

**Title:**

Valorization of proteins from co- and by-products from the fish and meat industry

**Authors:**

Aspevik, T.<sup>1</sup>, Oterhals, Å.<sup>1</sup>, Rønning, S. B.<sup>2</sup>, Altintzoglou, T.<sup>3</sup>, Wubshet, S. G.<sup>2</sup>, Gildberg, A.<sup>3</sup>, Afseth N. K.<sup>2</sup>, Whitaker, R. D.<sup>3</sup>, Lindberg, D.<sup>2</sup>

**Affiliations:**

<sup>1</sup> Nofima AS, P.B. 1425 Oasen, NO-5828 Bergen, Norway

<sup>2</sup> Nofima AS, P.B. 210, NO-1431 Ås, Norway

<sup>3</sup> Nofima AS, P.B. 6122 Langnes, NO-9291, Tromsø, Norway.

New permanent address A. Gildberg: Ildervegen 27, 9017 Tromsø, Norway

**Corresponding author:**

email address: diana.lindberg@nofima.no;

telephone nr: +47 913 00 486

**ORCID**

Diana Lindberg: 0000-0002-4015-4827

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## **Abstract**

Large volumes of protein-rich residual raw materials, such as heads, bones, carcass, blood, skins, viscera, hooves and feathers, are created as a result of processing of animals from fisheries, aquaculture, livestock and poultry sectors. The residuals contain proteins and other essential nutrients with potentially bioactive properties, eligible for recycling and upgrading for higher-value products, e.g. for human, pet food and feed purposes. Here, we aim at covering all the important aspects involved in reaching an optimal utilization of proteins in the residual raw materials, and naming those eligible for human consumption as co-products, and feed applications as by-products. Strict legislations regulate the utilization of various animal-based co- and by-products, and represent a major hurdle if not addressed properly. Thorough understanding and optimization of all parts of the production chain, including conservation and processing are important prerequisites for successful upgrading and industrial implementation of products. This review will encompass industrially used technologies such as freezing/cooling, acid preservation, salting, rendering and protein hydrolysis. In this aspect, it is important to accomplish a stable production and quality through all the steps in the manufacturing chain, preferably supported by at- or online quality control points in the actual processing step. If aiming for the human market, knowledge of consumer trends and awareness are important in the production and successful introduction of new products and ingredients.

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## List of abbreviations

Animal by-products	ABP
Bioactive peptides	BAP
Bovine spongiform encephalopathy	BSE
European Union	EU
Fourier Transform Infrared Spectroscopy	FTIR
Transmissible spongiform encephalopathy	TSE

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## 1. Introduction

The rapid growth, urbanization and increasing prosperity of the world population is demanding an improved utilization of existing protein sources along with the development of new and sustainable food production. The global demand for protein is expected to double by year 2050. This is not only due to the population growth, but also by an increased recognition of the important role of proteins in a healthy diet in general and especially for children and the growing elderly population [1]. Fish and meat products are important protein sources in the human diet and contains essential amino acids, minerals and vitamins. In industrial fish and meat processing the main goal is to process the *main products*, such as filets, chops and mince. However, these processes generate huge amounts of protein-rich residual raw materials. About 40-60% of the total weight of animal and fish are classified as residuals. This includes heads, bones, carcass, blood, skins, viscera, hooves and feathers, depending on the specie. Much of this have a great potential for higher-value applications within food and feed. In this review, we are focusing on how to increase the value of this material derived from the livestock and poultry, fisheries and aquaculture sectors. The residuals can be divided into *co-products*, which can be used for human consumption, and *by-products*, that cannot (Fig. 1). Applications of by-products are strictly regulated and we will in this review give an overview of the most important European regulations. Moreover, we will present more established industrial processes, e.g. ensilage and rendering, and emerging processes, i.e. enzymatic hydrolysis, for processing of fish and meat co- and by-products. When selecting the most suitable process for each specific situation, there is a possible trade-off. Established processes, with relatively easy production and low investment costs, can have a somewhat restricted product segment. On the other hand, a more complex technology often represents a higher investment cost, but allow for more end markets and possibly higher market price for some of the products. The available volume, its quality and nutritional properties, together with current legislations, regulate the potential use and processing demands for the residual raw materials. In addition, market demand, consumer acceptance, feasibility, technology awareness, needed level of process control, and available infrastructure will influence the choice of the most beneficial and cost-effective process.

## 2. Volumes and nutritional values

The total global production of fish from aquaculture and fisheries is estimated as 128 million tons for 2014 (Table 1) [2]. About 70 million tons of fish is being processed as main products globally, indicating that the residual raw materials constitute more than half of the round fish weight. It is important to stress that in some cases discarded fish is not included into the reporting of volumes of residual raw materials and hence represents large lost values, estimated to 7.3 million tons [3]. In Norway, white fish and pelagic fisheries resulted in 43% and 12% residual raw materials in 2015, respectively [4]. Here, the most underutilized materials consist of heads, intestines and roe from whitefish, and blood from aquaculture processing [4]. Another example is tuna processing, where values reported range from 50–55%, and up to 70% [5].

Looking at meat, the total production has reached more than 263 million tons annually [6]. In Table 2, production of some major livestock is presented together with the percentage of by-products resulting from processing of these species in Norway [7-9]. Other figures from USA on the portion of residuals generated, given in live weight of livestock, are 49% from cattle, 44% from pigs, and 37% from broilers [10]. Independent of percentage, as seen for fish above, the amount of residual raw material are directly related to the physiology of each specie. For example, pig slaughter result in low amount of residuals because large parts of the skin and fat contains substantial amounts of easily solubilized collagen that are used as a binding-ingredient in sausages and other mixed products. For cattle, the residual materials consists of almost 20% of bones. The bone marrow contains large amounts of proteins and was considered an excellent feed ingredient before the bovine spongiform encephalopathy (BSE) scandal in 1992. Nowadays, the marrowbones are regulated as specified risk material for destruction.

All these residual raw materials represent a different set of possibilities and challenges that it is important to investigate and solve before a successful industrial processing. Although a large part of this biomass in many countries are utilized today, there are still significant quantities under- or unutilized. Both animal and fish residuals have excellent nutritional values, and this material contains large amounts of high-quality proteins, lipids, micronutrients and minerals that could be better utilized towards human consumption and other kinds of products. The protein content of meat-based residuals are generally 10-23 g per 100 g raw material, with porcine chitterlings and brains at the lower end and ears, feet and liver at the top end [11]. The proteins in these different organs have different nutritional values and are furthermore more or less easily accessible, which is important from a processing perspective.

### 3. Regulatory frames

The laws and regulations governing the collection, transport, storage, handling, processing, use and disposal of residual raw materials are often overlooked factors that severely influence the number of applications available for processing of this material. It is outside the scope of this review to cover all regulations worldwide, and we will exemplify the importance by reference to European Union (EU) legislations. When a material is found unfit for food, but suitable for feed, it is classified as an animal by-product (ABP) and must be processed at appropriate by-product plants, according to EU regulation. After the material has reached this facility, it can no longer be upgraded for food use. It is therefore imperative that as much as possible of the residual raw material is handled according to food hygiene regulations if the intended use is in processing for human consumption.

