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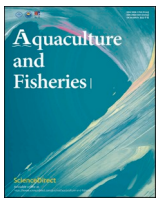

Review article

State of knowledge about biotechnological uses of digestive enzymes of marine fishery resources: A worldwide systematic review

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ABSTRACT

The characterization of digestive enzymes presents in fish and their potential biotechnological uses is a well-developed and studied field. However, there has not been a systematic review that analyzes the state of knowledge of these enzymes at a global level. Therefore, a systematic literature search on three platforms was carried out to review and analyze existing knowledge about digestive enzymes of marine fishes from fisheries and aquaculture and their potential application in industrial processes. Using the PRISMA method for selecting journal manuscripts, we found 112 scientific articles published between 1984 and 2020 studying different digestive enzymes from 87 fish species. Most studies were carried out in Tunisia and Mexico and only 6 articles were published in South American countries. The most studied digestive enzymes were alkaline proteases, mainly trypsin, and the proposed uses for these enzymes were mainly as additives in commercial detergents. There is a vacancy in the characterization of other digestive enzymes as pepsins, lipases and amylases and the study of species that are distributed in the Southwest Atlantic Ocean. It is necessary to expand the knowledge about other digestive enzymes and to carry out new studies in regions with an important fishery development.

1. Introduction

Fish consumption is increasing on a global scale, it is estimated that by 2030 this consumption will be 18% higher than that recorded in 2018. This increase was mainly driven by marine capture fisheries, whose annual production from 2017 to 2018 increased from 81.2 to 84.4 million tons.

In turn, total fisheries and aquaculture production reached an all-time high of 179 million tonnes in the same year (FAO, 2020) and this production was maintained during 2020· (FAO, 2022). Finfish accounted for the largest share of the total production (85%), mainly conforming to small pelagic species followed by gadiformes and tuna. Along with this increase, a large amount of fish waste rich in digestive enzymes is produced. Approximately 20–80% of the total fish catch is waste and this amount will depend on the species and the level of processing of the fish (e.g. gutting, scaling, filleting) (Arnaud, de Lamballerie, & Pottier, 2018; Coppola et al., 2021; Olsen et al., 2014).

In order to revalue the digestive enzymes, present in fishery residues, it is necessary to first know their functional properties. These enzymes are responsible for the hydrolysis of nutrients such as proteins, polypeptides, amino acids, lipids of all kinds and carbohydrates in the physiological digestion process of fish (Eroldoğan et al., 2008). Digestive enzymes such as amylase, proteases and lipases may be secreted within the lumen of digestive organs such as stomach and intestine, intestinal membrane enzymes (proteases, peptidases and carbohydrase) bound to the microvilli or contained in supranuclear vacuoles within the enterocyte (aminopeptidase and alkaline phosphatases) (Guillaume & Choubert, 2001; Noaillac-Depeyre & Gas, 1973; Rust, 2003; Segner et al., 1989).

Protein digestion begins in the stomach with a high activity of acid proteases such as pepsin and protein hydrolysis continue and ends in the intestine with the release of amino acids and peptides where alkaline proteases (Merino-Contreras et al., 2018; Tao, Zhao, Wang, Yang, Cui, Zhao, Wu, 2008). In turn, lipid and carbohydrate digestion is carried out by lipases and carbohydrases and occurs from extracellular hydrolysis of lipids and carbohydrates in the stomach, intestine and cecal lumen (Rust, 2003). The main digestive enzymes present in the digestive tract of most fish are categorized in: proteolytic enzymes (pepsin, chymotrypsin, trypsin), carbohydrate enzymes (chitinases, maltase and amylase), lipolytic enzymes (lipase) and phosphatases (alkaline

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phosphatase) (Bone & Moore, 2008). In general, digestive enzymes are stored in cells in an inactive form called zymogens and are activated only after they are secreted, when digestion or acid is present, or when activated by another enzyme (Moraes & de Almeida, 2020). The activity of these enzymes in each digestive organ is influenced by several factors such as feeding habits, ontogeny, diet (Hassaan et al., 2020; Shi et al., 2016; Thongprajukaew et al., 2013), salinity (Liu et al., 2017; Moutou et al., 2004; Psochiou et al., 2007; Pujante et al., 2018) and light intensity (Wang et al., 2015; Cuvier-Péres et al., 2001).

A characterization of the biochemical parameters of these enzymes is necessary to understand their types, modes of action and activity values. From this information, it is possible to propose the incorporation of these enzymes in biotechnological processes, to promote the market and ensure its profitable use on an industrial scale with the main objective of obtaining value-added products from fish waste (Friedman et al., 2021). Several studies have proposed the revaluation of fish wastes through the incorporation of these enzymes in many processes such as laundry commercial detergents production (Rengasamy et al., 2016) hydrolysate preparation (Rios-Herrera et al., 2020), for shrimp waste deproteinization (Ktari et al., 2014), in gelatin production (Bkhairia et al., 2016) as well as in bioremediation processes (Mohanty et al., 2018).

To characterize these enzymes, the studies usually determine the optimum temperatures and pHs and their stability (Alarcón et al., 2005; Bougatef et al., 2007; González-Félix et al., 2018). Proteases, the most widely used group of enzymes in industrial bioprocesses, generally have high and stable levels of activity over a wide range of pH and temperature conditions (Shahidi & Kamil, 2001) and are inactivated at relatively low temperatures. In addition, the molecular weights of the enzymes are determined by electrophoresis and the effect of different inhibitors and organic compounds are studied as part of their characterization (Bkhairia et al., 2016; Córdova-Montejo et al., 2019; Rios-Herrera et al., 2020). Subsequently, the enzymes can be purified and/or isolated on different types of supports such as chitosan and alginate (Sáenz de Rodrigáñez et al., 2018; Salazar-Leyva et al., 2016) for their potential application in commercial products.

Although there is a large number of articles published on the study of fish enzymes, both from the point of view of digestive physiology from the characterization of different enzymes as well as the study of fishery and aquaculture residues as a source of raw materials for obtaining enzymes for industrial uses, the information available is fragmented and dispersed. Reviews summarizing the published literature on bioactive compounds and digestive proteases from marine animals (Klomklao, 2008; Kim, Seo, Byun, Heu, & Pyeun, 2002), fish digestive enzymes (Fernandes, 2016; Vilhelmsson, 1997), fish proteases (De Vecchi & Coppes, 1996; Huiping et al., 1998), trypsins from fish processing waste (Bougatef, 2013) and aspartic proteases in fishes (Gildberg, 1988) have been presented so far. However, there are no reviews that allow us to visualize a general overview of the state of the field on this type with a critical analysis about the journals where these articles are usually published, the species used to study the enzymes and their geographical distribution, the type of enzyme characterized and their potential biotechnological application. In this sense, this work aims to review and analyze existing and updated information at a global level on marine fish digestive enzymes and their potential application in different industries using PRISMA's best-practice protocols. These systematic and rarely used methods allow us to identify, select and evaluate the research relevant to our search, with the final objective of compiling the data and carrying a critical appraisal of published articles.

