significant intraspecific differences between hydrolysates, retentate, and permeate fractions when using β-Carotene and DPPH methods (Blanco et al., 2017). The presence of both omega-3 fatty acids and ACE-inhibitory peptides in squid hydrolyzate suggested its nutraceutical potential (Apostolidis et al., 2016). In order to use and commercialize bioactive hydrolysates and peptides as

TABLE 3 | Enzymes from seafood processing discards for seafood processing.

Enzymes	Discard source	Seafood processing applications
Proteases (pepsin, trypsin, chymotrypsin, collagenases, acid and alkaline proteinases, cathepsins, peptidases, and others)	Seafood processing discards (finfish intestines, shrimp heads, etc.)	Tenderization of squid, Isolation of seafood flavor Recovery of proteins including collagen and chitin Pigments from shell discards, Production of fish protein hydrolyzates Enhancement of digestibility of aquafeed protein ingredients
Lipases	Discards of Atlantic cod, seal, salmon, sardine, Indian mackerel, red sea bream.	Production of omega-3-enriched triglycerides, Improvement of flavor
Chitinases	Shellfish, squid liver, octopus	Deacetylation of chitin to chitosan
Amylase	Abalone	Peeling and deveining of shrimp
β-1,3-Glucanase β- galactosidsase	Abalone, scallop, tilapia, sea cucumber	Removal of anti-nutritive effects of non-starch polysaccharides in aquafeed
Lysozyme	Arctic scallop shell, crab shell	Bacteriostatic agent.
Catalase, glutathione peroxidase	Marine mussel and other organisms	Antioxidants, Preserve fish quality.in combination with lysozyme
Transglutaminases	Various fishery sources	Texturization, encapsulation of nutraceuticals, development of protein sheets and surimi-analogs, edible films

Summarized from Venugopal (2016).

food ingredients, a number of significant challenges must first be overcome. These include high production costs, likely negative sensory attributes in end products, taste modifications of carrier food products, and potential toxicity or allergenicity, among others (Lafarga and Hayes, 2017).

The enormous pool of biodiversity in marine ecosystems offers a reservoir of enzymes with potential biotechnological applications. Enzymes from aquatic animals, particularly from marine habitats, exhibit significant variations in their properties in comparison with enzymes from terrestrial sources. The factors responsible for the variations include molecular weights, amino acid compositions, optimal pH and temperature requirements, inhibition characteristics, and kinetic properties, which facilitate their novel uses for a variety of practical applications. Seafood discards such as viscera, liver, and head are sources of enzymes including proteases including pepsin, gastricin, trypsin, collagenase, elastase, and peptidases, transglutaminases, lipases, phospholipase, chitinases, β-1, 3-glucanase, carrageenases, and others. Methodologies for their isolations from various seafood processing discards have been summarized (Shah et al., 2016; Murthy et al., 2018). Enzymes from seafood discards can be used for various seafood processing operations, as shown in **Table 3**.

LIPIDS AND CO-PRODUCTS

The global production of fish oil is around one million tons, predominated by cod liver oil (Bimbo, 2007). Fish oils are rich in omega-3 PUFA and vitamins A and D. The oil of fish species, such as Atlantic mackerel, shark, anchovies, menhaden, and Atlantic sardine, can have up to 35% omega-3 fatty acids, with EPA and DHA at around 10% of the oil (Venugopal, 2009). Fish processing discards, particularly the livers of albacore, cod, salmon, shark, haddock, and tuna, are good sources of oil. An average production of 10,000 kg filets of cod will generate by-products with more than 1,000 kg marine lipids (Falch et al., 2006). Conventionally, fish oil is extracted by wet reduction method involving cooking, pressing, and filtration. The extracted oil is purified by carbon treatment, degumming, alkali refining, deodorization, and stabilization by antioxidants for prolonged storage, while the protein-rich press liquor is used as animal feed (Venugopal, 2009). There have been concerns fueled by ominous predictions of depletion of several oil-rich oceanic fishes, which necessitates better exploitation of fish discards as oil resources.

Whereas, conventional extraction methods can lead to oxidation of fish oils, rich in unsaturated fatty acids, as pointed out earlier, green processes can help with the recovery of fish oil with minimum oxidation (Ivanovs and Blumberga, 2017). Fish viscera are an important source of lipids, with a content ranging from 19 to 21%. Up to 85% of this could be recovered by natural fermentation. Fermentation using added lactic cultures did not show any advantage over natural fermentation with respect to recovery of oil. Activity of acidic, neutral, and alkaline proteases decreased during fermentation. Even though the degree of protein hydrolysis increased up to 62% during fermentation using Pediococcus acidilactici K7, no differences were observed in the amounts of recovered proteins (Rai et al., 2010). Catfish viscera, a by-product of catfish processing, are industrially used to produce edible oils (Shahidi et al., 2019). The liver of shark is 22–30% of its body weight and its liver may contain oil as high as 90% of its weight. Natural decomposition, ensilage in presence of formic acid, alkali digestion, and steam rendering recovered oil from shark liver (Venugopal, 2009). Salmon frames were hydrolyzed by a mixture of commercial proteases, which helped recovery of 77% of total lipids present in the salmon frames as EPA and DHA rich oil (Liaset et al., 2003).

The enzymatic process disrupts the tissue and membranes under mild conditions to release the oil from fish by-products, such as liver and roe (Dumay et al., 2004). Treatment of salmon heads and other byproducts by commercial proteases (alcalase, neutrase, and flavourzyme) for 2 h released 17% oil, which contained 11.6 and 5.6% of DHA and EPA, respectively (Routray et al., 2019). Oil from sardine was obtained at 5.5, the iso-electric

pH the fish meat, which was adjusted by citric acid. The separated oil had good n-3 PUFA contents and exhibited high oxidative stability (Okada and Morrissey, 2007). Alcalase-based extraction of oil from tuna was conducted for 120 min at 60°C and pH 6.5 at an enzyme–substrate ratio of 1:200. The enzyme- extracted oil had lowest acidity and peroxide values and higher levels of EPA and DHA contents than chemically refined oil (de Oliveira et al., 2016). Hydrolysis of shrimp waste with alcalase gave an oil yield of 28.6 μg per g waste (Sachindra and Mahendrakar, 2011). The hydrolyzate of squid processing by-products had EPA and DHA at 16.9 and 29.2% of oil, respectively. About half of the oil was comprised of phospholipids (Apostolidis et al., 2016).

Lipases have grown in importance due to their ease of availability and possibilities for product modifications. *Candida rugosa* lipase was used to concentrate fatty acids in the glyceride fraction of the oil. By controlling the degree of hydrolysis, two products were obtained, one having 50% n-3 PUFA, and the other having 40% DHA and 7% EPA. The glyceride from these reactions was converted back to triglycerides using *Rhizomucor miehei* lipase catalyzed partial hydrolysis and esterification (Moore and McNeill, 1996). Lipolysis of salmon oil by a commercial lipase gave a mixture of free fatty acids and acylglycerols. A hydrophobic membrane was used to separate high melting saturated fatty acids from low melting acylglycerols. The sum of total PUFA increased from 42% in the crude oil to 47% in the filtrate with increase of DHA and EPA contents from 9.9 to 11.6%, and from 3.6 to 5.6%, respectively (Linder et al., 2005).

Carotenoids

Carotenoids provide red and orange colors to some foods. The pigments are present in shellfish, krill, shrimp, crab, crayfish, and also in salmon and trout. The red-orange color of cooked crustaceans is attributed to partial or complete separation of astaxanthin from the protein moiety to which it is attached in the native state. Carotenoids may be hydrocarbons, such as β-carotene or xanthophylls, or oxygenated derivatives, such as astaxanthin, astacene, canthaxanthin, cryptoxanthin, lutein, neoxanthin, violaxanthin, and zeaxanthin. Astaxanthin (3,3′-dihydroxy-β, β′-carotene-4,4′-dione) and canthaxanthin (β,β-carotene-4,4′-dione) have been used in aquafeed for many years in order to impart the desired flesh color in farmed salmonids. The shells of shrimp, prawn, crawfish, krill, crab, and lobster are important sources of astaxanthin, bound to free protein or chitin, and range from 40 to 200 μg per g, dry weight. Canthaxanthin is present in crayfish, mytiloxanthin in mussel, and mactraxanthin and fucoxanthin are present in clams (Sowmya and Sachindra, 2015; de Carvalho and Caramujo, 2017).

Microbial fermentation-based bioconversion methods can extract carotenoids from crustacean shells, giving better yields than conventional solvent extraction. During bacterial fermentation processes, the proteins and minerals present in the shrimp shells are effectively removed, thereby increasing the extraction efficiency of the pigments without any change in quality. Current extraction methods make use of proteolytic enzymes such as trypsin and alcalase and fermentation by LAB and other microorganisms (Prameela et al., 2017; Routray et al., 2019). Fermentation of shrimp shell waste using the lactic acid bacterium *Pediococcus acidolactici* under optimal conditions resulted in 98% deproteination, 72% demineralization, and carotenoid recovery of up to 78% (Bhaskar et al., 2007). Shrimp waste was hydrolyzed with alcalase at optimal conditions of 0.75% of enzyme for 150 min at 37°C. The recovered carotenoids were extracted in sunflower oil at an oil to hydrolyzed waste ratio of 2:1 at 70°C for 90 min (Sachindra and Mahendrakar, 2011). Caroteno-protein from pink shrimp (Parapenaeus longirostris) waste was extracted by trypsin treatment for 1 h at 25°C. The recovered caroteno-protein fraction after freeze-drying contained about 71% protein, 16% lipid, 8% ash, 2% chitin, and 87 μg astaxanthin per g of the sample. Enzymatic hydrolysis of the protein–pigment complex allows studies on pigment absorption, stability, and application (Sila et al., 2012). Trypsin from bluefish was used to extract caroteno-proteins from black tiger shrimp shells. The extract also contained 70% protein, 20% lipid, 6.6% ash, 1.5% chitin, and 87.9 μg with total astaxanthin per g sample (Klomklao et al., 2009). Autolysis of shrimp heads resulted in the recovery of 195 μg carotenoids per g wet shells (Cahu et al., 2012).

Processing effluents can also be used as a medium for the production of carotenoids. Employing non-sterilized mussel processing wastewater as a low-cost substrate for yeast fermentation, the green microalga (*H. pluvialis*) was cultivated in fish effluents for the production of astaxanthin with significantly greater antioxidant capacity than the synthetic one (Shah et al., 2016). Similarly, mussel processing water was used for the production of astaxanthin by *Xanthophyllomyces dendrorhous* (Amado and Vazquez, 2015). With shrimp waste, being highly perishable and seasonal, the fermented carotenoid-rich liquor can be prepared as per the availability of the waste and can be stored up to 75 days under normal storage conditions. Carotenoids from the liquor can be extracted by ultrasonic or supercritical CO_2 extraction, or can be extracted in palm oil or other vegetable oils or by high pressure chemical extraction (Sowmya and Sachindra, 2015). Astaxanthin is stable at 70–90°C in rice bran, ginger, and palm oils. Astaxanthin has important applications in the nutraceuticals, cosmetics, food, and aquaculture industries (Ambati et al., 2014; Shah et al., 2016).

POLYSACCHARIDE-BASED COMPOUNDS

The dry shell discards of crab, shrimp, and lobster may contain up to 70% chitin; dry squid skeleton pen and krill shells have a lower chitin content of 40%. The commercial process of extraction of chitin from crab and shrimp shells involves three steps: demineralization of dried and pulverized shells by dilute hydrochloric acid; deproteinization by dilute alkali; and decoloration, washing, and drying. Chitin is deacetylated to chitosan using 30 to 60% (w/v) sodium or potassium hydroxide at 80–140°C. The yield of chitin is about 25% of dry shell, and the yield of shrimp chitosan about 77% of the crude chitin (Dima et al., 2017). The chemical methods and the high treatment temperatures have influence on molecular weight, degree of deacetylation, and the functional properties of chitosan (Venugopal, 2009). Biological processes give products of better

quality, require less energy, and consume less fresh water, unlike chemical processes (Arbia et al., 2013; Kaur and Dhillon, 2015; Mao et al., 2017; Lopes et al., 2018).

Microbial Extractions of Chitin

Fermentation is beneficial for extraction of chitin from seafood processing discards (Yadav et al., 2019). Fermentation by lactic acid bacteria (LAB) has advantages over conventional methods for chitin extraction. One beneficial LAB is Lactobacillus plantaru. Other LABs include *L. paracasei, L. acidophilus, L. lactis, L. paracasei, S. marcescens*, and *T. turnirae*. Non-LAB organisms can also be used for fermentation (Vázquez et al., 2013, 2019). An epiphytic *L. acidophilus* is isolated from rapidly fermented shrimp waste. The chitin released in the fermented product can be easily transformed by a bleaching treatment. The product had better quality than chemically extracted chitin (Duan et al., 2012). Fermentation of shrimp head by a consortium of LAB for 48 h gave chitin and protein rich liquor; the latter can be used as aquafeed supplement (Ximenes et al., 2019). Jung et al. (2007) employed *L. acidophilus* for chitin extraction from crab shell waste by two-step fermentation, involving *L. paracasei* in the first step, followed by a protease producing bacterium *Serratia marcescens*. The process removed 94% $CaCO_3$ and 70% proteins. The highest deproteinization of 96% and demineralization (68%) were achieved through the combination of two-stage solid state culture by *Lactobacillus brevis* and *Rhizopus oligosporus*. Lactic acid was the main organic acid produced along with acetic, succinic, and oxalic acids. The purified chitin presented a molecular weight of $1{,}313 \times 10^3$ Da, preserving a high crystalline index and acetylation of 94% (Aranday-García et al., 2017). Sieber et al. (2018) suggested the use of natural microbial isolates as well as *Serratia* spp. and *Lactobacillus* spp. in fermentations that can realize a demineralization of 97%. Younes et al. (2016) used fermentation to extract highly acetylated chitin from crustacean shells, which were initially subjected to demineralization and enzymatic deproteinization prior to the treatment.

Fermentation by LAB for a maximum period of 7 days resulted in extensive deproteination and demineralization of crustacean shells, facilitating chitin recovery. The process can be conducted under conditions such as anaerobic, solid-stat, semi-continuous, or co-fermentation (Vázquez et al., 2020). Ghorbel-Bellaaj et al. (2011) optimized fermentation variables in accordance with Plackett–Burman design, which resulted in 96% demineralization and removal of 89% protein. Fermentation of shrimp head by *Bacillus licheniformis* released appreciable amounts of polysaccharides and other compounds in the fermented medium (Mao et al., 2017). *Bacillus cereus* and *Exiguobacterium acetylicum* accomplished 90% demineralization and deproteination during chitin extraction from shrimp waste (Sorokulova et al., 2009). Autolysis of shrimp heads could recover not only chitin and chitosan, but also protein hydrolyzate, carotenoids, sulfated-, and amino-polysaccharides. An amount of 25 mg of chitin and 17 mg chitosan (60–80% deacetylated) per g of wet shells were recovered (Cahu et al., 2012). A pilot plant study by Vázquez et al. (2017) employed a combination of enzymatic, acid, and alkaline processes for the recovery of chitin and also protein and carotenoprotein from the cephalothorax of Penaeus vannamei. The sequential treatment yielded 30% chitin with 92% acetylation. In another combination process, protease was used to remove Ca^{2+} and protein, followed by fermentation by *Bacillus coagulans* to extract chitin from crayfish shell waste, which resulted in recovery of chitin as high as 94% (Dun et al., 2019). Most of these studies reported deproteination and demineralization in the range of 45–90%.

Enzymatic Extractions of Chitin

Extraction of chitin from shellfish waste is facilitated by initial deproteinization and demineralization. One of the biological alternatives proposed is the use of proteases for deproteinization of crustacean shells, avoiding alkaline treatments. When using enzymatic deproteinization, previous demineralization is more convenient since it increases enzyme permeability of the tissues and reduces the presence of potential enzyme inhibitors. Crude proteases from fish discards such as viscera can be used for deproteination, which can lower the cost of treatment (Shah et al., 2016). Instead of the conventional hydrochloric acid, the use of organic acids (lactic and acetic) produced by cheese whey fermentation has been suggested for demineralization of shrimp shells. Organic acids were comparable to hydrochloric acid but less harmful when helping to maintain the integrity of chitin (Mahmoud et al., 2007). Proteolytic enzymes remove up to 90% of the protein and carotenoids from demineralized shrimp waste. Hamdi et al. (2017) extracted chitin from blue crab and shrimp shells by crude crab viscera alkaline protease digestion at pH 8.0 and 60°C. The treatment caused up to 91% deproteinization, facilitating the release of chitin.