The EU has developed an elaborate legislation framework governing the use of residual raw material from the fisheries, aquaculture, livestock, and poultry industries. The type and quality of the residuals is of utmost importance for further processing possibilities and defines their use for food or feed applications, given in the hygiene rules for food of animal origin [12]. Raw materials that does not meet the general rules for food hygiene or are classified as not suitable for human consumption are regulated by the rules given in the ABP regulation [13] and implementation of health rules as regards animal by-products and derived products not intended for human consumption [14]. The ABPs are divided into three categories (1-3; Fig. 2) based on their origin and potential risk for public and animal health and the environment. None of the ABPs can be used for human consumption and only the low risk category 3 by-products can be used for feed production. Some parts of the animals are not eligible for neither food nor feed use and are classified as category 1 specified risk materials. These include bone marrow, spinal cords and brains from cattle, sheep and goats, and sick and dead animals [13]. The category 3 by-products are further classified according to their origin, i.e. ruminants or non-ruminants, due to the risk of transmissible spongiform encephalopathies (TSE). A compilation of regulations for use of different category 3 ABPs in feed is given in Table 3. The ABP regulation [13], together with the TSE regulation [15], are major components of the EU's strategy to eradicate feed-borne crises such as BSE in cattle, foot-and-mouth disease and dioxin contamination. The main objective of the regulations is to protect the population and farmed animals from any health risks related to the contamination by infectious microorganisms or heat-stable bacteria-derived toxins (e.g. histamine and enterotoxins).

Production plants that are processing category 3 by-products shall comply with the general hygiene requirements provided in the ABP regulation [13] and have a documented pest control program. Materials that have not received the specific heat treatment during start up or leakage must be either recirculated through the used heat treatment step, collected and reprocessed or discarded. The health rules regarding ABPs [14] describes several processing methods approved for heat treatment of category 3 by-products; material originating from domestic animals based on methods 1 to 5 and 7, aquatic animals based on methods 1 to 7 (Table 4). The different heat treatment operation condition are based on the critical control points: 1) raw material particle size, 2) achieved core particle temperature level, 3) pressure, 4) duration of heat treatment and 5) in case of chemical treatment the achieved pH level. In case of fish processing, the material rapidly becomes tender as muscle proteins coagulate and disintegrate when exposed to mechanical forces in cooker, strainer, screw-press and screw conveyer. By experience, reduction of particle size prior to the heat treatment step is not required to achieve a uniform temperature throughout the fish material [16]. Moreover, high shear forces in a grinding step might give fat separation problems due to the formation of emulsions and should be avoided if not needed.

### 4. Post-harvest handling, industrial processing and analysis of meat and fish residual raw materials

Both meat and fish residual raw materials represent a rich supply of easily available nutrients. It has a high moisture content and are therefore easily spoiled in presence of microorganisms. Microorganisms can contaminate the materials via the fish and animals themselves, e.g. from the gastrointestinal tract or by contamination from hooves and/or hide and skin. The contamination can also come from the processing environment, e.g. due to poor employee hygiene or process facility cleansing routines. The most common contaminant is bacterial, but yeast and molds can also contaminate the meat products. There exist a broad battery of techniques used post-harvest in preservation of meat- and fish-based products [17], which also is valid for the by- and co-products [18]. In industrial processing, conserving can be done before processing, but it can also be coupled with a processing methods, e.g. in the case of fish silage. Conservation can also be minimized or potentially excluded in cases where the slaughterhouse is close-by or even connected to the processing facility for the residual rest materials. This ensures valorization of absolutely fresh residual raw materials.

#### 4.1 Chilling and freezing

Chilling or freezing are the preferred methods for the meat and poultry industry to preserve the quality of the main product. Cooldown is performed after processing and removal of the main product. By- and co-products from both fish and meat are handled in the same way to be able to preserve the quality, and chilling/freezing is easily facilitated in established slaughterhouses. Plate freezing equipment is applied to block-freeze mixed residuals sold as wet feed to the fur animal industry.

Fish residuals present some additional problems associated to conservation. Psychrotrophic microorganisms, able to grow at temperature as low as  $-5\text{ }^{\circ}\text{C}$ , are present on fish from cold waters, affecting the temperature needed for effective conservation. Furthermore, the amount of microorganisms present in the digestive system of many fish species is dependent on season. Some smaller fish species as capelin, herring, anchovy and sprat have in periods of the year a high content of hydrolytic enzyme activity caused by active feeding on zooplankton. Such species are prone to autolytic degradation and enhanced bacterial spoilage due to tissue softening and belly burst and this can be prevented by chilling.

Chilling of fish and residual raw materials onboard the fishing vessel is challenging. Many chilling techniques involve the addition of water or ice to obtain heat transfer. Modern large scale fishing vessels is normally equipped with active chilling systems based on circulation of refrigerated seawater or in combination with refrigerated fresh-water to reduce the salt uptake in the fish. A low salt uptake is important to comply with fishmeal specifications for salt and ash content. Disinfection of the pipes and chilling system by ozone injection [19] or other antimicrobial agents has improved the quality of the recirculating water before adding of the captured fish. This industrial practice has significantly improved the quality of pelagic fish caught for production of fishmeal and oil for use in animal feed, especially when combined with the optional addition of acetic acid. Chilling by crushed ice addition is also possible but is difficult to apply in largescale operations. A promising option is the production of an ice-slurry by partial freezing of the seawater [20]. This technology gives a pumpable ice and a very high chilling rate due to the content of small ice crystals and high cooling capacity. Excess blood-water generated by the above chilling technologies is generally drained off and pumped at sea. A novel cooling approach, avoiding the addition of water, is the use of solid carbon dioxide [21]. The choice of cooling medium and technology will depend on several factors including scale of operation, need for chilling to secure quality before further processing, investments and energy consumption.

#### 4.2 Organic acid preservation and fish silage

The preservation of meat- and fish-based foods by low molecular weight acids has been known for centuries [22]. Lactic, acetic, propionic, citric and benzoic acid are all organic acids used as food preservatives while are also being food ingredients, which add to their value and usefulness. The conservation principle is based on the dissociation of the organic acid after diffusion through the microbial cell wall. The cytoplasmic pH is reduced, and in combination with the accumulation of acid anions, this give inhibition of the cellular functions [22-23].

Lactic and acetic acid are used to prevent contamination of freshly slaughtered beef carcasses after removal of hides and before or after evisceration [17]. The production of fish silage using organic acids is a well established method in Scandinavia for preserving proteins and gaining value-added products from fish raw material that are otherwise not utilized. Fish silage can be described as a liquid product that develops when whole fish or parts of the fish are treated with an acid [24]. The use of fish silage in fish and animal feed has been widely studied and it is regarded as a well-suited protein source [25-26]. For the preparation of fish silage, the raw material must preferably be crushed or grinded and the mixture stirred to secure a good contact between the raw material and added acid. At an acidic  $\text{pH} < 4.5$ , the enzymes naturally present in the fish viscera will degrade and liquefy the fish tissue without risk of bacterial spoilage [26]. A schematic overview of the production of crude silage is given in Fig. 3. Further downstream processing of crude silage is covered in chapter 4.5.2.