2. Methods

2.1. Search strategy and database

We performed an intensive bibliographic search using three databases: Google Scholar, Scopus and ScienceDirect. Studies were collected systematically following PRISMA's best-practice protocols until October 2020. Through these searches, it is possible to identify trends and growth of knowledge in an area and in a specific time period, to study the dispersion and obsolescence of scientific literature, the type of journal chosen to publish, the countries where a specific topic is researched, the species most studied as sources of enzymes, the enzymes of greatest commercial interest and the types of methodologies used in the laboratory (Malesios & Abas, 2012). The keywords used were: "fish" AND "digestive enzymes" OR "lipase" OR "lipases" OR "protease" OR "proteases" OR "amylase" OR "amylases" AND "activity" OR "activities" AND "biotechnological applications" OR "biotechnology" OR "application". Juni et al. (2002) suggested that the requirement of including or not including studies that are in languages other than English, it depends on the subject of the review. In the present work, we wanted to include publications carried out in the Iberoamerican region where we assume that species from the Southwest Atlantic are studied and, for this reason so this search was also conducted in Spanish and Portuguese besides English.

We reviewed the first 1000 results of each search ordered by relevance to include only those articles that mentioned information concerning fish digestive enzymes. Selected articles that were found to be duplicates or triplicates in the databases were eliminated before further analysis. In addition, we did not include unpublished studies called "grey literature" (bachelor's, master's and doctoral theses) that may have quality and data reliability issues, and can compromise our research (Kelly & Jennions, 2011). Manuscripts with freshwater fish species as enzyme sources were excluded from the analysis. This exclusion was due to the fact that we aimed to focus the review only in marine species that are of interest for our future studies. We used the information described in the study area mentioned in the methodology of each manuscript and/or the experimental conditions in which the fish was used in bioassays to determine the habitat of the species. Next, manuscripts that studied the effect of ontogeny and/or diet modulation on digestive enzymes were excluded from the analysis. All the steps of our bibliographic search are summarized in Fig. 1 (Moher et al., 2009).

2.2. Inclusion criteria and data extraction

To be included in the analysis, the articles must: (i) be performed on marine fish species from fishing and aquaculture (ii) report data of digestive enzymes from marine fish species: their enzymatic activity and characterization by biochemical parameters (optimum pH and temperature conditions and stability, molecular weights and effects of inhibitors), (iii) include whether the enzymes were treated: as crude extracts or underwent a semi-purification or a purification process and (iv) include information about the biotechnological applications of the characterized digestive enzymes.

The selected articles were reviewed and divided in three groups according to the focus of each study: 1) enzyme characterization, 2) enzyme characterization and biotechnological applications and 3) biotechnological uses only (Supplementary information for full listing and references). From each article we extracted information about: article publication date, country where the study was conducted or place of work of the first author, the disciplinary category of the journal where the article was published (aquaculture, physiology, biotechnology, food, fisheries), species studied, taxonomic classification (order and family), conservation status (not evaluated, deficient data, least concern, vulnerable, near threatened or critically endangered), area of geographical distribution (North/South/East/West Atlantic Ocean, North/South/East/West Pacific Ocean and East/West Indo-Pacific Ocean), origin of the fishery resource (commercial aquaculture, experimental aquaculture, commercial fishing, sport fishing, aquarium use), type of digestive enzyme (acid or alkaline protease, lipase, amylase), biochemical parameters for characterization (optimum pH and temperature, stability at different pH and temperature conditions and molecular weight), if the enzymes were semi-purified or purified, and the biotechnological applications. The Search FishBase database was used to

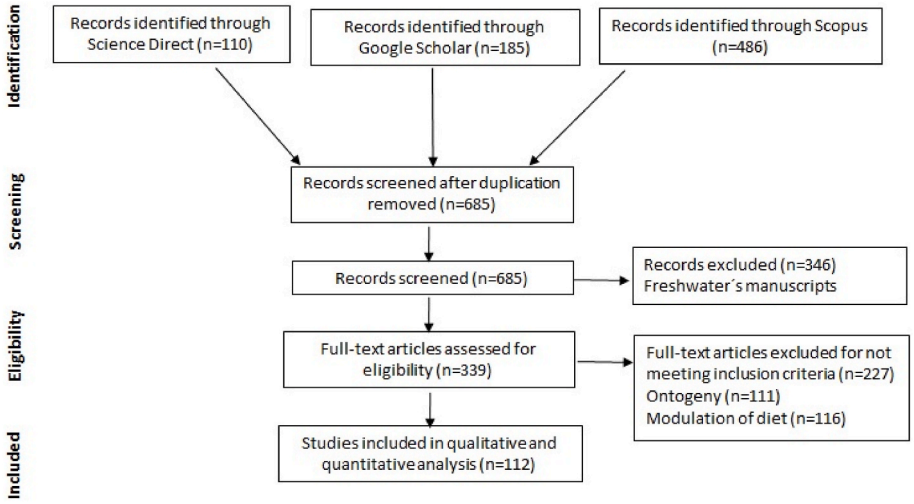

Fig. 1. Process used to select articles presented as a PRISMA flow diagram. Number of studies is indicated by "n" in each box.

extract information on: distribution of each species, taxonomic classification, conservation status and origin of the resource.

2.3. Statistical analysis

To analyze the differences in the number of articles that studied species with different areas of distribution, the Kruskall-Wallis nonparametric test was performed. Then, the results for the frequency of publication were compared between the articles of group 1) enzymatic characterization with articles of group 2) enzymatic characterization and biotechnological application, considering the type of enzyme treated and the techniques used to obtain them (not purified or purified) using a Chi-square test and the likelihood ratio test ($P < 0.05$). All statistical analyses were carried out using R software version 3.6.1 (R Development 215 Core Team 2019).

Specific enzyme activity values were not compared among species due to the fact that the methodology to obtain these values was very diverse. The articles used different substrates and great variability on the enzymatic activity expression and obtaining techniques (some enzymes were not purified, others were semi-purified or purified, reporting activity values at each purification step).

3. Results

The number of articles on marine and freshwater fish species was very similar. Of the total number of articles found in the three databases (n = 685), 346 studied freshwater fish enzymes and 339 articles studied marine fish enzymes (includes fisheries and aquaculture fish). Of these 339 articles, diet modulation and ontogeny studies were also removed and the qualitative and quantitative analysis was performed by including information from 112 manuscripts (see Supplementary information for full listing and references), which were grouped into the three groups previously established: 86 were about enzyme characterization (group 1), 22 related to enzyme characterization and biotechnological applications (group 2) and 4 about biotechnological uses only (group 3). Articles of group 1 are focused on characterize enzymes for general knowledge about the digestive physiology of fish while articles of group 2 are focused on characterizing digestive enzymes in order to propose a potential biotechnological use. Of these papers, 10 propose to study viscera (includes intestines and pyloric caecae, stomachs, pancreas and liver as a source of digestive enzymes to revalorize fishery wastes. The few articles of group 3 do not present a characterization of digestive enzymes but only present an application for fish enzymes. The selected articles were published between 1984 and 2020. However, during the last 10 years the number of articles published on the topic increased considerably. In 2015 the largest number of articles on this subject were published.

3.1. General information from manuscripts

The disciplinary categories of the journals, where most of these articles were published, were: food (32 of which 7 included technology topics), physiology (22), biology (13), aquaculture (9) and biotechnology (9). Most of the articles were published in the journals Food Chemistry (n = 13) followed by Comparative Biochemistry and Physiology (B) (Elsevier) and Fish Physiology and Biochemistry (Springer Netherlands) (n = 9 articles in each journal); 26.1% of the articles were conducted in Asia, 22.5% in North America, 20.7% in Africa, and the same percentage in Europe, mainly in Spain. Only five articles were published in Oceania and 6 in South America (3 from Uruguay, 2 from Brazil and 1 from Argentina). If we analyze by country, approximately 40% of the total number of articles were published in Tunisia (19.8%) and Mexico (18%) followed by China (9%) and Spain (8.10%).