In recent times there has been increased interest in using broad-specificity chitinases for chitin extraction. These novel enzymes possess two or three different catalytic activities, functioning as exochitinase, endochitinase, and N-acetyl-glucosaminidase. Endochitinases cleave chitin at internal sites, thereby generating low-molecular weight chitin oligosaccharides (COG), while exochitinases or chitobiases catalyze the progressive release of chitin dimmers by cleaving the polysaccharide at external sites (Suresh, 2012). Recently, a cold-adapted chitinase from a marine bacterium was characterized by broad pH stability, high thermo-stability, low K_m value, and optimal activity at 30 °C, with 35% activity at 0°C.The enzyme completely degraded colloidal chitin into N-acetyl glucosamine (GlcNAc). The enzyme was suggested to be a superior candidate for producing bioactive oligosaccharides (Fu et al., 2020). A mass production of chitonolytic enzymes by cultures of micro-organisms, such as *Trichoderma hamatum, T.viride*, *Aspergillus niger*, and *Carica papaya,* will be beneficial for large scale extraction of chitin and its transformation into valuable commercial products as a solution to waste management (Yadav et al., 2019). Chitin-degrading enzymes from *Serratia marcescens*, *Amantichitinus ursilacus*, and *Andreprevotia ripae* have been used on a pilot scale to degrade chitin into monomers with yields up to 95% (Sieber et al., 2018).

Chitin deacetylase from fungi such as such as *Mucor rouxii, M. mechei*, and *Aspergillus niger* catalyzes the hydrolysis of N-acetylamido linkage of chitin to give chitosan. The crystallized chitin, after pretreatment with 18% formic acid, is amenable to

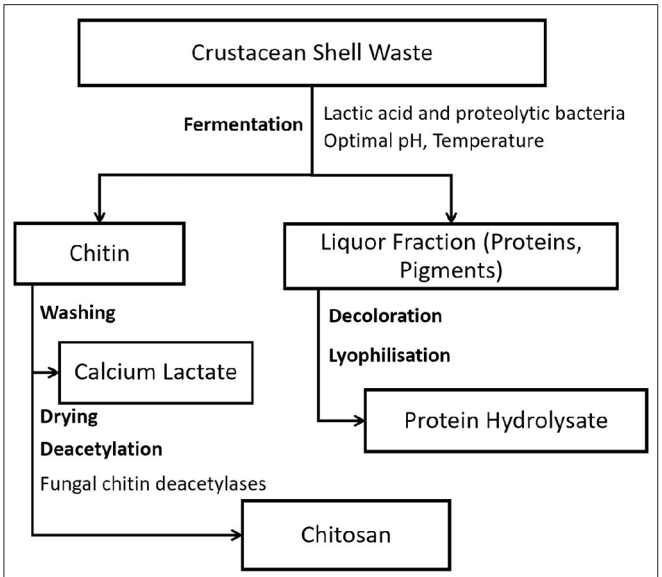

FIGURE 3 | Bioconversion processes for extraction of chitin, chitosan, and protein hydroluzate (adapted from Vázquez et al., 2013).

90% deacetylation by the fungal deacetylase (Suresh, 2012). To enhance the accessibility of chitin deacetylase to acetyl groups of natural crystalline chitin, pretreatment may be needed with physical or chemical methods such as sonication, grinding, heating, and derivatization (Yadav et al., 2019). The various applications of chitin and chitosan are not topics of discussion here.

Crude enzyme from *Bacillus cereus* was used to hydrolyze chitosan having 66% deacetylation in a membrane reactor, operated at 45 °C and pH 5. The major oligomers were chitobiose, chitotriose, chitotetraose, chitopentaose, and chitohexaose. The system could be operated for 15 h and still maintained a stable product composition (Kuo et al., 2004). Chito-oligomers (COS), the depolymerized products of chitosan, have attracted considerable interest due to their biocompatible, biodegradable, non-toxic, and non-allergenic natures, and potential applications in biomedical, food, pharmaceutical, agricultural, and environmental industries (Ngo et al., 2020). These suggest a need for commercial production of chitinases and chitosanases (Suresh, 2012; Zhou et al., 2019). Chitosan and its derivatives have been reported to possess various biomedical activities including free radical scavenging, antihypertensive, anticoagulant, antidiabetic, antiobesity, antiallergic, anti-inflammatory, antimicrobial, anticancer, and anti-Alzheimer effects. The antibacterial and antifungal properties of chitosan qualify it for use in food packaging films. Its mechanical, gas, and water vapor permeability properties can be enhanced by blending chitosan with other natural polymers such as starch, essential oils, and clay (Venugopal, 2011). **Figure 3** depicts the biological process for extraction of chitin and its conversion to chitosan.

Glycosaminoglycans (GAGs) are hetero-polysaccharides defined by a repeating disaccharide unit without branched chains, in which one of the two monosaccharides is an amino sugar (N-acetyl-galactosamine or N-acetyl-glucosamine) and the other one is a uronic acid. Based on the disaccharide composition, linkage type, and presence of sulfate groups, GAGs may be chondroitin sulfate (CS), hyaluronic acid (HA), dermatan sulfate, heparin, or keratan sulfate. CS chains have an important function in central nervous system development, wound repair, infection, growth factor signaling, morphogenesis and cell division, differentiation, and migration in addition to osteoarthritis and their conventional structural roles. CS from terrestrial and marine sources contains diverse chain lengths and sulfation. Shark cartilage may contain up to 29% CS, having a molecular weight of 40 kDa. Hyaluronic acid is a linear, high molecular weight linear, and non-sulfated GAG made by alternating disaccharide units of *N*-acetyl-D-glucosamine and D-glucuronic acids, linked by β-(1 → 3) and β-(1 → 4) glycosidic bonds.). Autolysis of shrimp head waste gave about 8 mg sulfated GAGs per g that exhibited electrophoretic migration similar to mammalian standards. The degradation products of the GAGs suggested the presence of C6-sulfated heparan sulfate (Cahu et al., 2012). Vázquez et al. (2013) reviewed environmentally friendly processes combining microbial, enzymatic, and other strategies to produce CS, HA, chitin, and chitosan. Bacterial production of HA using Streptococci has been industrially developed (Vázquez et al., 2013). Chondroitin sulfate and hyaluronic acid are commercially valuable. The structural similarity of microbial capsular polysaccharides to these biomolecules makes bacteria ideal candidates as non-animal sources of glycosaminoglycan-derived product GAGs because of their high bioactivities and physiological functions. Fish cartilage products, such as shark cartilage and chondroitin sulfate, glucosamine, and other glucosaminoglycans, are able to alleviate rheumatoid arthritis (Venugopal, 2009).

Mineral-Based Components

Finfish discards, which contain significant amounts of bone, are rich sources of minerals. The bone is composed of up to 70% minerals, followed by collagen, certain carbohydrates, and lipids. Hydroxyapatite and calcium phosphate have attracted attention for biomedical applications such as implant materials. Grass fish bones were subjected to flavourzyme treatment followed by fermentation with *Leuconostoc mesenteroides*, giving a preparation with a high content of soluble calcium lactate, calcium acetate, and also small peptides containing calcium. The calcium is bioavailable and therefore can promote growth, as shown by animal studies, suggesting its use as a calcium supplement (Tang et al., 2018). Salmon frames were hydrolyzed by a mixture of commercial proteases. After the procedure, the frames were separated by centrifugation into a bone fraction, which contained 62% of total ash present in the salmon frames. The fraction was high in calcium, phosphorus, and magnesium and also in various trace elements such as copper, iron, selenium, and zinc (Liaset et al., 2003). A fish bone phosphopeptide (FBP) containing up to 24% of phosphorus has a molecular weight of 3.5 kDa and a high calcium-binding activity. The FBP has potential nutraceutical value as a calcium binding agent (Jung et al., 2005). A combination of micro and nano-structured

TABLE 4 | Bioconversions of seafood processing discards by microorganisms.

Seafood discards	Microorganism	Products	References
Shell waste	*Serratia marcescens*	Chitinases, proteases, prodigiosin pigment	Wang et al., 2020
Shrimp head	*Bacillus licheniformis*	Antioxidant	Mao et al., 2017
Shrimp waste	Lactobacillus acidophilus	Chitin, protein hydrolyzate	Duan et al., 2012
Shell waste	Symbiotic lactic acid bacteria	Calcium, carotenoids peptides	Shan et al., 2011
Shrimp head	Lactic acid fermentation	Chitin and aquafeed	Ximenes et al., 2019
Shrimp waste	Pediococcus acidolactici	Carotenoids	Bhaskar et al., 2007
Crab shell	L. paracasei, and S. marcescens	Chitin	Jung et al., 2007
Fish waste	Mixed microorganisms	Liquid fertilizer	Dao and Kim, 2011
Shrimp shell waste	Pseudomonas aeruginosa	Chitin	Ghorbel-Bellaaj et al., 2011
Shell waste	Fermentation	Astaxanthin	Routray et al., 2019
Shrimp waste	Bacterial fermentation	Protein and chitin	Sorokulova et al., 2009
Shell waste	*P. monocertillium*	α-Chitin	Suresh, 2012
Crayfish waste	Fermentation with protease	Chitin	Dun et al., 2019
Seafood effluents	Microalgae	SCP	Shahid et al., 2020
Mussel processing effluents	S. zooepidemicus fermentation	Hyaluronic acid	Vázquez et al., 2013
Aquaculture solid waste	Aerobic microbial bioconversion.	Liquid fertilizer	Khiari et al., 2019
Squid pens waste	*Paenibacillus sp.*	Chitosanases, Chitosan oligosaccharides	Doan et al., 2020
Tuna waste	*L. plantarum; B. licheniformis*	Aquafeed	Hena et al., 2009
Tuna waste	*Staphylococcus epidermidis*	Lipase	Esakkiraj et al., 2010
Cod by-products	Proteolysis	Oil	Dumay et al., 2004
Fresh water viscera	*Pediococcus acidilactici*	Oil	Rai et al., 2010
Grass fish bone	Proteolysis and L. mesenteroides fermentation	Calcium supplement	Tang et al., 2018
Tuna condensate	Candida rugosa and Lactobacillus futsaii	Glutamic acid, GABA	Sanchart et al., 2018
Fish discards	Various lactic acid bacteria	Peptones	Vázquez et al., 2020
Shrimp waste	Successive Lactobacillus brevis and Rhizopus oligosporus	Chitin	Aranday-García et al., 2017
Shrimp shell powder	*B. cereus*	Reducing sugar, DNA protective compounds antioxidants	Rashid et al., 2018

hydroxyapatite (HAp) was isolated from tuna bone. The isolated Hap had comparable physicochemical characteristic with that of standard Hap and was also less toxic (Pallela et al., 2011). Pepsin hydrolyzate of channel catfish bones has antibacterial activity, suggesting that the fish bones are promising resources for generating antibacterial components (Ren et al., 2012).

The various bioconversion processes employing microorganisms and enzymes for the recovery of components from seafood processing discards and effluents are summarized in **Tables 4**, **5**. **Table 4** summarizes bioconversion processes using LAB and other microorganisms, while **Table 5** summarizes enzymatic bioconversion processes.

THE BIOREFINERY CONCEPT

Bio-Refinery Approach for Valorization of Seafood Discards

The above discussions pointed out various biological processes for the extractions of important compounds from seafood discards. While individual processes may not be economically feasible, an integrated refinery-type process for the extractions of multiple products is more practical. A "refinery" generally means conversion of raw materials into products of higher values, petroleum oil refinery being the most popular example. The International Energy Agency defined bio-refinery as the "sustainable processing of biomass into a spectrum of bio-based products (food, feed, chemicals, and materials) and bio-energy (de Farias and Barbera, 2018). The bio-refinery concept visualizes bio-waste as a potential renewable feedstock that can be valorized through a cascade of various biotechnological processes to produce marketable products and bioenergy on par with petro-chemical refineries. It involves stepwise refining processes using biological methods for the extraction of various high value biomolecules. Such downstream strategies could reduce overall production costs (Das, 2015; Mohan et al., 2016; Mitra and Mishra, 2019; Dineshkukar and Sen, 2020). Bio-refineries, which aim at valorizing biomass from agriculture and aquaculture, into a wide spectrum of products and bio-energy, have been recognized as part of a sustainable economy (Dragone et al., 2020).

Fish processing waste can be a promising renewable biomass for bio-refineries. The bio-refinery approach envisages conversion of fish waste into value-added products such as

TABLE 5 | Bioconversions of seafood processing discards by enzymes.

Seafood discards	Enzyme	Components	References
Fin, head, scales	Collagenase, trypsin	Collagen	Pal and Suresh, 2016; Shah et al., 2016
Fish waste	Proteases	FPH	Vijaykrishnaraj and Prabhasankar, 2015
Salmon frame	Proteases	Oil, peptides	Liaset et al., 2003
Salmon heads	Proteolysis, lipase	PUFA-rich oil	Linder et al., 2005
Fish oil	*C. rugosa* lipase	PUFA-rich oil	Moore and McNeill, 1996
Tuna head	Alcalase	Deodourized oil	de Oliveira et al., 2016
Chitin waste	Chitinase	Oligosaccharides	Fu et al., 2020
Lobster waste, shrimp waste	Papain, alcalase, pancreatin	Astaxanthin	Routray et al., 2019
Shrimp shell	Enzymatic, acid, alkaline	Protein, chitin, carotenoprotein	Vázquez et al., 2017
Marine waste	Enzyme and microorganism	astaxanthin	Vázquez et al., 2013
Crude chitin	Chitinase	N-acetyl-D-glucosamine	Zhang et al., 2016
Crude chitin	Chitinase	Chitin oligomers	Suresh, 2012
Crab shell	Crab viscera protease	Chitin	Hamdi et al., 2017
Shrimp heads	Brevibacilluys alkaline protease	Chitin	Doan et al., 2019
Shrimp, crab shell, squid pen	*P. aeruginosa* protease	Protein and chitin	Wang et al., 2019
Shrimp waste	*Bacilluscereus* protease	Chitin, chitosan, FPH	Manni et al., 2010
Black tiger shrimp shells	Bluefish trypsin	Caroteno-protein, lipid	Klomklao et al., 2009
Fish bone	intestine crude proteinase	Bone oligo-phospho-peptide	Jung et al., 2005
Shrimp shell	Bacterial protease	Astaxanthin	Sachindra and Mahendrakar, 2011
Shrimp shell	Alcalase	Protein, chitin, astaxanthin	Holanda and Netto, 2006

biofuels, industrial chemicals, animal feed, organic fertilizer, nutraceuticals, and others. Low cost and simplicity of operation by reducing the cost of material, energy consumption, and labor, but maintaining high productivity are some of the important attributes of the process (Sahu et al., 2016). An example is shell refinery, where crustacean shell waste is subjected to sequential treatment to recover chitin, proteins, lipids, carotenoids, calcium carbonate, and chitin monomers (Hülsey, 2018). Vázquez et al. (2019) coupled alcalase hydrolysis with bacterial fermentation to extract gelatin, oils, fish protein hydrolysate including bioactive peptides, and fish peptones from heads, skin, and bones of fish discards. Cahu et al. (2012) reported an integrated process employing autolysis of shrimp heads to recover chitin and chitosan, protein hydrolyzate, and sulfated- and amino-polysaccharides. Lactic acid fermentation followed by green extraction processes including filtration and centrifugation can lead to sequential or simultaneous extractions of astaxanthin, hydrolyzed protein, and chitin from crustacean shell waste (Vázquez et al., 2017; Routray et al., 2019). Similarly, anaerobic fermentation of fish waste resulted in methane and liquid fertilizer as primary products. The purchase price of methane is a crucial factor influencing the economics of the bio-refinery (Ratky and Zamazal, 2020). Another bio-refinery deals with extraction of oil from fish waste, its transesterification with ethanol, and concentration of n-3 PUFA. Fishmeal, glycerol, and saturated and short chain unsaturated fatty acids as liquid bio-fuel are the other products of the refinery. The process can significantly supply thermal energy and reduce CO_2 discharge (Fiori et al., 2017). A bio-refinery developed within an EU-funded project combines chitin demineralization by *Serratia* spp. and *Lactobacillus* spp. and an enzymatic degradation of chitin by chitin-degrading enzymes from *Serratia marcescens*, *Amantichitinus ursilacus*, and *Andreprevotia ripae*. The resulting N-acetylglucosamine monomers could be used for novel bio-based polymers. Proteins and lipids could be used as feed for biogas production (Sieber et al., 2018). Eurofish processes roughly 200 tons of tuna a day with discharge of at least 1,300 m^3 effluents. The company coupled seafood waste-to-energy technology, generating 1,300 m^3 methane daily. This reduced wastewater treatment costs by 50% and energy consumption by 35–40%. The plant has been in operation since March 2016, suggesting economic feasibility of bio-refineries based on seafood waste valorization (Fluence, 2019).

