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A Comprehensive Review of Fish Protein Hydrolysates Targeting Pet Food Formulations

Tânia Bragança Ribeiro[a], Margarida R. G. Maia[b,c], António J. M. Fonseca[b], Bianca Marques[d], Cristina Caleja[e,f], Ana Rosa[g], Rui Martins[h], André Almeida[h], Maria J. Mota[i], Tiago Aires[i], Cristina M. R. Rocha[d,j], José A. Teixeira[d,j], Ana R. J. Cabrita[b], Lillian Barros[e,f], and Manuela Pintado[a]

[a]Universidade Católica Portuguesa, CBQF Centro de Biotecnologia e Química Fina-Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal; [b]REQUIMTE, LAQV, ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, Porto, Portugal; [c]LEAF, TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, Lisboa, Portugal; [d]CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal; [e]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal; [f]Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal; [g]SEBOL, Comércio e Indústria de Sebo, S. A, Rua Padre Adriano nº 61, Olivais do Machio, Santo Antão do Tojal, Portugal; [h]Indústria Transformadora de Subprodutos, S.A. Herdade da Palmeira - Olheiros do Meio - São José da Lamarosa Agolada, Coruche, Portugal; [i]SORGAL, Sociedade de Óleos e Rações, S.A., Estrada Nacional 109, Lugar da Pardala, S. João de Ovar, Portugal; [j]LABBELS - Associate Laboratory, Braga, Guimarães, Portugal

ABSTRACT

The fish industry generates significant amounts of fish by- and co-products (FBCPs) annually, projected to reach 160.8 million tonnes by 2030. This growth highlights the urgent need for sustainable FBCP management and an opportunity to improve the sector's environmental sustainability. Fish protein hydrolysates (FBCPHs) and bioactive peptides (BPs) derived from these FBCPs are gaining recognition in the pet food sector for their nutritional and bioactives benefits. FBCPHs, primarily sourced from category 3 by-products unsuitable for human consumption, could significantly enhance the economic viability of both industries. This review analyzes production processes, highlighting the benefits and challenges of enzymatic hydrolysis and reviewed emerging technologies such as subcritical water hydrolysis (SWH), which are promising sustainable alternatives by enhancing extraction efficiency and reducing energy consumption. The review explores FBCPHs' applications in pet food, focusing on beneficial biological activities (e.g. antioxidant, prebiotic, neuroprotective). Findings show FBCPHs have significant potential in pet food formulations, providing palatability, hypoallergenic benefits, and addressing health concerns like gastrointestinal disorders and stress-related behaviors. However, further research is required to optimize production processes, scale industrial application, and ensure regulatory compliance. In conclusion, FBCPHs present a valuable solution for promoting sustainability, improving pet nutrition, and supporting the circular economy.

KEYWORDS

Bioactive peptides; companion animals; animal nutrition; fish waste valorization; sustainable ingredients; bioactive ingredients

Introduction

The food industry generates high amounts of animal-based waste annually. In 2022, the fish and aquaculture industry produced an estimated 185.4 million tons, being up to 35% lost or wasted every year. This waste includes heads, skins, trimmings, fins, bones, viscera, scales, and shells from crustaceans, which account for approximately 60% of the total fish processing volume. Additionally,

CONTACT Tânia Bragança Ribeiro tribeiro@ucp.pt Universidade Católica Portuguesa, CBQF- Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

by-catches and undersized fish are often rejected, further contributing to waste[1–3]. Traditionally, most of these were discarded as wastes with high costs and significant environmental impact, mostly due to their high organic load and rapid deterioration, especially when containing viscera.[1–3]

Despite being underutilized and generally low-valued, the use of fish by- and co-products (FBCPs) has been increasing for the production of food and non-food products, such as fishmeal, fish oil, and fertilizers. These food products, rich in proteins and oil, have begun to account for a growing share of fish-derived resources, with FBCPs now representing 34% of fishmeal and 53% of fish oil production.[1] While the recovery of other by-products, like chitin and chitosan, has been explored, their uptake by the industry remains limited. New sustainable extraction and conversion routes are being researched to develop industrially feasible and high-quality polymers, as conventional methods are energy-intensive and require strong acids, bases, and solvents. Potential applications for these polymers include agrochemicals, water treatment agents, packaging, coatings, biomedical devices, and dietary supplements.[4] Marine-sourced collagen and gelatin are other examples of valuable by-products; although still underutilized compared to terrestrial sources, they are gaining attention in food, cosmetic, and biomedical applications. Their future use as food ingredients, pharmaceuticals, and biomedical devices is also promising due to their texturizing, film-forming, and biocompatibility properties.[5]

The European Commission's goal of achieving zero waste by 2030, combined with the growing global population and concerns over food security, is forcing the fish industry toward more circular, sustainable solutions. The valorization of FBCPs, particularly proteins, supports these objectives by meeting nutritional demands while reducing waste and environmental impact. Adopting a circular bioeconomy approach improves resource efficiency by transforming waste into high-value products.[2,3,6]

In the pet food industry, the European Pet Food Industry Federation (FEDIAF) identified protein as a key ingredient essential for growth, muscle maintenance, digestion, and energy production.[6] While the pet food industry already utilizes a wide range of by- and co-products (BCPs) as protein sources, including fish, meat, bone meal, poultry, and poultry by-product meal,[6,7] there is a trend towards replacing and/or enriching these rendered meals with novel functional ingredients.[8] Pet owners' increasing concern for their animals' health and well-being has driven research toward new ingredients, such as plant and algae extracts, protein isolates, and hydrolysates (e.g., from soy or fish). These functional ingredients offer additional health benefits such as improved immune function, satiety, and digestive health, while also addressing specific health concerns such as halitosis, neuro-protection, or anti-carcinogenic activities. They also provide hypoallergenic properties, making them suitable for pets with sensitivities.[9]

Protein hydrolysates, like soy-based hydrolysates, are already used in the pet food industry to produce hypoallergenic formulations and improve palatability, although scientific data on their broader use remains limited.[2,3] Companies like BRF Ingredients offer commercially available hydrolyzed proteins, such as BioActio, for functional feed and pet food formulations. Among these, fish-based hydrolysates fish by- and co-products hydrolysates (FBCPHs), and their bioactive peptides (BPs) have attracted considerable attention for their nutritional benefits and biological properties.[2,3,6,10]

Numerous studies have reported that fish-based hydrolysates and purified BPs exhibit a variety of biological activities, namely antimicrobial, antidiabetic, antihypertensive, antioxidant, neuroprotective, anti-inflammatory, and immunomodulatory.[6,11,12] These properties make FBCPHs promising ingredients for functional pet food. Research by Folador et al.[11] demonstrated the potential of FBCPHs in canned foods and dry extruded kibbles. Other studies have reported that incorporating fish-based hydrolysates may improve digestion and enhance palatability in pet food.[6,11–13] For example, two recent studies found that incorporating FBCPHs into commercial dog food formulations was well accepted by beagle dogs and did not negatively impact food intake, digestibility, or fecal characteristics.[14,15] However, despite these advantages, data on their application in commercial pet food and their effects on companion animals remain limited.

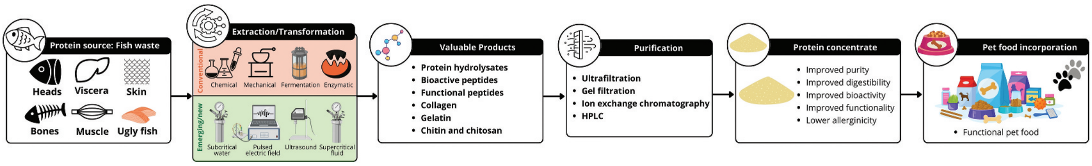

Figure 1. Summary of the main goals of the present review work.

Bioactive peptides (BPs) are inactive within their parent protein structure and require proteolysis to be released. This process produces fish-based hydrolysates.[2,3] Most fish BPs consist of 2 to 20 amino acid sequences with a molecular weight lower than 6000 Da [2,10]. Several methods have been used to hydrolyze protein and produce fish-based protein hydrolysates and BPs, including chemical hydrolysis, microbial fermentation, and enzymatic hydrolysis. Enzymatic hydrolysis is the preferred method due to its specificity and control.[2,3] While chemical hydrolysis is cheaper, it is unspecific and difficult to control, and the extreme alkali or acidic conditions can lead to the destruction of certain amino acids as well as to the formation of D – amino acids and toxic substances, leading to reduced nutritional quality.[3] In contrast, enzymatic hydrolysis occurs under moderate and controllable reaction conditions, high substrate specificity, and minimal toxic residues, though the high cost of enzymes limits its industrial-scale use.[2,3] Fermentation offers a more cost-effective alternative but introduces additional complexity in production.[2,3]

This review aims to consolidate the available knowledge on the sustainable upcycling of fish waste and FBCPs into valuable FBCPHs, highlighting their potential as functional ingredients in pet food formulations. By focusing on their biological properties, production methods, and application in the pet food industry, this review pursues to present new insights that can help researchers and industry overcome the challenges of producing high-value ingredients from undervalorized biomass, such as FBCPs. Furthermore, this approach supports the sustainable management of protein resources and aligns with the broader goals of reducing waste and environmental impact within the food production system.[2] Therefore, the following sections include (1) Describing the FBCPs generated by the fish processing industry, (2) Exploring various methods used for producing and purifying FBCPHs and bioactive peptides, including innovative technologies, (3) Analyzing the reported biological properties of these hydrolysates and peptides; (4) Highlighting the potential of FBCPHs and purified peptides for pet food formulations and (5) Discussing pet food demands, trends, and relevant EU legislation regarding functional ingredients and fish hydrolysate application in pet food (Fig. 1).

The relevant scientific data was collected by searching the Web of Science, Scopus, and Google Scholar using keywords like "fish hydrolysates," "fish by-products," "fish wastes valorization," "valorization of seafood," "fish hydrolysates in pet food," "fish protein hydrolysate," "hydrolyzed diets," and "upcycled foods." Information on pet food legislation was retrieved from FEDIAF and Global Alliance of Pet Food Associations (GAPFA) resources.

Fish by-products and co-products

Over the past decades, global fish production and consumption have significantly increased. Fish production increased from 138 million tonnes in 2000 to 179 million tonnes by 2019, with a corresponding rise in per capita fish consumption from 15.1 kg to 20.5 kg [16]. This trend is expected to continue, with FAO predicting a 13% increase in fish production by 2030, reaching 201 million tons.[16] Consequently, the fishing sector generates an estimated 20 million tons of FBCPs, annually.[17]

Around 70% of the fish consumed undergoes processing before reaching the consumer. Depending on factors like type of processing, fish species, and geographical area, fish processing generates FBCPs ranging from 20% to 80% of the total fish weight.[17] These FBCPs are rich in valuable nutrients such as proteins (49 to 57% dry weight, e.g., collagen and gelatin and derived peptides), minerals (22 to 30% dry weight of ash, e.g., calcium and phosphorus), lipids (7 to 19% dry weight, e.g., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), and enzymes (pepsin, trypsin, collagenase, and chymotrypsin).[2,3,18]

FBCPs are derived from various parts of the fish, each offering distinct bioactive compounds. For example, muscle contains protein, while viscera are rich in oil, enzymes, and protein.[19,20] Bones contain calcium and chondroitin sulfate,[19] while heads provide protein, oil, and chondroitin sulfate.[19] Scales are a source of collagen, calcium, and gelatin[19] and skin could provide collagen, gelatin, and protein.[19] Fins can be used for chondroitin sulfate production[19] (Fig. 2). These compositional differences and specificities allow for the recovery of various valuable products depending on the FBCPs processed. For instance, fish skin can be enzymatically hydrolyzed to produce hydrolysates rich in collagen peptides,[21] and fish viscera can be used to recover omega-3 fatty acids rich in EPA and DHA,[22] or even digestive enzymes.[20] Overall, all FBCPs are suitable to be valorized by enzymatic hydrolysis, a versatile technique that allows the production of FBCPHs by recovering the protein present in all types of FBPCs, or even whole fish,[19,23] while also recovering fatty acids or minerals present in FBCPs.[17,19,23]

BCPs hold significant potential for higher-value applications in food and feed, including pet food. However, international regulations strictly govern the treatment and handling of animal BCPs. In this context, co-products refer to residual raw materials remaining from the slaughtering process that retain a quality suitable for human consumption and can be employed in food production. Conversely, materials considered unsuitable for human consumption due to commercial, safety, and/or regulatory considerations are designated by-products. The European Union categorizes non-edible animal by-products into three categories based on their potential risk (Regulation (EC) 142/2011).[24] Category 1 (highest risk) is unsuitable for food or feed, while Category 2 can be repurposed for non-food applications like fuel. Category 3 (lowest risk) includes materials safe for human consumption but excluded for commercial or cultural reasons, such as packaged fish with minor damage or expired shelf-life.

Regulations regarding the processing of BCPs as animal feed have forced producers to adapt, introducing novel by-product processing strategies. Where BCP producers once freely disposed of these products, European Regulations (Regulation (EC) 142/2011 [24]) now mandate specific, detailed processing methods.[24] Despite the permitted applications of processed BCPs under European Regulations, the economic costs of implementing these techniques have increased. Therefore, there is a growing demand for novel methods that recover more valuable fractions from BCPs, increasing their economic value and offsetting processing costs.