3.2. Species

Enzymes from 87 fish species were studied in the selected publications. The following species were recorded in more than three articles: sea bream (*Sparus aurata*), Monterey sardine (*Sardinops sagax caerulea*), thornback ray (*Raja clavata*), totoaba (*Totoaba macdonaldi*), grass goby (*Zosterisessor ophiocephalus*), sardinella (*Sardinella aurita*), Atlantic salmon (*Salmo salar*), milkfish (*Chanos chanos*), yellowfin tuna (*Thunnus albacares*), skipjack tuna (*Katsuwonus pelamis*) and longtail tuna (*Thunnus tonggol*). The total studied species belong to 39 different families, represented mainly by Scombridae (11.6%), Sparidae (10.5%), Clupeidae (9.30%) and Sciaenidae (5.80%). The species are grouped into 15 different orders, most of the species belong to the order Perciformes (60.9%) followed by the orders Clupeiformes (10.3%) and Pleuronectiformes (6.90%). The rest of the species belong to the orders Mugiliformes, Gonorynchiformes, Siluriformes, Salmoniformes, Gadiformes, Tetraodontiformes, Beryciformes, Scorpaeniformes, Rajiformes, Carcharhiniformes, Myliobatiformes and Lamniformes (Table 1).

Related to the provenance of studied fishes, 93.1% of studied species are commercial fishery resources, of which 42.5% are also used for sport fishing; 31% provided by aquaculture, and 16.1% from ornamental species.

According to the IUCN (International Union for Conservation of Nature), most of the species are classified as Least concern (n = 52), and they are mainly studied in Asia. However, the species common dentex (*Dentex dentex*), smoothhound shark (*Mustelus mustelus*), cod (*Gadus*

Table 1
Orders and families of the species studied in the selected articles.

ORDER	FAMILY	Species
BERYCIFORMES	Trachichthyidae	*Hoplostethus atlanticus* [43]
CARCHARHINIFORMES	Triakidae	*Mustelus mustelus* [9]
CLUPEIFORMES	Clupeidae	*Sardinops sagax caerulea* [4,14,15,76,77,107]; *Sardina pilchardus* [10]; *Sardinops caeruleus* [10]; *Sardinops sagax caeruleus* [13,14]; *Sardinella aurita* [30,34,74]; *Alosa sapidissima* [47]; *Sardinella longiceps* [52,107]; *Brevoortia tyrannus* [63]
	Engraulidae	*Engraulis encrasicholus* [46]
GADIFORMES	Gadidae	*Gadus morhua* [27,41,79]
	Merlucciidae	*Macruronus novaezealandiae* [89]
GONORYNCHIFORMES	Chanidae	*Chanos chanos* [17]
LAMNIFORMES	Lamnidae	*Isurus oxyrinchus* [53]
MUGILIFORMES	Mugilidae	*Mugil cephalus* [5,63,80,84]; *Liza subviridis* [52]; *Chelon labrosus* [65]
MYLIOBATIFORMES	Dasyatidae	*Dasyatis pastinaca* [7]
PERCIFORMES	Sparidae	*Sparus aurata* [1,3,49,64, 50]; *Dentex dentex* [1,24,25]; *Pagrus pagrus* [22]; *Pagellus erythrinus* (22); *Boops boops* [22]; *Sarpa salpa* [22,41]; *Diplodus annularis* [26,103]; *Archosargus probatocephalus* [48]; *Lithognathus mormyrus* [91]
	Mullidae	*Mullus barbatus* [12]; *Pseudupeneus maculatus* [78]
	Centropomidae	*Centropomus undecimalis* [19]
	Sciaenidae	*Totoaba macdonaldi* [19, 29,66,82]; *Micropogonias furnieri* [62]
	Moronidae	*Dicentrarchus labrax* [20]; *Morone saxatilis* [66]
	Scombridae	*Thunnus thynnus* [23]); *Thunnus orientalis* [35,66]; *Thunnus albacares* (62); *Katsuwonus pelamis* (62); *Thunnus tonggol* (39,40,66,67); *Euthynnus affinis* (45); *Rastrelliger kanagurta*; 57. *Scomberomorus concolor* [52]; *Thunnus alalonga* [106]; *Scomberomorus Guttatus* [108]
	Gobiidae	*Zosterisessor ophiocephalus* [104,105,111]; *Boleophthalmus pectinirostris* [86]
	Sciaenidae	*Cynoscion othonopterus* [30]); *Cynoscion parvipinnis* [30]; *Cynoscion xanthulus* [30];
	Carangidae	*Megalaspis cordyla* [33]; *Trachinotus falcatus* [44]; *Trachinotus ovatus* [56]; *Parona signata* [63]
	Serranidae	*Epinephelus coioides* [43,84,85]
	Lutjanidae	*Lutjanus malabaricus* [47]; *Lutjanus guttatus* [64]; *Lutjanus vitta* [97]
	Siganidae	*Siganus canaliculatus* [68,69];
	Labridae	*Tautogolabrus adspersus* [73]
	Rachycentridae	*Rachycentron canadum* [77]
	Nemipteridae	*Nemipterus virgatus* [81]; *Nemipterus marginatus* [110]
	Coryphaenidae	*Coryphaena hippurus* [95]
	Priacanthidae	*Priacanthus tayenus* [97]
	Blenniidae	*Salaria basilisca* [98,99,100]
	Latidae	*Lates calcarifer* [110]
PLEURONECTIFORMES	Soleidae	*Solea solea* [18]
	Scophthalmidae	*Scophthalmus maximus* [32,50,83]
	Paralichthyidae	*Paralichthys orbignyanus* [21]; *Paralichthys californicus* [28]
RAJIFORMES	Rajidae	*Raja clavata* [51,102,105];
SALMONIFORMES	Salmonidae	*Salmo salar* [25,63,67]; *Oncorhynchus tshawytscha* [89,88]
SILURIFORMES	Ariidae	*Bagre panamensis* [109]
SCORPAENIFORMES	Sebastidae	*Sebastes mentella* [54]
	Scorpaenidae	*Scorpaena scrofa* [105,112]
TETRAODONTIFORMES	Trachichthyidae	*Fugu obscurus* [59];
	Balistidae	*Balistes capriscus* [35,96]

The numbers in the superscript indicate the number of the article in which they were studied.

morhua), gulf corvina (*Cynoscion othonopterus*), grey triggerfish (*Balistes capriscus*), bluefin tuna (*Thunnus orientalis*), Monterey Spanish mackerel (*Scomberomorus concolor*) and golden threadfin bream (*Nemipterus virgatus*) are Vulnerable, yellowfin tuna (*T. albacares*), thornback ray (*R. clavata*), shortfin mako ¡ (*Isurus oxyrinchus*) and albacore tuna (*Thunnus alalonga*) are Near Threatened, Atlantic bluefin tuna (*Thunnus thynnus*) is Endangered and totoaba (*T. macdonaldi*) is Critically Endangered. Fig. 2 shows the number of species studied according to their conservation status and the continent where the study was carried out.

The distribution of the species used for enzymatic studies was significantly different in the total articles (Kruskal-Wallis chi-squared = 27.3, df = 13, p-value = 0.01). The 56% of articles included species distributed in the Atlantic Ocean, mostly in the Eastern Atlantic from the north of the United Kingdom to Senegal, including the Mediterranean Sea. The rest of the articles included species distributed in the Western Atlantic from Canada to Argentina. However, only three articles studied species distributed exclusively in the Southwest Atlantic Ocean: white-mouth croaker (*Micropogonias furnieri*), parona leatherjacket (*Parona signata*) and Brazilian flounder (*Paralichthys orbignyanus*). The 18.3% included species distributed in the Indo-Pacific Ocean specifically on the west coast of east African countries, Japan and Australia and 7.43% included species distributed in the Pacific Ocean mostly on the east coast of the USA and Mexico. Only 8.25% included cosmopolitan species that are distributed in tropical, subtropical and temperate waters of all oceans.