Algal biotechnology can be a promising platform for bio-refining of seafood discards. The cultivation of microalgae in bio-wastes is known to produce SCP, as mentioned earlier. The recovery of products from algal biomass is a matter of constant development and progress (Sosa-Hernández et al., 2018). Whereas, the exploitation of SCP for a single product such as biofuel is not economically viable, multiple products such as pigments, antioxidants, and n-3 fatty acids can be extracted from SCP to make the process cost-competitive. The various possibilities are depicted in **Figure 4**. The approach offers novel ways to utilize wastewater and also help in the promotion of microalgae in the commercial market (Koyande et al., 2019; Mitra and Mishra, 2019). The increasing resource limitations are expected to drive SCP production and improve the economic feasibility in the future (Puyol et al., 2017). On the basis of increasing demands, recently seaweed has been cultivated with improved traits to harvest more than one

product through a bio-refinery. Three combination routes have been suggested for production of microalgae-based biodiesel, bio-hydrogen, and SCP (Banu et al., 2020). Cultivation of the microalga *H. pluvialis* for both SCP and astaxanthin can be an economically sustainable process (Shah et al., 2016; Khoo et al., 2019). food producers must now address environmental concerns, social responsibility and economic viability when designing their food processing techniques food producers must now address environmental concerns, social responsibility and economic viability when designing their food processing techniques. Systematic improvement of the technology readiness level (TRL) could be successful if applied to microalgae cultivation and processing (Caporgno and Mathys, 2018). Recent text mining tools on articles and patents published on algal biotechnology during the period 2012–2017 identified *Reinhardtius* sp. for wastewater treatment and a *Chlorellum* strain for biofuel and fatty acids (Parkavi et al., 2020). At present, downstream processing, and in particular the fractionation of microalgal components, remains the most expensive step of the algal processes, demanding novel technologies for SCP processing (Gifuni et al., 2019). Cho and Park (2018) observed that commercialization of the microbial route for fuel production remains uncertain due to the high cost of feedstock or low lipid yield. However, considering the low cost of seafood discards and effluents and potentials for enhancing fuel production through algal technology, as mentioned earlier, this observation may not be realistic. **Table 6** summarizes recent bio-refinery approaches for seafood valorization.

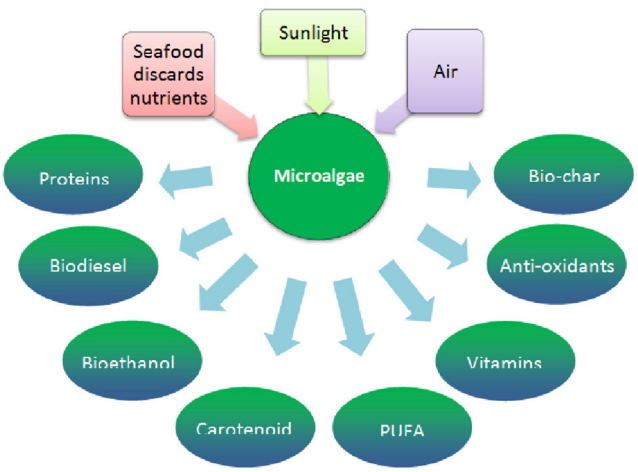

FIGURE 4 | Microalgae based bio-refinery for various products from seafood discards (adapted from Koyande et al., 2019).

CONCLUSIONS

The article pointed out the major problems associated with discards and process effluents, generated during industrial seafood processing. Besides being responsible for environmental pollution, the discards and effluents represent heavy losses of nutrients and other valuable compounds. There problems can be addressed by biological treatment processes, involving bioconversions of components of the waste by microorganisms and enzymes. Unlike conventional chemical treatments, biological processes are environmentally friendly, safe and economical. Further, biological processes do not adversely affect functional properties of isolated compounds, unlike the chemical processes. Biomass fermentations-using microalgae are emerging as green and economical processes to recover functionally active compounds and also biofuel. Fermentation is ideal to recover functionally active chitin from crustacean shell. Similarly, fermentation or lipase-based processes can replace hazardous solvent extraction techniques for the recovery of fish oil Microorganisms-mediated processes are highly desirable due to ease of handling, lower energy requirements and costs. With the development of tailor made bio-catalysts and advances in

TABLE 6 | Bio-refinery approaches for valorization of seafood discards.

Bio-refinery	Products	References
Cultivation of alga, *Haematococcus pluvialis*	Astaxanthin, SCP	Shah et al., 2016; Khoo et al., 2019
Lactic fermentation	Astaxanthin, hydrolyzed protein and chitin	Routray et al., 2019
Sequential treatment of crustacean shells	Chitin, proteins, lipids, carotenoids and $CaCO_3$.	Hülsey, 2018
Demineralization and enzymatic degradation of N-acetylglucosamine	Chitin monomers	Sieber et al., 2018
Sequential enzymatic, acid–alkaline extraction of shrimp cephalothorax	Chitin, chitosan, protein and astaxanthin	Vázquez et al., 2017
Integrated autolysis of shrimp head	Chitin, protein hydrolyzate, sulfated glycosaminoglycans	Cahu et al., 2012
Sequential extraction by ISP followed by enzyme	Collagen, myofibrillar proteins	Abdollahi et al., 2018
Sequential treatment of marine cartilage	Chondroitin sulfate, fish meal	Vázquez et al., 2013
Oil extraction, ethanol transesterification, n-3 PUFA concentration	Fish proteins, Glycerol, Lquid biofuel	Fiori et al., 2017
Hydrolysis of fish waste by protelytic enzymes	Food, Feed, Fertilizer ingredients	Sahu et al., 2016
Anaerobic fermentation with cow dung	Methane, Liquid mineral fertilizer	Ratky and Zamazal, 2020
Algal bio-refinery	Various products	Mitra and Mishra, 2019
Coupled alcalase hydrolysis and bacterial fermentation	Gelatin, oils, FPH, bioactive peptides, and fish peptones	Vázquez et al., 2019

green extraction techniques, it is possible to take up challenges of successful bio-processing of seafood discards and effluents. Genetic engineering of microorganisms, enzyme engineering, reactor designs and process optimizations offer strategies leading to a new manufacturing paradigm for successful valorization of seafood waste. The interesting features of microalgae such as their rapid growth, their photosynthetic ability, nutrient-rich characteristics of the cells make microalgae promising bio-platform for seafood waste re-cycling and energy transformation. Although process identification, scaling up and implementation of resource recovery solutions at an industrial level is a challenge, developments in these fields can favor successful waste utilization for sustainable fish processing towards a circular bioeconomy.

AUTHOR CONTRIBUTIONS

The author is the sole contributor of this work and has approved it for publication.

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Use of Polyphenols as Modulators of Food Allergies. From Chemistry to Biological Implications

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The multifactorial process of aging predisposes humans to infections and inflammatory disorders, thus affecting their quality of life and longevity. Given this reality, the need to increase the consumption of bioactive compounds, like dietary polyphenols emerges in our daily basis mostly due to their health related effects in slowing-down the incidence of chronic and degenerative diseases and even food allergy, which has been growing rapidly in prevalence currently affecting 5% of adults and 8% of children. Polyphenols embrace a large family of secondary metabolites from plant-derived foods and food wastes and are considerable of interest since they have attracted special attention over the years because of their reported anti-inflammatory and antimicrobial properties along with their high antioxidant capacity. These compounds are claimed as nutraceuticals with protective effect in offsetting oxidant species over-genesis in normal cells, and with the potential ability to stop or reverse oxidative stress-related diseases. Plant-derived foods represent a substantive portion of human diet containing a significant amount of structurally diverse polyphenols. There is a need to understand the polyphenolic composition of plant-derived foods mainly because of its chemistry, which discloses the bioactivity of a plant extract. However, the lack of standardized methods for analysis and other difficulties associated to the nature and distribution of plant polyphenols leads to a high variability of available data. Furthermore, there is still a gap in the understanding of polyphenols bioavailability and pharmacokinetics, which clearly difficult the settlement of the intake needed to observe health outcomes. Many efforts have been made to provide highly sensitive and selective analytical methods for the extraction (liquid-liquid; solid-liquid; supercritical-fluid), separation (spectrophotometric methods) and structural identification (chromatographic techniques, NMR spectroscopy, MS spectrometry) of phenolic and polyphenolic compounds present in these extracts. Liquid chromatography coupled to mass spectrometry (LC-MS) has been a fundamental technique in this area of research, not only for the determination of this family of compounds in food matrices, but also for the characterization and identification of new polyphenols classified with nutraceutical interest. This review summarizes the nature, distribution and main sources of polyphenols, analytical methods from extraction to characterization to further evaluate the health effects toward immune reactions to food.

Keywords: plant polyphenols, polyphenols characterization, polyphenols intake, protein/polyphenols interaction, food allergy, polyphenols from wastes

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PAST, PRESENT AND FUTURE IN POLYPHENOLS UPCYCLE

In the last years, the agricultural and food sector has been challenged with a continuous growing demand for nutritious foods, owing to the dramatic increase of world's population and changes in human dietary habits (Mathys et al., 2018). At present, it is estimated that more than 800 million people around the globe are still suffering from hunger, even though recent numbers indicate that almost one third of the food destined for human feeding is lost or wasted (Garcia-herrero et al., 2019). The agricultural sector generates, by now, ∼140 billion tons of organic wastes per year (Dedousi et al., 2017; Zuin and Ramin, 2018; Gullón et al., 2020). In an attempt to control such trend, the agri-food sector is now starting to implement cleaner, and sustainable, production strategies that seek to combine process efficiency to food quality and safety (Esparza et al., 2020). As a result, agro-industrial wastes such as stalks, leaves, bark, roots, straw residues, bagasse, wood and seeds that were once seen as unimportant materials and, as a consequence, discarded, are revealing themselves to be an outstanding source of bioactive and health-promoting compounds (Dedousi et al., 2017; Panzella et al., 2020).

The consumption of plant-based foods, including not only fruits and vegetables, seeds or cereals but also derived foodstuffs and beverages, has been claimed to be beneficial for human health by the scientific community and general public (Atta ur and Iqbal Choudhary, 2015). Consumers are progressively interested in foods that not only meets nutritional requirements but also improves physical performance and promote health and well-being while reducing environmental stress (Tresserra-rimbau et al., 2014; Gowd et al., 2019; Serino and Salazar, 2019; de Araújo et al., 2020). This challenge has led the agri-food sector to develop novel processes capable of recovering waste by-products generated during collection, processing, and storage of food items (Zhu et al., 2020). Regarding this, a growing interest has emerged in the valorization of these agri-food by-products in order to improve the sustainability of the food industry and reduce environmental problems involved in the management of these residues (Barba et al., 2017; Zuin and Ramin, 2018). Apart from their functionality as a source of energy, agri-food by-products should also be considered as value-added residues due to their chemical heterogeneity and content of bioactive compounds for subsequent applications in pharmaceutical, food and cosmetic sectors (Piccolella et al., 2019). These applications may range from functional food ingredients to nutraceuticals or even for obtaining other valuable bio-products, contributing not only for a sustainable and circular economy but also for the implementation of zero waste politics (Mirabella et al., 2014; Esparza et al., 2020).

Among all bioactive compounds found in agro-industrial wastes, polyphenols have become an emerging field of interest and research in several areas, not only for nutritionists but also for food scientists (Perez-Gregorio and Simal-Gandara, 2017; Santhakumar et al., 2018; Dias et al., 2020). Polyphenols, organic bioactive compounds known as secondary metabolites of plants are of considerable physiological and morphological importance in plants. In the last century, several clinical and epidemiological studies revealed that they possess a strong antioxidant capacity and anti-inflammatory properties that could have preventive or/and therapeutic effects for degenerative diseases, cardiovascular diseases, neurodegenerative disorders, cancer, obesity and food allergy (Kobernick and Burks, 2016; Mrduljaš et al., 2017; Cory et al., 2018; Gullón et al., 2020). Given these health benefits, the determination and characterization of polyphenols in foods to evaluate their bioavailability and bioactivity is becoming one of the most important research areas in food analysis (Lucci et al., 2017; Perez-Gregorio and Simal-Gandara, 2017). Conducting and adequate polyphenols extraction and characterization is crucial to further correlate the health outcomes. Indeed, significant efforts have been made in recent years to develop extraction methods for polyphenol recovery either directly from food products or from agri-food wastes (Domínguez-Rodríguez et al., 2017; Pinela et al., 2017; Panja, 2018; Routray and Orsat, 2019; Altinok et al., 2020; Esposito et al., 2020; Gómez-mejía et al., 2020). However, these techniques present several issues related to polyphenol stability during extraction process and to solvent toxicity, raising concerns about environmental damage and human health (Kelly et al., 2019; Yu et al., 2020). Regarding this, in the last decade, a demand for new extraction techniques has increased searching for higher extraction efficiency while reducing the extraction time, but also considering the ecological footprint of extraction procedures (Maroun et al., 2018; Panja, 2018; Piccolella et al., 2018; Kelly et al., 2019; Ballesteros-Vivas et al., 2020; Pimentel-Moral et al., 2020). Moreover, the analysis and characterization of polyphenols in food samples is quite complex not only due to their high diversity in plant based-foods and beverages but also because of the high complexity of food matrices (Manach et al., 2004; Lucci et al., 2017; Vuolo et al., 2018). Given the relevance of the subject, this review summarizes the main extraction methods as well as the main analytical techniques employed in the polyphenols characterization to further use these polyphenols as modulators of immune reactions to food. The biological mechanisms as well as application to use polyphenols to prevent food allergies was adopted through a nutritional point of view in a circular economy approach.

POLYPHENOLS IN AGRICULTURAL BYPRODUCTS AND FOOD WASTE: CHEMISTRY AND OCCURRENCE

Valorization of agri-food wastes has become a major priority to improve the sustainability of the food chain, minimizing environmental impacts and contributing to a circular economy based on zero waste policies (Rodriguez et al., 2020). In order to obtain potentially marketable polyphenols from agri-food wastes, sustainable, environmentally friendly and feasible low-cost processes need to be developed (Papaioannou et al., 2020). Understanding the chemistry and nature of polyphenols is the key for these purposes. They are present in almost all foods from plant origin such as fruits, vegetables and beverages, and include more than 8,000 structural variants (Belščak-Cvitanović

et al., 2018). Polyphenols are plant secondary metabolites, synthesized through different pathways such as the shikimate or phenylpropanoid as well as pentose phosphate one. They are generally involved in plant defense and protection (Vuolo et al., 2019), contribute to the growth and reproduction of plants and have an important role in the organoleptic properties of vegetables and fruits (de Araújo et al., 2020). Polyphenols are classified into two main groups depending on their structural features such as the number of phenol rings and structural elements that links these rings to one another: non-flavonoids (phenolic acids, stilbenes and lignans) and flavonoids (flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins) (Libro et al., 2016). Polyphenols classification is summarized in **Table 1** and the chemistry and nature of each group are described as follows:

Non-flavonoids

Phenolic Acids

Phenolic acids are broadly found all over the plant kingdom and can be divided into benzoic and cinnamic acids (Stalikas, 2007). These non-flavonoid compounds represent almost 30% of total dietary polyphenols (Belščak-Cvitanović et al., 2018). Benzoic acid derivatives are characterized by one carboxylic group (COOH). The most common ones are gallic, protocatechuic, vanillic and ρ-hydroxybenzoic acids. Usually, the amount of benzoic acids found in edible plants is not high, but in certain red fruits and onions they can reach several tens of milligrams per kilogram of fresh weight (Lucci et al., 2017). The most common cinnamic acid derivatives are ρ-coumaric (the most abundant isomer), ρ-hydroxycinnamic, sinapic, caffeic and ferulic acids. Despite their occurrence in nature as both cis and trans isomers, in plants, cinnamic acids appears mainly as trans isomers (Manach et al., 2004; de Araújo et al., 2020). Furthermore, phenolic acids should appear in nature free or conjugated to sugars and low-molecular-mass components.

