CHAPTER SEVEN

Challenges and opportunities regarding the use of alternative protein sources: Aquaculture and insects

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Abstract

The world population is constantly growing so that the needs of food, including protein sources, will also increase considerably in the coming years. Animal farming has been related to numerous environmental consequences such as soil erosion, exaggerated water consumption, generation of large quantities of waste and accumulation of greenhouse gases. This is a situation that demonstrates the suitability and importance of finding more sustainable protein alternatives without losing the quality and the

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nutritional benefits of current common protein sources. In this context, it is worth highlighting the potential of insects and products derived from aquaculture. Particularly, farmed aquatic food products can reduce the impact on wild fish stocks, whose overfishing may end up in an ecological collapse, and insects are easy to be reared and efficient in converting feed into biomass. However, there are still several challenges like the need to adapt technologies and methods for the production and well-characterization of the new ingredients, careful evaluation of the introduction of such new proteins in the diet and its safety of use, including potential allergies, and the acceptance by consumers.

1. Introduction

Scarcity of nutritious foods is directly linked to the development of many diseases and is a leading indicator for determining the quality of public health within the community (Musina et al., 2017). On the other hand, it is foreseen (FAO, 2009) that food production will increase considerably (70%) by 2050, which involves issues related to the environment and human health. In fact, providing food for the entire population of the future under the same conditions as today will mean an unsustainable pressure on water, land and energy (Verneau et al., 2016). In this context, meat production as a valuable protein supply that has been reported as the main cause of the impact discussed in the previous paragraph (Smetana, Palanisamy, Mathys, & Heinz, 2016). In addition, global meat consumption, especially in relation to fish production, is expected to rise by 50% above 2006 levels to satisfy the expected demand by 2050 (Nugroho & Nur, 2018). Almeida et al. (2015) indicated that most protein that contributes to an adequate balanced diet for the majority of the human population is obtained from farm animal products, such as milk and meat, and also increasingly from aquaculture products.

Even though current food science is directed toward exploitation of sustainable sources of protein, e.g., whey (Musina et al., 2018), still animal farming induces more than 50% of soil erosion worldwide, which results in more and more desertification. Moreover, it must be borne in mind that more than 65% of human infectious diseases are transmitted by animals and livestock production, which in turn account for 70% of all the farming lands. According to Stockholm International Water Institute, agriculture consumes 70% of water, a majority part is employed for meat production, and livestock and animal wastes originate at least 51% of all greenhouse gases. These issues demonstrate the importance of looking for alternative protein sources (Kostecka, Konieczna, & Cunha, 2017).

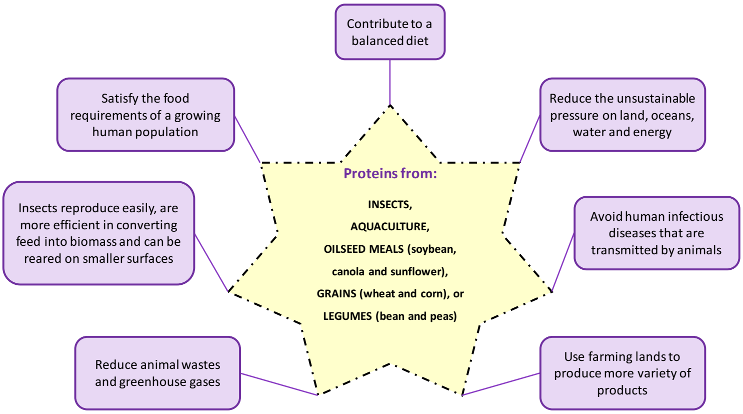

Fig. 1 Advantages of using alternatives to industrial farm animal proteins.

The results obtained by Van Mierlo, Rohmer, and Gerdessen (2017) suggested that it is possible to decrease the impact on the environment throughout a change in the use of meat to plant-based products, without losing the known nutritional benefits of meat. In this context, the ability of insects to be used as both, feed and food, has been extensively recognized. Fig. 1 shows numerous advantages of using alternatives to industrial farm animal proteins. However, in the 2013 "Edible insects" report published by the Food and Agriculture Organisation of the United Nations it was pointed out that, despite the noticed potential of insects, "insect rearing for food and feed remains a sector in its infancy, and key future challenges will likely emerge as the field evolves" (Verbeke et al., 2015). Insects have been cataloged as more efficient in generating food from biomass (Fig. 1), which is considered as an advantage when compared to the livestock for human consumption. Besides, insects can reproduce easily and are also able to breed on smaller surfaces, as well as on bio-waste streams, leading to a higher yield per hectare than other common crops. In addition, they produce a lower emission of greenhouse gases and ammonia per kg meat than pigs or cattle (Makkar, Tran, Heuzé, & Ankers, 2014; Verbeke et al., 2015).

Therefore, insects are increasingly considered as good substitutes for traditional food from animals, such as eggs, milk, fish and meat, in human nutrition, especially due to their significant content in protein and essential amino acids, as well as in other nutrients that must be taken into account (Fig. 2).

Nevertheless, they account for a low percentage in the total food intake in Europe. On the other hand, regarding the consumption by food producing animals, insects can be an interesting ingredient of their usual feed,

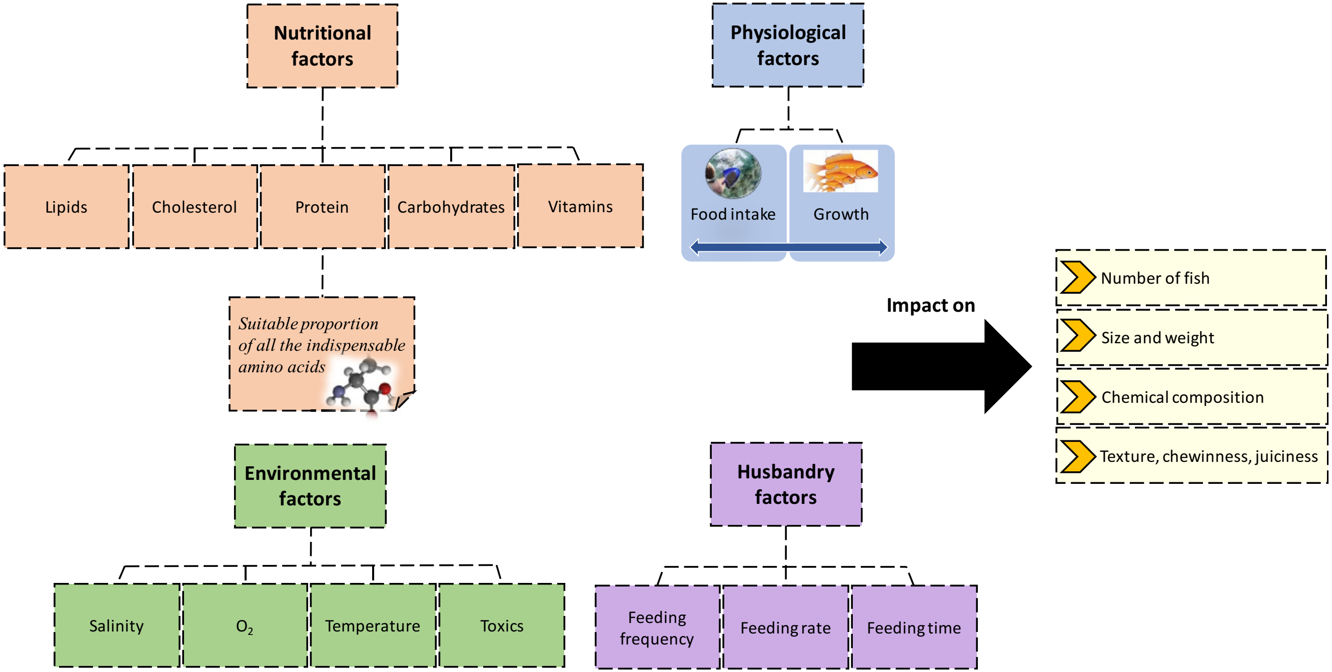

Fig. 2 Factors to consider in aquaculture feeding. *Modified from Sun, M., Hassan, S. G., & Li, D. (2016). Models for estimating feed intake in aquaculture: A review.* Computers and Electronics in Agriculture, 127, *425–438.*

linked to other sources of protein or even replacing them. In this sense, it is not about insects replacing 100% of the usual feed ingredients, but their introduction as a proportion of the feed (Scientific Committee, 2015). In fact, several publications summarizing animal feeding with insects describe this potential (Makkar et al., 2014; Riddick, 2014; Sánchez-Muros, Barroso, & Manzano-Agugliaro, et al., 2014; Veldkamp et al., 2012).