The rate of autolysis is determined by the content and activity of digestive enzymes, pH and temperature. Most commonly, formic acid is used, which produces a stable silage at a pH of about 4.0-4.5. Antioxidants, such as ethoxyquin, is added to prevent oxidation of the fish oil. The production of fish silage is a relative simple and low cost technology, however, demands strict process control to avoid growth of spoilage bacteria. Moreover, the capacity can be fitted to any scale of operation and the final products are stable and can be stored for long periods. In Norway, the production of fish silage is the main technique used for preserving marine by-products [4]. The final product is not suitable for human consumption and silage technology should preferably be based on fish and residuals found unfit for food production. Although there have been reports of ensiling of chicken

intestines [27], we are not aware of examples on the industrial implementation of this technology for preservation of ABPs.

#### 4.3 Salting and production of fish sauce

Fish sauce is used as a condiment in large areas of South-East Asia. Conservation during production is achieved through addition of sea-salt to fish raw materials. The mixture is stored for several months (6 – 12) at ambient (normally tropical) temperatures, until a clear, amber water solution, rich in hydrolyzed protein and salt can be recovered. Very few microorganisms can survive and grow at such high salt concentration, and after a couple of months of storage, only low numbers of harmless halophilic bacteria are present. In addition to hydrolyzed protein and free amino acids, this liquid also contains short chain fatty acids and aldehydes adding cheesy and meaty aroma to the dominating sharp and salty taste [28]. To obtain good product stability the amount of salt added to fresh raw material must be in the range 1:3 to 1:2 by weight. Normally industrial production is performed in squared concrete tanks covering large flat areas close to fishing harbors. In principle fish sauce can be produced from most kinds of fish raw materials. Lean raw materials are more suitable than fat raw materials, since the oil fraction does not contribute significantly to the volume of sauce recovered. Although fish sauce is normally produced from tropical fish species, pilot scale experiments in Spain, Canada and Norway have shown that good quality fish sauce can be obtained also from temperate- and cold water fish species. The presence of intestinal tryptic enzymes, however, is of premium importance to achieve good protein hydrolysis and sauce recovery. There are considerable variations in the level of intestinal enzymes in small pelagic species, particularly in species from temperate- and cold waters. To compensate for low levels of tryptic enzymes, suitable amounts of minced intestines from carnivore white fish can be added, since intestines of such fishes always contain high levels of tryptic enzymes [29]. The major application of fish sauce is as a salting and flavoring condiment to vegetable dishes.

#### 4.4 Rendering

Processing of animal and fish by-products is based on a common main principle called rendering technology. The raw material is heated to a temperature defined by legislations to eliminate any pathogenic bacteria, i.e. >70 °C for fish raw material and >100 °C for ABPs [14]. The main products from the rendering process are a protein powder and fat/oil. In the process the raw material is heat coagulated followed by mechanical dewatering and separation steps to extract the oil phase, and succeeding thermal dewatering steps to concentrate the solubles and obtain a dried high protein powder [30-31]. The applied industrial scale unit operations are fairly standardized worldwide although some technology and process layout differences exist depending of type of raw material and target product quality [32].

A general outline of the unit operations used in the conventional fishmeal and oil process is given in Fig. 4. After heat treatment to 90-95 °C in a continuous screw cooker the fish raw material is run over a strainer to remove free water and oil phase before entering the screw press. The compression ratio, normally 1:3.5 to 1:4 in a fish press, causes fish oil and water to be squeezed out of the coagulated material and through the sieve plates. The water and oil together with solubles and fine particles are collected in the bottom of the press and mixed with the oil/water phase removed by the strainer. The combined liquid streams are heated to above 90 °C and run over a decanter centrifuge to remove suspended fine solids before oil separation by a disc centrifuge.

#### 4.5 Protein hydrolysis

Protein hydrolysis is a commonly used method to extract proteins from meat and fish residuals. It involves a breakdown of proteins to smaller and more water-soluble peptides and free amino acids. The word *hydrolysis* literally means reaction with water and protein hydrolysis require the presence of water molecules. The main purpose of a hydrolysis process is increased protein recovery and yield of valuable components. Protein hydrolysis can be obtained using chemical or enzymatic processes.

##### 4.5.1 Chemical protein hydrolysis

Chemical processing includes the use of acid or alkali to cleave the peptide bonds. The acidic protein hydrolysis is most common and frequently used to produce flavor enhancers from vegetables. Hydrolyzed vegetable protein is produced by treating the protein source with a mineral acid (usually 4-6 M HCl) at 100-130 °C for 4-24 hours followed by neutralization with NaOH [33-34]. Alkaline hydrolysis is a straight forward process starting with solubilization of the protein by heat treatment followed by addition of alkaline agents (calcium, sodium or potassium hydroxide), adjustment of the temperature to desired set point (usually 25-55 °C) and performing the hydrolysis for several hours to reach a desired hydrolysis product. The alkaline hydrolysis is less

common in food and feed applications because of negative effects on the nutritive protein quality using alkali. Thermal processing at an alkaline pH may cause formation of toxic substances like lysinoalanine, leads to racemization of L-amino acids to undesired D-amino acids and partly destroys the amino acids, arginine, tyrosine, lysine, cysteine and threonine [35-36].

Even though acid hydrolysis are preferred over alkali hydrolysis, the acid process will also influence the nutritive value of the proteins: the essential amino acids tryptophan and cysteine are destroyed and glutamine and asparagine are converted to glutamic acid and aspartic acid [37]. Moreover, neither acid nor alkali hydrolysis are specific and generate large amounts of salt in the final product after a neutralization process. In general, a process based on chemical hydrolysis yield hydrolysates with reduced nutritional qualities, poor functionality and are restricted to use as flavor enhancers [36]. Chemical hydrolysis involve the use of highly corrosive acid or base and requires glass-lined stainless steel reactors that can withstand high pressure and temperatures [37].

#### *4.5.2 Enzymatic protein hydrolysis*

The production of protein hydrolysates using protein-digesting enzymes, i.e. proteases, is a very promising alternative for valorization of proteins from meat and fish by- and co-product for different markets. The processing technology is regarded as a mild process and results in products with high product yield without prejudicing the nutritional quality by e.g. destroying the amino acids as seen in chemical hydrolysis. An enzymatic hydrolysis process will decrease the molecular weight of the intrinsic proteins and peptides and increase the number of ionizable groups, leaving the new peptides smaller and more water-soluble than the intact proteins [38]. A typical hydrolysis process is characterized by an initial rapid burst phase where the substrate is in excess. As the enzymatic hydrolysis reaction progresses, the reaction rate levels off. This can be explained by reduced enzyme activity caused by one or a combination of the following factors: a change in reaction pH, fewer peptide bonds available for cleavage [39], substrate [40] and product inhibition [41], as well as possible presence of protease inhibitors in the substrate [42].

Enzymatic hydrolysis can be performed both by use of enzymes that are naturally occurring in the substrate (endogenous enzymes) and by addition of commercially available proteases (exogenous enzymes). The production of fish silage is essentially an endogenous enzyme protein hydrolysis using the visceral digestive enzymes. As described in chapter 4.2, the organic acid preserves the fish by-products, but the lowering of the pH also serves to activate the digestive proteases of the fish. The use of endogenous enzymes is seen as an inexpensive and mild process; however, it usually requires long hydrolysis times and gives an unspecific hydrolysis. Hence, the use of exogenous enzymes is considered the best choice for producing food-grade protein hydrolysates as the process is highly specific and reproducible and may enable the tailoring of well-defined hydrolysate products. Generally, the enzymatic hydrolysis industry finds the additional cost associated to the use of the latter enzymes as justified. This is because the specificity and higher reproducibility allows for production of products that potentially can reach higher-paying markets, as compared to products based on e.g. rendering or ensiling. There are a vast array of commercially available proteases and a judicious choice should be made with regards to enzyme performance and cost [43].