Stilbenes

Stilbenes are produced by plants in response to injury and infections, but very low quantities are found in the human diet (Manach et al., 2004; Belščak-Cvitanović et al., 2018). Only resveratrol is considered important to human health, being grape skins, red wine, peanuts, blueberries and cranberries the main sources of this compound. Pterostilbene, a compound chemically related to resveratrol, is also found in grapes and blueberries; due to high antioxidant capacity, it is also considered important to human health (Mrduljaš et al., 2017).

Lignans

Lignans are composed by two phenylpropane units. This small class of compounds are mainly found in linseed, which predominantly encloses secoisolariciresinol. Lower concentrations of lignans can also be found in grains and cereals such as oat, wheat, rye and barley; some fruits such as strawberries or apricots and certain vegetables from the *Brassica* genera like cabbage or broccoli (Calderón-Oliver and Ponce-Alquicira, 2018). Despite being a small family, it should be noticed that the intake of food containing lignans has been linked to prevention of cardiovascular diseases and cancers (Durazzo et al., 2019). Lignin is a random oxidative polymerization of lignans and have been considered as the most naturally abundant and important biopolymer substance in plant cell walls, exceeded only by cellulose.

Flavonoids

Flavonoids are widely distributed in plants, especially in fruits and vegetables. They have been extensively studied over the past few years due to their important role in synthesis of enzymes and vitamins, in minimizing lipid peroxidation and in influencing the organoleptic characteristics of foods (Del Rio et al., 2013; Belščak-Cvitanović et al., 2018; Durazzo et al., 2019). Structurally, they consist in an oxygenated heterocycle (C) linked to two aromatic rings (A and B) with a three-carbon bridge. This 15-carbon skeleton structure is usually referred to as C6-C3-C6. Flavonoids can be divided in six subclasses according with the degree of oxidation of the C ring and the position and number of hydroxyl groups: flavanols, flavones, flavonols, anthocyanidins, flavanones, and isoflavones (Durazzo et al., 2019). Each group contains a huge number of structural variations due to chemical substitutions such as glycosylation, acylation or alkylation (Birt and Jeffery, 2013; Calderón-Oliver and Ponce-Alquicira, 2018). The content of flavonoids in fruits and vegetables depend on several factors, including genetic factors, climatic and agronomic conditions or ripening. Usually, flavonoids are found in plants under the form of glycosides. Despite their high bioactivity, their bioavailability when ingested is very low (Vuolo et al., 2018).

Flavonols

Flavonols are one of the most abundant flavonoids in plant kingdom. They are usually glycosylated with glucose or rhamnose residues but other mono or di-saccharides may also be involved (El Gharras, 2009; Del Rio et al., 2013). Quercetin, kaempferol, and myricetin are the main flavonols found in vegetables such as broccoli, onions, kale and tomatoes and fruits like apricots, cranberries, grapes and apples (Lucci et al., 2017).

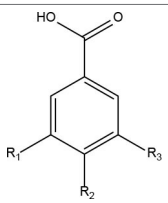
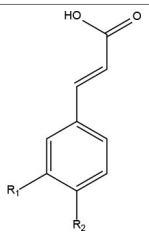
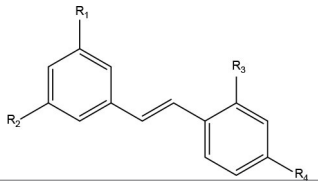
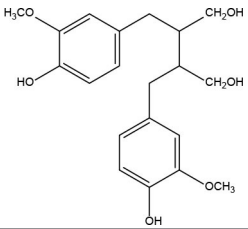
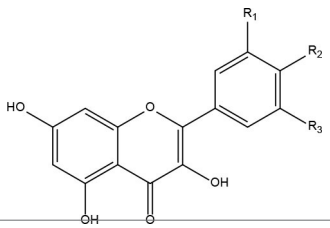
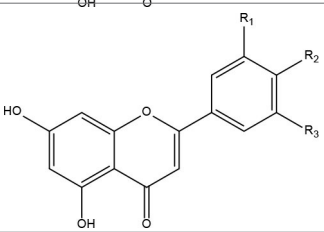
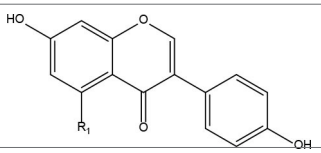
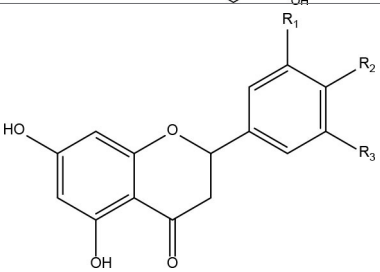
Flavones

In general, flavones are not found in fruits and vegetables, but in aromatic herbs such as parsley or even celery (Manach et al., 2004). Structurally, they can occur in hydroxylated, methylated, -glycosylated and/or alkylated forms (Del Rio et al., 2013). Examples of flavones include luteolin, apigenin, tangeritin, chrysin, scutellarein, 6-hydroxyflavone, wogonin, and baicalein. Some of these compounds are mainly found in citrus fruits such as tangerine and orange and are highly hydrophobic (El Gharras, 2009).

Isoflavones

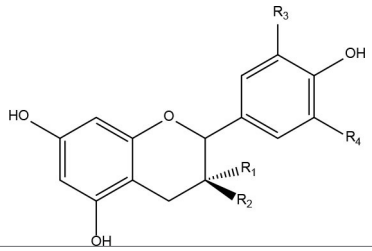
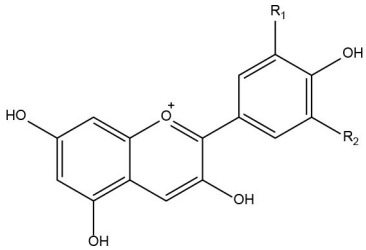
Isoflavones have been identified almost exclusively in leguminous and grain plants such as soya and soybeans. Its main representatives are daidzein, genistein, glycitein, and biochanin A. These flavonoids have an estrogen-like structure, and thus, are classified as phytoestrogens. They have the potential to be used as natural alternatives to traditional hormone therapies (Lucci et al., 2017; Durazzo et al., 2019).

TABLE 1 | Polyphenols classification.

Non-flavonoids	Phenolic acids	 Benzoic acids R1 = R2 = OH, R3 = H: Protocatechuic acid R1 = R2 = R3 = OH: Gallic acid	 Cinnamic acids R1 = OH: Coumaric acid R1 = OCH3, R2 = OH: Ferulic acid	Citrus peels (Ma et al., 2009) Grapes (seed and skin) (Maier et al., 2009) Potato peel (Zeyada et al., 2008)
	Stilbenes		R1 = R2 =R3 = R4 = OH: Oxyresveratrol R1 = R2 = R4 = OH, R3 = H: Resveratrol R1 = R2 = R3 = R4 = H: Trans-stilbene	Grape by-products (Casas et al., 2010)
	Lignans		Secoisolariciresinol	Triticale straw (Monteil-Rivera et al., 2012) Flaxseed cake (Boussetta et al., 2013)
Flavonoids	Flavonols		R1 = R2 = OH, R3 = H: Quercetin R2 = OH, R1 = R3 = H: Kaempferol R1 = R2 = R3 = OH: Myricetin	Onion skin (Ko et al., 2011) Mango peel (Berardini et al., 2005)
	Flavones		R1 = R2 = OH: Luteolin R1 = H, R2 = OH: Apigenin	Lemon and orange peel and pulp (Russo et al., 2015)
	Isoflavones		R1 = OH: Genistein R1 = H: Daidzein	Soy seeds (Popa and Rusu, 2017)
	Flavanones		R1 = OH, R2 = OCH3: Hesperidin R1 = R2 = OH: Eriodictyol R1 = H, R2 = OH: Naringenin	Citrus peel (Giannuzzo et al., 2003) Tomato peels and seeds (Kelebek et al., 2017)

(Continued)

TABLE 1 | Continued

Flavanols		R1 = H, R2 = R3 = R4 = H: (+)-Gallocatechin R2 = H, R1 = R3 = R4 = OH: (-)-Epigallocatechin R1 = R3 = H, R2 = R4 = OH: (+)-Catechin R1 = R4 = OH, R2 = R3 = H: (-)-Epicatechin	Grape seeds (Yilmaz et al., 2011) Green tea leaves (Chang et al., 2000)
Anthocyanidins (flavilium cation)		R1 = R2 = OCH3: Malvidin R1 = R2 = OH: Delphinidin R1 = OCH3, R2 = H: Peonidin R1 = OCH3, R2 = OH: Petunidin R1 = OH, R2 = H: Cyanidin	Grape skin (Cvjetko Bubalo et al., 2016) Wine lees (Bosiljkov et al., 2017) Grape pomace (Panić et al., 2019)

Flavanones

Flavanones are highly present in citrus fruits but can also be found at lower concentrations in tomatoes, spices and in some aromatic plants such as mint (El Gharras, 2009). Usually, this group of polyphenols is bound to one or two sugars moieties, and less frequently found as aglycones (Del Rio et al., 2013). Solid parts of citrus fruits have a very high flavanone content; they contain up to five times as much as orange juice serving. Generally, flavanones include aglycones such as hesperetin, naringenin, eriodictyol, and their glycosidic forms, namely naringin, neohesperidin, narirutin, and hesperidin (Lucci et al., 2017; Calderón-Oliver and Ponce-Alquicira, 2018).

Anthocyanidins

Anthocyanidins are responsible for providing color to plant tissues such as stems, leaves, roots, flowers and fruits. The chemistry of anthocyanins is a little bit complex varying according with the pH. Their color range from red to purple and blue depending on the species in equilibrium (Manach et al., 2004). Besides their role as water-soluble pigments, anthocyanidins also contribute to taste modeling astringent sensations, even though they are odorless and flavorless. Anthocyanidins are considered one of the most important flavonoids classes and can be found in colored fruits like berries, cherries, red cabbage, eggplant, red onion, and red wine (Lucci et al., 2017; Calderón-Oliver and Ponce-Alquicira, 2018). Due to their instability in the aglycone form, they are usually present in fruits and vegetables in the form of glycosides (anthocyanins), such as malvidin, delphinidin, peonidin, petunidin, cyanidin, among others (Del Rio et al., 2013). In this form, anthocyanins in fruits and vegetables provide resistance to light, pH and oxidation (Mrduljaš et al., 2017).

Flavanols

Flavanols are the most complex class of flavonoids, ranging from simple monomers to oligomers and polymers (also known as condensed tannins or proanthocyanidins). Contrary to other flavonoids, flavanols do not appear in plant-based foods and beverages glycosylated (Del Rio et al., 2013). Monomeric flavanols such as catechin and epicatechin can be found in high concentrations in red wine, green tea and chocolate, whereas epigallocatechin, gallocatechin and epigallocatechin gallate are found in certain seeds, grains or grapes, and in green tea (Manach et al., 2004; El Gharras, 2009). Proanthocyanidins made of catechin units are named procyanidins. Usually these compounds are the main responsible for the astringency of some fruits and beverages are the most ubiquitous type of proanthocyanidins found in plants (Mrduljaš et al., 2017).

Despite the great structural diversity among polyphenols (**Figure 1**), little isolated compounds or extracts have been assayed as modulators of food allergy. Indeed, related information is summarized in **Table 2**. As shown, different mechanisms were proposed, as further in-detail discussed, but little is known regarding the structure-activity relationship. Both polyphenol-rich extracts as well as isolated compounds were tested. A higher anti-allergic activity was generally observed in extracts instead of pure compounds (Tokura et al., 2005; Shim et al., 2009a; Zuercher et al., 2010). Likewise, flavonoid aglycones appeared to present a stronger histamine release-inhibitory activity and cytotoxicity than glycosides. Among flavonoids, it has been highlighted that flavonols require a 3-OH group for inhibitory activity while a 3-O-glycoside group may sterically hinder the active site of the aglycone. At the same, the presence of a 4'-hydroxyl group enhanced the activity of flavones while c-glycosilated flavones are less active. Overall, the glycosylation and methylation patterns was probed as able to influence the immunomodulatory effect of polyphenols in flavonols and flavones. However, the activity of anthocyanins, as well as the polymerization grade in flavan-3ols or structural key motifs in non-flavonoid compounds have not been studied jet.

Innovative Green Extraction Technologies Applied to Agri-Food By-Products

Sample treatment is considered a crucial step before the extraction, isolation and characterization of compounds. The

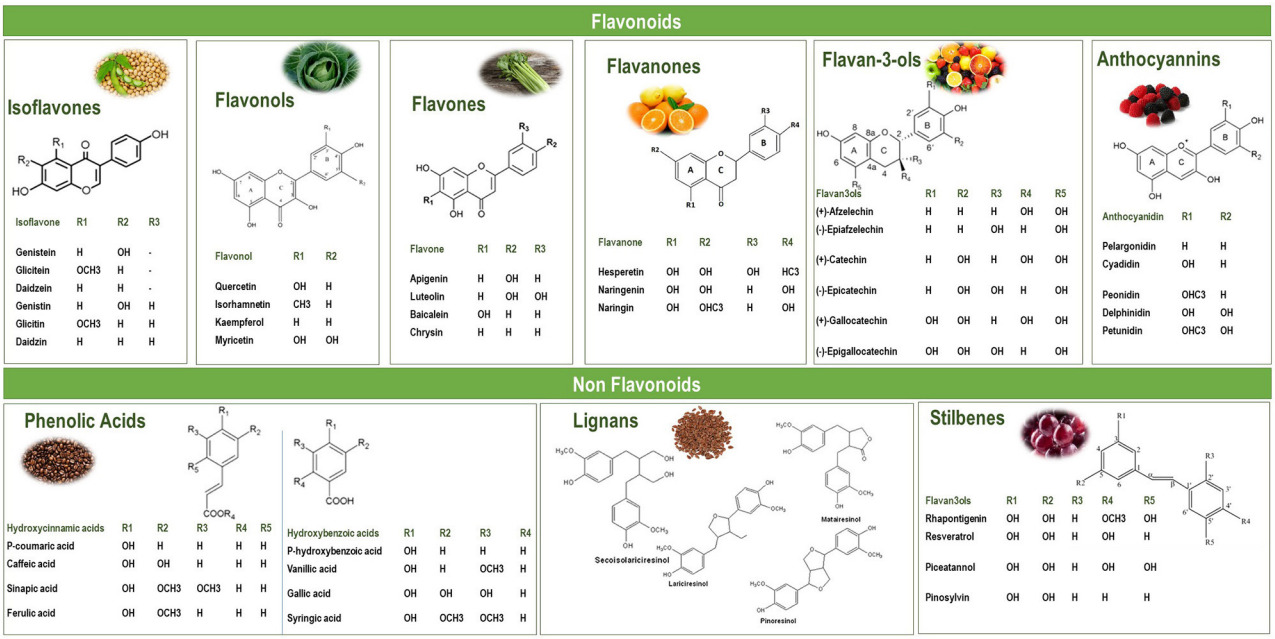


FIGURE 1 | Diversity in polyphenols structures and families.

major objectives of this process are to improve sample stability, to increase extraction efficiency, to remove impurities and to convert the analytes into a suitable form for detection, separation or quantification (Kontogianni, 2014; Pyrzynska and Sentkowska, 2019).

Solid samples usually follow pretreatment processes such as milling, grinding or sieving and homogenization. The latter are generally preceded by air drying or freezing with liquid nitrogen and freeze-drying in order to prevent or stop enzymatic activities. On the other hand, liquid samples can be treated by filtration, centrifugation, and purification before extraction (Khoddami et al., 2013; Kontogianni, 2014). Usually, solid phase extraction (SPE) is used as a pre-cleaning step to remove interfering elements from samples, allowing polyphenol isolation and determination (Pyrzynska and Sentkowska, 2019). The most used material for SPE is chemically bonded silica, typically with a C8 or C18 organic group, but other type of resins such as Sephadex LH-20, polyamide and amberlite have also been used to remove interfering compounds or to concentrate analytes (Kontogianni, 2014).

