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Fish Viscera Hydrolysates and Their Use as Biostimulants for Plants as an Approach towards Circular Economy: A Review

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Abstract: Crop production has become a priority issue in the last few years due to the exponential growth of the world population and the need to substitute chemical fertilizers. This last matter is under the spotlight to achieve a more sustainable approach in a cost-effective way. Biostimulants have gained attention as an alternative to chemical fertilizers. Although they are not considered fertilisers as inputs of nutrients, they stimulate plants' nutrition and tolerance to stress, among other characteristics. Amino acid-based biostimulants have been found to be effective in literature. This review focuses on the effectiveness of biostimulants, their presence in the global market, and mainly in their production with fish by-products as a source, using enzymatic hydrolysis and autolysis, focusing especially on fish viscera, their possibilities in the agricultural sector and their availability in Europe for possible opportunities. Fish viscera protein hydrolysates for biostimulant production seems a feasible alternative to fishmeal production in Europe, mainly in areas located far away from fishmeal plants.

Keywords: fish viscera; enzymes; hydrolysis; autolysis; biostimulant; amino acids

1. Introduction

Fisheries can be economically the most important sector in many countries. Among them we can find China, Chile, Norway, Egypt, and Nigeria [1]. According to FAO [1], worldwide fishing and aquaculture production increased in 2020, reaching 90.3 million tonnes and 87.5 million tonnes, respectively. In parallel to fish, the volume of fish by-products is also growing consequently. Fish by-products (head, skin, scales, bones, and viscera) constitute between 60 and 70% of the whole fish's weight [2]. By-products can be disposed or used for other purposes, due to their high protein and oil content, such as production of fishmeal or fish protein hydrolysates with bioactive peptides. If not used, they may cause environmental, health, and economic problems [3]. Therefore, the valorisation of fish by-products, especially viscera, must be fully implemented to meet societal needs. As another example, transformed by-products can be used as ingredients for the formulation of bio-based fertilizers or biostimulants. This alternative may be interesting in areas where there are no fishmeal production plants in the nearby. Thus, nowadays there are already some fertilizers produced from fish by-products in the market, some of them even authorized for organic agriculture [4]. Additionally, the combination of hydroponics and biostimulants presents a promising environmentally friendly production strategy for the next decades for vegetable growth that requires further research [5].

2. Bio-Based Fertilizers for Plants

Due to the exponential growth of the human population, the exploitation of natural resources is rising. These resources are, in most cases, non-renewable and perishable. Therefore, the use of

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alternative sources for food production should be focused to maintain the stability of food availability [6].

In the case of fertilizers, most of them need phosphate rock to be produced. Around 90% of the phosphate rock is imported, which makes the EU very vulnerable with the rise of prices of raw materials. It is expected that the demand of phosphorus and nitrogen will increase in the next years, which could lead to geopolitical problems [6,7]. Also, the use of phosphorus and nitrates in fertilizers provokes leaky losses in soil, and nutrients can run off into surface waters, producing pollution of environments and causing harm to aquatic ecosystems [6]. To reduce the number of toxic nitrates in the soil, the EU Council Directive of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources (91/676/EEC) called for a significant reduction in the number of nitrogen-containing fertilizers used in agriculture and horticulture. Therefore, it is necessary to find new alternatives to minerals, like recycled organic matter, and to improve the efficiency in the fertilization, reducing its environmental impact. There is also a need to recirculate nutrients, closing the loop and preventing their dissipation into the environment. For fertilizers, typically composed of minerals, part of the raw materials could be substituted with residual biomass coming from animals [8].

In this way, animal by-products that might otherwise end up in a landfill could be revalued and repurposed, enhancing resource efficiency. In fact, the availability of raw materials to produce bio-based fertilizers (BBFs) is abundant, and farmers from different countries within Europe have common preferences for BBFs with similar nutrient content but lower prices than chemical fertilizers [9]. BBFs are a promising alternative to traditional synthetic fertilizers and can help to promote sustainable agriculture practices.

However, several disadvantages and barriers have been identified with bio-based fertilizers. These include unpleasant odours, time-consuming processing, variable composition, and the slow mineralization and release of nutrients [6,10]. Additionally, BBFs face challenges related to the presence of antibiotic and microplastic residues, toxicity of heavy metals, pathogen exposure, and accumulation of salts. Despite these issues, BBFs offer opportunities to improve crop quality, enhance micronutrients uptake in plants, promote soil biodiversity, and aid in soil remediation [6].

2.1. EU Fertilizer Product Regulation

In the fertilizing product regulation 2019/1009 of the European Commission [11], broader types of products are covered apart from fertilizers. Also, end-of-waste status is laid down, with which material that constitutes waste can cease to be waste if it is contained in a compliant EU fertilizing product.