3.3. Enzymes

For the study of digestive enzymes, different samples, enzymes, techniques and forms of expressing enzymatic activity were reported in the selected articles. The samples used were fish viscera including: intestines and pyloric caecae, stomachs, pancreas and liver. All species studied have a true stomach with acid digestion and secretion of HCl and 82.7% of the species have pyloric caecae.

In order to characterize the protein extracts, the electrophoresis technique was used and the kinetic properties and the effect of the following biochemical parameters on enzyme activity were determined: substrates, pH and temperature conditions, inhibitors, organic solvents, metal ions and salts. According to the methodology of the articles in the laboratories, the obtaining of semi-purified or purified digestive enzymes was carried out through different procedures. The most commonly used methods of semi-purification in decreasing order were: ammonium sulfate precipitation and dialysis, salt precipitation and acetone precipitation. After the semi-purifications in some articles, the purification process was completed by different chromatographies: exchange affinity, size exclusion columns, affinity for specific ligand and gel filtration.

The types of digestive enzymes studied differed significantly among manuscripts in groups 1) enzyme characterization and 2) enzyme characterization and biotechnological applications (Pearson's Chi-squared test = 15.108, df = 5, p-value = 0.009913). 29.8% of the

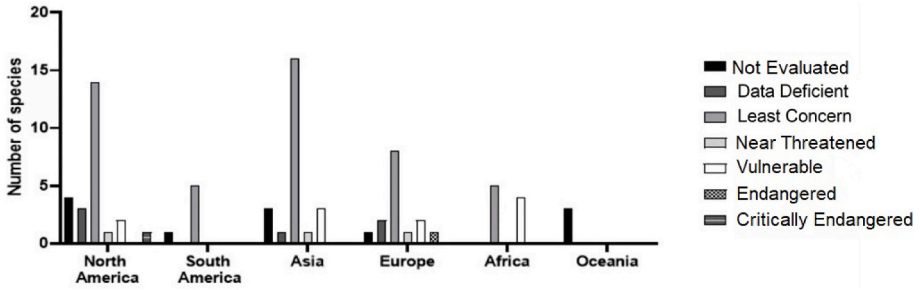

Fig. 2. Number of species studied according to their conservation status and the continent where the study was carried out.

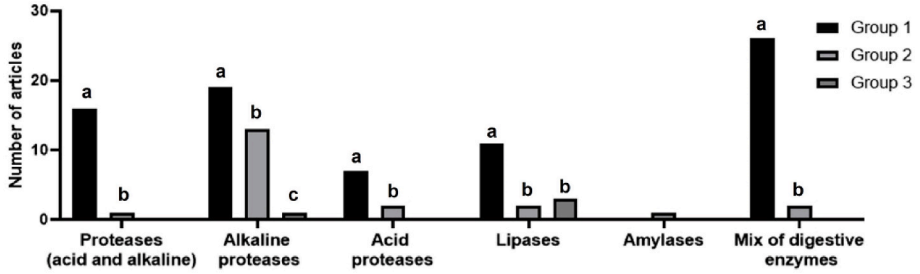

Fig. 3. Number of articles where each type of enzyme was studied according to the group. Lowercase letters indicate significant differences between the groups of items for each type of enzyme.

articles in group 1), studied different types as proteases, lipases, amylases, carboxypeptidases and esterases: 22.6% only alkaline proteases (trypsin and/or chymotrypsin), 20.2% acid and alkaline proteases, 13.1% lipases, 9.50% acid proteases (pepsins) and 4.80% amylases (Fig. 3). In 68.2% of the articles in group 2), the most studied digestive enzymes were alkaline proteases.

Table 2 shows the characteristics of the most studied enzymes of group 1) such as optimum and stable pH and temperature conditions and their molecular weight. The average value of the optimum pH found was 9; 8; 8.8; 7 and 2, and of the optimal temperature was 50; 55; 45; 42; 35 and 42 °C for intestinal proteases in general, trypsin, chymotrypsin, lipases, amylases and stomach proteases, respectively. The molecular weights of trypsins and intestinal proteases in general have been the most reported. The molecular weights for intestinal proteases are from 8 to 42 kDa (with an average of 25 kDa), for trypsins 22–24, for chymotrypsins 26 kDa, for lipases 35–70 kDa (with an average of 47 kDa) and for stomach proteases 17–31 kDa.

Another parameter to characterize digestive enzymes is through enzyme kinetics. Values of K_m (Michaelis–Menten constant) and Vmax (the maximal velocity) are usually indicated. Since the kinetic parameters reported were established at different temperatures, in different extracts (stomach, intestinal and pyloric cecum) and expressed in different units, reported K_m values were grouped together in similar conditions for different digestive enzymes. The substrates used to carry out these assays were: sAAPFpna (succinyl-l-Ala-l-Ala-l-Pro-l-Phe-p-nitroanilide) for serine proteases, BAPNA (Nα-benzoyl-dl-arginine-p-nitroanilide) for trypsins, hemoglobin for aspartic proteases, SApNA (succinyl-l-alanine p-nitroanilide) for chymotrypsins and p-NPP (p-Nitrophenyl Phosphate) for lipases. Further information on the enzyme kinetics of trypsins was reported. Most of the Km values for all enzymes were in the range of 0.03–0.08 mM.

Table 3 shows these same parameters together with the proposed biotechnological uses for the most studied enzymes of group 2. The proposed uses for these enzymes were mainly as additives in commercial detergents. It was reported that intestinal proteases, trypsins, lipases and α-amylases were stable in the presence of the solid detergents Dixan (Henkel, Spain), Ariel (Procter & Gamble, Switzerland), New Det (Sodet, Tunisia), and Axion (Colgate-Palmolive, France) at 30 °C.

In addition to testing the compatibility of enzymes with different detergents, the articles completed the enzymatic characterization by determining the stability of digestive enzymes in the presence of different compounds. The most commonly used were organic solvents (methanol, diethyl ether, hexane, acetone, and isopropanol), surfactants (SDS, Triton X-100, Tween 80) and oxidizing agents (sodium perborate). The assays were mostly performed with alkaline protease extracts for the species common dolphinfish (*C. hippurus*) (dos Santos et al., 2020), goby (*Z. ophiocephalus*) (Nasri, Sila, Ktari, Lassoued, Bougatef, Karra--Chaâbouni & Nasri, 2012 and Sila, Nasri, Bougatef, & Nasri, 2012) and thornback ray (*R. clavata*) (Lassoued, Hajji, Mhamdi, Jridi, Bayoudh, Barkia & Nasri, 2015) and these enzymes showed strong stability in the presence of all compounds.