The extraction of polyphenols from agri-food by-products and food-based products is fundamental to obtain the desired compounds for further analytical characterizations (Kontogianni, 2014; Chemat et al., 2019). As already referred, extraction techniques have been classified into conventional techniques and green technologies (Pimentel-Moral et al., 2020). The conventional extraction methods include solid-liquid extraction and liquid-liquid extraction, using heat and/or mixing with organic solvents such as acetone, hexane, methanol, ethanol or ethyl acetate. These extraction methods depend on several factors such as pressure, extraction time and pH (Louis et al., 2019; Gullón et al., 2020) and presents some difficulties associated with the thermal degradation of target compounds due to the high temperature employed (Pimentel-Moral et al., 2020). Considering this, great efforts have been made to develop emerging extraction techniques based in clean and sustainable practices focused on minimizing the use of solvents, energy and materials that are unsafe to human health and to the environment (Zuin and Ramin, 2018; Chemat et al., 2019). Recent technologies have been recently developed in order to control undesirable effects on the bioactivity and structure of polyphenols during extraction processes, such as microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE) among others (Mourtzinos and Goula, 2019; Kumar, 2020). These greener extraction techniques are known for their short extraction time, reduced volume of organic and unsafe solvents and high efficiency with lower energy consumption when compared to conventional extraction methods (Belwal et al., 2018).

Ultrasound-Assisted Extraction

This extraction method is based on the creation of cavitation bubbles when ultrasound waves pass through the extraction system, creating alternate decompression and compression cycles, which result in the compression and expansion of bubbles. When this bubbles grow too large to be contained by the surface tension force, they collapse, thus creating localized high pressure and temperature zones that cause plant tissues rupture and improve the release of intracellular compounds into the solvent (Magaton et al., 2020; Pimentel-Moral et al., 2020). This extraction method can be affected by several factors such as

TABLE 2 | Polyphenols assayed and mechanisms probed as modulators of food allergies.

Polyphenol	Experimental study	Biological action	Food Allergy	References
Epigallocatechin gallate	Protein-polyphenol complexation	Conformational changes	Milk allergy (lactalbumin)	Wang et al., 2014; Al-Hanish et al., 2016
Epigallocatechin gallate	Protein-polyphenol complexation	Conformational changes	Peanut allergy	Vesic et al., 2015
Apple extract Pomactiv HFV and quercetin	Mouse model	Impair the presentation to dendritic cells	Ovalbumin model	Zuercher et al., 2010
Red wine and coffee polyphenols	*In vivo*-gut microbiota	Increase bacterioides	Inflammation biomarkers-rhinitis allergic asthma	Singh et al., 2017
Fruit, seed, wine and tea polyphenols	*In vivo*-gut microbiota	Decrease clostridium	Inflammation biomarkers-general health status	Singh et al., 2017
Apple crude extract, apple condensed tannins, epicatechin, chlorogenic acid, phlorizin	Mast cell degranulation *in vitro* (RBL-2H3 cells and rat mast cells)	Histamine release	Allergy universal model	Kanda et al., 1998
Psidium guajava extracts	Mouse model-C57BL/6 mice	Inhibition Treg cells	–	Seo et al., 2005
Polyphenol-enriched apple extracts	Mast cell degranulation *in vitro*	Binding between polyphenols and IgE - FcεRI	Allergy universal model	Tokura et al., 2005
Ferulic acid, chlorogenic acid, caffeic acid	Western blot	IgE binding	Peanut allergy	Chung and Champagne, 2009b
Ecklonia cava extract	Mast cell degranulation *in vitro* (KU812F cells)	Binding between polyphenols and IgE - FcεRI	Allergy universal model	Shim et al., 2009a
Perilla frutescens extract, apigenin	*In vivo*-BALB/c mice Mast cell degranulation *in vitro* - RBL-2H3 cells	Mast cell degranulation Histamine release FcεRI cross-linking	Japanese cedar pollinosis	Kamei et al., 2017
Green tea polyphenols	Protein-polyphenol complexation	Digestion process	Egg allergy (ovalbumin and lysozyme)	Shen et al., 2014
Ferulic acid	Mouse model	Inflammation biomarkers	Atopic dermatitis	Zhou et al., 2020
Cocoa enriched diet	*In vivo*-gut microbiota	Inflammation biomarkers	–	Camps-Bossacoma et al., 2019
Polyphenol-rich diet	*In vivo*-gut microbiota	Metabolic parameters	–	Singh et al., 2011; Mine et al., 2020
Green tea, black tea, oolong tea	*In vivo*-gut microbiota Polyphenols Bioavailability (caco-2 cell model)	Metabolic parameters Epithelium transport Gut bacteria proliferation	–	Sun et al., 2018

ultrasound power and solvent composition or extraction time and temperature (Vieira da Silva et al., 2016). Usually, two types of equipment are employed, namely, ultrasonic probe and water bath system fitted with horn transducers (Panja, 2018). UAE is a green and efficient way to increase mass transfer, requiring reduced quantities of solvent and without specific solvent requirements (Dedousi et al., 2017; Contreras et al., 2020). Additionally, this extraction method is considered a rapid and sustainable alternative to conventional methods and it can be scaled up to industrial scales (Briones-Labarca et al., 2015; Meregalli et al., 2020; Saifullah et al., 2020).

Microwave-Assisted Extraction

Microwave-assisted extraction (MAE) is an emerging technology, that consists in the insertion of solvent and sample in a closed vessel using radiation to raise the temperature of the solvent above its boiling point. This heating process causes the rupture of the cell membrane, increasing polyphenol extraction (Kelly et al., 2019). Many aspects may affect this extraction method such as extraction time or temperature, solvent, sample-to-solvent ratio and microwave power. The capacity to absorb microwave radiation is the key factor when choosing the solvents to be used for extraction. This extraction process is quick, uses lower

solvent volume, can be performed in the absence of light and allows the extraction of polyphenols with similar yields when compared to conventional methods. Therefore, MAE is a useful methodology to extract and concentrate polyphenols in a single step (Mourtzinos and Goula, 2019; Kumar, 2020).

Pressurized Liquid Extraction

PLE occurs inside a closed and inert reaction vessel at high pressures (3.3–20.3 MPa), allowing the use of temperatures well above the boiling point of some common solvents (40–200°C). This improves the solubility and mass transfer of compounds, consequently raising the extraction efficiency (Ballesteros-Vivas et al., 2020; Pimentel-Moral et al., 2020). Matrix properties, type of solvent, extraction time and temperature are fundamental factors that determine PLE efficiency. When water is used as a solvent, PLE is commonly called pressurized hot water extraction, being this process entirely environmentally friendly and economically viable. Therefore, PLE is considered an energy saving methodology and environmentally safe as the most used solvents are water and ethanol, which are considered GRAS (Generally recognized as Safe) solvents (Kelly et al., 2019).

Supercritical Fluid Extraction

This extraction method is based on the use of gases, mostly CO_2, above or near critical temperature and pressure (Panzella et al., 2020). In these conditions, fluids behave like a single phase, displaying the properties of both liquid and gas at the same time. Fluid disperses into the solid matrix like a gas and dissolves target compounds like a liquid (Maroun et al., 2018). This extraction process is highly dependent on temperature and pressure. CO_2 is the most attractive fluid for SFE since it is non-toxic, non-flammable, chemically inert, cheap and widely (Kelly et al., 2019). The addition of a co-solvent can modify the polarity of the $scCO_2$ allowing the extraction of more polar molecules (Panzella et al., 2020). Usually, SFE uses extraction temperatures between 30 and 70°C. Also, this extraction process can be scaled up to an industrial size (Carabias-Martínez et al., 2005; Herrero et al., 2010). Although this technology involves easy operation, low production costs and high extraction efficiency, there has to be a significant investment in equipment need to be made to make the process viable (Wang et al., 2016).

Despite the great diversity in extraction methods little is known regarding the impact in the biological activity. A recent study reveals that solvent extracts had a remarkable influence on polyphenols characterization and activity, confirming the strong correlation between phytochemical constituents and antioxidant activities (Bouasla et al., 2021). However, most studies focused in analyzing the anti-allergic activities of polyphenols have not deep in the extracts characterization and effects of extraction methods. In the face of the priority to obtain sustainable agri-food systems, is mandatory to evaluate the gap between the extraction methods and their impact in bioactive compounds characterization and further biological effect.

CHARACTERIZATION OF POLYPHENOLS

The analysis of polyphenols in complex extracts requires efficient separation methods prior to their detection. Despite a high number of investigations, the separation and quantification of polyphenols in food matrices remain difficult, particularly if there are polyphenols from different groups (Naczk and Shahidi, 2004; Khoddami et al., 2013). High performance liquid chromatography (HPLC) has been the main technique to separate, characterize, and quantify polyphenols in the last 20 years. Some other relevant techniques include spectrophotometric assays (Kontogianni, 2014).

Spectrophotometric Assays

The Folin-Denis and Folin-Ciocalteu methods are relatively simple and have been commonly used to measure total phenolics in foods and plant materials for many years. The theoretical basis behind these methods are based on the chemical reduction of tungsten and molybdenum-containing salts, whose products have a broad light absorption around 760 nm (Khoddami et al., 2013). Besides the low cost and simplicity of these methods, the results are prone to overestimations because the Folin reagent reacts not only with polyphenols but also with compounds with reducing groups like sugars. These methods are suitable to obtain a global estimation of total phenolic compounds; yet, they do not offer a quantitative measurement of individual compounds. Therefore, more precise and exact methods such as chromatographic techniques are needed for qualitative and quantitative analyses (Naczk and Shahidi, 2004; Domínguez-Rodríguez et al., 2017).

Chromatographic Assays: High Performance Liquid Chromatographic

High performance liquid chromatographic techniques are now most widely used for both separation and quantitation of polyphenols (Naczk and Shahidi, 2004). This technique has not only the capacity to analyze all components of interest simultaneously but also their derivatives and degradation products. The chromatographic conditions of the HPLC methods include the use of a reversed-phase C18 column or alternatively a C8, a UV-Vis diode array detector and a binary solvent system containing acidified water (solvent A) and a polar organic solvent (solvent B) (Ignat et al., 2011). Generally, the most used organic solvents are acetonitrile and methanol. A common strategy to suppress the ionization of phenolic hydroxyl groups is to acidify solvents using acetic or formic acid, which also improves the resolution and reproducibility of the retention characteristics (Stalikas, 2007). Chromatographic separation depends mainly on the molecular weight, stereochemistry, polarity of the analytes and on matrix complexity. Reverse phase (RP) HPLC-DAD is the mostly used tool for the separation and determination of polyphenols. However, because the UV spectra of polyphenols are often very similar to each other, one needs to apply modern high-performance chromatographic techniques combined with instrumental analysis like NMR an MS for structural identifications (Ignat et al., 2011; Khoddami et al., 2013; Dzah et al., 2020).

Mass spectrometry coupled to liquid chromatography is a very useful analytical technique not only for quantitative analysis but also to elucidate the chemical structure of known and unknown compounds (Khoddami et al., 2013). This technique relies on the ionization of chemical compounds to generate charged

molecules or molecular fragments whose mass-to-charge ratios are subsequently measured. Analysis with MS detector can be performed with different types of ionization in the ion source such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) (Yang et al., 2009). ESI is the most used interface in LC-MS configurations, because it combines efficient separation capabilities of HPLC with the exact structural characterization of MS. Several scanning modes can be used to give additional structural information such as fragmentation of the pseudo molecular ion, selected ion monitoring (SIM), selected reaction monitoring (SRM) or multiple reaction monitoring (MRM) (Pyrzynska and Sentkowska, 2019). In order to improve the sensitivity and minimize the matrix effects, several MS/MS parameters can be optimized such as the capillary voltage, collision energy and ion mode (López-fernández et al., 2020). Therefore, LC-MS is considered as a standard method for the identification and characterization of polyphenols and presents several advantages such as selectivity, rapid method development, cost effectiveness, among others (Kumar, 2017).

Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance (NMR) is a complementary spectroscopic technique that provides complete structural elucidation of polyphenols either extracted from complex mixtures or without any previous sample preparation (Ajila et al., 2011). Several techniques have been employed to analyze polyphenols including ^{1}H NMR and ^{13}C NMR, two-dimensional homonuclear (2D ^{1}H–^{1}H), heteronuclear chemical shift correlation NMR (C–H HECTOR), correlated NMR spectroscopy (COZY), totally correlated NMR spectroscopy (TOCSY), rotating frame of reference (ROESY) and nuclear Overhauser effect in the laboratory frame (NOESY) (Naczk and Shahidi, 2006; Ye et al., 2015).

NMR instrument coupled to other analytical instruments such as HPLC, UV-Vis and MS to achieve an on-line separation and structural elucidation is a useful tool when target compounds are difficult to isolate or are unstable (Ye et al., 2015). Although expensive, NMR has many advantages over other techniques such as accuracy, precision, simplicity of the sample preparation, among others (Kontogianni, 2014).

In the last years, a huge number of analytical advances and applications have been applied for food analyses. Yet, there is still several concerns that need to be solved in this field of research. Overall, developing appropriate methods for the proper extraction, purification and characterization of polyphenols is necessary to achieve higher accuracy in the obtained results for each type of analytes.

USE OF POLYPHENOLS AS MODULATORS OF IMMUNE REACTIONS TO FOOD. THE CASE OF FOOD ALLERGY

On the basis of cross-sectional, prospective and intervention studies concerning polyphenols and human health and well-being, several experimental papers in the literature have tried to understand the molecular mechanisms behind their bioactivity. Moreover, *in vitro* studies have reported the potential of polyphenols to modulate several diseases such as cancer, cardiovascular diseases or metabolic disorders (Fernandes et al., 2017). Polyphenols have been demonstrated to inhibit cell proliferation in cancer studies, improve insulin secretion, stimulate vasodilatation and influence cell signaling and function (Del Rio et al., 2010). But what about food allergies? Are polyphenols able to influence the process? Food allergy (FA) is defined as "an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food" (Panel et al., 2010). Noteworthy, the prevalence of FA has increased over the past decades; nevertheless, therapeutic options remain limited. A better understanding of the key nutritional mechanisms involved in such immune responses will likely be vital for disease prevention and development of new therapies. Scientific knowledge has therefore to be improved to establish the basis for new treatments and prevention methods (Sicherer and Sampson, 2018). Hence, the effect of polyphenols as natural attenuators of FA has to be studied. This section summarizes the scientific evidences and future challenges in this field.

Nature and Prevalence of Food Allergy

The prevalence of FA is affected by lifestyle and food availability, particularly in developed western countries (Prescott et al., 2013; Tang and Mullins, 2017; De Martinis et al., 2019). In general, FA are more common in children than in adults due to the immaturity of their immune system. The great majority of affected children "outgrow" FA with age (Prioult and Nagler-Anderson, 2005; Prescott et al., 2013; De Martinis et al., 2019). Additionally, studies are showing that the incidence of FA among elderly is increasing with aging all around the world (Ventura et al., 2010; Mohrenschlager and Ring, 2011).

The immune responses caused by FA can be broadly categorized in two major types: Immunoglobulin E (IgE)-mediated disorders and non-IgE mediated food allergies (though a mix between IgE and non-IgE mechanisms is also common) (Sicherer and Sampson, 2009; Anvari et al., 2019). In IgE mediated FA, the allergen activates a rapid T helper (Th) 2 cell response characterized by Th2 cell proliferation, production of pro-allergic cytokines, interleukin-4 (IL-4), IL-5, and IL-13; and the release of allergic mediators, such as histamine and β-hexosaminidase, by effector cells (mast cells and basophils) (Anvari et al., 2019). In IgE-mediated reactions the symptoms occur shortly after food ingestion and may result in a potentially life-threatening allergic reaction that affects multiple organs (anaphylaxis) (Anvari et al., 2019). Conversely, non-IgE dependent reactions are typically restricted to the gastrointestinal tract and include a wide spectrum of chronic disorders, where allergen-specific T cells are thought to have a prominent role (Yu et al., 2016). However, non IgE dependent food allergies are more difficult to diagnose, and they generally reverse in infancy, usually before the age of 6 years (Connors et al., 2018).