According to Regulation 2019/1009 [11] and as shown in ***Error! Reference source not found.***, products are categorized into 7 Product Function Categories (PFCs). Fertilizers are products which function is to provide nutrients to plants or mushrooms, and they can be organic, organo-mineral, or inorganic. A liming material's function is to correct soil acidity. Soil improvers (inorganic or organic) improve or protect the physical or chemical properties, structure, or biological activity of the soil to which it is added. Growing medium's function is to maintain, improve or protect the physical or chemical properties, structure, or biological activity of the soil to which it is added. Inhibitors improve the nutrient release patterns of a product providing plants with nutrients by delaying or stopping the activity of specific groups of microorganisms or enzymes, related with nitrification, denitrification, or urease activity. Plant biostimulants' function is to stimulate plant nutrition processes independently of the product's nutrient content with the aim of improving one or more characteristics of the plant or plant rhizosphere (nutrient use efficiency, tolerance to abiotic stress, quality traits, and availability of confined nutrients in soil or rhizosphere), and they can be microbial or non-microbial. Fertilizing product blends are made of two or more fertilizing products that are already CE-marked.

An EU fertilizing product will consist only of component materials complying with the requirements for one or more of the Component Material Categories (CMCs) listed in ***Error! Reference source not found.***. Products are composed of at least one CMC or might contain more than

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one CMC. CMC 3, 4, 5, 12, 13, 14, and 15 must undergo a defined recovery operation and resulting recovered materials must comply with relevant requirements.

Table 1. Product Function Category (PFC) of products according to Regulation 2019/1009 of the European Commission [11].

Product Function Category (PFC)
1. Fertilizer
a) Organic
b) Organo-mineral
c) Inorganic
2. Liming material
3. Soil improver
4. Growing medium
5. Inhibitor
6. Plant biostimulant
7. Fertilizing product blend

Table 2. Component Material Category (CMC) of the products according to Regulation 2019/1009 of the European Commission [11].

Component Material Category (CMC)
1) Virgin material substances and mixtures
2) Plants, plant parts or plant extracts
3) Compost
4) Fresh crop digestate
5) Digestate other than fresh crop digestate
6) Food industry by-products
7) Microorganisms
8) Nutrient polymers
9) Polymers other than nutrient polymers
10) Derived products within the meaning of Regulation (EC) No 1069/2009 (animal by-products)
11) By-products within the meaning of Directive 2008/98/EC (industrial by-products)
12) Precipitated phosphate salts and derivates (struvite)
13) Thermal oxidation materials and derivates (ash)
14) Pyrolysis and gasification materials (biochar)
15) Recovered high purity materials

Regarding safety of plant biostimulants, according to Regulation 2019/1009 of the European Commission [11] contaminants must not exceed the limit values established for certain elements (***Error! Reference source not found.***). The information provided by the producer must be the physical form of the biostimulant, the production and expiry date, application method, relevant instructions related to the efficacy, and the effect claimed in the target plants. The regulation 2019/1009 obliges to demonstrate with evidence the effects claimed on the label of the biostimulant, and such evidence can be that published in the literature or come from experimental data from field trials [12].

Table 3. Contaminant limit values (mg/kg dry matter) for plant biostimulants according to Regulation 2019/1009 of the European Commission [11].

Contaminant	Limit value (mg/kg dry matter)
Mercury (Hg)	1
Cadmium (Cd)	1.5
Hexavalent chromium (Cr VI)	2
Inorganic arsenic (As)	40

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Nickel (Ni)	50
Lead (Pb)	120
Copper (Cu)	600
Zinc (Zn)	1500

2.2. Biostimulants

As an alternative to chemical fertilizers we can find biostimulants, formulated products of biological origin which can be used to stimulate plant growth and increase yields [12]. Unlike bio-based fertilizers, they do not provide nutrients directly to the plants, but they facilitate acquisition of nutrients though. Biostimulants act on the metabolic and enzymatic processes of plants improving productivity and crop quality [13]. Animal sources for biostimulants are usually hydrolysates of by-products, normally produced using alkaline or enzymatic hydrolysis [13] and food waste streams are considered important precursors for biostimulant development.

Depending on the composition and the expected results, biostimulants can be soil-applied or leaf-applied, and their effects are species-specific and product-specific [14]. For addressing this specificity, information and data are required that would enable farmers to discriminate among products with different levels of effectiveness [15]. Leaf permeability is a crucial factor as well as the penetration of biostimulants into plant tissue is a necessary condition for a reliable efficiency [16]. Also, foliar application increases amino acid and peptide availability for plant uptake by reducing the competition with microorganisms comparing to soil application [17].

The biggest consumer and producer of biostimulants globally is the United States [18]. Farmers, investors, regulators, consumers, and scientists are still learning about biostimulants and their role in agriculture [19]. Among the most important barriers to introduce bio-based fertilizers and biostimulants in Europe are the low level of technological readiness, the biological wastes collection system (to ensure the availability of secondary raw materials) and the legislation [8].

