DEGREE IN BIOCHEMISTRY AND MOLECULAR BIOLOGY

FINAL DEGREE PROJECT

Valorising by-products from brewery industry as an alternative protein source for sustainable aquaculture by promoting a circular economy

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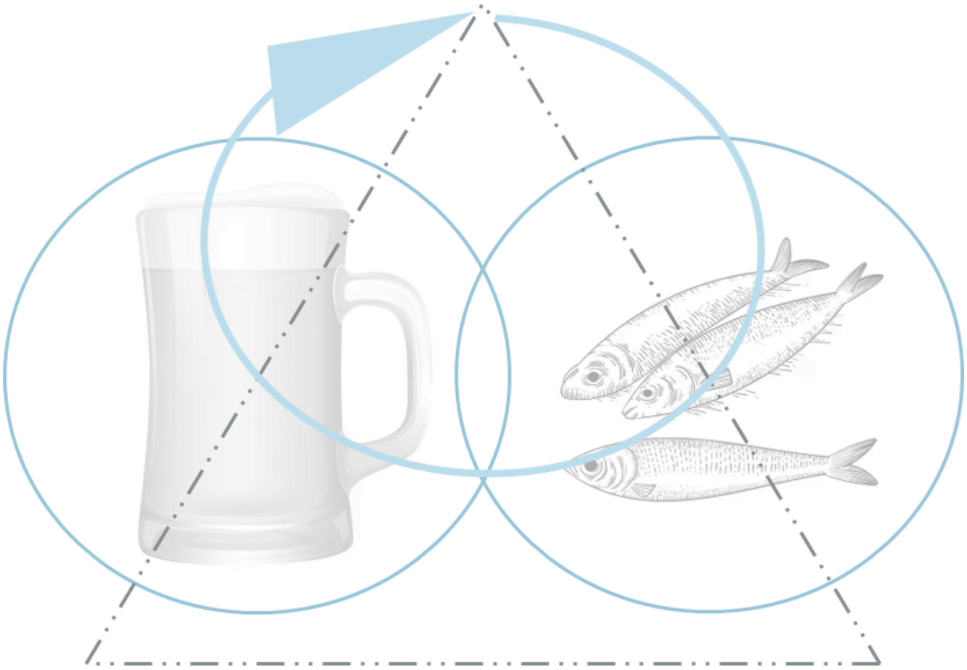

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> Would you improve a nation? Give it, instead of declamations against sin, better food. Man is what he eats.
>
> (Ludwig Feuerbach, Germany philosopher, 1850)

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List of Abbreviations

AA: Amino acid

ADC: Apparent digestibility coefficient

ANF: anti-nutritional factor

BSG: brewer’s spent grain

BSY: brewer’s spent yeast

D-BSG: dried brewer’s spent grain

D-BSY: dried brewer’s spent yeast

EAA: essencial amino acid

FAO: Food and Agriculture Organization

FDR: feed conversion ratio

FM: fish meal

FO: fish oil

H-BSG: hydrolised brewer’s spent grain

H-BSY: hydrolised brewer’s spent yeast

NEAA: non essencial amino acid

RGR: relative growth factor

PER: protein efficiency rati

PR: Experimental protein released concentration

SGR: specific growth factor

UN: United Nations

WOS: Web of Science

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Abstract

The exponential population growth and climate crises make it unsustainable to continue with our current food production methods. United Nations has set a series of goals and targets for 2050 if we want to continue living on this planet as we know it today. In this context, the Food and Agriculture Organization is working towards achieving a "blue transformation" in aquaculture production, making it more sustainable and eco-friendlier. In recent years, various studies have been conducted to find alternative protein sources for fish feed. Achieving nutritional formulations independent of fish meal (FM) and fish oil (FO) is one of the current challenges in aquaculture nutrition. This would help reduce fish catches, promote the conservation of seas and oceans, and promote an efficient use of marine resources. Researchers are studying some kind of protein sources, such as algae, plant oils and meals, insects, Single Cell Proteins, and industrial by-products.

In this review, we have focused on using by-products from the food industry in our region to promote a circular economy with zero kilometre. Spain has many breweries around the territory. It has been observed that brewer’s spent grain (BSG) and brewer's spent yeast (BSY) are by-products with very high protein content. Therefore, the possibility of reducing animal and plant protein levels in fish feed has been studied. We focused on the sea bream as a marine carnivorous specie and the rainbow trout as an omnivorous freshwater specie, both of which have high commercial value in the Mediterranean Sea.

It has been concluded that around 30% of BSY and 15% BSG could be included in sea bream diets without compromising growth rates or protein digestibility coefficients. In contrast, rainbow trout could have a 20% of BSY and no more than 15% BSG without any hydrolysis treatment. Additionally, the use of BSY in feed formulation significantly reduces CO2 emissions.

Key words: Aquaculture, fish oil, fishmeal, protein source, by products, brewer waste, circular economy, gilthead seabream, rainbow trout, Brewers’ Spent Yeast, Brewers’ Spent Grain, apparent digestibility coefficient

Resum

L'augment poblacional exponencial i les crisis climàtiques fan insostenible seguir en una forma d'alimentació com l'actual. La ONU ha marcat un seguit d'objectius i metes per al 2050 si volem seguir vivint en un planeta tal i com el coneixem actualment. Dintre d'aquest context la FAO lluita per aconseguir una transformació blava dintre de la producció aqüícola, més sostenible i ecològica. Durant els darrers anys, s'han realitzat diferents estudis per trobar fonts de proteïna alternativa per a l'alimentació dels peixos. Poder aconseguir formulacions nutricionals independents a FM i FO és un dels reptes actuals dintre de l'àmbit de la nutrició en l'aqüicultura. Aquest fet ajudaria a disminuir les captures pesqueres promovent una conservació dels mars i oceans, així com un ús més eficient dels recursos marins. S'estan estudiant fonts de proteïna molt diverses, com les algues, olis i farines vegetals, insectes, *Single Cell Proteins* o subproductes de la indústria.

En la següent revisió ens hem focalitzat en l'ús de subproductes de la industria alimentaria del nostre territori per tal de fomentar una economia circular de quilòmetre zero. España disposa d'un gran nombre de fàbriques de cervesa repartides al llarg del seu territori. S'ha vist com el bagàs de cervesa (BSG) i el llevat gastat de cervesa (BSY) son subproductes amb un contingut de proteïna molt elevat. Per tant, s'ha estudiat la possibilitat de disminuït els nivell de proteïna animal i vegetal en l'alimentació dels peixos. Ens em centrat en la dorada com a espècie carnívora d'aigua salada i la truita irisada com un omnívor d'aigua dolça, dues espècies d'alt valor comercial al mediterrani.

S'ha conclòs que en les dietes de dorada es podrien incloure fins un 30% de BSY i un 15% de BSG sense comprometre les taxes de creixement ni els coeficients de digestibilitat proteics. Mentre que en la truita irisada es podrien afegir un 20% de BSY i no més d'un 15% de BSG sense ser necessari un tractament d'hidròlisi previ. També s'ha vist com l'ús de BSY en la formulació dels pinsos redueix notablement les emissions de CO2.

Paraules clau: *Aqüicultura; oli de peix; farina de peix; proteïna alternativa; subproductes; residus de cervesa; economia circular; bagàs gastat de cervesa; llevat gastat de cervesa; dorada; truita irisada; coeficient aparent de digestibilitat*

1. Introduction

1.1 The current state of food security

In September 2015, 193 member countries of the United Nations approved what we now know as the 2030 Agenda, which includes 17 Sustainable Development Goals and 169 targets to improve people's lives. It has been a global challenge that affect all areas, the world population and the environment and planet.
All the goals are interconnected, and in many cases, achieving one of them could impact the others. In the following review, we will focus particularly on Goal 2, which is working for "Zero Hunger," and Goal 14, which deals in "Life Below Water."

The pandemic and recent war conflicts explain this bad situation, where the lack of food and primary resources have developed in increasing cost of living, becoming the main obstacle to achieve one of the principal goals of the 2030 Agenda.

On the other hand, the Objective 2 has a very clear goal, which is to achieve a world free of hunger by 2030. However, it has been observed that since 2015, the situation has been drastically worse. Since 2022 (figure 1.1), 9.2% of the population is in a state of chronic hunger, and it is estimated that by 2030, more than 600 million people will be in a situation of food vulnerability.

The pandemic and recent war conflicts explain this bad situation, where the lack of food and primary resources have developed an increasing of the cost of living, becoming the main obstacle to achieve one of the principal goals of the 2030 Agenda (1).

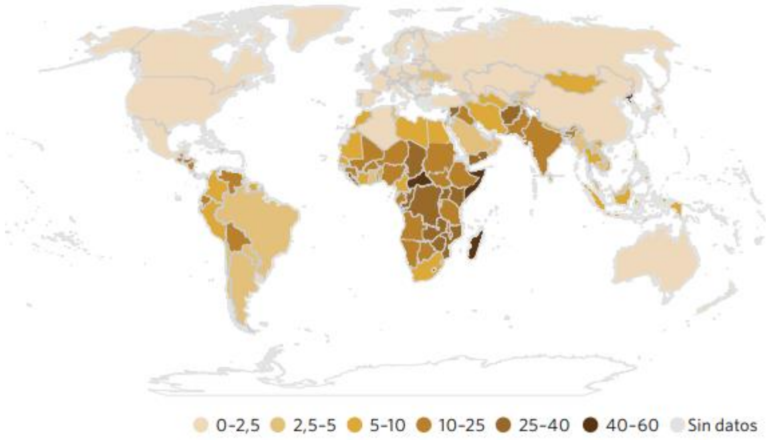
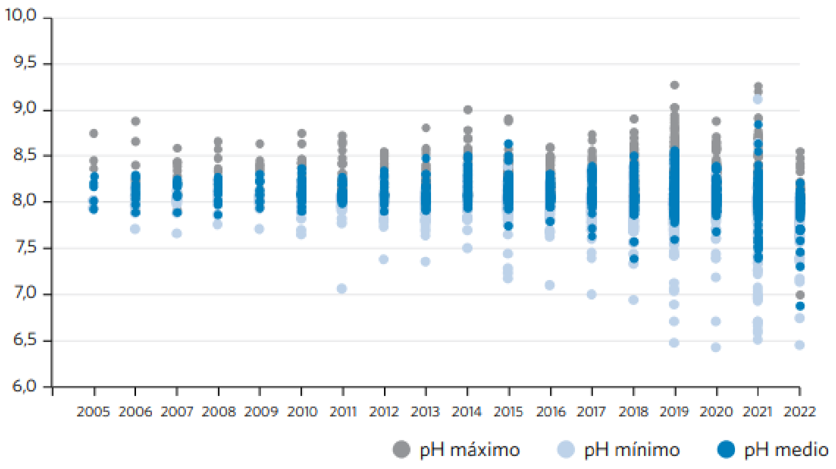


Figure 1.1 Prevalence of undernutrition. Average: 2020-2022 (%) (2).

On the other hand, Goal 14 works for the conservation of seas and oceans and the efficient and sustainable use of marine resources. Three-quarters of the planet are covered by oceans, so it is the biggest ecosystem that exists. Coastal eutrophication caused by the increasing of nutrients and the global ocean acidification (**figure 1.2**) caused by raising of the greenhouse gases are some of the risks to the supervenience of all marine life forms. It is a source of a big number of natural resources, some of which are not discovered. Moreover, it works as the biggest organ for eliminating and decomposing waste. Despite this, levels of marine pollution are out of control putting some emphasis on the presence of plastics. At the level of food security, ocean health is a crucial issue, as it constitutes the main source of protein for developing countries (1, 2).

Figure 1.2 Surface pH values based on representative sampling stations between 2005-2022 (pH total). (2).



1.2 Current state of aquaculture

In recent years, aquaculture has experimented an exponentially growth to reach the global demand for seafood products (3).

As shown in **figure 1.3**, one of the latest reports from the Food and Agriculture Organization (FAO) indicates that in 2022, aquaculture surpassed capture fisheries for the first time in history as a producer of aquatic animals. While both production methods increased by 4.4% compared to 2020, it is remarkable that aquaculture has achieved a total record of 94.4 million tons of aquatic animals produced.