In the same way, aquaculture is becoming a very important and recurrent source of protein available in human diet (Rodrigues et al., 2018). Aquaculture has grown in the past three decades, and it is expected to continue with an average annual growth rate of 4.5% during the period 2010–2030 (Sun, Hassan, & Li, 2016). In fact, in the past decade, aquaculture has increased at a rate of 7–9% per year, making it the fastest growing food production industry in the world. Currently it already produces more biomass than either wild seafood or beef, making it a fundamental part of future food production (Froehlich, Runge, Gentry, Gaines, & Halpern, 2018; Oken et al., 2012). Fisheries and aquaculture products will represent predominant sources in the near future, meeting the nutritional needs of a growing human population. A wide diversity of species of crustaceans and fish can transform feed to protein in a much more efficiently way than cattle, poultry and swine (Gamboa-Delgado & Márquez-Reyes, 2018). According to FAO (2016) over the past decade and even today, aquaculture supplies over half of the fish consumed globally.

Some studies are revealing the potential benefits of shifting human diets away from meat and directing them toward other protein sources, including seafood. Seaweeds are included in the food of some human consumers and is used for industrial purposes, as the production of hydrocolloids (Xiao et al., 2017). Now, the majority of seafood is farmed (i.e., aquaculture), and will continue this way in the near future. In recent years, seafood supplies and demands have increased exponentially and such growth is expected to continue in the future. Regarding this fact, aquaculture provided in 2012 over 50% of all the seaweed and fish food supplies, which translated into 90 million tons and worth of the US$144 billion (Alfaro & Young, 2018).

Freshwater resources in some regions, as the majority of the area of the Gulf Cooperation Council Countries (GCC-Bahrain, Kuwait, Oman, Qatar, Saudi Arabia and UAE), are becoming threatened by agricultural water overuse and mismanagement. Moreover, there are many scientists who think that overfishing of wild fish stocks is not sustainable and may end up in an ecological collapse (Brown, Das, & Al-Saidi, 2018). The growing demand for fish is progressively harming the marine biodiversity, and the fact that the fishing catch is lower than the mentioned demand, made it indispensable to expand the fish farming (Rizzo & Baroni, 2016). A sustainable aquaculture is a good strategy to produce food and can potentially reduce the overfishing of wild stocks. It has been reported that an efficient aquaculture team should exert appropriate mechanical and electronic skills, a wide experience and strong working knowledge of water chemistry, fish nutrition and their health management. In addition, to maintain sustainability and lower costs, industries should try to develop their local ingredients to feed fish (Brown et al., 2018).

A negative side in aquaculture is that over 50% of the production cost corresponds to feed. The main responsible for this high cost are fish oil and fishmeal (FM), both considered as very expensive products. The nutritive value of fish feed depends in large part on the quality of the proteins used, namely, to include an appropriate proportion of the indispensable amino acids. Not only because of the high price of FM, but also for its limited availability, is the aquafeed industry looking for cheaper and abundant alternative protein sources. Additionally, recent articles as well as detailed scientific reports have simultaneously addressed both the nutritional and toxicological aspects of fish consumption. Environmental pollutants are contaminating, to a greater or lesser degree, almost all fish. For this reason, the more fish consumed, on average, the more likely an individual is to be exposed to different toxicants, as methylmercury. Therefore, consumers who usually eat fish or accidentally consume highly contaminated species may exceed exposure thresholds (Oken et al., 2012). Aquaculture companies are trying to improve features associated with food quality, such as disease resistance, growth rate, conversion of feed into muscle, or fertility. However, one of the main challenges to solve by this industry is its effect on environmental sustainability. Different efforts to reduce the environmental impacts of aquaculture intensification are likely to be required (Little & Bunting, 2016). In aquaculture, proteomics application is mainly focused on three factors that, according to Rodrigues, Silva, Dias, and Jessen (2012), have proven to be the major constraints in order to get an efficient production: nutrition, welfare and health management. Aquaculture must face several challenges for being able to continuously deliver a high-quality farmed fish by a sustainable production system. Achieving this goal is not easy and, according to Rodrigues et al. (2018), new management strategies are needed and state-of-the-art technologies as proteomics are being applied to investigate different factors like nutrition, welfare, diseases and safety, which are directly related with the end-product quality.

Meanwhile, Goldberg (2016) studied issues associated with the industrial farm animal protein, such as animal welfare, human health consequences, environmental deterioration and cost, with the aim of continuing with the production but through less aggressive ways, within the concept of sustainable intensification (SI).

2. Aquaculture products nutritional composition

Aquatic products are known to be high in protein and rich in micronutrients (Little & Bunting, 2016). Fisheries contribute 20% of the protein for 3.1 billion people's diets and 17% of global protein consumed, representing a crucial contribution to global food (Teneva, Schemmel, & Kittinger, 2018). Both fish and seafood products provide the only readily available dietary source of long-chain omega-3 polyunsaturated fatty acids for direct human consumption (including eicosapentaenoic acid or EPA and docosahexaenoic acid or DHA). Oken et al. (2012) mentioned that fish is a product rich in protein, with low content in saturated fats, and with a variety of other healthful compounds such as selenium, iodine, and vitamin D. Specifically, tocopherols (α-, β-, γ -, and δ-tocopherol) can be obtained from aquaculture products and seafood. Moreover, it is proven that the fish contains the hepatic α-TTP protein, which is necessary to maintain plasma and tissue α-tocopherol levels (Afonso, Bandarra, Nunes, & Cardoso, 2016). Nevertheless, a number of industrial by-products and toxic chemicals released into environment like heavy metals are also present in fish, particularly in its fatty tissues, due to their lipophilic properties. Additionally, fish products represent the main source in human diet of PCBs (67%). In the same way, fish products also seems to be an important source of methylmercury (Rizzo & Baroni, 2016).

In this context, the increasing role played by farmed aquatic food products toward global fish and seafood supply is clearly evident. According to Tacon and Metian (2018) at the global level fish and seafood products constitute the third major source of dietary protein consumed by humans after cereals and milk, representing 6.4% of total protein supply (19.8% of total animal protein supply), 1.4% of total fat supply, and 1.2% of total calorie supply. From a health perspective, the excess consumption of these products, in combination with a sedentary lifestyle, can have negative effects on human health as increased risk of coronary heart disease, stroke and diabetes. In fish farming products the methylmercury residue is not found, but other unhealthy compounds such as dioxins and PCBs can be present in higher concentrations than in caught fish (Rizzo & Baroni, 2016).

Regarding the feed intake of aquatic animals (see Fig. 2), there are different factors to consider: physiological, nutritional, environmental, or husbandry factors (Sun et al., 2016). Among others, nutritional factors include protein, lipids, cholesterol, carbohydrates and vitamin E. In regard to optimal dietary level and the protein-to energy ratio, the growth of grouper (*Epinephelus malabaricus*) was investigated. In this case, by increasing the protein content in the diet also increased the feed efficiency and, at the same time, the dietary protein level was proportional to the weight gain of the grouper (Shiau & Lan, 1996). It is also important to take into account the feeding time, feeding rate and feeding frequency.

Fish require diets containing 30–55% of crude protein and an amino acid supply precisely adapted to meet the needs for optimal growth. In this sense, FM is an excellent protein source, because it has a high protein content (65–72%; see Table 1) joined to a suitable proportion of all 10 essential

Table 1 Protein contents of different aquaculture products.