2.3. Amino Acid-Based Biostimulants

Studies showed that organic compounds like amino acids promote the growth of several horticultural plants [20–23]. Synthesis of amino acids demands a high energy consumption in plants, so their foliar application in agriculture is a usual practice, as it allows plants to save energy on amino acid synthesis by regulating nitrogen acquisition in roots to increase the pace of their reconstruction, especially during critical times like transplantation or climatic stress [24,25]. Amino acids are also used as chelators of metal ions. Combination of essential micronutrients with amino acids in form of aminochelate fertilisers can accelerate their absorption and transport within the plant and thus enhance plant growth [26,27].

Glutamic acid plays an important role in the biosynthesis of proline and other nitrogen-containing compounds, apart from having a positive effect on the photosynthetic activity and major production of fruits in plants [22,28]. In literature, glutamic acid is found as the most abundant amino acid in fish protein hydrolysates produced from both enzymatic hydrolysis and silage [29–31].

Apart from glutamic acid, other amino acids have beneficial effects in crops. According to the work reviewed by Ashraf et al. [32], proline stimulates plant defences to biotic and abiotic stress. In fact, several studies have shown that proline accumulation was high in cells adapted to high concentrations of salt [33] that it mitigates the effect of sodium chloride on cell membrane disruption [34], and that proline neutralizes the increased ethylene production in stressed plants suffering from drought [35]. Flores et al. [36] reported that arginine plays an important role in nitrogen storage and transport in plants during both biotic and abiotic stress. However, tyrosine, methionine and lysine are the amino acids usually applied in biostimulants [23].

Some examples of commercially available amino acid-based plant biostimulants are Radifarm produced by Valagro (Atessa, Italy), which stimulates root growth, Megafol produced by Valagro (Atessa, Italy), that stimulates plant growth during abiotic stress, and C-BIO SeaActiv, produced by C-BIO (Dunkineely, Ireland) and derived from fish, it increases plant resistance to insects, heat, and drought).

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3. Fish Protein Hydrolysates

After the production of fishmeal, the production of fish protein hydrolysates (FPHs) is the most common valorisation route for fish by-products. Fish protein hydrolysates are rich in soluble proteins, with high digestibility, and sometimes even antioxidant and antimicrobial properties [37], among others. FPHs contain mainly free amino acids and low molecular weight peptides, and hydrolysis can be alkaline, acidic, or enzymatic.

3.1. Chemical Hydrolysis

Alkaline and acidic hydrolysis require strong experimental conditions and extreme precautions [38,39], and such conditions attack all peptide bonds, getting a very high degree of hydrolysis and releasing free amino acids. However, it also brings the destruction of several amino acids: tryptophan is usually destroyed with acidic hydrolysis, cysteine, serine, and threonine are partially lost, and asparagine and glutamine are converted into their acidic forms [17]. Large use of acids and alkalis can lead to an increase in salinity in the FPH too. In addition, during chemical hydrolysis there is racemisation. This means that there is a conversion of the free amino acids from the L-form to D-form, which may cause some problems in terms of the effectivity of the FPHs, for example as biostimulants, as plants cannot directly use D-form amino acids in their metabolism [17].

3.2. Enzymatic Hydrolysis

Given the disadvantages associated with the chemical hydrolysis, enzymatic hydrolysis is the most widely used due to its mildness, ease of control and lack of residual organic solvents [40]. Enzymes hydrolyse proteins more gently than chemicals, allowing the production of low molecular weight peptides, dipeptides, or even free amino acids depending on the experimental conditions. A schematic flowchart of the production of fish protein hydrolysates using enzymes is shown in Figure 1.

Enzymes used in hydrolysis can be classified into two types based on their origin. Exogenous enzymes, which are commercially available, are added to the sample to be hydrolysed, and they are typically extracted from plants, animals, and microbes. Among these, Alcalase is the most referenced in literature [41–45], but others like Protamex [41,46] Flavourzyme [29,46] and Protana Prime [30] are also used. Some examples of published works on enzymatic hydrolysis of fish with commercial enzymes are shown in Error! Reference source not found..