Proteins are complex substrates containing peptide bonds with different accessibility to enzymatic cleavage [44]. Proteases are ubiquitous and exhibit a huge diversity in action, e.g. in substrate selectivity, where individual proteases cleave the peptide bond, and in pH and temperature preferences [45]. In processing of protein-rich materials, the selection of protease is based on both influence on product parameters, such as peptide size distribution, and protein source [37]. Processing parameters also determine product outcome, e.g. enzyme-to-substrate ratio, pH and process time. A careful optimization of these parameters allows for development of products with selected properties.

A simplified protein hydrolysis process flow diagram is given in Fig. 5. The raw material is minced and diluted with water to allow good mixing and enzyme access [46]. Proper dilution can prevent product inhibition and maximize product yield, but added water is also a factor influencing processing costs in the drying process. A compromise between desired product yield and water that needs to be removed is imperative. The pH of the enzymatic hydrolysis process are often selected based on the optimum for a given protease [47]. However, adjusting the pH requires acid or base that will introduce high levels of salt to the final hydrolysate. This might reduce the nutritional value of the product and, if possible, this should be avoided. At the end of the reaction, the enzyme activity is terminated by irreversible denaturation of the enzyme by heating to above 90 °C for at least 10 minutes. The crude hydrolysate is separated by a 3-phase decanter into an oil, water and solid phase. The water phase constitutes the water-soluble protein hydrolysate that can be concentrated in an evaporator followed



by drying in a spray dryer. The solid phase contains bones and insoluble proteins that can be used in the production of a bone meal.

#### 4.6 Analysis

Valorization of proteins from by- and co-products is, in most cases, based on heterogeneous raw materials with a substantial and uncontrollable variation in quality. It is crucial to develop a production process that is robust towards raw material quality and variations in order to produce a stable product. An essential first step is thus evaluation of the raw material composition (e.g., fat, protein, moisture and ash). The Kjeldahl Nitrogen method for protein determination is a commonly used analytical approach based on classical wet-chemistry. The method provides figures of the total nitrogen in the sample, which gives the content of protein based on multiplication with a suitable nitrogen-to-protein conversion factor. Food matrices contain other nitrogenous organic compounds that will influence the determination of the true conversion factor, such as non-protein amino acids and nucleotides. Substrate-specific nitrogen to protein conversion factors for poultry by-products, meat and bone meal, and Atlantic salmon head and backbone residuals have been calculated [43, 48]. Another important raw material characterization parameter is the amino acid composition. Amino acid analysis is typically performed using chromatographic methods and can provide important knowledge about the nutritional properties of the raw material.

The above analytical measurements are performed offline and typically on a small representative sample from a batch of raw material, and the heterogeneity of meat- and fish-based products poses a significant challenge in representative sampling. Rapid and non-destructive spectroscopic techniques such as fluorescence, Raman and near-infrared spectroscopy have been demonstrated to be valuable tools that allows the characterization of raw materials and products in terms of gross composition [49-50]. Such approaches are expected to be essential in future enzymatic protein hydrolysis, where novel strategies for optimization and monitoring is used to obtain robust processes and products of defined qualities.

One of the main process control parameters in protein processing is measurements on to which extent the proteins has been degraded. In this respect, measurement of the degree of hydrolysis, i.e. the percentage of cleaved peptide bonds, is a widely used approach in the monitoring of an enzymatic protein hydrolysis. Another valuable parameter used for characterization of protein hydrolysates is the molecular weight distribution of the peptides. Unlike the degree of hydrolysis, which is always relative to the starting material, the molecular weight distribution is a direct measure of the peptides and proteins present. A major limitation of both of these measurement techniques is the laborious sample treatment and lengthy analysis time. This limits their use in industrial settings and as potential online monitoring tools. Recently, it has been shown that Fourier Transform Infrared Spectroscopy (FTIR) spectra are useful for characterization of enzymatic protein hydrolysates [51-52]. This technique holds a promising potential as an on- or at- line process monitoring tool for measurements of degree of hydrolysis. FTIR could thus be a valuable future tool in industrial hydrolysate production by providing on-line process control, optimization possibilities and thus stable product qualities.

A thorough characterization of individual peptides is also a very important aspect, especially if the chemistry of a given product is to be related to a specific sensory or biological activity. In such cases, chromatographic fractionation and mass spectrometry has been shown as a powerful technique for an unequivocal elucidation of peptides [53].

#### 4.7 Scale-up of bioprocesses

Many of the processes used to convert fish and animal residuals into higher-value products are developed in laboratory scale, and demonstrated in pilot scale. When developing new products from these biomasses, many processes fail to meet significant challenges in the transition from laboratory to industry scale. Thus, the upscaling steps need to be carefully planned already at the start of the product development. The scale-up phase is commonly called the demonstration phase, where a prototype product can be produced, capital and operating costs of the process can be calculated, and the product can be tested in the market. It is an important step in the bioprocess development. The reason for the challenges is in part due to the process chemistry of scale-up, where differences in mixing, shear rate, and mass and heat transfer causes differences in how the biomass is processed compared to in small-scale processes. In addition, the production of process inhibitors that are effective in large scale and not in small scale can be experienced [54-55]. Another challenge can be the economics of the demonstration phase. The demonstration phase is referred to the “valley of death” in product development. There is a lack of available risk capital to perform this step, and a limited access to demonstration plants that can be

used to test the process. Demonstration plants are needed in order to reduce the risk and cost of the demonstration phase. It is too risky for most developers to invest in a full scale facility before the process or the market acceptance has been demonstrated. A solution that has been successful in many countries is the establishment of publicly financed demonstration plants. Here, flexible plants are built and constructed to be able to accommodate a large variety of different processes. In such plants, many different developers can test and demonstrate their process and their product in the market. Due to the flexibility of the plants, operating and capital costs can be estimated; however, there is likely a loss of biomass and process yield when using flexible plants that will be improved in a plant specially designed for one process. Still publicly financed demonstration plants have and will be important in the continued commercialization of new biomasses towards new products [56].

## 5. Applications

Co- and by-products from fish and animal processing has a great potential for use in food and feed products, as well as for other markets. In use for human consumption, it is important that the residuals are not classified as ABPs. As described under chapter 2, in general the co- and by-product material contains high amounts of protein, with essential amino acids, vitamins and minerals.

### 5.1 Food ingredients

Fish and meat co-products have several applications within food ingredients, given that the process have implemented systems such as Good Manufacturing Practice (GMP) and Hazard Analysis and Critical Control Point (HACCP) [57]. Variety meats and other parts of the animals that by tradition are considered edible, such as kidneys, liver and oxtail, can be used directly for human consumption. Collagen-rich material like animal hides and bones and fish skin are used for gelatin production. In Norway, dried cod heads, co-products from the stock- and klippfish industry, and meat-rich salmon trimmings and backbones are sold for different food applications. There are also many different applications of blood in food production, such as emulsifier, stabilizer, and as a clarifier [58-59]. However, in order to use animal blood for human consumption it needs to be extracted by special equipment in direct contact with a cooled tank to avoid contamination [60].