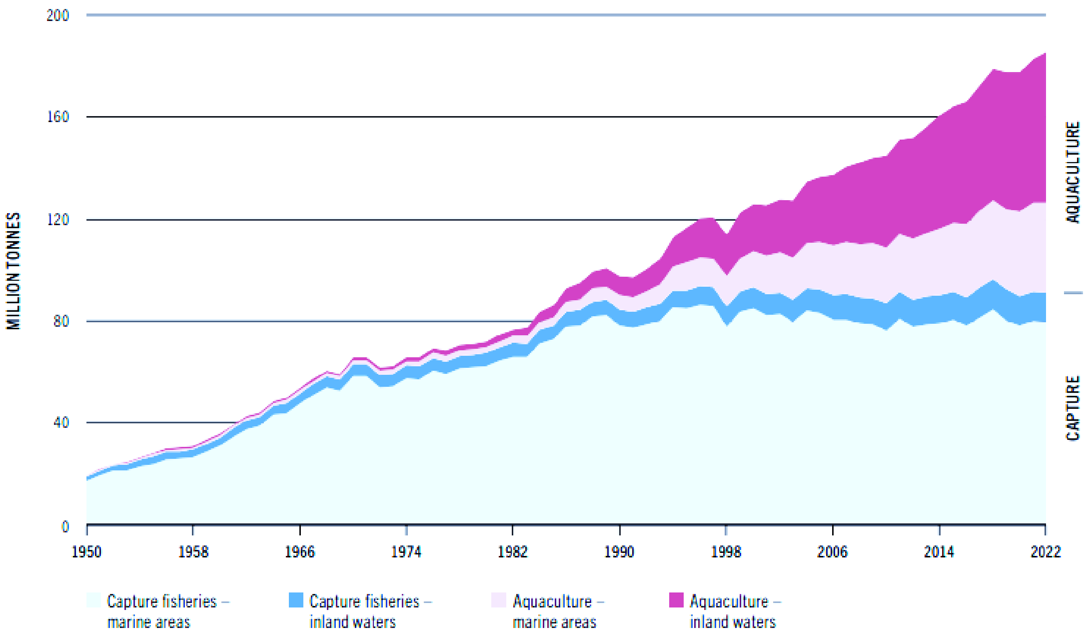


Figure 1.3 Total production of fisheries and aquaculture between 1950-2022. (except algae, aquatic mammals, caimans and crocodiles) (4).

The rapid growth of the sector has developed the creation of a program known as "Blue Transformation" (FAO). The main objective is to achieve sustainability between capture fisheries and aquaculture, ensuring an ecological transformation within the agri-food chain and current food security.

Additionally, **figure 1.4** shows that global per capita consumption has doubled since 1961, increasing from 9.1kg per capita annually to 20.7 kg in 2021.

The consumption of aquatic animals promotes a healthy diet as they provide high-quality proteins. They also represent a very valuable source of essential nutrients such as omega-3 fatty acids, minerals, and vitamins. Nearly all the production is directly going to human consumption. However, about 11% is used for other purposes, mainly for producing fish oil and fishmeal for animal feed.

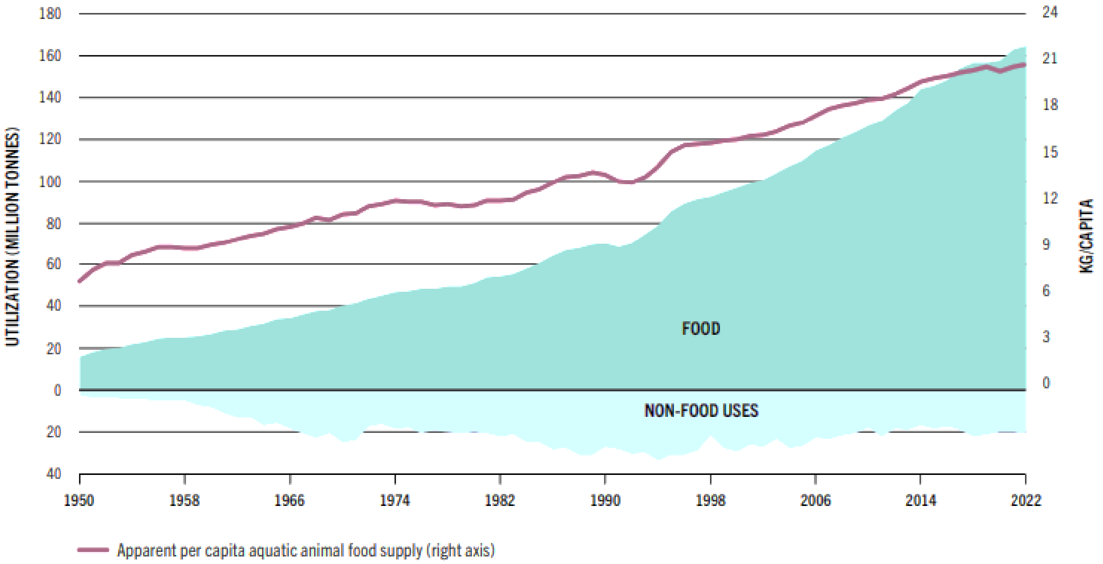


Figure 1.4 Utilization of fisheries and aquaculture production and individual consumption between 1950 and 2022. (except algae, aquatic mammals, caimans and crocodiles) (4).

China, Indonesia and India figure as the first consumers and producers of marine species. Worldwide, in 2021, aquatic animals used for food consumption became around 15% of animal protein and 6% of total protein. However, in **figure 1.5**, we can highlight how countries with low incomes have more availability and affordability of protein sources from aquatic species than from terrestrial animals (4).

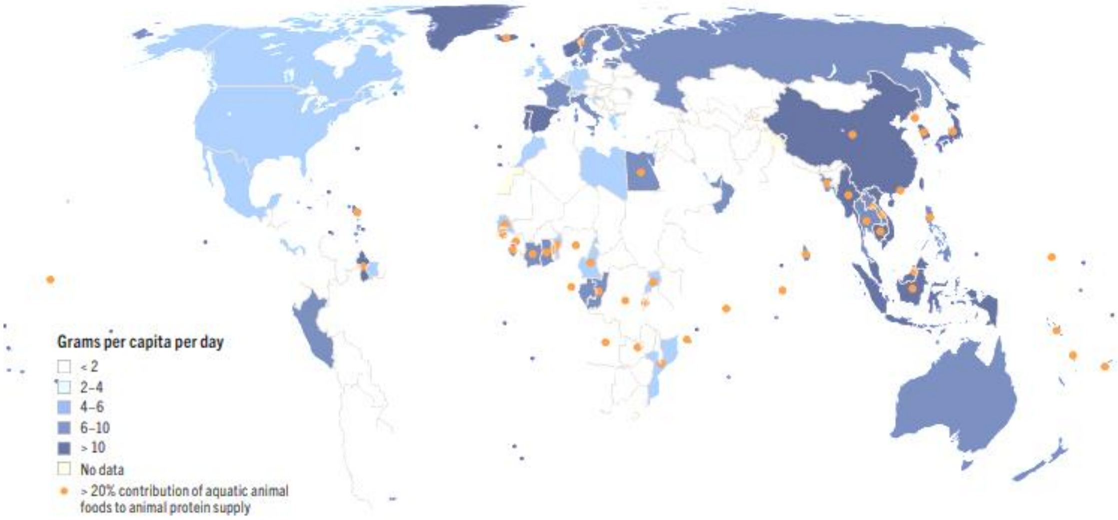
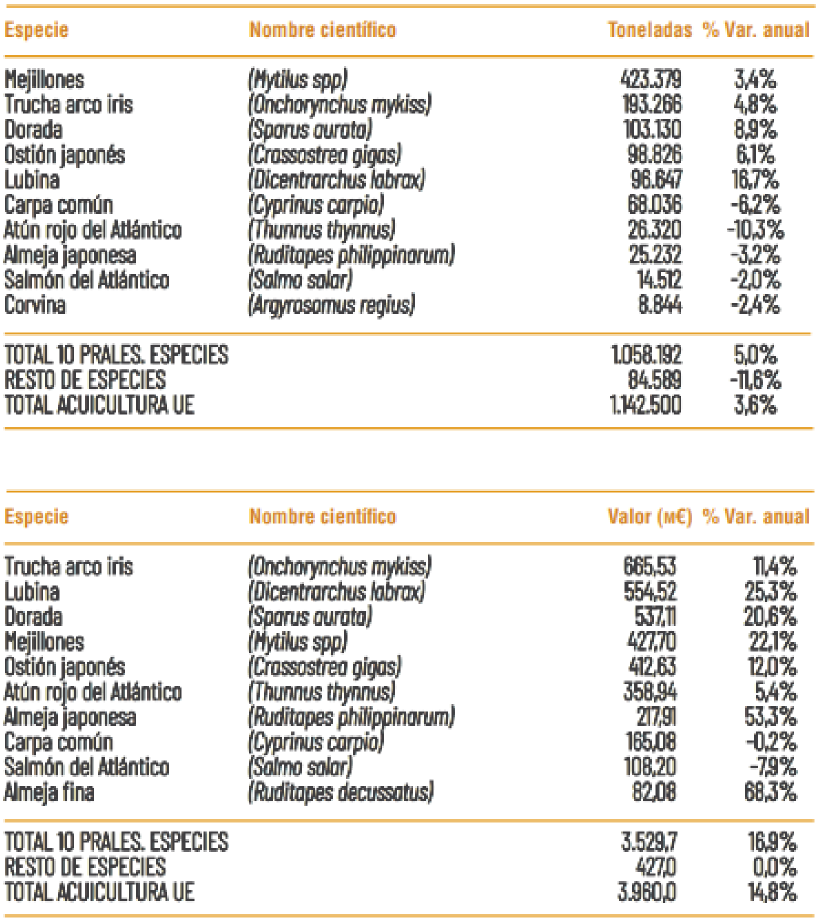


Figure 1.5 Per capita consumption of animal protein from aquatic resource compared with the total animal protein. Average: 2019-2021 (4).

Aquaculture productions are represented by a little number of species. The kombu Japanese algae (*Saccharina japonica*) and Eucheuma seaweed (genus *Eucheuma* and *Kappaphycus*) are the main ones. The list is followed by Japanese oyster (Crassostrea gigas), white shrimp (*Litopenaeus vannamei*) and Chinese carp (*Ctenopharyngodon idella*).

In the European Union, aquaculture production and consumption have not reached the expected levels compared to the global revolution that the sector has experimented over the past decade. Nevertheless, Spain leads the list of producing countries. Considering aquaculture species groups, 70% are fish and 30% are molluscs, while the cultivation of crustaceans and seaweeds have been insignificant.

Therefore, **table 1.1** highlights the fact that the mussel (*Mytilus spp*) is the main cultivated species, followed by rainbow trout (*Oncorhynchus mykiss*) and gilt-head bream (*Sparus aurata*). Nevertheless, fish groups represent a significantly higher monetary contribution (5).



Especie	Nombre científico	Toneladas	% Var. anual
Mejillones	*(Mytilus spp)*	423.379	3,4%
Trucha arco iris	*(Onchorynchus mykiss)*	193.266	4,8%
Dorada	*(Sparus aurata)*	103.130	8,9%
Ostión japonés	*(Crassostrea gigas)*	98.826	6,1%
Lubina	*(Dicentrarchus labrax)*	96.647	16,7%
Carpa común	*(Cyprinus carpio)*	68.036	-6,2%
Atún rojo del Atlántico	*(Thunnus thynnus)*	26.320	-10,3%
Almeja japonesa	*(Ruditapes philippinarum)*	25.232	-3,2%
Salmón del Atlántico	*(Salmo salar)*	14.512	-2,0%
Corvina	*(Argyrosomus regius)*	8.844	-2,4%
TOTAL 10 PRALES. ESPECIES		1.058.192	5,0%
RESTO DE ESPECIES		84.589	-11,6%
TOTAL ACUICULTURA UE		1.142.500	3,6%

Especie	Nombre científico	Valor (M€)	% Var. anual
Trucha arco iris	*(Onchorynchus mykiss)*	665,53	11,4%
Lubina	*(Dicentrarchus labrax)*	554,52	25,3%
Dorada	*(Sparus aurata)*	537,11	20,6%
Mejillones	*(Mytilus spp)*	427,70	22,1%
Ostión japonés	*(Crassostrea gigas)*	412,63	12,0%
Atún rojo del Atlántico	*(Thunnus thynnus)*	358,94	5,4%
Almeja japonesa	*(Ruditapes philippinarum)*	217,91	53,3%
Carpa común	*(Cyprinus carpio)*	165,08	-0,2%
Salmón del Atlántico	*(Salmo salar)*	108,20	-7,9%
Almeja fina	*(Ruditapes decussatus)*	82,08	68,3%
TOTAL 10 PRALES. ESPECIES		3.529,7	16,9%
RESTO DE ESPECIES		427,0	0,0%
TOTAL ACUICULTURA UE		3.980,0	14,8%

Table 1.1 Main production species in aquaculture in UE, in tones (top) and in value (down) in 2021 (5).