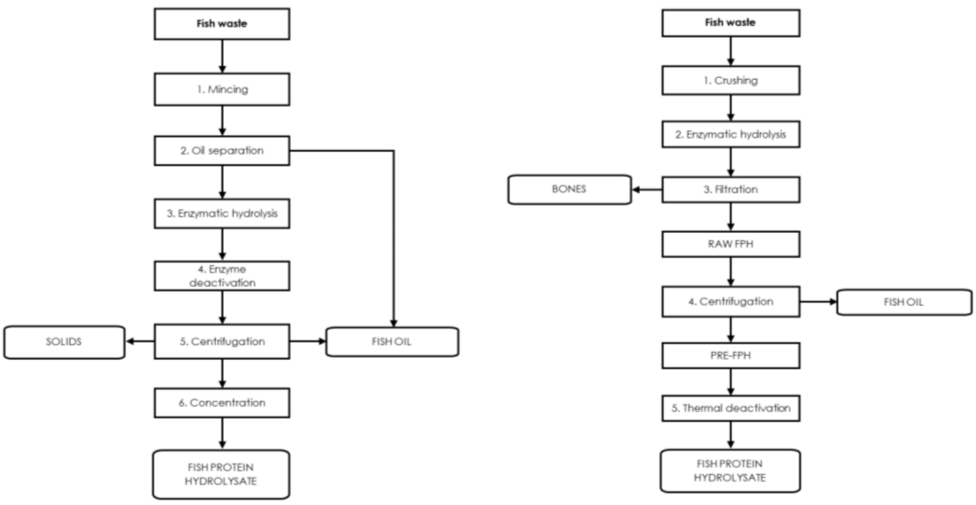

Figure 1. Different schematic flowcharts of enzymatic hydrolysis of fish waste. Compiled from Domínguez *et al*. [30] on the left and Vázquez *et al*. [37] on the right.

Table 4. Examples of literature on the use of commercial enzymes to produce fish protein hydrolysates and the experimental conditions applied.

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Reference	Raw material	Enzyme	Dose (% of Protein)	pH	Temperature (°C)	Time
Valcarcel *et al.* [44]	Seabream heads Seabass heads	Alcalase	0.2	8.2 8.5	57.1 58.4	3 3
Garofalo *et al.* [42]	Tuna viscera	Alcalase	1	8.5	55	2
Vázquez *et al.* [45]	Rainbow trout frames and trimmings Salmon heads	Alcalase	0.1 0.2	8.3 9	56 64	3 3
Aspmo *et al.* [41]	Cod viscera	Alcalase	1 g/100 g of sample	Unadjusted	55	24
Ramakrishnan *et al.* [43]	Mackerel waste	Alcalase	0.5	7.5	55	1
Domínguez *et al.* [30]	Rainbow trout viscera	Alcalase + Protana Prime	1	7	60	7
Korkmaz *et al.* [46]	Trout waste	Alkaline protease	1 (enzyme /substrate)	8	60	1
Aspmo *et al.* [41]	Cod viscera	Protamex	1 g/100 g of sample	Unadjusted	55	24
Alahmad *et al.* [29]	Bighead carp	Flavourzyme	4% (enzyme /substrate)	6.5	50	6

3.3. Endogenous Enzymes of Fish

Enzymes can also be naturally present in fish, known as endogenous enzymes. The largest group of enzymes in fish are proteases, and they catalyse the hydrolysis of peptide bonds using different mechanisms [47]. Proteases are classified into two groups depending on the substrate specificity. Exoproteases or peptidases cleave the peptide bond of the terminal amino acid (amino/carboxyl end) of the polypeptide chain, whereas endoproteases or proteinases cleave the internal bonds of the chain [47,48].

As explained by Vannabun *et al.* [49] and Sriket [50], fish viscera contain different types of digestive proteases: serine, aspartic/acid, cysteine/thiol and metallo-proteases. Inside the serine type, trypsin can be found, which is stable and active at a pH range between 7.5 and 8.5 and is in the fish pyloric caeca [51]. Trypsin not only cleaves ingested proteins but also activates precursor forms of several other digestive proteases like chymotrypsin [52]. Meanwhile, in the group of aspartic/acid, pepsin can be found, which is secreted in the fish stomach and its peak activity is under acidic conditions [52].

The use of fish proteases can reduce the economic cost of the process, as the price of commercial enzymes is high. The use of trypsin is rising due to its unique features: stability and activity in a wide range of pH (8-11) and temperature (38-70 °C) [47].

The process of hydrolysis using endogenous enzymes is known as autolysis, and it can be performed in two ways. The most common is silage or acid autolysis, typically used in areas with high densities of fisheries but where fishmeal processing plants are not economically viable [53]. As an example, in 2014 more than 250,000 tonnes of fish by-products were preserved by silage only in Norway, representing 41% of the total fish by-products generated in the country [54]. Silage is the process of liquefaction and stabilization of minced fish at room temperature (see flowchart of the process in Figure 2). Typically, formic acid is added to achieve a pH range of 3.5–4.5 to prevent microbial growth [55]. Protein hydrolysis occurs thanks to the aspartic endoproteases present in the fish viscera, particularly pepsin [52], which has been reported to be highly stable at pH values between 1 and 5 [49]. Pepsin triggers the breakdown of proteins and makes it possible to obtain low molecular weight peptides [56]. Acid-preserved fish silage can completely or partially replace fishmeal in feed for fish, as a 6-day-old silage can contain a similar protein content to good quality fishmeal [57]. However, due to chemical reactions between *α* amino and aldehyde groups present in amino acids, the concentration of some amino acids can lower over time [3].