For some time now, the production of enzymatic protein hydrolysates has gained interest for use within human nutrition. The process is mild and does not impair the nutritional quality of the original protein substrate. Protein hydrolysates may have several applications as food ingredients, such as for emulsifiers and foaming agents. Other applications are in specialized adult nutritional formulas, e.g. in diets for elderly who need extra protein supplements to maintain their body weight, formulas for infants with allergies towards intact food proteins or with inborn errors of metabolism and nutraceuticals [61-64]. A current drawback with production of protein hydrolysates is the formation of bitter and unpalatable tastes generated during the hydrolysis process. Bitter taste is mainly ascribed to small peptides of less than 1000 Da with hydrophobic and/or aromatic amino acids [65]. Not only the presence of hydrophobic and aromatic amino acids, but also the amino acid peptide sequence is important for the bitter taste intensity. Based on the hydrolytic specificity of the chosen protease it may be possible to produce hydrolysates of different bitter potency from the same substrate [66]. It can be possible to remove bitter taste using different debittering techniques [67]; however, these techniques may be challenging in industrial relevant applications [68]. In general, restricting the hydrolysis to reach a low degree of hydrolysis with a broad molecular weight distribution will reduce the formation of bitter taste [66]. In addition to the formation of bitter tastes, protein hydrolysates will have raw material related flavors, such as fish, chicken, meat etc. depending on raw material that will influence the overall flavor profile. These flavors are not related to the protein, but rather water-soluble compounds that are present in the substrate [66].

### 5.2 Feed

The use of processed animal proteins in fish feed have several advantages compared to current uses of plant proteins, due to the fact that plant-derived feed ingredients can contain anti-nutrients and allergenic proteins [69]. Meat and bone meal and fishmeal are the final products from rendering of animal and fish by-products, respectively (chapter 4.4). These are excellent feed sources due to a high content of essential proteins, minerals and vitamins. However, especially the meat and bone meal may be subjected to large variations in nutritional quality. This is mainly caused by variations in composition of raw material and the harsh rendering temperatures [70-71]. In addition, raw materials rich in bone will result in a meal with high ash content, which is associated with low protein digestibility.

As described in chapter 4.5.2, after enzymatic protein hydrolysis there are two resulting fractions with usable peptide content. One is a water phase with soluble peptides, and the other is a solid phase containing insoluble proteins and minerals. Both these are frequently used as feed, although with large price differences. The use of protein hydrolysates for animal feed have favourable formulation properties, such as a high solubility over a wide pH range and ionic strengths, and a set of positive nutritional properties, including feeding stimulation and palatability enhancement, facilitated adsorption of e.g. labile and insoluble amino acids, and lastly, presence of beneficial hormone-like peptides [72]. As mentioned, feed production is an area surrounded by a strict regulatory framework, and it has recently been published a relevant review of the European regulatory framework and potential uses of ABPs for feed [73].

The potential that lies in the valorization of proteins from fish residuals is not fully fulfilled [74]. In general, fish protein products have many applications within feed for the aquaculture sector and monogastric land animals, such as weanling pigs, poultry and pets. The use and importance of fishmeal in the aquaculture sector has grown substantially over the last decades [75], and there is huge potential for increased fishmeal production from underutilized sources towards this sector. Meat and bone meal from pigs and poultry may also have an increasing potential as feed ingredient within the aquaculture sector. In Europe, the use of ABPs in fish feed has been restricted due to the risk of TSE, but the restrictions was lifted in 2012 for non-ruminant protein meal [76]. Recent studies have evaluated the effects of poultry and porcine by-products as feed ingredients for Atlantic salmon and found that the ABP material could cover about 50% of the protein in the diet without negative effect on their growth. Moreover, the use of ABP protein did not show any severe negative effects on gut health, that is often a problem with plant-based diets [77-78].

Piglets have shown higher preference for feed that included either dried hydrolyzed porcine protein or fish meal compared to other protein-rich feeds, e.g. soybean protein, wheat gluten and sweet milk whey. The fish meal based feed got the highest score at inclusion level of 50 g per kg, while the feeds including hydrolyzed porcine proteins gave a high preference over a larger inclusion span (50 – 200 g per kg) [79].

### 5.3 Pet food

Companion animals, such as cats and dogs, represent additional consumption of protein via human purchasing. Thus, as such it also represent an additional indirect protein need for humans. To ensure a sustainable pet ownership in the future, pet food needs to be sustainable and affordable and most effectively satisfy the needs for good health and well-being of the animals [80]. In general, all category 3 by-product material is suitable for pet food production (Table 3). In the production of wet pet food, only residuals that are eligible for human consumption (i.e. co-products), but is found unfit for various reasons, can be used [13]. Pet food formulas are either dry, semi dry or wet, and protein content varies between 10-50%, with wet foods at the lower, and dry food in the upper end [81].

Meat and bone meal products derived from rendered ABP are used by many pet food producers. However, its popularity is declining due to several reasons, including the name of the product and its perceived association with TSE risk. Also, poultry meal is a widely used protein ingredient in pet foods. In general, poultry protein meals are well utilized by dogs and cats and makes up a big share of the total protein in many premium pet foods [82]. Protein hydrolysates of both animal and fish origin are also increasingly utilized for pet food applications. The hydrolysates contain short peptides and free amino acids that might act as feeding stimulants and palatability enhancers [71, 83]. Moreover, protein hydrolysates might have hypoallergenic [84] and bioactive properties (discussed below), which makes it an interesting pet food ingredient for companion animals with special needs.

### 5.4 Health-promoting products

By- and co-products from the fish and meat industry are rich sources of biologically active molecules with potential health-promoting effects. Such bioactive molecules can be included in, e.g., food (nutraceutical) and as active ingredient in cosmetics (cosmeceuticals). Bioactive peptides (BAPs) are short chains of amino acids with hormone- or drug like activity that modulate physiological function through interactions with specific therapeutic target [85]. Numerous BAPs derived from co-products have been proven to exhibit a wide range of positive health effects, and most of the research focus have been on blood pressure lowering, blood sugar regulating, anti-microbial and antioxidant activities [86-88]. In addition to a specific therapeutic function, peptides may have other beneficial effects, for example, rats fed with hydrolyzed fish protein showed reduced visceral adipose tissue mass [89], and peptides derived from collagen were shown to increase muscle mass and strength in elderly

men [90]. BAPs from by-products can also have a positive effect on collagen production, which makes them attractive ingredients in cosmeceuticals for wound healing and skin aging [91].

A standard process of evaluating health-promoting potential of BAPs comprises several stages of analysis involving both *in vitro* and *in vivo* experiments. Ideally, in order to validate bioactivity of a given bioactive peptide, human intervention studies are necessary. However, these studies are expensive and typically conducted after selecting a potent candidate through a rigorous screening process. The majority of the screening experiments for BAPs are performed using *in vitro* assays including enzymatic assays, cell cultures, genomic tests and *in vitro* digestion stimulation. Candidates from such screenings are further evaluated using animal models and eventually human intervention (Fig. 6).