Protein source	**Analysis**	**Crude protein (%)**	**Reference**
Diets fed to juvenile Atlantic cod and haddock			
– Low protein	–	42.26	Pérez-Casanova, Lall, and Gamperl (2009)
– High protein		55.03	
Requirements of different farm fish species	–	30–55	Médale and Kaushik (2009)
FM		66–72	
Cynoglossus semilaevis (tongue sole) fed to 100% satiation at 22°C (better growth than other treatments)	Nitrogen content was determined by Vario ELIII Elemental Analyzer (Elementar, Germany). Protein was calculated as multiplying nitrogen by 6.25	71.38 ± 0.80	Fang, Tian, and Dong (2010)

Table 1 Protein contents of different aquaculture products.—cont'd

Protein source	Analysis	Crude protein (%)	Reference
Raw, edible seafood (including bones, cartilage and shells)			
– Cod	–	17–19	Anal, Noomhorm, and Vongsawasdi (2013)
– Herring		18–20	
– Sardine		15–17	
– Salmon		19–21	
– Skate		14–16	
– Tuna		22–24	
– Crab		19–20	
– Prawn		17–18	
– Octopus		29–30	
– Crayfish		16–17	
– Mussel		23–24	
– Oyster		18–19	
Crawfish whole meal		35.8	
Crawfish shell		16.9	
Fish fed diets replacing FM protein with cottonseed meal of different sources: GI = genetically improved (glandless), GMO = genetically modified (glandless)			
– 50% GI-CSM	Crude protein was analyzed with a Labconco Kjeltec System (Rapid Digestor, Distilling Unit-Rapid Still II and Titration Unit, Labconco Corporation, Kansas City, MO, USA) using boric acid to trap ammonia by the Kjeldahl method	15.9 ± 0.14	Alam et al. (2018)
– 75% GI-CSM		16.2 ± 0.28	
– 100% GI-CSM		15.7 ± 0.09	
– 50% GMO-CSM		15.8 ± 0.34	
– 75% GMO-CSM		16.0 ± 0.39	
– 100% GMO-CSM		16.2 ± 0.27	

Continued

Table 1 Protein contents of different aquaculture products.—cont'd

Protein source	Analysis	Crude protein (%)	Reference
Microalgae and cyanobacteria:			
– *Chaetoceros sp.*	–	33	Brown et al. (2018)
– *Dunaliella sp.*		25.7	
– *Isochrysis sp.*		47.9	
– *Nannochloropsis sp.*		30.3	
– *Phaeodactylum sp.*		49.5	
– *Synechococcus sp.*		63	
– *Tetraselmis sp.*		30.7	
– *Chroococcidiopsis sp.*		60.3	
Whole body/muscle tissues from juvenile Black Sea Bass fed diets with different percentages of FM protein replaced by poultry by-product meal protein after 56 days			
– 50%	All experimental diets were produced using a meat grinder and analyzed at the UNCW Center for Marine Science Aquaculture Facility	15.8 ± 0.14 19.3 ± 0.17	Dawson, Alam, Watanabe, Carroll, and Seaton (2018)
– 100%		16.2 ± 0.34/ 18.2 ± 0.35	
Microalgae FM	–	6–62 59–74	Gamboa-Delgado and Márquez-Reyes (2018)
Best diet for significantly improving the lipid profile in tilapia fillets (algae meal 8.77%)	–	36.5	Stoneham et al. (2018)

amino acids (see Table 2) that satisfy the requirements of the different fish species (Médale & Kaushik, 2009). Specifically, FM is a powder enriched in protein, widely used internationally, which results from the industrial processing of small fish such as sardine, herring, anchovy or capelin. It is an important substance of the aquafeed of trout, salmon, shrimp and other farmed marine species, including fatty acids, essential amino acids and other

Table 2 Essential amino acids profile of some aquaculture products.

Protein source	Units	His	Ile	Leu	Lys	Met	Thr	Tryp	Val	Cys	Phen	Arg	Reference
Southern flounder whole bodies after 8 weeks of feeding diets replacing FM protein with CSM													
– 50% GI-CSM		1.02	1.93	3.57	4.01	1.49	2.20	–	2.19	–	2.40	3.38	Alam et al. (2018)
– 75% GI-CSM		1.05	1.96	3.62	4.07	1.51	2.24	–	2.24	–	2.44	3.46	
– 100% GI-CSM		0.97	1.80	3.37	3.83	1.40	2.09	–	2.05	–	2.27	3.17	
– 50% GMO-CSM		1.00	1.92	3.51	3.92	1.49	2.14	–	2.40	–	1.98	3.26	
– 75% GMO-CSM		1.03	1.95	3.61	4.02	1.51	2.21	–	2.45	–	2.02	3.38	
– 100% GMO-CSM		0.97	1.82	3.36	3.77	1.42	2.08	–	2.31	–	1.89	3.20	
Proteins from shrimp (Penaeus vannamai) head	–	11.74	23.57	37.50	34.20	13.57	19.20	5.63	28.75	2.14	26.16	31.43	Anal et al. (2013)
FM (Herring)	g/100 g protein	2.4	4.5	7.5	7.7	2.9	4.3	1.2	5.4	–	3.9	–	Brown et al. (2018)
Microalga Nannochloropsis sp.	g/100 g protein	1.5	3.5	6.7	4.8	1.8	3.6	1.7	4.6	–	3.9	–	Brown et al. (2018)
Cyanobacterium Chroococcidiopsis sp.	g/100 g protein	0.8	2.6	7	3.8	0.4	6.1	–	7.8	–	6.3	–	Brown et al. (2018)
Range of amino acid requirements of farm fish species	g/16 g, N	1.6	2.3	3.2	4.6	2.7[a]	2.5	0.6	2.9	–	4.8[b]	4.1	Médale and Kaushik (2009)
FM	g/16 g, N	2.4	4.3	7.2	7.5	3.7[a]	4.2	1.0	5.1	–	7.0[b]	5.8	Médale and Kaushik (2009)
Best diet for significantly improving the lipid profile in tilapia fillets (algae meal 8.77%)	%Basis	0.78	1.24	2.25	1.92	0.44[a]	1.36	0.26	1.42	–	1.54	2.43	Stoneham et al. (2018)

[a] Value corresponding to methionine + cysteine.
[b] Value corresponding to phenylalanine + tyrosine.

micronutrients. However, the limited supply of both FM and oil from wild catches and their efficient use is a major issue for the aquaculture industry (Leduc et al., 2018; Merino et al., 2012). Consequently, the continuity and growth of aquaculture will require to act in a sustainable way and to develop new highly functional and nutritive sources efficient to replace FM. There is a recent trend toward diets-containing vegetable protein and oil sources instead of the traditional use of marine-harvested resources. Proteins obtained from canola, pea, or soy are good examples of appropriate ingredients to substitute animal protein in the formulation of feeds. On the other hand, protein hydrolysates generated from fish farming by-products are also cataloged as likely candidates to replace FM in aquaculture feeds, without damaging animal metabolism and performances (Leduc et al., 2018). However, the nutritional and functional properties of the protein hydrolysates could be highly dependent on the methodology utilized for their manufacture (Leduc et al., 2018).

Although the substitution of animal protein in the formulation of feeds in aquaculture can be a suitable strategy to decrease existing consequences such as the over-exploitation on the water-origin food source, the feed efficiency, growth rates, and body composition are compromised. Nevertheless, proteomics is contributing to a better knowledge and understanding of the metabolic pathways influenced by such dietary modifications (Almeida et al., 2015). Furthermore, a number of substances derived from natural by-products can be cataloged as antinutrients because they can reduce nutritional or functional properties of feed for the aquatic creatures. For instance, saponins, phytosterols, tannins, phytic acid and protease inhibitors of protein origin could be found in such by-products. Protease inhibitors have been classified as the most important antinutritional agents due to their negative affect to protein digestion and the amino acids assimilation (Azevedo, Amaral, Ferreira, Espósito, & Bezerra, 2018). Alam et al. (2018) evaluated the effects of substituting FM protein at different levels by low-gossypol cottonseed meal protein from genetically-improved (glandless) and genetically-modified (GMO) plants on feed utilization, growth performance, body composition and dietary protein digestibility of southern flounder. And Zhou, Ringø, Olsen, and Song (2018) reviewed the effects of soybean products on the immunity and microbial ecology of the gastrointestinal tract of aquatic animals, concluding that the appropriate mixture of plant-based substances can limit harm as well as provide an interesting option to enhance GI immunity and disease resistance.