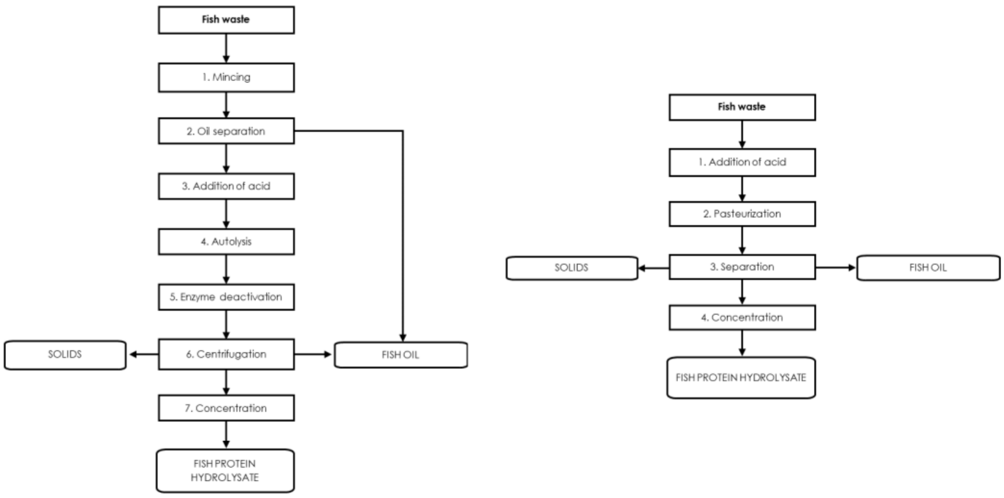

Figure 2. Schematic flowcharts pf acid autolysis or silage of fish waste. Compiled from Domínguez *et al*. [30] on the left and Arason *et al*. [58] on the right.

The other alternative method is autolysis, which requires higher temperatures than silage and not necessarily an acidic pH. The autolysis conditions (pH, solid: liquid ratio and temperature) usually need to be optimized for each raw material used. Response surface methodology is a common method used to optimize the experimental parameters of fish autolysis [59–61]. However, the efficiency in the production of free amino acids of both silage and autolysis can be low to medium compared with enzymatic hydrolysis.

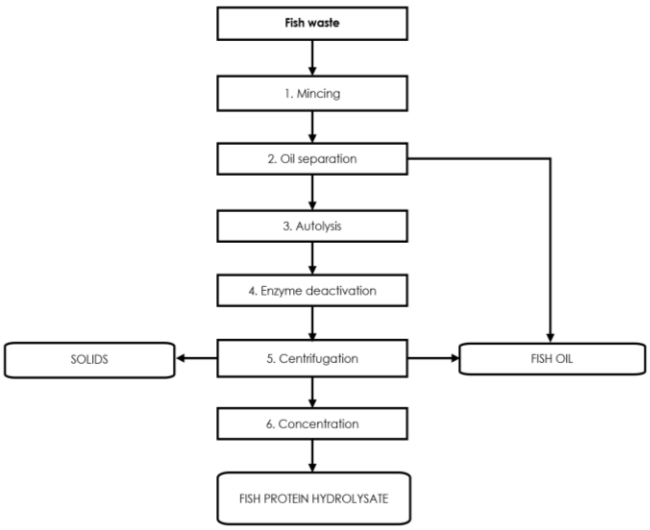

Figure 3. Schematic flowchart of autolysis of fish waste. Compiled from Domínguez *et al.* [60].

3.4. Use of Fish Protein Hydrolysates in Agriculture

When fish byproducts do not meet the standards to be processed into fishmeal, they can be converted into liquid or solid forms of fertilizers, as when using fish viscera protein hydrolysates as ingredients. Historically, civilizations like Egyptians, Incas, Mayans and Norwegians have used fish by-products to fertilize their crops [62]. Fish protein hydrolysates can be used as biostimulants, enhancing plant nutrition, fruits and vegetables quality, and crop productivity [63,64]. However, phytotoxic effects and growth depression have also been reported [65]. In addition, there was some

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concern about the use of animal-derived protein hydrolysates as biostimulants, leading to the implementation of European Regulation 354/2014 and later European Regulation 2021/1165, which prohibit the application of these products on the edible parts of organic crops. However, Corte *et al.* [66] concluded in their work that protein hydrolysates did not negatively affect eukaryotic cells and soil ecosystems, and that they could be used in farming without causing any harm to the environment and human health.

Among the CMCs mentioned in the Regulation (EU) 2019/1009 [11], composting (CMC 3) is one of the most relevant methods to valorise fish by-products. Composting is a biotransformation process of organic materials into stable and complex macromolecules under the action of microorganisms such as fungi, bacteria, or enzymes [4]. It was reported that soil fertilization with compost produced with fish by-products increased leaf yield of lettuce and increased the content of nitrogen, phosphorus, potassium, sodium, calcium, and magnesium in leaves [4].