With the global population constantly growing and major protein sources declining, aquaculture has emerged as one of the most relevant alternative sources of animal protein (3). Consequently, the intensification of its production has also increase in terms of waste generation.

Nowadays, the focus on the implementation of more sustainable aquaculture models and systems. On one hand, this involves researching methods for treating and utilizing by-products generated during cultivation. On the other hand, it is necessary look for new feeding formulas using by-products from other industries (7).

1.2.1 Traditional aquaculture: Fishmeal and fish oil

As we have seen in **figure 1.3**, it is thought that fish catches will tend to decline, while aquaculture production will star to play a very important role in the global production of fish for human consumption (8).

Feeds represent one of the main costs in aquaculture production. In 2018, 40.1 million metric tons of aquafeed were produced (9).

Throughout the history, aquaculture feeds have included fishmeal (FM) and fish oil (FO) as their principal ingredients. The most important reason is their high nutritional value and excellent levels of polyunsaturated fatty acids. It gives potential benefits for the aquaculture animals and, subsequently, for the health of consumers. FO and FM are processed raw materials made from the fishing of small oily fishes, principally anchovy (*Engraulis encrasicolus*) and sardine (*Sardinella aurita*). These ingredients insure a quick growth rate due to improved palatability (10). However, about 12% of total fish production, which means around 20 million tons, was used to obtain these products, with Peru being the main producer (9).

The latest information assumed that by 2050, when the population increase will be at historic maximum, more than the double of the amount of food and as a result, raw materials. By the way, aquatic animal production will be needed to increase (10). This will put a significant pressure on the inclusion of FM and FO in aquaculture feeds, in terms of economy and ecology.

Nowadays, it has been possible to reduce FM and FO levels by half guarantying production levels and animal health. The issue become from the fact that FM is obtained by drying and grinding the whole fish, using between 4 and 5 tons of the fish to produce 1 ton of dry FM. Furthermore, FO continue being the only source of essential fatty acids in the diet of aquatic animals. All these factors put in danger the possibility of obtained a diet completely independent of these marine animal products (9, 10).

1.2.2 Present and future challenges of aquaculture

Today, as we are living in constant uncertainty changes of raw material prices, there is a huge interest to find new alternative sources that can substitute traditional ones without compromising the nutritional values and growth rates of fishes. This would give to the sector an excellent flexibility in terms of feeding the animals.

On the other hand, there is a focus on more sustainable growth by studying the most efficient ways to use the planet's resources trying to replace raw materials with more eco-friendly alternatives. Thus, reducing the inclusion levels of FM and FO, making the feeding of carnivorous aquaculture species independent of wild fish catches.

The principal strategy of the aquaculture sector is based on decrease the use of natural resources for feed production and growing the production of farmed animals for consumption, without compromising animal health, the final product's quality or consumer safety and acceptance (8, 10).

Currently, the main lines of research in the field of aquaculture nutrition focus on using alternative nutritional sources for fish feed while reducing FM and FO levels in feed formulations.

During the experiments, the impact on growth rates and the coefficients are studied such as the potential effects on the immune system, microbiota and gut health. Also, there is an interest in oxidative stress and disease resistance in animals fed with alternative diets compared to a control diet which includes common levels of FM and FO (8).

1.2.3 New protein sources for aquaculture feed

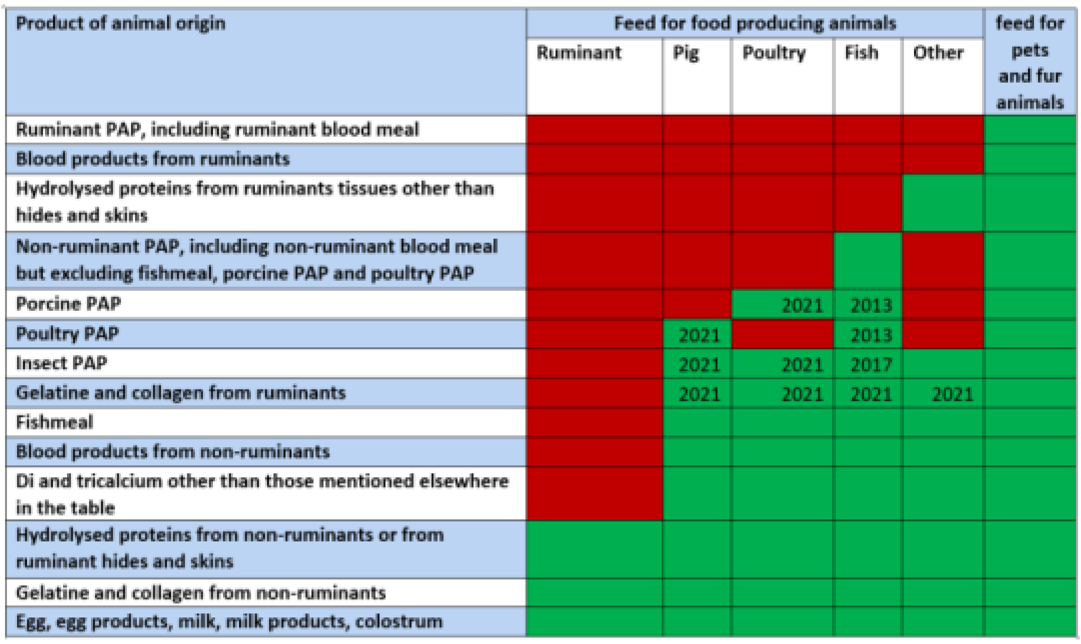
Based on the evidence that feeding routines are constantly changing, there is extensive and dynamic legislation regulated by the European Community which controlled animal feed. Feeds can be found as raw materials, compound feeds, feed additives, premixes or medicated feeds.

Different regulations must be followed depends on the use and type of the feed. Raw materials are animal or plant-based products used just to achieve the basic needs of animals and must include essential nutritional components. In contrast, compound feeds are made from two or more raw materials and may or may not include additives. In addition, additives and medicated products must be chemically defined and scientifically justified. Therefore, attached to the Regulation (EC No 767/2009), there is a list of raw materials for feed and compound feeds that are prohibited or restricted to guarantee food safety. Therefore, the use and commercialization of additives must obey with Regulation (EC No 1831/2003), and the use of medicated feeds must follow to Directive 90/167/EEC (11).

The FEFAC (European Compound Feed Manufacturers' Federation) assure that Spain was considered the leading producer of compound feeds within the EU in 2022. Despite of that, its contribution to the field of aquaculture is deeply insignificant.

Focusing on protein terms, BSE (Bovine Spongiform Encephalopathy) was the main reason for the EU's prohibition on the use of processed animal proteins (PAP) in the production of compound feeds. This led to a 70% decline in the use of these substances as an alternative protein source by 2001. In 2013, the use of insect meal was authorized as a substitute in aquaculture feeds. Over the past decade, sustainability goals and the search for new alternative sources have mandatory to legislation to re-approving the use of some materials (**table 1.2**).

However, they have established standards to prevent cross-contamination that could develop in a food crisis like that of the 1990s (13).



Product of animal origin	Feed for food producing animals					feed for pets and fur animals
	Ruminant	Pig	Poultry	Fish	Other	
Ruminant PAP, including ruminant blood meal	🟥	🟥	🟥	🟥	🟥	🟩
Blood products from ruminants	🟥	🟥	🟥	🟥	🟥	🟩
Hydrolysed proteins from ruminants tissues other than hides and skins	🟥	🟥	🟥	🟥	🟩	🟩
Non-ruminant PAP, including non-ruminant blood meal but excluding fishmeal, porcine PAP and poultry PAP	🟥	🟥	🟥	🟩	🟥	🟩
Porcine PAP	🟥	🟥	🟩 2021	🟩 2013	🟥	🟩
Poultry PAP	🟥	🟩 2021	🟥	🟩 2013	🟥	🟩
Insect PAP	🟥	🟩 2021	🟩 2021	🟩 2017	🟩	🟩
Gelatine and collagen from ruminants	🟥	🟩 2021	🟩 2021	🟩 2021	🟩 2021	🟩
Fishmeal	🟥	🟩	🟩	🟩	🟩	🟩
Blood products from non-ruminants	🟥	🟩	🟩	🟩	🟩	🟩
Di and tricalcium other than those mentioned elsewhere in the table	🟥	🟩	🟩	🟩	🟩	🟩
Hydrolysed proteins from non-ruminants or from ruminant hides and skins	🟩	🟩	🟩	🟩	🟩	🟩
Gelatine and collagen from non-ruminants	🟩	🟩	🟩	🟩	🟩	🟩
Egg, egg products, milk, milk products, colostrum	🟩	🟩	🟩	🟩	🟩	🟩

Table 1.2 Processed animal proteins (PAP) authorized by UE to produced compound feeds (13).

When discussing alternative protein sources in fish feed, aside from animal-based proteins such as by-products from animals or insect meal, there are other options. The most common include plant-based proteins, microalgae or macroalgae, and single-cell proteins (SCP) such as yeasts (14).

In all cases, these products are derived from organisms at lower trophic levels, highlighting the need to move away from FM and FO in order to succeed environmental and food safety challenges (15).

1.2.4 The role of proteins in fish diets

Fish require a very high level of protein for their growth and development. The main energy source is amino acids (AAs), which can be classified into essential (EAA), those that fish cannot synthesize on their own or they have in insufficient quantities and must obtain from external sources, and non-essential (NEAA), the ones that they are able to produce (18).

Fish need 10 EAAs: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (19).

We can highlight glutamate as one of the most abundant AAs since it works as a neurotransmitter, promotes food intake and activates nucleotide synthesis. On the other hand, arginine, taurine, methionine, and lysine are also relevant because their contribution in the feed's aroma, encouraging food consumption. Moreover, glutathione stands out as a bioactive compound.

So, functional AAs must be carefully taken in count when formulating feeds to ensure optimal growth rates and health (18).

Additionally, it should be remarketed that the nutritional value of a product in terms of protein level is measured by its amino acid profile and its richness in EAAs. Moreover, it must have high digestibility, meaning the fish should absorb a high concentration of the nutrients present in the feed to obtain energy (19).

To determine if an ingredient is effective and meets nutritional requirements based on protein levels, we need to look at the apparent digestibility coefficient (ADC), which is calculated according to Maynard et al., 1979.

First, it is needed to calculate the ADCs of experimental diets following the next formula:

ADC (%) = 100 x (1 − dietary Y_2O_3level / feces Y_2O_3level) x

(faeces nutrient or energy level / dietary nutrient or energy level)

The next step to know ADCs of ingredients is:

$\mathrm{ADC}_{\mathrm{ing}}$ (%) = $\mathrm{ADC}_{\mathrm{test}}$ [($\mathrm{ADC}_{\mathrm{test}}$ – $\mathrm{ADC}_{\mathrm{ref}}$) x ((0,85 x D_{ref}) / (0,15 x D_{ing}))]

D_{ref} = g/Kg nutrient of the reference diet

1.2.5 Food industry by-products in aquaculture feeding

The term by-product has recently been introduced in the UE legislation. According to Article 4 of Law 7/2022, a by-product is considered as a qualified substance derived from a production process when its primary purpose does not follow the production of that product. Furthermore, it must be able to be used directly without need any further processing in the normal production chain and must follow all human and environmental health requirements (12).

We have seen how the food industry generates large quantities of waste. In many cases it is discarded in landfills or incinerated, with the potential impact this has on climate change and global warming.

However, most of them could be reused as by-products for the production of aquaculture food due to their high protein value and as a substitute for FM and FO. Some examples would be the residues derived from brewers' grains, soya meal, sunflower meal or rapeseed meal.

To sup up, nowadays there is a huge interest looking for a solution to find ways of formulating feeds that valorise this waste and return it to the market through a circular economy (20).