A large proportion of the reported bioactivities from meat and fish protein hydrolysates are based on either a chemical, or an enzyme-based bioassay. BAPs in food must be absorbed in the intestine during digestion, and enter blood stream to exert a physiological effect, although some peptides may act locally in the stomach or intestine. After absorption, BAPs can act on a given therapeutic target as a single molecule or synergistically [92]. BAPs from a different meat and fish processing co-products have been shown to possess bioactivities towards key therapeutic targets related to diseases such as diabetes, obesity and coronary heart disease [53, 93-95]. Cell models can provide additional knowledge to the preliminary bioactivity-screening assays, including endogenous effect, dose-response and target organ. Some peptides might exert their effect on muscle development, while other can influence liver, bone, angiogenesis, inflammation etc. It is therefore vital to use different cell model types when examining for bioactivity effects.

## 5.5 Other applications

It is important to stress that there are a huge number of applications for the use of products based on meat and fish residual raw materials that are not covered by the above. Protein hydrolysates based on both chemical and enzyme production can be used as growth media in areas of fermentation and biotechnology for the production of pharmaceuticals and recombinant protein, as well as in diagnostic media [96]. Blood from various animals has also shown antioxidant and antimicrobial activity [97]. Also, in microbiology and many enzyme assays, bovine serum albumin extracted from blood is an important tool. Various enzymes extracted from livestock livers, used in many biotechnology applications, e.g. several types of dehydrogenases and catalase from bovine liver and porcine liver esterase. Aside from being an excellent feed source, poultry feathers can be used for a range of non-food applications [98]. Examples span electrical and electronic applications, composite materials, oil adsorbents, and generation of micro- and nanoparticles. The meat and bone meal and fat from the rendering process may also be used as fertilizers and biodiesel or as raw material in chemical industry, respectively [99].

## 6. Utilization of co-products towards consumer products

In valorization of meat and fish by- and co-products, the human consumption market has been specifically challenging to reach. In the meantime, consumers are being increasingly concerned about the environment and sustainability. This trend is expected to remain, or even increase, in the future with consumers aiming to influence industries with their consumption pattern, i.e. food, and particularly protein production [100]. Being an integral part of the value chain, consumers are influencing both the levels of food waste and the acceptance of value-added co-products [101]. Still, there are challenges regarding regulations and feasibility of products when trying to introduce products for human consumption in terms of supply, control and economics [56]. Only minor efforts have been done so far to understand the best approaches for upgrading fish and animal-based co-products towards lucrative products for human consumption [102].

Fish- and animal-based by- and co-products can be used in the development of new products or as replacement of ingredients in existing products. Much of this work is following an 'industry push' strategy, where products are tested with consumers after most product development decisions are already made [103]. There is a current lack of awareness about the need for consumer acceptance. The consumer acceptance is of utmost importance for a potential market success for products based on ingredients from new sources [104-105]. Thus, a poor product development strategy could lead to product failure. In order to improve the strategy, implementation of a systematic product testing will likely increase the chances of success in the market [106].

Information about products and production methods have repeatedly been shown to influence consumer choices [107]. Studies on the use of added-value compounds usually rely on analytical and sensory testing that do not take into account the consumer perspective [108]. In rare cases, consumer acceptability studies are included, however usually on the end products and without adding any information about the origin of the ingredients

[109]. When excluding information about origin in use of trained panels or consumers for tasting of food products, it will be hard to estimate the actual acceptability of a product by consumers on the real market. If the relevant information is provided in a balanced communication strategy, it could act to increase consumers demand for products with proteins from a production method with a reduced environmental impact [110].

Consumers have adequate knowledge on how to use food ingredients and supplements, but besides segments that show particular interest, they are rarely aware of the product production methods [111-112]. When new products or ingredients are presented in the market, consumers become more alert and curious towards its origin [113]. This concern has been one of the main barriers for e.g. use of ABPs or genetically modified ingredients in salmon feed, despite the potential benefits [114]. Considering the fact that consumer acceptance varies and changes with exposure to products and information, it is possible to achieve potential improvement of products without sacrificing quality [115].

When consumers are analysing their food choices, they are confronted with trade-offs that may challenge their final decisions between convenience, health and sustainability [116]. Thus, if communicating one positive element of a product, such as “sustainability” or “using the whole animal”, it should not come in the way of aspects such as “food safety”, “health”, “convenience”, “hedonic expectations” etc. In fact, it is more probable that products will be chosen by consumers if all positive elements of the product are combined with production information in a holistic and transparent reputation-building strategy. Such a strategy could be successful when used in combination with the targeting of consumers that are interested in functional foods and are likely to seek additional information about the product [117].

Despite continuously improvements, there has been an increase in public concerns about the livestock and aquaculture production, due to food crises and the environmental impact of production practices [118]. This has led consumers away from consumption of meat proteins in favour of plant proteins [119]. An expected positive trend in the near future is that consumers will demand fish that is farmed under safe and controlled conditions in clean waters [120]. Therefore, any information towards the consumers should focus on trust building, with transparent and balanced communication. This type of approach could establish a fertile ground for introduction of new products and ingredients for human consumption in the market, such as products based on sustainable co-products from fish and animals.

## 7. Challenges and future trends

There is a growing number of initiatives in the context of the foreseen transition from an oil-based economy to a bioeconomy. This includes a concomitant awareness of consumers, producers and governments about the importance of recovery and recycling of what was previously regarded as waste. In many countries worldwide, research funding are directed towards finding new methods of optimized recovery and exploitation of the intrinsic raw material components, preferably with a biorefinery approach to enable maximum utilization. There is an immense potential for growth when it comes to increased valorization of fish- and animal-based co- and by-products. As more products reach the market, producers will start competing for what is now inexpensive start materials and one can expect higher prices for the residual materials in the future. At the same time, producers must be able to count on a stable supply of raw material to obtain needed predictability in the production.

Many of the described technologies form the basis in established industries, with well-established processing operations and well-known markets. Enzymatic protein hydrolysis represent an up-and-coming, relatively new industry based on a very enabling technology, showing great promise based on published research. However, when implemented into industrial practice, results show that there are challenges related to controlling the number of variables that affects the final product properties and quality. This includes raw material variations, processing parameters, such as pH, time, choice of enzyme and inactivation method in protein hydrolysis, choice of downstream unit operations and the choice of drying method. Current industrial practices rely on traditional and established analytical tools for quality parameter evaluation. This methodology is unable to provide the necessary fast feedback required for production of products with specified properties. Nondestructive spectroscopy-based technologies could be valuable future tools in the industrial production of protein hydrolysates, by providing on-line process control, new optimization possibilities and reduced product quality variation.

Considering the predictions of a future food shortage, it makes sense to strive to use as much as possible of the high-quality meat and fish co-products for human consumption. There is an increasing interest in upgrading of this residual material for human consumption; however, knowledge of consumer acceptance and preferences

should be known and targeted before the products reach the market. Sometimes the desired market growth is hindered by a lack of synergy among stakeholders, and a lack of common vision. A perfect collaboration among all stakeholders is challenging, but increased cooperation will lead to shared vision, strategic planning, targeted communication and an overall image improvement.

Image improvement form the foundation for societal acceptance and vice versa, societal perceptions could also inform the strategic decisions of stakeholders towards image improvement and positive reputation. Based on a systematic research, stakeholders will reach a point where there is less of “waste products” and “residual raw materials” and rather an optimized utilization and processing of all resources.