Meanwhile, fish protein is expected to play an indispensable role in China's food security. There are diverse farmed species, and a great amount of "trash fish" is directly used as feed, or is manufactured to obtain FM for fish feed. Trash fish is considered as the small fish with low-value in the commercial catches that in China is specially composed for small benthic and mesopelagic fish, crustaceans and cephalopods. A promising alternative that has been suggested to tackle the problem of sourcing safe and sustainable feed consist of valorizing the locally available food waste as protein source to produce fish feed (Mo, Man, & Wong, 2018).

On the other hand, food products from the sea and aquaculture are quite perishable being much more spoiled than other food groups. Among the factors that promote alterations related with the product quality, causing rejection by consumers and therefore, important economic losses, are: the high moisture level, the protein profile, the presence of many nitrogen-containing compounds with a low molecular weight, the high content in ω3-PUFA in fatty fish, and the presence of several bacterial groups. These modifications happen even at low temperatures, namely, refrigeration conditions. Oxidative and hydrolytic reactions during manufacturing and storage are the leading cause of quality deterioration in protein rich animal products (Domínguez, Barba, Gómez, et al., 2018). They originate hydroperoxides (Lorenzo et al., 2018), free fatty acids and rancidity (Afonso et al., 2016) as well as protein hydrolysis and oxidation.

Fish raised in captivity are sensitive to a wide range of viral, bacterial, fungal and parasitic infections. These losses currently involve a prominent impact on the volume and quality of the fish produced in Europe and around the world (Adams & Thompson, 2006; Hill, 2005). An effective and lasting health management program must consider different elements included in the aquaculture activity. In this context, biotechnology has meaningful uses in connection with fish health and many novel technologies that are now available to help solve these issues. For instance, luminex technology offers the opportunity for both pathogen detection and vaccine development, and molecular technologies, such as the polymerase chain reaction (PCR), real time PCR and nucleic acid sequence-based amplification (NASBA), have enabled detection, identification and quantification of very low levels of aquatic pathogens. Meanwhile, microarray applications supply a new way to multiplex screening for pathogens and host response (Adams & Thompson, 2006).

High market demand for protein foods requires various analytical tools to evaluate the food quality (Domínguez, Barba, Centeno, et al., 2018). Additionally, the texture of the fillet of fish is an overriding aspect affecting the eating quality. This factor includes juiciness, firmness flakiness, fibrousness and oiliness (Almeida et al., 2015). Jessen, Wulff, Mikkelsen, Hyldig, and Nielsen (2012) identifying diverse proteins in rainbow trout muscle linked to textural properties.

3. Insects nutritional composition

At present, insects are a fundamental alternative of protein sources in different places of South East Asia, Central and Western Africa, and Central and South America. However, Western consumers' intention to introduce insect-based food and/or insect-derived proteins as part of their diet is very low, and is perceived with skepticism and disgust (Vanhonacker, Van Loo, Gellynck, & Verbeke, 2013). Barsics et al. (2017) reported that for several populations throughout the world, insect intake is already an usual or even traditional habit. However, in Western societies, insects are habitually regarded as inedible and disgusting, except in desperate situations.

Insects can contribute in the human diet not only with protein and important amino acids, but also with fatty acids. Furthermore, as mentioned previously, they are efficient to be reared and the wastes from their rearing are suitable to be used as organic fertilizer, like a close circle, within the concept of biorefinery (Verbeke et al., 2015). In addition to the possibility of being cultivated by reusing organic wastes, they need six times less feed than cattle. Other reasons to consider are that insects have high feed conversion rate, low intake of water and energy, and are a good source of essential protein for animal feed (Nugroho & Nur, 2018). In fact, insects are already being used as feed in aquaculture and poultry. Particularly, about 1900 insect species seem to be an interesting edible resource with health benefits that are consumed around the world. These protein-rich insects are an appropriate election instead of classic protein sources, reducing feed costs and environmental pollution (Borrelli et al., 2017). Some relevant points to take into account in case of leading an insect production farm are shown in Fig. 3.

In accordance with FAO (2006), among the benefits stemming from incorporating insects in the human diet, there are two distinct points. On one side, many edible insects present an ideal nutritional profile, which brings multiple health benefits. For instance, the oils extracted from different insects contain unsaturated fatty acids in higher amounts than meat, and

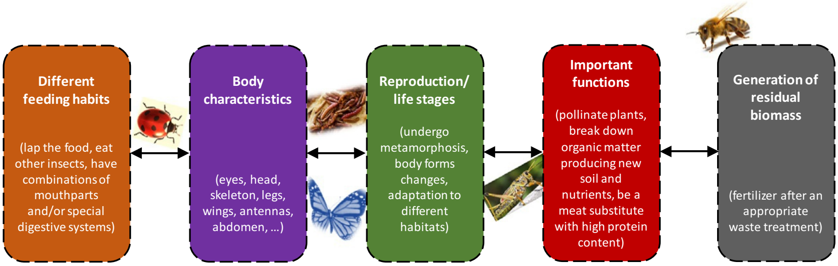

Fig. 3 Relevant aspects for considering in an insect production scenario.

usually include the well-known omega 3, that are globally recognized for their healthy properties (DeFoliart, Dunkel, & Gracer, 2009). On the other side, there are remarkable environmental and social benefits, as to water consumption, greenhouse gas emissions, waste reduction, animal welfare, feed conversion efficiency, and prevention of the risk of suffering from infections (Van Huis et al., 2013).

In addition, most insects include a significant and suitable proportion of minerals, trace elements and vitamins. Finke (2004) collected the mineral content of 32 species and observed the following ranges, provided in g/kg dry matter for Mg: 0.3–27.4; Ca: 0.4–24.8; P: 1.2–14.3; and provided in mg/kg dry matter for Se: 0.3–400; Mn: 3–39; Cu: 9–265; Zn: 21–390. It was reported that most insects present higher levels of phosphorus compared with calcium. In fact, they seem to be appropriate sources for iron, zinc, copper, manganese and selenium, but not for calcium (EFSA, 2015). The iron content is similar or even higher than the iron amounts found in beef, and the relevant concentration of zinc is a positive feature since its lack is a noticeable health problem, especially for certain groups like children and the pregnant women. In the same way, other indispensable compounds for the correct activity of metabolic processes and immune system are vitamins, which have been detected in most edible insects in quantities even higher than in meat (Verneau et al., 2016).

Among the insects that could be an interesting option as a nutritional source for both animals and humans, *Hermetia illucens* has significant abundance of proteins and chitin, representing a promising diet replacement in the case of laying hens. Because there is scarce information about the impact of an insect- rich diet on the gut microbiota and the metabolites generation, Borrelli et al. (2017) evaluated the effects of *H. illucens* larvae meal supply on cecal microbiota and short chain fatty acids production in laying hens. Meanwhile, Oonincx and De Boer (2012) indicated that the manufacture of mealworms leads to a lower climate change value and needs less water and surface than the meat production.

Makkar et al. (2014) discussed the nutritional relevance of house fly maggots, black soldier fly larvae, locusts-grasshoppers-crickets, silkworm meal and mealworm and their use as an alternative of FM and soymeal in the eating habits of pigs, poultry, ruminants and fish species. The amount of crude protein (CP) in these potential resources are high (Table 3): 42–63% and so are the lipid contents (up to 36% oil) (Bußler et al., 2016; Churchward-Venne et al., 2017; Ghosh et al., 2017; Hall et al., 2017; Yang et al., 2014; Yi et al., 2013). However, CP extracts have limitations for functional food applications in terms of color, taste, and weak gel forming ability, hence purification of proteins from CP extract could be used to enhance the physical and sensory properties of functional foods. Another limitation is that nutritional composition of insects can significantly vary among species and within the same insect species even with sex (Kulma et al., 2019).