According to Ahuja et al [62], OMRI (Organic Materials Review Institute) has allowed 154 commercial fish fertilizers until 2020, but only a few have been deeply investigated in scientific research, most of them in the United States and Canada. Some examples are given by Madende & Hayes [12]. These fertilizers are produced in the form of pellets, hydrolysed powder, and emulsion-based liquid.

4. Fish Viscera

Viscera constitute 8-15% of the whole fish weight and contain high-quality proteins, long-chain omega-3 fatty acids, vitamins A and D, and minerals like Fe, Zn, and Se [55]. In Europe alone, it is estimated that between 200 and 400 million tonnes of fish viscera are generated annually from aquaculture, with over half originating from salmon produced in Norway [67]. In Error! Reference source not found., it can be observed that in the whole picture of Europe, in 2021, more viscera were produced from captures rather than aquaculture (607,000-1,140,000 tonnes vs 216,000-406,000 tonnes, respectively). However, viscera obtained from captures can sometimes be thrown into the sea by fishermen in order to preserve better the captured fish and to save space, so that the real number of viscera weight landed is much lower than the one proposed in Error! Reference source not found.. Fish gutting at home, restaurants, and hotels instead of in processing plants can make the collection of viscera difficult logistically.

Table 5. Quantification of captures, aquaculture production, and the estimated range of viscera weight for each case in every country of Europe. Data obtained from FishStatJ software [67,68].

Tonnes by country, 2021	Captures	Estimated range of by-products weight	Estimated range of viscera weight	Aquaculture	Estimated range of by-products weight	Estimated range of viscera weight
Albania	7,589	4,553 – 5,312	607 – 1,138	8,048	4,829 – 5,634	644 – 1,207
Austria	350	210 – 245	28 – 53	4,875	2,925 – 3,413	390 – 731
Belgium	13,805	8,283 – 9,664	1,104 – 20,171	223	134 – 156	18 – 33
Belarus	605	363 – 424	48 – 91	8,504	5,102 – 5,953	680 – 1,276
Bosnia-Herzegovina	305	183 – 214	24 – 46	3,819	2,291 – 2,673	305 – 573
Bulgaria	55,484	33,291 – 38,839	4,439 – 8,323	12,565	7,539 – 8,796	1,005 – 1,885
Croatia	59,960	35,976 – 41,982	4,797 – 8,994	25,970	15,582 – 18,179	2,078 – 3,896
Cyprus	1,357	814 – 950	109-204	7,845	4,707 – 5,492	628-1,177
Czechia	3,314	1,988 – 2320	265 – 497	20,991	12,595 – 14,694	1,679 – 3,149
Denmark	415,261	249,157 – 290,683	33,221 – 62,289	32,100	19,260 – 22,470	2,568 – 4,815
Estonia	63,189	37,913 – 44,232	5,055 – 9,478	849	509 – 594	68 – 127
Faroe Islands	532,282	319,369 – 372,597	42,583-79,842	115,650	69,390 – 80,955	9,252-17,348
Finland	124,835	74,901 – 87,384	9,987 – 18,725	14,399	8,639 – 10,079	1,152 – 2,160
France	362,379	217,427 – 253,665	28,990 – 54,357	47,910	28,746 – 33,537	3,833 – 7,187
Germany	176,847	106,108 – 123,793	14,148 –26,527	18,294	10,976 – 12,806	1,464 – 2,744
Greece	46,764	28,058 – 32,735	3,741 – 7,015	130,171	78,103 – 91,120	10,414 – 19,526
Hungary	4,601	2,761 – 3,221	368 – 690	17,847	10,708 – 12,493	1,428 – 2,677
Iceland	1,027,250	616,350 – 719,075	82,180 – 154,088	53,136	31,882 – 37,195	4,251 – 7,970
Ireland	184,761	110,857 – 129,333	14,781 – 27,714	13,381	8,029 – 9,367	1,070 – 2,007
Italy	94,016	56,410 – 65,811	7,521 – 14,102	60,484	36,290 – 42,339	4,839 – 9,073