The most common diagnostic procedures for FA are skin prick tests (SPTs) and serum food-specific IgE tests. However, there are numerous diagnostic procedures whose employment is

chosen based on the medical history and physical examination (Sicherer and Sampson, 2010). The standard of care of FA includes strict dietary elimination of the allergen and ready access to injectable epinephrine to avoid anaphylaxis reactions (Bird et al., 2015); however, no active, definitive therapeutic options exist for food-allergic patients (Muraro et al., 2014; Sicherer and Sampson, 2018). Although the mechanisms underlying the development of FA are still under study, FA appears to be the direct result of a breakdown in oral tolerance, defined as the unresponsiveness of the immune system after food antigen exposure (Yu et al., 2016). Oral tolerance is an active process that occurs at the gut-associated lymphoid tissue (GALT) and is mediated predominately by regulatory T cells (Yu et al., 2016). The lack of tolerance initiates when the food antigen crosses the gut epithelial barrier and is processed by antigen presenting cells (APCs) into peptides, which are displayed on APCs surface to be recognized by antigen-specific Th cells. In the presence of inflammatory stimuli, Th cells differentiate into allergen-specific Th2 cells that orchestrate allergic sensitization mechanisms. Accordingly, sensitization mediated by Th2 cells includes the differentiation of B cells into plasmocytes, which secret antigen-specific systemic IgEs. Then, after the re-exposure to the food antigen, IgEs bind the surface receptor FcεRI expressed in mast cells and basophils. This IgE-FcεRI interaction promotes mast cells and basophils degranulation as well as the release of allergen-induced cytokines (IL-4 and IL-13) (Yu et al., 2016).

Currently, therapeutic approaches to FA are focused on oral food challenges (OFCs) that is to say, modulating the immune response to allergens promoting the induction of oral tolerance (Muraro et al., 2014; Sicherer and Sampson, 2018). Although OFCs are promising, tolerance to allergens has to be induced, posing a danger to patients (e.g., anaphylaxis). Overall, to date the guidelines for controlling a FA are to avoid allergens and be prepared in case of exposure, for example with epinephrine to counteract a potential fatal reaction (Lieberman et al., 2015). Hence, further research must be performed in order to find safer alternatives to prevent or control FA epidemic.

Dietary Effects in Food Allergy Incidence—Microbiota Role

Dietary factors influence gut microbiota and consequently the immune system (Chistiakov et al., 2015). Not surprisingly, this crosstalk between gut bacteria and immune cells was found to be closely related to the development of allergic disorders. Commensal microbiota colonizes mucosal surfaces, such as respiratory tracts, skin, vagina, and gastrointestinal tracts. These microorganisms are in symbiosis with the host, and influence nutrient absorption, resistance to pathogens, immune defense, and tissue repair (Hong et al., 2017). In the intestine, these bacteria have important immunoregulatory properties, as they provide a large variety of harmless antigens that continuously stimulate intestinal immune cells to be tolerant, not only to molecules produced by commensal bacteria, but also to exogenous proteins derived from food (Chistiakov et al., 2015). Moreover, microbiota derived signals have a direct impact on T cell differentiation, with some favoring the regulatory T cell subset, which is vital for the maintenance of the intestinal homeostasis (Arroyo Hornero et al., 2020).

Notwithstanding, some factors including the mode of delivery (van Nimwegen et al., 2011; Mitselou et al., 2018), use of antibiotics (Hirsch et al., 2017), a western diet (Myles, 2014), and improved hygiene patterns (Prioult and Nagler-Anderson, 2005; Hong et al., 2017), can lead to dysbiosis, which is characterized by changes in the microbial communities and their metabolic reactions. Altogether, this imbalance can diminish the mucosal immune tolerance to commensal bacteria and food antigens leading to a less protection against allergic diseases (Pascal et al., 2018).

There are a few studies relating the presence of allergies with changes in bacterial ecology and diversity early in life (Prioult and Nagler-Anderson, 2005; Hong et al., 2017). Furthermore, different diets lead to different microbial compositions, as shown in **Figure 2**. Protein, fats, carbohydrates, probiotics, and polyphenols induce changes in commensal microbiota, interfering with immune responses. The intake of animal protein induces the increase concentration of bile-tolerant microorganisms and decrease butyrate-producing bacteria. Carbohydrates suppress the growth of butyrate-producing bacteria and increase the anaerobic bacteria concentration. The saturated fat ingestion contributes to increase the number of anaerobic bacteria. Probiotics contain beneficial living microorganisms to balance the gut microbiota (Singh et al., 2017). Moreover, probiotics and polyphenols increase lactic-acid bacteria concentration.

The study of different diets seems to be important to understand how it interferes with gut microbiota. Western and Mediterranean diets are very popular worldwide and differ widely. **Table 3** compares the two types of diet. The association between the mostly consumed components in these diets and their interference in gut microbiota, allows to conclude that Western diet contributes to the reduction of flora diversity, particularly of the beneficial species, whereas Mediterranean diet provides a more varied and balanced microbiota. Accordingly, considering the influence of intestinal microbiota on immune responses, the adoption of Mediterranean diet provides greater protection against allergic diseases (Singh et al., 2017).

Proving the importance of adopting a varied diet to maintain a balanced flora (**Figure 2**), it is also necessary to relate dietary habits with the digestive processes and how this can influence the development of FA (Ortega, 2006; Pali-Schöll et al., 2018). Food intake determines the composition of microbiota and therefore, the maintenance of a healthy physiological epithelial barrier and function and, consequently, oral tolerance (Tappenden and Deutsch, 2007). The effect of several diet components in FA development has been highlighted. Epidemiologic studies suggest that selenium; zinc; vitamins A, C, D, and E deficiencies, low fiber consumption and high fat intake may be associated with the development of allergic disorders (Kamer et al., 2012; Allen et al., 2013; Jonsson et al., 2016; Tan et al., 2016). Fruits and vegetables are important sources of dietary antioxidants, mainly polyphenols, which have been widely described as containing antiallergic properties (Kanda et al., 1998; Gruber et al., 2004;

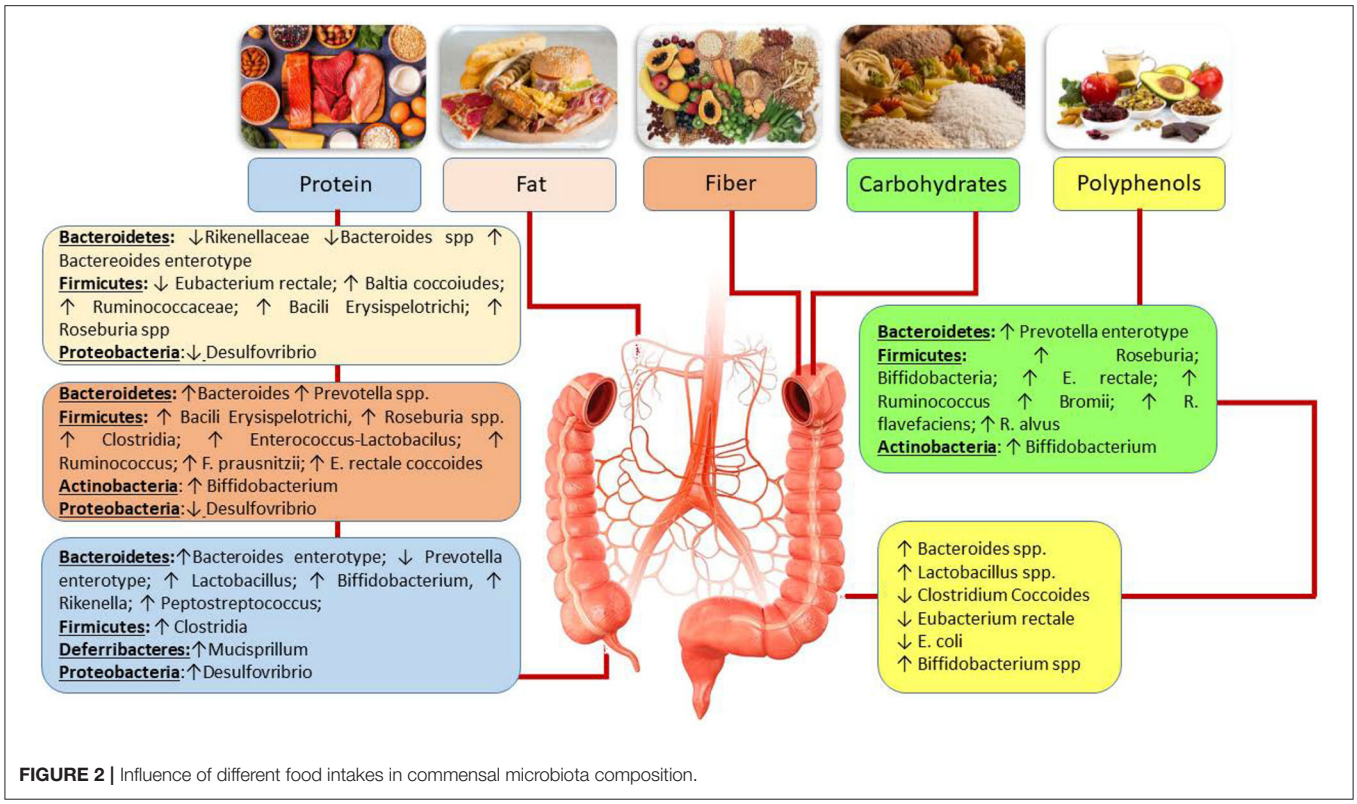


FIGURE 2 | Influence of different food intakes in commensal microbiota composition.

TABLE 3 | Comparison between Mediterranean diet and Western diet.

		Western diet intake	Mediterranean diet uptake	Food sources examples	References
Food macronutrients	Lipids (saturated)	High	Moderate	Fast-food, animal oil, butter	Mirmiran et al., 2021
	Carbohydrates (sugar)	High	Moderate	Bread, cookies, ultraprocessed food	Gómez-Donoso et al., 2021
	Proteins	High	Moderate	Meat, fish, legumes	Altomare et al., 2013
Food micronutrients	Vitamins	Low	High	Fruits and vegetables	De Pergola and D'Alessandro, 2018
Bioactive compounds	Polyphenols	Low	High	Fruits and vegetables	Bonaccio et al., 2017
	Carotenoids	Low	High	Fruits and vegetables	Marhuenda-Munoz et al., 2019
	Phytosterols	Low	High	Fruits and vegetables	Escurriol et al., 2009
	Phytoestrogens	Low	High	Fruits and vegetables	Ogce et al., 2008
	Glucosinolates	Low	High	Fruits and vegetables	Del Bo et al., 2019

Tokura et al., 2005; Nakano et al., 2008; Chung and Champagne, 2009a; Shim et al., 2009b; Zuercher et al., 2010; Kamei et al., 2017). Promisingly, *in vitro* and *in vivo* studies, including in humans, have suggested that polyphenols can act on the sensitization as well as on the re-exposure immune mechanisms to allergens (Singh et al., 2011). For instance, polyphenols can impair the presentation of food antigens by APCs, namely dendritic cells, to allergen-specific T cells, promote a decrease in intestinal mast cell proteases release (Zuercher et al., 2010), induce the reduction of local intestinal mRNA expression of several Th2 associated and pro-inflammatory genes (MacDonald and Monteleone, 2005; Zhu, 2017) and impact IgE production by B cells (Singh et al., 2011).

Despite growing research are being conducted regarding the role of polyphenols in food allergies by direct antiallergenic mechanisms or indirectly through modulating gut microbiota some concerns must be considered. Most *in vitro* studies are performed by using isolated polyphenols or extracts but only a large minority of these compounds reach the colon intact. Under this context, metabolomics approaches must be conducted to understand the immunomodulatory action of polyphenols metabolites. Likewise, the high structural variability as well as

the already mentioned diversity in extraction methods claim for further studies correlating the structure/activity relationships and effects on extraction methods in polyphenols functionality.

Polyphenol/Protein Interactions as a Mechanism to Modulate Food Allergy

Besides their anti-inflammatory and anti-allergic properties, polyphenols possess a significant binding affinity for proteins, which can lead to the formation of soluble and insoluble protein–phenolic complexes (Perez-Gregorio et al., 2014; Dias et al., 2015, 2016; Fernandes et al., 2016; Brandão et al., 2017). Antigens that elicit FA reactions are usually proteins. Indeed, recent studies revealed the interaction between polyphenols and food allergens (Shen et al., 2014; Wang et al., 2014; Vesic et al., 2015; Al-Hanish et al., 2016) which could induce conformational changes in the allergen (Wang et al., 2014; Vesic et al., 2015). However, whereas the polyphenol binding capacity was already proved in different food allergens, the influence in each step that leads to FA development have not been studied yet. Furthermore, polyphenol-protein interactions have already described as have potentially significant biochemical implications in the digestion (Soares et al., 2015) nevertheless is still a gap in the polyphenols effect in allergen digestion.

Overall, it was already described that polyphenols may bind to food antigens and conformational changes are consequently induced. Protein digestibility, conformation, and aggregation might be important for biological activities of dietary proteins that elicit hypersensitivity reactions in humans. IgE-binding capacity and activation of effectors cells, uptake by antigen-presenting cells and sensitizing potential of food allergens in allergy are the biological factors key for the disease onset. However, there is still a gap in the knowledge as how allergen-polyphenol complexes could influence the immune response. Little studies tentatively proved the inhibition of the IgE-binding activity through allergen-polyphenol interaction (Chung and Champagne, 2009a; Gray et al., 2015). It was also studied that some insoluble complexes could block the allergen availability (Chung and Champagne, 2009a). However, these studies should be interpreted with caution as were based the native unmodified forms of allergen that might suffer previous digestive and metabolic steps (Cardona et al., 2013). Several mechanisms prompted by polyphenols seem to be effective in allergic sensitization, a) their ability to bind with allergenic proteins (Li and Mattison, 2018; Bansode et al., 2019; Plundrich et al., 2019) or to IgE (Bansode et al., 2019; Yousef et al., 2020) b) the protective effect as anti-inflammatory and antioxidant compounds (Zhou et al., 2020) and/or c) the ability to modulate cell functions either dampening MHC-II and co-stimulatory molecule expression or inhibiting cytokine production (Zuercher et al., 2010), or increasing their expression on Treg cells (Kim et al., 2007), thus hampering the antigen presentation process. Polyphenols could also influence FA during elicitation. Indeed, polyphenols inhibited the activation, proliferation and function of Th-2 cells during re-exposure to allergen in sensitized individuals (Singh et al., 2011). The polyphenol ingestion also attenuates the allergenic re-exposure by inhibition of adhesion and migration of peripheral B-cells, IgA-attenuating (Camps-Bossacoma et al.,

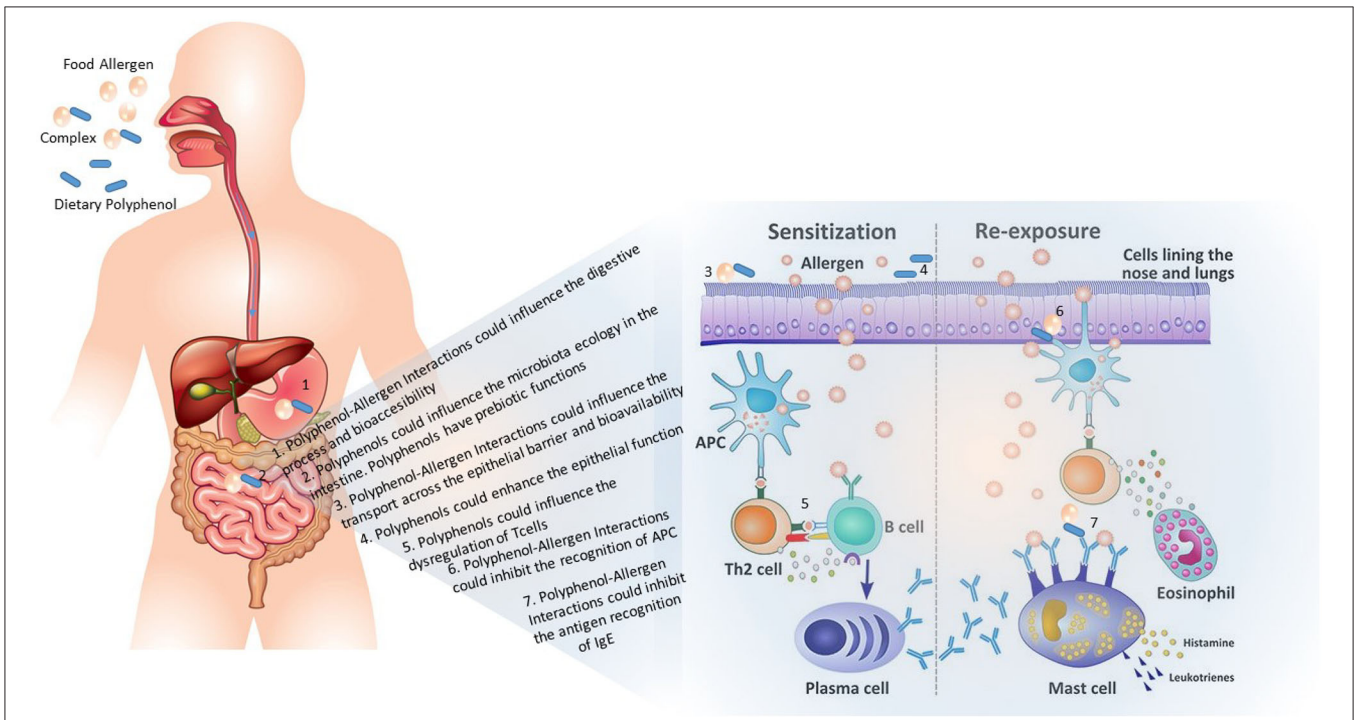


FIGURE 3 | Schematic representation on the polyphenol mechanisms to modulate the immune response in food allergy.