Insects are rich in essential amino acids, especially in those amino acids which are limiting like lysine, methionine and leucine usually from vegetable protein sources. Insects proteins contain high proportions of all essential amino acids, as can be observed in Table 4. Although insects' proteins profiles are rich in essential amino acids, they are still below fishmeal (Sánchez-Muros, Barroso, & Manzano-Agugliaro, 2014). The protein of black soldier fly larvae is particularly rich in lysine (6–8% of the CP). Black soldier larvae meal resulted to be a valuable ingredient in growing pig diets, especially due to its good contents in amino acids, calcium and lipids. Nevertheless, its relative deficiency in methionine+ cystine and threonine demands for the addition of those amino acids to obtain balanced diets. In addition, several assays have proven that black soldier fly larvae could substitute partially or fully the FM as feed of aquatic organisms. The CP content of housefly maggots varies between 40% and 60%, while older larvae were found to content less CP and more lipids. Housefly maggots, as in the case of black soldier larvae, contain enough lysine (from 5 to 8.2g/100g CP). Live maggots can be a promising ingredient to the diet of rural chickens. In the same way, poultry farms are likely consumers of housefly maggot meal, and this meal has been incorporated in broiler diets as a replacement for traditional protein raw materials, notably FM. On the other hand, high amounts of CP (47–60%) and fat (31–43%) were detected in mealworms, and like other insects, they were characterized by a low Ca content and a very low Ca:P ratio. Therefore, an exclusive feeding of mealworms could promote Ca deficiency and symptomatic metabolic bone disease.

Table 3 Protein content of several edible insects and edible insects based-products.

Protein source	Analysis	Crude protein (%)	Reference
Tenebrio molitor (larvae)	Dumas (Thermo Quest NA 2100 Nitrogen and Protein Analyser, Interscience, Breda, the Netherlands)	19.1 ± 1.3	Yi et al. (2013)
Zophobas morio (larvae)		20.6 ± 0.1	
Alphitobius diaperinus (larvae)		20.7 ± 0.3	
Acheta domesticus (adult)		21.5 ± 0.5	
Blaptica dubia (adult)		19.3 ± 0.9	
Holotrichia parallela Motschulsky (adult)	Kjeldahl method (984.13)	70.57 ± 0.10	Yang et al. (2014)
Gryllodes sigillatus Control sample	Standard AOAC method 984.13 (A-D)	56.8 ± 0.01	Hall, Jones, O'Haire, and Liceaga (2017)
Hydrolyzed with alcalase	–	65.7 ± 0.01	
Acheta domesticus (Cricket protein powder 2050)		65.0	Churchward-Venne, Pinckaers, van Loon, and van Loon (2017)
Acheta domesticus (Cricket flour)		57.5	
Bombyx mori (Silkworm flour)		53.8	
Gryllus bimaculatus (Cricket flour)		59.4	
Locusta migratoria (Locust flour)		69.4	
Tenebrio molitor (Mealworm protein powder 2050)		58.1	
Alphitobius diaperinus (EntoPure sports protein concentrate)		70.0	
Allomyrina dichotoma (larvae)	Amino Acid analyzer S433 (Sykam GmbH, Germany) following the standard method of AOAC (1990)	48.74	Ghosh, Lee, Jung, and Meyer-Rochow (2017)
Protaetia brevitarsis (larvae)		39.16	
Tenebrio molitor (larvae)		44.50	
Teleogryllus emma (adult)		49.95	
Gryllus bimaculatus (adult)		53.83	
		g/g d.m.	

Continued

Table 3 Protein content of several edible insects and edible insects based-products.—cont'd

Protein source	Analysis	Crude protein (%)	Reference
Hermetica illucens (larvae)	Aqueous extraction (60 °C for 30 min) followed by a removal of fat by a two-step extraction with hexane. Kjeldahl method (KjeldathermTurbosog, Titrino plus 48, Gerhardt Analytical Systems, Königswinter, Germany), according to DIN EN 25663 and as described by the Association of German Agricultural Investigation and Research Institutions	64.6 ± 0.3	Bußler, Rumpold, Jander, Rawel, and Schlüter (2016)
Hermetica illucens (insect fluor)		57.8 ± 1.2	
Hermetica illucens (defatted insect fluor)		64.6 ± 0.3	
Tenebrio molitor (larvae)		31.7 ± 0.5	
Tenebrio molitor (insect fluor)		34.7 ± 0.2	
Tenebrio molitor (defatted insect fluor)		44.9 ± 1.4	

Similarly, based on an investigation about the nutritive interest of eight insect species usually eaten in Manipur (India), Gope and Prasad (1983) reported that insects are the cheapest source of animal protein in that area and their intake should be encouraged because many poor people cannot afford fish or other meat. Meanwhile, Ramos-Elorduy and Pino (1990) determined the energy values of 94 of different edible insect species, finding that 50% of the analyzed species showed a higher caloric value than soybeans; 63% were higher than beef; 87% were superior to maize, and 70% were better than fish, beans and lentils. In fact, only nine of the analyzed species showed less than 30% protein. More recently, DeFoliart (2002) has summarized the gist of the research published until that time on the nutritive benefits of insects. This review and subsequent works (Banjo, Lawal, & Songonuga, 2006; Cerritos & Cano-Santana, 2008; DeFoliart et al., 2009) concluded that the content of fat and proteins in the insect species is frequently higher than in common sources of protein such as dairy products, meat and a variety of seeds. In addition to the significant amount of proteins found in insects, some studies have made obvious their high quality in many species. From a nutritional point of view, the phrase "a protein of high quality" means that it includes different types of essential amino acids in

Table 4 Essential amino acids content of several edible insects.

Protein source	Units	His	Ile	Leu	Lys	Met +Cys	Thr	Tryp	Val	Reference
Tenebrio molitor (mealworm)	mg/g crude protein	29	43	73	54	26	39	12	61	Payne, Scarborough, Rayner, and Nonaka (2016)
Zophobas morio (larvae)	mg/g crude protein	31	46	71	54	24	40	14	63	Payne, Scarborough, Rayner, and Nonaka (2016)
Alphitobius diaperinus (larvae)	mg/g crude protein	34	43	66	61	26	39	12	58	Payne, Scarborough, Rayner, and Nonaka (2016)
Acheta domesticus (adult)	mg/g crude protein	21	36	66	53	25	35	9	55	Payne, Scarborough, Rayner, and Nonaka (2016)
Blaptica dubia (adult)	mg/g crude protein	23	31	56	43	23	32	8	52	Payne, Scarborough, Rayner, and Nonaka (2016)
Acheta domesticus (house cricket)	mg/g crude protein	16	28	48	36	11	27	–	46	Kulma et al. (2019)
Hermetica illucens (larvae)	mg/g	15	12	37	29	115	22	1	22	Al-Qazzaz, Ismail, Akit, and Idris (2016)
Samia ricinii (adult)	mg/g dry weight	27	44	66	65	28	48	–	54	Longvah, Mangthya, and Ramulu (2011)
Pachilis gigas (adult)	mg/g dry weight	70	42	69	45	60	36	6	62	Ramos-Elorduy et al. (1997)
Boopedon flaviventris (adult)	mg/g dry weight	24	47	88	55	38	44	6	57	Ramos-Elorduy et al. (1997)
Scyphophorus acupunctatus (adult)	mg/g dry weight	15	48	78	55	42	40	8	62	Ramos-Elorduy et al. (1997)

suitable proportions besides being highly digestible by the individuals that consume it. Vitamins and minerals can be also found in insect-based foods in great amounts (Premalatha, Abbasi, Abbasi, & Abbasi, 2011).