Latvia	116,413	69,848 – 81,489	9,313 – 17,462	901	541 – 631	72 – 135
Liechtenstein	0	0	0	0	0	0
Lithuania	91,253	54,752 – 63,877	7,300	5,135	3,081 – 3,595	411 – 770
Luxembourg	0	0	0	0	0	0
Moldova	0	0	0	12,900	7,740 – 9,030	1,032 – 1,935
Malta	2,353	1,412 – 1,647	188 – 353	16,433	9,860 – 11,503	1,315 – 2,465
Montenegro	753	452 – 527	60 – 113	640	384 – 448	51 – 96
Netherlands	261,571	156,943 – 183,100	20,926 – 39,236	5,540	3,324 – 3,878	443 – 831
North Macedonia	514	308 – 360	41 – 77	3,169	1,901 – 2,218	254 – 475
Norway	2,115,496	1,269,298 – 1,480,847	169,240 – 317,324	1,662,675	997,605 – 1,163,873	133,014 – 249,401
Poland	201,321	120,793 – 140,925	16,106 – 30,198	44,786	26,872 – 31,350	3,583 – 6,718
Portugal	156,076	93,646 – 109,253	12,486 – 23,411	8,671	5,203 – 6,070	694 – 1,301
Romania	3,476	2,086 – 2,433	278 – 521	11,714	7,028 – 8,200	937 – 1,757
Serbia	2,354	1,412 – 1,648	188 – 353	7,308	4,385 – 5,116	585 – 1,096
Slovakia	1,815	1,089 – 1,271	145 – 272	2,304	1,382 – 1,613	184 – 346
Slovenia	241	145 – 169	19 – 36	1,256	754 – 879	100 – 188
Spain	743,530	446,118 – 520,471	59,482 – 111,530	70,285	42,171 – 49,200	5,623 – 10,543
Sweden	155,925	93,555 – 109,148	12,474 – 23,389	11,796	7,078 – 8,257	944 – 1769
Switzerland	1,486	892 – 1,040	119 – 223	2,334	1,400 – 1,634	187 – 350
Ukraine	34,507	20,704 – 24,155	2,761 – 5,176	16,882	10,129 – 11,817	1,351 – 2,532
United Kingdom	523,488	314,093 – 366,442	41,879 – 78,523	219,198	131,519 – 153,439	17,536 – 32,880
TOTAL	7,587,527	4,552,516 – 5,311,269	607,002 – 1,138,129	2,700,987	1,620,592 – 1,890,691	216,079 – 405,148

Among the fish species most farmed in Europe, salmon tops the list followed by trout, gilt-head bream, seabass, carp, and turbot (Table 6). Only from these species, between 1,554,000 and 1,813,000 tonnes of by-products and between 207,000 and 388,500 tonnes of viscera are generated in total. The production of salmon only in Norway supposed almost 1,600,000 tonnes of live weight in 2021 [67], which means that between 128,000 and 240,000 tonnes of viscera that can be valorised.

Table 6. Quantification of carp, seabass, gilt-head bream, salmon, trout, and turbot from aquaculture in every country of Europe. Data obtained from FishStatJ software [67] and APROMAR website [69]. The estimated weight of the viscera was calculated assuming that viscera account for 8-15% of the total weight.

Tonnes by country, 2021	**Carp**	**Seabass**	**Gilt-head bream**	**Salmon**	**Trout**	**Turbot**
Albania	-	2,463	3,724	-	1,861	-
Austria	666	-	-	8	3,205	-
Belgium	7,557	-	-	-	127	-
Belarus	356.2	54	82	-	3,317	-
Bosnia-Herzegovina	-	-	-	-	-	-
Bulgaria	5,986	-	-	1	5,468	-
Croatia	3,630	9,039	7,519	-	350	-
Cyprus	-	2,680	5,097	-	52	-
Czechia	18,709	-	-	-	1,070	-
Denmark	-	14	-	1,668	28,476	-
Estonia	-	-	-	-	712	-
Faroe Islands	-	-	-	115,650	-	-
Finland	-	-	-	-	13,551	-
France	1,470	2,290	1,850	-	38,800	-
Germany	4610	-	-	-	8725	-
Greece	1	51,232	66,891	-	1,911	-
Hungary	12,707	-	-	-	74	-
Iceland	-	-	-	46,458	6,341	-
Ireland	-	-	-	12,844	537	-
Italy	199	7,394	8,176	-	41,971	30
Latvia	564	-	-	-	183	-
Liechtenstein	-	-	-	-	-	-
Lithuania	3,734	-	-	-	131	-
Luxembourg	-	-	-	-	-	-
Moldova	-	221	2,640	-	-	-

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Malta	10,580	-	-	-	-	-
Montenegro	-	43	36	-	561	-
Netherlands	-	-	-	-	50	100
North Macedonia	299	-	-	-	2,828	-
Norway	-	-	-	1,562,415	97,774	-
Poland	18,941	-	-	-	19,298	-
Portugal	-	834	3,091	-	857	3,538
Romania	7,369	-	-	-	2,747	-
Serbia	5,649	-	-	-	1,556	-
Slovakia	740	-	-	-	800	-
Slovenia	131	-	-	-	921	-
Spain	-	23,037	7,823	-	-	7,629
Sweden	-	-	-	-	11,703	-
Switzerland	-	-	-	162	1,230	-
Ukraine	13,450	-	-	-	312	-
United Kingdom	168	-	-	205,000	13,253	-
Total fish weight	117,516	99,301	106,929	1,944,206	310,751	11,297
Estimated range of by-products weight	70,510 – 82,261	59,581 – 69,511	64,157 – 74,850	1,166,524 – 1,360,944	186,451 – 217,526	6,778 – 7,908
Estimated range of viscera weight	9,401 – 17,627	7,944 – 14,895	8,554 – 16,039	155,536 – 291,631	24,860 – 46,613	904 – 1,695