Statistical analysis not showed significant differences in the number of studies where enzymes were purified or not purified between the manuscripts of group 1) enzyme characterization and those of group 2) enzyme characterization and biotechnological applications (Pearson's Chi-squared test = 0.60, df = 1, p-value = 0.44). 63% of the articles in group 1) the digestive enzymes were not purified, in 30.6% the enzymes were purified by different techniques and in the rest semi-purifications were performed. 50% of the articles in group 2) the digestive enzymes were not purified, in 40% they were purified and, in the remaining articles, semi-purification techniques were used. Only 4articles were included in group 3) technological uses only. In these articles an enzyme characterization was not performed but only biotechnological applications of lipases and alkaline proteinase were developed. The species studied were Atlantic bluefin tuna (*T. thynnus*), chinook salmon (*Oncorhynchus tshawytscha*) (in two of these articles), New Zealand hoki (*Macruronus novaezealandiae*) and annular sea bream (*Diplodus annularis*) and enzymes extracted from these species were used to hydrolyze and recover enzymes from other fish waste, to generate flavor compounds in milk and as thermostable compounds in detergents, respectively.

In only 4 articles digestive enzymes were immobilized for biotechnological applications. Proteases from Monterey sardine (*S. sagax caeurelea*) were immobilized into chitin and chitosan materials extracted from shrimp head waste (Salazar-Leyva et al., 2013, 2016) and lipases from chinook salmon (*O. tshawytscha*) to generate flavour compounds in

Table 2
Summary of the biochemical and kinetic parameters reported in the articles of group 1) enzymatic characterization for digestive enzyme. Opt. optimum. Stab. stability. K_m. Michaelis–Menten constant.

Enzymes/Species	pH		Temperature (°C)		Molecular weight (kDa)	K_m	Reference (detailed in Supplementary Information)
	Opt.	Stab.	Opt.	Stab.		(mM)	
Intestinal proteases							
sheepshead (*Archosargus probaacephalus*)	9	10		25–45	34.9; 27.8; 21.2		48
common snook (*Centropomus undecimalis*)	7; 11	4–10	65	35–45	25; 35		18
common dentex (*Dentex dentex*)	10	5–11	50	30	24.5; 69.5		1
skipjack tuna (*Katsuwonus pelamis*)	10	10	50	37–40	42		62
spotted rose snapper (*Lutjanus guttatus*)	9		50; 55				60
grey mullet (*Mugil cephalus*)	7.5	7–9	60	40	21.6		72
golden threadfin bream (*Nemipterus virgatus*)	9; 10		55				81
sardinelle (*Sardinella aurita*)	8	8–10	60	<40	14.2	0.033	30
turbot (*Scophthalmus maximus*)	9.5; 10						50
Canadian redfish (*Sebastes mentella*)	9.5; 10		35–40				50
Senegalese soles (*Solea senegalensi*)	9.5–10		37–40	50			2
gilthead sea bream (*Sparus aurata*)	10	5–12	50	30; 50	24.5		1; 3;22; 50
yellowfin tuna (*Thunnus albacares*)	10	10	50	37–40	21		62
tonggol tuna (*Thunnus tonggol*)	9	9	50	37–40	21		62
Atlantic bluefin Tuna (*Thunnus thynnus*)	10; 12		60		16.8–26.8		23
totoaba (*Totoaba macdonaldi*)	9; 11	8–12	45	35–55			19
Trypsins							
grey triggerfish (*Balistes capriscus*)	10.5	7–12	40	40	23.2	0.068	33
menhaden (*Brevoortia* spp.)	9.5	6–10	63	50	24		58
milkfish (*Chanos chanos*)	8		55–60		24.8; 22		16
gulf corvina (*Cynoscion othonopterus*)	7–9		55–65		24.4		24
shortfin corvina (*Cynoscion parvipinnis*)	8–9		55–65		23.6		28
orangemouth corvina (*Cynoscion xanthulus*)	7–9		55–65		23.7		28
grouper (*Epinephelus coioides*)	8–10	6–8	50	≤50	24		43
white croaker (*Micropogonias furnieri*)	9.5	5–11	60	55	24	0.081	56
mullet (*Mugil* spp.)	7–9	7–10	60	60	24		58
smoothhound shark (*Mustelus mustelus*)	8.5	7–9	50	40	24	0.387	8
palometa (*Parona signata*)	8.5	3–11	65	50	24		59
spotted goatfish (*Pseudupeneus maculatus*)	9		55	45	24.5		77
sardine (*Sardina pilchardus*)	8	6–9	60	<40	25		9
Monterey sardine (*Sardinops sagax caerulea*)	8	7; 8	30; 50	<30	25	0.13 and 0.051	4:10; 14
Monterey Spanish mackerel (*Scomberomorus concolor*)	9		50	40			55
tamilnadu (*Siganus canaliculatus*)	8		55				69
cunner (*Tautogolabrus adspersus*)	8.5		45				73
totoaba (*Totoaba macdonaldi*)	8		65		24.1		82
Chymotrypsins							
milkfish (*Chanos chanos*)	8		60				16
Monterrey sardine (*Sardinops sagax caeruleus*)	8	7–8	50	<30		0.074	12
tamilnadu (*Siganus canaliculatus*)	8		30				69
totoaba (*Totoaba macdonaldi*)	8		45		25.9		82
Stomach's proteases							
sheepshead (*Archosargus probaacephalus*)	2		45	25			48
snook (*Centropomus undecimalis*)	2	2–8	75	25–45			18
common dentex (*Dentex dentex*)	2–2.5	2–7	40	40			1
cod (*Gadus morhua*)	2–5		20				41
orange roughy (*Hoplostethus atlanticus*)	2.5; 3.5	2–6	37		33.5; 34.5		40
spotted rose snappe (*Lutjanus guttatus*)	2; 3		45				60
golden threadfin bream (*Nemipterus virgatus*)	2; 3		50				81
sardinelle (*Sardinella aurita*)	3	2–5	40	50	17	0.073	34; 86
Monterey sardine (*Sardinops sagax caerulea*)	2–2.5	3–6	45	30	30		7; 13
turbot (*Scophthalmus maximus*)	2		40	40	42		5; 83
beaked redfish (*Sebastes mentella*)	2		35–40				50
Senegal sole (*Solea senegalensis*)	2–2.5		37–40	50			2
gilthead sea bream (*Sparus aurata*)	2–2.5	2–7	40	50	67		1; 3;22; 50
Atlantic bluefin Tuna (*Thunnus thynnus*)	2; 3.5		50				23
totoaba (*Totoaba macdonaldi*)	2	2	35	35			19
α-amylase							
bogue (*Boops boops*)	7	12	30	30			24
annular seabream (*Diplodus annularis*)	6; 9	9; 12	45	60			24
cod (*Gadus morhua*)	5		20				41
common pandora (*Pagellus erytrhinus*)	7; 9	10; 12	35	30; 40			24
red porgy (*Pagrus pagrus*)	7	9; 12	45	50			24
red pandora (*Paguellus bogaraweo*)	4; 6; 8	11; 12	40				24
Brazilian flounder (*Paralichthys orbignyanus*)	7.5		40			0.054	21
rabbitfish (*Siganus canaliculatus*)	6.5		25	45; 50			68

(*continued on next page*)

Table 2 (*continued*)

Enzymes/Species	pH		Temperature (°C)		Molecular weight (kDa)	K_m	Reference (detailed in Supplementary Information)
	Opt.	Stab.	Opt.	Stab.		(mM)	
Lipases							
shortfin corvina (*Cynoscion parvipinnis*)	8		40–46		61.5; 36.0		28
gulf corvina (*Cynoscion othonopteru*)	8		40–45		65.8		28
orangemouth corvina (*Cynoscion xanthulus*)	8		40–45		69.5		28
cod (*Gadus morhua*)	7		25–35	<40			79
skipjack tuna (*Katsuwonus pelamis*)	10	10	60	37–40			62
golden grey mullet (*Chelon auratus*)	8	7.5–9	50		35		76
stripped bass (*Morone saxatilis*)	8		36–45				66
grey mullet (*Mugil cephalus*)	8	4–10	50	10–50		0.22	5
chinook salmon (*Oncorhynchus tshawytscha*)	8–8.5		35	30			39
Brazilian flounder (*Paralichthys orbignyanus*)	8.5–9		37				21
cobia (*Rachycentron canadum*)	7		20				77
sardinelle (*Sardinella aurita*)	9		37		43		74
yellowfin tuna (*Thunnus albacares*)	10	10	60	37–40	60		62
Pacific bluefin tuna (*Thunnus orientalis*)	8		35–45				66
tonggol tuna (*Thunnus tonggol*)	9	9	60	37–40			62
totoaba (*Totoaba macdonaldi*)	8		37–45		70.4; 47.5		66

Table 3
Summary of the biochemical parameters reported in the articles of group 2) enzymatic characterization and biotechnological applications for digestive enzyme. Opt. pH or temperature optimum. Stab. Stability at the different conditions of pH and temperature.