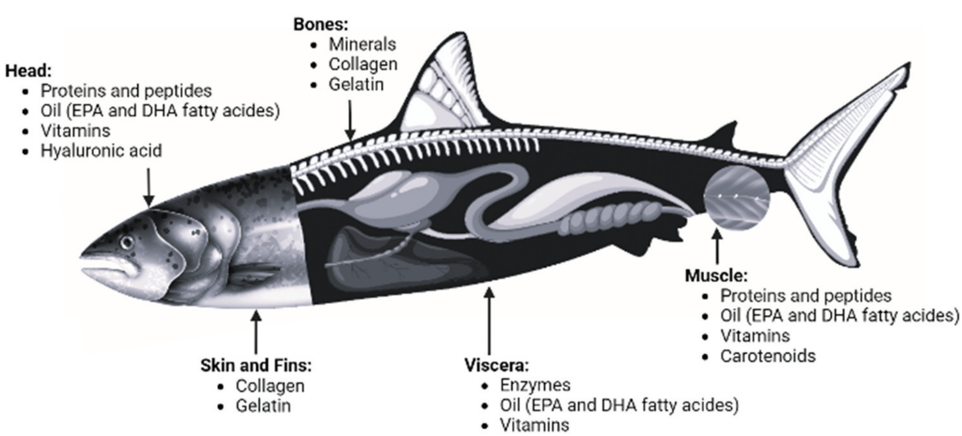

Figure 2. Fish by- and co-products (FBCPs) and their composition. Created with BioRender.com.

Numerous investigations have shown that valorizing animal BCPs (category 3) for animal feed, including pet food, is an environmentally beneficial approach.[25] The European Union generates between 88 and 129 million tons of food waste annually, emitting 170 million tonnes of CO_2, with half of the waste generated in the production chain.[26] Reducing this waste is critical to lowering CO_2 emissions and mitigating global warming. Recycling BCPs can significantly reduce CO_2 emissions in animal and pet food production.[25,26] For example, a study has shown that incorporating recycled meat (e.g., beef, pork, and poultry) from packaged food waste into pet food has significantly reduced the industry's environmental footprint.[25]

Similarly, life cycle assessment (LCA) studies on fish meal production plants have shown that valorizing FBCPs into feed and pet food ingredients can decrease the environmental impact of fish processing.[27–29] Laso and colleagues studied the environmental management of FBCPs in the canned anchovy industry using LCA methods. Their analysis demonstrated that valorizing anchovy heads and bones for fishmeal and oil production was significantly more environmentally advantageous compared to incineration or landfilling, mainly due to the avoided environmental burdens from traditional processes such as fishing fresh anchovy for fishmeal production. According to the authors, this valorization approach resulted in the emission of 37,8 kg of CO_2 equivalents per ton of anchovy heads and bones, while landfilling led to emissions of 810 kg of CO_2 equivalents (with gas recovery) and up to 830 kg without gas recovery. Although the study did not deeply approach the financial aspects, the authors noted that valorizing FBCPs exhibits clear economic advantages over landfilling or incineration, as it produces a final product with commercial value and demand.[28] In another study, Maiolo and colleagues assessed the Italian rainbow trout (Oncorhynchus mykiss) supply chain and concluded that valorizing FBCPs for pet food production resulted in slightly higher emissions than incineration. However, the authors stated that this result was primarily due to the reliance on fossil fuels for energy, and using renewable energy could lower emissions significantly, offering a strong alternative to reduce the industry's environmental footprint.[29] Furthermore, Vàzquez and colleagues explored the application of enzymatic treatments to FBCPs, finding that these treatments produce high-quality protein products with excellent digestibility and bioactive properties, such as antioxidant and antihypertensive activities. Although preliminary assessments indicate that enzymatic processing is energy-intensive, this process presents a more sustainable, scalable, and flexible option compared to traditional methods.[30]

FBCPHs are particularly interesting for pet food due to several factors. They provide a sustainable protein source, rich in easily absorbable amino acids in the small intestine.[13] Additionally, FBCPHs possess hypoallergenic properties,[12] making them suitable for pets with sensitivities. Furthermore, they may act as palatability enhancers,[11] improving the taste and acceptance of pet food. Besides these benefits, FBCPHs also exhibit antioxidant and antihypertensive activities,[6] enhancing their nutritional and functional value for pets. These combined properties make FBCPHs an interesting option for pet food formulation. However, careful consideration must be given to the processing methods, source materials, and energy consumption involved in FBCPH production to ensure sustainability and cost-effectiveness.[23,29]

Overall, alternative methods for FBCPs valorization may be promising solutions to reduce the environmental impact of the fish processing industry by lowering the CO_2 emission and producing valuable products such as fishmeal and FBCPHs. Besides environmental benefits, these products increase economic value, while landfilling or incineration represents an expense for producers. However, these methods still need improvement, particularly in terms of energy efficiency. However, addressing energy efficiency challenges and performing further optimization studies are essential to fully exploit their potential benefits and accelerate their use in industry, such as pet food industry.

Peptides production from fish by-products

Enzymatic hydrolysis

FBCPHs can be produced by chemical hydrolysis, physical treatment, microbial fermentation, or enzymatic hydrolysis. The choice of hydrolysis method significantly influences the purity, yield, and overall cost of the process. Chemical hydrolysis is a more cost-effective method, using acids or bases to break down proteins. However, this process is less specific resulting in the degradation of sensitive amino acids (tryptophan degradation) and the formation of side products such as salts, which reduce the purity and bioactivity of the FBCPHs.[6,31] Fermentation presents a more sustainable and economical alternative, utilizing microbial enzymes to generate bioactive FBCPHs. However, lower-purity FBCPHs were normally obtained due to the broader range of molecular weights in the peptides generated.[6,31] In contrast, enzymatic hydrolysis allows more control over protein breakdown, producing peptides with higher bioactivity and specificity. This method employs mild reaction conditions, which protect the integrity of sensitive amino acids. However, it is also more expensive due to the cost of enzymes and the complexities involved in scaling up the process.[6,31] Preliminary assessments suggest that valorizing FBCPs via enzymatic hydrolysis or fermentation may offer environmental advantages compared to conventional fish meal production.[30]

Among the available methods, enzymatic hydrolysis is the most widely used due to its many advantages. The selectivity of enzymes allows the controlled and specific release of peptides, and the use of mild temperature and pH conditions results in hydrolysates with higher nutritional quality (e.g., less loss of amino acids).[32,33] In enzymatic hydrolysis, a proteolytic enzyme, or an enzyme mixture, breaks specific peptide bonds (H^+) of proteins, producing smaller (3–20 AA residues) and more water-soluble peptides (with increased ionizable groups) than the intact proteins.[33,34]

The enzymatic hydrolysis process of FBCPs typically involves four steps: (1) Homogenization of FBCPs through grinding and water addition, which ensures thorough mixing and enzyme access while adjusting the protein concentration to 8–12%; (2) Establishment of hydrolysis parameters, such as temperature, pH, enzyme-to-substrate ratio, water-to-substrate ratio, and processing time, followed by enzyme addition; (3) Enzyme inactivation post-hydrolysis, usually through heating (85–95°C for 5–20 min) or pH adjustment; (4) Separation of the hydrolysate (liquid fraction) from solids (unhydrolyzed proteins, bones, skin, scales, and others) and oil fraction via three-phase decanter, filtration, or centrifugation. The hydrolysate can then be concentrated through evaporation or dried using a spray or freeze-dryer.[3,33–36] A schematic representation of the enzymatic hydrolysis process of FBCPs is illustrated in Fig. 3.

Although enzymatic hydrolysis may appear straightforward, many factors must be considered to produce high-yield, high-quality FBCPHs. These factors include enzyme selection and extraction conditions, such as water-to-substrate ratio, pH, temperature, and processing time. Selecting an appropriate enzyme is crucial, as it determines the cleavage pattern of peptide bonds.[32,33] Enzymes can be sourced from vegetables (papain and bromelain), animals (pepsin and trypsin), bacteria (alcalase and neutrase), or fungi (fungal protease), and they target different peptide bonds based on their endo- or exopeptidase activities.[31–33] The choice of enzyme should be tailored to the protein source and the desired peptide size distribution.[33] Other important considerations include enzyme concentration, pH, and temperature. Increasing enzyme concentration speeds up hydrolysis but raises production costs.[31] Enzyme efficiency is also highly dependent on maintaining optimal pH and temperature ranges, as deviations can reduce hydrolysis efficiency and peptide yield[31,33] However, adjusting the optimum pH may cause high salt levels in the final hydrolysate, negatively impacting the FBCPH's nutritional value.[33] Similarly, while higher temperatures increase enzyme activity, temperatures outside the optimal range can denature enzymes and slow down reactions, reducing efficiency.[6] Other important factors include the hydrolysis time and the water-to-substrate ratio. Longer hydrolysis times can increase peptide yield but excessive time may cause over-hydrolysis, reducing the bioactivity.[31] A proper water-to-substrate ratio is essential to prevent product inhibition and maximize yield, though adding more water increases drying costs.[33,37]

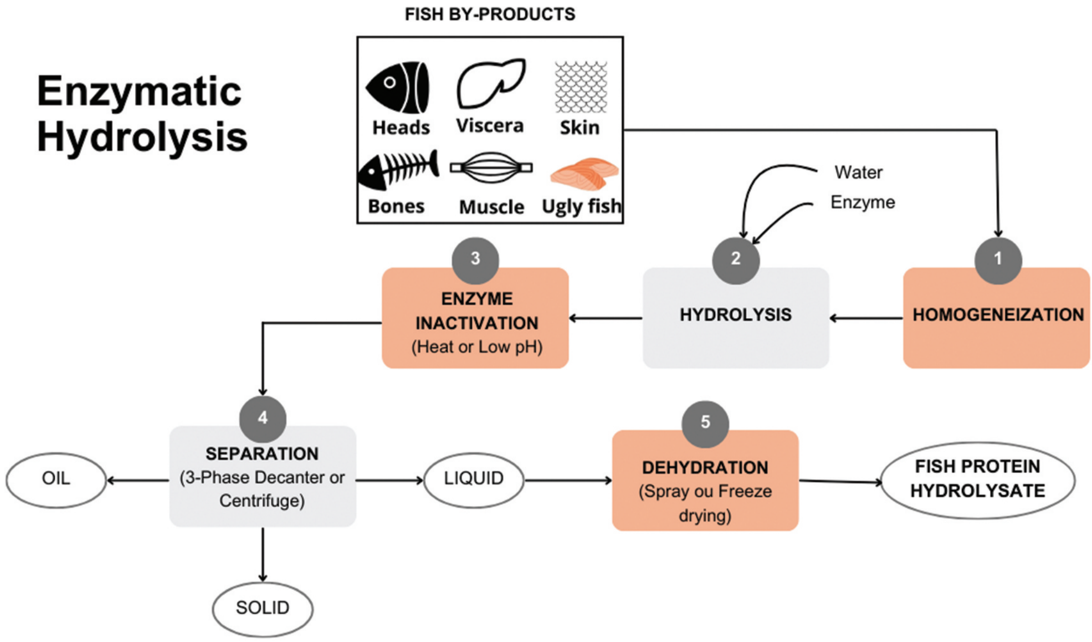

Figure 3. Schematic representation of the enzymatic hydrolysis process of fish by- and co-products (FBCPs).

Processing FBCPs presents additional challenges due to the complexity and heterogeneity of the material. Therefore, controlling their proximate composition (e.g., fat, protein, moisture, and ash) and amino acid composition is crucial for producing quality, stable FBCPHs.[33] For example, high lipid and solid content (e.g., skin, bones, scales) can lead to significant lipid oxidation and negatively affect enzymatic hydrolysis.[35,36] Suitable antioxidants can be added, or lipids can be removed before hydrolysis to prevent lipid oxidation.[35] Mechanical deboning can address the removal of solid components. Autoclaving and hydrothermal (85–135°C for 15–120 minutes) pre-treatments have been effective in softening hard FBCP components such as heads[38] and scales.[39] Other pre-treatments, like microwaves, offer benefits such as shorter hydrolysis times, higher antioxidant activity, and lower immunochemical reactivity.[40] Lower pH values and higher temperatures also enhance oxidation and should be avoided by selecting the proper enzyme.[33]

Regarding control parameters, the extent of protein cleavage is a primary consideration. A common method for monitoring enzymatic protein hydrolysis is measuring the degree of hydrolysis (%DH), i.e., the percentage of cleaved peptide bonds. A higher %DH typically means higher protein recovery.[33,36] The MW distribution of peptides is another important parameter for ensuring protein hydrolysate quality, directly measured in the final hydrolysate rather than relative to the starting material, as with %DH. However, both methods are labor-intensive, limiting their industrial use. Recently, Fourier-transform infrared (FTIR) spectra have shown promise in monitoring enzymatic protein hydrolysates by predicting the degree of hydrolysis and the average MW of protein hydrolysates.[41]

Emergent technologies

As mentioned above, different methods can release BPs, including enzymatic hydrolysis, chemical hydrolysis, and fermentation. However, these methods have some disadvantages, including long processing times, high concentration of solvents, high energy consumption, and/or high production costs (e.g., enzymes).[42] Moreover, extreme conditions, including high temperatures, long reaction times, and/or high alkalinity/acidity, can affect or alter the functional properties of potential bioactive compounds.