Fish Viscera Protein Hydrolysates

Generally, fish viscera are disposed to landfill, to the sea, or used in the production of fishmeal where rendering plants exist in the nearby. All the problems surrounding climatic change and the rise of the circular economy make the first two choices unacceptable. However, their processing in fishmeal plants can be difficult because they deteriorate very fast due to the high microbial load of the gastrointestinal tract, together with the storage and transportation to the fishmeal plants in inappropriate conditions, and because of their content of water, which can be as high as 80% [40], resulting in high energy costs in the rendering process [53,70]. For these reasons and due to their protein content, the production of fish viscera protein hydrolysates may be a suitable route for their valorisation. Moreover, the main advantage of using viscera for hydrolysis is the possibility of using the endogenous enzymes (pepsin, trypsin…) contained in the digestive system, which are very effective and can reduce the economic cost of the process by producing silage and autolysates, as mentioned in the previous Section. Among some studies that have used endogenous enzymes for autolysis and even optimized the experimental conditions are Domínguez *et al.* [60] and Nikoo *et al.* [61]. Enzymatic hydrolysis of fish viscera seems a promising and profitable process to valorise these by-products.

Viscera of some species may have a high oil content which can be used for feed and the protein content can be used for getting protein hydrolysates. There is no published work on the production of fish viscera hydrolysates for biostimulant purposes. In fact, some specific words related with this topic were searched in Scopus and the results are displayed in Fig. 4, where it can be observed that in the last 25 years, the publications about fish viscera hydrolysis and autolysis increased until reaching its peak during the last 5 years. However, publications about fertilizers produced from fish viscera did not increase and publications about biostimulant fertilizers produced from fish viscera are none. Meanwhile, viscera hydrolysates seem a feasible option to produce amino acid-based biostimulants as they can more than comply with the legislation. For example, the Spanish legislation (RD 506/2013) establishes a minimum of 6% of free amino acids in dry matter for the composition of a biostimulant labelled as amino acids, and the viscera hydrolysates obtained with enzymatic hydrolysis and silage by the authors of the present review achieved a percentage of free amino acids of 50.3 ± 3.7 and 49.2 ± 1.2 (D.M. basis) respectively [30]. Biostimulants formulated with viscera hydrolysates must also to comply with the European Regulation 2019/1009, mentioned in the Section “EU fertilizer product regulation”.

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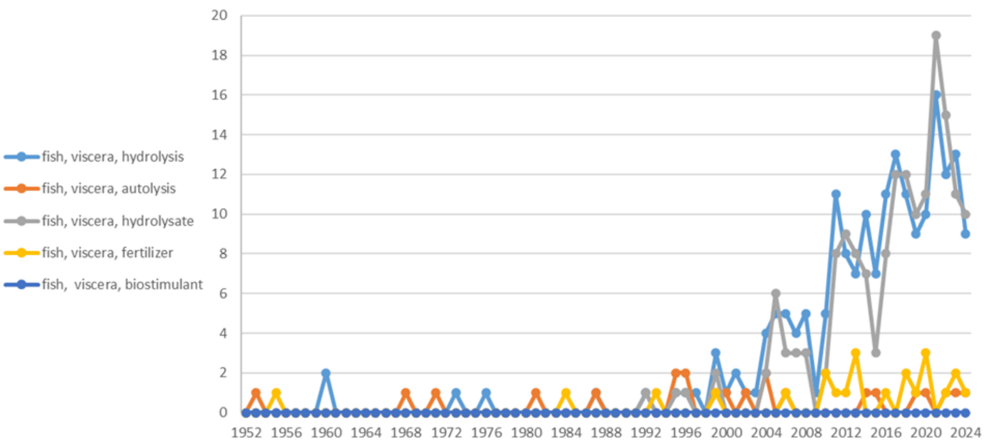

Figure 4. Results of the search in Scopus of different keyword combinations from 1952 to 2024.

5. Conclusions and Future Outlook

Although fish viscera are mainly used for the production of fishmeal or simply disposed to landfill, using them for the production of protein hydrolysates, which can be used as ingredients for biostimulants, seems a promising alternative for areas where fishmeal plants are not accessible. Moreover, this option allows promoting the circular economy, as free amino acid-rich hydrolysates can enhance plant nutrition and crop productivity. Availability of raw material is assured for the next decades and the use of other fish by-products can also be brought to this value chain. The profitability of the production of fish viscera hydrolysates should be considered compared to the production of fishmeal in some cases. Amino-acid based biostimulants produced from fish by-products appear as promising and effective products that will grow in the global market in the next years. However, more studies on the biostimulant effect of the fish derived products are needed. The sustainability of the different ways to produce biobased fertilisers from fish by-products will have to be compared in terms of environmental, economic and social impact, also with the nowadays used fertilisers of mineral origin.

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