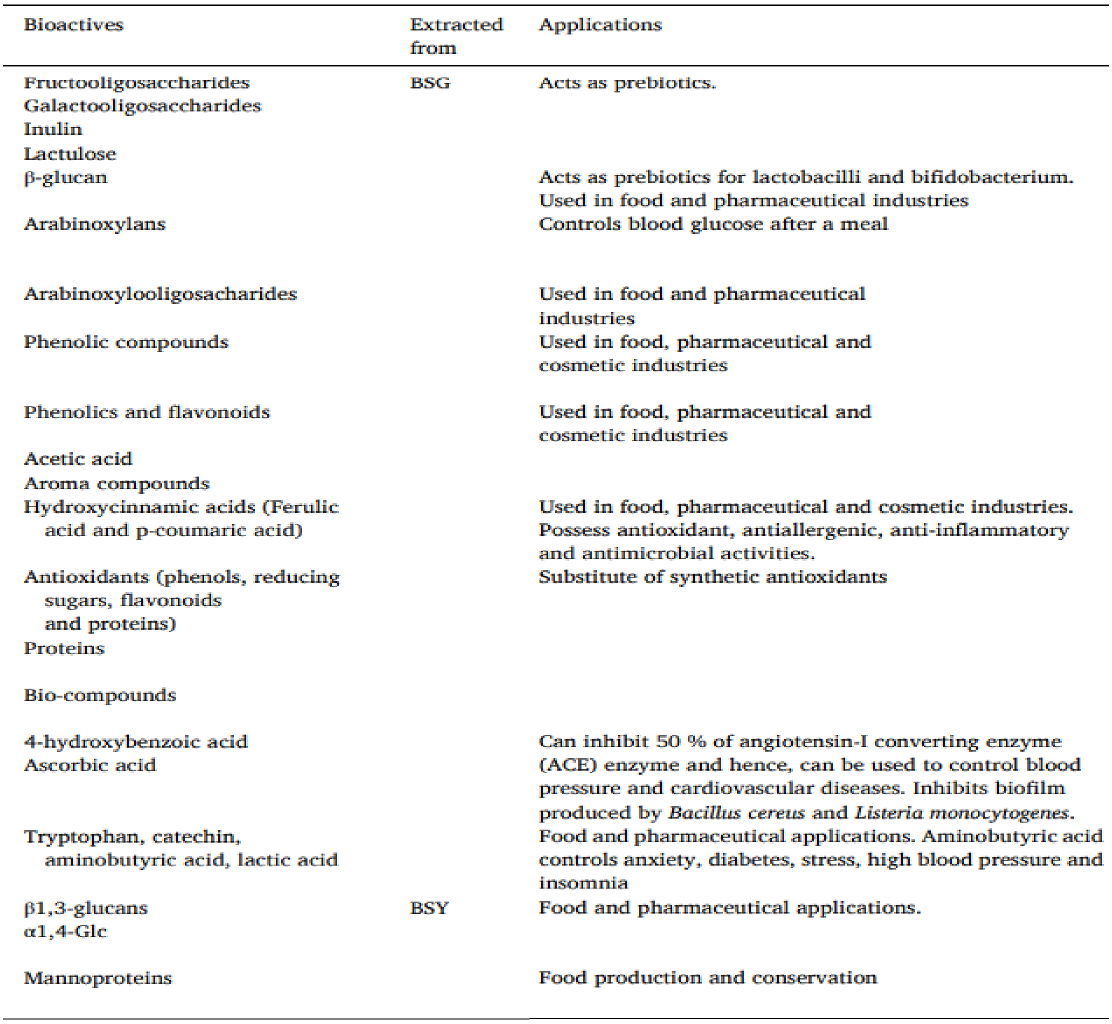
1.2.5.1 Brewery waste

Beer is the most consumed alcoholic drink in the world. Globally, around 2 billion litres are produced, with a hidden facet due to the large quantities of waste it generates. Many of these can be considered by-products. Brewer’s spent grain (SGB), which represents 85% of the waste, and brewer’s spent yeast (SGY) are the ones to highlight.

Only 10% of this waste is disposed of in landfill. Around 70% is destined for the agrifood industry and 20% for biogas production (16).

BSY is a biomass residue of *Saccharomyces sp*. obtained after the fermentation process in brewing beer. Some countries require prior treatment before disposal, others discard it directly into the environment causing harmful effects on the fauna and flora (17).

As it is shown in **table 1.3**, BSG and BSY are nutrient rich residues, with a high protein content and beneficial bioactive for the health (16).



Bioactives	Extracted from	Applications
Fructooligosaccharides Galactooligosaccharides Inulin Lactulose	BSG	Acts as prebiotics.
β-glucan		Acts as prebiotics for lactobacilli and bifidobacterium. Used in food and pharmaceutical industries
Arabinoxylans		Controls blood glucose after a meal
Arabinoxylooligosacharides		Used in food and pharmaceutical industries
Phenolic compounds		Used in food, pharmaceutical and cosmetic industries
Phenolics and flavonoids		Used in food, pharmaceutical and cosmetic industries
Acetic acid		
Aroma compounds		
Hydroxycinnamic acids (Ferulic acid and p-coumaric acid)		Used in food, pharmaceutical and cosmetic industries. Possess antioxidant, antiallergenic, anti-inflammatory and antimicrobial activities.
Antioxidants (phenols, reducing sugars, flavonoids and proteins)		Substitute of synthetic antioxidants
Proteins		
Bio-compounds		
4-hydroxybenzoic acid		Can inhibit 50 % of angiotensin-I converting enzyme (ACE) enzyme and hence, can be used to control blood pressure and cardiovascular diseases. Inhibits biofilm produced by *Bacillus cereus* and *Listeria monocytogenes*.
Ascorbic acid		
Tryptophan, catechin, aminobutyric acid, lactic acid		Food and pharmaceutical applications. Aminobutyric acid controls anxiety, diabetes, stress, high blood pressure and insomnia
β1,3-glucans	BSY	Food and pharmaceutical applications.
α1,4-Glc		
Mannoproteins		Food production and conservation

Table 1.3 Bioactive compounds extracted from BSG and BSY, the most important brewery waste (16).

The nutritional value of BSY is comparable to FM, with a crude protein level around 49% and an excellent amino acid profile. Furthermore, very favourable results have been obtained by including these by-products in fish feed, improving the digestibility and growth index compared to terrestrial animals (17).

Figure 1.6 confirms that the beer industry is very well consolidated in Spain, being the second largest producer of beer in Europe and the ninth largest worldwide. In 2023, it is produces 41.5 million hectolitres Additionally, it generates a large quantity of BSG and BSY. Todays, according to the Spanish Brewers' Association, it is really importance to be able to achieve a reduction of up to 3% of non-recovered waste. Promoting the use of BSG and BSY as ingredients in fish feed is one of its priorities (17).

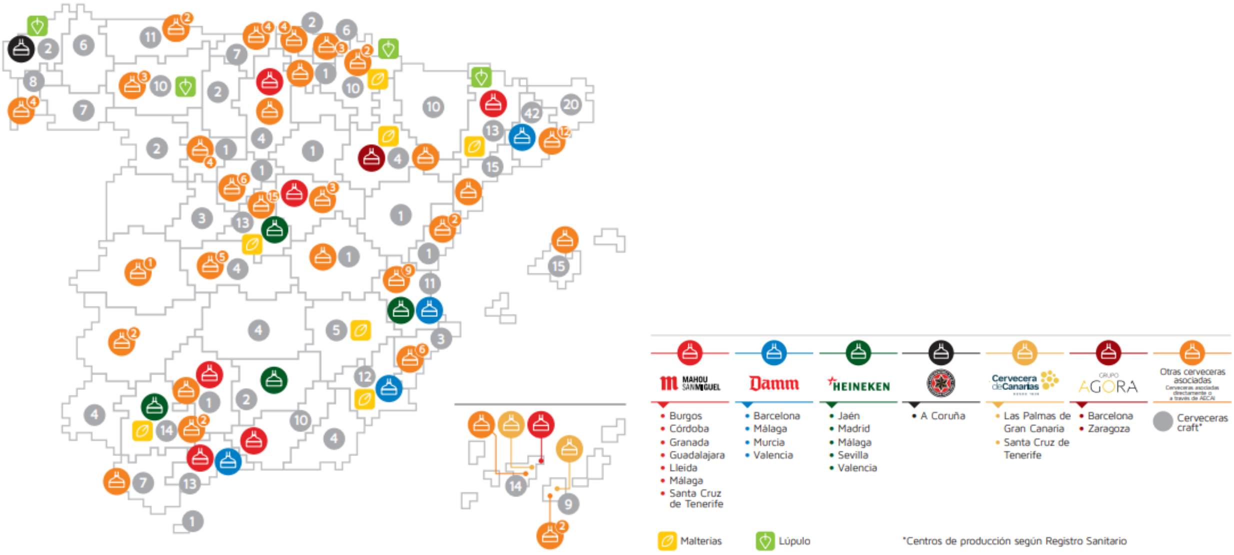


Figure 1.6 Brewery industries around Spanish territory (21).

In the following **figure 1.7** it is shown the beer brewery process with the most relevant steps. In addition, it is remarked in blue which and where are added supplementary ingredients and in red it is observed by-products generation.

The first step is malting where barley grains are germinated in order to produce hydrolytic enzymes used to cut down starches and proteins. Moreover, grains are milled to increase the available of these components.

The next step is mashing where milled malt is mixed with hot water to generate sugars from starches and amino acids from proteins by enzyme activity. Through this process it is produce brewer's spent grain (BSG), the major by-product in the beer production. The final product of these process is a sweat liquid called worth.

In the next stage the worth is boiled adding sugar and hops to achieve a good flavour.

Finally, there is a fermentation of the medicated worth to produce the primary beer product. Through this step it is produced brewer's spent yeast (BSY).

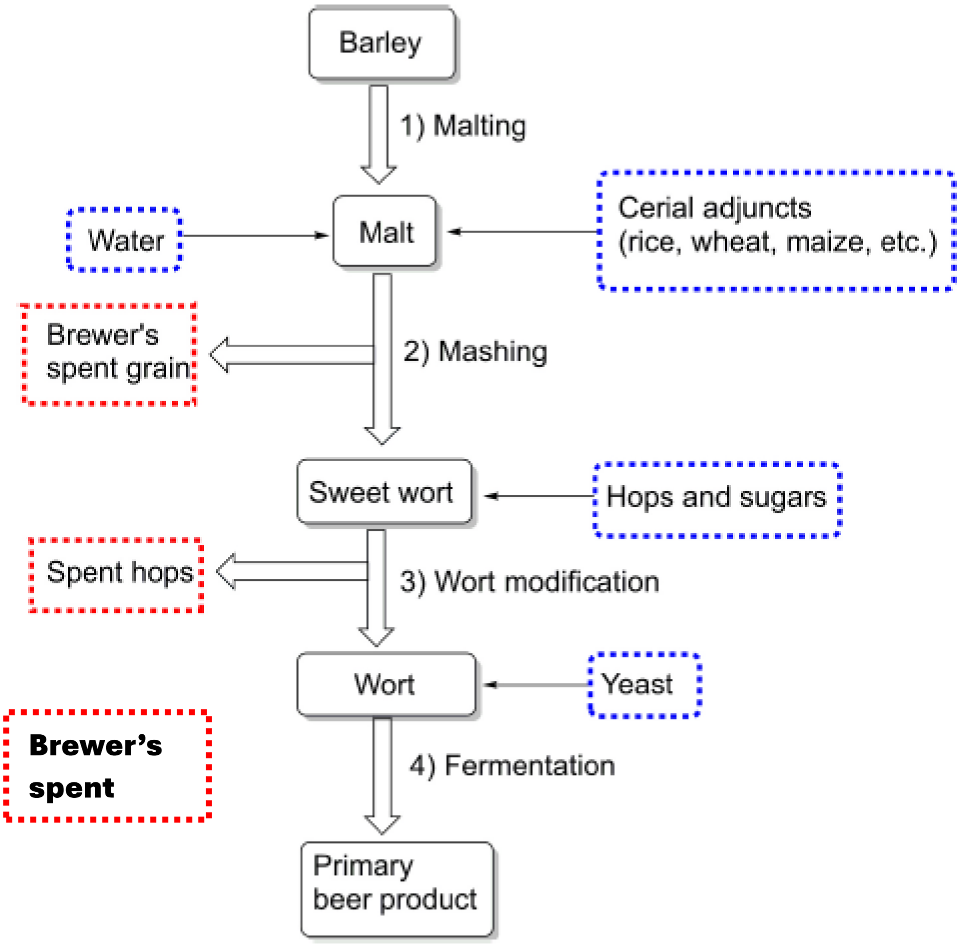


Figure 1.7 Beer brewery process showing where are produce by-products and where are adding supplementary ingredients (24).