Enzymes/Species	pH		Temperature (°C)		Molecular weight (kDa)	Biotechnological uses	Reference (detailed in Supplementary Information)
	Opt.	Stab.	Opt.	Stab.			
Proteases							
grey triggerfish *(Balistes capriscus)*	8	5–9	40			Oligosaccharides production	92
striped Seabream *(Lithognathus mormyrus)*	10	5–10	50	30–40		Deproteinization of shrimp waste and laundry detergents	87
red snapper *(Lutjanus vitta)*	8		60; 65		24	Gelatin hydrolyzate	93
golden grey mullet *(Chelon auratus)*	8	4–10	60	40		Deproteinization of shrimp waste and laundry detergents	88; 89
Priacanthus taandenus	8		60		22	Laundry detergents	93
thornback ray *(Raja clavata)*	8	8–11	50	30; 40		Liquid laundry detergents	98; 107
zebra blenny *(Salaria basilisca)*	8	6–11	55–60	30; 40		Gelatin hydrolyzate	96
Indian oil sardine *(Sardinella longiceps)*	9; 10	9	50	60	60; 65	Laundry detergents	103
Spanish mackerel *(Scomberomorus guttatus)*	10	8–12	60	10–40		Laundry detergents	104
large red scorpionfish *(Scorpaena scrofa)*	10	5–12	55			Detergent additive and deproteinization	108; 107
grass goby *(Zosterisessor ophiocephalus)*	8; 10	6–11	50; 55	20–50	23.2	Chitin extraction, detergent additive and deproteinization of shrimp waste	101; 107
Trypsins							
chihuil sea catfish *(Bagre panamensis)*	11	9–12	45; 50		29	Hydrolyzate production	105
dolphinfish *(Coryphaena hippurus)*	8	5–10	40		26	Detergents additive	91
zebra blenny *(Salaria basilisca)*	9.5	7–12	60	30; 40	27	Detergents additive	95
albacore tuna *(Thunnus alalunga)*	9	6–11	55	50	30	Proteolytic degradation of fish muscle	102
grass goby *(Zosterisessor ophiocephalus)*	9	7–11	60	40; 30		Detergent additive	100
Lipases							
Tunisian barbel *(Barbus callensis)*	8	5–9	50			Oligosaccharides production	92
α-amilases							
barramundi *(Lates calcarifer)*	8		50	<50	60	Defatting of fish skin	106
zebra blenny *(Salaria basilisca)*	3	2–7	50			Gelatin extraction	94
sardinella *(Sardinella aurita)*	6; 8	5–9	40			Oligosaccharides production	92

milk. All biochemical parameters that were studied for each enzyme in the selected articles are detailed in Supplementary Table 1A.

The Fig. 4 summarizes the number of articles in which different studies were carried out with different digestive enzymes. The most studied digestive enzymes for the three groups of articles was alkaline proteases, especially trypsin followed by acid proteases. The most used biochemical parameters to characterize these enzymes was the effect of pH, temperature and inhibitors on enzyme activity and the electrophoresis technique to know their molecular weight. The alkaline proteases, in turn, were the most studied for their biotechnological application.

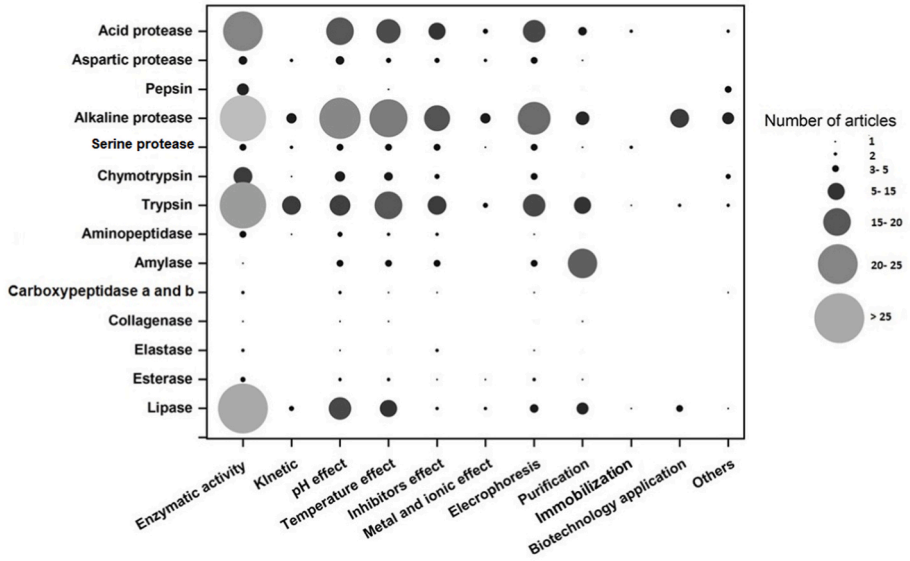

Fig. 4. Number of articles per enzyme characterized according to different biochemical parameters and biotechnological applications.

4. Discussion

4.1. General information from manuscripts

Even though we found a trend in the analysis of the data obtained, it was important to conduct a systematic review in order to carry out the literature review in a rigorous and transparent manner. Systematic reviews are considered the most reliable method through which all available evidence can be identified, synthesized and evaluated, in addition to ensuring future replication of the search through a protocol (Mallett et al., 2012).

According to the selected articles and the years of the publications, it can be concluded that from approximately twenty years ago to the present, the interest in using biomolecules such as digestive enzymes present in fishery residues giving them an added value to incorporating them in some type of industry is increasing. This same increase was reported by the annual State of World Fisheries and Aquaculture 2020 report (FAO, 2020).