To address some of these problems, innovative processing technologies have emerged and been exploited for producing FBCPHs and extracting peptides,[42,43] aiming at faster, more efficient, and highly selective processes with decreased consumptions (solvents/chemicals and energy). These technologies include subcritical water hydrolysis (SWH), pulsed electric fields (PEF), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE).[43]

Subcritical water hydrolysis

Subcritical Water Hydrolysis (SWH) is an extraction and hydrolysis method that uses subcritical water (SCW) to extract and hydrolyze target compounds or fractions from several matrices. SCW is defined as water under a range of pressures and temperatures (i.e., ranging from 100°C at 0.1 MPa to 374.5°C at 22.06 MPa), above its boiling temperature at the selected pressure and below its critical point, with pressure high enough to maintain its liquid state. Under subcritical conditions, the physicochemical features of water change, namely: (i) the ionic product increases, which allows water to act as an acid or base catalyst (significant, for instance, in hydrolytic reactions); and (ii) the dielectric constant decreases, which allows water to behave as a less polar solvent[42] (Fig. 4). Furthermore, due to the high temperature applied, viscosity decreases, solubility of some target compounds increases, and reaction rates increase, leading to faster and more efficient processes.

SWH properties are particularly relevant when aiming to hydrolyze proteins and produce peptides. This is because, when high pressure and high temperature are applied separately to proteins, they experience denaturation, i.e., the hydrogen bonds are broken and proteins loss their quaternary (if present), tertiary, and even secondary structures.[42,44] In fact, it is known that when applying high hydrostatic pressure at room temperature or high temperature under atmospheric pressure, the denaturation process of proteins occurs without altering their molecular size.[44] The protein's hydrolysis only occurs when using higher temperatures, only possible when combined with high pressures, which is what happens when SWH is used.[42,44] Once the SCW is present, proteins break down into peptides, amino acids, and, ultimately, organic acids, depending on the reaction's extensiveness.[42,44] The main parameters influencing the extension of hydrolysis are temperature, pressure, and time of reaction. Up to now, the primary limitation of using this technology for protein hydrolysis is the lack of complete understanding regarding protein cleavage specificity and how it can be controlled or predicted.[42]

The main applications of SWH are related to the extraction of bioactive compounds (polyphenols, flavonoids, and anthocyanins) and hydrolysis of biopolymers (lignin, polysaccharides, and protein) from several food matrices. In particular, SWH has been exploited for protein extraction and hydrolysis in a wide range of food processing wastes, from both vegetable and animal sources, including deoiled rice bran, soy pulp, onion waste, bean dregs, poultry waste, and other meat by-products and, more specifically, FBCPs/wastes.[44]

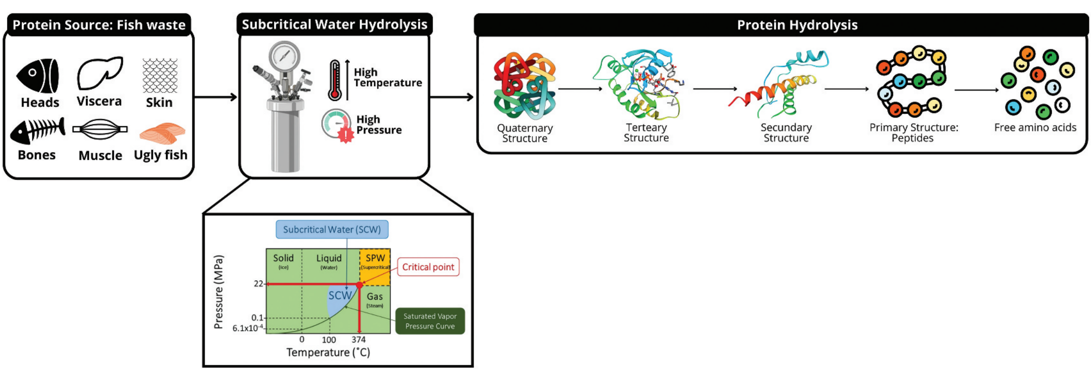

Figure 4. Schematic representation of the technique subcritical water hydrolysis (SWH) of fish by- and co-products (FBCPs).

Regarding the use of SWH to extract and hydrolyze protein from fish wastes, conditions tested include temperatures ranging from 110 to 300°C and times ranging from five minutes to six hours. These hydrolysates displayed important biological activities, including antioxidant, antimicrobial, antiproliferative, and anti-hypertensive.[42,45–52] Examples of the use of SWH to obtain protein-enriched fish hydrolysates and bioactive peptides are described below.

Hao et al.[45] used abalone viscera. Several temperatures (110 to 230°C) during 1 h were tested. The results showed that 170°C was the temperature with the best extraction yields. Hydrolysates' protein content increased gradually with temperatures up to 200°C, ranging from 57 to 69%. The peptide profile of hydrolysates showed that SWH at low and medium temperatures (110–170°C) produced peptides with MW >5000 Da, but for SWH at high temperatures (200–230°C), the peptides were not produced. Nevertheless, most peptides produced were between 1000 and 5000 Da and 180 and 1000 Da, respectively. All hydrolysates showed antioxidant activity by scavenging free radicals, inhibiting lipid oxidation, and exerting reducing power. Hydrophobic AAs, such as phenylalanine, leucine, methionine, and tyrosine, were correlated with the bioactive features of hydrolysates produced.

Melgosa et al.[46] applied SWH at sardine and deoiled sardine wastes aiming to produce fish protein hydrolysates with bioactive properties. Results showed that prior defatting by supercritical CO_2 of sardine BCPs increased protein yields in hydrolysates. The bioactive properties of fish hydrolysates obtained were affected by temperature. Hydrolysates exhibited antioxidant activity by scavenging DPPH radical and presented antiproliferative effects against HT-29 adenocarcinoma cells, the 250°C temperature producing the most bioactive hydrolysate.

Another example of SWH application in fish proteins was reported by Ahmed and Chun,[47] who used tuna skin and collagen previously extracted from tuna skin by supercritical CO_2 as raw materials. SWH was performed using a ratio sample: water of 1:200 and 1:50 for collagen and skin, respectively. The time of SWH was 5 min, and temperatures ranged from 120 to 300°C, with a stirring rate of 150 rpm. The maximum %DH was achieved at 250°C for both raw materials. Hydrolysates exhibited antioxidant activity by scavenging radicals, chelating metals, and exhibiting reducing power, the best antioxidant features found in hydrolysates produced at 280°C. Hydrolysates also exhibited antimicrobial activities against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus cereus*. Functional properties of hydrolysates vary with temperature and protein source, collagen-derivative hydrolysates being higher than tuna skin-derivative hydrolysates. These functional properties arose from small peptides (MW <600 Da) and free AAs.

Other authors have also investigated the SWH of fish wastes from different origins, such as shrimp,[48] blue mussel,[49] mackerel,[50] oyster,[51] and squid viscera,[52] and reported antioxidant and anti-hypertensive activity of hydrolysates obtained.

The advantages of these technologies include their sustainability, reproducibility, and low toxicity when compared with other organic solvents and chemical extraction methodologies, as well as the lower extraction times and lower costs when compared with enzymatic hydrolysis.[44] The main drawbacks are the degradation of some heat-sensitive compounds, the lack of information about cleavage specificity, and the costs of specific equipment. So, additional studies are needed to implement this technology in industry, including energetic and economic cost evaluation. Nevertheless, SWH has been proven to be an effective and green technology for protein hydrolysis and valorization of fish wastes.

Pulsed electric fields

Pulsed Electric field (PEF) is a non-thermal technology that submits a matrix to high-voltage electrical pulses during short periods. Further, applying PEF disturbs cell membranes' structure and facilitates the extraction of entrapped bioactive compounds, with minimal changes in product sensorial and nutritional properties.[53–55] In fact, when a cell membrane is placed in an electric field, the electric potential causes the separation of membrane molecules based on their dipole nature and charge within the membrane. Once the transmembrane potential reaches around 1 V, there is a repulsion between the charged molecules creating pores in the membrane's weak spots, leading to a dramatic increase in permeability[56] The effectiveness of extraction processes is greatly influenced by the temperature of treatment, pulse shape, and amplitude, frequency, intensity, and duration of the electric pulses.[56,57]

PEF technology has been widely used in the food industry for food dehydration, sterilization, and preservation.[58,59] PEF technology provides various benefits when compared to traditional pasteurization methods in the food sector, including prolonging shelf-life, maintaining nutrients, preserving quality, and being cost-effective[59] ore recently, this technology has been exploited to extract bioactive compounds (such as polyphenols, anthocyanins, carotenoids) from plants and fruits, and to extract and hydrolyze proteins from meat wastes and, more specifically, fish wastes.[53,56,57,60] Though it was demonstrated that PEF treatment could also cause protein hydrolysis, it has been mainly used as a pretreatment in enzymatic hydrolysis processes to extract and hydrolyze protein form fish wastes.[58,61] Being a non-thermal and non-chemical process, it may also have the advantage of lower degradation and better bioactivity preservation.

Regarding the valorization of protein from fish wastes, the uses of PEF are still scarce, but some examples are described below.

Zhou et al.[62] studied PEF for protein extraction using mussels as raw material. PEF protein extraction yielded faster than those obtained for enzymatic and alkali extraction methods. The maximum protein extraction yield was 77.08% and was achieved using an electric field intensity of 20 kV/cm, a pulse number of 8, and an enzymolysis time of 2 h.

Li et al.[58] evaluated PEF-assisted enzymatic extraction of the protein from abalone viscera and resulted in a hydrolysate fully hydrolyzed, with high protein yield and good emulsifying properties, compared to purely enzymatic extraction. Moreover, water extraction assisted by PEF of fish residues seemed to improve the antioxidant capacity of resulting protein extracts when compared to water and methanol conventional extraction treatments alone.[53] PEF-assisted enzymatic extraction also showed to be promising for extraction of taurine from mussel.[61] Other studies used PEF to valorize fish wastes, but they are focused more on valorizing fishbone.[43,54,63–67]

Despite the increasing exploitation of PEF to hydrolyze proteins, the mechanism behind PEF's effects on proteins is still not well understood. It has been suggested that proteins' polar groups may take in the energy of the PEF treatment, producing free radicals and that those created radicals could impact the intramolecular interactions in protein molecules, including hydrophobic and electrostatic interactions, disulfide bridges, hydrogen bonds, salt bridges, and Van der Waals forces. Furthermore, the apparent charge of proteins may be altered by PEF as a result of changes in their ionic interactions. Thus, further research is required to accurately assess the impact of PEF treatments on the structural and techno-functional characteristics of proteins. Nevertheless, PEF is gaining interest in this field due to its sustainability, reduced operational costs, high scalability, low time consuming, and, particularly, because it seems to not affect the quality of the products.[68]

Ultrasound-assisted extraction

Ultrasound-assisted extraction (UAE) consists of submitting a sample matrix at frequencies above human hearing levels, ranging from 20 to 10 MHz. UAE efficiency is due to the creation of acoustic cavitation and mechanical impact in the sample matrix, which increases the surface area of contact between the solvent and the biomolecules present in the sample matrix, facilitating the penetration of the solvent into the sample matrix and releasing the desired extractible bioactive compounds.[43,63]

Based on the frequency and amplitude of the applied ultrasound waves, there are two types of UAE, which are high intensity and low intensity.[43,69,70] Low-intensity ultrasounds (LUS) with high frequencies (100 kHz to 1 MHz) are manly used to assess the physical and chemical characteristics of food products without causing damage. High intensity ultrasound (HIUS) with low frequencies (20 kHz-100 kHz) is employed to accelerate and enhance the effectiveness of sample preparation by modifying the physical or chemical characteristics of food.[43,69–71]

Recently, HIUS's capability to hydrolyze biopolymers has been increasingly exploited, and UAE has shown to be also a useful technology to depolymerize/hydrolyze polysaccharides like dextran, xanthan, carrageenan, chitosan, and starch.[71]

Though it has been described to hydrolyze macromolecules and may have the potential to hydrolyze proteins, it is usually more associated with protein extraction. Currently, UAE alone or combined with other technologies has been explored for extracting valuable compounds from fish wastes, including gelatin, collagen, and proteins.[43,63,64,72–74]

Gelatin is a polypeptide derived from insoluble collagen denaturation with valuable functional properties, and it is widely used in food and pharmaceuticals.[43,64] FBCPs/wastes, especially skin and bones, have been exploited as novel sources of gelatin. For instance, Huang et al.[64] used fresh bighead carp (*Hypophthalmichthys nobilis*) scales as raw material and studied the gelatin extraction by conventional water bath (CWB) and UAE-assisted conventional extraction methods. The results suggested that UAE-assisted extraction can extract fish gelatin with better rheological properties, higher gel strength, and emulsifying properties than the CWB extract. Another study using the same raw material[72] also showed that UAE gelatin extraction resulted in better extraction yields and quality of the gelatin than the one extracted using CWB. However, UAE parameters must be adjusted to prevent acoustic cavitation from causing protein degradation. In fact, the combination of high ultrasound intensity (over 200 W) with longer extraction times (5 h or more) led to a decrease in gel strength and melting points of gelatin extracted by UAE.[72]

Collagen is a structural protein also found in the skin and bones of fish, which is valuable and extensively used in the cosmetic, food, and pharmaceutical industries.[51,73] So, Kim et al.[73] evaluated the collagen extraction by UAE using the skin of sea bass (*Lateolabrax japonicus*) as raw material and showed that UAE at 80% amplitude, 0.1 mol L^{-1} acetic acid, and 3 h could successfully extract collagen from this fish by-product and could be an alternative to the existing methodology for collagen extraction, which imply soaking the fish skin on a solution of 0.5 mol L^{-1} of acetic acid for approximately three days. [74]

Moreover, Álvarez et al.[74] studied protein extraction from mackerel wastes and showed that when combining UAE with alkaline extraction and isoelectric solubilization precipitation, protein extraction yields could be higher, using lower extraction times and less solvent. Besides, when using abalone viscera as raw material, UAE combined with alkaline extraction also showed to improve the protein extraction rate by around 17% when comparing with alkaline extraction alone, and the produced peptides demonstrated iron chelating properties. [49]

UAE has been recognized as a fast, clean, reproducible, and alternative non-thermal extraction method when compared to traditional extraction methods. [34] Nevertheless, and similarly to the other innovative processing technologies, UAE is still not a standard technology, and more studies are required to establish standardized protocols for each desired application in order to make full use of this technology, including but not limited to protein extraction from food wastes.