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**Table 1. A summary of the production of freshwater, marine and diadromous (can live in fresh and salt water) fishes in 2014. Data is based on FAO global production statistics [2], and is divided into continental and global data and with a subset showing the aquaculture production.**

Continent	Inland waters		Marine areas		All production (tonn)	Aquaculture (tonn)	Value Aquaculture (USD 000)
	Sub-total all production (tonn)	Sub-total aquaculture (tonn)	Sub-total all production (tonn)	Sub-total aquaculture (tonn)			
Africa	4 527 980	1 682 039	5 455 976	12 814	9 983 955	1 694 852	3 573 168
Americas	1 607 879	1 076 073	13 870 982	1 018 460	15 478 861	2 094 533	11 112 732
Asia	47 557 457	40 319 666	38 759 848	3 388 124	86 317 305	43 707 790	73 760 477
Europe	831 604	477 051	14 205 697	1 820 109	15 037 301	2 297 160	12 099 014
Oceania	20 165	4 432	1 281 690	63 124	1 301 856	67 557	756 601
<b>Summary</b>					<b>128 127 276</b>	<b>49 861 891</b>	<b>101 301 993</b>

**Table 2. Summary over production year 2014 of major livestock in the separate continents in based on FAOSTAT data [8]. At bottom, percentage of residual raw materials resulting from slaughter of some livestock, based on Norwegian data [9].**

<b>Continent</b>	<b>Cattle</b>	<b>Pigs</b>	<b>Sheep</b>	<b>Chickens</b>	<b>Turkeys</b>
	1000 Head	1000 Head	1000 Head	1000 Head	1000 Head
Africa	312 327	34 332	340 749	1 809 059	23 658
Americas	508 942	169 902	86 074	5 436 151	312 477
Asia	491 020	590 548	536 251	11 923 472	14 575
Europe	122 011	185 546	130 118	2 114 988	110 786
Oceania	40 226	5 346	102 432	126 014	1 377
<b>Summary World</b>	<b>1 474 526</b>	<b>985 673</b>	<b>1 195 624</b>	<b>21 409 683</b>	<b>462 873</b>
By-product (%)*	60	37	63	51	45

\* The figures are based on assumption that everything not sold as meat from the animals is to be considered as a by-product.

**Table 3: Overview of category 3 animal by-product material suitable for feed for different animals according to the TSE regulation [15].**

	Ruminants	Non-ruminants	Fish	Pets and fur animals
Processed protein from ruminants	✘	✘	✘	✓
Processed protein from non-ruminants	✘	✘	✓	✓
Blood from ruminants	✘	✘	✘	✓
Blood from non-ruminants	✘	✓	✓	✓
Hydrolyzed protein from ruminants	✘	✘	✘	✓
Hydrolyzed protein from non-ruminants	✓	✓	✓	✓
Collagen and gelatin from ruminants	✘	✘	✘	✓
Collagen and gelatin from non-ruminants	✓	✓	✓	✓
Fishmeal	✘	✓	✓	✓

**Table 4. Approved alternative methods for heat treatment of category 3 animal by-products [14].**

Method (#)	Particle size (mm)	Core temperature (°C)	Time (min)	Pressure (bars)	pH	Batch	Continuous
1	50	>133	20	3		x	x
2	150	>100	125	NS		x	
	150	>110	120	NS		x	
	150	>120	50	NS		x	
3	30	>100	95	NS		x	x
	30	>110	55	NS		x	x
	30	>120	13	NS		x	x
4 <sup>a</sup>	30	>100	16	NS		x	x
	30	>110	13	NS		x	x
	30	>120	8	NS		x	x
	30	>130	3	NS		x	x
5 <sup>b</sup>	20	>80	120	NS		x	x
	20	>100	60	NS		x	x
6 <sup>c</sup>	50	>90	60	NS	<4.0	x	x
	30	>70	30	NS	<4.0	x	x
7 <sup>d</sup>	NS	>76	20	NS		x	x
	NS	>70	20	NS		x	x

NS – not stated

- a) Carver-Greenfield process; i.e. heated in a vessel with added oil.
- b) The by-products must be heat coagulated and mechanical pressed to remove water and fat before final heat treatment.
- c) Animal by-products originating from aquatic animals or aquatic invertebrates only.
- d) Method approved by Norwegian authorities (wild fish >70 °C and aquaculture fish >76 °C; Nygaard, 2010), or any method authorized by the competent authorities complying with the following microbiological standards: *Clostridium perfringens* absent in 1 g of product after heat treatment, *Salmonella* absent in final product (n=5; c=0; m=0; M=0), Enterobacteriaceae (n=5; c=2; m=10; M=300 in 1g).

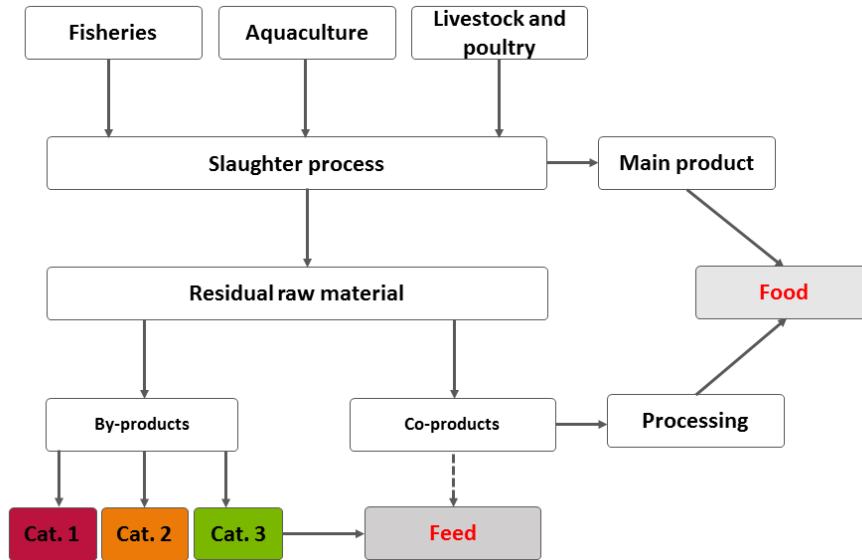


Figure 1: The progress from fisheries and farming of fish and livestock, to co-products and by-products. The co-products are in this review defined as residual raw materials from the slaughter process that still have a food grade quality and can be used for food. When the rest material is not suitable for human consumption, for commercial or safety and/or regulatory reasons, it is defined as a by-product. The by-products are separated in three categories (1-3) based on their origin and potential for feed applications.