2019), suppression of IgE and IgG1 levels and abrogation of Th-2 cytokines (Singh et al., 2011; Mine et al., 2020). Growing scientific research also related the consumption of dietary polyphenols with the composition of gut microbiota and function (Sun et al., 2018). Indeed, the ingestion of polyphenols have been related with the growth of specific gut microbiota that have been shown to modulate Treg production (Turroni et al., 2020).

Altogether, although the data collected during the last years could help to understand the polyphenol's potential mechanisms of action (summarized in **Figure 3**), the metabolism, interactions with host and/or with other dietary factors as well as intrinsic variations within individuals remain largely unknown. According to the diversity of mechanisms proposed, the biological effect of polyphenols is likely the interplay between all of them. Furthermore, the digestion, metabolism and bioavailability of polyphenol, allergens and allergen-polyphenol complexes have to be well-understood when studying their immunomodulatory effects. Scientific knowledge must be improved to establish the basis for nutritional recommendations which could help preventing or minimize the prevalence and symptoms of FA.

CONCLUSIONS

Polyphenols are bioactive compound usually found in agri-food wastes with widely proven potential to be incorporated in the formulation of functional foods designed to prevent the increase of non-communicable diseases. Healthy constraints must go hand in hand with climate constraints claiming for the use of bioactive compounds from wastes in a circular economy approach. The epidemic increase in immune reactions to food allergens claim for stablishing nutritional recommendations able to control this rise. Based in aforesaid evidences, there is an unmet need to evaluate new therapeutic modalities in a nutritional approach (functional foods or supplements) that may decrease the risk of food-induced anaphylaxis and improve patients' quality of life. However, there is still a lack in the real intake of polyphenols through diet given the lack of standardized methods to characterize them from both, food components and agri-food wastes. Some missing links need to be addressed to actively modulate the immune system through diet and food systems via polyphenols. Active components and metabolites within different polyphenols extracts must be identified considering the inter-personal variability. Besides, complex *in vitro* systems must be designed for a better understanding of mechanistic studies and the cellular impact of polyphenols depending on anatomical locations (gut vs. blood) or intervention window (prevention vs. treatment). Furthermore, safe concerns must be explored in terms of maximum recommended daily intake, presence of toxic substances from extraction methods and overall cytotoxic activities. Whether used as therapy or as dietary interventions, their long-term safety profile needs to be addressed in different age groups. This review summarizes the pros and cons of the existent analytical methods to properly characterize polyphenols as the basis for the study in the use of polyphenols as modulators of immune reactions to food. Furthermore, the polyphenols bioavailability and metabolism, the polyphenols effect in gut microbiota and the ability to bind to proteins and cell receptors have to be studied in order to recommend the consumption of polyphenols to prevent the rise of food allergy.

AUTHOR CONTRIBUTIONS

TF and RP-G conceived and planned the overall review. RP-G devised the project, the main conceptual ideas, and proof outline. TF and CB took the lead and supervise in writing the manuscript, in consultation with all authors. TF carried out the documentation and writing in the analysis of polyphenols from agri-food wastes. CB planned and wrote the scientific section of the review related with the nature and food allergy related to immunology area. RD aided in interpreting the documents consulted and worked on the manuscript. NM and VF developed the theoretical formalism and were in charge of overall direction and planning. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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Polyphenols Extraction From Vegetable Wastes Using a Green and Sustainable Method

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Food systems have the potential to support human health, respecting the environmental sustainability principles. Food sustainability, enclosed in the concept of environmental sustainability, involves different aspects, including the recycling of food waste derived from the agri-food production chain, the use of biotechnologies ensuring the sustainability of the recovery processes of bioactive compounds from food waste and, last but not least, the awareness of having to consume and waste less food. Food loss and waste is generated during the whole supply chain, from production to household utilization. The utilization of agricultural wastes as an abundant, renewable and low-cost source for the production of high value-added products is currently explored. The bioactive compounds present in these sources have been proved to possess a wide range of biological activities; therefore, research is needed into the application of environmentally friendly traditional and advanced techniques with low production costs in the extraction, isolation and purification of phytochemical compounds from agricultural wastes in high yields and at maximal quality. Authors of this manuscript propose and discuss an innovative and sustainable extraction system of polyphenols from vegetable waste, based on an enzymatic pre-treatment coupled with a solid-liquid extraction by using a particular extractor (Naviglio Estrattore®). This extraction system, organic solvent free, allowed to extract relevant amount of polyphenols (flavonoids in particular) from several vegetable waste products.

Keywords: food system sustainability, food waste, food waste recycling, bioactive compounds, enzymatic extraction, polyphenols

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INTRODUCTION

Food Loss and Waste in the Food Supply Chain

The reduction of food loss and waste is considered by the United Nations and many international institutions as one of the main ways to proceed toward the protection of the environment and for the well-being of humanity, as also reaffirmed by the UN 2030 Agenda for Sustainable Development [ODD–ONU Italia (unric.org)]. Furthermore, food waste is a key determinant, inter alia, of the loss of biodiversity, the dispersion of greenhouse gases in the atmosphere, the pollution of water, soil and other resources (www.isprambiente.gov.it). The main global factors, responsible of the large amount of food waste, are:

- the increase in the world population and urbanization,
- the great availability of fossil energy sources,
- the economic and cultural diffusion of mass agro-industrial systems,
- the transformation of (food) lifestyles
- assigning a relatively low economic and socio-cultural value to food (Clapp, 2002; FAO, 2011; Gille, 2012).

Several evidences also show that the increase of food waste, especially when produced during sales and consumption, is directly proportional to the increase of the levels of economic development (29, 30). It is extremely hard to exactly quantify the lost and wasted food in the world today and, even more difficult, is to prevent the food losses. Moreover, currently, there is not much research in the area, which is quite surprising considering that food production must significantly increase to meet future global demand. Not much attention appears to be paid to current global food supply chain losses (Gustavsson et al., 2011). The issue of food loss and waste represents an important topic in the efforts to reduce hunger, raise income and improve the food security in the world's poorest countries. Food losses have a relevant impact on food security for poor people, on food quality and safety, on economic development and on the environment. According to the Food and Agriculture Organization (FAO), 1.300.000.000 tons of food gets yearly lost or wasted in the all the world, representing approximately one-third of the edible parts of food produced for human consumption.

Food loss and waste is generated throughout the entire supply chain: the 54% of total loss and waste occurs during the upstream processes (including production and postharvest) and 46% of total loss and waste occurs during the downstream processes (including processing, distribution, and consumption) (Mirabella et al., 2014). In particular, in developing countries more than 40% of these food losses occur at post-harvest and processing levels, while in industrialized countries, around the 45% of the food losses occurs at retail and consumer levels. In Europe every year, 280–300 kg per capita of food at different stages are wasted and lost, mainly concerning the vegetables and fruit sector (Gustavsson et al., 2011; FAO et al., 2014). According to the report by the FAO of the United Nations (FAO et al., 2014), 45% of fruit and vegetable wastes and by-products from the fruit and vegetable processing industry are generated around the world throughout the entire food supply chain. Most of fruit and vegetable wastes is disposed in landfills or rivers, which represents a threat to the environment due to their high biodegradability, leachate, and methane emissions (Misi and Forster, 2002). However, these resources have a great potential to be used for the recovery of value-added products (Wadhwa and Bakshi, 2013).

Food Waste Recovery Improves Sustainability of Food System

A food system assembles different aspects (environment, people, inputs, processes, infrastructures, institutions, etc.) and activities related to the production, processing, distribution, preparation, and consumption of food, and their socioeconomic and environmental outcomes (HLPE, 2014). As mentioned before, food waste has an important impact on food and nutrition security, food quality and safety, natural resources, and environmental protection. For these reasons, management of food loss and waste, with their co- and by-products have already attracted the attention of food scientists and industry over the last decades (Galanakis, 2015). In fact, recently several scientific reports, relevant to food waste and treating methods, were published, especially including strategies for reduction of waste production, the valorisation of co- and by-products, and improvement of waste management. This increasing attention is mainly due to the following reasons:

a) the growing environmental concerns,
b) the necessity to minimize the impact of waste on human health,
c) the high costs of waste disposal that are limiting the profits of the food industry,
d) the growing interest toward benefits deriving from potentially marketable components present in food wastes and co-products (Laufenberg et al., 2003).

For all these reasons, unlike the traditional linear economic model, based on a "take-make-consume-throw away" pattern, a circular economy model is concepted and based on sharing, leasing, reuse, repair and recycling, in an (almost) closed loop, where the contained products and materials are highly valued (Bourguignon, 2016).

Agriculture not only is ensuring global food security for over 7 billion people around the world, but also plays an important role in supporting and promoting the development of other industries, such as nutraceuticals, pharmaceuticals and cosmetics. In particular, agri-food industry produces a large amount of wastes and residues along the whole supply chain, still containing a significant quantity of valuable bioactive compounds, such as polyphenols (phenolic acids, flavonoids, proanthocyanidins, anthocyanins, glycosides…), carotenoids, saponins, tannins, alkaloids, sterols, steroids, triterpenes, peptides and carbohydrates properties (Moure et al., 2001; Llorach et al., 2002; Zhang et al., 2017), which, as largely demonstrated, possess several biological activities, including antioxidant, antibacterial, antifungal, antiviral, antimicrobial, antidiabetic, anticancer, antidiarrhoeal, antihypertensive, antimutagenic, anti-inflammatory, anticholesterol and protective properties of cardiocirculatory system (Balasundram et al., 2006; Santana-Méridas et al., 2012; Nguyen, 2017). Increasing the yield of the target compounds adopting the most appropriate food waste recovery strategies, the economic value gained from them could be maximized. Moreover, the recovery of high added-value material could result in the development of innovative products and/or materials (Galanakis, 2015) and highlights the importance of science-based innovation to improve the sustainability of food system (Defra, 2006). There is another important aspect to consider: the recovery of food wastes and its valuable bioactive compounds could also help in promoting the viability and diversity of rural and urban economies, contributing to create new job opportunities (Galanakis, 2015). Finally, reducing food waste, by recovering compounds with biological activity present in the waste, represents an important solution to increase the

sustainability of the food production system, also contributing to the reduction of waste management costs which can represent a serious problem, especially for smaller producers.

Sustainability of Extraction Processes

Food by-products and wastes contain highly complex components with a relevant biological and economical value, although these residual materials generally present a lower concentrations of valuable compounds respect to the initial sources. Fruit and vegetable wastes, in particular, contain a significant amount of biologically active compounds (value-added products) like polyphenols, glucosinolates, dietary fibers, essential oils, pigments, organic acids, etc. (Baiano, 2014; Kumar et al., 2017). In addition, in some vegetable materials, the content of bioactive compound in by-products is higher than in their major parts. These compounds can be extracted, purified, and characterized using emerging technologies, allowing to develop new commercial applications in food and non-food (pharmaceutical, biomedical, cosmetic, etc.) areas (Galanakis et al., 2012). Even in the process of recovering food waste, the reduction of energy and the optimization of raw materials can significantly reduce costs and at the same time increase the environmental sustainability of the food system. An efficient food waste recovery process has two meanings; firstly, it is necessary to use only that type of material that otherwise would have been thrown away and, secondly, to use / process that material as efficiently as possible, using technologies and extraction methods functional to the type of compounds to be extracted. Food wastes are generated in different forms and compositions, according to regional, seasonal, and processing characteristics in each case (Gustavsson et al., 2011). This implies that the cost for the processing could increase, as well as the recovery yield can decrease. Moreover, food wastes are susceptible to microbial contamination and require both proper preservation and fast treatment. Following all these considerations, the development of an economically feasible, sustainable, and safe recovery of bio-active compounds from food residue must take into account several parameters such as the abundance and distribution of food wastes (related to the presence of industry), the set-up of methodologies providing the highest recovery yield of different compounds, the utilization of green solvents, the preservation of the biological properties of selected compounds from source to final product, waste minimization prior to the recovery process, the application of environmentally friendly traditional and advanced techniques with low production costs in the extraction, isolation and purification of phytochemical compounds from agricultural wastes in high yields and at maximal quality (Varzakas et al., 2016; Nguyen, 2017). Hence, technological advances in extraction, separation and identification have been developed to produce natural products with potent biological activity (Van Lanen and Shen, 2006; Wang and Weller, 2006). The biggest research challenge is now the identification and set up of the best "extraction" conditions, i.e., the condition that improve release of the bioactive compounds from the vegetable matrix in which they are encased. The recovery process of bioactive compounds from fruit and vegetable waste includes several important stages in succession: preparation of dried samples, extraction process, production of the powder extract, isolation and purification by chromatography (Pham, 2017). Each of these different phases involves the use of different technologies, aimed at the recovery of specific molecules with different physico-chemical characteristics; however, it is important that, in compliance with sustainability criteria, each of these processes has a reduced environmental impact both in terms of energy and the use of solvent. Regarding the extraction methods, conventional methods, commonly found in literature, are very numerous and long to list but the main used are liquid–liquid or solid–liquid extraction, primarily based on the use of organic solvents. Nowadays, in respect with "Circular Economy" principles, alternative and innovative extraction systems are used, depending on the characteristics of target compounds; the main emerging techniques, considered innovative, rapid, reproducible, cost/benefit, and clean, are the following:

- Ultrasound Assisted Extraction (UAE)
- Pulsed Electric Field (PEF)
- Microwave assisted Extraction (MAE)
- Pressurized Liquid Extraction (PLE)
- Supercritical Fluid Extraction (SFE)
- Enzyme Assisted Extraction (EAE)

Except for solvent extraction, whose sustainability is depending on the type of used solvent, the other listed technologies are considered "green," although the cost of some equipment used in the extraction plants is sometimes high. Enzyme Assisted Extraction is perhaps the most environmentally and economically sustainable of those listed above. The main mechanism behind EAE involves cell wall degrading enzymes such as glucanase and pectinase which weaken or deconstruct the cell wall, making intracellular compounds more accessible for extraction.

STRATEGY FOR POLYPHENOLS EXTRACTION FROM VEGETABLE WASTE MATERIAL—AN EXPERIMENTAL EVIDENCE

An eco-friendly extraction system, alternative to conventional solvent-based and complex physical methods, was set up by the authors of this papers and it will be described below.

Some vegetable waste, obtained from manufacturing industry, were used as source of bioactive compounds such as polyphenols. Since many polyphenols are found to form complexes with plant cell-walls structures (proteins and carbohydrates), often recalcitrant to degradation, the treatment of plant materials with enzymatic mixtures containing cellulases, hemicellulases and pectinases, promotes the hydrolytic degradation of cell wall polymers, thereby favoring the release of secondary metabolites (Khandare et al., 2011; Puri et al., 2012). Cell wall polysaccharides, in fact, are known to interact with various polyphenols, modifying their bioaccessibility and bioavailability. The EAE treatment of the food waste is then complemented by a solid-liquid (water) extraction with the

Naviglio Estrattore® (NE), a system based on the generation of a negative pressure gradient between the outside and inside of solid matrix, followed by a sudden reinstatement of the initial balanced conditions. This pressure gradient leads to the forced extraction of compounds from the solid matrix in the aqueous phase. The preceding enzymatic treatment is expected to enhance the amount of extracted polyphenolic compounds.