Supercritical fluid extraction

Supercritical fluid extraction (SFE) uses solvents above their critical point (temperature and pressure) to separate compounds from the matrix. Under supercritical conditions, the solvents have intermediary properties between gases and liquids, facilitating the extraction of the desired compounds. Supercritical fluids possess diffusion, viscosity, and surface tension similar to those of gases, thus accelerating mass transfer phenomena to and from the solid matrix, and liquid-like density and solvation power, thus facilitating the penetration of the solvent into the solid matrix. Carbone dioxide (CO_2) is the most widely used SFE solvent in food applications (GRAS), and it is usually used to extract non-polar compounds.[43,75] SFE is reported as a fast, efficient, selective, and environmentally friendly technology for the extraction of valuable compounds from several matrices.[43,75] However, this technique has some drawbacks, namely the need for specific and expensive equipment to operate at elevated pressures, the non-selectivity for extracting polar substances, and the high energy consumption.[43]

Regarding the valorization of fish wastes, SFE has been mainly exploited as a pre-treatment for oil valorization and extraction of fatty acids.[75] Nevertheless, AA recovery from *Todarodes pacificus* squid is reported using SFE, followed by SWH.[52]

Purification of the peptide fraction

Several authors include a purification step in the process of extracting bioactive compounds. Purification is crucial for obtaining peptide fractions with specific composition or biological properties. Composed by several processes, purification aims to isolate a molecule from a mixture, allowing the characterization of the structure, function, and interactions of the peptide or protein of interest. Thus, expressing peptides often involves the fusion of tags to these molecules, which is essential in the purification process.[76] After this purification step, the peptides show a high degree of purity, a crucial factor in obtaining reliable results in the evaluation of their bioactive effects. In addition, this purity becomes mandatory for their intended industrial application, particularly in the pharmaceutical sector.[77]

Purifying and characterizing peptides have been a major challenge. Classical purification methods, namely dialysis, membrane separation, gel chromatography, ion exchange chromatography, and reverse-phase high-performance liquid chromatography (RP-HPLC), have been applied and combined to purify the peptides.[78] However, these methods perform a separation based on the difference in molecular polar interaction or weight, which has been pointed out as a limitation of these methods.

Ultrafiltration and nanofiltration are widely used after enzymatic hydrolysis to fractionate peptides by molecular weight. These membrane-based techniques are scalable and cost-effective, being a good option for applications that balance purity with economic efficiency.[79,80] However, additional steps may be needed for higher purity.[81] In contrast, RP-HPLC offers high peptide purity by separating them based on hydrophobicity, but its high cost and limited scalability restrict its use to high-value products.[79,80] Gel chromatography, frequently combined with ultrafiltration or RP-HPLC, separates peptides by molecular weight and is valuable for producing peptides with reliable bioactivity, though it is time-consuming and less efficient for large-scale production[79,80]. Ion exchange chromatography separates peptides based on charge, yielding high-purity peptides, but it is more costly and less efficient for industrial-scale operations.[79,80] Affinity separation, which is based on reversible interactions between molecules, provides high specificity but is also costly and complex, limiting its industrial use.[79,80,82]

BPs obtained from FBCPs, such as skin, viscera, and scales with antioxidant properties, may be a potential alternative to synthetic antioxidants, benefiting human and animal nutrition.[83] Thus, several studies are dedicated to extracting, characterizing, and purifying bioactive compounds from different fish parts. Liu et al.[84] proved the tyrosinase inhibitory activity *in vitro* of the peptide derived from zebrafish phosvitin. Scale peptides from tilapia, a tropical fish originally from Africa, were reported by Chai et al.,[85] highlighting its advantages as natural ingredients, low MW, and easy absorption by the human body.

Cholecystokinin-releasing peptides contained in FBCPHs (from blue whiting (*Micromesistius poutassou*) and brown shrimp (*Penaeus aztecus*)) were partially purified and characterized by size (of apparent molecular weight ranging from 1000 to 1500 Da) exclusion chromatography using a Toyopearl HW-40F column. This study pioneered the potential use of peptide molecules from FBCPs and crustacean FBCPs for application in appetite suppressant products.[86]

In a study developed by Ko et al.,[87] two peptides derived from the muscle protein of the olive flounder (*Paralichthys olivaceus*) were purified and showed a strong antioxidant action. The authors prepared the hydrolysates by enzymatic reactions of flounder fish muscle using eight commercial proteases such as papain, pepsin, trypsin, neutrase, alcalase, kojizyme, protamex, and α-chymotrypsin. Further separation of the α-chymotrypsin hydrolysate was performed by ultrafiltration, gel filtration, and RP-HPLC.

A study developed by Saidi et al.[88] intended to produce and fractionate an FPH from the FBCPs of tuna dark muscle, highlighting the performance of ultrafiltration and nanofiltration processes in purifying these hydrolysates. In this study, Alcalase proved to be the most suitable protease for producing peptide fractions, with optimal conditions: temperature 55°C, pH 8.5, time 60 min, enzyme-to-substrate ratio 1%. The study also verified that fractionation is improved when combining membranes.

Another study subjected a proteolysate generated by alcalase from *Stichopus horrens*, a popular sea cucumber species, to fractionation based on the peptide hydrophobicity. In this study, peptides were fractionated using a gradient elution of acetonitrile in a percentage between 0% and 31.3%.[89] The results proved that the hydrolyzed protein components have bioactive peptides with potential application in the food industry as functional ingredients. Similar studies on peptide fractionation using RP-HPLC showed the elution of peptides with acetonitrile in percentages below 50%. Peptides isolated from proteolyzed cuttlefish were purified in the third step with an acetonitrile concentration below 29%.[90] The most active hydrolysate was obtained with the crude protease extract from the hepatopancreas of cuttlefish (64.47 ± 1.0% at 2 mg of dry weight/ml) with a degree of hydrolysis of 8%. Likewise, BPs isolated from catfish muscle hydrolysate were purified with the aim of ultrafiltration, gel filtration, and RP-HPLC with a C18 column with acetonitrile at a concentration below 50%.[91]

The production of FBCPHs presents an environmentally sustainable and valuable solution for the fish processing industry. Among hydrolysis methods, enzymatic hydrolysis is preferred for producing highly bioactive and pure peptides, despite its higher costs. Emerging technologies like SWH, PEF, and UAE offer more efficient alternatives by reducing processing times and energy consumption. For peptide extraction/purification, ultrafiltration and nanofiltration are considered ideal for industrial use due to their cost-effectiveness and molecular weight separation, while RP-HPLC and ion exchange chromatography are better for high-purity needs, especially in research and pharmaceutical applications. Overall, optimizing hydrolysis, integrating novel technologies, and improving purification techniques are crucial for fully disclosing the potential of FBCPHs in the nutraceutical and pet food industries. More research is required to enhance energy efficiency and scalability.

Fish by-products protein hydrolysates as functional ingredients

Peptides biological activities

FBCPHs and their peptides have a wide range of applications across several industries, including food, nutraceuticals, pharmaceuticals, and cosmetics, due to their biological activities and nutritional value. In recent years, FBCPHs and their peptides have gained significant importance in animal nutrition.[12,92] FBCPHs deliver a rich source of peptides with a complete amino acid profile, superior bioavailability, and higher levels of essential amino acids compared to peptides derived from mammalian or plant proteins[92,93] Besides that, FBCPH peptides have demonstrated the potential to enhance human and animal health preventing and alleviating symptoms of various diseases.[6,12,92] FBCPHs and their peptides have been shown to possess several biological activities, such as antioxidant, anti-inflammatory, anti-hypertensive, immune-modulatory, antimicrobial, or hormone-regulating properties.[34,92,94] The development of FBCPHs through enzymatic hydrolysis has been essential in showing these biological activities, providing sustainable, bioactive, and nutrient-rich ingredients that support the health and well-being of animals.[6,12,31] Each of the above-enunciated biological activities will be discussed in detail in the following sections.

Oxidative stress control

Several FBCPHs and peptides exhibit potent antioxidant activity, countering reactive oxygen species (ROS) to mitigate oxidative stress.[95] Uncontrolled ROS generation contributes to health disorders such as diabetes, cardiovascular issues, neurodegenerative diseases, and inflammation.[6,96] On the other hand, Food deterioration is linked to lipid oxidation and secondary lipid peroxidation products.[97] FBCPs peptides are suggested as alternative additives to synthetic antioxidants.[87]

The antioxidant efficacy of FBCPHs and peptides depends on production parameters like enzymatic hydrolysis specificity and degree of hydrolysis (%DH). These parameters influence peptide structure and, consequently, antioxidant activity.[6,94,98] Antioxidant peptides generally have shorter chain lengths (0.5–1.5 kDa), hydrophobic amino acid-rich composition, and aromatic amino acids like tyrosine and tryptophan, with valine and leucine at the N-terminus.[99–103]

Several authors commonly correlate low MW and antioxidant activity of FBCPHs.[47,104,105] Low-molecular-weight antioxidant peptides interact more easily with free radicals and have a higher capacity for elimination.[101,105] However, Sierra et al.[99] reported higher antioxidant activity in hydrolysate fractions from red tilapia scales with MW of 10–100 kDa and 3–10 kDa compared to the < 3 kDa fraction. However, these hydrolysates presented AAs with hydrophobic character at the C-terminal position (glycine and proline), which, when in high proportion, contribute to higher antioxidant activity.[99,103] Moreover, aspartic and glutamic acids were also identified as contributors to the antioxidant activity of FBCPHs (scales and viscera) due to an excess of electrons that could be donated by interacting with ROS/free radicals.[102]

High metal ion-chelating activity of Stripped weakfish FBCPHs was detected by Lima et al.[101] Furthermore, metal ion-chelating activity is related to the presence of basic (arginine and lysine) and aromatics AAs (e.g., phenylalanine) at the C-terminal position, together with the high content of hydrophobic AAs (leucine). On the other hand, tryptophan at the N-terminal position was associated with its capacity to act as a hydrogen donor in the sequestration of hydroxyl radicals.[101] Positively charged AAs as lysine at the *N*- and C-terminal is another valuable feature detected in antioxidant FBCPHs.[104,105]

The mechanisms of antioxidant activity studied to FBCPHs include in vitro radical scavenging [2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)], superoxide and OH-radical scavenging,[104,105] but also ferric reducing antioxidant power (FRAP), metal chelating activity.[47,101,103] Cell oxidative stress reduction has recently been assessed.[99,102] Excessive ROS and oxidative stress are associated with several diseases, emphasizing the significance of antioxidant peptides.[12,99]

The antioxidant activity of FBCPHs is commonly evaluated regarding in vitro radical scavenging activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS); superoxide (O2-) and OH-radical scavenging activities,[104,105] but also by evaluation of ferric reducing antioxidant power (FRAP) and metal chelating activity.[47,101,103] Cell oxidative stress reduction has recently been assessed in red tilapia viscera and scales hydrolysates.[99,102] Red tilapia viscera hydrolysate (0.1 mg mL-1) and its < 1 kDa fraction (0.25 mg/mL) demonstrated no cytotoxic effect and cytoprotective effects preventing the decrease of the cell viability and ROS level accumulation in Caco-2 cells treated with 5 mm H2O2 to induce oxidative stress, being potential effective antioxidant agents against ROS-mediated intestinal injuries, principally the < 1 kDa fraction. In another study, red tilapia scales hydrolysate and fractions (2 mg/mL) reduced oxidative stress in A7r5 cells stimulated by 1 µM of Ang II. Ang II activated ROS production in A7r5 vascular cells, which can react with nitric oxide and trigger inflammation and proliferation, leading to systemic vascular dysfunction and accelerating hypertension/atherosclerosis development.[99]

Prevention of obesity-associated diseases

Obesity is a global public health problem, affecting both humans and their pets, with associated risks such as hypertension, type 2 diabetes, cardiovascular disease, osteoarthritis, and cancer.[106–108] Besides the capacity to reduce oxidative stress induced by obesity, FBCPHs are reported to possess other vital activities against several obesity-associated diseases, such as angiotensin I-converting enzyme (ACE) and dipeptidyl peptidase IV (DPP-IV) inhibitory activities, offering potential benefits against hypertension and type 2 diabetes,[103,109–111] but also satiating, lipid-lowering, and antiatherogenic potential effects.[108,112–114]

ACE inhibitory peptides, which typically have low molecular weight (8–20 AA residues) and moderate hydrophobicity, have been identified in FBCPHs from shortfin scad (*Decapterus macrosoma*)[115]; sturgeon skin[103] and rainbow trout frames.[116] Besides the low molecular weight (<3 KDa), the antihypertensive hydrolysate (IC_{50} 2.20 mg/mL) from shortfin scad exhibited greater hydrophobicity at the *C*-terminal (e.g., alanine, leucine) with an aliphatic AA (glycine) at the

N-terminal. On the other hand, the ACE inhibition activity of sturgeon skin peptides was mainly attributed to strong hydrogen bonds with lysine at the *C*-terminal and aliphatic AAs (two proline) at the *N*-terminal.[103] Moreover, a study of selective fractionation (electrodialysis with ultrafiltration membrane) of rainbow trout frames hydrolysates highlighted the role of hydrophobic and positively charged AAs in the ACE inhibitory activity.[116] Cationic peptide fraction showed a potent inhibitory activity (IC_{50} 0.0036 mg/mL) compared to initial FBPH (0.015 mg/mL).