2. HYPOTHESES and OBJECTIVES

2.1 Hypotheses

The hypothesis of the review focuses on determine whether we could replace fish oil and fish meal in feed formulations with a by-product from the brewery industry, while ensuring or improving the growth, development and health of the fish.

2.2 Main objective

The main objective of this Final Degree Project is to review the effect of using alternative proteins derived from food industry by-products, specifically the waste from the brewing industry, in aquaculture feed.

2.3 Specific objectives

- Studied if it is necessary any previous treatment for nutrition optimization of brewery waste.
- Determinate the impact of these new protein sources in terms of nutrient digestibility of using by-products to feed the main species produced in our region, such as gilthead sea bream (*Sparus aurata*) as a carnivore marine fish and rainbow trout (*Oncorhynchus mykiss*) as an omnivore freshwater animal.
- Know the optimal replacement of BSG and BSY studying the growth parameters and apparent digestibility coefficient for protein and amino acids in rainbow trout and gilthead seabream feeding.
- Estimate the environmental gains in terms of reduction CO2 emissions.

3. MATERIAL AND METHODS

To carry out this project, a literature review had been done to answer the initial objectives.

Various databases were used, including Web of Science and Science Direct. In addition, other sources, such as the journal "Aquaculture", were also consulted. Basic Boolean Operators as AND, OR and NOT were used in database searches to obtain more precise information.

The keywords used in the WOS database were aquaculture as a topic; brewer*, seabream and rainbow trout contained in the title; and by-product and protein in the abstract. It is used the operator OR between seabream and rainbow trout to find information from both species.

In the Science Direct platform, Aquaculture was the journal where information was looked for. Moreover, it is used the terms brewer waste and by-products.

3.1 Inclusive Criteria

The same inclusion criteria were followed for all the articles:

1. The main objective must be the use of the brewery by-product as an alternative protein source.
2. It must be an application for the following species: gilthead sea bream (*Sparus aurata*) or rainbow trout (*Oncorhynchus mykiss*).
3. Studies must examine growth rates and digestibility index.
4. Year of publication must be after 2020.
5. English language.

3.2 Exclusive Criteria

1. The main objective was not the use of the brewery by-product as an alternative protein source.
2. The study was not carried out with the interest specie.

4. RESULTS

At the end, twelve articles were found in the WOS database where seven of them were discarded. Three of them because of the first exclusive criteria and the other four due to the second exclusive criteria.

In Science Direct, three articles were found. Two of them were the same as it was found in WOS.

It is request for a no-available article due to the interest in the review.

In the following table there is a summarize about the articles used in the results of the review.

Author	Main objective of the study	By-product	Speacies	Bibliographic Resource
J. Nazzaro *et al*., 2021 (23)	Protein aparent digestibility coeficients	Brewer's spent yeast (BSY) Brewer's spent grain (BSG)	Gilthead seabream and rainbow trout	Web of Science Science Direct
D.San Martin *et al*., 2020 (12)	Effectiveness in brewey waste enzimatc hydrolisis method	Brewer's spent yeast (BSY) Brewer's spent grain (BSG)	Gilthead seabream	Web of Science
A.Estévez *et al*. 2022 (6)	Optimal inclusion of brewery waste	Brewer's spent yeast (BSY) Brewer's spent grain (BSG)	Rainbow trout	Web of Science
M. Gokulakrishnan *et al*., 2023 (17)	Sustainablility viewpoint of using brewery waste in aquafeed	Brewer's spent yeast (BSY)	Gilthead seabream and rainbow trout	Science Direct
A.Estévez *et al*., 2021 (20)	Optimal inclusion of brewery waste	Brewer's spent yeast (BSY) Brewer's spent grain (BSG)	Gilthead seabrem	Web of Science Science Direct *Request
Karlsen, Freja, y Peter V. Skov., 2022 (24)	Potencials and limitations from using brewery waste in aquafeed	Brewer's spent yeast (BSG)	Not reported	Web of Science

Table 4.1 Summarize about the articles used in the review including the author, the main objective of the study, the by-product, the specie and the bibliography resource.

4.1 The best previous treatment for nutrition optimization of brewery waste

Karlsen, Freja, and Peter V. Skov (2022) studied the potential limitations and benefits of using BSG as an alternative protein source for feeding aquatic animals. As the main by-product of beer production, BSG is notable for its high protein content, essential amino acids (EAA), cost, and availability. However, as it is shown in **figure 4.1**, it is an ingredient that contains high concentrations of lignin and fibre, which are known as anti-nutritional factors (ANFs) due to their very low digestibility rates. These factors limit the use of BSG in fish feed, affecting the growth and development of the animals.

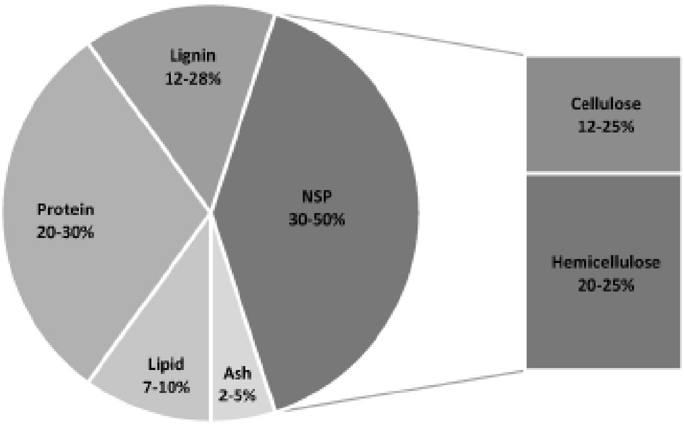


Figure 4.1 Biochemical composition of BSG before any treatment (%) (24).

Looking for a solution, Karlsen, Freja, and Peter V. Skov (2022) reviewed different methods to refine the BSG. The first method (**figure 4.3**) was a fractionation process where it is used a chemical separation to remove the ANFs from BSG. The other technique (**figure 4.2**) was a conversion treatment realizing chemical, biological, and enzymatic reactions, transforming the ANFs into components with higher digestibility.

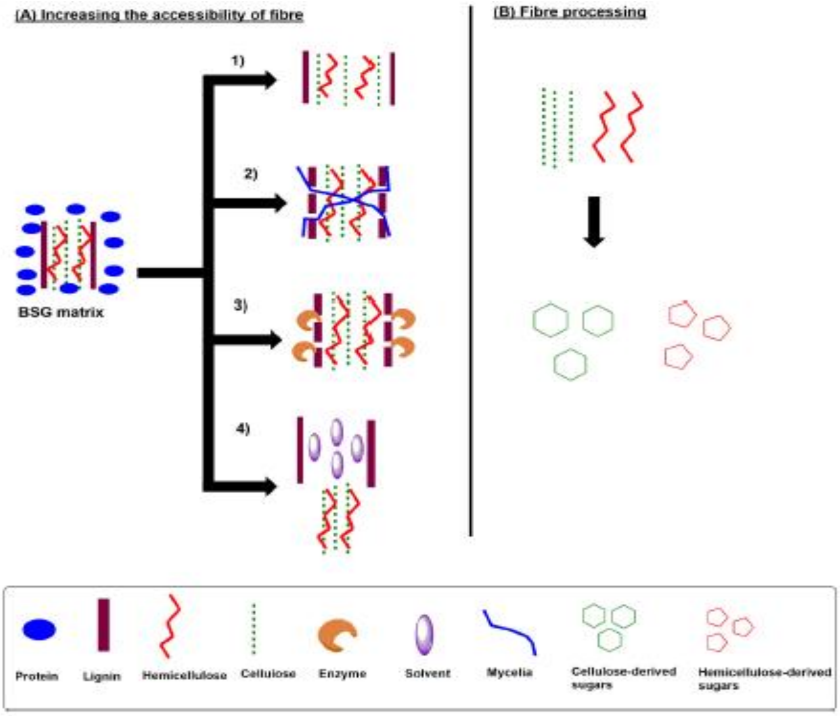


Figure 4.2 Conversion of the lignocellulose to increase the digestibility of fibre (24).

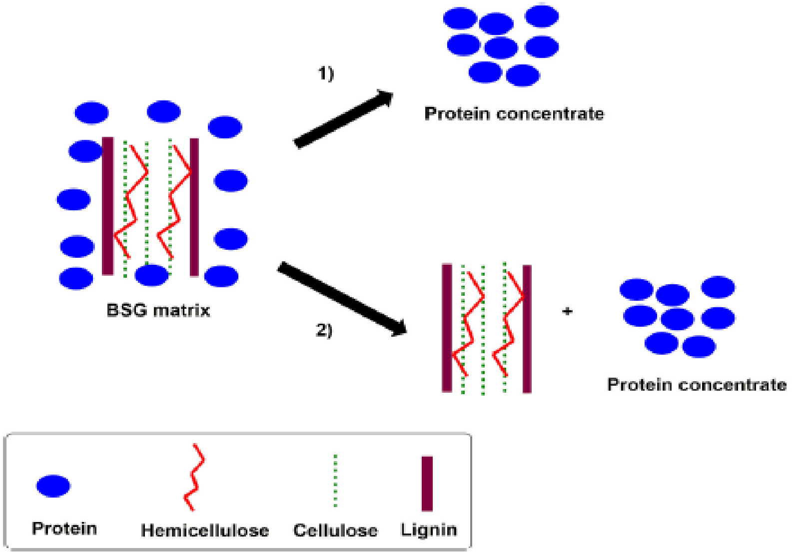


Figure 4.3 Chemical fractionation to remove the protein content from the anti-nutritional factors (24).

Also, different preservation techniques were also analysed to achieve the highest energy efficiency and health benefits for the animals. Three methods were compared: freeze-drying, oven-drying, and LAB (lactic acid bacteria) treatment.

D. San Martin *et al*. (2020) developed an innovative method to improve the digestibility of BSY (Brewer's spent yeast) and BSG (Brewer's spent grain) as alternative protein sources to FM (fish meal) and FO (fish oil). The by-products were exposed to a process of hydrolysis and drying to increase the inclusion rates of these ingredients in the feeds tested on gilthead seabream (*Sparus aurata*). The goal was to achieve the same level of intestinal digestibility as when using other ingredients of marine origin, such as FM and FO.

In order to evaluate the effectiveness of the proteolytic activity in the hydrolysis of BSY and BSG, three treatments based on different combinations of commercial enzymes (Protamex®, Flavourzyme®, and Celluclast®) were used.

Different hydrolysis conditions such as commercial enzyme concentration, temperature and reaction time, were tested in BSY and BSG to determine the optimal conditions for maximum proteolytic activity.

Firstly, the hydrolysis kinetics of each treatment were studied by analysing the total protein content using the Kjeldahl method. Results were obtained for PR (experimental protein released concentration), where K1 and K2 indicated the maximum concentrations of protein released during the first and second phases of the enzymatic kinetics, respectively. Subsequently, the effectiveness of the enzymatic hydrolysis was analysed by SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis), that developed the molecular profile of the different protein hydrolysis treatments by measuring the release of free amino acids (AAs) and peptides.

At the end, the tests confirmed that for BSY, the simultaneous combination of Protamex® and Flavourzyme® was more effective. On the other side, the sequential addition of Celluclast® and Protamex® provided better hydrolysis results for BSG.

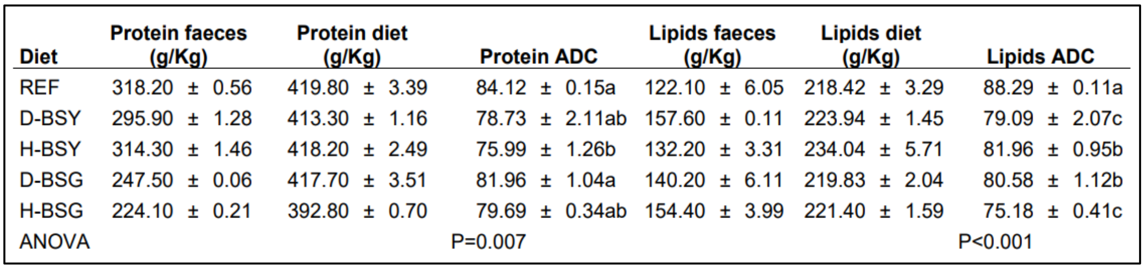
4.2 Is it possible to use brewery waste to feed gilthead seabream and rainbow trout?