Vegetable wastes [*Petroselinum crispum* (Mill.) (parsley), *Brassica oleracea* L. italica var. (broccoli), *Brassica oleracea* L. botrytis var. (cauliflower), *Spinacia oleracea* L. (spinach), *Allium ampeloprasum* L. (leek), *Eruca vesicaria* L. (rocket salad), *Cichorium intybus* L. (chicory)] were collected from farms and food processing industries (AOP Unolombardia consortium), located in Lombardia region (Italy). Enzymatic mixtures were obtained from Novozymes®.

Preparation of Samples, Enzymatic Pre-treatment and Extraction System

The enzymatic mixtures (around 10 U/ml) used in this work include hemicellulases, mixture of different cellulases and pectinase mixture, optimized after preliminary experiments in smaller scale. Fragments of the vegetable residues (0.5 cm), obtained by using a mill, were placed in the plastic trays with distilled water and an aliquot of each commercial enzymatic mixture in stirring conditions.

After this pre-treatment (50°C for 3 h), the solid residue (around 5 kg) from each material is placed in Naviglio Estrattore® (N.E.), filled with only water, to complete the extraction process of polyphenols. The total amount of polyphenols and flavonoids was then measured using classical colorimetric methods (Folin-Ciocolteau and aluminum chloride methods, respectively), and the identification of the main polyphenols was performed by HPLC.

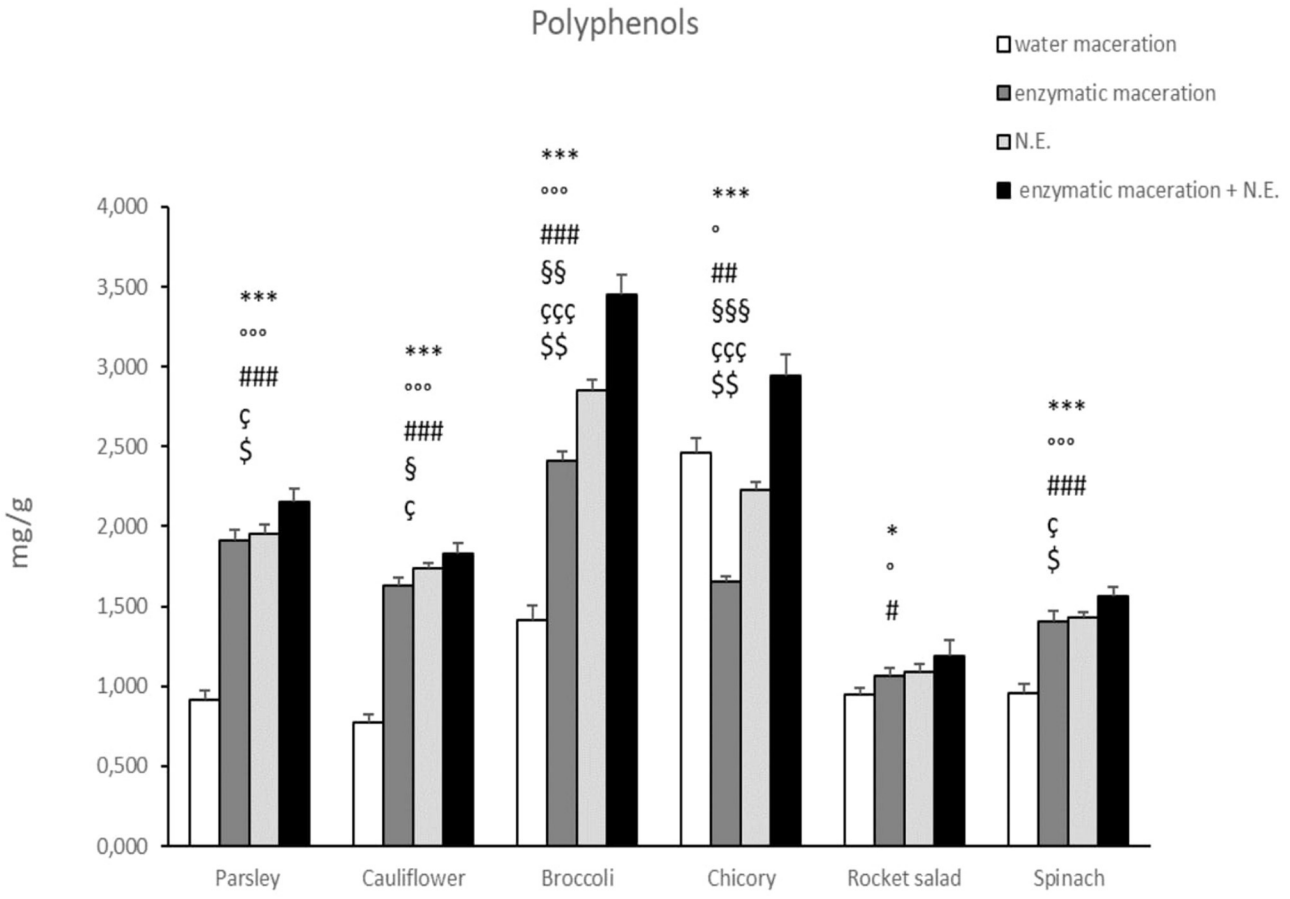

FIGURE 1 | Polyphenolic content (mg/g) in the different extracts obtained from vegetable waste. Data are expressed as mean values ± standard deviation. Statistical analysis: within-group comparison (Polyphenols at different extraction methods) carried out by repeated measures analysis of variance. Level of significance: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ expressed with comparison symbols: (*) water maceration vs. enzymatic maceration, (°) water maceration vs. N.E., (#) water maceration vs. enzymatic maceration + N.E., (§) enzymatic maceration vs. N.E., (ç) enzymatic maceration vs. enzymatic maceration +N.E., ($) N.E. vs. enzymatic maceration +N.E. N.E., Naviglio Estrattore®. Statistical analysis: Continuous variables were reported as mean values ± standard deviation (SD), and were analyzed using nonparametric tests (i.e., Friedman and Wilcoxon test, as appropriate), since these variables were not normally distributed (based on the Shapiro-Wilk statistic). All tests were two-sided and a $p < 0.05$ was considered as statistically significant.

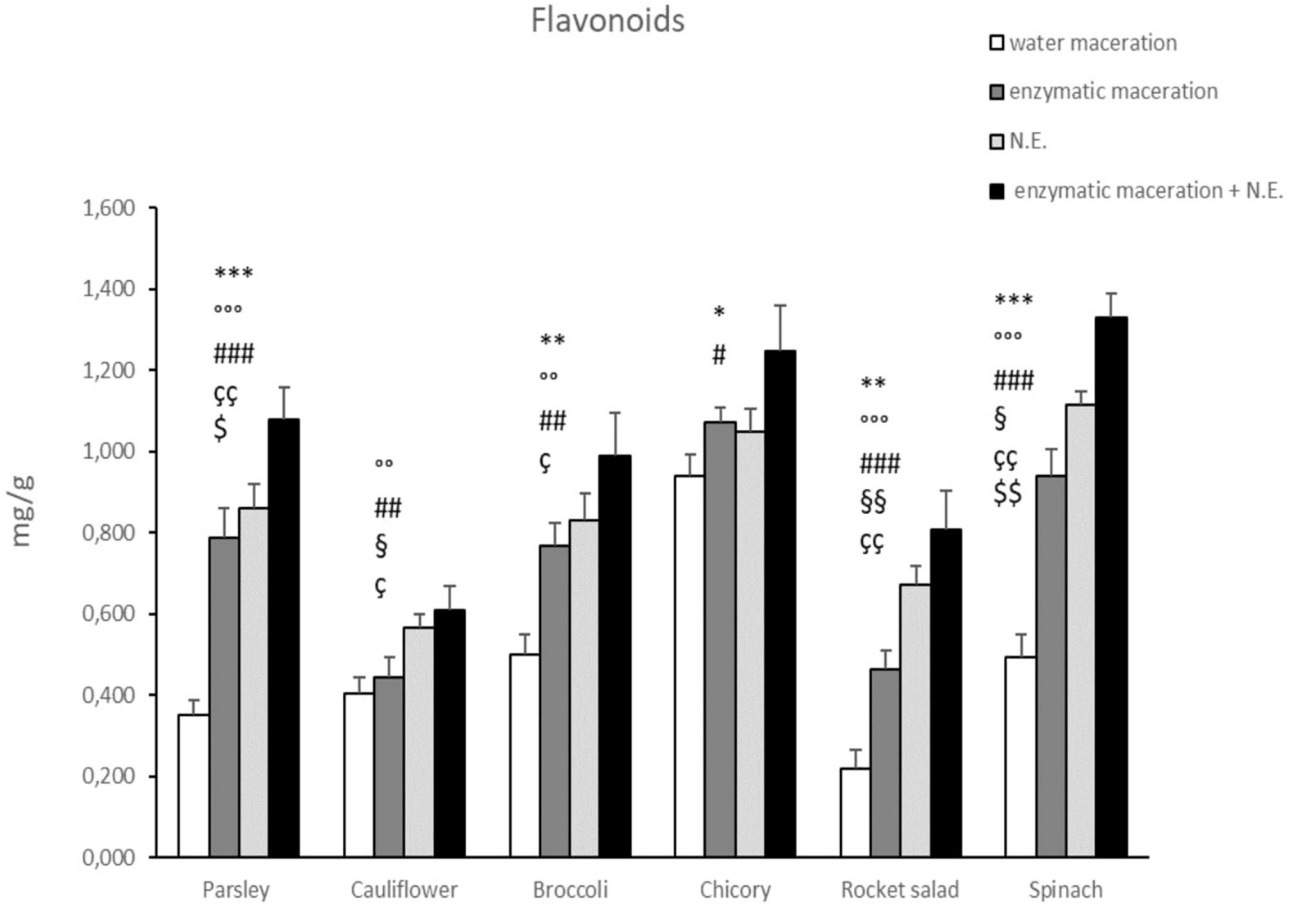

FIGURE 2 | Flavonoids content (mg/g) in the different extracts obtained from vegetable waste. Data are expressed as mean values ± standard deviation. Statistical analysis: within-group comparison (Flavonoids at different extraction methods) carried out by repeated measures analysis of variance. Level of significance: $p < 0.05$ expressed with comparison symbols: (*) water maceration vs. enzymatic maceration, (°) water maceration vs. N.E., (#) water maceration vs. enzymatic maceration + N.E., (§) enzymatic maceration vs. N.E., (ç) enzymatic maceration vs. enzymatic maceration +N.E., ($) N.E. vs. enzymatic maceration +N.E. N.E., Naviglio Estrattore®. Statistical analysis: Continuous variables were reported as mean values ± standard deviation (SD), and were analyzed using nonparametric tests (i.e., Friedman and Wilcoxon test, as appropriate), since these variables were not normally distributed (based on the Shapiro-Wilk statistic). All tests were two-sided and a $p < 0.05$ was considered as statistically significant.

RESULTS

The total polyphenols content (TPC) and the total flavonoids content (TFC) contents are showed in **Figures 1** and **2**, respectively. The data are presented as mg/g fresh weight (FW), mean ± standard deviation (SD, $n = 3$). It is possible to observe as the enzymatic pre-treatment followed by the extraction with Naviglio Estrattore® (N.E.), allowed to recover a higher concentration of polyphenols in all the plant waste material and especially from broccoli and chicory residues. In these two types of material, the amount of extracted polyphenols was clearly higher compared to that one observed using the only N.E. or the only enzymatic maceration. When the flavonoid concentration was tested, in all the examined material a noticeable increase using the N.E. after the enzymatic pre-treatment was measured (**Figure 2**). This observed difference in pre-treatment efficacy and in the phenolics extraction from vegetable waste is principally due to the biochemical characteristics and the cell wall composition of each material.

By using HPLC analysis, many phenolics were identified after the complete extraction system (enzymatic pre-treatment + N.E.) (**Table 1**).

The data obtained in this work are difficult to compare to those found in literature for different reasons. There are huge differences in vegetables polyphenol quantification depending on seasonal harvesting (Arabbi et al., 2004; Hertog et al., 2007), different cultivar (Heimler et al., 2007; Koh et al., 2009), the climate where they grow (Podsedek, 2007), the cultivation site (D'Acunzo et al., 2017) endogenous circadian rhythms (Soengas et al., 2018), soil and pest-control treatment (Valverde et al., 2015). Moreover, the use of solvent is a variable that greatly affects the extraction efficiency. In all the scientific papers regarding the extraction of polyphenols from the same vegetable matrix, several

TABLE 1 | Phenolic compounds found in the vegetable extracts, obtained after the extraction process by N.E. following the enzymatic pre-treatment ($n = 3$).

Polyphenols	Broccoli (mg/kg)	Cauliflower (mg/kg)	Chicory (mg/kg)	Rocket salad (mg/kg)	Spinach (mg/kg)	Parsley (mg/kg)
Chlorogenic acid	70 ± 0.5	320 ± 15	150 ± 11	100 ± 13	80 ± 7	10 ± 0.9
Catechins	90 ± 3	130 ± 8	380 ± 10	20 ± 2	9 ± 0.9	70 ± 5.5
Caffeic acid	40 ± 5	–	170 ± 6	–	6 ± 0.5	3 ± 0.6
Cumaric acid	–	5 ± 0.5	–	–	5 ± 0.3	16 ± 4
Vitexin	3 ± 0.4	–	–	–	–	30 ± 7.5
Orientin	190 ± 8	16 ± 2	–	–	160 ± 15	22 ± 5
Rutin	12 ± 0.9	9 ± 0.8	–	–	–	50 ± 5
Quercetin	–	–	8 ± 0.6	–	–	13 ± 0.8
Cinnamic acid	–	3	–	–	–	1 ± 0.3
Luteolin	–	–	6 ± 0.5	–	–	8 ± 0.5
Kaempferol	–	–	7 ± 0.3	7 ± 0.5	5 ± 0.4	–

solvents like alcohols (methanol, ethanol), acetone, diethyl ether and ethyl acetate, often mixed with different proportions of water, were used.

DISCUSSION

The recovery of food by-products is a way to re-use the waste of the agri-food production chain, recovering their precious compounds, complying with the requirements of a circular economy, increasing the sustainability of the food production system. The set-up of innovative and sustainable extraction systems of natural products is currently a hot research topic involving different areas. The aim is to reduce or to eliminate the use of hazardous extraction solvents and ensure a safe and high quality of extract/product, in order to protect both the environment and consumers. Consequently, a sustainable and eco-friendly recovery of bioactive compounds from fruit and vegetable by-products for application in food, medical, cosmetic, pharmaceutical or agrochemicals industries is crucial to enhancing their added value and to reducing the pollution risk for the environment. For many years, the conventional techniques based on the use of solvent have been widely accepted, mainly because of their ease of use, efficiency, and wide-ranging applicability (Stalikas, 2007). In recent years, the use of organic conventional solvent is highly discouraged and legal limitations are becoming more and more rigorous, especially for food and pharmaceutical industry. Processes involving the use of organic solvents have a high negative impact on health and environment; beside this important aspect, the residues of solvent may also remain in the final products. This requires long additional purification steps and has repercussions on the total process cost. Additionally, by using pure organic solvents, very polar phenolic acids (benzoic, cinnamic acids) cannot be extracted completely. Therefore, the possibility to use important amounts of industrial by-products of agri-food chain to extract bio-active compounds as commercial goods with high marketing potential, in compliant with the current European legislation that strongly encourages the food industry to find new end-uses for by-/co-products, was explored in the experimental work presented in this paper. Since the biggest research challenge is the identification of the best "extraction" conditions, in order to improve the release of bioactive compounds from the vegetable waste matrix reducing the use of organic solvents and respecting the principles of sustainability of the whole extraction process, the enzymatic pre-treatment coupled with aqueous extraction using Naviglio Estrattore® represent a sustainable strategy allowing to recover a good amount of phenolics, in particular. Due to the use of water as extraction solvent, phenolics compounds represent the main phytochemicals extracted and analyzed from the selected vegetable by-products.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

ED: writing—original draft. ED and DB: work planning. EB and AM: performed research. MV, DB, and ED: data curation. DB: statistical analysis. MD and DB: funding acquisition. All authors contributed to the article and approved the submitted version.

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