The mechanism of action of ACE inhibitory activity of FBCPHs is linked to the modulation of the renin-angiotensin system (RAS), relaxing blood vessels, and decreasing blood pressure.[103] Besides that, the inhibition of ACE may increase nitric oxide availability and improve endothelial function, as observed in subjects with high cardiovascular disease after ingesting an FBCPH from Nile tilapia FBCPs.[117] An enhancement of muscle O_2 desaturation and resaturation parameters, with no changes in blood pressure, was also detected.[117] In an *in vivo* study using a model of high-salt and -fructose diet-induced hypertension in Wistar rats, a reduction of the systolic blood pressure and consequent hypertension attenuation was verified after administration of a viscera FBPH and its < 1 kDa peptide fraction.[114]

Regarding the DPP-IV inhibitor activity of peptides obtained from FBCPs, the lower MW and presence of hydrophobic AAs at the *N*-terminal position have been reported using salmon trimmings,[115] and skin,[118] tilapia FBCPs[111] and sturgeon skin.[103] The hydrolysates obtained enzymatically from salmon skin gelatin and trimmings with higher DPP-IV inhibitory activity exhibited a higher proportion of peptides < 1 kDa.[118] Hydrophobic AA residues such as phenylalanine, arginine, and tyrosine were also detected. More recently, the molecular docking studies of DPP-IV inhibitory peptides obtained from sturgeon skin revealed that their activity is mainly attributed to hydrogen bonds and hydrophobic interactions involving tyrosine, arginine, and serine residues.[103] Besides that, the hydrolysates obtained from salmon skin gelatin and trimmings also showed glucagon-like peptide-1 (GLP-1) secretory activity and a positive effect on insulin release from BRIN- BD11 cells. Other FBCPHs also increase insulin secretion by stimulating GLP-1 secretion, further increasing insulin release and improving glucose homeostasis.[94,111] Theysgeur et al.[111] identified new peptides from enzymatic hydrolysis of tilapia FBCPs that stimulate GLP-1 secretion after canine gastrointestinal simulated digestion. GLP-1, as an intestinal anorexigenic hormone, also exerts a satiating effect via different pathways. After a transport study through the Caco-2 cell monolayer, peptides with DPP-IV inhibiting activity were also found in the hydrolysate obtained from the tilapia FBCPs.

Regarding the effects of FBCPHs on body weight regulation, hydrolyzed fish collagen peptide obtained from tuna skin by subcritical water was tested in a mouse model of obesity induced by high-fat diet feeding.[112] The hydrolysate reduced body weight gain and improved the serum lipid profiles of obese mice. Besides that, differentiation inhibition of 3T3-L1 preadipocytes into adipocytes by decreasing the expression of adipogenic master genes was also noticed.

Reduction of anti-inflammatory response

The hydrolysates from FCBPs have been reported to possess potent anti-inflammatory properties.[119] Inflammation is a typical response of the immune system to lesions and infection, modulated by inflammatory mediators and pro-inflammatory compounds such as cytokines released by activated macrophages or other cells. Excessive inflammation (uncontrolled production of pro-inflammatory compounds) can contribute to various acute and chronic diseases, such as metabolic syndrome and inflammatory bowel disease.[120,121] Several FBCPHs have shown significant modulatory effects against inflammatory reactions through inhibition of the production of pro-inflammatory markers, such as nitric oxide (NO), inducible nitric oxide synthase (iNOS), and cytokines (e.g., Tumor necrosis factor-α (TNF-α)).[122] Among them, the FBCPHs from FBCPs of canned sardine,[122] herring,[123] sturgeon caviar muscle,[119] tuna fishery,[124] mixed-species fish skin,[125] and Atlantic salmon heads and backbones[126] can be highlighted.

An anti-inflammatory hydrolysate was obtained from canned sardine FBCPs hydrolysis using a brewing spent yeast protease extract. According to Vieira et al.,[122] the desalted sardine protein hydrolysate (<10 kDa) decreased all inflammation markers (2.0 mg peptides/mL) in the endothelial cell line (EA.hy926), but also the co-culture model with an intestinal cell line (Caco-2), compared to TNF-α-treated control. The potential anti-inflammatory effects during endothelial inflammation detected in sardine protein hydrolysate were related to the presence of the AAs glycine, histidine, and cysteine despite its high content in glutamic acid, glutamine, aspartic acid, and alanine (51.2%). Durand et al.[123] separated by electrodialysis with ultrafiltration membrane (EDUF), a herring milt hydrolysate obtaining two anionic (higher acidic AAs content) and two cationic fractions (higher presence of basic AAs). Positively charged peptides characterized both cationic fractions, with arginine as one of the main AAs described as characteristic of effective anti-inflammatories. Both cationic fractions revealed the potential to prevent metabolic syndrome. These fractions exhibited anti-inflammatory effects by reducing iNOS activation in the lipopolysaccharides. NO production was induced in J774 mouse macrophage cells. Furthermore, FPH from the kingfish frame[127] ameliorated the adverse effects of juvenile barramundi's poultry by-product meal-based diet in aquaculture. Chaklader et al.[127] showed the positive impact of FPH supplementation at 10% in terms of modulation of disease resistance and pro-inflammatory and inflammatory cytokines post-*Vibrio harveyi* infection.

FCBP hydrolysates' potential to prevent gastrointestinal inflammation-related diseases such as ulcerative colitis has also been reported. Ulcerative colitis is a primary form of inflammatory bowel disease.[119] Skin collagen of Pangas catfish and sturgeon FBCPHs significantly decreased the severity of dextran sodium sulfate (DSS) -induced damage mice model.[119,121] Besides that, the *in vitro* experiments performed by Sivaraman and Shanthi[121] revealed that skin collagen hydrolysate also attenuates TNF-α induced tight junction barrier disruption. On the other hand, the suppression of DSS-induced activation of the NF-κB and MAPK pathways in the colon was detected after the administration of sturgeon FBCPH.[119] Gao et al.[119] also noticed the restoration of gut microbiota of colitic mice, namely an increase in the *Bacteroidetes/Firmicutes* ratio and the relative abundance of other benefic bacteria while decreasing the abundance of potentially harmful bacteria such as *Enterococcaceae*. Positive modulation of distal intestinal microbiota with an enrichment of the *Firmicutes* and *Fusobacteria* was reported in juvenile barramundi with a poultry BCPs meal-based diet after tuna BCPs hydrolysate supplementation.[124] The upregulation of pro-inflammatory cytokines (IL-1β and TNF-α) and downregulation of the anti-inflammatory cytokine IL-10 were also reported by Siddik et al.[124]

Role in microorganisms' control and modulation

Several studies have highlighted the role of microbiota in health and diseases. Gut microbiota dysbiosis is associated with obesity and other metabolic diseases such as diabetes, asthma, inflammatory bowel disease, and rheumatism.[119,128] Some studies showed that peptides could beneficially modulate gut microbiota. However, the microbiota regulation by peptides obtained from FBCPs has barely been studied. Wang et al.[129] valorize Walleye pollock skin through the production of collagen peptides, which significantly increased the levels of some beneficial bacteria and decreased the levels of bacteria involved in inflammation in the high-fat diet (HFD) mouse model. Besides, significant anti-obesity effects were also described.

The role of FBCPHs in microbiota regulation is also described relative to wound healing. The oral administration of fish skin collagen peptides to the murine wound model promoted wound healing by regulating microflora colonization in the wound tissues, controlling the inflammatory reaction, and increasing angiogenesis and collagen deposition.[130] Until now, peptides from FBCPs have not been evaluated regarding their potential benefits in oral microbiota modulation, but they could be promising agents in the equilibrium of oral microbiota. Oral microbiota equilibrium has not been investigated as much as gut microbiota. However, its imbalance and dysbiosis are linked to teeth and oral diseases, exerting relevant influence in systemic diseases such as rheumatoid arthritis, inflammatory bowel disease, and chronic kidney disease.[131]

Besides their microbiota modulation role, FBCPHs have been described as potential antimicrobial compounds. Antimicrobial peptides (AMPs) are produced in fish as part of their immune systems.[132] AMPs exhibit activity against Gram-positive and Gram-negative bacteria, fungi, viruses, and unicellular protozoans.[34] AMPs such as piscidin, defensin, hepcidin, cathelicidin, and histone-based peptide families have been isolated from several kinds of fish.[133] Besides that, AMPs have been produced enzymatically from FBCPs, such as yellowfin tuna viscera,[134] mixed FBCPs of the distribution market,[104] tuna skin collagen,[47] and stripped weakfish FBCPs.[101] However, its exploitation and application have been less studied than antioxidant and anti-hypertensive activity.

Most antimicrobial peptides identified in FBCPs are cationic with low MW and hydrophobic properties. According to a study of fractionation of yellowfin tuna viscera hydrolysate, the shortest peptides (<3 kDa fraction) are the most active against Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram-positive (*Listeria monocytogenes*, *Staphylococcus aureus*) with a Minimum Inhibitory Concentration (MIC) of 0.5 mg/mL.[134] Furthermore, the higher concentration of positively charged and hydrophobic AAs was < 3 kDa fraction. The presence of AAs with a positive charge (lysine, arginine, and histidine), in addition to the hydrophobic AA content (60%), such as alanine, leucine were also associated with the antimicrobial activity of 10–30 kDa fraction of collagen hydrolysate derived from mixed FBCPs against *E. coli* (10 mg/mL).[104] Lima et al.[101] also reported a higher proportion of hydrophobic AAs in the highest effective Stripped weakfish FBCPHs against *E. coli* (50 mg/mL) and *S. aureus* (100 mg/mL) compared to the other hydrolysates. Moreover, Ahmed and Chun[47] reported that a hydrolysate of tuna skin collagen obtained by subcritical water hydrolysis at 280°C exhibited either the highest content of hydrophobic AA and antimicrobial activity (against *Bacillus cereus*, *Pseudomonas putida*, *P. aeruginosa*). The hydrophobic character of peptides facilitates the interaction between the positively-charged peptide with the negatively-charged surface of the bacteria, and the AAs charged positively penetrate bacterial membranes interacting with the negative charges of the cell wall of phospholipids, promoting cellular lysis.[47,104]

Besides their broad antimicrobial spectrum, AMPs have been pointed out as promising alternatives to antibiotics due to their low level of induced resistance and potential capacity to modulate the inflammatory response and gut microbiota.[135] The overuse of antibiotics is a severe problem in animal production, including aquaculture, leading to increasing antimicrobial resistance, microbiota dysbiosis, and the development of other diseases. Recently, a novel AMP from rainbow trout (*Oncorhynchus mykiss*) with antibacterial activity against *Streptococcus iniae* (zoonotic pathogen) was identified using an *in silico* approach.[136] Regarding its immunomodulatory activity, treating rainbow trout cells with the AMP did not upregulate the immune genes. However, it stimulated the cell Line RTS11 (rainbow trout macrophage/monocyte-like cell line) and significantly upregulated the TNF-α gene.