J. Nazzaro *et* al. (2021) carried out an experiment to determine the apparent digestibility coefficients (ADCs) of the protein fraction in the use of BSY and BSG in the formulation of feeds for rainbow trout (*Oncorhynchus mykiss*) and gilthead seabream (*Sparus aurata*).

A nutrition assay was developed with 4 ingredients: dried spent yeast (D-BSY), hydrolysed spent yeast (H-BSY), dried spent grain (D-BSG), and hydrolysed spent grain (H-BSG). The hydrolysis process for BSY and BSG had been done according to the previous study by D. San Martin *et al*. (2020).

The test took 30 days while 4 experimental diets were tried against a reference diet. The experimental diets included 20% of spent grain (H-BSG and D-BSY) and 30% of spent yeast (H-BSY and D-BSY) in their two forms, hydrolysed and dried, respectively. The reference diet was a commercial feed with FM and FO levels typically used to achieve the nutritional needs of the two species. The diets included yttrium oxide (Y_2O_3), an inert marker, that allowed the quantification of protein digestibility by biochemical analyses of the feeds and faeces.

In the case of rainbow trout, groups of 20 fishes were distributed in 15 tanks. Faeces were also collected by stripping. As shown in **table 4.2**, the analyses concluded that the apparent digestibility coefficients (ADCs) of protein and lipids in the tested diets goes from 75% to 82%, with a little higher ADCs in the reference diet, where protein ADC was 84% and lipid ADC was 88%.

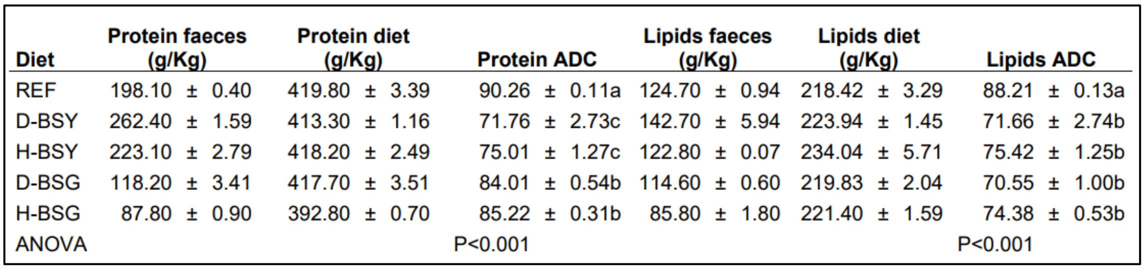


Diet	Protein faeces (g/Kg)	Protein diet (g/Kg)	Protein ADC	Lipids faeces (g/Kg)	Lipids diet (g/Kg)	Lipids ADC
REF	318.20 ± 0.56	419.80 ± 3.39	84.12 ± 0.15a	122.10 ± 6.05	218.42 ± 3.29	88.29 ± 0.11a
D-BSY	295.90 ± 1.28	413.30 ± 1.16	78.73 ± 2.11ab	157.60 ± 0.11	223.94 ± 1.45	79.09 ± 2.07c
H-BSY	314.30 ± 1.46	418.20 ± 2.49	75.99 ± 1.26b	132.20 ± 3.31	234.04 ± 5.71	81.96 ± 0.95b
D-BSG	247.50 ± 0.06	417.70 ± 3.51	81.96 ± 1.04a	140.20 ± 6.11	219.83 ± 2.04	80.58 ± 1.12b
H-BSG	224.10 ± 0.21	392.80 ± 0.70	79.69 ± 0.34ab	154.40 ± 3.99	221.40 ± 1.59	75.18 ± 0.41c
ANOVA			P=0.007			P<0.001

Table 4.2 Apparent digestibility coefficients (ADC, average and SD in %) of the protein and lipid in the experimental and reference diet in rainbow trout (23).

On the other hand, for gilthead seabream, groups of 25 fishes were distributed in 15 tanks, and faeces were collected in sedimentation columns at the end of the tank.

In this case, the following **table 4.3** shows that the ADCs of protein in experimental diets ranged from 71% to 85% compared to a protein ADC of 90% in the reference diet. On the other side, the ADCs of lipids in the RE was 88% and for experimental diets between 71% and 75%.

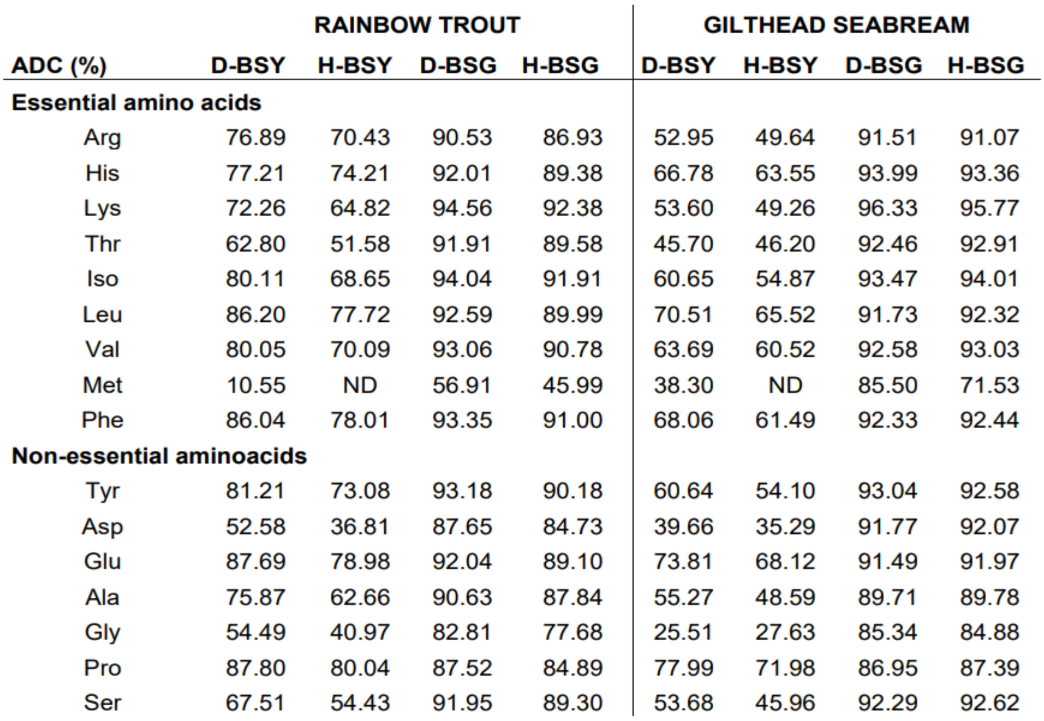


Diet	Protein faeces (g/Kg)	Protein diet (g/Kg)	Protein ADC	Lipids faeces (g/Kg)	Lipids diet (g/Kg)	Lipids ADC
REF	198.10 ± 0.40	419.80 ± 3.39	90.26 ± 0.11a	124.70 ± 0.94	218.42 ± 3.29	88.21 ± 0.13a
D-BSY	262.40 ± 1.59	413.30 ± 1.16	71.76 ± 2.73c	142.70 ± 5.94	223.94 ± 1.45	71.66 ± 2.74b
H-BSY	223.10 ± 2.79	418.20 ± 2.49	75.01 ± 1.27c	122.80 ± 0.07	234.04 ± 5.71	75.42 ± 1.25b
D-BSG	118.20 ± 3.41	417.70 ± 3.51	84.01 ± 0.54b	114.60 ± 0.60	219.83 ± 2.04	70.55 ± 1.00b
H-BSG	87.80 ± 0.90	392.80 ± 0.70	85.22 ± 0.31b	85.80 ± 1.80	221.40 ± 1.59	74.38 ± 0.53b
ANOVA			P<0.001			P<0.001

Table 4.3 Apparent digestibility coefficients (ADC, average and SD in %) of the protein and lipid in the experimental and reference diet in gilthead seabream (23).

Moreover, the apparent digestibility coefficients of essential (EAA) and non-essential amino acids (NEAA) for the different tested ingredients were also shown in the **table 4.4** (23).

M. Gokulakrishnan *et al*. (2023) also determined the amino acid values corrected for total protein digestibility in the ingredients. It is compared spent yeast (BSY) with fishmeal (FM) included in feeds.

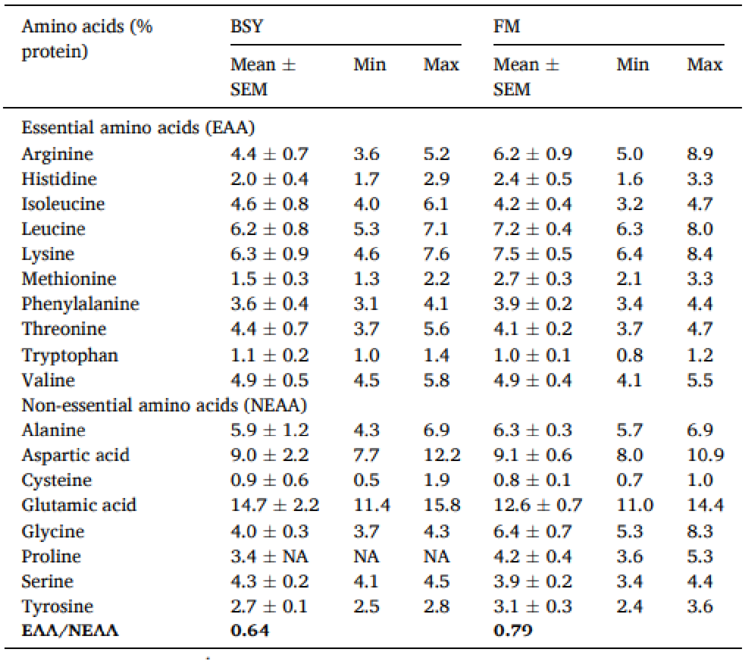


	RAINBOW TROUT				GILTHEAD SEABREAM			
ADC (%)	D-BSY	H-BSY	D-BSG	H-BSG	D-BSY	H-BSY	D-BSG	H-BSG
Essential amino acids								
Arg	76.89	70.43	90.53	86.93	52.95	49.64	91.51	91.07
His	77.21	74.21	92.01	89.38	66.78	63.55	93.99	93.36
Lys	72.26	64.82	94.56	92.38	53.60	49.26	96.33	95.77
Thr	62.80	51.58	91.91	89.58	45.70	46.20	92.46	92.91
Iso	80.11	68.65	94.04	91.91	60.65	54.87	93.47	94.01
Leu	86.20	77.72	92.59	89.99	70.51	65.52	91.73	92.32
Val	80.05	70.09	93.06	90.78	63.69	60.52	92.58	93.03
Met	10.55	ND	56.91	45.99	38.30	ND	85.50	71.53
Phe	86.04	78.01	93.35	91.00	68.06	61.49	92.33	92.44
Non-essential aminoacids								
Tyr	81.21	73.08	93.18	90.18	60.64	54.10	93.04	92.58
Asp	52.58	36.81	87.65	84.73	39.66	35.29	91.77	92.07
Glu	87.69	78.98	92.04	89.10	73.81	68.12	91.49	91.97
Ala	75.87	62.66	90.63	87.84	55.27	48.59	89.71	89.78
Gly	54.49	40.97	82.81	77.68	25.51	27.63	85.34	84.88
Pro	87.80	80.04	87.52	84.89	77.99	71.98	86.95	87.39
Ser	67.51	54.43	91.95	89.30	53.68	45.96	92.29	92.62

Table 4.4 Apparent digestibility coefficients (ADC, average in %) of amino acids in the experimental and reference diet in rainbow trout and gilthead seabream (23).

The protein digestibility of BSY in gilthead seabream was 71.8%, while in trout was 78.7%. In contrast, the digestibility values for FM are around 87.7%. Additionally, the protein content of BSY is 49% with a similar amino acid profile of FM. As it is shown in **table 4.5**, the ratio between essential amino acids (EAA) and non-essential amino acids (NEAA) in BSY is 0.64 and in FM is 0.79. Despite some essential amino acids like threonine and isoleucine are higher in BSY, there is a low level of arginine, lysine, leucine, and methionine compared to the availability in FM.