The species used as a source of these enzymes are very diverse, although some groups are more common, due to their high production values and therefore large amounts of waste. Although the world production of marine fish is increasing, this boom is not the same all over the world. Based on the data reported by FAO (2020), fish wastage rates are highest in North America and Oceania, where about half of all fish captured is discarded at the consumption stage. In Africa and Latin America, fish wastage is mainly due to lack of infrastructure and inadequate technical knowledge of fish preservation, so it would be important to encourage and support this type of studies that propose the recovery of biomolecules present in these wastes and their potential reutilization. Of all the research included in this review, most was conducted on the Asian continent and this is related to the fact that China was the largest producer of fisheries and marine aquaculture in 2020 (FAO, 2022). However, we found a higher concentration of publications in only four countries: Tunisia, Mexico, China and Spain, where, although a great number of studies were published, the species studied tended to be repeated. For example, in Tunisia the studies were carried out with the species zebra blenny (*Salaria basilisca*), golden grey mullet (*Mugil auratus*), sardinella (*S. aurita*) and grass goby (*Z. ophiocephalus*), in Mexico with different species of sardines and totoaba (*T. macdonaldi*). Despite the fact that there are important fishing ports in South America, such as Brazil and Argentina, we only found 5 articles out of 112 that were carried out in this region, where species distributed in the Southwest Atlantic were studied. This may be due to the fact that although at a local level there are species that represent an important volume of production, when the comparison is at a global level (as the objective of this work) there are other regions where species with much higher values are produced. For example, the common hake (*Merluccius hubbsi*) is the main fishing resource of the Argentinean Sea with annual catches of 291 thousand tonnes (MAGYP, 2022) and in other regions, such as the North Atlantic and the Indo-Pacific, the main marine resources are species such as Atlantic salmon (*S. salar*) and milkfish (*C. chanos*) whose production reaches 2 719.6 and 1 167.8 thousand tonnes respectively (FAO, 2022).

Although biotechnological applications of enzymes recovered from waste is a growing topic of interest (Andler & Goddard, 2018; Kannah et al., 2020; Nayak & Bhushan, 2019; Osorio et al., 2021; Zhong et al., 2020), in this systematic review the number of articles on food and basic biology of marine fish digestive enzymes was higher than the number of articles on applied biology and this was also reflected in the scope of the journals where these investigations are published. The articles were published mainly in food journals since there is a large market in the food industry for enzymes recovered from fish. Enzymatic processing can improve physical, chemical and organoleptic properties. physical, chemical and organoleptic properties of foods so that these enzymes can be used in numerous forms, such as milk replacers, beverage stabilizers, protein supplements and animal feeds (Wangkheirakpam et al., 2019).

4.2. Species

Regarding the taxonomic classification of the species used in the manuscripts, the most represented order was Perciformes, this was to be expected since it is the largest vertebrate order, including 40% of all fish species (Nelson et al., 2016). In the articles reviewed, some species were used more than others as enzyme sources and this may be related to the availability and access to samples of each species, exploited as fishery resources, and/or the commercial interest of them; some species are usually more well-known and chosen by consumers, therefore, they are fished more than others. Alarmingly, several of these species used in various investigations, have a conservation status of concern according to IUCN categories (IUCN, 2020). This can be related to the fact that at least 34% of marine fish stocks in the world's fisheries are currently classified as overexploited (FAO, 2020) which leads the reduction of fish size, lower catches, scarcity of hydrobiological resources and places the species at risk of extinction (Narváez et al., 2013). It is important to note

that the studies with enzymes extracted from totoaba (*T. macdonaldi*) the species with the most worrying conservation category, are recent; they have been conducted during the last four years. Totoaba is a large endemic species in Mexico, which has been under extreme poaching pressure due to its valuable swim bladder. One of the conservation strategies is through the cultivation of this species to compete against illegal sales (González-Félix et al., 2021). Several articles have characterized the digestive enzymes of this species and proposing dietary changes to produce high-value totoaba swim bladders from aquaculture at substantially lower costs (Villanueva-Gutiérrez et al., 2020; Córdova-Montejo et al., 2019; González-Félix et al., 2018; Rueda-López, Martínez-Montaño, & Viana, 2017).

Tuna (Thunnus spp.) and tuna-like species play a very important role in the economy of many countries because they are widely consumed species worldwide. However, they are in a worrying state of conservation due to heavy fishing pressure (Pons et al., 2017). In turn, in the tuna canning process, only one third of the whole fish is used, so this industry generates up to 70% of solid waste. These protein-rich by-products are usually used to obtain products of low commercial value, such as fishmeal and fertilizer (Herpandi et al., 2011). Fortunately, a large number of authors (Poonsin et al., 2019; Rueda-López et al., 2017; Kihara, 2015; Prasertsan & Prachumratana, 2008; Klomklao, Benjakul, & Visessanguan, 2004; Kim, Jeon, Byeun, Kim, & Lee, 1997) have characterized the digestive enzymes of tuna species and have proposed an alternative use of these by-products as functional food ingredients by obtaining protein hydrolysate.

It is necessary to know the conservation status of the species that we study in order to avoid incentives for their fishing exploitation and to promote the management and implementation of different regulatory measures from other areas to protect them. One way could be to study the ecology and behavior of the species and fishing (Pereira et al., 2021). This information is also relevant for those species that are exploited but there is still insufficient data to assign them a conservation category.

4.3. Enzymes

From the information collected, it was possible to visualize that most of the enzymatic characterization of fish digestive enzymes is carried out through physiological and biochemical parameters. Before proposing a potential biotechnological use for the enzymes, optimal and stable conditions of enzyme activity at different pH values and temperatures are determined. In turn, to know the identity of the enzymes in the extracts, the electrophoresis technique is used to establish the molecular weights. There are a large number of current articles that have focused on characterizing the digestive enzymes of different fish species using these parameters (Nolasco-Soria, 2021; Champasri et al., 2021; Friedman et al., 2021; González-Félix et al., 2020; Villanueva-Gutiérrez et al., 2020).

To reduce processing costs, the articles do not generally purify digestive enzymes, but rather make determinations from protein extracts. In some cases, digestive enzymes were semi-purified, however, according to the general count, in a few articles of group 1 (enzyme characterization), enzyme purification was performed. As for the articles in group 2 (characterization and biotechnological uses), it was assumed that in most of the articles the enzymes would be purified before being used for the formulation of a value-added product. Semi-purification and purification processes provide certain advantages such as avoiding side effects produced by the presence of other unknown enzymes, increasing catalytic efficiency and maintaining the activity of the chosen enzyme stable and is a promising option as they can contribute to the development of a green and sustainable industry (Mardina et al., 2020) by reducing the environmental impact caused by the inadequate disposal and treatment of these wastes. However, in 50% of the publications corresponding to this group, the enzymes were not purified. Although these enzymes are raw materials that are available in fish waste and can be obtained at no extra cost (Khangembam & Chakrabarti, 2015), the process of purifying them is time-consuming, costly and difficult to scale up to an industrial level (Labrou, 2014). The recovery of biological products has an important impact on the cost of the product due to the fact that separation and purification represents an important fraction (70–80%) of the total cost of the bioprocess (Aguilar & Rito-Palomares, 2010Aguilar & Rito-Palomares, 2010). A possible alternative could be to integrate separation and purification techniques with a minimum number of possible steps (Schoemaker et al., 2003). Is for these reasons that in the articles where the enzymes were not purified there were incorporated as crude extracts since it is a more cost-effective way (Niyonzima & Más, 2015).

In the manuscripts where purifications were performed, the affinity chromatography method was the most specialized for efficient protein purification compared to other separation methods by offering high selectivity, resolution and capacity in many protein purification procedures (Premetis & Labrou, 2020).

The most studied enzymes in the articles reviewed were the alkaline proteases, mainly trypsin. Among the digestive proteases involved in the digestive physiology of fish, trypsin plays a crucial part in the activation of other zymogens and is therefore considered a central enzyme in protein digestion (Azevedo et al., 2018). In addition, trypsin was the first enzyme discovered and one of the first proteolytic enzymes isolated in pure form in sufficient quantities for accurate chemical and enzymological studies. For these reasons, its conformation, properties and mechanism of action have been studied with sufficient detail (Bougatef, 2013) and there is a large amount of work where the study of this enzyme has been given relevance over other digestive enzymes.