Neuroprotective ability and behavioral issues control

Peptides or protein hydrolysates have been described as promising neuroprotective agents that act in neuronal survival via modulating signaling pathways but also inhibit neuroinflammation and consequently suppress cognition decline.[137,138] FBCPHs have been exhibited to be potential candidates for memory and learning-improving functions.[137,139,140] Anchovy hydrolysates proved to be a potential improver for memory in scopolamine-induced amnesia mice. The anchovy hydrolysate obtained enzymatically after 8 h exhibited better results, probably due to its high glutamate content.[137] Potential positive effects in memory and learning deficiency were described for peptides from lantern fish (*Benthosema pterotum*). Lantern fish peptides revealed efficacy in neuron protection in human neuroblastoma SH-SY5Y cells against H_2O_2-induced apoptosis and improved D-gal-induced deficit of memory and learning ability in aging ICR mice. Lower levels of D-gal-induced thiobarbituric acid reactive substances (TBARS) and endothelial NOS were detected in mice fed with lantern fish hydrolysate and higher levels of glucose-6-phosphate dehydrogenase and brain-derived neurotrophic factor in comparison to the control group.[139] Sea cucumber (*Cucumaria frondosa*) hydrolysate also ameliorates learning and memory deficits of D-galactose-induced aging mice.[140] In Lin et al.[140] study, peptides' neuroprotective activity seems to be associated with the capacities to inhibit lipid peroxidation and protein oxidation, increase antioxidant enzyme activity (superoxide dismutase and glutathione peroxidase), and down-regulate acetylcholinesterase. The expression of Klotho, which acts as an anti-aging humoral factor, is also upregulated. More recently, peptides isolated from round scad hydrolysate exhibited neuroprotection action against glutamate-induced neurotoxicity in PC12 cells.[138] Tyrosine and tryptophan also proved to be related to the neuroprotection activity of peptides obtained from round scad.

Until now, few studies have assessed the neuroprotective ability of FBCPHss.[141,142] In Chataigner et al.[141] study, the supplementation with low MW peptides from FBCP hydrolysate combined with *omega-3* long-chain polyunsaturated fatty acids prevented the age-related spatial short-term memory deficits and modulated navigation strategies adopted during spatial learning in aged mice. Besides that, FBCP peptides also reduced anxiety-like behavior, restored plasmatic corticosterone levels like those of adult animals following acute stress, and modulated the hypothalamic stress response. A sardine byproduct hydrolysate (Peptidyss®) also decreased stress reactivity in Balb/c mice by reducing corticosterone levels 30 min after stress induction compared to control mice.[142] The sardine BCPs hydrolysate supplementation modulated stress-responsive gene expression, especially in the hippocampus. Similar positive effects on stress and anxiety responses were found in dogs supplemented with FBCPs.[143,144] According to Landsberg et al.,[144] the mackerel and cod hydrolysates (Gabolysat® PC60) exhibited anxiolytic properties, decreasing hyperactivity and reducing cortisol response to stress. On the other hand, a new dietary supplement (derived from fish hydrolysate and melon juice concentrate rich in superoxide dismutase) promoted dog-human interactions and tended to reduce subtle stress behaviors.[143]

Lastly, opioid-like peptides such as glyprolines (PGP and GP) are other peptides that present the capacity to relieve the behavioral problems caused by stress in rats subjected to a forced swimming test (10 min). Peptides Pro-Gly-Pro and Gly-Pro intranasal[145] and intraperitoneal[146] were administrated 15 minutes after the end and before the stress test, respectively. Both studies demonstrate that Pro-Gly-Pro and Gly-Pro peptides prevent and mitigate stress-induced anxiety and behavioral disorders, potentially through interactions with CNS structures involved in stress responses. However, Pro-Gly was less effective, highlighting the importance of specific peptide sequences in conferring antistress properties. Despite this, the opioid-like activity of peptides or hydrolysates from FBCPs has not yet been explored.[147]

Overall, FBCPHs exhibit a wide range of bioactivities. Their ability to mitigate oxidative stress, regulate lipid metabolism, inhibit enzymes linked to obesity-related diseases, and control inflammation highlights their potential as functional ingredients in both human and animal health. In addition, FBCPHs present novel insights into the modulation of gut microbiota and stress-related behaviors, particularly in neuroprotection and stress reduction, showing their potential role in improving cognitive function and emotional well-being. These findings highlight the FBCPHs' potential as an environmentally sustainable source of proteins and bioactive peptides that support overall health. However, some limitations must be addressed for their effective application, namely the variability in peptide efficacy due to molecular weight, amino acid composition, and hydrolysis conditions emphasizing the need for standardized production methods to improve bioactivity. Additionally, further in vivo studies are needed to elucidate the underlying mechanisms, validate their therapeutic potential, and establish safety, efficacy, and acceptable use levels.

Future research on the potential of FBCPHs as alternatives to antibiotics in animal nutrition, as well as their effects on oral microbiota and neuroprotection in humans and animals, needs to be addressed. Understanding these aspects will contribute to the development of innovative bioactive ingredients from FBCPs.

Fish by-products hydrolysates application on pet food

Pet food demands and trends

Pet ownership is steadily increasing globally, including in emerging economies, and according to the European Pet Food Federation,[148] 90 million European households (46%) own a pet, with cats (113 million) and dogs (92 million) being the most popular companion animals in Europe. Higher-income, increased urbanization, demographic changes such as family size, millennial couples having children late in life, people living alone, high level of education, and the recent COVID-19 pandemic constitute drivers for this trend in the pet population.[149,150] Consequently, sales of pet food have increased dramatically, reaching 27.7 billion euros in Europe in 2021 with 3.1% annual growth for the pet food industry,[148] and expected to progress at a compound annual growth rate of 7.22% during the period 2022–2032.[151]

Dry pet food is the most popular among the three commercially available pet foods (dry, semi-dry, and wet) because of its long shelf life.[152] Pet food markets offer various commercial diets formulated to meet nutritional requirements according to life stage, health status, and activities. In Europe, regulatory guidelines and recommendations are published by the FEDIAF, based primarily on research data published by the National Research Council.[153]

To ensure sustainable pet ownership, pet food must be simultaneously sustainable and affordable while assuring the requirements for optimum animal health and well-being.[154] Market research has been reporting a trend towards premiumization,[155] with pet owners demanding high-quality ingredients in line with the increasing humanization of pets that are now considered family members.[156,157] Concurrently, pet owners increasingly favor food's functional value to promote pets' health and well-being. Among the top health-related claims by pet food brands, high protein is the most common and natural ingredient, the second being vitamins, antioxidants, and immune system health.[155] Additionally, pet owners' sociocultural factors and eating habits have been suggested to influence their companion animals' choice of pet foods and eating habits.[158]

Protein requirements for dogs and cats are relatively high, set at 18–25% and 25–33.3% (dry weight basis), respectively.[148] Compared to vegetable sources, animal-based ingredients present several advantages, such as high protein content and digestibility and provision of significant amounts of vitamins (e.g., vitamin B_{12}) and minerals in organic forms, thus making them more bioavailable for the animal.[8,159] A growing trend in the pet food industry is replacing rendered animal meals, traditionally representing the most common protein source in pet food, with raw animal proteins,[160] more in line with ancestral and unprocessed diets. However, this trend exacerbates the pressure on natural resources[161] and increases the pet food industry's carbon footprint.[162] A sustainable alternative lies in using BCPs from meat and fish processing, which is negatively perceived by owners who demand human-grade meat as the main ingredient for their pets.[163] The inclusion of BCPs under the new food category 'upcycled food' can contribute to these products rebranding and improve public acceptability while promoting industry sustainability and repurposing food that otherwise would be wasted.[164,165] "Upcycled ingredients" have been gaining popularity among pet food and treat brand manufacturers. However, consumer consciousness remains limited. In a recent survey, 66% of pet owners reported unfamiliarity with the term "upcycled ingredients".[166] However, only 7% indicated that they have already fed their pets with "upcycled ingredients," while 54% expressed willingness to try them. For 24% of respondents, acquiring more information would be the initial step toward "Upcycled ingredients" adoption and only 7% of pet owners indicated no interest in the concept. Besides that, the highest awareness was registered among UK consumers and the lowest among US pet owners. Furthermore, younger generations were more likely to recognize the term than older generations. In this context, FBCPHs are particularly interesting to pet food. Indeed, FBCPHs provide AAs more easily absorbed in the small intestine,[13] with hypoallergenic[12] and bioactive properties, acting as palatability enhancers[11] and contributing to pet food sector sustainability and circular economy.[163] However, despite the high interest in these ingredients, studies on companion animals are scarce.[13]

European pet food legislation on functional ingredients

When developing novel pet food products enriched with functional ingredients, it is essential to consider that appropriate regulatory requirements must be complied with to place the product on the market. Both pet food manufacturing and commercialization are highly regulated worldwide, with legislation covering the ingredients, the production process, and the marketing and sales. The aim is to ensure that pet food products are safe, fulfill the nutritional needs of pets, and that the information provided to consumers is accurate and truthful.[167]

The legal requirements for pet food are established explicitly by country and/or region. This work will focus on the European Union (EU) legislation, where the EU Commission regulates pet food, the EU Parliament, and the Council of the EU.[167] The main legal requirements for pet food production and commercialization are outlined in Table 1.

For the use of functional ingredients in pet food, EU Regulation 767/2009 is particularly relevant since it provides basic rules for product claims (including functional claims), labeling, and packaging. It also provides a framework for establishing and marketing PARNUTs (products for particular nutritional purposes), i.e., legally "dietetic" pet food products.

Aiming to explain and clarify how EU Regulation 767/2009 requirements work in practice, FEDIAF developed the Code of Good Labelling Practice in 2011, revised in 2019.[168] Both versions of the document were endorsed and recognized by the EU authorities. The Code intends to offer clear guidance to the EU Member States, avoiding different interpretations and applications of the defined rules. Therefore, the FEDIAF Code must be considered and analyzed when novel pet food products with functional claims are under development.

According to the Code, functional claims describe the effect of a complete or complementary pet food or a nutrient, substance, characteristic, or additive in the pet food on the body's growth, development, or normal functions. It provides a specific physiological benefit and may concern "optimization of the nutrition and support or protection of the physiological conditions" (R. 767/2009, Art. 13.2). These effects must go beyond meeting the basic nutritional needs of the animals. Functional claims can be considered as nutrient function claims, enhanced function claims, or health maintenance and decreased disease risk claims, according to the general requirements indicated in Table 2.[168]

Table 1. List of EU legislation concerning pet food safety and labeling and claims.

Scope	Legislation applied	Description
Pet food safety	Regulation 178/2002	General principles and requirements of food law (which also applies to feed)
	Regulation 183/2005	Requirements for feed hygiene
	Regulation 1069/2009 Regulation 142/2011	Animal by-product regulations with detailed rules on the safety of raw materials of animal origin used in pet food, their processing requirements, and model health certificates for imports to the EU
	Regulation 999/2001	Rules for the prevention, control, and eradication of certain transmissible spongiform encephalopathies
	Regulation 1831/2003	Requirements on additives for use in animal nutrition; all approved additives are listed in the Register published by the EU Commission
	Directive 2002/32	Directive dealing with undesirable substances in feed, which sets maximum limits for contaminants, with specifications for raw materials and finished feeds
Labeling and Claims	Regulation 767/2009	Rules for labeling, claims, and other forms of marketing communication
	Directive 2008/38	List of all approved PARNUT (products for particular nutritional purposes) indications

Table 2. Different types of functional claims and their general requirements are according to the FEDIAF Code.[168]

Functional claims	General requirements
Nutrient function claims	Simply links the presence of a nutrient or combination of nutrients in a product to the physiological role in the body's growth, development, and normal functions, without any further detail about the effect's level or degree/mechanism.
Enhanced function claims	Describes the specific beneficial effect of nutrients or other substances, alone or in combination, on physiological functions or biological activities in the body. Enhanced function means an effect that either exceeds its usual role in maintaining normal metabolic functions, including growth and development or is related to a substance that is not essential for the animal but provides a benefit beyond nutrition. No reference should be made to particular diseases or pathological states.
Health maintenance and decreased disease risk claims	Related to the optimization of nutrition and the support or protection of physiological conditions. It can also be related to health maintenance and reducing the risk of disease development resulting from nutritional imbalances in a healthy animal. Such claims relate to the consumption of a product containing a nutrient or other substances, alone or in combination, that helps to reduce the risk of disease development or maintain physiological functions or health. Claims referring to the treatment or curing of a disease are considered medicinal claims and would cause a product to be medicinal by presentation. However, words such as prevent may be used if not related to a disease treatment. The Code provides a guideline with some examples of words that may be avoided since they are usually associated with authorized medicinal products. When using substances supporting health maintenance and decreased disease risk claims, operators shall ensure that the relevant substance is properly classified as an additive, a feed material, or a veterinary medicinal product; if it is classified as a veterinary medicinal product, it may not be used in pet food. In case of unclear classification, the Commission Guidelines shall be consulted.

Functional claims (as well as other types of product claims) shall be objective, verifiable to the competent authorities, and understandable by the pet food user, thus meeting the following requirements[168]:

(i) Must be substantiated at the time of putting on the market;
(ii) Must not confuse or mislead purchasers;
(iii) Must not denigrate other pet foods or suggest that other pet foods do not possess such characteristics when it is not true.