Karlsen, Freja, and Peter V. Skov (2022) also provided the ratio EAA/NEAA between fishmeal, soybean meal and BSG. The results showed 0.97, 0.82 and 1,84, respectively.



Amino acids (% protein)	BSY			FM		
	Mean ± SEM	Min	Max	Mean ± SEM	Min	Max
Essential amino acids (EAA)						
Arginine	4.4 ± 0.7	3.6	5.2	6.2 ± 0.9	5.0	8.9
Histidine	2.0 ± 0.4	1.7	2.9	2.4 ± 0.5	1.6	3.3
Isoleucine	4.6 ± 0.8	4.0	6.1	4.2 ± 0.4	3.2	4.7
Leucine	6.2 ± 0.8	5.3	7.1	7.2 ± 0.4	6.3	8.0
Lysine	6.3 ± 0.9	4.6	7.6	7.5 ± 0.5	6.4	8.4
Methionine	1.5 ± 0.3	1.3	2.2	2.7 ± 0.3	2.1	3.3
Phenylalanine	3.6 ± 0.4	3.1	4.1	3.9 ± 0.2	3.4	4.4
Threonine	4.4 ± 0.7	3.7	5.6	4.1 ± 0.2	3.7	4.7
Tryptophan	1.1 ± 0.2	1.0	1.4	1.0 ± 0.1	0.8	1.2
Valine	4.9 ± 0.5	4.5	5.8	4.9 ± 0.4	4.1	5.5
Non-essential amino acids (NEAA)						
Alanine	5.9 ± 1.2	4.3	6.9	6.3 ± 0.3	5.7	6.9
Aspartic acid	9.0 ± 2.2	7.7	12.2	9.1 ± 0.6	8.0	10.9
Cysteine	0.9 ± 0.6	0.5	1.9	0.8 ± 0.1	0.7	1.0
Glutamic acid	14.7 ± 2.2	11.4	15.8	12.6 ± 0.7	11.0	14.4
Glycine	4.0 ± 0.3	3.7	4.3	6.4 ± 0.7	5.3	8.3
Proline	3.4 ± NA	NA	NA	4.2 ± 0.4	3.6	5.3
Serine	4.3 ± 0.2	4.1	4.5	3.9 ± 0.2	3.4	4.4
Tyrosine	2.7 ± 0.1	2.5	2.8	3.1 ± 0.3	2.4	3.6
EAA/NEAA	**0.64**			**0.79**		

Taula 4.5 Comparation of the amino acids profile between brewer spent yeast (BSY) and fish meal (FM) (% protein) (17).

4.3 Optimal replacement of BSY and BSG in rainbow trout feeding.

Once it was determined the digestibility of the products and known the reasonable nutritional profile, A. Estévez *et al*. (2022) analysed the optimal inclusion levels of BSY and BSG, both hydrolysed and dried, in feeds for rainbow trout (Oncorhynchus mykiss).

The study was carried out to test two inclusion levels of each ingredient. Brewery's spent grain (hydrolysed and dried) was tested at 7.5% and 15%. On the other side, brewery's spent yeast (hydrolysed and dried) was tested at 10% and 20% inclusion in the feeds. Moreover, a reference diet (CTRL) and a diet including 20% of commercial hydrolysed or dried yeast (ABN) were used to compare the results.

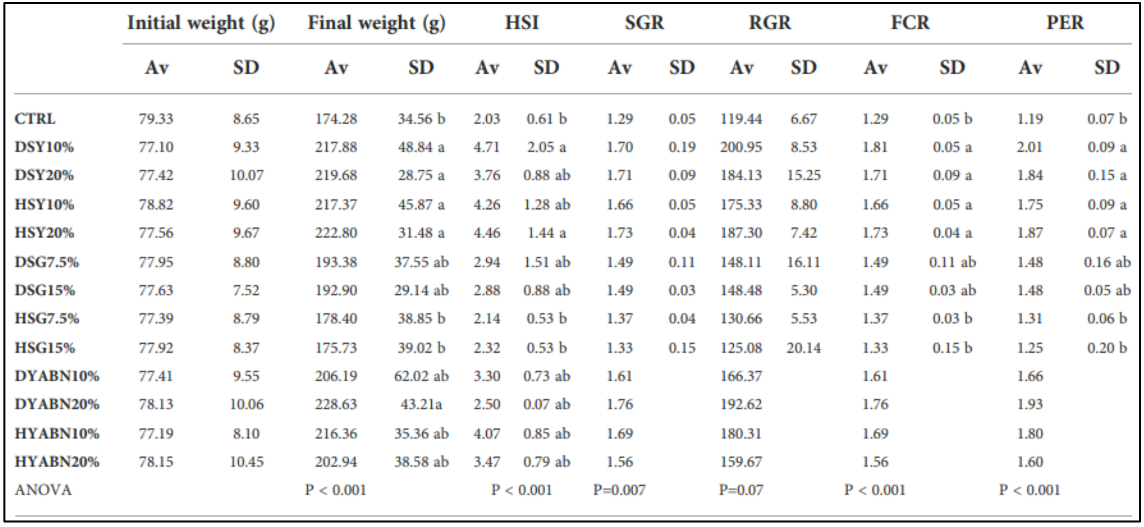
The nutritional test took 60 days. Each treatment was done by triplicate. So, there were a total of 22 tanks with 15 fishes in each. The diets included yttrium oxide (Y_2O_3) as a marker to determine digestibility by analyses of the feeds and faeces.

The **table 4.6** remarked the digestibility coefficients for the highest inclusion levels of different ingredients that were tested. The protein ADC was around 75% for 20% BSY (hydrolysed and dried) and the reference diet. In contrast, both 15% of H-BSG and D-BSG 15% had ADCs going from 49% to 65%, respectively.

Apparent Digestibility Coefficients of Ingredients						
	D-BSY 20%	**H-BSY 20%**	**D-BSG 15%**	**H-BSG 15%**	**DY-ABN 20%**	**HY-ABS 20%**
Protein	74.52 ± 1.62a	75.37 ± 1.47a	65.10 ± 2.90b	49.93 ± 3.32c	78.62 ± 1.27a	76.09 ± 1.35a

Taula 4.6 Apparent digestibility coefficients (ADC, average and SD in %) of protein contented in the experimental and reference ingredients in rainbow trout (6).

On the other hand, growth parameters such as SGR (specific growth factor), RGR (relative growth factor), feed conversion ratio (FDR) and protein efficiency ratio (PER) were analysed. As it is shown in **table 4.7** Fishes fed with 20% BSY (dried or hydrolysed) grew more and had a higher PER than CRTL.



	Initial weight (g)		Final weight (g)		HSI		SGR		RGR		FCR		PER	
	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD
CTRL	79.33	8.65	174.28	34.56 b	2.03	0.61 b	1.29	0.05	119.44	6.67	1.29	0.05 b	1.19	0.07 b
DSY10%	77.10	9.33	217.88	48.84 a	4.71	2.05 a	1.70	0.19	200.95	8.53	1.81	0.05 a	2.01	0.09 a
DSY20%	77.42	10.07	219.68	28.75 a	3.76	0.88 ab	1.71	0.09	184.13	15.25	1.71	0.09 a	1.84	0.15 a
HSY10%	78.82	9.60	217.37	45.87 a	4.26	1.28 ab	1.66	0.05	175.33	8.80	1.66	0.05 a	1.75	0.09 a
HSY20%	77.56	9.67	222.80	31.48 a	4.46	1.44 a	1.73	0.04	187.30	7.42	1.73	0.04 a	1.87	0.07 a
DSG7.5%	77.95	8.80	193.38	37.55 ab	2.94	1.51 ab	1.49	0.11	148.11	16.11	1.49	0.11 ab	1.48	0.16 ab
DSG15%	77.63	7.52	192.90	29.14 ab	2.88	0.88 ab	1.49	0.03	148.48	5.30	1.49	0.03 ab	1.48	0.05 ab
HSG7.5%	77.39	8.79	178.40	38.85 b	2.14	0.53 b	1.37	0.04	130.66	5.53	1.37	0.03 b	1.31	0.06 b
HSG15%	77.92	8.37	175.73	39.02 b	2.32	0.53 b	1.33	0.15	125.08	20.14	1.33	0.15 b	1.25	0.20 b
DYABN10%	77.41	9.55	206.19	62.02 ab	3.30	0.73 ab	1.61		166.37		1.61		1.66	
DYABN20%	78.13	10.06	228.63	43.21a	2.50	0.07 ab	1.76		192.62		1.76		1.93	
HYABN10%	77.19	8.10	216.36	35.36 ab	4.07	0.85 ab	1.69		180.31		1.69		1.80	
HYABN20%	78.15	10.45	202.94	38.58 ab	3.47	0.79 ab	1.56		159.67		1.56		1.60	
ANOVA			P < 0.001		P < 0.001		P=0.007		P=0.07		P < 0.001		P < 0.001	

Taula 4.7 Growth parameters SGR (specific growth factor), RGR (relative growth factor), FDR (feed conversion ratio) and PER (protein efficiency ratio) (average and SD in %) of protein contented in the experimental and reference groups in rainbow trout trial (6).

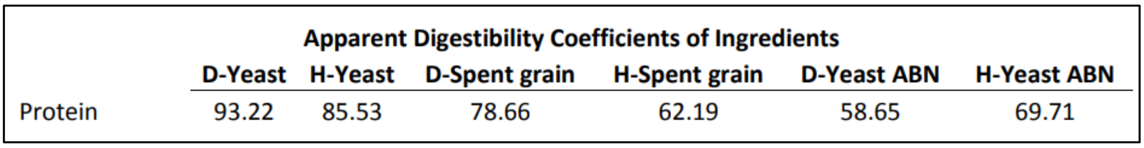
Furthermore, protein levels in liver and muscle were examined. Muscle protein content was higher in fish fed 20% H-BSY and 10% commercial yeast (HY-ABN) diets. In opposition, the highest protein content in the liver was found in fish fed with 7.5% D-BSG and 15% H-BSG diets.

4.4 Optimal replacement of BSY and BSG in gilthead seabream feeding.

In the same way, A. Estévez *et al*. (2021) determined the most effective inclusion levels of BSY and BSG for fed gilthead seabream (*Sparus aurata*).

Two trials were done. The duration of the first was 70 days. 22 fishes were distributed per tank with a total of 22 tanks. It was used the same diets tested in rainbow trout. In the second assay, they increase the inclusion levels of byproducts, using 30% BSY and 20% BSG for 60 days of trial. Moreover, they reduce fishmeal (FM) levels from 15% to 10%. There was a total of 15 tanks, each with 15 fishes.

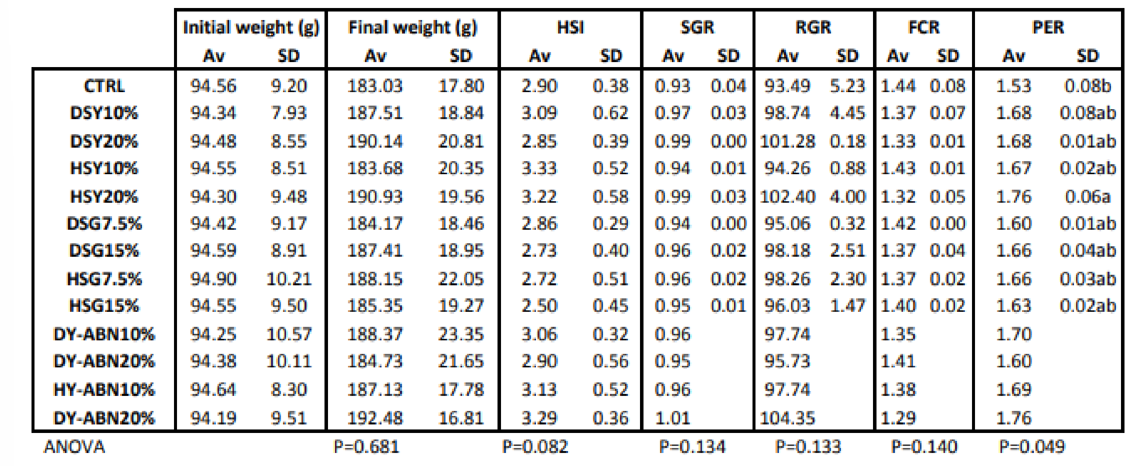
In the **table 4.8** it is shown the digestibility coefficient of the first trial. Diets with dried spent yeast (D-BSY) with the highest inclusion level was 93%, dried spent grain at 15% (D-BSG) was 78% and commercial yeast DY-ABN was 58%. In addition, the ADCs of the hydrolysed forms were 85%, 62%, and 69%, respectively.