Regarding the disposition and acceptance of citizens and especially of people directly related to the field of agriculture and food industry toward the introduction of insects in animal feed, Verbeke et al. (2015) found that they are frequently favorable, especially in case of fish and poultry. Foods derived from animals fed on insect-based feed were considered as more sustainable, healthier and with a better nutritional value. By contrast, the presence of both allergens and off-flavors was associated with the resulting foods. On the other hand, there are several current studies showing that the habit of eating insect-based foods is determined by a variety of personal interests and attitudes, such as food neophobia or fear to try new foods, concern about the environmental impact of personal food choices, and being open mind to change the usual diet, as well as cultural exposure, familiarity or past experience and knowledge.

The results of the study carried out by Hartmann, Ruby, Schmidt, and Siegrist (2018) revealed that consumers of vegetarian and insect products were considered as more environmentally friendly, health-conscious, brave, interesting, imaginative and knowledgeable than habitually consumers of meat. Actually, meat option was perceived as unhealthier than the vegetarian and insect alternatives. Given the good image of people who is in favor of consuming alternatives to traditional meat proteins, their social influence may be high and important to expand such products.

4. Extraction methods to recover proteins

The development and application of suitable technologies in handling, processing and storage of insects subsequent to harvesting are a crucial challenge to face in the introduction of insects in animal feed. In this sense, the current technologies and systems used for the animal feed industry should be reviewed and adapted to this new ingredient, preserving the product quality and efficiency, at the same time that there are no losses in terms of guaranteeing hazard identification, risk assessment and traceability.

da Rosa Zavareze et al. (2014) performed a study in which the Whitemouth croaker muscle and its industrialization by-product were hydrolyzed by using microbial protease (Flavourzyme 1000L) under the following reaction conditions: 2 g/100 g enzyme/substrate, pH 7, and

120 min of reaction at 50 °C. The aim was to encapsulate the obtained protein hydrolysates and to produce healthy capsules with antioxidant activity.

There are a number of methods adequate to fractionate insects and this approach is increasingly usual because the insect production industry is growing. Nevertheless, accurate details of the processes employed are complicated to know, as these (combined with insect rearing practices) are intellectual property of the manufacturing companies. Both solvent extraction and mechanical separation are utilized in order to produce fractionated insect products. For instance, it is possible the mechanical separation of large insoluble chitin particles from ground insects by water or steam extraction technologies. Similarly, the separation of protein from fats/oils can be performed with organic solvent extraction using, for example, hexane. Other advanced technologies such as microwave assisted accelerated solvent extraction or super critical fluid extraction using CO_2 are emerging and practicable technologies for the large-scale production of fat and protein isolates. The improvement of solvent-free protein/fat extraction methodologies appears to be the main purpose of the industry (EFSA, 2015).

In case of current and future advances for aquaculture animals, it is important to bear in mind the amount and the heterogeneity of starting material. Total or selective protein extraction and purification should not induce protein modification, and endogenous enzymatic activities need to be avoided by controlling temperature or adding inhibitors. Classic biochemical techniques in combination with protein precipitation are considerably employed for sample preparation before proteomics approaches (Almeida et al., 2015). More specifically, both protein hydrolysates and bioactive peptides can be obtained from marine sources by solvent extraction, enzymatic hydrolysis or microbial fermentation. In the food and pharmaceutical industries, enzymatic hydrolysis is preferred to other methods since less residual solvents and toxic products are used (Anal et al., 2013). In this sense, there are novel extraction methods used in some industries with the aim of reducing traditional solvents and processing-time: ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), or supercritical-fluid extraction (SFE).

5. Analysis of the extracts

Proteomics are among the more widely applied techniques in fish nutrition for determining the biological effects caused by specific nutritional elements or dietary modifications. The addition of alternative compounds in fish feed formulations (such as vegetable oils, plant proteins, and manufactured animal proteins) is becoming a significant issue in aquaculture industry, and so several proteomic investigations focus on this topic.

Technologies like MALDI-TOF-MS, 2-DE and PCR have played a relevant role in the field of fish authentication. Among the proteins that appear to be affected by the use of vegetable substances instead of FM include fatty acid-binding proteins, apolipoproteins, nitric oxide synthase, heat shock proteins, homogentisate 1,2-dioxygenase, and methionine/homocysteine metabolism proteins (Rodrigues et al., 2018).

The study of the presence of proteins in a particular tissue or fluid (the proteome) can be carried out by proteomics. It must be considered that this type of procedures is of great importance to different scientific areas, including veterinary and animal disciplines. However, proteomics has limitations in these specific sciences because of numerous reasons such as lack of good genomic information from several species of interest, cost and a lack of knowledge of the possibilities of this technology. In this context, proteomics approaches have been contributing with the development of new disease diagnostics and vaccines. Additionally, it is a highly rewarding tool in estimating fish welfare. As a rule, the liver is the principal target organ to explore because it can provide data about the metabolic status, as well as body fluids like blood plasma (Almeida et al., 2015).

Regarding the ever-changing feed formulations, it is indispensable to know the impact of dietary micronutrient levels on fish health and metabolism. With this in mind, some studies of the dietary effects of micronutrient administration such as vitamin K and phosphorus by using proteomic techniques have been performed (Richard et al., 2014; Ye et al., 2016). This accentuates the ability of proteomic approaches to face a variety of subjects as general instruments in the study of fish nutrition, even to detect potential effects at the level of cellular stress or metabolism (Rodrigues et al., 2018).

The growing concern of society about the impact of the consumed ingredients on health has led to variations in their food routine, demanding high-quality products, safe and nutritious. In this sense, the current valuable omics methodologies are forming part of an important advance on modern food science. As a matter of fact, a new discipline, called Food omics, had been described, for example, in the specific question of fishery products. According to Carrera, Cañas, and Gallardo (2013), the applications of the proteomics techniques for the evaluation of both safety and quality of fishery can be classified in four major topics, namely, (i) fish authentication;

(ii) allergen identification; (iii) changes of fish quality during processing and storage; and (iv) control of spoilage and/or pathogen microorganisms.

Metabolomics constitutes a very powerful tool to examine the dietary performance. For instance, the use of a protein-rich zygomycetes fungus (*Rhizopus oryzae*) was checked as replacement for the habitual FM protein in Arctic charr (*Salvelinus alpinus*) diets (Abro, Moazzami, Lindberg, & Lundh, 2014). Specifically, they carried out metabolite profiles of fish fed with three different diets: one of them consisting in mostly FM protein; a second one with an unknown composition; and finally, another containing mostly zygomycetes protein. The study of metabolite profiles from liver samples showed that there were no differences between diets including FM protein or zygomycetes protein, suggesting similar physiological responses. Nevertheless, the commercial diet did exert significant metabolite differences in comparison with fish fed each of the other two protein-based diets. The use of both nuclear magnetic resonance (^{1}H NMR) and statistical analyses, including orthogonal projection to latent squares discriminate analysis (OPLS-DA), demonstrated to be a potent procedure to determine the similarities and differences among the metabolite profiles to assay the effects caused by a particular diet.

Technological advance and innovation are necessary points that will be required to improve feed formulation and nutrition, food quality and safety, reproduction and conditioning, immunology and disease diagnostics, cultivation systems performance and larval rearing. Meanwhile, Alfaro and Young (2018) reported that recent biotechnological development has derived in advances in all of these disciplines. For instance, molecular technologies, such as PCR and nucleic acid sequence-based amplification (NASBA), have been essential to detect, identify and quantify extremely low amounts of pathogens affecting aquatic animals. While microarray procedures allow a new dimension to multiplex screening for pathogens and host response (Adams & Thompson, 2006). A major drawback is the lack of information relating to aquaculture in the databases. Besides, although 2D electrophoresis is a modern gold standard for identifying changes in the expression of proteins, it presents reproducibility issues and other handicaps such as being a time-consuming and expensive procedure. Even in combination with mass spectrometry, only the proteins that are in greater quantity can be detected, thus highlighting the suitability for boosting advances in new technologies (Adams & Thompson, 2006).