All claims must be substantiated and verifiable, but the degree of substantiation will depend on the type of claim made. In the case of functional claims, the level of substantiation depends on whether the claim is considered generic or innovative. Generic claims are considered when well-established and recognized knowledge exists, typically functions of approved additives and/or nutrient functions. In that case, substantiation is based on general (scientific) knowledge, with published literature and documentation demonstrating the beneficial effects of the pet food product. On the other hand, innovative claims are not yet widely recognized. Their substantiation may be based on a comprehensive review and evaluation of all available scientific data related to the claim's validity, whether published or in-house, irrespective of whether its impact is favorable or otherwise.[168]

As a final remark, it is important to note that products with functional claims should be clearly separated from PARNUTs, which correspond to "dietetic" pet food products. These are conceived to meet the specific nutritional needs of animals whose process of assimilation, absorption, or metabolism is or could be temporarily or irreversibly impaired and which can benefit from ingestion of the feed appropriate to their condition. In this case, the claims are strictly controlled, and thus, diets may only be marketed as PARNUTs if their indication and nutritional characteristics are listed explicitly in Directive 2008/389.[167,168]

Fish hydrolysates in pet food

In the last few years, the life expectancy of companion animals has increased due to advances in pet nutrition and veterinary care. Simultaneously, a higher risk of developing chronic diseases by pets was also verified. As a result, protein hydrolysates have been rising as promising nutraceuticals of interest in the animal health market.[12] In the pet food sector, protein hydrolysates are among the most popular palatability enhancers, being considered to exert satiety effects, and commonly used to manage adverse food reactions, gastrointestinal and dermatological diseases, and to reduce stress.

The increasing demand for premium diets that promote nutritional and functional benefits, natural and sustainable ingredients, and organic and clean-label products has been amplifying the fish protein hydrolysate industry. Indeed, a recent report from Global Market Insights Inc. (2024)[169] outlined that the fish protein hydrolysate market size for animal feed and pet food was valued at USD 233.08 million in 2023, being projected to grow at 5.5% CAGR from 2024 to 2032, with the enzymatic hydrolysis segment comprising the main market share due to its functional benefits. The valorization of fish by and co-products and their reintroduction into food and feed chains, namely on pet food, is economically advantageous and contributes to counteracting their negative environmental impact while adding value for fisheries and aquaculture sectors under a circular economy approach.

Despite the wide usage of protein hydrolysates, the development of FBCPHs designed for formulating pet food has been scarcely studied compared to aquaculture and animal feed.[12] Folador et al.[11] conducted one of the first studies assessing the potential utilization of FBCPHs in pet formulations based on the chemical composition characteristics and protein quality indices. In this study, different FBCPs were minced (sole, pink salmon, red salmon, and pollock late season) after being cooked and deboned, and the decanted liquid was hydrolyzed using commercial papain with the addition of 0.01% ethoxyquin (antioxidant). FBCPHs' protein quality indices were affected by the specific part of the fish used. More recently, the production of enzymatic hydrolysates achieved the valorization of FBCPs (heads, viscera, trimmings, and frames) generated from aquaculture turbot (*Scophthalmus maximus*) filleting. The optimized FBCPHs were obtained using 0.2% (v/w) of alcalase for 3 h at 60°C and pH 8.5. The application as an ingredient in the formulation of human protein concentrates, pet food diets, and aquaculture feeds was proposed based on the concentration of soluble protein and the adequate balance of AA, protein digestibility, and bioactivities of FBCPHs attained.[30]

Cats

Scientific studies evaluating functional foods or supplements including fish hydrolysates in cats are almost inexistent (Table 3). Commercially available elimination diets with the inclusion of partially hydrolyzed salmon[170] and hydrolyzed fish protein[171] were shown to reduce the pruritus visual analog scale and scoring feline allergic dermatitis, thus demonstrating their benefit to diagnose and manage cutaneous adverse food reaction, a common disease affecting cats. Moreover, the study conducted by Jeusette et al.[172] with a prescription diet supplemented with 0.1% of fish peptides fed for 10 weeks, showed a reduction in stress biomarkers, with a decrease in the average 24-hour urinary cortisol/creatinine ratio, and an increase in serotonin levels. However, the supplement complexity which also included L-tryptophan and lemon balm extract with a minimum of 5% rosmarinic acid, and oligofructose, does not allow us to attribute the effects observed solely to fish hydrolysate. Similarly, effects on reduction of the joint degeneration and spondylitis deformans through a supplement with collagen peptides from fish scales and D-glucosamine from crabs are impossible to be only attributed to fish peptides.[173] Although the authors remain optimistic about the use of FBCPHs on cats' diets, more research is needed to support their benefits on animal health and welfare.

Dogs

Despite the considerable interest in hydrolyzed protein and its use in commercial diets, studies with functional foods and diets containing fish hydrolysates in dogs are scarce (Table 4).

Table 3. Studies of functional foods and diets containing fish hydrolysates in cats.

Animal species/ model	Functional food/diet	Inclusion level	Study duration	Effects	Reference
Cats with allergic dermatitis (*n* = 12; 7 females, 5 males; 2-12 years)	Partially hydrolyzed salmon and pea commercial hypoallergenic diet	85% hydrolyzed salmon	2, 4, 6, 8, and 10 weeks	Partially hydrolyzed salmon and pea diet reduced pruritus and clinical symptoms in all animals after 8 weeks, showing to be useful for feline adverse food reaction diagnosis and treatment	[170]
Nonseasonally pruritic cats (*n* = 32; 22 females, 10 male; 0.4-14 years)	Hydrolyzed fish protein and rice starch commercial hypoallergenic diet	No information available	56 days followed by a challenge with the previous diet	Hydrolyzed fish protein and rice starch diet reduced pruritus in 77% of cats, showing to be useful for feline adverse food reaction diagnosis	[171]
Healthy cats (*n* = 10; 5 males, 5 females, 3-5 years)	Prescription diet with L-tryptophan and supplemented with fish peptides (from sardines), lemon balm extract, and oligofructoset	Supplement contained 0.1% fish peptides plus 0.1% lemon balm (minimum 5% rosmarinic acid) and 0.5% oligofructose	10 weeks supplementation	Supplementation of fish peptides, lemon balm, and oligofructose reduced the average 24-hour urinary cortisol/creatinine ratio, a stress marker in cats, and increased serotonin. Overall effects highlight its potential use in cats suffering from mild stress-related conditions.	[172]
Cats with lameness caused by orthopedic diseases and spondylitis deformans (*n* = 3)	Collagen peptide (from fish scales) and D-glucosamine (from crabs) supplement	Oral administration of 1 g each/animal/ day, mixed with food	1, 2 and 3 months	Simultaneous administration of collagen peptide and D-glucosamine supplements from marine origin was effective in the recovery of several joint degeneration and spondylitis deformans in dogs after one month. The absence of side effects suggests the potential for long-term administration	[173]

A significant effort in diet formulation is focused on palatability as it strongly affects intake and, thus, nutrient provision. Protein hydrolysates are among commercial pet food's most popular palatability enhancers,[11,12] but research studies are scarce. Sensorial characteristics have been associated with a protein source and mixture of peptides,[180] being short peptides and the amino acids taurine, glycine, arginine, glutamic acid, and alanine considered feeding stimulants for companion animals.[12] Additionally, bitterness was earlier associated with the molecular weight of peptides, with increased bitterness reported when the molecular weight of the peptides of soy protein hydrolysates ranged from 4 to 2kDa and decreased bitterness with peptides < 1kDa.[181] When comparing a diet with salmon hydrolysate with a chicken-based control diet, dogs showed a preference for the salmon hydrolysate

Table 4. Studies of functional foods and diets containing fish hydrolysates in dogs.

Animal species/model	Functional food/diet	Inclusion level	Study duration	Effects	Reference
Healthy adult dogs ($n = 20$; 10 Beagle, 10 pointers; 7.9-32.8 kg BW)	Salmon hydrolysate-supplemented diet	10% salmon protein hydrolysate	2 days	10% hydrolyzed salmon protein increased the consumption ratio compared to a chicken-based control diet, showing to be highly palatable to dogs	[11]
Healthy Beagle dogs ($n = 12$; 6 females, 6 males; 5.4 ± 0.57 years; 11.8 ± 2.20 kg BW; BSC of 4.3 ± 0.69)	Fish hydrolysate and fish oil (from fish waste) diet	5% fish hydrolysate and 3.2% fish oil	2 periods of 6 weeks (crossover design)	Fish hydrolysate and oil diet increased intake of EPA and DHA without affecting palatability, digestibility, and coat quality.	[15]
in vitro static gut model of dog digestion	Tilapia byproduct protein hydrolysate	2 g of hydrolysate as sole substrate	2 min oral digestion, 2 h gastric digestion, 4 h intestinal digestion	*In vitro* digestion of tilapia byproduct hydrolysate released several peptides with potential bioactive activity that may be involved in the regulation of food intake, glucose metabolism, intestinal hormones secretion, and dipeptidyl peptidase IV inhibitory activity, suggesting to exert satiety effects	[111]
Dogs with atopic dermatitis signs ($n = 13$)	Partially hydrolyzed salmon and pea commercial hypoallergenic diet	85% hydrolyzed salmon	2, 4, 6, 8, and 10 weeks	Partially hydrolyzed salmon and pea diet reduced pruritus and clinical symptoms in all animals after 10 weeks, showing to be useful for canine adverse food reaction diagnosis and treatment	[170]
Nonseasonally pruritic dogs ($n = 50$; 24 females, 26 males; 0.4-14 years old)	Hydrolyzed fish protein and rice starch diet commercial hypoallergenic diet	No information available	56 days followed by a challenge with the previous diet	Hydrolyzed fish protein and rice starch diet reduced pruritus in 63% of dogs, showing to be useful for adverse food reaction diagnosis, even in dogs allergic to fish and rice	[174]
Dog with chronic clinical otitis symptoms ($n = 30$; 14 females, 16 males; 6.0 ± 0.15 years; 32.0 ± 1.17 kg BW)	Commercial diet (93-94%) supplemented with therapeutical microcapsules (6-7%) with fish and vegetable hydrolysates	Microcapsules contained 60-80% fish and vegetable hydrolysates, minerals, and 20-40% natural substances (*Melaleuca alternifolia*, *Tilia platyphyllos scapoli et cordata, Allium sativum, Rosa canina*, and zinc)	90 days	Supplementation of hydrolyzed fish and vegetable-based microcapsules relieved the intensity of otitis externa-related symptoms in combination with topical drugs, suggesting the efficacy of combined dietary and conventional drug therapy	[175]
Healthy Labrador Retriever puppies ($n = 42$)	Fish meal-based commercial diet (93-94%) and nutraceutical supplement (6-7%) with fish and vegetable hydrolysates	Supplement contained hydrolyzed fish and vegetable protein, supplemented with glucosamine, chondroitin sulfate, chitosamine, *Boswellia serrata*, *Harpagophytum procumbens*, green-lipped mussel, and fish-oil omega-3/6 polyunsaturated fatty acids (1:1 ratio)	From 3 to 12 months of age	Nutraceutical supplement with fish and vegetable hydrolysates did not affect the prevalence of hip and elbow dysplasia but reduced osteoarthritis severity at 12 months of age, showing to have beneficial effects on severe osteoarthritis development	[176]

(*Continued*)

Table 4. (Continued).

Animal species/model	Functional food/diet	Inclusion level	Study duration	Effects	Reference
Dogs with lameness caused by orthopedic diseases and spondylitis deformans ($n = 68$)	Collagen peptide (from fish scales) and D-glucosamine (from crabs) supplement	Oral administration of 1 g each/animal/day, mixed with food	1, 2 and 3 months	Simultaneous administration of collagen peptide and D-glucosamine supplements from marine origin was effective in the recovery of several joint degeneration and spondylitis deformans in dogs after one month. The absence of side effects suggests the potential for long-term administration	[173]
Dogs with immune-mediated keratoconjunctivitis sicca ($n = 50$; 19 females, 31 males; 6.5 ± 0.7 years)	Commercial diet (93-94%) supplemented with nutraceutical tablets (6-7%) with fish and vegetable hydrolysates	Tablets contained 60-80% fish and vegetable hydrolysates, minerals, and 20-40% botanical substances	60 days	Supplementation of hydrolyzed fish and vegetable-based tablets combined with immunosuppressive therapy reduce the immune-mediated ocular symptoms of keratoconjunctivitis sicca-affected dogs that had poor or no response to classical immunosuppressive drugs, unveiling its potential immune modulation effect	[177]
Healthy senior pointer dogs ($n = 12$; 2 female, 10 male; 7.9 ± 1.24 years; 24.0 ± 2.4 kg BW)	Hydrolyzed pink salmon-supplemented diet	20% pink salmon hydrolysate	26 days	Pink salmon hydrolysate at 20% inclusion had no negative effect on the palatability, nutrient digestibility, and immune function of healthy senior dogs, revealing its potential as an alternative ingredient	[178]
Healthy Beagle dogs ($n = 12$; 6 females, 6 males; 5.4 ± 0.57 years; 11.8 ± 2.20 kg BW; BSC of 4.3 ± 0.69)	Fish hydrolysate and fish oil (from fish waste) diet	5% fish hydrolysate and 3.2% fish oil	2 periods of 6 weeks (crossover design)	Fish hydrolysate and oil diet decreased plasma triglycerides and angiotensin-converting enzyme activity while no negative impact on systemic inflammation markers, cardiac structure, and function was observed. Fecal microbiome modulation towards health-promoting bacterial genera suggests the potential of a functional diet	[179]
Adult Beagle dogs ($n = 45$; female and male; 2.7-17 years)	Fish hydrolysate capsules	750 and 1500 mg/day	35 days	Fish hydrolysate supplementation reduced hyperactivity response and cortisol response, supporting its use to reduce dogs' fear and anxiety	[144]
Dogs of several breeds (29) and crossbreeds ($n = 39$; 26 females, 13 males; 1-6 years)	Fish hydrolysate capsules (500 mg GABOLYSAT PTP 55, 11 mg of SOD B Primo-Peptidyss®-M, 5 IU/mg)	Less than 10 kg BW: 1 capsule/day More than 10 kg BW: 2 capsules/day	30 days	Fish hydrolysate supplement promoted dog-human interactions and tend to reduce subtle stress behaviors, suggesting being effective in situations of mild stressors	[143]

diet with significant effects on the first approach and taste and intake ratio, suggesting that salmon hydrolysate provided a taste that the dogs preferred.[11] Additionally, in a recent study performed with adult Beagle dogs fed diets differing on protein hydrolysate and oil sources, with the experimental diet containing fish hydrolysate and oil in substitution of shrimp hydrolysate and salmon oil, no effect was observed on the first diet approached and tasted, and on intake ratio.[15] Both diets presented the same kibble size, shape, and texture, and similar amino acids profile, but contained a different fatty acids profile and peptide size was not evaluated, thus making it difficult to draw conclusions.