	Apparent Digestibility Coefficients of Ingredients					
	D-Yeast	H-Yeast	D-Spent grain	H-Spent grain	D-Yeast ABN	H-Yeast ABN
Protein	93.22	85.53	78.66	62.19	58.65	69.71

Taula 4.8 Apparent digestibility coefficients (ADC, average and SD in %) of protein contented in the experimental and reference ingredients in gilthead seabream (20).

On the other hand, growth parameters (**taula 4.9**) were analysed in which SGR, RGR, FDR and PER were determined. No significant differences were observed between the alternative diets and the control except for the PER of the group fed 20% H-BSY which had a significantly higher value than the control and equal to DY-ABN, 1.76, 1.53 and 1,76, respectively.



	Initial weight (g)		Final weight (g)		HSI		SGR		RGR		FCR		PER	
	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD
CTRL	94.56	9.20	183.03	17.80	2.90	0.38	0.93	0.04	93.49	5.23	1.44	0.08	1.53	0.08b
DSY10%	94.34	7.93	187.51	18.84	3.09	0.62	0.97	0.03	98.74	4.45	1.37	0.07	1.68	0.08ab
DSY20%	94.48	8.55	190.14	20.81	2.85	0.39	0.99	0.00	101.28	0.18	1.33	0.01	1.68	0.01ab
HSY10%	94.55	8.51	183.68	20.35	3.33	0.52	0.94	0.01	94.26	0.88	1.43	0.01	1.67	0.02ab
HSY20%	94.30	9.48	190.93	19.56	3.22	0.58	0.99	0.03	102.40	4.00	1.32	0.05	1.76	0.06a
DSG7.5%	94.42	9.17	184.17	18.46	2.86	0.29	0.94	0.00	95.06	0.32	1.42	0.00	1.60	0.01ab
DSG15%	94.59	8.91	187.41	18.95	2.73	0.40	0.96	0.02	98.18	2.51	1.37	0.04	1.66	0.04ab
HSG7.5%	94.90	10.21	188.15	22.05	2.72	0.51	0.96	0.02	98.26	2.30	1.37	0.02	1.66	0.03ab
HSG15%	94.55	9.50	185.35	19.27	2.50	0.45	0.95	0.01	96.03	1.47	1.40	0.02	1.63	0.02ab
DY-ABN10%	94.25	10.57	188.37	23.35	3.06	0.32	0.96		97.74		1.35		1.70	
DY-ABN20%	94.38	10.11	184.73	21.65	2.90	0.56	0.95		95.73		1.41		1.60	
HY-ABN10%	94.64	8.30	187.13	17.78	3.13	0.52	0.96		97.74		1.38		1.69	
DY-ABN20%	94.19	9.51	192.48	16.81	3.29	0.36	1.01		104.35		1.29		1.76	
ANOVA			P=0.681		P=0.082		P=0.134		P=0.133		P=0.140		P=0.049	

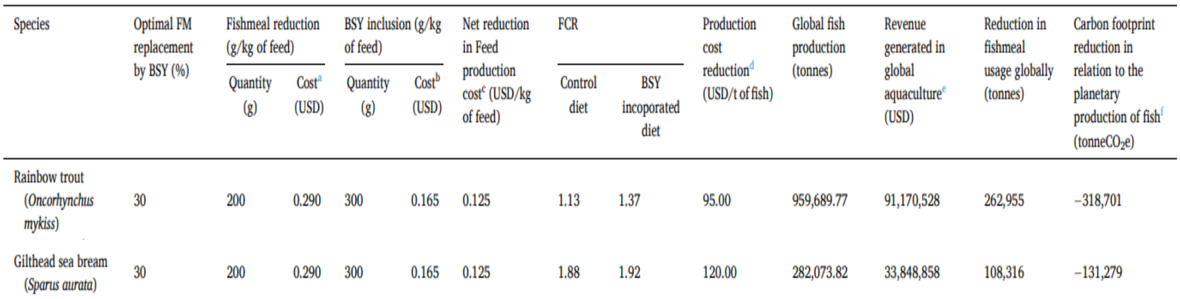
Taula 4.9 Growth parameters SGR (specific growth factor), RGR (relative growth factor), FDR (feed conversion ratio) and PER (protein efficiency ratio) (average and SD in %) of protein contented in the experimental and reference groups in gilthead seabream first trial (20).

E. Estévez *et al.* (2021) informed that in the second trial, better growth results and higher protein levels in the fillet and liver were succeeded with 30% BSY compared to the reference diet, even with reduced FO levels on it.

4.5 Environmental gains

In the last point, M. Gokulakrishnan *et al.* (2023) reviewed the effectiveness in terms of production and sustainability of substituting FM with BSY in different marine species.

The **table 4.8** suggests that reducing CO_2 emissions in relation with marine animal production, from aquaculture or wild capture, would be 318.000 tons if rainbow trout were fed with 30% BSY instead of FM. Additionally, 131.279 tons of CO_2 emissions would be keep away from planet in the case of gilthead seabream.



Species	Optimal FM replacement by BSY (%)	Fishmeal reduction (g/kg of feed)		BSY inclusion (g/kg of feed)		Net reduction in Feed production cost[c] (USD/kg of feed)	FCR		Production cost reduction[d] (USD/t of fish)	Global fish production (tonnes)	Revenue generated in global aquaculture[e] (USD)	Reduction in fishmeal usage globally (tonnes)	Carbon footprint reduction in relation to the planetary production of fish[f] (tonneCO_2e)
		Quantity (g)	Cost[a] (USD)	Quantity (g)	Cost[b] (USD)		Control diet	BSY incoporated diet					
Rainbow trout (*Oncorhynchus mykiss*)	30	200	0.290	300	0.165	0.125	1.13	1.37	95.00	959,689.77	91,170,528	262,955	−318,701
Gilthead sea bream (*Sparus aurata*)	30	200	0.290	300	0.165	0.125	1.88	1.92	120.00	282,073.82	33,848,858	108,316	−131,279

Taula 4.8 Economic and environmental impact of substitution of FM with BSY in rainbow trout and gilthead seabream (17).

5. DISCUSSION

This review has shown that by-products of the brewing industry, BSG (brewer's spent grain) and BSY (brewer's spent yeast), are good candidates due to their high protein content to potentially replace or reduce the inclusion rates of fishmeal (FM) and fish oil (FO) in aquaculture feeds, specifically for the two most farmed species in the Mediterranean, gilthead seabream and rainbow trout.

Firstly, we focused on reviewing studies that explored the pretreatment of these by-products to maximize protein availability. Karlsen, Freja, and Peter V. Skov highlight the importance of removing anti-nutritional factors from the protein portion to increase the digestibility of essential amino acids (EAAs). They studied conversion and fractionation methods to eliminate components that hinder protein hydrolysis.

On the other hand, D. San Martin *et al*. determined that the best pretreatment using commercial enzymes for BSY is the simultaneous combination of Protamex® and Flavourzyme®, and for BSG, the sequential addition of Celluclast® and Protamex®.

It is crucial to determine the nutritional quality of an ingredient by measuring its digestibility before calculating the optimal inclusion rate. The apparent digestibility coefficients (ADCs) for protein and lipids are slightly lower in the experimental diets that include brewing by-products (BSG and BSY) compared to the reference diet in both species.

Notably, in rainbow trout, BSY and BSG have higher ADCs in their dried form than in their hydrolysed form. In contrast, in gilthead seabream, the apparent digestibility coefficients are higher in diets that include hydrolysed BSG (H-BSG) and hydrolysed BSY (H-BSY) compared to dried BSG (D-BSG) and dried BSY (D-BSY).

Comparing the two by-products, in all cases, brewer's spent grain (BSG), both dried and hydrolysed, has better digestibility than brewer's spent yeast (BSY).

At the species level, rainbow trout has higher digestibility levels than gilthead seabream with diets that include BSG in any of its forms. However, the ADC for gilthead seabream with feeds containing dried brewer's yeast (D-BSY) is significantly higher, and the hydrolysed form is similar to that of rainbow trout.

Regarding AA data, it is important to note the low digestibility levels of methionine in both by-products, due to the low levels present in the ingredients, particularly in brewer's spent yeast (BSY). However, the ADC for the rest of the essential amino acids is above 85% in both forms of brewer's spent grain (BSG).

Moreover, if we consider the protein digestibility values of BSY, they are very similar to those of FM in both species. However, although the EAA/NEAA ratio does not differ significantly between BSY (0.64) and FM (0.79), having such low levels of certain AAs, especially methionine and arginine, makes BSY unsuitable as the primary protein source in the diet. Therefore, it is impossible to completely replace FM with BSY as the sole protein source.

It is also important to compare the EAA/NEAA ratio between FM, soybean meal and BSG. BSG has a higher value, although the lack of some essential AAs still makes a complete substitution for FM impossible. Nevertheless, it could be a good alternative to soybean meal.

As shown by the results of the experiment conducted by E. Estévez *et al*. (2022), regarding the trial with rainbow trout, the apparent digestibility coefficient of 20% BSY is similar to that of the diet that also uses 20% commercial yeast. Similarly, it is true that there are no significant differences between using yeast that is mechanically dried or treated by enzymatic hydrolysis. However, the ADC of H-BSG at 15% is notably lower, making prior hydrolysis unnecessary.

On the other hand, in the experiment with gilthead seabream, much higher digestibility was obtained with 20% D-BSY, followed by 15% D-BSG and 20% commercial yeast (DY-ABN). It is important to note that with both by-products, better digestibility is achieved without prior hydrolysis. However, in the case of commercial yeast, the results are better with the hydrolysed ingredient (HY-ABN) than with the dried one (DY-ABN). There is also important to remarkable that PER is higher in 20% H-BSY and HY-ABN without growth differences with the control group.

In the study on the efficient use of BSY conducted by M. Gokulakrishnan et al., it is highlighted that the production of rainbow trout is significantly higher than that of gilthead seabream, making the use of BSY as a by-product for their feed more optimal, both because the reduction in greenhouse gas emissions is higher and due to the reduced use of fishmeal in the formulation of the feeds that nourish them.

6. CONCLUCION

The global population is on the rise, and the UN and FAO have set goals to eradicate hunger and combat the effects of climate change. Within this context, sources of animal protein are in decline and are a limiting factor in achieving these goals. Similarly, large amounts of by-products are wasted daily in landfills and incinerators, which is a limiting factor in this race towards sustainability. Knowing that Spain is a territory where the brewing industry is very present, and the residues of this industry are continue growing can be repurposed within the aquatic food sector. The viability of their use has been determined for the two most produced species in aquaculture in our territory.

In the case of gilthead bream (*Sparus aurata*), the results confirmed in terms protein digestibility the ability to include up to 30% of brewer’s spent yeast (BSY) and 15% of brewer’s spent grain (BSG) as a protein source, as the protein digestibility results were similar to feeds that include fishmeal (FM). In all cases, the dried by-products had better results that hydrolysed ingredients.

Focusing on the use of brewery by-products for the formulation of feeds for rainbow trout (*Oncorhynchus mykiss*), it would be viable in terms of growth parameters and protein digestibility to use feeds with an inclusion of 20% spent brewer's yeast (BSY), regardless of whether it is hydrolysed or dried. However, it is recommended not to include brewer’s spent grain (BSG) in concentrations higher than 15%.

In both cases, they would be a good protein source to reduce the use of marine-derived ingredients like fishmeal (FM) and fish oil (FO) and other less sustainable plant-based ingredients promoting the decrease of CO_2 emissions. However, both BSG and BSY have an AA profile that makes impossible to achieve a diet completely independent of FM and FO due to the low levels of some essential AAs, such as methionine.

It will be interesting in the near future to continue investigating different challenges to increase the inclusion quantity of these by-products. More trials will be needed to achieve a better quality of protein content. Also, reviewing the use of brewery waste to produce aquafeed for other species it will be a good option.

To conclude, the valorisation and reuse of these industrial by-products would be a clear option to increase the sustainability of the brewing sector and aquaculture. At the same time, it would be an alternative to promoting a circular economy within a field that, in recent years, aims to achieve the goals set by the FAO's Blue Transformation and the 2030 Agenda to end malnutrition and food insecurity while achieving efficient use of marine resources.

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