Despite the evaluation of zootechnical performances of original formulations remains necessary, the developments of modern tools and procedures for biochemical and molecular experiments can help to better understand animals' responses to novel diets. Leduc et al. (2018) combined zootechnical and transcriptomic approaches to test the yield and effects of protein hydrolysates of different origins (shrimp, tilapia, and a combination of the two) as substitutes of traditional FM in European seabass. The transcriptomic response of the intestine revealed that genes and metabolic pathways were modified in a hydrolysate-dependent way, the combined hydrolysates regulated more genes and metabolic pathways than by testing each hydrolysate independently. Authors concluded that the assayed protein hydrolysates are potential candidates to help the replacement of FM in aquaculture feeds.

Regarding insects, taking into account the existing variety in this group, not many studies were found in which the methods of analysis were specified. For instance, Finke (2002) reported a complete nutritional analysis of different insect species commercially raised as food for animals, but the author did not detail the methodology followed for each determination ("materials were shipped to a commercial analytical laboratory for nutrient analysis"). More recently, the nutritive value of grasshopper was investigated after being pre-treated by placing it into boiled water for 5–10 min, sun-dried, milled and sieved using a 595 mm sieve. Then, the homogenous product was subjected to several chemical analyses, among them the total nitrogen (micro-Kjeldahl procedure), the crude protein (estimated as N × 6.25), and the essential amino acids profile determined after hydrolyzing the sample with 6 N HCl for 22 h at 110 °C (Alegbeleye, Obasa, Olude, Otubu, & Jimoh, 2012). Similarly, most of the amino acids from maggots were measured after an acidic hydrolysis using 6 N HCl for 24 h at 110 °C (Hwangbo et al., 2009).

To determine the amino acids profile of these maggots, authors used an automatic amino acid analyzer (Hitachi L-8800, Japan). In the same way, the nutritional value of the larva meal of *Hermetia illuscens* L. was evaluated by Arango Gutiérrez, Vergara Ruiz, and Mejía Vélez (2004) by means of moisture content, protein, fat, fiber, nitrogen-free extract, and total minerals (ash). The methods used for these analyses were those recommended by the Association of Official Analytical Chemists (AOAC, 1995), in the case of the protein 981.10, the same as St-Hilaire et al. (2007) for determining the proximate composition of the flies and other protein feedstuffs used with the aim of finding alternative rainbow trout diets. On the other hand, Hall et al. (2017) determined the protein content with the current standard AOAC method 984.13 using HPLC with a Waters AccQ Tag amino acid analysis column for the free amino acids' composition. Two years later, in a study about the effect of sex on the nutritional value of house cricket (Kulma et al., 2019), the nitrogen content was calculated using the Kjeldahl method ISO 5983-1:2005 and the amino acid profile by acid and oxidative hydrolysis of the samples, followed by their evaluation through an amino acid analyzer (with Na-citrate buffers and ninhydrin detection.

Meanwhile, Pretorius (2011) indicated the variation found in nutritional composition of insects analyzed by different authors and provided information of the analytical methodologies used to determine the composition of common house fly. In particular, the crude protein determination was performed after measuring the total N content according to the official method 4.2.07 (AOAC, 2002) in a LECO FP528 apparatus, and multiplying by a factor of 6.25. The amino acids profile was determined through acid hydrolysis of the samples and using HPLC with a fluorescence detector.

6. Purification and fractionation stages

Purification stages are normally required to obtain an adequate product to be used as food ingredient. Before their use in formulated diets, all types of waste materials should be properly processed. The quality of raw food waste could be the most critical factor at the time of preparing food waste-origin feeds, due to the difficulty of removing chemical contaminants, as the ones migrated from packing materials (Mo et al., 2018). For this purpose, membrane separation (already used in a number of fields such as the beverage, food, biotech, or pharmaceutical industries) can be an excellent methodology. This technology employs a gradient, either pressure or electrostatic, to force the passage of certain components of a solution through a semipermeable porous membrane. Consequently, separation is obtained based on the size and/or the molecular charge of the chemical species (Chacón-Villalobos, 2006). There is a great diversity of membranes, both in configurations and in materials and pore sizes. In relation to the material of manufacture (Vaillant et al., 2005), membranes can be made of acetate cellulose (e.g., cellulose), organic polymers (such as polysulfones, teflon, propylenes, polyamides, polysulfides, polypropylenes), or composed of inorganic materials (e.g., ceramics).

Besides being a simple technology, the use of membranes offers numerous advantages. These include energy efficiency, operation without harmful organic solvents, possibility of modifying the operating conditions, such as pressure, temperature, agitation or feed flow. Additionally, they do not require excessive space for the use, while at the same time upscaling is relatively easy (Michelon, Manera, Carvalho, & Maugeri Filho, 2014; Pinelo, Jonsson, & Meyer, 2009). The main drawback in the use of a membrane bioreactor is the continuous decrease of permeate flux due to the progressive fouling, and Van der Bruggen, Mänttäri, and Nyström (2008) identified other challenges for nanofiltration such as chemical resistance and limited lifetime of membranes.

On the other hand, ion-exchange processing with a variety of commercial cation- and anion-exchange resins has also been described to reduce the concentration of undesirable compounds from a heterogeneous mixture (Buruiana, Gómez, Vizireanu, & Garrote, 2017). In the food industry, ion exchange is used to demineralize sugary liquids and syrups; to control acidity, smell, color and taste; as well as, to isolate or purify an additive or a food component. In addition, on several occasions, it is advisable to remove certain parts of the insects, such as the legs and wings of crickets, with the aim of improving the taste and texture (experimenting a better eating moment), and reduce choking risks (EFSA, 2015).

Finally, after the purification stages the use of certain fractionation techniques permits obtaining defined fractions that would even facilitate the creation of final products with specific functional characteristics. Membranes, in addition to their applications in purification and concentration, are also used to fraction. As potential fractionation techniques could be cited the size exclusion chromatography and the high-performance anion exchange chromatography coupled to an amperometry pulse detector (HPAEC-PAD).

7. Development of new products based on insect proteins and aquaculture products

There are two different ways to prepare insects, in pastes by milling when frozen or without treatment, or in ground powders by milling after drying. Then, these products are adequate to be used as ingredients in both feed and food (EFSA, 2015).

Megido et al. (2016) prepared four different burger patties containing three principal ingredients: green lentils, unflavored ground beef, and mealworms. In the mealworm/beef burger a proportion of 50% insects and 45% ground beef was used. The remaining 5% of each burger consisted of a mixture of common ingredients that enrich the flavor and aroma (salt, onions, garlic, carrots, tomato and pepper). Insects were fasted for

24 h before they were harvested by freezing, to ensure that they have excreted all feces. This protocol resulted in a reduction of the bacteria present in the insect gut, thus offering a safer product suitable for human consumption. Authors observed a decrease in the insect food neophobia, since participants marked the burgers' appearance and taste with higher scores than neutral ones, situating them between a fully meat burger and a fully vegetable burger.

Meanwhile, in relation to aquaculture products, da Rosa Zavareze et al. (2014) investigated the production and characterization of encapsulated antioxidant protein hydrolysates from Whitemouth croaker muscle and by-product. Their results showed high encapsulation efficiency, reaching values around 80%, and the evaluation of the antioxidant activity of the capsules indicated an activity that compares favorably with that of α-tocopherol. On the other hand, several authors focused their research in replacing FM by other renewable and efficient sources in fish diets. In this sense, Zhou et al. (2018) reviewed the effects of soybean meal on finfish species and crustaceans. They highlighted the negative nutritional balances produced by the consumption of plant based-diets, which lack some nutrients and contain harmful compounds able to damage gut microbiota, intestinal mucosa or immune system. The ideal would be to use a correct proportion of different additives. According with this, Gajardo et al. (2017) investigated the impact of alternative dietary protein sources in the distal intestine of Atlantic salmon finding important differences between the intestinal digesta and mucosa in the presence and abundance of bacteria. In the same way, poultry by-product meal protein demonstrated to be a promising substitute of FM in the diet of juvenile Black Sea Bass (Dawson et al., 2018) and experimental feeds containing a variety of levels of n-3 fatty acids from either fish oil or algae meal contributed to improve the lipid profile in tilapia fillets (Stoneham et al., 2018). In addition, this last study also suggested that tilapia fed omega-3 enriched diets could result in new value-added by-products, by using n-3 enriched rib meat, liver and mesenteric fat tissues in future processed foods.