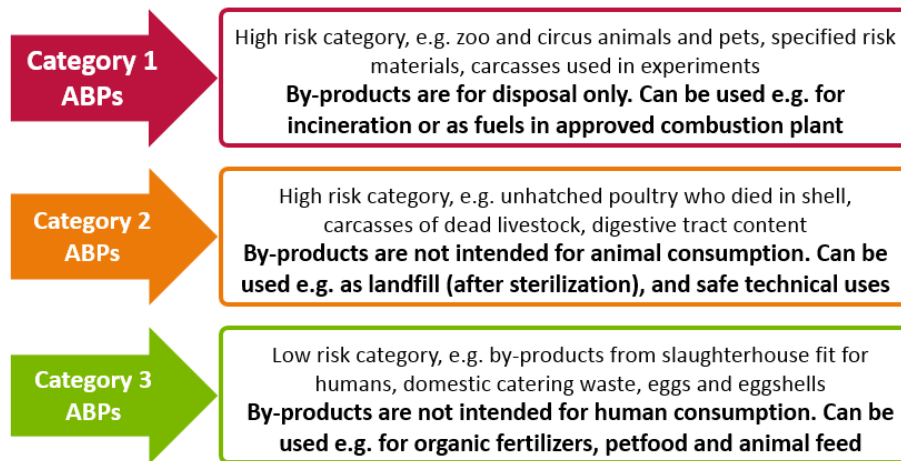
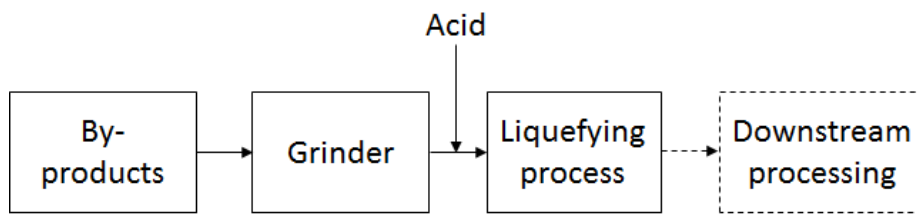


Figure 2. A brief summary of the by-products that are included into each of the three animal by-products (ABP) categories regulated within the EU, and examples of some approved uses of the animal by-product in each category [13].



**Figure 3: Simplified illustration of the silage process. The raw material must be ground so that the raw material can be in contact with the acid. At an acidic pH, the enzymes present in the fish viscera will degrade and liquefy the fish tissue without risk of bacterial spoilage due to the low pH. The silage gradually liquefies due to the activity of tissue degrading enzymes which are naturally present in the fish, mainly the viscera. The silage can be used as is (crude silage) or be further processed (Figure 5)**

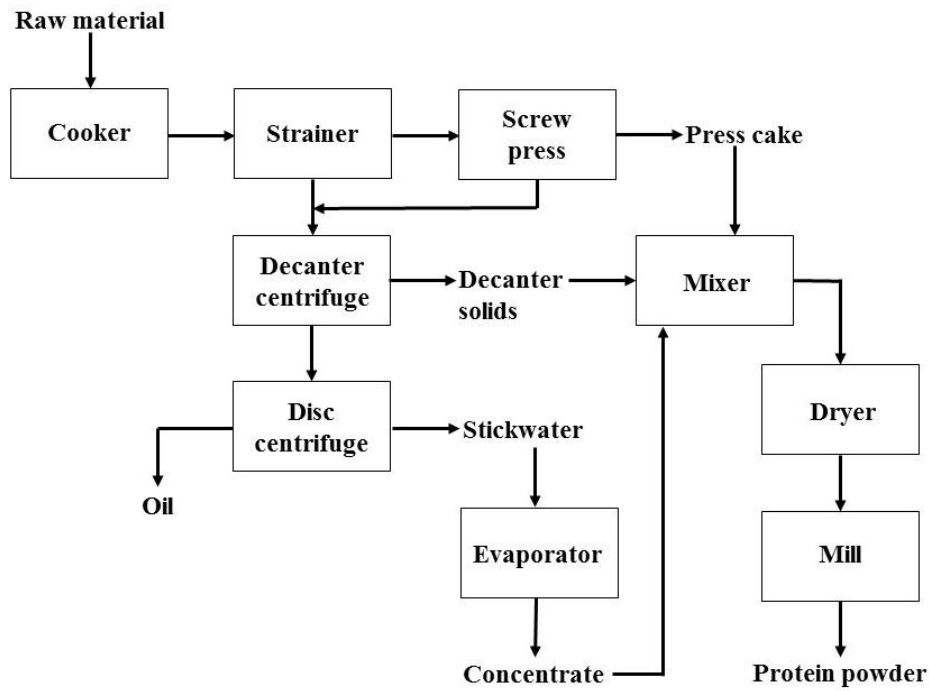


Figure 4. Simplified process flow diagram representing the main unit operations applied in the wet rendering process. The raw material is cooked before entering the strainer. The decanter and disc centrifuges separates the solids, oil and stickwater phases. The stickwater is evaporated to a concentrate, mixed with the solids (press cake and decanter solids) and eventually dried to a protein powder. The process conditions used are dependent on raw material and listed in table 4.

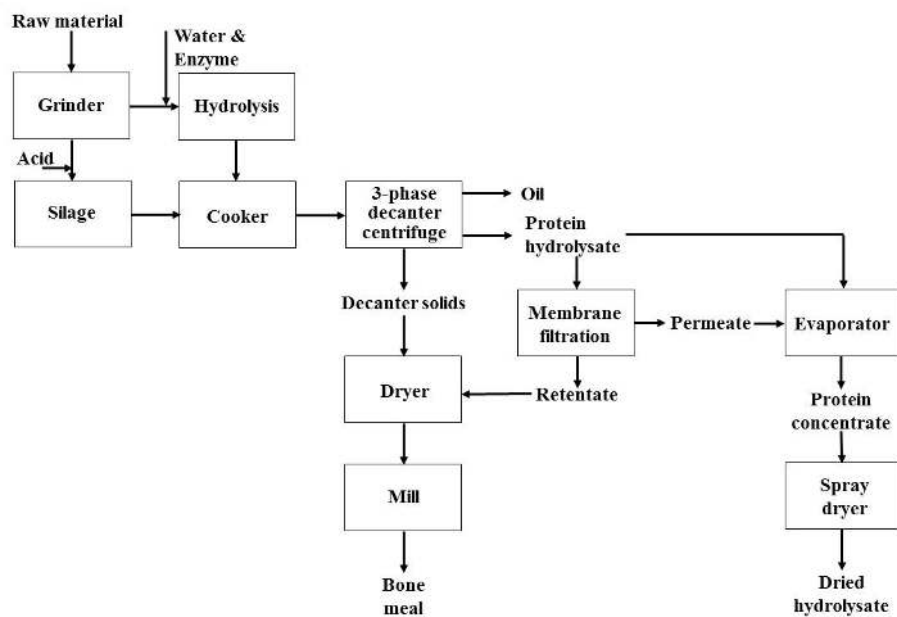


Figure 5. Simplified process flow diagram representing the main unit operations applied in a hydrolysis process. The raw material is ground and mixed with water and enzyme and the hydrolysis process run at predefined time and temperature conditions. In case of silage production, the raw material is added acid and hydrolysed using the inherent proteolytic enzyme activity at ambient temperature. The enzyme activity is terminated by cooking and the crude hydrolysate separated by a 3-phase decanter centrifuge into oil, soluble peptides and amino acids, and solid phases. The solids is dried and milled into a bone meal. The protein hydrolysate can be membrane filtrated to achieve desired molecular weight distribution of the peptides and eventually evaporated and dried to a dry protein hydrolysate.



**Figure 6: Comprehensive method platform for testing health-promoting ingredients, from preliminary in vitro digestion models, bioactivity screening assays to extensive human interventions studies.**