Alkaline proteases are considered the most important group of industrial enzymes with extensive uses in the leather, food and pharmaceutical industries although their main application is as additives in the detergent industry (Bougatef, 2013). This corresponds with the results obtained from the present review where the biotechnological applications for these enzymes were biased towards one type of use as additives in commercial detergents. These enzymes are characterized by being stable over a wide pH and temperature range and inactivated at relatively low temperatures (Poonsin et al., 2019). The high stability of digestive enzymes in the presence of detergents has also been reported by the following authors El-Hadj Ali et al. (2011), dos Santos et al. (2020) and Rios-Herrera et al. (2020) for the species striped seabream (*Lithognathus mormyrus*), common dolphinfish (*Coryphaena hippurus*) and chihuil sea catfish (*Bagre panamensis*), respectively.

The use of proteases, especially alkaline proteases with lipases, is a safer and more sustainable alternative than the use of non-biodegradable and traditional chemicals present in detergents (surfactants, oxidants and other additives) because they offer several advantages as lower energy cost, specificity, removal of difficult stains and ability to degrade before entering watercourses, thus avoiding water contamination. Upon addition of these enzymes, protein material or insoluble triglycerides are liberated from the stains by catalyzing the breaking of chemical bonds when water is added (Mardina et al., 2020; Naganthran et al., 2017). Although most of the papers in group 2 propose the use of fish alkaline enzymes for their application in detergents, we found few papers on the potential application of digestive enzymes in industrial processes.

It is important to know which compounds are present in the industrial processes and products where fish digestive enzymes are to be incorporated as functional ingredients. In addition to studying the compatibility of enzymes in the presence of detergents, favorable results were found for alkaline enzymes in the presence of surfactants such as the family of Tween (20 and 80). Digestive enzymes stable to these compounds could be included in the food industry (e.g. milk, cream, beverages and mayonnaise), in the cosmetic industry for their optimal dispersion and encapsulation properties and in pharmaceutical formulation, where these surfactants are used to stabilize proteins (Salvia-Trujillo et al., 2017). Enzyme stability in the presence of these compounds was reported for different species such as red scorpionfish

(*Scorpaena scrofa*) (Younes et al., 2015) and Indian oil sardine (*Sardinella longiceps*) (Ramkumar et al., 2018).

Although enzyme kinetics assays were scarcely considered in the selected articles, they are important for understanding the mechanisms of enzymatic hydrolysis that are part of enzyme characterization. Processes such as enzymatic hydrolysis provide a considerable result in producing bioactive compounds, allowing the conversion of proteins into functional ingredients (Haryati et al., 2021). According to the information obtained from the articles, there were coincidences between K_m data for the same enzymes present in other species. Similar K_m values for aspartic enzymes were reported for sea bream (*S. aurata*) (Zhou et al., 2007), and Atlantic cod (*G. morhua*) species (Gildberg, Olsen, & Bjarnason, 1990). For the trypsins and chymotrypsins from sardines, similarities were found with the K_m of the trypsins from anchovy (*Engraulis japonica*) and japanese sardine (*Sardinops melanostictus*) (Castillo-Yáñez et al., 2005). The enzymatic kinetics have been more studied for trypsins, so the comparison between other species was easier to carry out since there is more information available. In contrast, no information was available to compare the K_m of lipases and amylases. Only one data for lipases from chinook salmon (*O. tshawytscha*) and New Zealand hoki (*M. novaezelandiae*) but the K_m values were much lower (0.078 and 0.068 mM, respectively) (Kurtovic et al., 2010).

The number of publications that exclusively studied acid proteases (pepsins) or other alkaline enzymes such as chymotrypsin, elastase, lipases and amylases were low. There is a vacancy in the characterization of digestive enzymes other than only alkaline proteases, so, in order to expand the field of knowledge about fish digestive enzymes, the study of these enzymes and their application as bioactive compound in various industrial uses can be explored in depth. According to research over the past few years, it has been reported that pepsins can be used in the production of gelatins (Bkhairia et., 2016), to extract collagen from skins (Gomez et al., 2018) and to replace conventional milk coagulation enzymes, such as chymosin (Osuna-Ruiz et al., 2019); lipases are used in the food and dairy industry for the hydrolysis of milk fat contributing to improve the flavor of cheeses, creams and other dairy products (Sae--Leaw & Benjakul, 2018) and amylases can be used in the production of oligosaccharides (Hmidet et al., 2013). In addition, lipases and amylases are used in different industries such as pharmaceutical, leather, textile, cosmetics and paper (Houde et al., 2004).

In a few articles the enzymes were immobilized for later use in some of the biotechnological processes mentioned above. The immobilization process improves the stability of the fish enzymes with the aim of enhancing the applicability of these valuable biocatalysts (Salazar-Leyva et al., 2013). This immobilization can be carried out by means of natural and easily obtained polyaminosaccharides such as chitosan and chitin, the latter being one of the most abundant and renewable organic resources in the world. As functional materials, chitin and chitosan offer a unique set of environmentally friendly characteristics such as biocompatibility, biodegradability into harmless products, chelation of heavy metal ions and remarkable affinity with proteins (Krajewska, 2004). Authors immobilized of digestive enzymes to treat fishery by-products. Salazar-Leyva et al. (2013) immobilized semi-purified acid proteases from Monterey sardine stomachs on chitin and chitosan materials extracted from shrimp head waste and Liu and Dave (2022) used Alcalase immobilized on chitosan-coated nanoparticles to extract oil from Atlantic salmon (*S. salar*) by-products.

Regarding the articles (group 3) it was not usual to find articles about only technological uses, since before applying an enzyme in an industrial process at laboratory scale, the biochemical parameters of the enzymes involved and their stability in the presence of different agents (organic solvents, surfactants and oxidizing agents, among others) are usually described in detail. These agents are present in the formulations of the commercial products in which it is potentially desired to incorporate the enzymes and thus obtain a value-added product.

5. Conclusions

Based on the information obtained from the selected publications and their subsequent analysis, we conclude that most of the reviewed articles are based on the knowledge of fish physiology through the characterization of digestive enzymes. However, there is an area of vacancy in research focused on the biotechnological application of these enzymes. In farmed fish species, it is important to know the feeding habits, nutritional requirements and digestive capacities of the different species since through the functional characteristics of the enzyme, valuable information can be obtained on the factors that affect the net efficiency of food processing. At the same time, fishing is an activity that is increasing on a global scale and, consequently, the amount of solid waste is also increasing, so the knowledge and use of the enzymes present in the waste will allow proposing new viable, productive and environment friendly alternatives in order to provide novel useful products of higher value and improve the economic returns of the fishing industry without increasing overfishing. Only a few papers on the study of digestive enzymes from species of the Southwest Atlantic Ocean were found, so this is another area of vacancy.

Since proteases are the most widely used group of enzymes in the bioprocess industry, those species with carnivorous/omnivorous habits will have a high content of these enzymes, so they may be good candidates for technological uses. In turn, it is very important to take into account the production in tons of this species and its consequent volume of waste generated by said processing. Taking these factors into account and the global survey on the main group of species produced in 2020 (FAO, 2022), the species of marine fish that could be suggested as susceptible to biotechnological use are Atlantic salmon (*S. salar*), milkfish (*C. chanos*), anchovy (*Engraulis ringens*), yellowfin tuna (*T. albacares*), skipjack tuna (*K. pelamis*) and sardines (*Sardina pilchardus* and *S. sagax*).

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. Literature reviewed for this study are available in the Supplementary Information.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aaf.2023.01.002.

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