Finally, development of new technologies for the extraction, purification and characterization of valuable functional compounds is currently considered for wastes from marine-processing materials and can be a good strategy to increase the viability of the fishing industry. So, extensive information on extraction and characterization of bioactive compounds of interest, especially on protein hydrolysates and bioactive peptides from seafood and crustacean waste, have been reported (Anal et al., 2013). In fact, recent works have demonstrated the viability of fish by-products as a source of peptides with relevant bioactivity like antioxidant and ACE-inhibitory peptides in sardinelle hydrolysates fermented by *Bacillus subtilis A26* and *Bacillus amyloliquefaciens An6* (Jemil, Mora, Nasri, et al., 2016), such hydrolysates demonstrating hypolipidemic, antiobesity and cardioprotective effects (Jemil et al., 2017), and also antidiabetic and anti-hyperlipidemic effect of proteins hydrolysates from *Octopus vulgaris* (Ben Slama-Ben Salem et al., 2018), and antimicrobial activity in protein hydrolysate from zebra blenny (*Salaria basilisca*) obtained by fermentation with *Bacillus mojavensis A21* (Jemil, Mora, Abdelhedi, et al., 2016).

8. Challenges and future perspectives of aquaculture products as protein sources

Aquaculture industry has been meeting and still has great challenges, such as profitability, product quality, and environmental sustainability (Sun et al., 2016). Like other livestock production practices, a variety of chemical substances, which types and amounts are established in Official Regulations, may be used in aquaculture to fight against pathogens and optimize performance and welfare of the stock. The principles of sustainable intensification (SI) indicate that any chemicals should be minimally toxic in order to have insignificant environmental consequences and no harm human health (Little et al., 2018).

A worry regarding SI is that food obtained from industrialized structures has worse nutritional characteristics. Specifically, a balanced micronutrient profile is being sacrificed for obtaining higher yields. Little et al. (2018) advised that SI cannot be tested only on the farm, since a thorough value chain perspective is needed, preferably integrated by Life Cycle Assessment (LCA) and other procedures to evaluate overall social and environmental consequences. Negative effects on land and water resources are boosting expansion and integration of aquaculture with other uses. The great challenge of satisfying food and energy needs in the next decades will force formation of ever more efficient mechanisms.

The capacity of aquaculture and marine fisheries to supply food for the coming population will depend, in part, on the performance of fisheries management and on the ability to reduce the environmental impact of the aquaculture. It is expected that future options to feeds obtained from wild fish will decrease not only the current price, but also the strong pressure that is being caused on marine stocks (Merino et al., 2012).

This fact is important because abundance of wild fish stocks is expected to reduce further in coming decades with the stress exerted by climate change and habitat alteration (Oken et al., 2012). Nowadays, fish from aquaculture accounts for 23% of the total fish consumed. Only one-third of the production of farmed fish is employed directly for human intake, using the remaining fish as meal in other farming activities. The global maximum sustainable fishing yield has been already exceeded by a factor of 3–4 (Pauly et al., 2002), and future requirements will probably be even more devastating. By 2050, it will be required to increase fish production in an estimated 50% to face the basic protein needs of a demanding and rapidly growing population (Rice & Garcia, 2011). Hence, more economical and easily available different protein sources are indispensable.

In the last few years, new interesting advances have been reached on this direction. In fact, it is already possible to find some commercial fish feeds with even less than 10% of expensive FM, which is replaced by oilseed meals (canola, soybean, and sunflower), legumes (bean, lupine, and peas), or grains (wheat and corn). However, numerous nutritional problems are related to the utilization of plant substances, since these ingredients can present unbalanced amino acid profile, as well as anti-nutritional factors (phosphorous-rich phytic acid, saponins, fibers or protease inhibitors). Meanwhile, animal proteins are rich in most essential amino acids and include great amounts of water-soluble proteins, which present the advantage of being highly digestible. In this context, since June 2013, the FM can be partially replaced by a variety of mixtures of non-ruminant animal proteins, being one of the most favorable and attractive strategies to produce fish feed formulations, the poultry by-product meal (Rimoldi, Terova, Ascione, Giannico, & Brambilla, 2018).

Nevertheless, the inclusion of different ingredients in the food routine must be neatly evaluated, since gut microbiota is readily affected by changes in the diet. This complex bacterial community is associated with the host metabolism, growth, and disease resistance. For instance, microbiota contributes in the synthesis of digestive enzymes, vitamins and important metabolites such as short-chain fatty acids, which represent the major energy source for intestinal epithelial cells. In this sense, Rimoldi et al. (2018) evaluated the impact of partial substitution of traditional FM with a blend of animal by-product meals and plant-derived proteins on gut microbiota composition of rainbow trout. They concluded that good results were obtained in terms of growth yields and without inducing significant variations in intestinal microbial richness. On the other hand, microalgae biomass offers proteins with better quality than rice, vegetables and wheat, but not so valuable as the animal proteins, such as those find in milk or meat (Rizwan, Mujtaba, Memon, Lee, & Rashid, 2018).

Last years, proteomics has been playing a key role as a fundamental technique in the aquaculture sector to achieve high-quality end products. These emerging approaches have been utilized to enhance the global understanding about potential biomarkers for environmental monitoring, risk assessment, including allergens' detection, traceability, and authenticity. An interesting element that can promote an adequate interpretation of proteomic results is the co-measurement of supplementary information, from easy-to-measure zootechnical details (such as body length, fish body weight, condition factor or hepatosomatic index), to other biological data acquired from high-throughput profiling procedures (transcriptomics, metabolomics, chemometrics) (Rodrigues et al., 2018).

9. Challenges and future perspectives of insects as proteins source

Since meat is a heterogeneous and high complex product characterized for a particular and estimated texture and flavor, its imitation is an authentic technological challenge. Several new environmentally friendly sources of protein have been proposed in last years, among which it is worth highlighting the alternative of insects. Despite they seem to be valuable candidates, nowadays there is a lack of knowledge of how their administration can affect the human and animal physiology. Nonetheless, due to the growing worldwide concern about adequate diets including insects both for humans and animals, further investigation is required. In this sense, it is very important to evaluate how the insect-based foods might modulate the intestinal microbiota of the host, and which are the main metabolites produced (Borrelli et al., 2017).

A positive point to value is that plant and insect proteins can be obtained more sustainably than ones from beef and pork, although consumer acceptance is a rather large challenge for the later (Hartmann et al., 2018). Kostecka et al. (2017) found that representatives of Polish consumers did not exhibit open-mindedness toward introducing insect-based foods into their eating habits. The most frequently cited reasons by previous research include irritation that European manifest for insects consumption originated in neophobia and disgust (La Barbera, Verneau, Amato, & Grunert, 2018). Therefore, good communication, educational programs and information provision are advisable to enhance consumers' knowledge about the consequences of food choices on the environment and themselves (Megido et al., 2016).

It would be desirable for insect-based foods to be produced and processed on a large scale by the feed industry, and as a part of regular animal diets. Nowadays, insect rearing is performed at a small scale; therefore, it will be a good idea to create cost-effective and well-optimized mass insect rearing facilities able to utilize specific substrates to manufacture insects or insect meals with a differentiated quality. Moreover, the production of appropriate insect meals susceptible to be used as feed depends on the introduction of safe procedures before the use of bio wastes, as well as the control of the presence of heavy metals, pesticides and the possibility of developing diseases. Namely, there is a need to establish a regulatory framework to guide the incorporation of insects in our diets and to improve risk assessment methodologies (Makkar et al., 2014).

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