Additionally, the bitterness of protein hydrolysates has been suggested to potentially reduce consumption in companion animals. As referred above bitterness has been associated with the molecular weight of peptides.[181] Recent advancements regarding the reduction of bitterness in FBCPs-derived peptides have been explored.[182] However, high palatability has been reported for fish protein hydrolysates as pet food ingredients in studies with dogs.[11,15] These findings may be explained by the fact that pets have a distinct sensitivity to bitterness compared to humans, and are likely less sensitive to bitterness overall, or at least to certain bitter compounds. Research assessing the bitter taste receptors (Tas2rs) of dogs' receptive range has revealed a reduced sensitivity to bitter compounds relative to humans.[183] Although fish hydrolysates and peptides have not yet been specifically analyzed, palatability studies suggest that bitterness is a less significant issue in pet food applications than in other sectors.

Animal protein hydrolysates can also exert satiety effects,[184] and although scarcely studied, Theysgeur et al.,[111] using an *in vitro* simulated dog gastrointestinal digestion model, found stimulation of cholecystokinin and glucagon-like peptide 1 secretion and inhibition of the dipeptidyl peptidase IV activity with a tilapia FBCPHs.

Adverse food reactions involve non-immune (food intolerance) and immune mechanisms (food hypersensitivity), and their diagnosis is often performed through an elimination diet with novel protein sources or hydrolyzed proteins. As dogs are exposed to a wide variety of protein sources, it is not easy to identify a novel protein source; thus, hydrolyzed proteins are increasingly considered. Indeed, by disrupting the protein structure, protein hydrolysis removes existing allergens and allergenic epitopes, preventing an immune response even by animals sensitized to the intact protein.[185] Therefore, protein hydrolysates may play a role in preventing hypersensitivity in individuals who are at risk or already sensitized.

Although studies with chicken hydrolysates[186,187] have reported benefits when used as an elimination diet for diagnosing and treating adverse food reactions in dogs, results in conflict with allergic reactions reported in other studies.[188] Peptide size can contribute to these conflicting results as it conditions immunoglobulin (Ig) E-mediated reactions. Indeed, IgE recognizes protein allergens with low MW,[189] suggesting that hydrolyzed proteins with MW lower than 5 kDa should be used in elimination diets.[190] However, Bizikova and Olivry[188] reported no induced hypersensitivity in dogs fed a hydrolyzed poultry feather meal containing 95% hydrolyzed proteins with MW ≤ 1 kDa, whereas 78% hydrolyzed chicken liver proteins with MW ≤ 1 kDa induced food reactions in 40% of the dogs. Additionally, a recent study[190] suggested that hydrolyzed diets might contain proteins that stimulate helper T-lymphocytes, thus making them inadequate for treating food hypersensitivity in all situations. Studies available on dogs fed commercial hypoallergenic diets with partially hydrolyzed salmon[170] and fish protein hydrolysate[174] reported a reduction in pruritus and the extent and severity of atopic dermatitis, being thus considered to be useful for adverse food reaction diagnosis.

Hydrolyzed protein diets have also been considered to manage inflammatory bowel disease, an immunologically mediated intestinal disorder, and one of the most common dog gastrointestinal diseases.[191] Marks et al.[192] reported the resolution of clinical signs in dogs with inflammatory bowel disease fed a commercially hypoallergenic diet containing an enzymatically hydrolyzed defatted soy globulin as the only protein source, and Ambrosini et al.[193] found improved intestinal membrane integrity in dogs with inflammatory bowel disease fed a commercial hydrolyzed diet. However, to the best of the author's knowledge, scientific studies with FBCPHs in dogs with inflammatory bowel disease are lacking.

Hydrolyzed diets are highly digestible and present reduced antigenicity; they have also been considered interesting in managing exocrine pancreatic insufficiency, mainly when associated with dermatological disease. Indeed, despite the likelihood that endopeptidases from the intestinal brush border compensate for the loss of pancreatic proteases, adverse food reactions have been reported in dogs with exocrine pancreatic insufficiency.[194] Studies with FBCPHs to manage exocrine pancreatic insufficiency in dogs were not found, but a study with a limited number of dogs (three German Shepherds),[195] found improvement in dermatological signs of dogs with exocrine pancreatic insufficiency fed a soy and chicken-based-hydrolysate diet.

Even though FBCPHs have been reported to improve the clinical signs of dogs, namely reducing the intensity of chronic otitis externa,[175] osteoarthritis severity,[176] joint degeneration and spondylitis deformans[173] and might have a role in the treatment of keratoconjunctivitis sicca,[177] it is impossible to consider the FBCPHs as the sole responsible for the reported improvements due to the complexity of the dietary treatments.

Regarding effects on immune function, Zinn et al.[178] demonstrated that the dietary inclusion of pink salmon hydrolysate (20%) in replacement of poultry by-product meal does not dramatically impact the immune function of healthy, senior dogs. Authors suggested that the lack of an effect may have resulted from the short length of the treatment period (26 days) and that FBCPs may more effectively affect immune function in diseased or challenged animals. Similarly, feeding adult Beagle dogs for 6 weeks with a diet including fish hydrolysate and oil from FBCPs in replacement of shrimp hydrolysate and salmon oil did not affect systemic inflammatory markers, cardiac structure, and function, but potentially benefited bacterial genera associated with healthy microbiome.[179] However, it is difficult to distinguish the effects of protein hydrolysates and oil sources.

Stress associated with fear and anxiety adversely affects animal health, and related behaviors can negatively affect human-pet relationships.[196,197] Pet owners are reluctant to use psychotropic drugs for anxiety treatment, and despite several natural products being marketed for behavior therapy, very few have unequivocal efficacy. Animal protein hydrolysates may include opioid-like peptides that can act as anxiolytic agents.[198] Indeed, bovine αs1-casein hydrolysate decreased anxious disorders[199,200] and caseinate hydrolysate alleviated stress in dogs.[201] Regarding the use of FPH supplements, Landsberg et al.[144] reported benefits through decreased hyperactivity and cortisol responses, while Titeux et al.[143] observed the promotion of dog-human interactions and reduced subtle stress behaviors.

The scarce studies available in the literature along with the experience of the authors on the practical use of fish hydrolysates, support the potential of these feed resources in dog diets (Fig. 5). Indeed, due to their high protein content, amino acids profile, high digestibility, and attractive taste, they are advantageous to be included in high digestible and palatable diets. In addition, their effects on satiety make these ingredients useful in diets for body weight control. Due to their novelty and peptide size, they are commonly used in elimination diets to manage adverse food reactions and in managing dermatological diseases. Finally, the eventual presence of opioid-like peptides might play an important role in reducing anxiety and stress-related behaviors improving the dogs' welfare and the relation human-dog.

However, more studies are needed to fully disclose the potential of FBCPHs as functional ingredients in pet food, even because the amino acid and peptide profiles of protein hydrolysates differ according to the fish source, enzyme source, and hydrolysis conditions,[202–204] thus impacting their functionality. This information is essential to take advantage of these resources in pet food fully.

Overall, FBCPHs represent a valuable and sustainable resource for pet food formulations due to their high protein content, digestibility, palatability, and bioactive properties. These ingredients are particularly useful for managing adverse food reactions, dermatological issues, satiety, and stress-related behaviors in pets, making them ideal for premium and functional diets. However, additional research is required to fully explore their potential, as amino acid and peptide profiles of FBCPs vary with fish source and hydrolysis conditions, affecting their bioactivity. The development of standardized production methods is crucial to improving the reproducibility of FBCPHs' bioactivity and consequently expanding their application in the pet food industry. The growing trend towards personalized pet nutrition presents opportunities to tailor FBCPHs for specific health needs, while

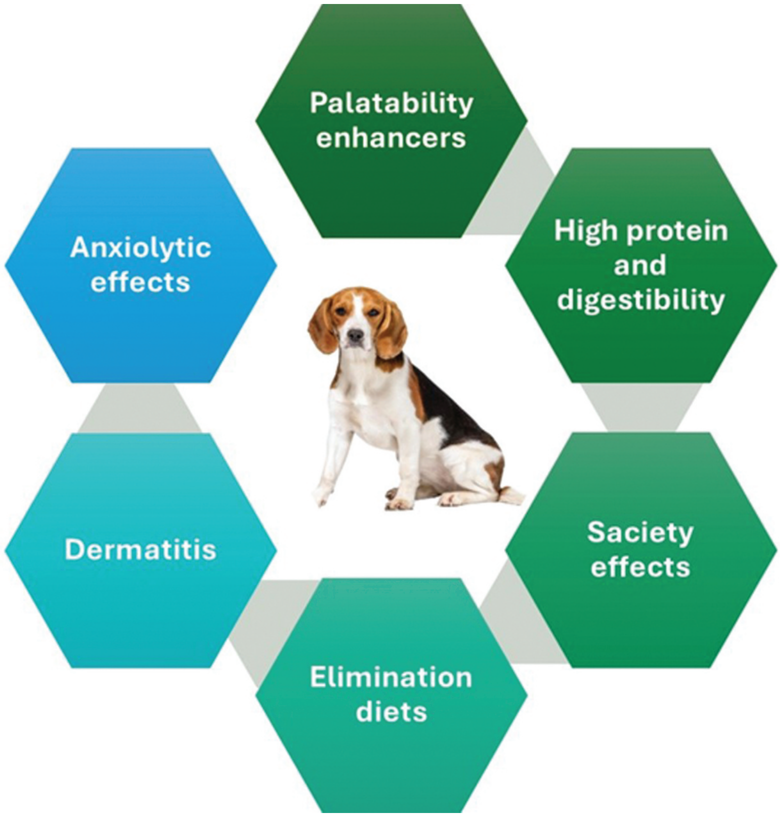

Figure 5. Potential effects of hydrolysates from fish by-products and co-products (FBCPs) on dog nutritional and health parameters.

combining them with other bioactive compounds like prebiotics and probiotics could further enhance gut health and immune function. These future research pathways, combined with efforts to optimize production processes and improve scalability, will contribute to developing innovative, sustainable ingredients from FBCPs, supporting both the circular economy and environmental sustainability in the pet food and fish industries.

Conclusions

The fish processing industry faces the challenge of managing increasing amounts of FBCPs, which have been a significant contributor to both environmental and economic inefficiencies in the sector. FBCPHs represent a promising and sustainable solution, emerging as valuable functional ingredients for pet food. These hydrolysates provide numerous advantages, including high digestibility, essential amino acids, and potential bioactive properties, making them effective for addressing pet health concerns such as allergies, gastrointestinal issues, and anxiety-related behaviors. Their application in premium and functional diets highlights their versatility and value in pet nutrition.

However, to maximize the potential of FBCPH, further research is essential to optimize hydrolysis and extraction processes. This includes exploring various approaches, such as FBCP pretreatment techniques, enzyme selection, hydrolysis parameter optimization, utilization of novel technologies, and even the integration of renewable energy sources. Besides, process optimization, the development of standardized production methods is crucial to enhance the yield, quality, and reproducibility of BCPHs' bioactivities, ensuring their effective and seamless application in pet food formulations.

While preliminary studies demonstrated the positive effects of FBCPHs in pet food formulations, more comprehensive research is needed to confirm these benefits establish their benefits, and ensure optimal efficacy in formulations. Additionally, regulatory considerations must be addressed to ensure compliance as these ingredients are integrated into the market. Despite all these challenges, FBCPHs align with the growing trend towards premium and health-focused pet foods. Future research will be crucial in improving FBCPH production, incorporating renewable energy, ensuring bioactivity, and meeting regulatory standards, all while addressing pet owners' expectations for high-quality, functional nutrition.

Disclosure statement

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